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Contaminantes emergentes en matrices ambientales acuáticas. Desarrollo de metodologías para el análisis químico y determinación de su prevalencia

Tesis para optar al grado de doctor en Ciencias y Tecnología Analítica

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RESUMEN

Un amplio número de compuestos de diversa naturaleza química, conforman el grupo de los contaminantes de preocupación emergente (CECs): los productos farmacéuticos, los productos de cuidado personal, pesticidas, nanomateriales, contaminantes perfluorados, retardantes de llama, ftalatos, entre otros. Muchos de estos a la vez, pueden ser sub-agrupados como disruptores endocrinos (DEs): compuestos que interfieren mimetizando, antagonizando o bloqueando la función hormonal endógena de los organismos vivos. Los productos farmacéuticos, están diseñados para producir una respuesta biológica en un organismo objetivo, por lo tanto, aunque estos se encuentren a concentraciones trazas en las aguas medioambientales, pueden producir la misma respuesta en entidades no objetivo tras una exposición crónica. El amplio uso en todo el mundo de los CECs como parte de la actividad antropogénica, ha generado efectos tóxicos y de bioacumulación indeseables, en los ecosistemas acuáticos y terrestres. Algunos de ellos, reportados en humanos y otras especies, como alteraciones al sistema endocrino, toxicidad crónica y la resistencia a antibióticos. Desafortunadamente, las plantas de tratamiento de aguas residuales (PTAR) no fueron diseñadas para eliminar completamente a los CECs, y en consecuencia, estos se han encontrado en ambientes acuáticos como efluentes, aguas superficiales y subterráneas alrededor del mundo, en rangos de concentración de mg/L y ng/L, incluso en el continente Antártico. Chile se encuentra dentro del grupo de países donde el estudio de los CECs es insuficiente en la actualidad, por lo tanto, existe una brecha de conocimiento sobre cuán expuestos nos encontramos a ellos, a pesar de que la OMS declaró en el año 2012, que las investigaciones sobre CECs resultan primordiales, en aras de poder evaluar sus posibles efectos negativos sobre el medio ambiente y poder emitir futuros lineamientos y regulaciones que permitan asegurar la

calidad de las aguas. Por tanto, el desarrollo de nuevas técnicas analíticas que permitan el análisis de un amplio grupo de contaminantes en las aguas chilenas es fundamental. Los métodos multirresiduales han ocupado un importante lugar en el estudio medioambiental de CECs. Estos métodos, permiten el análisis de un amplio grupo de compuestos, con diversas características físico-químicas, a través de la cromatografía líquida de ultra alta eficiencia (UHPLC) acoplada a detectores de espectrometría de masas (MS) de alta (HRMS) y baja resolución (LRMS), y empleando estrategias de análisis dirigido (*target)*, no dirigido (*non target)* y análisis de sospechosos (*suspects*). Adicionalmente, los métodos multirresiduales pueden ser acoplados de modo offline con técnicas de microextracción para la preconcentración de la muestra, con la finalidad de aumentar la sensibilidad durante la detección de CECs a bajas concentraciones en aguas reales. La microextracción en fase sólida, ha ganado un importante lugar en las últimas décadas, debido a ciertas ventajas como el bajo volumen de solvente y cantidad de muestra requerida en el proceso extractivo. Un ejemplo es la extracción con disco rotatorio (RDSE).

Como se ha mencionado, las PTAR no logran remover los CECs en su totalidad, por tanto, resulta indispensable el desarrollo de métodos alternativos, con vistas a ser aplicados como tratamientos terciarios en la remoción y/o degradación de los CECs, de manera más eficiente en las PTAR, evitando que lleguen al consumo humano a través del ciclo del agua.

Los procesos electroquímicos de oxidación avanzada (EAOP) han recibido gran atención en los últimos años para la remoción de contaminantes en aguas residuales. En especial, la Oxidación Anódica (AO) es uno de los EAOP más comunmente empleados debido a su versatilidad y fácil escalabilidad. A través de este proceso, la materia orgánica se mineraliza por completo a CO_2, mediante la oxidación mediada por especies reactivas de oxígeno (fundamentalmente los radicales hidroxilos (•OH, $E^o = 2.80$ V/SHE) electro generados *in situ* en la superficie del ánodo

a altas densidades de corrientes) y otras especies oxidantes como las activas de cloro, el radical sulfato y el ion persulfato, que se originan en presencia de estas sales.

Tomando en consideración estos antecedentes, este trabajo se propone como objetivos, establecer una metodología multirresidual basada en microextracción en fase sólida por RDSE acoplada offline a UHPLC-MS/MS y UHPLC-Orbitrap Q-Exactive, para la determinación de CECs en matrices acuosas chilenas, así como estudiar la transformación y/o remoción de los contaminantes inducidas por AO.

CAPÍTULO I: INTRODUCCIÓN Y OBJETIVOS

1. INTRODUCCIÓN

1.1 Contaminantes de preocupación emergente (CECs)

Desde la década de los 90' la contaminación antropogénica de las aguas por CECs, ha ganado un gran interés en el campo de las investigaciones ambientales (Jakimska et al., 2017; Montes-grajales et al., 2017). Esta tendencia ha tenido lugar, debido a la introducción y desarrollo de nuevos equipamientos y metodologías analíticas, que resultan más sensibles y selectivas para la detección y cuantificación de dichos contaminantes a bajas concentraciones en matrices ambientales (Wille et al., 2012; World Health Organization, 2012), así como, por el aumento en materia de conocimiento acerca de sus efectos toxicológicos (Söderström et al., 2009). El primer estudio que reporta la presencia de fármacos como residuos en el medioambiente fue llevado a cabo por Higaite y Azarnoff en el año 1977, con el descubrimiento de ácido clorfíbrico en efluentes en una Planta de Tratamiento de Aguas Residuales (PTAR) en Kansas City, Missouri (Higaite and Azarnoff, 1977).

Los CECs se definen como sustancias químicas sintéticas o de origen natural que no son comúnmente monitoreadas en el ambiente pero que tienen el potencial de entrar en él y causar efectos adversos ecológicos o sobre la salud humana, ya sean conocidos o sospechosos (Geissen et al., 2015). El grupo de los CECs abarca diversos compuestos dentro de los que se pueden encontrar: (1) pesticidas (herbicidas, fungicidas, insecticidas, rodenticidas, acaricidas, etc.), (2) nanomateriales, (3) ftalatos, (4) aditivos para plásticos, (5) compuestos no halogenados, (6) productos para el cuidado personal, (7) compuestos fluorados, (8) parafinas cloradas (retardadores de llama, selladores, aditivos plásticos), (9) almizcles sintéticos, (10) compuestos bromados, (11) fitoestrógenos, (12) productos farmacéuticos y/o sus metabolitos, (13) drogas de abuso y/o sus metabolitos, (14) edulcorantes, entre otros (Barrios-Estrada et al., 2018;

Hernández et al., 2015; Wille et al., 2012). Muchos de estos compuestos a su vez se pueden sub-agrupar como disruptores endocrinos (DEs), que no son más que aquellos CECs que ejercen un impacto ecológico interfiriendo con la función hormonal normal, a través de tres posibles mecanismos (1) pueden imitar o imitar parcialmente las hormonas naturales del cuerpo humano, como andrógenos, estrógenos u hormona tiroidea, produciendo mayormente sobrestimulación (2) pueden actuar como antagonistas dentro de las células, uniéndose a receptores de hormonas endógenas, conduciendo a una falla en la señal hormonal y por tanto a una falla del organismo y (3) pueden interferir o bloquear las hormonas naturales o sus receptores, por ejemplo, alterando su metabolismo en el hígado (Kabir et al., 2015). Dentro de los DEs se incluyen compuestos producidos naturalmente, como los fitoestrógenos y los estrógenos y andrógenos naturales, así como una amplia gama de productos químicos industriales y domésticos que incluyen hormonas sintéticas, hidrocarburos aromáticos policíclicos (PAH), compuestos policlorados (bisfenoles, dioxinas y furanos), compuestos alquilfenólicos, productos farmacéuticos y pesticidas (Fauzan et al., 2016).

1.2 Vías de entrada de los CECs al medioambiente acuático

Los CECs llegan al medioambiente acuático e ingresan al ciclo del agua a través de múltiples vías, como se representa en la Figura. 1: a través de las aguas residuales domésticas, incluyendo las provenientes de las actividades recreativas en piscinas, excreción humana y de la ducha. A través de descargas hospitalarias e industriales, de prácticas agrícolas y ganaderas, así cómo a partir de fosas sépticas y vertederos (Yang et al., 2017). Sin embargo, la liberación de los efluentes de las PTAR en las aguas superficiales, se considera la principal causa de la aparición de CECs en estas aguas y otros ecosistemas (Rout et al., 2020), llegando a reportarse en niveles de concentración desde ng L^{-1} a mg L^{-1}, incluyendo en las aguas del continente Antártico (Alygizakis et al., 2020; Ferrer and Thurman, 2012; Hernández et al., 2019). Esta problemática

se debe, a que las PTAR no logran remover en su totalidad a los CECs, pues no fueron diseñadas con esta finalidad (Patel et al., 2019).

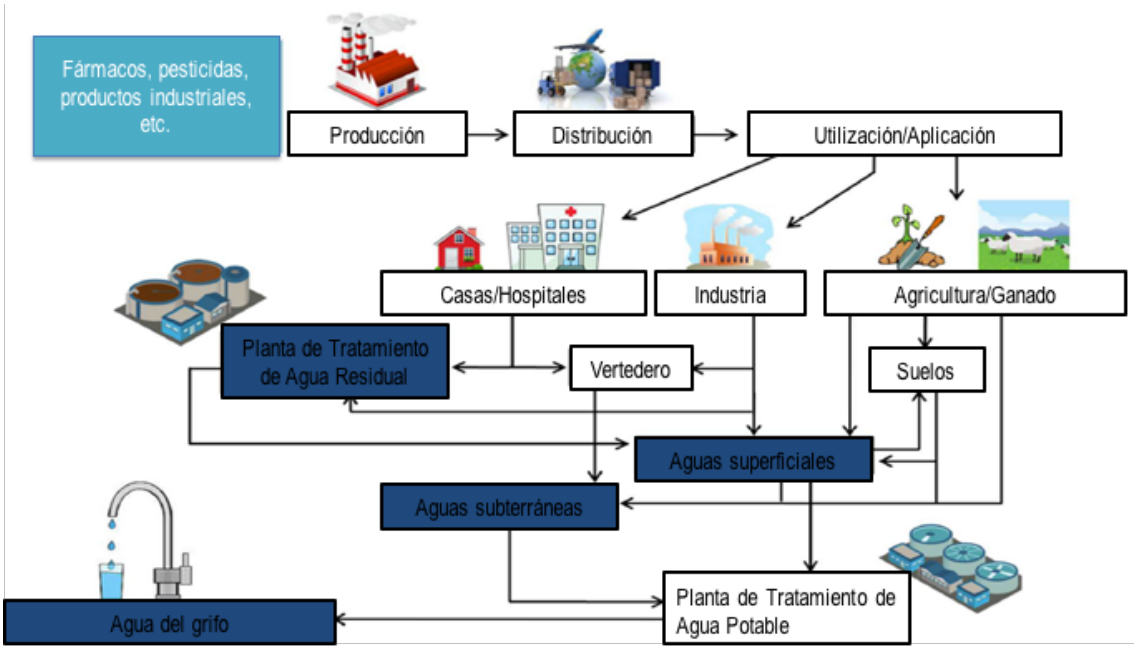


Figura. 1. Fuentes y vías de entrada de los CES al medioambiente. Tomado y adaptado de (Barbosa et al., 2016).

1.3 Efectos toxicológicos de los CECs sobre el medioambiente y seres humanos

Los productos farmacéuticos constituyen uno de los grupos más grandes e importantes dentro de los CECs. Estos compuestos fueron diseñados para producir una respuesta biológica en un organismo determinado, sin embargo, también pueden producir la misma respuesta en otras entidades después de una exposición crónica incluso a concentraciones traza de estos compuestos (Rasheed et al., 2018).

Por otra parte, tanto los fármacos como otros CECs, pueden actuar como DEs mimetizando e impidiendo los efectos producidos por hormonas en los organismos, tal y como se ha explicado anteriormente. En un review publicado por Patel et al en 2019, se resume una serie de efectos

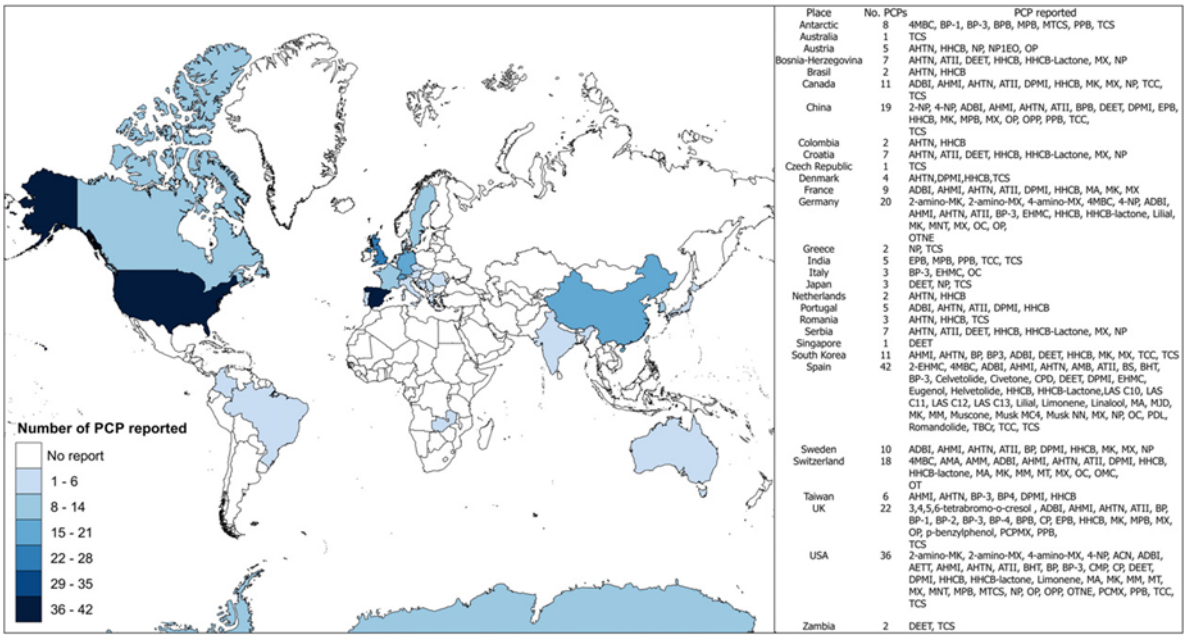
tóxicos provocados por CECs a los ecosistemas y al medioambiente. Algunos ejemplos son la alteración en la expresión de genes y en la actividad de enzimas y proteínas, así como malformaciones en el crecimiento de ratas, peces y ranas. Efectos dañinos a las poblaciones de insectos del estiércol e invertebrados acuáticos ocasionados por la invemectina, feminización de peces machos provocada por el etinilestradiol, disminución de las poblaciones de buitres en el sudeste asiático ocasionado por el diclofenaco, así como la toxicidad inducida por los antibióticos oxitetraciclina y trimetoprim en *Daphnia magna*, en el alga verde *Pseudokirchneriella subcapitata* y en cianobacterias *Anabaena Flos-aque*. Por otra parte, pueden ocasionar la resistencia a antibióticos en bacterias o poblaciones microbianas (Patel et al., 2019), fenómeno este último que indirectamente tiene un significativo impacto sobre la salud humana y animal. En este sentido, Grenni et al,. 2018 plantearon que en presencia de tetraciclina, detectada en muestras ambientales a concentraciones de µg/L, puede ocurrir transferencia horizontal de genes de resistencia en *E. coli.* Además, se ha reportado que incluso a concentraciones por debajo de 1 ng/L ya puede existir resistencia a antibióticos (Grenni, Ancona, & Barra Caracciolo, 2018). En este mismo sentido, investigadores de la Universidad de Concepción reportaron la presencia de cepas de *E. coli* resistentes a antibióticos ß-lactámicos, aminoglucósidos, tetraciclinas y trimetoprim, encontradas en agua de mar y en una planta de tratamiento de agua residual local en la Península de Fildes en la Antártica (Rabbia et al., 2016).

De acuerdo al informe de la Organización Mundial de la Salud (OMS) en el 2012, existe poca evidencia de que a las concentraciones a las que se encuentran los CECs en las aguas ambientales (en su mayoría a concentraciones de ng/L) representen un riesgo directo para la salud humana (World Health Organization, 2012), aunque se desconoce los riesgos asociados por la exposición prolongada a los mismos (bioacumulación) y los efectos combinados o

sinérgicos de las mezclas de ellos. No obstante, varios autores han reportado la relación entre los DEs con riesgos de padecer trastornos metabólicos, neurológicos, daño al sistema inmunológico, trastorno de los niveles hormonales y alteraciones en el sistema reproductivo femenino y masculino en humanos (Barrios-Estrada et al., 2018).

1.4 Prevalencia de los CECs en Chile y el mundo

Según un estudio realizado por Montes-Grajales et al., en un periodo comprendido entre los años 1996 y 2016, se reportó la presencia de CECs en todos los continentes, incluyendo la Antártida, siendo los países con mayor prevalencia España, Estados Unidos y Reino Unido (Figura 2) (Montes-grajales et al., 2017). Cabe destacar que Chile no se encontró dentro de los países citados en este estudio y no por ello puede descartarse la presencia de CECs en sus aguas medioambientales (Alonso and Castro-díez, 2017).



Place	No. PCPs	PCP reported
Antarctic	8	4MBC, BP-1, BP-3, BPB, MPB, MTCS, PPB, TCS
Australia	1	TCS
Austria	5	AHTN, HHCB, NP, NP1EO, OP
Bosnia-Herzegovina	7	AHTN, ATII, DEET, HHCB, HHCB-Lactone, MX, NP
Brasil	2	AHTN, HHCB
Canada	11	ADBI, AHMI, AHTN, ATII, DPMI, HHCB, MK, MX, NP, TCC, TCS
China	19	2-NP, 4-NP, ADBI, AHMI, AHTN, ATII, BPB, DEET, DPMI, EPB, HHCB, MK, MPB, MX, OP, OPP, PPB, TCC, TCS
Colombia	2	AHTN, HHCB
Croatia	7	AHTN, ATII, DEET, HHCB, HHCB-Lactone, MX, NP
Czech Republic	1	TCS
Denmark	4	AHTN,DPMI,HHCB,TCS
France	9	ADBI, AHMI, AHTN, ATII, DPMI, HHCB, MA, MK, MX
Germany	20	2-amino-MK, 2-amino-MX, 4-amino-MX, 4MBC, 4-NP, ADBI, AHMI, AHTN, ATII, BP-3, EHMC, HHCB, HHCB-lactone, Lilial, MK, MNT, MX, OC, OP, OTNE
Greece	2	NP, TCS
India	5	EPB, MPB, PPB, TCC, TCS
Italy	3	BP-3, EHMC, OC
Japan	3	DEET, NP, TCS
Netherlands	2	AHTN, HHCB
Portugal	5	ADBI, AHTN, ATII, DPMI, HHCB
Romania	3	AHTN, HHCB, TCS
Serbia	7	AHTN, ATII, DEET, HHCB, HHCB-Lactone, MX, NP
Singapore	1	DEET
South Korea	11	AHMI, AHTN, BP, BP3, ADBI, DEET, HHCB, MK, MX, TCC, TCS
Spain	42	2-EHMC, 4MBC, ADBI, AHMI, AHTN, AMB, ATII, BS, BHT, BP-3, Celvetolide, Civetone, CPD, DEET, DPMI, EHMC, Eugenol, Helvetolide, HHCB, HHCB-Lactone,LAS C10, LAS C11, LAS C12, LAS C13, Lilial, Limonene, Linalool, MA, M3D, MK, MM, Muscone, Musk MC4, Musk NN, MX, NP, OC, PDL, Romandolide, TBC, TCC, TCS
Sweden	10	ADBI, AHMI, AHTN, ATII, BP, DPMI, HHCB, MK, MX, NP
Switzerland	18	4MBC, AMA, AMM, ADBI, AHMI, AHTN, ATII, DPMI, HHCB, HHCB-lactone, MA, MK, MM, MT, MX, OC, OMC, OT
Taiwan	6	AHMI, AHTN, BP-3, BP4, DPMI, HHCB
UK	22	3,4,5,6-tetrabromo-o-cresol , ADBI, AHMI, AHTN, ATII, BP, BP-1, BP-2, BP-3, BP-4, BPB, CP, EPB, HHCB, MK, MPB, MX, OP, p-benzylphenol, PCPMX, PPB, TCS
USA	36	2-amino-MK, 2-amino-MX, 4-amino-MX, 4-NP, ACN, ADBI, AETT, AHMI, AHTN, ATII, BHT, BP, BP-3, CMP, CP, DEET, DPMI, HHCB, HHCB-lactone, Limonene, MA, MK, MM, MT, MX, MNT, MPB, MTCS, NP, OP, OPP, OTNE, PCMX, PPB, TCC, TCS
Zambia	2	DEET, TCS

Figura. 2. Estudios de CECs en el mundo. Tomado de (Montes-grajales et al., 2017).

Según la bibliografía consultada hasta el momento, algunos investigadores determinaron la presencia de triclosán a 1.856,7 ng/L en efluentes que descargan a la cuenca del río Bio-Bio, en la ciudad de Concepción. Este mismo autor, además de triclosán encontró 15 fármacos, como ibuprofeno, gemfibrosil, carbamazepina, diclofenaco, atenolol, entre otros, en esta misma matriz (Moncada, 2015). En 2009 y 2011, Bertin et al. detectaron estrógenos en sedimentos de este mismo río (Bertin et al., 2011; Inostroza and Quinones, 2009), mientras que Henríquez en 2012, determinó un grupo de fármacos más amplio como cafeína, antidepresivos, beta bloqueadores, reguladores lipídicos y antibióticos, en afluentes y efluentes provenientes de las PTAR de las localidades de Santa Bárbara, Los Ángeles y Concepción en la región del Bio Bio (Villa, 2012). Por otra parte, el grupo de investigación de Richter y colaboradores ha reportado en PTAR en una en Santiago de Chile, la presencia de antiinflamatorios no esteroidales (AINEs) (Manzo et al., 2014), gemfibrozilo, ácido clofíbrico (Becerra-herrera et al., 2015), triclosán (Jachero et al., 2013), parabenos (Becerra-Herrera et al., 2020), ente otros.

Si bien es cierto que existen estudios sobre CECs en Chile, aún resulta insuficiente la información que se tiene al respecto, existiendo una brecha de conocimiento sobre cuán expuestos nos encontramos a estos contaminantes, que imposibilita que la agencia regulatoria nacional actúe en función de regular sus emisiones y vertimiento, así como su prevalencia, como parte del control de calidad de las aguas.

1.5 Normas para la regulación de los CECs

En la actualidad, el desarrollo de normas y directrices que regulen la presencia de productos farmacéuticos en matrices ambientales es limitado por la falta de evidencias relacionadas con la exposición prolongada a estos contaminantes, en bajas concentraciones (Patel et al., 2019). Sin embargo, algunas agencias medioambientales alrededor del mundo se han pronunciado al respecto y comenzado a adoptar algunas medidas.

La Unión Europea (UE) a través de la directiva 2008/105/ CE, establece las normas de calidad ambiental para sustancias prioritarias y otros contaminantes. Actualmente existen 45 sustancias prioritarias en el anexo X de la directiva 2013/39/ CE, dentro de las que se encuentran al nonilfenol, atrazina, ftalato de di(2-etilhexilo), entre otras. En esta última directiva además, se establece el marco de actuación comunitaria en el ámbito de la política integrada del agua, estableciendo que dichas sustancias deben ser controladas de forma prioritaria con acciones como la reducción progresiva de su vertimiento y emisiones (Directiva 2008/105/CE, 2008; Directiva 2013/39/UE, 2013). Además, en la UE se ha establecido tres listas de vigilancia para el seguimiento de sustancias preocupantes, dentro de las que se encuentran actualmente productos farmacéuticos como la venlafaxina, amoxicilina, ciprofloxacino, fluconazol, clotrimazol, entre otros (Decisión de Ejecución UE 2020/1161, 2020). Por otra parte, en 2007 se estableció en Europa el reglamento REACH (Restricción, Evaluación, Autorización y Restricción de Productos Químicos), cuyo objetivo es identificar las sustancias químicas peligrosas y los sustitutos menos peligrosos. La aplicación de este reglamento exige la eliminación de tres ftalatos (Dietil ftalato, Di-n-butil ftalato, y ftalato de butilbencilo) clasificados como cancerígenos, tóxicos para la reproducción o persistentes en el medio ambiente (Deblonde et al., 2011).

En los Estados Unidos de América, la Agencia de Protección Ambiental (USEPA), considera como primer paso para la regulación de contaminantes, su incorporación a una lista de candidatos a contaminantes (CCL). Posteriormente, estos son reevaluados, en base en una serie de criterios: potencialidad de ocasionar un efecto adverso sobre la salud humana y su presencia en los sistemas públicos de agua con una frecuencia y niveles de concentración preocupantes para la salud pública. Finalmente, al menos 5 de los contaminantes propuestos en las CCL pasan a ser regulados. En la actualidad se han creado 4 CCL, que incluyen un total de 97

contaminantes de naturaleza química y 12 microbiológicos, dentro de los que se encuentran algunos productos farmacéuticos como el antibiótico eritromicina, hormonas estrógenos, entre otros contaminantes. Una quinta lista se encuentra en proceso de revisión, para ser publicada próximamente (US EPA, 2021).

Por otra parte, el gobierno australiano ha dado un primer paso al introducir directrices para la gestión de riesgos en aguas recreativas en el año 2008. Estas directrices utilizan las dosis terapéuticas mínimas recomendadas para establecer los niveles aceptables, dividiendo estos valores por factores de seguridad para calcular las ingestas diarias de CECs tolerables (NHMRC, 2008).

Finalmente, Japón ha estado trabajando en el tema de los DEs a través de los Programas estratégicos sobre disruptores endocrinos ambientales (SPEED'98). En 2003, el ministerio de medio ambiente decidió emprender nuevas acciones a través del programa: Tareas ampliadas sobre la alteración endocrina 2010, con el propósito de establecer y acelerar metodologías para evaluar el riesgo y el efecto de los DEs (Environmental Health and Safety division, 2010).

1.6 Metodología analítica para el análisis de CEs en matrices medioambientales

1.6.1 Tratamiento o preparación de muestras líquidas

Previo al paso de extracción, es usual la filtración y/o centrifugación de la muestra, para eliminar los sólidos suspendidos (Jakimska et al., 2017; Wille et al., 2012). Además, se suele ajustar el pH para aumentar la afinidad de los analitos por el sorbente de extracción o añadir reactivos para mejorar la eficacia de la extracción. Los reactivos más utilizados son los agentes quelantes como el ácido etilendiaminotetraacético (EDTA) o sus sales, para evitar que los metales se unan a algunos analitos, en especial a las tetraciclinas (López-serna and Petrovic, 2011; Wille et al., 2012). En general, los productos farmacéuticos tienen un carácter polar y la

mayoría de ellos están presentes en las aguas ambientales en su forma ionizada. Por tanto, es recomendable que las muestras se coloquen en contenedores de plástico como el politetrafluoroetano (PTFE), polietileno de alta densidad (HDPE) o polipropileno (PP), en lugar de vidrio, para minimizar las pérdidas de analitos causadas por adsorción en las paredes del vidrio. Se recomienda congelar o enfriar las muestras siempre durante la transportación y almacenamiento de las mismas (Pazdro et al., 2016).

1.6.2 Metodologías extractivas para muestras líquidas

En la literatura se ha reportado el uso de varios procedimientos analíticos con diferentes metodologías de extracción para la limpieza y preconcentración de muestras líquidas medioambientales: microextracción en fase sólida (SPME) (Díaz and Peña-Alvarez, 2017), extracción adsortiva por agitación con barra (SBSE) (Aparicio et al., 2017), extracción con membrana (Luiz Oenning et al., 2017), microextracción en fase líquida (LPME) (Hashemi et al., 2017), extracción con fluidos supercríticos (SFE), QueEChERS (Division et al., 2017), extracción con líquidos presurizados (PLE) (Nieto et al., 2010), dispersión por matriz en fase sólida (MSPD) y extracción dispersiva en fase sólida (DSPE) (Rashvand and Vosough, 2016). Sin embargo, la extracción en fase sólida (SPE) en modo off-line sigue siendo la técnica de preparación de muestras utilizada con mayor frecuencia para la extracción de los analitos y limpieza de matrices líquidas (Aparicio et al., 2017; Gentili, 2017; Rodriguez-narvaez et al., 2017; Wille et al., 2012). Con el empleo de fases poliméricas la retención de productos farmacéuticos ha mejorado considerablemente mediante el desarrollo de nuevos polímeros adsorbentes, mayormente materiales que combinan equilibrios hidrofílicos-hidrofóbicos. La mezcla de copolímeros de divinilbenceno y n-vinilpirrolidona, comercialmente desarrollado como Oasis® HLB por Waters™, es actualmente el sorbente para SPE más utilizado en la extracción y preconcentración de CECs, debido a su capacidad para retener analitos de variadas

polaridades. Esta mezcla de copolímeros posee un alto grado de entrecruzamiento, una gran porosidad y estructura abierta, lo que permite interacciones hidrofóbicas (a través de interacciones π-π entre los anillos de benceno y las cadenas carbonadas con la parte menos polar de los analitos) y la retención hidrófila (a través de la formación de enlaces de hidrógeno entre el resto de pirrolidona y los grupos polares del analito) (Figura 3) (Cañas and Samuel, 2014). Oasis® PRiME HLB comercializada más recientemente por Waters™, es otra fase polimérica constituida estructuralmente también por los copolímeros de divinilbenceno y n-vinilpirrolidona, por lo que comparte características química-físicas con Oasis® HLB, sin embargo con respecto a esta última, el fabricante declara que Oasis® PRiME HLB minimiza el efecto matriz al entregar muestras más limpias, es más simple de usar y más robusta (Zhang, 2015). Su composición exacta aun se desconoce, pues se encuentra en fase de registro. Otras marcas comercializan este copolímero bajo el nombre de Strata-X (Phenomenex), Chromabond HLB (Macherey-Nagel), Supel™-Select HLB (Merck) entre otros.

Además de los ya mencionados, también se pueden encontrar los copolímeros Oasis®MCX (intercambiador catiónico para bases con fase reversa en modo mixto), MAX (intercambiador aniónico para ácidos con fase reversa en modo mixto), WCX (intercambiador catiónico débil para bases fuertes y aminas cuaternarias y fase reversa en modo mixto) y WAX (intercambiador aniónico débil para ácidos fuertes con fase reversa en modo mixto (Waters Oasis, 2016; Wille et al., 2012), además de los adsorbentes de Isolute ENV+ (combinación de poliestireno hidroxilado y divinilbenceno), Chromabond HR-X (combinación de poliestireno y divilbenceno), etc., (Wille et al., 2012).

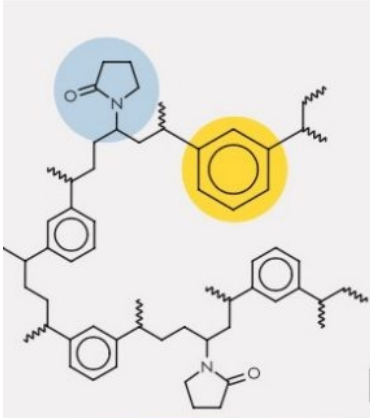


Figura. 3. Estructura química del copolímero Oasis® HLB. Tomado de (Waters Oasis, 2016).

En las últimas dos décadas, se ha producido un aumento en el uso de técnicas de microextracción, debido a ciertas ventajas como la simplificación, la fuerte reducción o incluso la eliminación del uso de disolventes orgánicos tóxicos y la reducción de la muestra requerida (Aparicio et al., 2017). Dentro de ellas podemos citar a la extracción adsortiva por agitación con barra (SBSE) y la extracción con disco rotatorio (RDSE), entre otras (Babic and Horvat, 2007; Zhang et al., 2007).

1.6.2.1 Extracción con disco rotatorio (RDSE)

La RDSE es una técnica integrada de extracción/agitación propuesta en 2009 por Richter et al (Vieira et al., 2019). Esta técnica ha sido aplicada en la extracción de CECs desde matrices acuosas, como fármacos antiinflamatorios, hormonas, triclosán, parabenos y un gran número de pesticidas (Arismendi et al., 2020; Becerra-Herrera et al., 2020; Donato et al., 2017; Manzo et al., 2014). RDSE funciona de manera similar a la extracción sortiva con barra de agitación (SBSE), ya que la unidad se agita en la muestra durante un período de tiempo definido para el aislamiento de los compuestos objetivo (Soledad and Lucena, 2017).

Sin embargo, los dispositivos de extracción RDSE se pueden agitar a una velocidad mucho mayor que la barra de agitación utilizada en SBSE, sin dañar la fase extractante, porque esta sólo está en contacto con la muestra líquida. Por lo tanto, una mayor velocidad de agitación facilita la transferencia de masa de analito desde el seno de la solución hacia la superficie de la fase estacionaria (Pereira et al., 2015).

Para la RDSE se ha propuesto dos tipos de dispositivos, (Figura 4A y 4B) (Jachero et al., 2014; Pereira et al., 2015; Soledad and Lucena, 2017). El dispositivo 4A, con polidimetilsiloxano (PDMS) inmovilizado en su superficie de politetrafluoroetileno (PTFE) ha jugado un papel fundamental en la extracción de compuestos apolares (Richter, 2011), sin embargo, esta fase extractiva presenta como desventaja que es una fase de baja polaridad, por lo que la extracción de compuestos polares, especialmente aquellos con valores log K_{ow} inferiores a 4 resulta compleja. Por tanto, el desarrollo del dispositivo 4B, que presenta una cavidad en su interior donde se puede incorporar material extractante de diversas naturalezas, ha venido a dotar a la RDSE de una gran versatilidad y ha abierto las puertas para la extracción de analitos de mediana y alta polaridad (Soledad and Lucena, 2017). Dentro de estos materiales se pueden encontrar no solo fases extractantes comerciales empleados en SPE como el Oasis HLB (Cañas and Samuel, 2014) y C18 (Ca and Richter, 2012), también algunas desarrolladas en laboratorio, como los polímeros impresos molecularmente (MIPs) (Pereira et al., 2015) o líquidos iónicos (Rosero-moreano et al., 2016).

Durante la etapa de optimización de este método extractivo, resulta fundamental una serie de variables tanto químicas como hidrodinámicas (Sandoval, 2015):

Variables químicas

- Efecto del pH del medio
- Efecto de la fuerza iónica

- Cantidad de metanol
- Disolvente y tiempo de desorción

Variables hidrodinámicas

- Velocidad de agitación
- Perfil de extracción y volumen de muestra

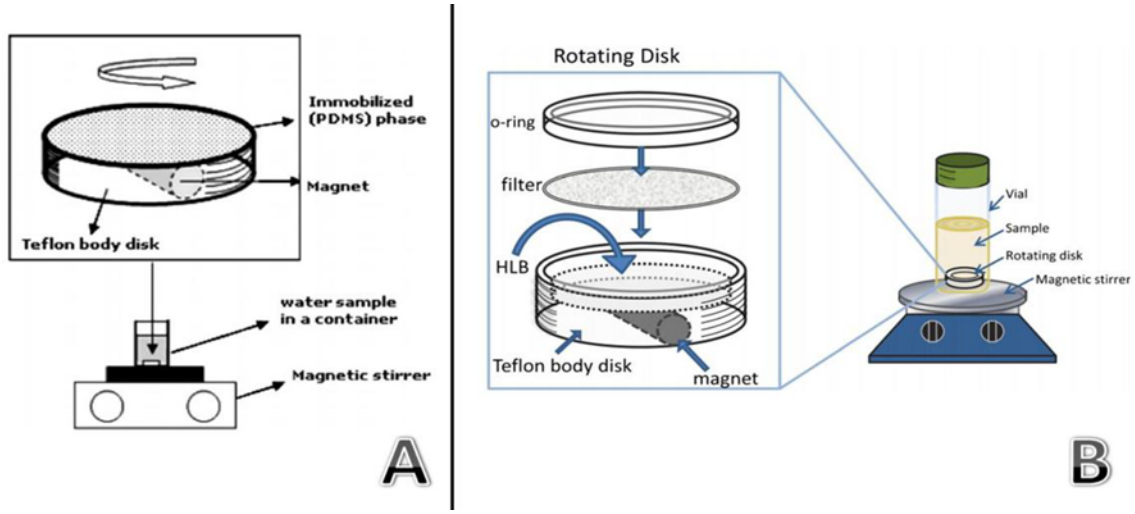


Figura. 4. Dispositivos empleados para la RDSE. **(A)** La fase estacionaria PDMS (polidimetilsiloxano) se inmoviliza en la superficie. **(B)** La fase estacionaria (diversa naturaleza química), se incorpora en el centro del disco, luego se sella con un filtro y con ayuda de un anillo de teflón. Tomado de (Soledad and Lucena, 2017).

1.6.3 Metodologías para la estimación de CECs

1.6.3.1 Métodos multiclases o multirresiduales

Los métodos multirresiduales, también conocidos como multiclases o multicomponentes, son métodos analíticos para determinar docenas o incluso cientos de analitos en un solo análisis (Nurmi and Pellinen, 2011). La principal ventaja que ofrecen estos métodos, es que proporcionan una información mucho más amplia que los métodos centrados en el análisis de

una única familia química. Asimismo, el tiempo de análisis y el coste que implicaría analizarlos mediante un método optimizado para cada grupo terapéutico es mucho menor. Sin embargo, debido a las distintas propiedades físico-químicas de los compuestos analizados, el desarrollo de un método multirresidual resulta un proceso complicado. A medida que aumenta el número de compuestos a analizar, la complejidad del método se hace mayor. Por tanto, se requiere encontrar un compromiso en la selección de las condiciones experimentales que permita el análisis simultáneo de todos ellos (Gracia-lor et al., 2011; Montes-grajales et al., 2017; Petrovic et al., 2010). Por otra parte, estos métodos están muy expuestos al efecto matriz con la consecuente supresión o mejora de la señal analítica, particularmente si se trabaja con matrices complejas como las aguas residuales (Petrovic et al., 2010).

Durante el desarrollo de un método multirresidual, inicialmente deben ser optimizadas las condiciones cromatografías con vistas a mejorar la resolución y a minimizar la coelución no deseada. En segundo lugar, se debe encontrar un compromiso entre la sensibilidad y los tiempos de permanencia seleccionados o *dwell time*, para mantener una forma de pico satisfactoria para todos los compuestos seleccionados. En ese sentido, los métodos MS/MS generalmente se dividen en diferentes ventanas de tiempo de elución que contienen las diferentes transiciones MRM con el *dwell time* apropiado (Gracia-lor et al., 2011). Cuando se emplean fuentes de ionización por electroespray (ESI) y/o ionización química a presión atmosférica (APCI), se puede trabajar simultáneamente en forma multimodo. Esto supone una alternación de polaridad positiva y negativa en una sola ejecución cromatográfica, y brinda la ventaja de analizar una amplia gama de compuestos independientemente de su polaridad, funcionalidad o estabilidad térmica (Gentili, 2017).

1.6.3.1.1 Métodos multirresiduales empleando cromatografía líquida acoplada a espectrometría de masas de baja resolución (LC-LRMS)

Desde finales de la década de los 80', la Cromatografía Líquida acoplada a la Espectrometría de Masas (LC-MS) ha crecido rápidamente y ganado en popularidad como técnica para el control ambiental. Comparado con la Cromatografía de Gases acoplada a Espectrometría de Masas (GC-MS), la LC-MS ofrece una serie de ventajas compatibles con la naturaleza polar de la mayoría de los contaminantes de problemática emergente: eliminación del paso de derivatización de compuestos no volátiles y termolábiles, aumento del número de productos químicos analizables y reducción del tiempo de análisis total (Gentili, 2017). Por otra parte, en los últimos años la Cromatografía Líquida de Ultra Alta Eficiencia (UHPLC), se ha convertido en una herramienta poderosa para el desarrollo de métodos multiclases para la determinación de CECs. Mediante el uso de columnas con un tamaño de partícula sub-2µm, esta técnica proporciona una mayor resolución y sensibilidad gracias a la obtención de picos cromatográficos más estrechos y de mayor altura. Además, la separación cromatográfica es más rápida (Gracia-lor et al., 2010; Jakimska et al., 2017; López-serna and Petrovic, 2011).

Actualmente la interface más utilizada en el análisis de fármacos en muestras ambientales es la ESI. La interface APCI y fotoionización (APPI) se ha empleado en un número de trabajos más reducido para el estudio de contaminantes de moderada y baja polaridad (Gentili, 2017).

Dentro de los analizadores de masas de baja resolución (LR), los más frecuentemente utilizados son el de triple cuadrupolo (QqQ) (Nurmi and Pellinen, 2011) y triple cuadrupolo con trampa de iones lineal (QqQ_{LIT}), ya que proporcionan una alta selectividad y sensibilidad, esta última aproximadamente de 1 a 2 órdenes de magnitud más altos cuando se trabaja en modo monitoreo reacción de múltiple (MRM) en comparación con instrumentos de alta resolución (HR).

Una de las mayores desventajas asociadas a los métodos LC-MS/MS es el efecto matriz, el cual tiene como resultado una supresión o un mejoramiento en la intensidad de la señal analítica, debido a la presencia de componentes coextraídos que pueden afectar la ionización de los analitos. Este efecto indeseable puede afectar la identificación y la cuantificación de los analitos, especialmente cuando se trabaja con matrices complejas (Gracia-lor et al., 2011; Sancho et al., 2012).

1.6.3.1.1.1 Estrategias de trabajo en LRMS. Análisis dirigido o target

El análisis *target* o análisis dirigido ha sido la estrategia de trabajo más comúnmente empleada en el análisis de muestras ambientales. Mediante este enfoque, tradicionalmente la presencia o ausencia de cada sustancia se determina individualmente mediante el cromatograma de iones extraídos (XIC), en el que solo ciertos compuestos previamente son elegidos y determinados mediante el uso de estándares de referencia. Por tanto este método es validado únicamente para esos compuestos (Gentili, 2017; Nurmi and Pellinen, 2011). En las técnicas de MS en tándem (MS/MS), los analitos se detectan a través de monitoreo de reacción seleccionada (SRM) o monitoreo de reacción múltiple (MRM), midiendo iones producto conocidos de iones precursores (Nurmi and Pellinen, 2011). Dado que, durante todo el tiempo del análisis se está midiendo una o varias transiciones específicas, este modo de trabajo proporciona una elevada selectividad (Gracia-lor et al., 2011).

La desventaja de las técnicas MS/MS es que conducen a información sesgada en las muestras, porque solo los datos definidos por el usuario obtenido a través de SRM o MRM se guardan. Todos los demás datos de la muestra se descartan permaneciendo desconocidos (Nurmi and Pellinen, 2011).

1.6.3.1.1.2 Confirmación de la identificación de compuestos en un análisis dirigido por LRMS

Un tema clave en el monitoreo de los contaminantes ambientales es la confirmación de hallazgos positivos. La creciente preocupación por la confirmación de los datos, favoreció el desarrollo de diferentes criterios para asegurar la calidad de los mismos y evitar el reporte de falsos positivos (Petrovic et al., 2010).

En 2002, las Guías de la Comisión Europea proponen la Decisión 2002/657/CE, donde se manifiesta que en el análisis de muestras ambientales basados en espectrometría de masas, la confirmación de contaminantes se realice en base al uso de puntos de identificación (IP) (Commission, 2002). Según lo planteado en las Guías, los compuestos se dividen en dos grupos: Grupo A, compuestos prohibidos, y Grupo B, compuestos legales. Para la identificación y confirmación de un compuesto del Grupo B, es necesario recolectar 3 IPs y de Grupo A 4 IPs para confirmar un resultado positivo (Jakimska et al., 2017). El número de IPs depende de la técnica utilizada, diferenciando entre MS y MS^n, y entre instrumentos de baja resolución y de alta resolución (Tabla 1). Así, para instrumentos de baja resolución en modo MS/MS, como es el caso de un analizador de triple cuadrupolo, se requieren al menos 4 IPs (un ion precursor y dos iones producto), es decir, dos transiciones por analito para una identificación fiable (Gracia-lor, 2013).

Tabla 1. Cantidad de IPs obtenidos por analito de acuerdo a la técnica MS empleada (Commission, 2002; Jakimska et al., 2017).

Técnica MS	IPs obtenidos por cada analito
QqQ-MS/MS ion precursor	1.0
QqQ-MS/MS ion producto	1.5
QTOF-MS ion precursor	2.0
QTOF-MS ion producto	2.5

Además, la desviación de la intensidad relativa de los iones registrados no debe exceder un cierto porcentaje con respecto al estándar de referencia (Tabla 2), y el tiempo de retención no debe desviarse más del 2.5%.

Tabla 2. Tolerancias máximas permitidas para la confirmación de contaminantes según la Decisión 2002/657/EC de la Comisión Europea (Commission, 2002).

Relación de intensidades (Q/q)	Desviación permitida LC-MS y LC MS^n
1 - 2	± 20%
2 - 5	±25%
5 - 10	±30%
> 10	±50%

1.6.3.1.2 Métodos multirresiduales empleando cromatografía líquida acoplada espectrometría de masas de alta resolución (LC-HRMS)

La evolución de la espectrometría de masas de alta resolución (HRMS) acoplada a la cromatografía de gases o líquidos (GC o LC) ha iniciado una nueva tendencia en el procesamiento de datos analíticos en los últimos años (Schymanski et al., 2015) en el campo de la investigación ambiental (Nurmi and Pellinen, 2011), para la detección y confirmación de productos farmacéuticos y sus productos de transformación y para la elucidación de contaminantes desconocidos (Jakimska et al., 2017). En este caso, los métodos analíticos dirigidos (*target*) se complementan a menudo, con métodos de adquisición de datos de sospechosos (*suspects*) y no dirigidos (*non-target*) y métodos de cribado (*screening*), en los que se utiliza la espectrometría MS/MS para obtener información sobre la fragmentación, que permite apoyar la identificación (Schymanski et al., 2015).

En cuanto a la instrumentación, los instrumentos híbridos como el cuadrupolo/ TOF (QTOF) o el de trampa de iones lineal/orbitrap (LTQ Orbitrap) han demostrado especialmente una excelente capacidad de detección e identificación de compuestos de bajo peso molecular en diversas matrices, basada en la medición de masa precisa de alta resolución de los iones precursores y del producto. Por lo tanto, la gran mayoría de los estudios relativos a las identificaciones non-target utilizan instrumentos de la familia Orbitrap o TOF. De igual modo que en los instrumentos LR, entre las posibles técnicas de ionización, la ESI es la más utilizada (Brack et al., 2019), mientras que la APCI o la fotoionización a presión atmosférica APPI, pueden complementar el espectro de sustancias, cubriendo compuestos menos polares que no son detectables con ESI (Gago-Ferrero et al., 2016; Gentili, 2017).

1.6.3.1.2.1 Estrategias de trabajo en HRMS. Análisis de sospechosos y non-target

Como se ha mencionado anteriormente, las sustancias químicas pueden identificarse mediante un análisis *target*, en el que se preseleccionan las sustancias químicas a analizar (analitos objetivo) y se utilizan estándares de referencia para su confirmación, sin embargo también mediante análisis de *suspects* y *non target*, empleando métodos de cribado (*screening*). En la Figura 5, se resume el esquema de trabajo para el screening dirigido (target), de sospechosos (suspect) y no dirigido de compuestos desconocidos (non-target) por HRMS.

El *screening* de *suspects* o sospechosos implica la búsqueda de compuestos conocidos o previstos, que se sospecha están presentes en la muestra. Así, aunque no se disponga de un patrón de referencia (porque estos estándares de referencia no están disponibles para una gran cantidad de potenciales contaminantes ambientales, en particular los productos de transformación), la masa exacta y el patrón isotópico calculados a partir de la fórmula molecular, sumados los aductos esperados de la sustancia sospechosa, pueden utilizarse para detectar esta sustancia en la muestra (Gago-Ferrero et al., 2016; Schymanski et al., 2015). Durante este proceso, un paso crucial es producir listas inteligentes de sospechosos. En ese sentido, se han desarrollado diferentes estrategias para la selección de sospechosos en muestras ambientales, para varias sustancias químicas y clases de compuestos, por ejemplo, para plaguicidas registrados y productos de transformación (PT) asociados, productos farmacéuticos, sus metabolitos predichos y productos de fotodegradación, tensioactivos, nuevas sustancias psicoactivas, entre otras (Gago-Ferrero et al., 2018).

Por otra parte, en el *screening non target* o no dirigido de compuestos desconocidos se incluyen todos los componentes restantes detectados en una muestra, de la que no se dispone de información previa. Dado que no se dispone de información estructural de antemano, es

necesario realizar una identificación completa de los componentes *non target* a partir de la masa exacta, el isótopo, el o los aductos y la información de fragmentación (Schymanski et al., 2015). En ambos casos (screening de sospechoso y non target), los estándares de referencia no se utilizan durante las mediciones iniciales, sino que se compran a posteriori en algunos casos, para confirmar la identificación (Gago-Ferrero et al., 2016).

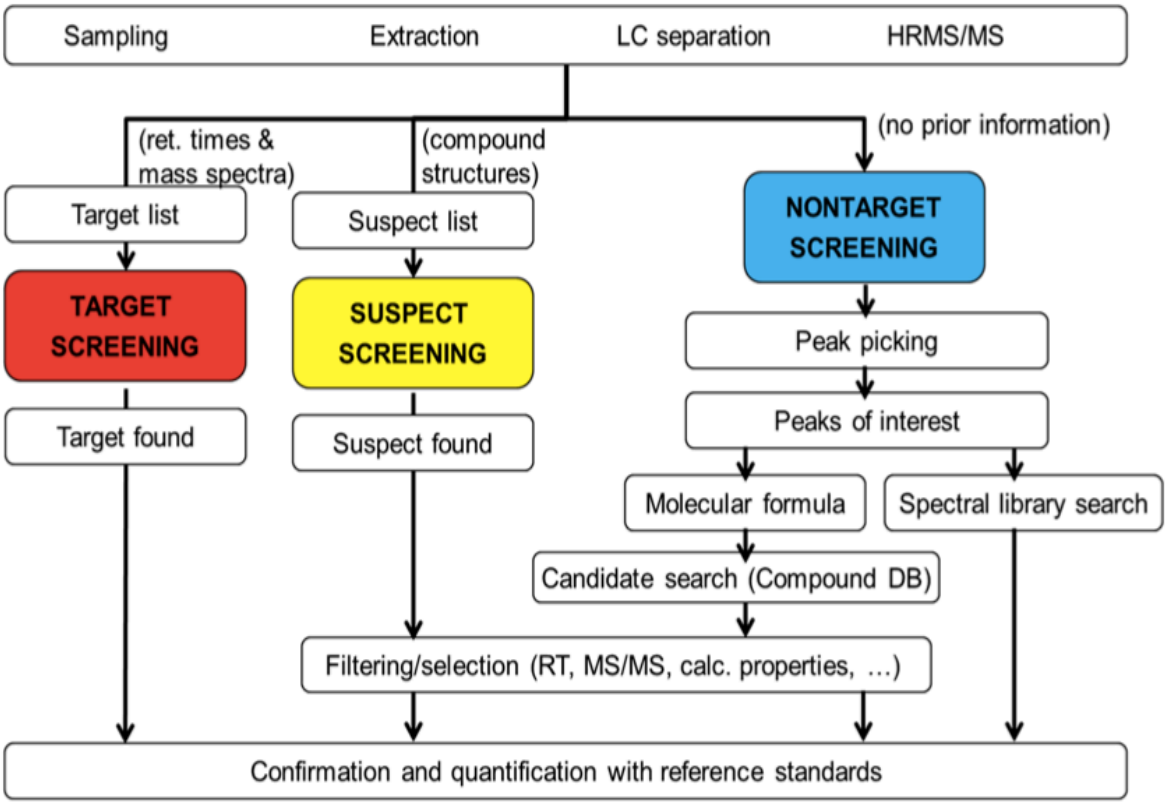


Figura. 5. Esquema de trabajo para el *screening* dirigido (target), de sospechosos (suspect) y no dirigido (non-target) por HRMS. Tomado de (Brack et al., 2019).

1.6.3.1.2.2 Softwares y bases de datos para el análisis non-target

En los últimos años se ha visto un incremento de las librerías espectrales MS/MS disponibles por LC-HRMS, con la finalidad de identificar la presencia de compuestos desconocidos en el medio ambiente.

Actualmente hay tres bases de datos MassBank disponibles, (www.massbank.eu) de Europa, (www.massbank.jp) Japón y (www.mona.ucdavis.edu) de Norteamérica. Los espectros de muchas de estas sustancias también están disponibles en mzCloud (www.mzcloud.org). Otras bases de datos disponibles son la METLIN (www.metlin.scripps.edu), que contiene 9083 compuestos únicos (4165 moléculas pequeñas y el resto son péptidos), incluyendo muchos productos farmacéuticos, y la NIST, que contiene 9159 compuestos (7447 moléculas pequeñas) (Gago-Ferrero et al., 2016). Por otra parte, están disponibles las bases de datos de estructuras moleculares ChemSpider (Royal Society of, 2021) o PubChem (NCBI, 2021), que contienen decenas de millones de compuestos, pero sin información espectral.

Las estrategias *in silico* para clasificar las estructuras químicas candidatas mediante el cotejo de los espectros de fragmentación medidos con los predichos de los compuestos consultados en las bases de datos químicas es ahora un enfoque popular (Gago-Ferrero et al., 2016). Este es el caso del software MetFrag (https://ipb-halle.github.io/MetFrag/), donde las moléculas candidatas de diferentes bases de datos se fragmentan *in silico* mediante la desconexión de enlaces y se comparan con los valores de masa y carga. Luego, una puntuación calculada a partir de las coincidencias de los picos de los fragmentos da pistas sobre la calidad de la asignación del espectro candidato (Ruttkies et al., 2016; Wolf et al., 2010).

También existen softwares para la asignación de fórmulas moleculares a compuestos desconocidos como el caso de MOLGEN-MS/MS (https://www.molgen.de/online.html), basado en la precisión de la masa, la presencia/ausencia de determinados elementos, el ajuste del patrón isotópico y la asignación de subfórmulas de fragmentos (Gago-Ferrero et al., 2016; Gugisch et al., 2015).

1.6.3.1.2.3 Análisis de sospechosos mediante la plataforma digital de congelación de muestras (DSFP) NORMAN

La DSFP es una plataforma de archivo de datos de LC-HRMS, desarrollada para el *screening* retrospectivo de miles de contaminantes ambientales sospechosos, con la ambición de convertirse en un estándar europeo y posiblemente mundial. Esta plataforma, incorpora los últimos desarrollos en métodos de *screening* por HRMS dentro de la Red NORMAN. Durante el flujo de trabajo, los datos espectrales de masas en bruto se convierten en archivos mzML, y a continuación se extrae la información espectral de masas y cromatográfica de miles de picos de cada muestra en plantillas de recogida de datos. Las muestras "congeladas digitalmente" pueden ser examinadas retrospectivamente para detectar la presencia de prácticamente cualquier compuesto susceptible de ser analizado por LC-MS, utilizando una combinación de información sobre su (i) masa exacta, (ii) ventana de tiempo de retención prevista en el cromatograma, (iii) ajuste isotópico e (iv) iones de fragmentos. La DSFP proporciona pruebas de apoyo para la identificación tentativa, no asigna niveles de identificación. Sin embargo, todas las identificaciones propuestas pueden ser consideradas técnicamente de nivel 3 (Figura 6), aunque las que tienen pruebas de apoyo sustanciales son claramente más fiables que las que sólo tienen una coincidencia de masa exacta. Todas las identificaciones plausibles deben ser verificadas con una coincidencia exacta en bibliotecas espectrales, para obtener un estatus de nivel 2a, o la confirmación del tiempo de retención y la información de los fragmentos con un estándar de referencia, para el nivel 1. La DSFP, no está diseñada para resolver los problemas de identificación de isómeros estructurales, ya que es una desventaja inherente a la instrumentación LC-HRMS actual. Los isómeros estructurales pueden tener un tiempo de retención similar y fragmentos comunes, por lo que es sumamente importante evaluar más a fondo el resultado utilizando los espectros de masas sin procesar, basándose en otras pruebas

como la relación de iones, o buscando iones de diagnóstico utilizando el conocimiento de los expertos (Alygizakis et al., 2019).

1.6.3.1.2.4 Niveles de confianza para la identificación de moléculas pequeñas por HRMS

El sistema basado en puntos de identificación propuesto en la Decisión 2002/657/EC de la Comisión Europea (Commission, 2002) puede ser empleado para presentar la evidencia disponible en una identificación, cuando se cuenta con un estándar de referencia y por tanto con información MS, MS/MS y tiempo de retención de los compuestos en estudio. Sin embargo, el creciente uso de la HRMS para la identificación *non target*, ha originado la necesidad de comunicar la confianza en la identificación de forma tal que refleje la evidencia disponible (Gago-Ferrero et al., 2016). En ese sentido en 2014, Schymanki *et al*, propusieron un sistema conformado por 5 niveles de confianza, que ayuda a comunicar la confiabilidad de la identificación de compuestos desconocidos en un análisis *non target*, cuando se emplea HRMS. A continuación se detallan estos niveles (Schymanski et al., 2014) y se presentan a modo de resumen en la Figura 6:

Nivel 1. Estructura confirmada: Constituye la situación ideal, donde la estructura propuesta puede ser confirmada a través de la medición de un estándar de referencia con coincidencia en la información de tiempo de retención, MS y MS/MS. Si es posible, también puede usarse un método ortogonal para la confirmación.

Nivel 2. Estructura Probable: Se asigna a aquellos compuestos para los que es posible proponer una estructura exacta empleando distintos tipos de evidencia. **Nivel 2a. Bibliotecas:** Implica la coincidencia de datos entre el espectro de la sustancia con bibliotecas espectrales, tomando en consideración que el grado de coincidencia espectro-estructura no debe ser ambiguo. Para garantizar la validez de la coincidencia cuando se comparan espectros, se debe tener en cuenta que los parámetros de adquisición sean los mismos (ej. Resolución, energía de

colisión, ionización, nivel MS), además el criterio de decisión debe ser presentado claramente.

Nivel 2b. Diagnóstico: Describe a aquellos compuestos para los que solamente una estructura se ajusta a la información espectral, sin embargo, no existe información bibliográfica o estándar disponible para su confirmación. La evidencia puede incluir fragmentación MS/MS y/o ionización, información del compuesto original y el contexto experimental.

Nivel 3. Candidatos tentativos: Describe a aquellos compuestos para los que existe evidencia de una o más estructura posible, pero esta resulta insuficiente para eliminar otros posibles candidatos estructurales, como, por ejemplo, los isómeros posicionales. Este es el caso de metabolitos o productos de transformación que han sufrido hidroxilaciones, nitraciones o desmetilaciones, pero no queda claro en que posición esto ha ocurrido.

Nivel 4. Formula molecular inequívoca: Se atribuye a aquellos compuestos a los que es posible asignar una fórmula molecular inequívoca, sin ambigüedades a partir de información espectral como, por ejemplo, información isotópica, aductos y/o fragmentación. Sin embargo, esta información es insuficiente para proponer sus posibles estructuras.

Nivel 5. Masa exacta: Se atribuye a compuestos a los que se le conoce su masa exacta, pero la evidencia no es suficiente para asignarle una formula molecular inequívoca. Puede resultar contraproducente etiquetar todas las masas de una muestra como nivel 5, por lo que este nivel se deberá aplicar a masas de interés específico. Por otra parte, deben usarse medidas de blanco para garantizar que la sustancia no surja de la preparación o la medición de la muestra.

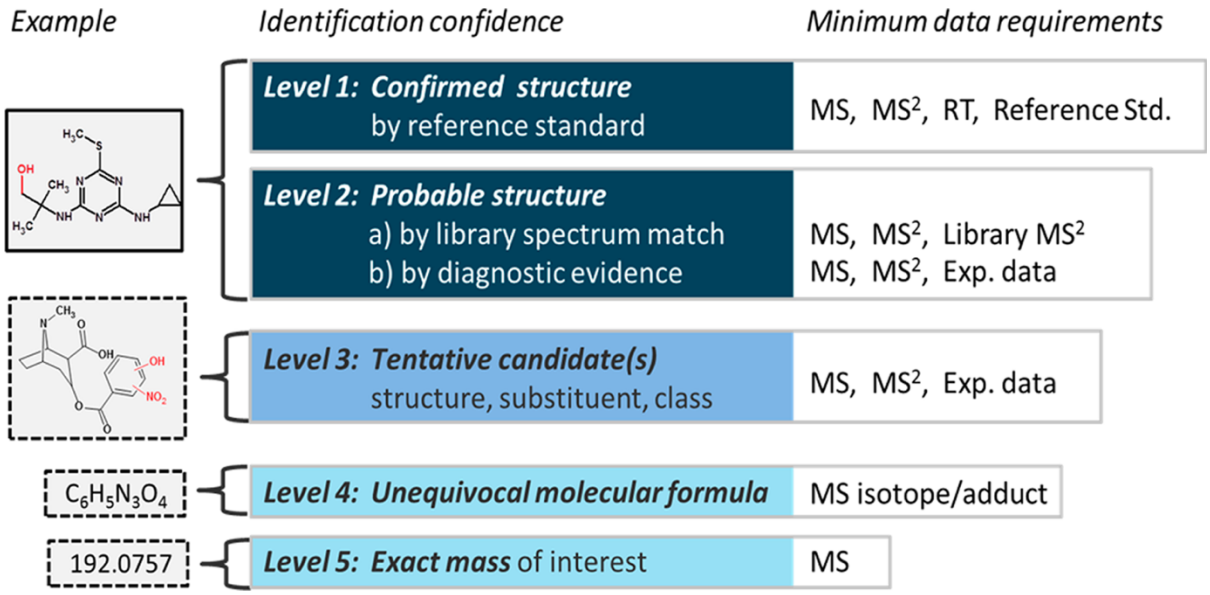


Figura. 6. Niveles de confianza propuestos para la identificación de compuestos por HRMS. Nota: MS^2 pretende representar cualquier forma de fragmentación en MS (ej. MS^e, MS^n). Tomado de (Schymanski et al., 2014).

1.7 Métodos para la remoción de CECs

Como se ha mencionado antes, las PTAR no logran remover en su totalidad a los CECs, pues no fueron diseñadas con esta finalidad (Boix et al., 2015; Patel et al., 2019). Como consecuencia, estos se han encontrado en diversos compartimentos ambientales, a niveles de concentración que van desde ng L^{-1} a mg L^{-}, incluso en las aguas del continente Antártico (Alygizakis et al., 2020; Ferrer and Thurman, 2012; Hernández et al., 2019). Por tal motivo, los investigadores han desarrollado diversas tecnologías alternativas que permiten la remoción más eficiente de los CECs en los afluentes de las PTAR. Las principales tecnologías de tratamiento se clasifican como tratamiento biológico, intercambio de fases y tecnologías avanzadas de oxidación (Rasheed et al., 2018; Rodriguez-narvaez et al., 2017).

1.7.1 Tecnologías de intercambio de fases

La remoción de CECs a partir de las tecnologías de intercambio de fases, ocurre a partir de la adsorción de estos a ciertos materiales. Por tal motivo, uno de los aspectos más importantes a considerar a la hora de elegir el intercambio de fase como proceso de remoción, es la selección del tipo de material a utilizar, principalmente porque este determina ciertas características como el tamaño de poro, la naturaleza metálica o no metálica y la capacidad de acoplar con un segundo tratamiento. A continuación se describen algunos de los materiales más empleados según (Rasheed et al., 2018; Rodriguez-narvaez et al., 2017):

- *Adsorción empleando carbón activado*

El carbón activado ha sido utilizado ampliamente para la remoción de CECs, debido a su capacidad de adsorción, dada por la gran superficie específica y la alta porosidad que posee este material. La fuente de materia prima de la que proviene el carbón activado, es uno de los aspectos más relevantes que influyen en las tasas de remoción de contaminantes obtenidas con este material. Se ha reportado por ejemplo, tasas superiores al 90% para una amplia variedad de compuestos como tetraciclina, ciflofloxacino, norfloxacino, etc. Esto confirma que este material adsorbente elimina selectivamente algunos CECs en el agua. Por otra parte, empleando carbón activado, es posible desarrollar sistemas combinados de forma secuencial con otras tecnologías como la ultrafiltración y la coagulación para lograr mejores tasas de remoción para ciertos compuestos.

- *Adsorción empleando biocarbón*

Los biocarbones son sustancias obtenidas a base de carbón vegetal que generalmente se usan para mejorar la condición del suelo. Se preparan a partir de biomasa, mediante pirólisis (proceso de calentamiento a alta temperatura sin oxígeno similar al proceso de producción de material carbonoso). La adsorción de los CECs en el biocarbón y por tanto la selectividad y la eficacia

del tratamiento de remoción, depende de varios factores como: las condiciones de pirólisis, la fuente de materia prima, así como del tratamiento térmico o químico empleado durante el proceso de producción del biocarbón. En comparación con el carbón activado, el biocarbón presenta una selectividad diferente y puede eliminar los CECs de manera más eficiente que el carbón activado. Sin embargo, el biocarbón no ha sido empleado en sistemas de tratamiento acoplados de manera similar al carbón activado a pesar de la semejanza en sus propiedades.

- *Adsorción asistida por nanomateriales (nanotubos de carbono)*

Los nanotubos de carbono (CNT) son un alótropo del carbono, con una configuración similar al grafito. Sus propiedades de adsorción dependen de sus características físico-químicas, geometría interna, diámetro, desarrollo de la hoja original, grado de curvatura y del método de tratamiento utilizado para su generación. Los CNT se clasifican como nanotubos de pared simple, con un diámetro interno de 1 nm aproximadamente y nanotubos de paredes múltiples, hechos de varios tubos o películas de grafeno laminado. Esta estructura (pared simple o múltiple), juega un papel importante en el desarrollo de la superficie y por tanto en la remoción de los contaminantes. Por ejemplo, se ha reportado la remoción de tetraciclina con una tasa de eliminación del 92% usando nanotubos de pared simple, mientras que, con nanotubos de pared múltiple, solo el 16% de tetraciclina se logró remover, manteniendo los mismos parámetros en ambos casos.

- *Adsorción empleando arcillas*

Los minerales arcillosos consisten principalmente en filosilicatos de aluminio hidratados y cationes como magnesio, hierro, metales alcalinos y alcalinotérreos con otros cationes en cantidades variables sujetos a la superficie planetaria. La eficacia del tratamiento con arcillas, depende en gran medida del material utilizado, debido a sus propiedades adsorbentes y a la cantidad de hierro, nitrógeno y otros minerales presentes en la matriz. Una aplicación

interesante de las arcillas con presencia de óxidos metálicos en su estructura, es el acoplamiento de estas a técnicas que utilizan sus características adsortivas combinadas con iones, para producir reacciones dentro de la estructura de la matriz porosa. Este sistema se ha utilizado con procesos de oxidación avanzados, en especial la reacción Fenton, con el fin de evitar la impregnación del catalizador en la matriz después de la reacción (arcilla mineral) y luego recuperar la matriz utilizando métodos convencionales (sedimentación) o procedimientos no convencionales (magnéticos).

- *Otros adsorbentes*

Se ha reportado el uso de otros materiales adsorbentes en la literatura para eliminar CECs. Estos incluyen zeolitas, materiales mesoporosos y microporosos, resinas y óxidos metálicos.

Uno de los inconvenientes del uso de la tecnología de adsorción, es la poca escalabilidad de las metodologías, que limita su aplicación práctica. Además de la poca sostenibilidad en la producción de materiales adsorbentes como las arcillas, entre otros materiales de origen natural. Esto constituye un problema significativo a largo plazo, por lo que las investigaciones deben ir encaminadas a reducir la huella ambiental asociada con el uso de estos materiales adsorbentes. Además de las tecnologías de adsorción, dentro de las tecnologías de intercambio de fases se encuentra la tecnología de membranas (Rodriguez-narvaez et al., 2017):

- *Tecnología de membranas*

Los procesos de remoción con membranas, se basan en el uso de presión hidrostática para eliminar sólidos en suspensión y solutos de alto peso molecular, dejando pasar agua y solutos de bajo peso molecular a través de una membrana. Las membranas se pueden producir a partir de diversos materiales, que dan lugar a sus características de filtrado (por ejemplo, tamaño de poro, carga superficial e hidrofobicidad), que determinan a su vez el tipo de contaminante que se puede retener.

La filtración a través de membranas se puede clasificar como: ultrafiltración (UF), nanofiltración (NF), microfiltración (MF), ósmosis directa (FO) y ósmosis inversa (RO). La Figura 7 muestra una representación esquemática de los diferentes tipos de membrana, así como ciertos contaminantes del agua, que pueden ser removidos de acuerdo a los diferentes rangos de tamaño de poro de estas. Excepto por la MF, los CECs pueden ser removidos por el resto de las membranas. La eficiencia de remoción, puede variar ampliamente dependiendo del tipo de membrana y el tipo de contaminante. Aunque a medida que el tamaño del poro disminuye la eficiencia de proceso de remoción mejora significativamente, por lo que los mejores resultados se han obtenido con FO y RO, alcanzando niveles de remoción entre 80 y 99% para ciertos analitos como carbamazepina y cafeína. Este proceso se considera de alto costo.

Aunque las tecnologías de intercambio de fases resultan una buena alternativa para la remoción de contaminantes emergentes, llegando a remover un alto porcentaje de ellos, sigue siendo un desafío la eliminación de estas sustancias en el medioambiente ya que los contaminantes que se eliminan irán a la fase sólida en el caso de los procesos de adsorción o fluirán con el efluente rechazado, en el caso de procesos de membrana. Por tanto, los CECs solo cambian de lugar, pero siguen permaneciendo como un problema para el medio ambiente (Barbosa et al., 2016; Rodriguez-narvaez et al., 2017).

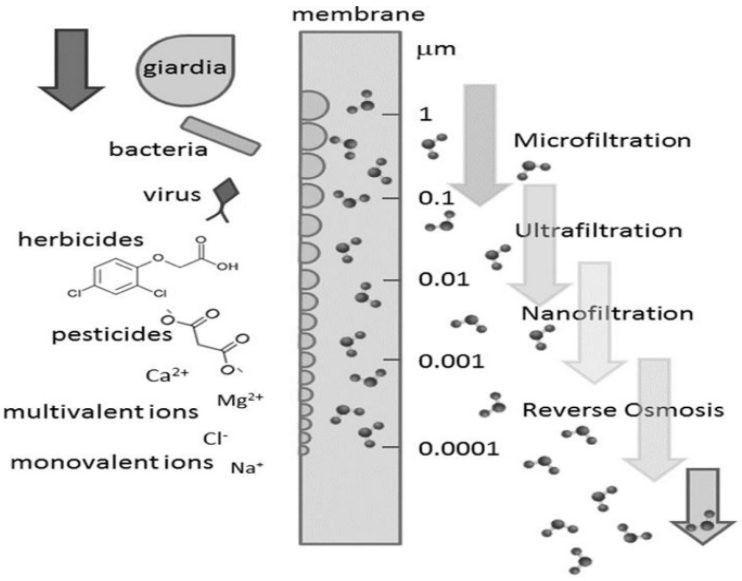


Figura. 7. Tipos de membranas, rangos de tamaño de poro y contaminantes removidos por cada tamaño de poro. Adaptado de (Rodriguez-narvaez et al., 2017).

1.7.2 Tratamiento Biológico

Existen diversos sistemas de tratamiento biológico para la remoción de CECs (filtración biológica, filtración de suelos y lodos activados), sin embargo, el uso de lodos activados es el más empleado, debido a su efectividad. Dependiendo del compuesto específico, las condiciones de tratamiento (aeróbico o anaeróbico) y las condiciones ambientales en diversos puntos geográficos, los valores de eficiencia de remoción pueden ir desde ninguna (por ejemplo, en el caso de carbamazepina o ácido diatrizoico usando lodo aeróbico activado en Alemania) hasta la casi completa eliminación (entre el 97-100% utilizando lodos activados aeróbicos/anaeróbicos para la eliminación de naproxeno en Finlandia). Estos procesos pueden ser acoplado secuencialmente con otros procesos de tratamiento terciario.

Una de las principales dificultades en la aplicación de procesos biológicos para la eliminación de CECs es la falta de metodologías analíticas precisas capaces de identificar y cuantificar estos

compuestos en una matriz tan compleja, así como la información relativamente limitada de la que se dispone sobre los microorganismos implicados en el proceso de degradación.

Por otra parte, algunos procesos interesantes han surgido, como parte de la búsqueda de procesos biológicos alternativos, como por ejemplo: sistemas bioelectroquímicos y biorreactores electroquímicos de membrana (Rodriguez-narvaez et al., 2017).

1.7.3 Procesos de Oxidación Avanzados (AOP)

Los AOP son muy eficaces en la oxidación de numerosos contaminantes orgánicos e inorgánicos. Todos estos procesos se basan en la generación de radicales libres ($^{\bullet}OH$, $O_2^{-\bullet}$, $HO_2^{\bullet}$, $SO_4^{-\bullet}$), especies altamente reactivas que pueden atacar con éxito la mayoría de las moléculas orgánicas, con constantes de velocidad de reacción elevadas que varían de 10^6 a 10^9 M^{-1} s^{-1}, en particular el radical hidroxilo ($^{\bullet}OH$) (Gautam et al., 2019; Rivera-Utrilla et al., 2013).

Los AOP comprenden una gran variedad de métodos, que presentan diferentes rutas de producción de estos radicales libres, con condiciones de trabajo específicas, que puede involucrar diferentes materiales, y pueden combinarse efectivamente con procesos convencionales para eliminar CECs (Rodriguez-narvaez et al., 2017; Zhu et al., 2016). Estos se pueden agrupar sobre la base de los procesos físico-químicos que se aplican en cada uno ellos (Figura 6).

1.7.3.1 Procesos Electroquímicos de Oxidación Avanzados (EAOP)

Los EAOP son AOP que utilizan electricidad para electrogenerar especies oxidantes. En las últimas décadas, han recibido especial atención cómo método para la remoción de contaminantes emergentes en aguas residuales (Moreira et al., 2017), debido a su alta eficiencia en la mineralización de compuestos refractarios (Özcan et al., 2008). En especial, la oxidación anódica es uno de los EAOP más comúnmente empleados para la remoción de contaminantes

en aguas (Tabla 3) (Flores et al., 2017; Martínez-Huitle et al., 2015) debido a su versatilidad y fácil escalabilidad (Garcia-Segura et al., 2018).

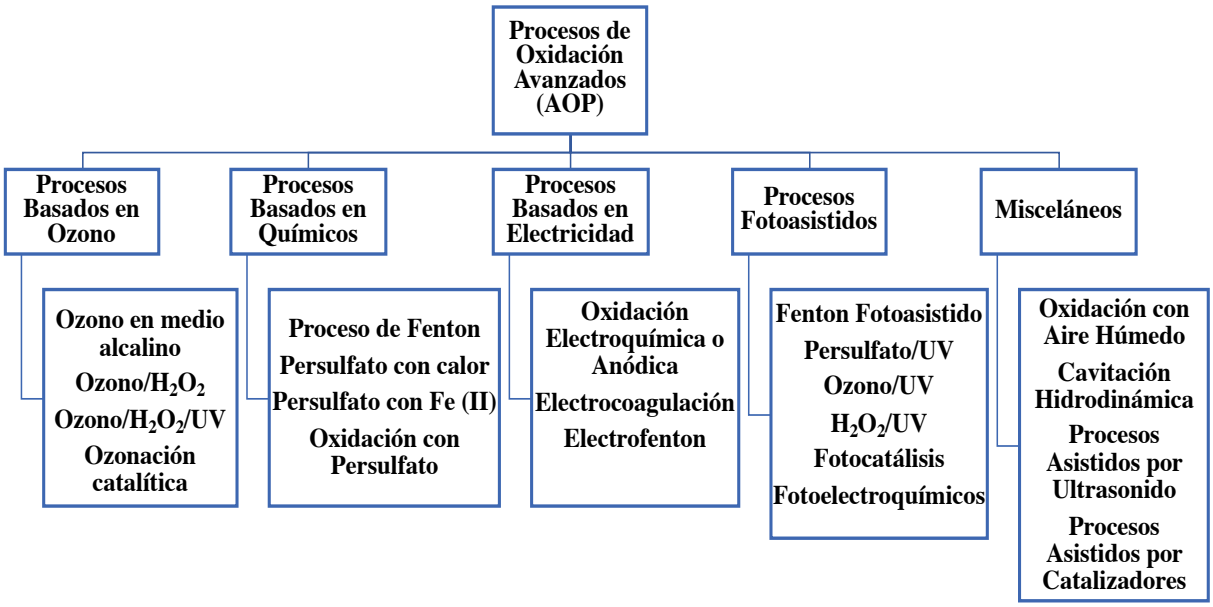


Figura. 8. Procesos de Oxidación Avanzados. Tomado y adaptado de (Gautam et al., 2019).

Tabla 3. Mineralización de fármacos por Oxidación Anódica.

Compuestos	Ánodo	Solución	Condiciones de electrólisis	Decaimiento del TOC (%)	Decaimiento del COD (%)	Referencias
Omeprazol	Pt BDD	100 mg $L^{-}1$ de fármaco en 100 mL de buffer compuesto por NaH_2PO_4 0.146 M + H_3PO_4 0.025 M, pH 7.0	100 mA cm^{-2} / 360 min	78	—	(Cavalcanti et al., 2013)
Estrona	BDD	500 mg L^{-1} de fármaco en 500 mL de Na_2SO_4 0.1 M, pH 7	10 mA cm^{-2} / 30 min	98	—	(Brocenschi et al., 2016)
Acetaminofeno y Diclofenaco	BDD	50 mg L^{-1} y 100 mg L^{-1} de fármaco en Na_2SO_4 0.05 M, pH 3	6.25 mA cm^{-2} / 3 h	51 and 54 ≈ 80	—	(García-Montoya et al., 2015)
Losartan	BDD	100 mg L^{-1} de fármaco en 100 mL de Na_2SO_4 0.05 M, pH 7	10 mA cm^{-2} /360 min	71	—	(Salazar et al., 2016)
Hidroclorotiazida	BDD	353 mg L^{-1} de fármaco en 100 mL de Na_2SO_4 0.05 M, pH 7	10 mA cm^{-2} /300 min	95	—	(Contreras et al., 2015)
Diclofenaco	Pt BDD	175 mg L^{-1} de fármaco en 100 mL: ▪ 0.05 M Na_2SO_4, pH 5.8 ▪ buffer compuesto por KH_2PO_4 0.05 M + Na_2SO_4 0.05 M + NaOH, pH 6.5	100 mA cm^{-2} /360 min	97.7 73 46 > 97	—	(Brillas et al., 2010)
Sulfametoxazol	Pt	52.7 mg L^{-1} de fármaco en 220 mL de Na_2SO_4 0.05 M, pH 3	30 mA /600 min	7	—	(Dirany et al., 2010)

Tabla 3. Continuación

Atenolol	Pt BDD	2.25 μM de fármaco en 250 mL a pH 6: ▪ Na_2SO_4 ▪ NaCl ▪ $NaNO_3$	8.88 mA cm^{-2} /25h	84.6 and 96 ≈ 80 < 65	—	(Murugananthan et al., 2011)
Amoxicillina	Ti/RuO_2–IrO_2 BDD	36.54 mg L^{-1} de fármaco en Na_2SO_4 0.05 M, pH 5.3	12.50–41.66mA cm^{-2} /6h	<25 ≈100	—	(Sopaj et al., 2015)
29 fármacos y pesticidas	BDD	100 μg L^{-1} de mix de fármacos y pesticidas en 114mL de efluente secundario a pH	196 Am^{-2}/ 6h	—	100	(Garcia-Segura et al., 2015)

Abrev: TOC, carbono orgánico total; DOC, demanda química de oxígeno; BDD, diamante dopado con boro

1.7.3.1.1 Oxidación Anódica (AO)

Durante la AO, los contaminantes orgánicos son oxidados por radicales hidroxilos heterogéneos M(•OH), formados a partir de la descarga del agua en la superficie de un ánodo (M) Ec. (1) (Martínez-Huitle and Ferro, 2006).

$$M + H_2O \rightarrow M(\bullet OH) + H^+ + e^- \qquad (1)$$

El radical hidroxilo es un poderoso oxidante (Sirés et al., 2014), con un alto potencial redox estándar (E^0 (•OH/H2O)= 2.80 V vs SHE) (Yu et al., 2014), capaz de oxidar a la mayor parte de los contaminantes orgánicos y organometálicos de forma no selectiva, hasta su completa mineralización a CO_2, agua e iones inorgánicos, a través de cuatro posibles mecanismos de ataque: (i) abstracción de un átomo de hidrógeno (deshidrogenación), (ii) adición electrofílica a un enlace insaturado (Hidroxilación), (iii) reacción de transferencia electrónica e (iv) ipso-sustitución de un átomo de halógeno (Mousset et al., 2018).

La reactividad de los radicales heterogéneos M (•OH) adsorbidos va a depender fuertemente del material del ánodo (M) empleado en el proceso oxidativo, el cual puede ser de naturaleza activa o no activa (Comninellis et al., 2008). Cuando el ánodo es "activo", los radicales interactuaran fuertemente con la superficie del ánodo pudiendo formar un superóxido MO, siempre y cuando el material del electrodo permita un estado de oxidación superior. Un cásico ejemplo de ánodo activo es el caso del IrO_2. Por otra parte, cuando el ánodo es "no activo" su superficie interactúa débilmente con los •OH, por tanto la oxidación de los contaminnates va a estar mediada por los •OH, puediendo llegar a obtener la completa mineralización (Martínez-Huitle and Ferro, 2006).

El ánodo BDD, es considerado un ánodo "no activo", capaz de producir un mayor grado de mineralización incluso que otros ánodos no activos como es el caso del PbO_2 y el de SnO_2, así como también que los activos Pt y RuO_2 (Rivera-Utrilla et al., 2013), por lo que se considera

ideal para el tratamiento de aguas residuales (Comninellis et al., 2008). Este mayor grado de mineralización alcanzado con BDD, se debe a la alta generación de •OH débilmente adsorbidos en su superficie (Ec. (2)) (Espinoza et al., 2018; Panizza and Cerisola, 2009) que favorece la alta reactividad de estos radicales con la materria orgánica.

$$BDD + H_2O \rightarrow BDD(\bullet OH) + H^+ + e^- \qquad (2)$$

De acuerdo con el potencial aplicado, la oxidación de la materia orgánica en el ánodo BDD puede seguir dos mecanismos: oxidación directa e indirecta (Comninellis et al., 2008). La oxidación directa ocurre en la superficie del ánodo e involucra reacciones de transferencia electrónica entre esta y la materia orgánica, cuando los potenciales se encuentran en la zona anterior a la del evolución del oxígeno. En este proceso, los contaminantes deben adsorberse en la superficie del ánodo inicialmente, lo cual constituye el paso limitante del mismo.

Por otra parte, en la oxidación indirecta median especies oxidantes electrogeneradas *in situ* en la superficie del ánodo como el •OH (cuando se alcanza la zona de potenciales de evolución del oxígeno). Otros ejemplos son las especies derivadas del carbonato (ion peroxodicarbonato $C_2O_6^{2-}$), fosfato (ion peroxodifosfato $P_2O_8^{2-}$), sulfato (radical sulfato $SO_4^{-\bullet}$ e ion persulfato $S_2O_8^{2-}$) (Martínez-Huitle and Brillas, 2009), y especies activas de cloro (Cl_2, $HClO/ClO^-$) (Contreras et al., 2015a) generadas cuando estas sales se encuentran en la solución. Estos oxidantes facilitan la oxidación de los contaminantes orgánicos cerca de la superficie del ánodo y/o en el seno de la solución, así como la desinfección en el caso de las especies de cloro (Candia-Onfray et al., 2018).

La generación del radical $SO_4^{-\bullet}$ y el ion $S_2O_8^2$ cuando en el medio hay presencia de la sal sulfato, tiene lugar a partir de los siguientes pasos descritos en las ecuaciones (Ec. (3-5)) (Espinoza-Cisternas, 2018).

$$HSO_4^- + \bullet OH \rightarrow SO_4^{-\bullet} + H_2O \qquad (3)$$

$$SO_4^{2-} + \bullet OH \rightarrow SO_4^{-\bullet} + OH^- \quad (4)$$

$$SO_4^{-\bullet} + SO_4^{-\bullet} \rightarrow S_2O_8^{2-} \quad (5)$$

Ambas especies actúan como oxidantes fuertes, eliminando la materia orgánica en la solución (Ec (6-8):

$$SO4^{-\bullet} + \text{organics} \rightarrow \text{Oxidation products} \rightarrow\rightarrow\rightarrow CO_2 + H_2O + SO_4^{2-\cdot} \quad (6)$$

$$S_2O_8^{2-} + \text{organics} \rightarrow \text{Oxidation products} \rightarrow\rightarrow\rightarrow CO_2 + H_2O + 2SO_4^{2-\cdot} \quad (7)$$

Por otra parte, las especies reactivas de cloro se generan a partir de una serie de pasos que tienen como inicio la combinación de radicales $Cl^{\bullet}$, originados a partir de la oxidación directa del Cl^- en la superficie del ánodo, para dar lugar a Cl_2 (aq) (Ecs. (8 and 9)) (Sirés et al., 2014). Luego, el cloro electrogenerado difunde fuera del ánodo y es hidrolizado dando cómo resultado ión Cl^- y ácido hipocloroso (HClO) (Ec. (10)). El HClO está en equilibrio con el ion hipoclorito ClO^- en el seno de la disolución (Ec. (11)) con un pKa de 7.5 (Garcia-Segura et al., 2018, 2015; Moreira et al., 2017).

$$Cl^- \rightarrow Cl^{\bullet} + e^- \quad (8)$$

$$2Cl^{\bullet} \rightarrow Cl_2\,(aq) + 2e^- \quad (9)$$

$$Cl_2\,(aq) + H_2O \rightarrow HClO + Cl^- + H^+ \quad (10)$$

$$HClO \leftrightarrow ClO^- + H^+ \quad (11)$$

Las especies activas de cloro (Cl_2, HClO/ClO^-) muestran diferencias significativas en su reactividad con los contaminantes orgánicos, dependiendo del valor de pH de la solución a tratar Deborde and von Gunten, 2008; Sales Solano et al., 2013). Deborde et al., 2008 plantean que en un rango de pH entre 6 y 9, HClO y ClO^- son las especies predominantes, mientras que a pH cercano a 7.5 estas especies se encuentran en una relación muy similar (Candia-Onfray et al., 2018).

Además, otras especies oxidantes pueden originarse a partir de la interacción de los •OH con los iones Cl^- (Ecs. (12-15) (Cotillas et al., 2017; Sirés et al., 2014). Estas especies son estables

y migran al seno de la disolución donde indirectamente oxidan la materia orgánica, aunque su poder oxidante es menor comparado con los •OH y HClO/ClO^- (Martínez-Huitle et al., 2015).

$$Cl^- + \bullet OH \rightarrow ClO^- + H^+ + e^- \quad (12)$$

$$ClO^- + \bullet OH \rightarrow ClO_2^- + H^+ + e^- \quad (13)$$

$$ClO_2^- + \bullet OH \rightarrow ClO_3^- + H^+ + e^- \quad (14)$$

$$ClO_3^- + \bullet OH \rightarrow ClO_4^- + H^+ + e^- \quad (15)$$

1.8 Métodos de análisis para monitorear la degradación y/o mineralización de los CECs durante la AO

1.8.1 Métodos para determinar el decaímiento de los niveles de contaminantes durante la AO

1.8.1.1 Determinación del carbono orgánico total

El carbón orgánico total (TOC) es una expresión directa del contenido orgánico total de una muestra, incluso más conveniente y directo que otros parámetros como la demanda bioquímica de oxígeno, la demanda química de oxígeno y el carbono orgánico asimilable. Es independiente del estado de oxidación de la materia orgánica, y no mide otros elementos ligados orgánicamente (por ejemplo, nitrógeno e hidrógeno) o inorgánicos que puedan contribuir a la demanda de oxígeno (American Public Health Association, American Water Works Association, n.d.). Por tal razón, la disminución del TOC en el tiempo, durante la AO de un agua residual o sintética, constituye un indicativo de la mineralización del contenido orgánico presente en ella y por tanto de la descontaminación de la misma.

Durante su determinación, el carbono orgánico es convertido en dióxido de carbono (CO_2), una forma molecular que se puede medir cuantitativamente, aplicando altas temperaturas (típicamente de 680 a 950 ° C) con catalizadores y oxígeno o aire, o temperaturas más bajas (<100 ° C) con irradiación ultravioleta y/o uno o más oxidantes químicos y catalizadores. El CO_2 se puede purgar de la muestra, secar y transferir a través de un gas portador a un analizador

de infrarrojos no dispersivo o valorador culombimétrico. Alternativamente, el CO_2 se puede separar de la muestra a través de una membrana selectiva de CO_2 en agua de alta pureza, donde el aumento de conductividad corresponde a la cantidad de CO_2 que pasa a través de la membrana (American Public Health Association, American Water Works Association, n.d.).

1.8.1.2 Espectrofotometría UV-vis

La espectrofotometría UV-vis constituye una poderosa herramienta para monitorear efluentes de aguas residuales y para evaluar la efectividad de procesos de tratamiento de aguas en la remoción de CECs. Basado en el principio de que la gran mayoría de estos contaminantes presentes en las aguas residuales, cómo fármacos, productos de cuidado personal, colorantes, entre otros, absorben fuertemente la radiación ultravioleta y visible (American Public Health Association, American Water Works Association, n.d.).

1.8.2 Métodos para la determinación de bioproductos y compuestos intermediarios generados durante la AO

1.8.2.1 Determinación de especies iónicas

La determinación de la evolución de aniones y cationes como bromuro, cloruro, fluoruro, nitrato, nitrito, fosfato, sulfato y amonio, durante un AO, es usada ampliamente como un indicador del estado de mineralización de una muestra que ha sido sometida a este proceso de tratamiento. Por ejemplo, el aumento del contenidos de aniones como el nitrito (NO_3^-) o sulfato (SO_4^{2-}) al final de una electrólisis, se atribuye a la degradación de contaminantes que contienen átomos de N o S en su estructura (Cavalcanti et al., 2013). Por otra parte, ha surgido la necesidad de medir la concentración de los subproductos de desinfección clorito, clorato, perclorato y bromato debido a los riesgos que estas especies representan para la salud de los organismos vivos.

Existen métodos colorimétricos, electrométricos o titrimétricos convencionales descritos para determinar aniones y cationes de forma individual, sin embargo, la Cromatografía Iónica (CI) proporciona una técnica instrumental única que puede utilizarse para su medición secuencial rápida, que elimina la necesidad de utilizar reactivos peligrosos y distingue eficazmente entre los haluros (Br^-, Cl^- y F^-) y los oxihaluros (ClO^{2-}, ClO^{3-} y BrO^{3-}) y los oxi-iones (PO_4^{3-} , SO_4^{2-}, NO_2^- y NO_3^-) (American Public Health Association, American Water Works Association, n.d.).

1.8.2.2 Determinación de intermediarios orgánicos

La determinación de la evolución de productos intermediarios como los ácidos carboxílicos de cadena corta u otros intermediarios de mayor peso molecular durante la AO, se realiza con la finalidad de corroborar la degradación y/o mineralización de los CECs presentes en la muestra, así como, con la intención brindar una posible vía o ruta de degradación de los compuestos en estudio a partir de los mecanismos de ataque de las especies oxidantes.

Los métodos más empleados actualmente para realizar el seguimiento de los intermediarios orgánicos, son la Cromatografía de Exclusión Molecular y la LC o GC con detectores altamente sensibles y selectivos como el MS de alta resolución (LC-HRMS o GC-HRMS), como el de tiempo de vuelo acoplado a un cuadrupolo (QTOF) u Orbitrap, o de baja resolución (LC-LRMS o GC-LRMS) como el triple cuadruplo (QqQ) o el de triple cuadrupolo con trampa de iones (QqQ_{LIT}).

1.9 Método para la determinación del consumo energético durante la oxidación de los contaminantes

El consume de energía por volume de solución electrolizada es un indicador de la eficiencia del tratamiento electroquímico y puede obtenerse a partir de la siguiente ecuación:

Consumo Energético (kW hm-3) = IEcell t / 1000 Vs (16)

donde I es la corriente aplicada (A), Ecell es el promedio del voltage de la celda (V), t es el tiempo de electrólisis (h), and Vs es el volumen de la solución tratada (m^3) (Candia-Onfray et al., 2018; Contreras et al., 2015b; Salazar et al., 2016a, 2017).

2. HIPÓTESIS Y OBJETIVOS

2.1 Hipótesis

Científica

El desarrollo de metodologías químico-analíticas basadas en microextracción en fase sólida acoplados off-line a LC-MS, permiten determinar contaminantes emergentes desde matrices acuosas.

Tecnológica

Los Procesos Electroquímicos de Oxidación Avanzada permiten la transformación y/o remoción de contaminantes en matrices acuosas.

2.2 Objetivo general

Establecer una metodología multirresidual basada en microextracción RDSE y HPLC-MS, que permita determinar los principales contaminantes emergentes en matrices acuosas, así como estudiar las vías de transformación y/o remoción inducidas por Procesos Electroquímicos de Oxidación Avanzada.

2.3 Objetivos específicos

1. Desarrollar una metodología analítica para la determinación multirresidual de CECs en aguas mediante UHPLC-MS/MS.
2. Aplicar una metodología basada en RDSE para la extracción de CECs e identificación por LC-HRMS.
3. Evaluar la transformación y mineralización de los principales CECs inducidas por Procesos Electroquímicos de Oxidación Avanzada.

3. ESTRATEGIA ANALÍTICA

La estrategia analítica para dar cumplimiento al objetivo general y los objetivos específicos se ilustra en la Figura 9.

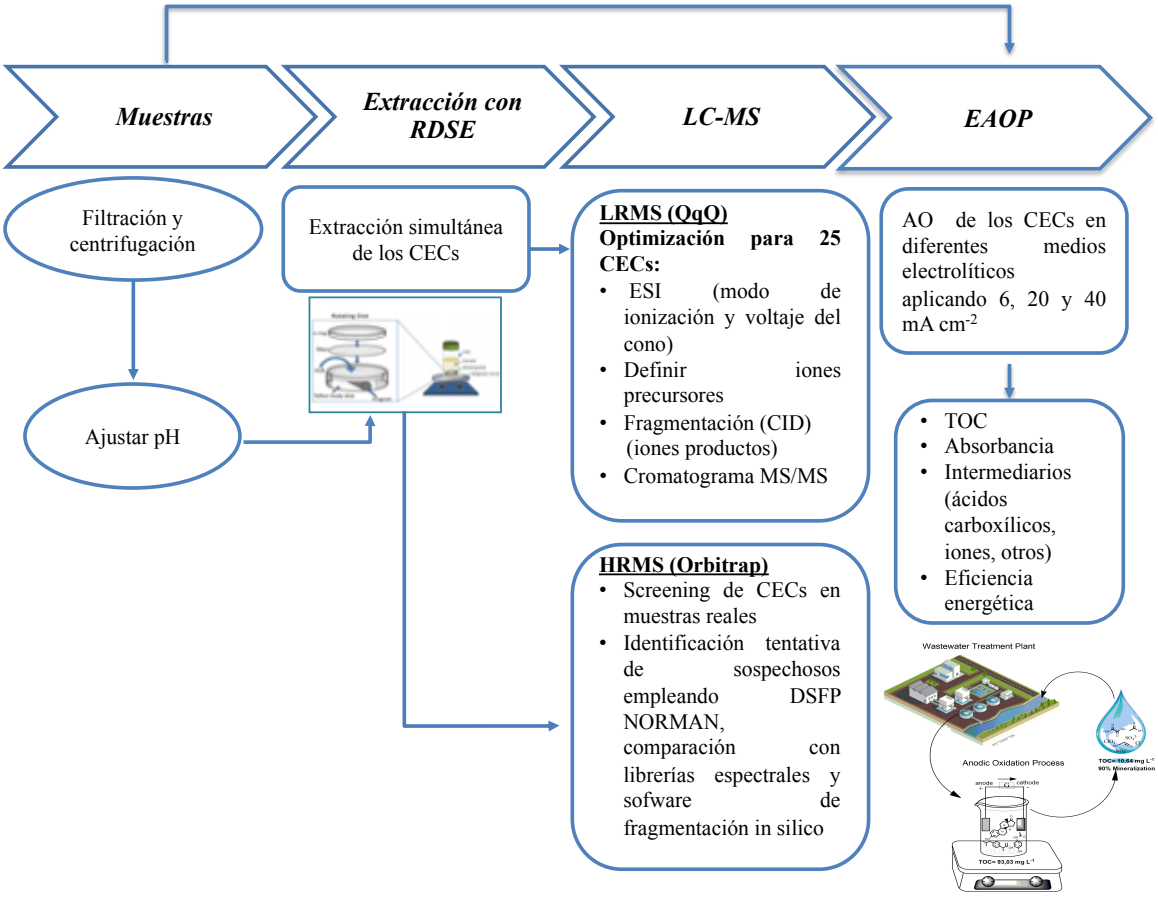


Figura. 9. Estrategia analítica propuesta

3.1 Desarrollo de una metodología analítica para la determinación multirresidual de CECs en aguas mediante UHPLC-MS/MS.

Se desarrolló un método multirresidual para la determinación de 25 productos farmacéuticos pertenecientes a varios grupos terapéuticos, en aguas superficiales, mediante cromatografía

líquida de ultra alta eficiencia acoplada a espectrometría de masas en tándem (UHPLC-MS/MS). Se seleccionaron tres transiciones de monitorización de reacción seleccionada (SRM) por compuesto, para garantizar una identificación fiable de los compuestos objetivo. Este método permite la determinación simultánea de compuestos ácidos y básicos en una sola inyección. La metodología desarrollada y los resultados obtenidos se detallan en el Capítulo II sección 1.

3.2 Aplicar una metodología basada en RDSE para la extracción de CECs e identificación por LC-HRMS.

Se evaluó preliminarmente un paso de preconcentración de la muestra mediante extracción con disco rotatorio (RDSE), empleando como fase extractiva el copolímero Oasis® PRiME HLB. La RDSE permitió la extracción simultánea de los 25 compuestos analizados con características físico-químicas diferentes, a pH 8, utilizando 15 mg de fase Oasis® PriME HLB, aplicando una velocidad de agitación de 1200 rpm y durante un tiempo de extracción de 120 min. La etapa de desorción se realizó con 10 mL de metanol acidificado al 1%, durante 30 min a una velocidad de agitación de 1200 rpm. Bajo estas condiciones, el factor de concentración para la mayoría de los compuestos no superó el 40 %. Los resultados obtenidos se detallan en el Capítulo II sección 1.

Cuatro muestras acuosas chilenas (tres de agua superficial y una proveniente de un afluente secundario de una PTAR), fueron procesadas empleando la metodología RDSE y analizadas empleando LC-HRMS en combinación con la DSFP. Se identificaron tentativamente 27 CECs diferentes al menos una vez, y 9 se identificaron en todas las muestras. Dentro de los contaminantes identificados tentativamente se encontraron pesticidas, plastificantes como los ftalatos, productos de cuidado personal, productos farmacéuticos, edulcorantes, retardantes de

llama, entre otros. La metodología implementada y los resultados alcanzados se explicitan en el Capítulo II sección 2.

3.3 Evaluar la transformación y mineralización de los principales CECs inducidas por Procesos Electroquímicos de Oxidación Avanzada

Se estudió la mineralización y transformación de 30 productos farmacéuticos mediante AO en medios electrolíticos diferentes y aplicando densidades de corriente de 6, 20 y 40 m A cm^{-2}. Se identificaron 25 intermediarios producidos durante la electrooxidación, obteniéndose claras diferencias en los compuestos formados cuando el electrolito soporte es NaCl o Na_2SO_4. En todos los medios electrolíticos, se produce la generación de ácidos carboxílicos, iones NO_3^-, SO_4^{2-} y NH_4^+. Adicionalmente, una muestra de agua proveniente de efluente secundario, cargada con los mismos 30 productos farmacéuticos fue tratada aplicando 6 m A cm^{-2} a pH natural, y sin la adición de electrolito de soporte, alcanzando un 88% de reducción del TOC. Todos los experimentos realizados, las metodologías implementados y los resultados alcanzados se explicitan en el Capítulo II sección 3.

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CAPÍTULO II: RESULTADOS Y DISCUSIÓN

CAPÍTULO II SECCIÓN 1

Title: Multiresidue method for determination of pharmaceuticals in water by UHPLC-MS/ MS and a preliminary study for their extraction by rotary disk sorptive extraction (RDSE)

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Abstract

A multiresidue method for the determination of 25 pharmaceuticals from various therapeutic groups, in surface water using ultra-high-performance liquid chromatography coupled to tandem mass spectrometry (UHPLC-MS/MS) was developed. Three selected reaction monitoring (SRM) transitions have been monitored per compound to ensure reliable identification of target compounds. This method allows the simultaneous determination of acidic and basic compounds in a single injection. In addition, a preconcentration step by rotating disk sorptive extraction (RDSE) with a hydrophilic-lipophilic balanced polymer for sample treatment was preliminarily evaluated.

1. Introduction

Emergent contaminants (ECs) are defined by Estrada et al., 2018 as "chemical substances that have no regulation, are suspected to affect the environment or whose effects are unknown" (Barrios-Estrada et al., 2018). A wide number of compounds of diverse chemical nature constitute the ECs group, among them pharmaceuticals, personal care products, endocrine disrupting compounds, nanomaterials and perfluorinated pollutants (Tijani et al., 2016). However, pharmaceuticals are considered one of the biggest and important subgroups within the ECs (Gómez et al., 2010). Pharmaceuticals are designed to produce a biological response in a target organism, however they can also produce the same response in non-target entities after chronic exposure even to trace concentrations of these compounds (Rasheed et al., 2018). Its extensive use worldwide has generated bioaccumulation and toxic effects in aquatic and terrestrial ecosystems (Ebele et al., 2017). Some of the adverse effects reported in humans and other ecological species are disruptions of the endocrine system, chronic toxicity, and antibiotic resistance (Grenni et al., 2018; Tijani et al., 2016).

Wastewater treatment plants (WWTPs) were not designed to completely remove ECs (Rout et al., 2020), consequently, pharmaceuticals have been determined in aquatics environments such as effluents, surface and ground waters around the world, at concentrations ranges of μg/L and ng/, including in Antarctic continent (Ferrer and Thurman, 2012; Hernández et al., 2019; López-Serna et al., 2010). Although several research groups have reported the occurrence of ECs in Chile (Jachero et al., 2013; Moncada, 2015; Sandoval, 2015; Villa, 2012), the available information is still insufficient, so it is considered very important to continue working in this direction with the development of new and more sensitive analytical techniques that allow the analysis of a broad group of contaminants. One of the increasingly used options in

environmental analysis is ultra-high performance liquid chromatography coupled to tandem mass spectrometry (UHPLC-MS/MS) (López-serna and Petrovic, 2011). UHPLC provides higher speed of analysis, better resolution, higher sensitivity and reduced matrix effects (Wille et al., 2012). Coupled to the MS/MS, it significantly improves its sensitivity, operating in SRM mode. (Jakimska et al., 2017). In addition, an offline preconcentration step is required to increase sensitivity for detecting the low concentrations of pharmaceuticals in real water samples (Gracia-lor et al., 2010). Rotating disk sorptive extraction (RDSE) is an integrated extraction/stirring technique proposed in 2009 by Richter et al (Vieira et al., 2019). This technique operates similarly to stir bar sorptive extraction (SBSE), since the disk is stirred into the sample for a defined period until the extraction of the target compounds (Soledad and Lucena, 2017), however in RDSE, the extracting phase is only in contact with the liquid sample, so the devices can be stirred at a much higher speed than the stir bar used in SBSE without damaging the phase. RDSE have been applied widely in the extraction of ECs from aqueous matrices, such as anti-inflammatory drugs, hormones, triclosan, parabens, pesticides, among others (Arismendi et al., 2020; Becerra-Herrera et al., 2020; Donato et al., 2017; Becerra-herrera et al., 2015). Nevertheless, few studies have been reported using this methodology for the simultaneous extraction of a broad group of contaminants with diverse physicochemical characteristics.

Multiresidue analytical methods have become preferred tools that allow the determination of a large number of pharmaceuticals in a single analysis, thus reducing their time and cost. In these methods, simultaneous analysis of compounds of several classes with different physicochemical characteristics is performed, therefore a compromise is required in the selection of experimental conditions (preconcentration, chromatographic separation, and MS

detection) which results in the method performance being not optimal for each compound, but acceptable for most (Petrovic, 2014).

In this study, a multi-residue method optimized by HPLC-MS/MS is proposed for the determination of a group of 25 drugs with different physicochemical characteristics such as analgesics, anti-inflammatory drugs, antidepressants, antibiotics, antihistamine hormones, among others, from water. In addition, a preliminary study by RDSE was carried out using Oasis® PRiME HLB as sorbent phase for the simultaneous extraction of these contaminants.

2. Experimental

2.1 Reagents and chemicals

Trimebutine maleate, caffeine, acetaminophen, chlorphenamine maleate, sodium diclofenac, ibuprofen (>99% purity) was supplied by Pasteur S.A Laboratory (Santiago, Chile). Mefenamic acid, venlafaxine, sertraline, escitalopram, fluoxetine, amoxicillin, losartan, enalapril, ergotamine, famotidine, omeprazole, and loratadine in commercial tablets, were acquired from the established trademarks in Chile. Norfloxacin, ciprofloxacin, naproxen, sulfamethazine (>98% purity) and enrofloxacin (>99% purity) were obtained from Sigma-Aldrich (Santiago, Chile). Salicylic acid (>99% purity) was purchased from Merck (Santiago, Chile). Table 1 shows the physicochemical properties of all the pharmaceuticals under study.

Methanol and acetonitrile employed were HPLC grade from Merck and Sigma-Aldrich (Santiago, Chile). Ultrapure water was obtained for a Millipore Milli-Q system (resistivity ≤18 mΩ cm). Sodium Hydroxide. formic acid (≥ 98%) and ammonium acetate was supplied by Merck (Santiago, Chile).

Stock standard and commercial tablets solutions were prepared in methanol for a final concentration of 500 mg/L. These stock solutions were used for spiking water samples in RDSE and SPE. Furthermore, the individual stock solution was mixed and diluted in methanol/water (80:20 v/v) for the LC-MS analysis. For better dissolution of quinolones (ciprofloxacin and norfloxacin) 100 µL of 1N NaOH was added taken to reference the procedure described by (Ziarrusta et al., 2017). Solution of Omeprazole was prepared monthly due to the low stability of this compound. All solutions were kept at -4 °C.

The rotating disks used in this work were provided by Dr. Pablo Richter of the University of Chile. To carry out the extractions and desorption, a 2 mag AG (Munich, Germany MIX 15 eco multi-position magnetic stirrer and WiseStir MS-MP4 multipoint magnetic stirrer were used. The pH was adjusted with a pH-meter HANNA Edge® HI2020. Oasis® PRiME HLB cartridges was supplied by Waters (USA).

2.2 Liquid Chromatography mass spectrometry

UHPLC analysis was carried out in the Nexera X2 UHPLC system (Shimadzu, Kyoto, Japan). This system was equipped with an LC-30 AD pump, DGU-20A5R degassing unit, SIL-30 AC autosampler, CTO-20 AC column oven, CBM-20A communication module and SPD-M20A diode array detector (DAD). Chromatographic separation was performed using a Kinetex® XB-C18 (100 x 4.6 mm. 2.6 µm) column, set at 40 ºC using a mobile phase composed of water: methanol (95:5 v/v) acidified with 0.01% formic acid (A) and methanol (B). The following gradient was applied at a flow rate of 0.5 mL min^{-1}: 0 min, 30% B; 1 min, 30% B; 2 min, 30% B; 3 min, 75% B; 6 min, 90% B; 9 min,100% B; 10 min, 100% B. The analysis run was 15 min and the sample injection volume was 10 µL.

MS/MS analysis was performed using an LCMS-8030 triple quadrupole (TQ) mass spectrometer with electrospray ionization (ESI) source applying the following settings: capillary voltages of +4.0 and -2 kV in positive and negative modes, nebulizer gas (N_2) 3 L min^{-1}, desolvation gas (N_2) 15 L min^{-1}, desolvation line temperature 250 °C and heat block temperature 400 °C. Data was acquired and analyzed employing Shimadzu LabSolutions 5.8 software .

2.4 Preliminary study to establish a procedure for extraction by RDSE

In order to establish a procedure for the extraction of the contaminants under study by RDSE, different variables were evaluated: pH sample, amount of sorbent phase, time and desorption solvent. Solvent and desorption time were evaluated by varying one parameter at a time. The significance of each parameter was defined through analysis of variance (ANOVA) with a confidence level of 95% take the signal of each compound as the response.

The stirring speed during all experiments was 1200 rpm, which is the highest speed achieved by the magnetic stirrer used. The pH of the sample were evaluated by keeping the stirring time constant at 120 minutes. Experiments were performed in triplicate at three pH values 2, 6 and 8. Variables such as stirring speed (1200 rpm), extraction solvent (methanol acidified with 1% formic acid), desorption time (30 minutes), and sorbent amount (15 mg) were kept constant.

The influence of the ionic strength of the medium is considered an important variable to study in microextraction procedures, since the salting out effect favors the extraction of high and medium polarity compounds (Aparicio et al., 2017). However, in this preliminary study it was not possible to incorporate salts to improve the extraction efficiency of these compounds, since due to the characteristics of the disk, part of the salt was retained in the cavity containing the stationary phase, passing then to the mass spectrometer and causing a matrix effect that

enhanced some analytical signals such as sertraline or losartan. Furthermore, the matrix effect caused by the addition of salt to the medium is a fact reported by Cañas et al., 2014. In this case, the sensitivity of the analytical signal of florfenicol was negatively affected with the addition of salt using the polymeric extracting divinylbenzene-N-vinylpyrrolidone phase (Cañas and Samuel, 2014). For these aforementioned reasons, no salts were added in the extractive methodology.

In addition, a comparative study was performed between the preliminary methodology by RDSE and a methodology described in the literature by SPE using the t-contrast for paired samples. The concentration factors of each methodology at pH 2, 6 and 8 were compared to determine if the methods produce statistically different results and according to the result continue to improve or not the RDSE procedure. The disk used in the RDSE procedure is shown in Fig 1.

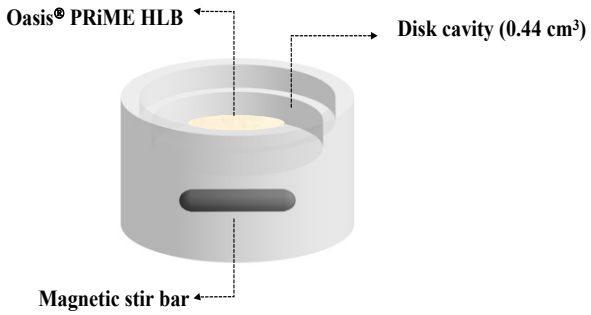

Figure. 1. Rotating disk used in the study. Adapted from (Cañas and Samuel, 2014).

2.5 Proposed procedure preliminarily by RDSE

They were taken 15 mg of Oasis® PRiME HLB phase was placed in the 0.44 cm^3 Teflon disk cavity, them the cavity was covered with a paper filter and sealed with a Teflon ring. The Oasis® PRiME HLB phase was conditioned with ethyl acetate, methanol and Milli-Q water for 5 minutes each before extraction following (Becerra-Herrera et al., 2020). Next, the disk was

immersed in an amber vial EPA of 40 mL contained 10 mL of ultrapure water spiked with 1mL of the standard mix (0.5 mg/L) (pH adjusted to 2,6 or 8 with 0.1N HCl or 0.1 N NaOH) and stirred at 1200 rpm on the magnetic stirrer for 120 minutes. After extraction, the disk was removed from the water and dried. Later, was placed in another vial and 10 mL of methanol 1% formic acid was added and stirred by 30 minutes at 1200 rpm to reach the desorption of analytes. Subsequently, the disk is out and the solvent was evaporated under N_2 stream. Finally, the extract was reconstituted with 1mL of methanol: water (80:20 v/v), filtered with a 0.22 µm syringe filter and injected into the UHPLC-MS/MS system.

2.6 Solid-phase extraction

The SPE procedure was adapted from (Gracia-lor et al., 2010). Briefly, Oasis® PRiME HLB cartridges (200 mg) were preconditioned with 3 mL of methanol and 3 mL of Milli-Q water. Then, 100 mL of Milli-Q water spiked with the standard solution of the contaminant to obtain a final concentration of 1µg/L and pH adjusted to 2, 6 or 8 was passed through the cartridge with the aid of vacuum. After drying, the analytes were eluted with 5 mL of methanol. The extract was evaporated to dryness under a gentle stream of nitrogen and finally reconstituted with 1 mL of methanol: water (80:20 v/v), filtered through a 0.22 µm syringe filter and injected into the UHPLC-MS/MS system.

Table 1. Physicochemical properties of the emergent contaminants studied.

Group	Compounds	Formula	pKa	Log P	pH 2[c]	pH 6[c]	pH 8[c]	References
AINEs	Diclofenac	$C_{14}H_{11}Cl_2NO_2$	4.00	4.26	neutral	anionic	anionic	
	Ibuprofen	$C_{13}H_{18}O_2$	4.85	3.84	neutral	anionic	anionic	
	Naproxen	$C_{14}H_{13}O_3$	4.19	2.99	neutral	anionic	anionic	
	Mefenamic acid	$C_{15}H_{15}NO_2$	3.89	5.40	neutral	anionic	anionic	
	Salicylic acid	$C_7H_6O_3$	2.79	1.98	neutral	anionic	anionic	
	Acetaminophen	$C_8H_9NO_2$	9.46	0.91	neutral	neutral	neutral	
Antispasmodic	Trimebutine	$C_{22}H_{29}NO_5$	8.68[b]	4.11	cationic	cationic	cationic	

Antidepressants	Escitalopram	$C_{20}H_{21}FN_2O$	9.78[b]	3.76	cationic	cationic	cationic	(ChemAxon, 2016)
	Sertraline	$C_{17}H_{17}Cl_2N$	9.56[b]	5.15	cationic	cationic	cationic	
	Fluoxetine	$C_{17}H_{18}F_3NO$	9.80[b]	4.17	cationic	cationic	cationic	
	Venlafaxine	$C_{17}H_{27}NO_2$	14.42[a] 8.91[b]	2.74	cationic	cationic	Equilibrium neutral-cationic	
Alkaloid	Ergotamine	$C_{33}H_{35}N_5O_5$	9.71[a] 8.39[b]	2.71	cationic	cationic	cationic	
Antihypertensive	Enalapril	$C_{20}H_{28}N_2O_5$	3.67[a] 5.20[b]	0.59	cationic	anionic	anionic	
	Losartan	$C_{22}H_{23}ClN_6O$	5.85[a] 3.85[b]	5.00	cationic	Equilibrium neutral-anionic	anionic	
Antihistamines	Loratadine	$C_{22}H_{23}ClN_2O_2$	4.33	4.55	cationic	neutral	neutral	
	Chlorphenamine	$C_{16}H_{19}ClN_2$	9.47	3.58	cationic	cationic	cationic	
Antiulcer agents	Famotidine	$C_8H_{15}N_7O_2S_3$	9.29[a] 8.38[b]	-1.95	cationic	cationic	cationic	
	Omeprazole	$C_{17}H_{19}N_3O_3S$	9.29[a] 4.77[b]	2.43	cationic	neutral	neutral	
Stimulant	Caffeine	$C_8H_{10}N_4O_2$	-1.16	-0.55	neutral	neutral	neutral	
Antibiotics	Amoxicillin	$C_{16}H_{19}N_3O_5S$	3.23[a] 7.22[b]	-2.31	cationic	zwitterionic	anionic	
	Ciprofloxacin	$C_{17}H_{18}FN_3O_3$	5.56[a] 8.68[b]	-0.85	cationic	zwitterionic	zwitterionic	
	Norfloxacin	$C_{16}H_{18}FN_3O_3$	5.58[a] 8.68[b]	-0.96	cationic	zwitterionic	zwitterionic	
	Enrofloxacin	$C_{19}H_{22}FN_3O_3$	5.69[a] 6.68[b]	1.15	cationic	zwitterionic	anionic	
	Sulfamethazine	$C_{12}H_{14}N_4O_2S$	6.99[a] 2.04[b]	0.65	Equilibrium neutral-cationic	neutral	anionic	
Hormones	Progesterone	$C_{21}H_{30}O_2$	18.92[a] -4.80[b]	4.15	neutral	neutral	neutral	

MW: molecular Log P: partition coefficient. pKa: acid dissociation constant. a: pKa strongest acidic. b: pKa strongest basic. c: species present at pH 2, 6 and 8.

3. Results and discussion

3.1 Chromatographic optimization

To obtained an adequate chromatographic separation (reduction of peak tailing and better resolution) and high sensitivity in the signals of the analytes ionized in negative and positive modes in the same run, different columns (XB-C18 (100 x 4.6 mm. 2.6 µm) and EVO-C18 (100

x 4.6 mm. 2.6 µm) both Kinetex®), mobiles phases (methanol and acetonitrile), the flow rate and additives (formic acid and ammonium acetate at various concentrations) were tested.

Initial experiments to establish the mobile phase were carried out by adding ammonium acetate at different concentrations (0.1 mM and 1 mM) only in water and in both solvents (water and methanol) since it has been reported in the literature that for certain compounds under positive ionization, sensitivity improves when NH_4Ac is added. However, signals of many compounds were obtained with an undesirable peak shape. Subsequently, formic acid (0.01% and 0.1%) was added because this additive reduces peak tails and improves the resolution (Gracia-lor et al., 2010), but signals of antibiotics such as ciprofloxacin, norfloxacin and amoxicillin still have an undesirable peak. When formic acid was added to the mobile phase at the highest concentration evaluated 0.1%, the responses of the negative ion of ibuprofen decreased. This phenomenon is attributable to the fact that when the acid concentration increases in the (-) ESI mode, the low pH does not favor the formation of the deprotonated analyte (Zengru Wu et al., 2004).

Methanol was replaced by acetonitrile in the same proportions used in the mobile phases evaluated, but a significant decrease in the signals of all analytes with a marked decrease in the signal of naproxen and ibuprofen. All the mobile phases proposed were tested with the EVO-C18 and XB-C18 columns at two flow rates of 0.3 and 0.5 mL /min, obtaining the best results in terms of sensitivity and peak shape with the mobile phase composed by (A) water containing 5 % (v/v) of methanol and 0.01 % (v/v) formic acid and (B) methanol, XB-C18 column and a flow rate of 0.5mL /min.

3.2 MS and MS/MS optimization

Full scan and product ion scan mass spectra were obtained from *m/z* 50 to 1000 by direct injection of 1 mg/L methanol/water (80:20 v/v) individual standard solutions at a flow rate 0.3 mL/min. All the compounds were ionized in both positive and negative modes. Only 4 compounds (ibuprofen, naproxen, diclofenac and salicylic acid) out of 25 studied had the highest ionization in negative mode, the others compounds ionized better in positive mode. In all cases the most abundant ion was $[M + H]^+$ or $[M - H]^-$ and they were selected as precursor ions. A MS/MS chromatogram of compounds studied in positive and negative ionization mode is shown in Figure 2.

For most compounds, three selected SRM transitions between the precursor ion and three most abundant fragment ions were monitored, except for ibuprofen, fluoxetine, naproxen and salicylic acid, which showed poor fragmentation and therefore only two transitions were obtained for them. This allows simultaneous detection, quantification and confirmation of all analytes, making it possible to reach more than 4 identification points (IPs), in accordance with the requirements proposed in the guidance of the European Union Decision 2002/657/EC1 (Commission, 2002). A full list of SRMs and the optimal instrumental conditions are given in Table 2.

To guarantee the sensitivity in SRM detection, transitions were separated into 11 segments for the acquisition. Each one collecting data for a maximum of 4 analytes because the detection capacity decreases as the number of monitored transitions per minute increases as reported by (Vasiljevi and Lauˇ, 2009). As the monitoring time of each transition was set to a time segment, it was necessary to reduce the *dwell time* of each transition from 0.1 s, which the software sets

by default, to 0.015 s and thus it was possible to obtain the appropriate number of points per peak to ensure well-defined chromatographic peaks

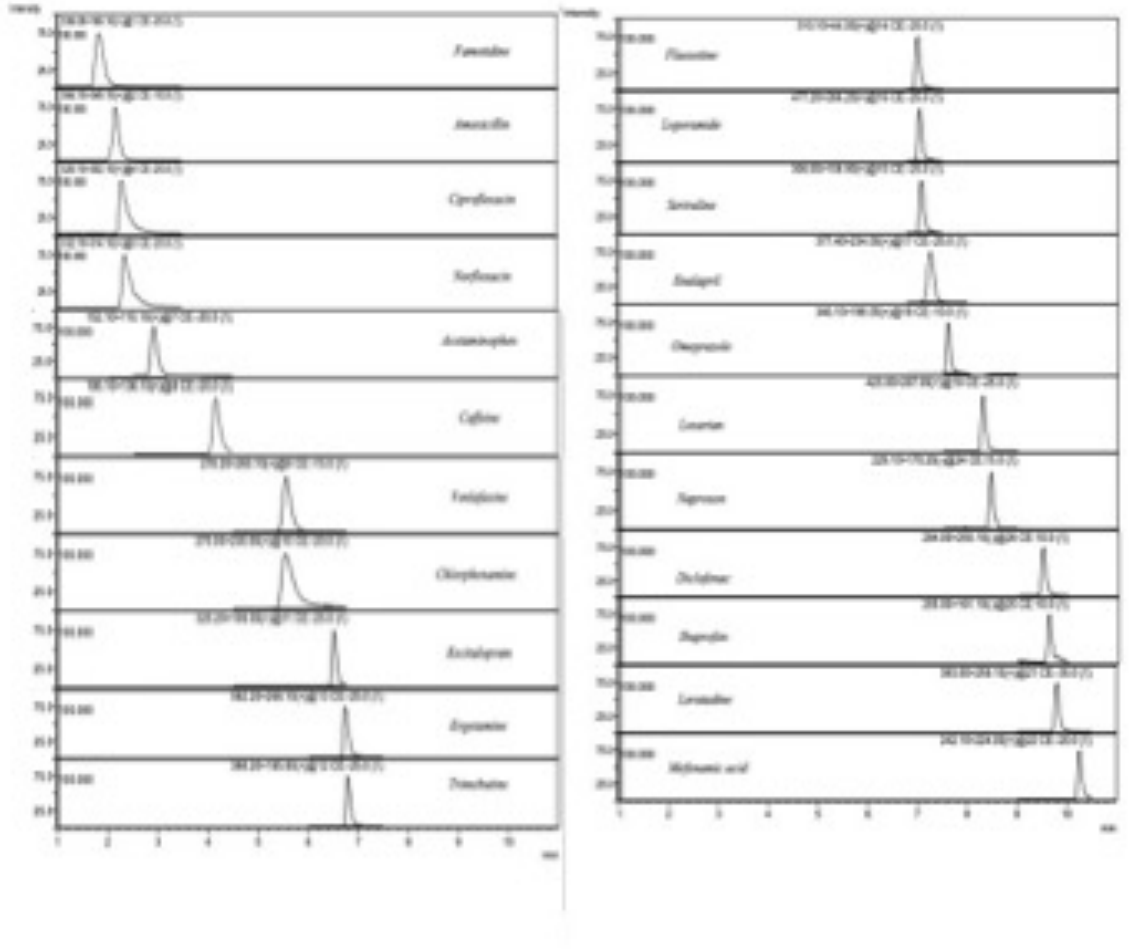

Figure. 2. Mass Chromatogram (quantification transition) of some compounds studied in positive and negative ionization mode (0.1 mg/L).

Table 2. LC-ESI-MS/MS optimized conditions.

Compound	Polarity (ESI)	MW	SRM_1	CE/CV	SRM_2	CE/CV	SRM_3	CE/CV
Acetaminophen	+	151.1	152.1>110.1	20/4	152.1>65.0	35/4	152.1>93.1	35/4
Amoxicillin	+	365.1	366.1>114.1	25/3.5	366.1>208.1	10/4	366.1>349.2	10/3.5
Caffeine	+	194.1	195.1>138.1	20/4	195.1>110.1	25/4	195.1>69.1	35/4
Ciprofloxacin	+	331.1	332.1>314.1	20/3.5	332.1>231.1	35/3.5	332.1>288.1	10/4
Chlorphenamine	+	274.1	275.1>230.0	20/4	275.1>167.1	45/4	275.1>188.0	45/4
Diclofenac	-	295.0	294.0>250.1	10/4	294.0>213.9	20/4	294.0>221.1	45/4
Enalapril	+	376.2	377.4>234.0	45/4	377.4>91.1	25/4	377.4>117.1	35/4
Enrofloxacin	+	360.2	360.2>245.1	35/3.5	360.2 >316.1	35/3.5	360.2 >342.1	35/3.5
Ergotamine	+	581.3	582.2>268.1	25/4	582.2>223.15	25/4	582.2>208.0	25/4
Escitalopram	+	324.2	325.2>109.0	25/4	325.2>116.1	25/4	325.2>234.0	25/4
Famotidine	+	337.0	338.0>189.1	25/4	338.0>155.0	35/4	338.0>113.1	45/4
Fluoxetine	+	309.1	310.1>44.0	25/4	310.1>148.1	25/4		
Ibuprofen	-	206.1	205.0>161.1	10/2	205.0>204.7	10/2		
Loratadine	+	382.1	383.0>259.1	35/4	383.0>337.1	25/4	383.0>267.1	35/4
Losartan	+	422.2	423.0>207.0	25/4	423.0>180.1	35/4	423.0>405.2	10/4
Mefenamic acid	+	241.1	242.1>224.0	20/4	242.1>180.1	45/4	242.1>209.1	35/4
Naproxen	-	230.1	229.1>170.2	15/2	229.1>185.3	15/2		
Norfloxacin	+	319.1	320.1>302.1	20/3.5	320.1>233.2	20/3.5	320.1>275.9	20/3.5
Omeprazole	+	345.1	346.1>198.1	10/4	346.1>136.1	25/4	346.1>151.1	25/4
Progesterone	+	315.2	315.2>109.1	25/3.5	315.2>97.15	20/4	315.2>123.15	25/3.5
Salicylic acid	-	137.0	137.0 > 93.0	20/4.5	137.0 > 65.0	30/4.5		
Sertraline	+	305.1	306.0>158.9	25/4	306.0>275.0	15/4	306.0>129.1	25/4
Sulfamethazine	+	279.0	279.0>186.1	20/2	279.0 >124.0	25/2	279.0 >156.0	20/2
Trimebutine	+	387.2	388.2>195.0	25/4	388.2>131.1	25/4	388.2>343.2	10/4
Venlafaxine	+	277.2	278.2>260.1	15/4	278.2>121.0	35/4	278.2>147.1	25/4

MW (monoisotopic molecular weight), SRM_1 (transition for quantification), SRM_2 and SRM_3 (transitions for confirmation), CE (collision energy), CV (cone voltage).

3.3 Preliminary study to establishment extraction conditions by RDSE

Time and solvent for desorption

Desorption conditions (time and solvent) were evaluated, keeping fixed the stirring speed (1200 rpm), extraction time (30 minutes), sample pH (natural pH 5.5 and 6.5), sample volume (10 mL), amount of sorbent phase (15 mg) and solvent of desorption (methanol 1% formic acid).

Figure 3 shows the results of the desorption time tests for those contaminants (16.7% of the total analytes studied) that presented significant differences in the analytical signal ($p<0.05$) at different extraction times evaluated (10, 15, 20, and 30 minutes). As can be seen, the signals are greater and therefore greater desorption when the extraction is carried out for 30 min. In this preliminary study, times longer than 30 minutes were not considered, since the procedure would be very time consuming and because it has been reported that with very long desorption times a pre-equilibrium process can take place, with some molecules returning to the sorbent and consequently a decrease in response (Vieira et al., 2018). Solvents and mixtures of solvents of different polarities, such as methanol, acetonitrile, ethyl acetate and water: methanol: acetonitrile mixture (1:6:3) were tested for desorption of contaminants. In the first stage, the best results were obtained with the mixture of water: methanol: acetonitrile, since this mixture includes high and medium polarity solvents that favor the extraction of pharmaceuticals with different Log P (Table 1). However, during the evaporation to dryness of the samples under a stream of nitrogen, it was difficult to define at which point all the organic solvent was evaporated and only the water with the concentrated analytes remained.

Methanol was the second medium with the best extraction efficiency. Nevertheless, the extraction yield for amoxicillin was very low with this solvent. Therefore, another extraction solvent consisting of methanol acidified with 1% formic acid was proposed. The objective of incorporating this acid into methanol was to promote the creation of extra hydrogen bonds between the analytes and the solvent and thus improve the solubility and the extraction of the compounds. Figure 4 shows that not only the extraction of amoxicillin (Log P -2.31) is improved with this solvent but also the extraction efficiency of other compounds with Log $P < 3$ such as acetaminophen, enalapril, famotidine and ergotamine and with Log $P > 3$ such as escitalopram, sertraline, fluoxetine and trimebutine. In addition, the lowest RDS values among

experimental replicates (RDS < 22%) were obtained with this desorption solvent. Therefore, the solvent selected was 1% acidified methanol.

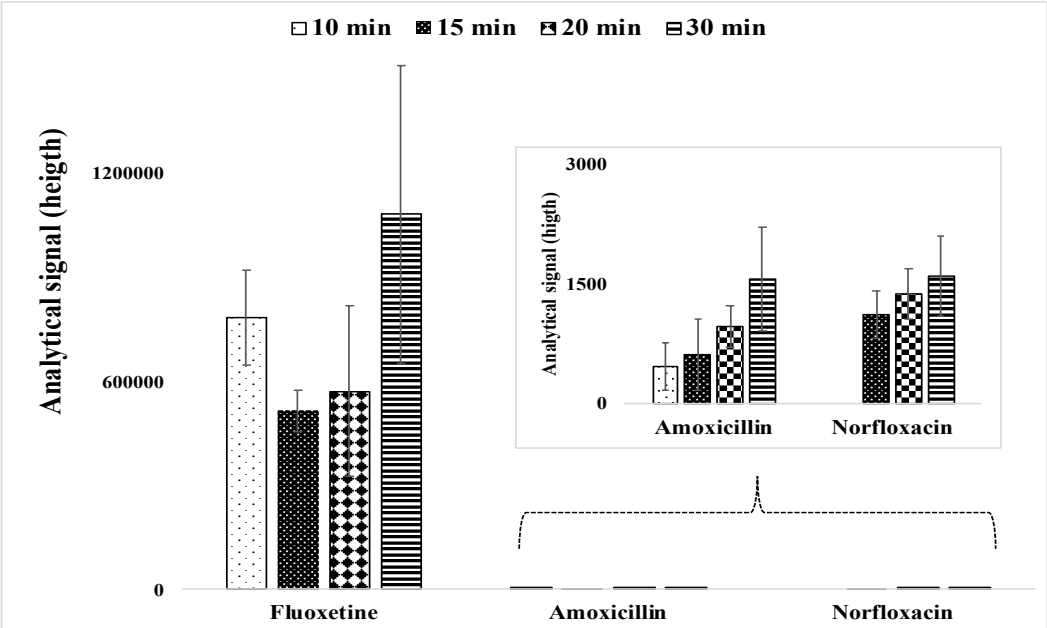

Figure 3. Influence of the desorption time in the extraction efficiency by RDSE for those contaminants that showed significant differences in the analytical signal ($p < 0.05$) (n=2).

In the desorption stage, in addition to the extraction time and solvent, the option of desorbing the contaminants from the polymeric phase by ultrasound or by disc rotation was evaluated. In the experiments (not shown) better results were obtained with disk stirring desorption than with ultrasound. This may be due to the fact that when rotation is applied, a small vortex is created that favors the release of contaminants from the stationary phase into the extraction solvent. This phenomenon does not occur when ultrasound is applied, so stirring was used in this work as a method to desorb contaminants from the sorbent phase. However, it is very important to consider that excessive vortexing can cause the opposite effect, hindering the desorption of analytes (Manzo et al., 2014).

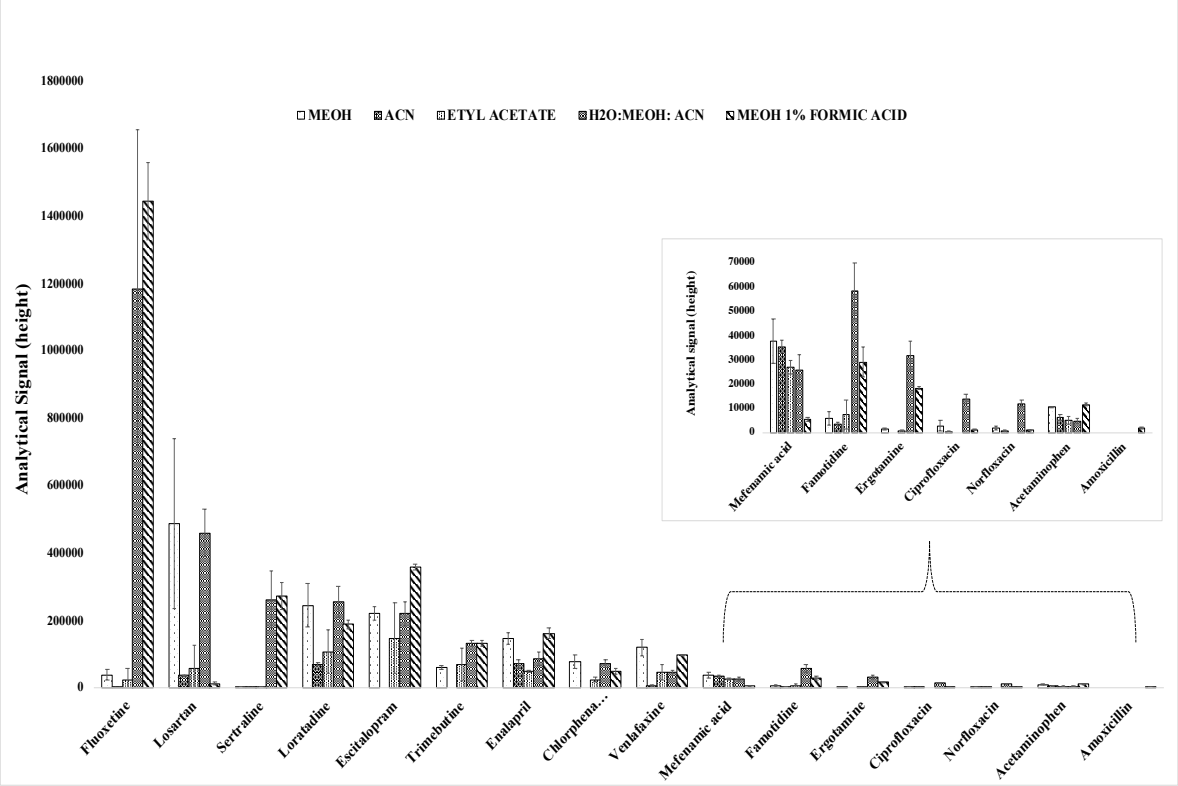

Figure 4. Influence of solvent in the extraction efficiency by RDSE for 76.19% of the contaminants studied

Screening at different pH conditions to determine the concentration capacity of RDSE and SPE

In order to obtain preliminary information on the extraction by RDSE of the broad group of contaminants with different physicochemical characteristics under study, a comparison was carried out between the concentration factors (CFs) provided by the RDSE procedure and an SPE procedure described in the literature, using the t-contrast for paired data. This contrast provides information on whether the methods produce statistically different results, since they operate under different principles. Both extractive procedures were performed under the same pH conditions (2, 6 and 8), however, factors such as extraction time, sample volume and amount of sorbent phase were different (Description in sections 2.4 and 2.5).

The calculated CFs expressed in % for each of the extraction methodologies with their difference (d), are shown in Table 3. CFs were expressed in % for a better understanding since theoretically the FCs in RDSE and SPE are 10 and 100 respectively. The t-contrast for paired data found statistically significant differences between the two extractive methods ($t_{cal} > t_{crit}$), with 95% confidence.

Although the experiments were performed using two completely different extractive procedures, it was expected that, at least working at the same pH values the trend of the results would be similar between experiments at the same pH conditions, since both the disk and cartridge employed the same sorbent phase and the same analytes were spiked into the water sample. For example, compounds such as fluoxetine, escitalopram, venlafaxine, sertraline, trimebutine, ergotamine and chlorphenamine, achieved the highest CFs with the RDSE procedure at pH 8, whereas with the SPE procedure the highest values for these compounds were obtained at pH 2. Therefore, the extraction yield of contaminants is affected not only by the pH of the medium using the same extractive procedure, but also by the different operating principle of each procedure performing at the same pH conditions.

Low FCs in RDSE, not exceeding 40% for most contaminants, may be largely due to factors that significantly influence extraction performance, such as stirring speed, sample volume or the amount of FE in the disk. In addition, they may be due to losses that could not be corrected by not adding isotope-labeled internal standards during extraction, as well as matrix effects that significantly affect analyte determination (Gracia-lor et al., 2010).

Stirring speed is one of the key factors in RDSE extraction, since without the aid of a stirring device, the process would depend entirely on the rate of diffusion of the analyte in the aqueous phase, resulting in extraction times that are too long and inefficient compared to currently developed techniques. By applying stirring, a greater exchange of the analyte from the solution

to the sorbent phase occurs, ultimately improving the speed of the extraction (Sandoval, 2015). Several authors report high extraction yields using high stirring speeds, with values close to 3000 rpm (Becerra-Herrera et al., 2020; Manzo et al., 2014; Rosero-moreano et al., 2016). Higher speeds results in turbulent flow that reduces the water boundary layer at the interface, which accelerates the mass transport of the analyte to the stationary phase and therefore the performance of the extraction process is favored and high CFs are achieved (Cañas and Samuel, 2014). The stirring speed used in our study was 1200 rpm, which was the highest speed reached by the magnetic stirrer used; therefore, this may be one of the reasons for the low yields obtained with the RDSE procedure.

The sample volumes used in the RDSE procedure (10 mL) could be a factor influencing the low CFs values obtained. According to Manzo et al., increasing the sample volume from 50 to 100 mL while keeping the analyte concentration constant improved sensitivity because the preconcentration factor increased for the same extraction time. However, equilibrium was not reached in the same time range because the larger the sample volume, the larger the space in which analytes can be present and the longer the residence time in the aqueous phase (Manzo et al., 2014). To corroborate whether the volume of sample tested directly influenced the low CFs obtained with the RDSE procedure, volumes greater than 10 mL of sample should be tested in subsequent experiments.

The amount of sorbent phase in the disk, was much lower (15 mg) than in the SPE cartridge (60 mg). To confirm whether the amount of extractant phase influences the extraction efficiency in the RDSE procedure, experiments using 15, 20 and 30 mg of Oasis® PRiME HLB were tested. Amounts higher than 30 mg were not considered, since the disk cavity used does not allow larger amounts and because with amounts of 50 and 65 the extraction efficiency decreased

with respect to 25 mg in the study of Pereira et al., while in the study of Donato et al., the best results were achieved (recoveries > 80%) with 20 mg instead of 30 and 40 mg, which may be related to the fact that the larger the amount of extracting phase, the more it tends to compact inside the disk cavity, avoiding the interaction of the analytes with the stationary phase and consequently decreasing the extraction efficiency (Donato et al., 2017; Pereira et al., 2015). Figure 5 presents the results for 35.7% of the analytes evaluated in this study, which did show significant differences in the analytical signal ($p < 0.05$) as the amount of sorbent phase varied. As can be observed, the highest efficiency is achieved with 15 and 30 mg of sorbent phase. Therefore, we can conclude on the basis of the evidence reported in other studies and the results achieved by us, that increasing the amount of stationary phase did not improve the extraction efficiency in the RDSE procedure.

The lower CFs in SPE for several compounds at the same pH conditions with respect to RDSE could be due to different factors affecting the recovery of analytes in SPE, such as the sample flow rate through the cartridge, which must be maintained between (0.2-1.5 mm/s) and can directly affect analyte retention in the stationary phase, because at higher flow rates the analytes are not completely retained by the sorbent phase (Poole, 2002). In RDSE procedure the analytes do not pass through the stationary phase, they are in contact with it for 120 minutes during the extraction. Another factor that could influence low CFs values with respect to RDSE is excessive drying of the cartridges, which can result in low analyte recovery by retention in poorly solvated regions of the sorbent (Poole, 2002). None of the above factors were controlled in SPE, so they could have affected the results obtained with this extraction methodology.

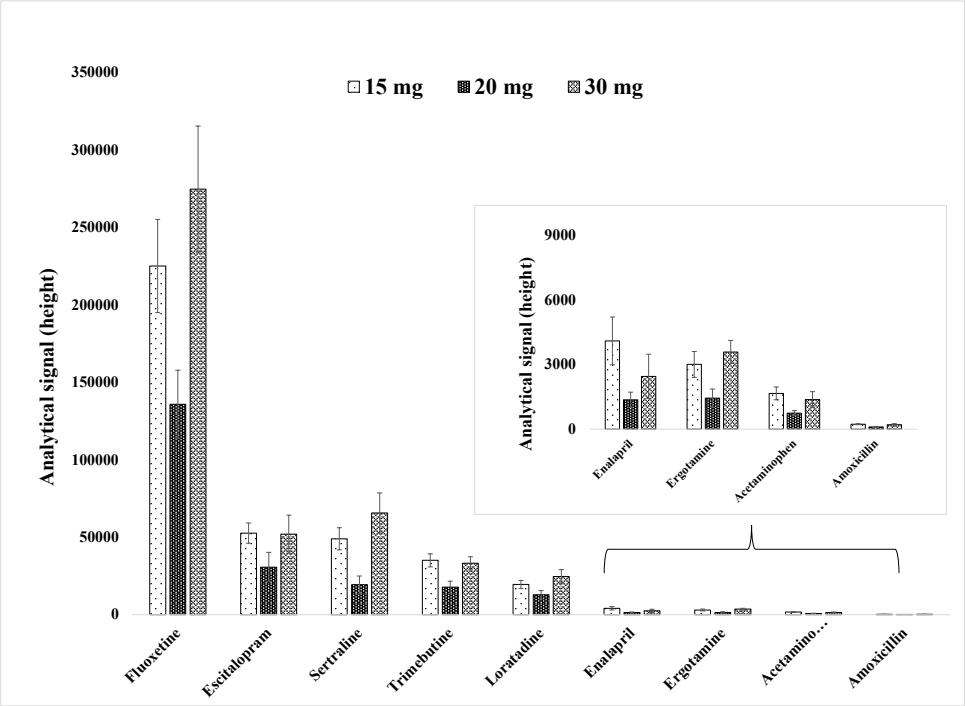

Figure 5. Influence of amount of extractant phase in the extraction efficiency by RDSE.

Among the compounds evaluated, at pH 2, 8 are present as neutral species and the remaining 16 as cationic species and 1 in equilibrium between the neutral and cationic specie. At pH 6, 6 are as neutral species, 8 as cationic species, 6 as anionic species, 4 as zwitterionic species and 1 in equilibrium between neutral and anionic specie. At pH 8, 5 are as neutral species, 7 as cationic species, 10 as anionic species, 2 as zwitterionic species and 1 in equilibrium between the neutral and cationic specie (See Table 1).

The Oasis® PRiME HLB polymeric phase is still in the patent process, hence its composition has not yet been detailed by the manufacturer. Nonetheless, it is known to be similar to hydrophilic-lipophilic balanced phase Oasis® HLB (presence of N-vinylpyrrolidone and divinylbenzene copolymers). These polymeric phases extended the applicability of solid-phase extraction to more hydrophilic compounds (log P <1) (Huntscha et al., 2012). The interaction between the analytes and the lipophilic divinylbenzene copolymer takes place via π-π

interactions with the less polar groups of the analytes and benzene rings in the polymer, while interactions via hydrogen bonds take place between the hydrophilic N-vinylpyrrolidone moiety and the more polar groups of the analytes (Waters Oasis, 2016). In addition, other studies suggest possible dipole-dipole interactions between the sorbent phase and the analytes, mediated by the pH of the solution. Li et al., propose that the C=O group of the N-vinylpyrrolidone moiety may undergo nucleophilic addition reactions in acidic, basic and neutral medium giving rise to different retention mechanisms of the analytes on the sorbent. At basic pH, the negative alkoxide ion is formed through hydration of the C=O, at acidic pH the C=O group is activated towards the attack of the weakly nucleophilic water molecule and forms the positively polarized conjugated acid, while at neutral pH a geminal diol originates, reinforcing the hydrogen bond between the solutes and the sorbent (Li et al., 2010).

At pH 8, as already mentioned, the retention mechanisms of the analytes are given by hydrogen bonds, π-π interactions and dipole-dipole interactions through the negative alkoxide formed and the cationic species. In these conditions, compounds with acidic characteristics such as ionized NSAIDs should be retained to a lesser extent due to the repulsion between the carboxylate in their structure and the negative alkoxide originating from the C=O of the N-vinylpyrrolidone, however, except for salicylic acid, the rest of the NSAIDs improve their CFs in both RDSE and SPE extraction procedures. These results agree with those reported by (Gracia-lor et al., 2010; Nosek et al., 2014), but cannot be explained according to the approach of Liu et al. On the other hand, protonated compounds at pH 8, such as antidepressants, trimebutine, ergotamine, famotidine and chlorphenamine, achieved the best CFs by RDSE and this can be explained considering the approach of Liu et al, however the CFs obtained for the same compounds using SPE were low and cannot be justified by the same logic. Some authors

have been reported high yields of antidepressants at basic or neutral pH by SPE (Gracia-lor et al., 2010; Martínez Bueno et al., 2007; Metcalfe et al., 2003; Weigel et al., 2004).

At pH 2, all the cationic species (Table 1) exhibit the lowest CFs in the RDSE procedure. This may be due to the fact that the positively polarized conjugated acid originating from hydration in acid medium at the C=O group of the N-vinylpyrrolidone moiety may weaken the retention of the protonated amino groups present in these compounds, producing a repulsion between the positive charges of the sorbent phase and these compounds leading to their lower retention and thus lower CFs at acidic pH. However this same approach cannot be applied in the SPE procedure, since antidepressant, ergotamine, trimebutine, chlorphenamine and famotidine showed higher CFs at pH 2.

At pH 6, the medium is still acidic, so that the positively polarized conjugated acid is still present in the sorbent phase; however, most of the species are affected by this pH change. NSAIDs and enalapril, which at pH 2 were found as neutral and protonated species respectively, change to anionic species and their retention by the sorbent phase improves compared to pH 2 with both extractive methods.

The antibiotics quinolones ciprofloxacin and norfloxacin at pH 6 and 8 are found to be zwitterionic species. The best CFs for these compounds in RDSE were achieved at pH 8, which makes sense according to the retention mechanisms explained above, however in SPE this is not fulfilled in the same way.

Norfloxacin and trimebutine reached very high CFs values at pH 2 (700.47 ± 478.69 and 228.00 ± 1. 79 respectively). In the case of norfloxacin this is probably due to random errors that affected the measurement, given the high standard deviation (SD) between one value and another, while in the case of trimebutine the SD was low, so it could be a matrix effect that boosted the analytical signal and therefore increased their CF.

As expected, neutral compounds within the pH range tested, such as caffeine, acetaminophen and progesterone were not influenced by pH variations in the SPE procedure (Weigel et al., 2004), However, in RDSE the FC values achieved at pH 8 for these three compounds were higher than those achieved at pH 2 and 6.

Table 3. CFs expressed in % and d values obtained with the RDSE and SPE extraction procedures for the contaminants under study, at three pH values.

pH values	Compounds	% CF RDSE ± SD	% CF SPE ± SD	Difference (d)
pH 2	Ibuprofen	9.20 ± 2.51	50.54 ± 17.22	41.30
	Naproxen	16.3 ± 7.97	61.51 ± 15.56	45.21
	Acetaminophen	3.9 ± 1.69	28.90 ± 3.60	24.98
	Diclofenac	16.4 ± 8.80	52.04 ± 3.60	35.65
	Mefenamic acid	2.90 ± 2.40	12.07 ± 4.37	9.16
	Salicylic acid	4.81 ± 2.60	40.76 ± 8.01	35.95
	Caffeine	3.28 ± 2.95	90.05 ± 0.93	86.76
	Sertraline	8.75 ± 4.58	43.65 ± 11.82	34.89
	Venlafaxine	6.13 ± 3.15	109.32 ± 1.72	103.19
	Escitalopram	8.91 ± 3.52	81.42 ± 5.10	72.50
	Fluoxetine	7.52 ± 3.54	57.01 ± 4.46	49.48
	Amoxicillin	0.46 ± 0.40	8.28 ± 6.49	7.81
	Ciprofloxacin	0.19 ± 0.32	31.83 ± 8.94	31.64
	Norfloxacin	0.49 ± 0.85	700.47 ± 478.69	699.98
	Enrofloxacin	0.20 ± 0.34	20.33 ± 11.96	20.12
	Sulfametazine	1.30 ± 1.13	12.60 ± 4.06	11.30
	Progesterone	12.79 ± 7.02	55.95 ± 20.72	43.15
	Famotidine	0.49 ± 0.27	7.69 ± 4.05	7.19
	Loratadine	8.06 ± 3.13	55.03 ± 1.07	46.97
	Chlorfenamine	2.53 ± 1.82	64.73 ± 1.66	62.21
	Omeprazole	0.01 ± 0.02	0.10 ± 0.07	0.09
	Trimebutine	29.30 ± 14.11	228.00 ± 1.79	198.71
	Ergotamine	6.33 ± 3.29	30.51 ± 3.79	24.18
	Enalapril	6.11 ± 1.79	93.94 ± 0.86	87.83
	Losartan	4.36 ± 2.59	26.28 ± 14.42	21.92
pH 6	Ibuprofen	14.68 ± 5.42	90.80	76.12
	Naproxen	18.74 ± 7.30	88.00	69.25
	Acetaminophen	4.63 ± 0.83	28.61	23.98
	Diclofenac	17.89 ± 2.99	84.77	66.88
	Mefenamic acid	4.98 ± 3.23	77.15	72.17
	Salicylic acid	0.96 ± 0.35	15.68	14.71
	Caffeine	4.58 ± 1.40	88.27	83.68
	Sertraline	10.83 ± 3.54	1.64	-9.18

	Venlafaxine	12.67± 5.81	54.64	41.96
	Escitalopram	12.81 ± 3.67	4.17	-8.64
	Fluoxetine	9.85 ± 4.39	1.30	-8.55
	Amoxicillin	0.21 ± 0.13	0.14	-0.07
	Ciprofloxacin	1.46 ± 1.08	15.61	14.15
	Norfloxacin	1.76 ± 1.06	28.31	26.55
	Enrofloxacin	1.08 ± 0.33	27.74	26.65
	Sulfametazine	6.15 ± 2.57	47.93	41.78
	Progesterone	15.12 ± 3.34	52.14	37.01
	Famotidine	3.55 ± 1.40	1.79	-1.76
	Loratadine	11.76 ± 3.11	36.73	24.97
	Chlorfenamine	11.53 ± 6.66	5.58	-5.95
	Omeprazole	0.13 ± 0.10	47.36	47.23
	Trimebutine	42.76 ± 13.64	7.13	-35.63
	Ergotamine	11.70 ± 1.57	< LD	-11.70
	Enalapril	9.97 ± 3.44	98.88	88.91
	Losartan	6.60 ± 1.86	64.04	57.43
pH 8	Ibuprofen	17.35 ± 5.37	89.47	72.11
	Naproxen	18.80 ± 3.30	78.91	60.12
	Acetaminophen	7.66 ± 1.36	30.44	22.78
	Diclofenac	18.76 ± 4.54	88.55	69.79
	Mefenamic acid	6.47 ± 2.37	78.18	71.71
	Salicylic acid	0.70 ± 0.16	4.65	3.95
	Caffeine	8.25 ± 2.31	86.12	77.87
	Sertraline	34.20 ± 5.03	2.16	-32.05
	Venlafaxine	30.67 ± 3.95	36.57	5.89
	Escitalopram	33.41 ± 4.15	0.07	-33.35
	Fluoxetine	36.01 ± 4.82	1.97	-34.04
	Amoxicillin	0.70 ± 0.41	0.50	-0.19
	Ciprofloxacin	4.66 ± 1.23	13.27	8.60
	Norfloxacin	6.69 ± 1.53	38.55	31.85
	Enrofloxacin	3.15 ± 0.82	22.23	19.08
	Sulfametazine	12.50 ± 2.23	70.48	57.98
	Progesterone	30.01 ± 3.26	61.00	30.99
	Famotidine	1.12 ± 0.11	5.61	4.49
	Loratadine	25.11 ± 3.41	44.61	19.49
	Chlorfenamine	29.42 ± 5.20	< LD	-29.42
	Omeprazole	0.83 ± 0.19	4.91	4.07
	Trimebutine	113.53 ± 13.48	0.17	-113.37
	Ergotamine	42.23 ± 8.45	< LD	-42.24
	Enalapril	20.20 ± 5.48	104.71	84.52
	Losartan	13.06 ± 7.19	82.31	69.25

According to the preliminary results achieved to date, as can be seen in Figure 6A and 6B, pH 8 greatly favored the extraction of most of the contaminants by the RDSE procedure, while SPE

is favored at pH 2. Although it is certain that the extraction of all compounds is not favored at these pH values, in multiresidual methods it is necessary to reach a consensus and select working conditions that are acceptable to the majority.

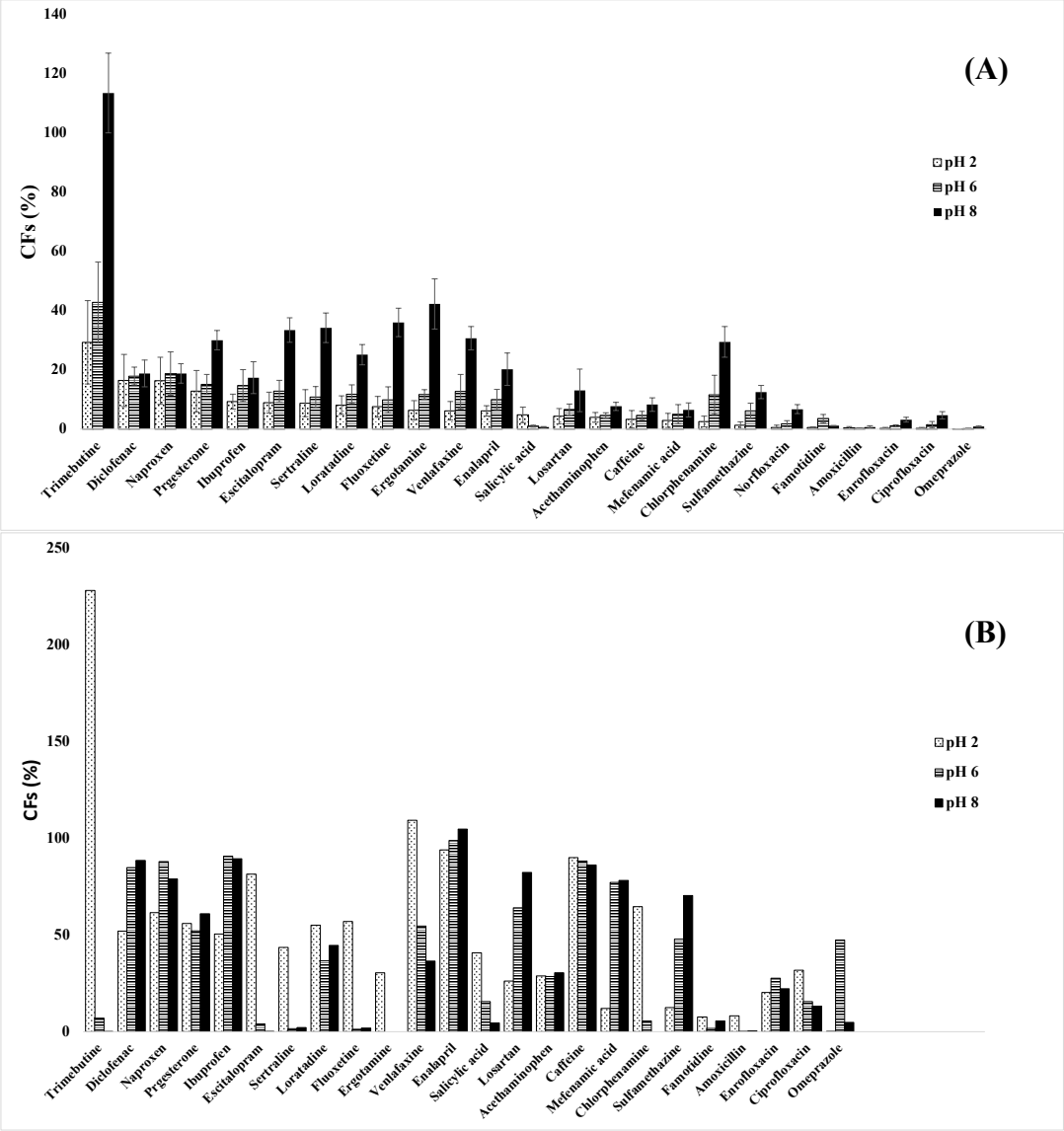

Figure 6. CFs achieved for the contaminants under study by (A) RDSE and (B) SPE extraction procedures at different pH values.

4. Conclusions

A method for the simultaneous determination of 25 acidic, basic and neutral ECs in a single injection was developed by UHPLC-MS/MS. Therefore, it is not necessary to perform two analyses for positive and negatively ionized compounds. In a preliminary study, RDSE allows the simultaneous extraction of the quite different physico-chemical characteristics compounds, at pH 8, using 15 mg of Oasis® PriME HLB phase, applying a stirring speed of 1200 rpm and during an extraction time of 120 min. The desorption step was performed with 10 mL of 1% acidified methanol for 30 min at a stirring speed of 1200 rpm. The t-test for paired samples showed statistically significant differences between the CFs achieved with the RDSE procedure and the SPE procedure adapted from the literature.

The CF values for most of the compounds did not exceed 40 % with the RDSE procedure, however with the SPE procedure values higher than 80 % were achieved for many of these same compounds. To improve the results achieved by RDSE, further steps could be performed by increasing the stirring speed range and sample volume, as well as incorporating isotopically labeled internal standards during the extraction to correct the analytical response of the compounds most affected by loss during the procedure or by the matrix effect. The pH influenced the FCs within both extractive procedures, but the main differences are given by the principle of operation of each method using the same pH conditions.

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CAPÍTULO II SECCIÓN 2

Title: Suspect screening of contaminants of emerging concern in Chilean surface and wastewaters

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Abstract

The analysis of suspected contaminants of emerging concern (CECs) in a secondary effluent and three surface water samples from Chile was performed using high resolution mass spectrometry (HRMS) combined with screening databases from the Digital Sample Freezing Platform (DSFP) developed by the NORMAN collaborative network. The samples were retrospectively screened to find matches using the EXPHRMSMSAVAL list (experimental fragments of CECs), composed of 6828 compounds in positive and 3042 compounds in negative ionization using the combination of information on exact mass, predicted retention time window, isotopic fit, and fragment ions. All potential matches were verified by scrutiny of raw mass chromatograms and visualization of MS/MS spectra. In addition, potential suspects found were supported by comparison with an exact match in spectral libraries or with *in silico* fragmentation software. Sixty-three suspects were tentatively identified and 2 were confirmed by comparison with reference standards, including pesticides, plasticizers like phthalates, personal care products, pharmaceuticals, sweeteners and flame retardants, among others. At least 27 of the 65 CECs were detected once and 9 were detected in all samples, demonstrating the ubiquity of CECs in the environment.

Keywords: Digital sample freezing platform; High-resolution mass spectrometry; Suspect screening; Contaminants of emerging concern; Chilean environmental waters.

1. Introduction

As a result of anthropogenic activity, the occurrence of contaminants of emerging concern (CECs) in environmental waters has been widely demonstrated (Castillo-Zacarías et al., 2021; Llorca et al., 2017; Sancho et al., 2012; Starling et al., 2019). CECs are a category of pollutants constituted by a wide group of compounds such as pharmaceuticals, personal care products, pesticides, sweeteners, plasticizers, steroid hormones and flame retardants, that has received special attention (Barrios-Estrada et al., 2018; Peña-Guzmán et al., 2019) from the scientific community due to the lack of knowledge about their medium and long-term impact on human health, and the environment (Deblonde et al., 2011). These contaminants enter the water cycle through various pathways, such as domestic wastewater from recreational activities in swimming pools, human excretion, and showering; hospital and industrial discharges, agricultural and livestock practices, and septic tanks and landfills (Gavrilescu et al., 2015; Vargas-Berrones et al., 2020; Yang et al., 2017). They are found at concentrations ranging from ng L^{-1} to mg L^{-1}, even in the waters of the Antarctic continent (Alygizakis et al., 2020; Ferrer and Thurman, 2012; Hernández et al., 2019; Valdez-Carrillo et al., 2020). Several regulatory agencies have started to establish some policies and watch lists aimed at regulating the discharge, emission, and monitoring of priority substances and potential CECs (Environmental Health and Safety division, 2010; NHMRC, 2008; US EPA, 2021; Directiva 2013/39/UE, 2013; Decisión de Ejecución UE 2020/1161, 2020). However, no legislation on this matter has been issued in Chile so far, mainly because of the lack of information about the prevalence of pollutants in the Chilean environment, and of the subsequent limited evidence on the potential effects after prolonged exposure to these contaminants at low concentrations (Patel et al., 2019). Only a few studies have warned about the presence of these

contaminants in the country, mainly in effluents from wastewater treatment plants (Bertin et al., 2011, 2009; Jachero et al., 2013; Moncada, 2015; Sandoval, 2015; Villa, 2012). The coupling of liquid chromatography (LC) to HRMS with high mass accuracy has emerged as a powerful tool (Brack et al., 2019; Hernández et al., 2015; Krauss et al., 2010) in environmental analysis. Currently, target analytical methods are often complemented with suspect and non-target screening methods (Gago-Ferrero et al., 2018; Schymanski et al., 2015) since performing target screening alone can result in bias due to the preselection of the substances and potential chemical stressors may be omitted. Suspect screening involves searching for known or predicted compounds suspected to be present in environmental samples. This includes lists of known substances or predicted transformation products (TPs), which may even be more persistent, more toxic or may be present in much higher concentrations than their parent compound (Gago-Ferrero et al., 2016). In suspect-screening workflows, there is no need for reference standards until the confirmation stage, thus saving time and money and allowing the inclusion of an extensive list of substances (Gago-Ferrero et al., 2018, 2016).

The NORMAN Digital Sample Freezing Platform (DSFP) is a key tool developed by NORMAN Association to support suspect and non-target screening. This platform enables retrospective suspect screening of tens of thousands of CECs and their transformation products in environmental samples from the large amount of environmental mass spectral information. DSFP incorporates all recent developments in HRMS screening methods within the NORMAN Network and offers an integrated tool for wide-scope screening of CECs in the environment. In addition, both single-substance and batch queries are possible across selected samples, or all samples stored on the platform. In the workflow, raw mass spectral data are converted into mzML; then, mass spectral and chromatographic information on thousands of peaks per sample is extracted into data collection templates (DCT). The “digitally frozen”

samples can be retrospectively screened for the presence of virtually any compound amenable to LC-MS using a combination of information on its (i) exact mass, (ii) predicted retention time window in the chromatogram, (iii) isotopic fit, and (iv) qualifier fragment ions. In brief, DSFP promotes automation of retrospective screening, improves the transparency of LC-HRMS data, and serves as a tool for the development of future policy recommendations for the management of chemicals in the environment (Alygizakis et al., 2019; Dulio et al., 2020). In this work, NORMAN DSFP was used to process, test and extract data from Chilean surface water and secondary effluent samples, with the purpose of identifying the presence of CECs in them, using the EXPHRMSMSAVAL list of the NORMAN Suspect List Exchange. This list is constituted by a total of 9,870 compounds, including pharmaceuticals and/or their transformation products, personal care products, drugs and/or their transformation products, colorants, sweeteners, flame retardants, plasticizers and other industrial products that have been commonly reported as positive findings in environmental matrices in different parts of the world. In addition, this report could help to update the screening lists used in the target analysis in Chile by applying the new findings.

2. Experimental

2.1 Reagents, chemicals and materials

Reagents, chemicals and materials used in this work are shown in SM1 section in Supplementary Material (SM).

2.2 Water samples

Samples of wastewater were collected from WWTP “Aguas Andinas, Mapocho/Trebal” in Padre Hurtado (33°32’82”S / 70°50’08”W), Santiago de Chile during July 2019. Samples of

river and lagoon water were collected from Bio-Bio region during October 2019, in Bio-Bio River (36°48'22"S / 73°06'33"W), Andalien river (36°48'22"S / 73°06'33"W) and Lo Galindo lagoon (36°47'17"S / 73°01'48"W). All samples were stored in polyethylene high-density bottles and refrigerated in darkness at -20 °C until further analysis.

2.3 Samples treatment

Fifteen mg of Oasis® PRiME HLB phase were placed in the 0.44 cm^3 Teflon disk cavity, and it was covered with a paper filter and sealed with a Teflon ring. The Oasis® PRiME HLB phase was conditioned with ethyl acetate, methanol and ultrapure water for 5 min each before extraction in accordance with (Manzo et al., 2014). Next, the disk was immersed in an amber vial EPA of 40 mL that contained 10 mL of sample (pH adjusted to 8.0 with 0.1M NaOH) and stirred at 1200 rpm on the magnetic stirrer for 120 min. After extraction, the disk was removed and dried. Later, the same disk was placed in another vial and 10 mL of methanol 1% formic acid were added and agitated for 30 min at 1200 rpm to reach complete analyte desorption. Subsequently, the disk was taken out and the solvent was evaporated to dryness under a gentle nitrogen stream. Finally, the extract was reconstituted with 1mL of methanol and injected.

2.4 Instrumental analysis

A mass spectrometer with a hybrid quadrupole–Orbitrap analyzer Q-Exactive (Thermo Fisher Scientific, San Jose, CA, USA) was used. The chromatographic and HRMS conditions for sample analysis are reported in Table SM2. Mass spectra were acquired in two full scan events, in data independent acquisition (DIA) mode. The first scan at low collision energy (10 eV) and the second scan at a high collision energy (40 eV), resulting in a full MS scan and MS/MS mode of all fragment ions, respectively.

2.5 Suspect screening processes

In the suspect screening process the NORMAN DSFP was used (Alygizakis et al., 2019). During the workflow, raw mass spectral data obtained using XcaliburTM4.1 software (Thermo Scientific), were converted into mzML files in the Msconvert 3.0 software (ProteoWizard), using peak picking and an intensity threshold of 5000 in ESI (+) and 1000 in ESI (-) as filters. These files were uploaded to DSFP on the norman-data.eu site together with information about the type of sample, origin, chromatographic and ionization conditions and procedural blank samples. In addition, a set of standards was introduced for the calibration of the chromatographic retention time index (RTI) obtained under working conditions (Table SM3).

The digitally frozen samples were retrospectively screened to find matches using the EXPHRMSMSAVAL list accessible online at NORMAN Suspect List Exchange (6828 compounds in ESI+ and 3042 compounds in ESI-), using the combination of information on exact mass (± 5ppm), predicted retention time window, isotopic fit and fragment ions.

Mass spectral and chromatographic information (identification proofs) on hundreds of suspected compounds of each sample were then extracted in a data collection template (DCT) .xlsx file and imported from the site. All potential matches were verified and supported by scrutiny of raw mass chromatograms and visualization of MS^n spectra; in addition, comparison with an exact match to the spectral libraries (when this information was available) MassBank (www.massbank.eu), Mona (www.mona.ucdavis.edu) or HMDB (Wishart et al., 2018) or to the in silico fragmentation software MetFrag (Wolf et al., 2010) was employed, as well as comparison with reference standards when they were available (Gago-Ferrero et

al., 2016; Alygizakis et al., 2019). Furthermore, commercial importance criteria (the number of references and data sources from ChemSpider and the number of patents from PubChem) (Gago-Ferrero et al., 2015; NCBI, 2021; Royal Society of, 2021) was also considered supporting information in tentative identification.

The confidence level for suspect identification was expressed from 1 to 5 according to (Schymanski et al., 2014), where level 1 corresponds to confirmed structures with available reference standards; level 2 to probable structures with data matching in spectral libraries (level 2a) or diagnostic evidence (level 2b), while level 3 is assigned to tentative candidates and the lowest confidence to unequivocal molecular formulas (level 4) or exact mass of interest detected (level 5).

3. Results and discussion

3.1 Identification of suspects

Identification was considered to have sufficient supporting evidence if three or more fragments were detected for compounds with available library spectra (unless the available library spectra contained fewer fragments) and if five or more fragments were detected for compounds with in silico predicted mass spectra, plus an exact match of the molecular ion mass and a plausible RTI. Compounds that meet this set of identification criteria or evidence can be considered technically as level 3 (Alygizakis et al., 2019). In this regard, all the tentative compounds given in the DCT from DSFP were verified by going back to the raw mass chromatograms by obtaining extracted ion chromatograms (XIC) and visualization of MS/MS spectra, and by comparison with an exact match with spectral libraries (when this information was available) and in silico fragmentation software. A hit number of 30 compounds in ESI+ and less than 7 in ESI- was achieved in all samples. This manual scrutiny

prevented the reporting of an erroneous confidence level, as for several contaminants with MS/MS information (more than three fragments) that were reported in the DCT theoretically as level 3, the evidence was not strong enough to demonstrate an identification level higher than 4. These compounds are reported in Table SM4.

A total of 63 suspects were tentatively identified (level 2a, 2b and 3) and 2 suspects were confirmed by comparison with reference standards (level 1). At least 27 of 65 were detected once and 9 were detected in all samples, demonstrating the ubiquity of CECs in the environment. Of these 9, 8 were plasticizers and flame retardants (diethyl phthalate, diisobutyl phthalate, benzyl butyl phthalate, dibutyl phthalate, diethylhexyl phthalate, tributyl phosphate, tris(2-butoxyethyl) phosphate and dibutyl adipate) and the metabolite of the synthetic musk galaxolide fragrance (galaxolidone). Others compounds tentatively identified include pesticides (aminocarb and dinoterb), personal care products (lauryl sulfate, 4-Dodecylbenzenesulfonic acid and (9Z)-Octadec-9-enamide), pharmaceuticals (norfloxacin, enrofloxacin, venlafaxine), sweeteners (acezulfame), and UV filters (benzophenone-3 and octinoxate), among others. Table 1 reports a list of these 26 suspect contaminants (22 hits in ESI+ and 4 hits in ESI-) confirmed and tentatively identified in water samples from the Trebal WWTP, Bio-Bio river, Andalien river and Lo Galindo lagoon. Additionally, the corresponding confidence level and all the evidence that led to the identification are included.

Table 1. Suspected contaminants confirmed and tentatively identified in water samples from the WWTP Trebal, the Bio-Bio river, the Andalien river and the Lo Galindo lagoon.

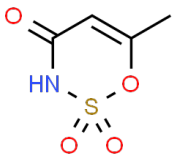
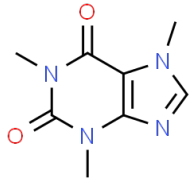
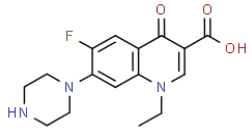
Suspect analyte	t_R (min)	Ion species/ Adducts	Accurate mass (m/z)	Mass error (ppm)	Evidence for identification	Annotation	Level of confidence	Location
 **Acesulfame** $C_4H_5NO_4S$	1.43	$[M - H]^-$	161.9867	0.299	▪ Presence of characteristic fragments *m/z:* 77.9656 [NO_2S]; 82.0299 [C_4H_4NO]; 161.9868 [$C_4H_4NO_4S$] ▪ Rt plausible ▪ Similarity 0.93 with MassBank (EA275659) ▪ Best match in MetFrag	▪ Food additives ▪ Artificial sweetener	2a	Andalien river
 **Caffeine** $C_8H_{10}N_4O_2$	4.24	$[M + H]^+$	195.0877	0.245	▪ Presence of characteristic fragments *m/z*: 83.0604 [$C_4H_7N_2$]; 110.0713 [$C_5H_8N_3$]; 123.0425 [$C_5H_5N_3O$]; 138.0662 [$C_6H_8N_3O$]; 195.0878 [$C_8H_{11}N_4O_2$] ▪ CONFIRMED with Reference Standard	▪ Central nervous and respiratory system stimulant ▪ Cardiac and respiratory stimulant ▪ Diuretic	1	Andalien river
 **Norfloxacin** $C_{16}H_{18}FN_3O_3$	4.35	$[M + H]^+$	320.1406	0.324	▪ Presence of characteristics fragments *m/z*: 205.0772 [$C_{11}H_{10}FN_2O$]; 219.0930 [$C_{12}H_{12}FN_2O$]; 233.1087 [$C_{13}H_{14}FN_2O$]; 276.1506 [$C_{15}H_{19}FN_3O_3$]; 320.1403 [$C_{16}H_{19}FN_3O_3$]; 321.1439 [$C_{15}{}^{[13]}CH_{19}FN_3O_3$] ▪ Similarity 0.75 with MassBank (AU103203) ▪ Rt and MSMS spectra plausible with the confirm compound Ciprofloxacin	▪ First generation fluoroquinolone antibiotic	2a	Bio-Bio river Lo Galindo lagoon

Table 1. continued

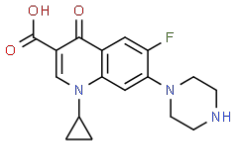
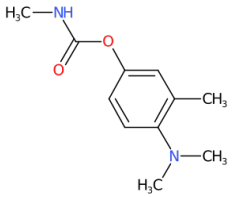
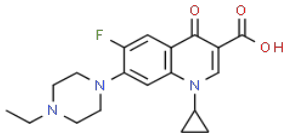
Suspect analyte	t_R (min)	Ion species/ Adducts	Accurate mass (m/z)	Mass error (ppm)	Evidence for identification	Annotation	Level of confidence	Location
 **Ciprofloxacin** $C_{17}H_{18}FN_3O_3$	4.46	$[M + H]^+$	332.1404	-0.290	▪ Presence of characteristic fragments *m/z*: 203.0614 [$C_{11}H_8FN_2O$]; 231.0566 [$C_{12}H_8FN_2O_2$]; 245.1084 [$C_{14}H_{14}FN_2O$] ▪ CONFIRMED with Reference Standard	▪ Second generation fluoroquinolone a ntibiotic	1	Lo Galindo lagoon
 **Aminocarb** $C_{11}H_{16}N_2O_2$	4.46	$[M + H]^+$	209.1285	0.218	▪ Presence of characteristic fragments *m/z*: 136.0759 [$C_8H_{10}NO$]; 152.1069 [$C_9H_{14}NO$]; 209.1282 [$C_{11}H_{16}N_2O_2$] ▪ Rt plausible ▪ Best match in MetFrag	▪ Insecticide	3	Bio-Bio river
 **Enrofloxacin** $C_{19}H_{22}FN_3O_3$	4.50	$[M + H]^+$	360.1716	-0.545	▪ Presence of characteristic fragments *m/z*: 203.0618 [$C_{11}H_8FN_2O$]; 245.1087 [$C_{14}H_{14}FN_2O$]; 316.1814 [$C_{18}H_{23}FN_3O$]; 360.1717 [$C_{19}H_{23}FN_3O_3$]; 361.1752 [$C_{17}H_{23}N_5O_4$] ▪ Similarity 0.75 with MassBank (AU102903) ▪ Rt and MSMS spectra plausible with the confirm compound Ciprofloxacin ▪ Best match in MetFrag	▪ Fluoroquinolone antibiotic for veterinary use	2a	Bio-Bio river Andalien river Lo Galindo lagoon

Table 1. continued

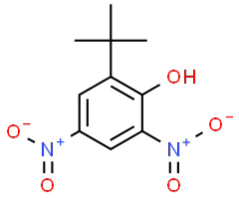
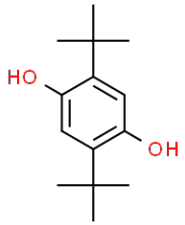
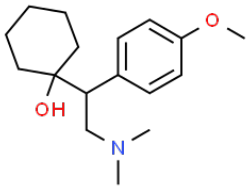
Suspect analyte	t_R (min)	Ion species/ Adducts	Accurate mass (m/z)	Mass error (ppm)	Evidence for identification	Annotation	Level of confidence	Location
 **Dinoterb** $C_{10}H_{12}N_2O_5$	6.17	$[M - H]^-$	239.0671	-1.024	▪ Presence of characteristic fragments *m/z*: 150.0562 [$C_8H_8NO_2$]; 151.0766 [$C_9H_{11}O_2$]; 162.0199 [$C_8H_4NO_3$]; 164.0719 [$C_9H_{10}NO_2$]; 176.0354 [$C_9H_6NO_3$]; 207.0412 [$C_9H_7N_2O_4$]; 239.0672 [$C_{10}H_{11}N_2O_5$] ▪ Similarity 0.76 with MassBank (EQ310953) ▪ Good match in MetFrag	▪ Herbicide and rodenticide	2a	WWTP Trebal
 **2,5-Di-tert-butylhydroquinone** $C_{14}H_{22}O_2$	6.48	$[M - H]^-$	221.1541	-2.728	▪ Presence of characteristic fragments *m/z*: 148.0532 [$C_9H_8O_2$]; 164.0844 [$C_{10}H_{12}O_2$]; 191.1080 [$C_{12}H_{15}O_2$]; 205.1233 [$C_{13}H_{17}O_2$]; 220.1467 [$C_{14}H_{20}O_2$]; 221.1547 [$C_{14}H_{21}O_2$]; ▪ Rt plausible ▪ Best match in MetFrag	▪ Cosmetics ▪ Antioxidant ▪ Enzyme Inhibitors	3	Bio-Bio river Lo Galindo lagoon
 **Venlafaxine** $C_{17}H_{27}NO_2$	6.67	$[M + H]^+$	278.2114	-0.200	▪ Presence of characteristic fragments *m/z*: 121.0648 [C_8H_9O]; 147.0804 [$C_9H_{11}O$]; 260.2006 [$C_{17}H_{26}NO$]; 278.2118 [$C_{17}H_{28}NO_2$] ▪ Similarity 0.91 with MassBank (SM832201) ▪ Good match in MetFrag	▪ Antidepressant ▪ Serotonin and n orepinephrine re uptake inhibitor	2a	WWTP Trebal

Table 1. continued

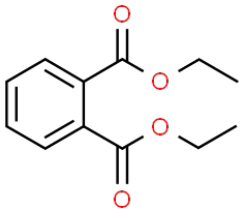
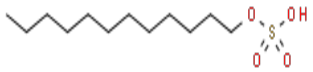
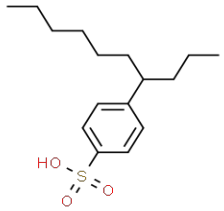
Suspect analyte	t_R (min)	Ion species/ Adducts	Accurate mass (m/z)	Mass error (ppm)	Evidence for identification	Annotation	Level of confidence	Location
 **Diethyl phthalate** $C_{12}H_{14}O_4$	7.62	$[M + H]^+$ $[M + Na]^+$ $[M - C_{10}H_9O_3]^+$	223.0965	0.065	▪ Presence of characteristic fragments *m/z*: 77.0388 [C_6H_5]; 92.0258 [C_6H_4O]; 99.0442 [$C_5H_7O_2$]; 111.0441 [$C_6H_7O_2$]; 121.0285 [$C_7H_5O_2$]; 149.0234 [$C_8H_5O_3$]; 163.039 [$C_9H_7O_3$]; 177.0546 [$C_{10}H_9O_3$]; 181.0496 [$C_9H_9O_4$] ▪ Rt plausible and consistent among the analogues series ▪ Similarity 0.83 with MassBank (SM835301) ▪ Good match in MetFrag	▪ Plasticizer	2a	Andalien river Bio-Bio river Lo Galindo lagoon WWTP Trebal
 **Lauryl sulfate** $C_{12}H_{26}O_4S$	7.66	$[M - H]^-$	265.1474	-1.898	▪ Presence of characteristic fragments *m/z:* 79.9574 [O_3S]; 95.9525 [HO_4S]; 96.9603 [HO_4S] 98.9561 [$HO_4{}^{34}S$]; 265.1475 [$C_{12}H_{25}O_4S$] ▪ Rt plausible ▪ Similarity 0.82 with MassBank (AU240762) ▪ Best match in MetFrag	▪ Detergent and protein denaturant	2a	WWTP Trebal
 **4-(decan-4-yl)benzenesulfonic acid** $C_{16}H_{26}O_3S$	7.66	$[M - H]^-$	297.1525	1.946	▪ Presence of characteristic fragments *m/z:* 79.9575 [O_3S]; 119.0504 [$C_7H_6O_3S$]; 183.0123 [$C_8H_7O_3S$];; 197.0281 [$C_9H_9O_3S$]; 297.1528 [$C_{16}H_{25}O_3S$] ▪ Rt plausible ▪ Good match in MetFrag	▪ Surfactant	3	WWTP Trebal

Table 1. continued

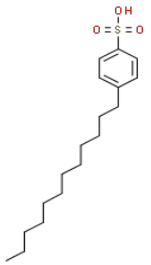
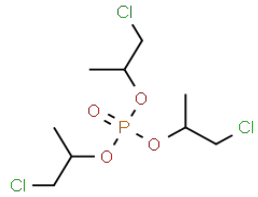
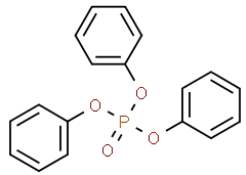
Suspect analyte	t_R (min)	Ion species/ Adducts	Accurate mass (m/z)	Mass error (ppm)	Evidence for identification	Annotation	Level of confidence	Location
 **4-Dodecylbenzenesulfonic acid** $C_{18}H_{30}O_3S$	8.09	$[M - H]^-$	325.1840	-0.888	▪ Presence of characteristis fragments *m/z:* 79.9574 $[O_3S]$; 119.0504 $[C_7H_6O_3S]$; 170.0044 $[C_7H_6O_3S]$; 183.0121 $[C_8H_7O_3S]$; 184.0199$[C_8H_8O_3S]$; 197.0277 $[C_9H_9O_3S]$; 325.1842 $[C1_8H_{29}O_3S]$ ▪ Rt plausible ▪ Best match in MetFrag	▪ Surfactant ▪ Emulsifier	3	WWTP Trebal
 **Tris(1-chloropropan-2-yl) phosphate** $C_9H_{18}Cl_3O_4P$	8.54	$[M + H]^+$ $[M + Na]^+$ $[M + K]^+$	327.0080	-0.322	4. Presence of characteristic fragments *m/z*: 98.9843 $[H_4O_4P]$; 174.9922 $[C_3H_9ClO_4P]$; 251.0003 $[C_6H_{14}Cl_2O_4P]$; 252.9975; $[C_5{}^{[13]}CH_{14}Cl_2O_4P]$; 327.0080 $[C_9H_{18}C_{l3}O_4P]$ ▪ Rt plausible ▪ Similarity 0.75 with MassBank (SM881002) ▪ Best match in MetFrag	▪ Adhesives and sealant chemicals ▪ Finishing agents ▪ Flame retardants ▪ Paint additives and coating additives not described by other categories	2a	Andalien river Lo Galindo lagoon
 **Triphenyl phosphate** $C_{18}H_{15}O_4P$	8.93	$[M + H]^+$ $[M + Na]^+$	327.0780	-0.220	▪ Presence of characteristic fragments *m/z*: 152.0620 $[C_{12}H_8]$; 168.0570 $[C_{12}H_8O]$; 233.0363 $[C_{12}H_{10}O_3P]$; 327.0777 $[C_{18}H_{16}O_4P]$ ▪ Rt plausible ▪ Similarity 0.83 with MassBank (SM824902) ▪ Best match in MetFrag	▪ Flame retardant agent ▪ Plasticizer for cellulose acetate and nitroc ellulose ▪ Lubricating oil and hydraulic fluids	2a	Bio-Bio river Lo Galindo lagoon

Table 1. continued

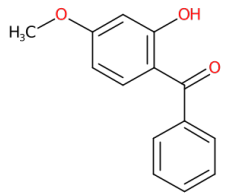
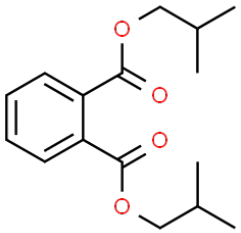
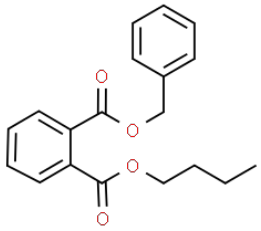
Suspect analyte	t_R (min)	Ion species/ Adducts	Accurate mass (m/z)	Mass error (ppm)	Evidence for identification	Annotation	Level of confidence	Location
 **Benzophenone-3** $C_{14}H_{12}O_3$	9.02	$[M + H]^+$	229.0858	-0.527	▪ Presence of characteristic fragments *m/z:* 105.0336 $[C_7H_5O]$; 108.0206 $[C_6H_4O_2]$; 151.0391$[C_8H_7O_3]$; 229.0860 $[C_{14}H_{13}O_3]$ ▪ Rt plausible ▪ Similarity 0.62 with MassBank (SM862902) ▪ Good match in MetFrag	▪ Ingredient in sunscreen and other cosmetics ▪ Absorbs UVA ultraviolet rays.	2a	WWTP Trebal
 **Diisobutyl phthalate** $C_{16}H_{22}O_4$	9.32	$[M + H]^+$ $[M + Na]^+$	279.1590	-0.307	▪ Presence of characteristic fragments *m/z*: 92.0256 $[C_6H_4O]$; 93.0335 $[C_6H_5O]$; 95.0491 $[C_6H_7O]$; 111.0440 $[C_6H_7O_2]$; 121.0284 $[C_7H_5O_2]$; 149.0233 $[C_8H_5O_3]$; 163.0388 $[C_9H_7O_3]$; 167.0338 $[C_8H_7O_4]$; 181.0494 $[C_9H_9O_4]$; 205.0857 $[C_{12}H_{13}O_3]$; 279.1589 $[C_{16}H_{22}O_4]$ ▪ Rt plausible and consistent among the analogues series ▪ Good match in MetFrag	▪ Plasticizer	2b	Andalien river Bio-Bio river Lo Galindo lagoon WWTP Trebal
 **Benzyl butyl phthalate** $C_{19}H_{20}O_4$	9.35	$[M + H]^+$ $[M + Na]+$	313.1432	-0.752	▪ Presence of characteristic fragments *m/z*: 77.0386 91.0542 $[C_7H_7]$; 121.0284 $[C_7H_5O_2]$; 149.0233 $[C_8H_5O_3]$; 163.0388 $[C_9H_7O_3]$; 167.0338 $[C_8H_7O_4]$; 181.0494 $[C_9H_9O_4]$; 205.0859 $[C_{12}H_{13}O_3]$ ▪ Rt plausible and consistent among the analogues series ▪ Similarity 0.71 with MassBank (SM836901) ▪ Best match in MetFrag	▪ Additive to make plastics flexible	2a	Andalien river Bio-Bio river Lo Galindo lagoon WWTP Trebal

Table 1. continued

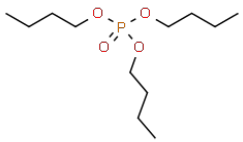
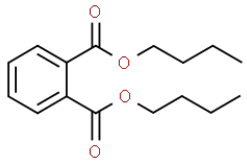
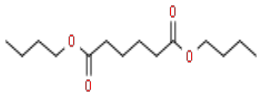
Suspect analyte	t_R (min)	Ion species/ Adducts	Accurate mass (m/z)	Mass error (ppm)	Evidence for identification	Annotation	Level of confidence	Location
 **Tributyl phosphate** $C_{12}H_{27}O_4P$	9.37	$[M + H]^+$ $[M + Na]+$	267.1719	-0.271	5. Presence of characteristic fragments *m/z*: 98.9842 $[H_4O_4P]$; 155.0467 $[C_4H_{12}O_4P]$; 211.1093 $[C_8H_{20}O_4P]$; 267.1718 $[C_{12}H_{27}O_4P]$ 6. Rt plausible 7. Good match in MetFrag	▪ Plasticizer for cellulose esters, lacquers, plastics, and vinyl resins	3	Andalien river Bio-Bio river Lo Galindo lagoon WWTP Trebal
 **Dibutyl phthalate** $C_{16}H_{22}O_4$	9.42	$[M + H]^+$ $[M + Na]^+$	279.1590	-0.307	▪ Presence of characteristic fragments *m/z*: 92.0256 $[C_6H_4O]$; 93.0335 $[C_6H_5O]$; 95.0491 $[C_6H_7O]$; 111.0441 $[C_6H_7O_2]$; 121.0284 $[C_7H_5O_2]$; 149.0233 $[C_8H_5O_3]$; 163.0389 $[C_9H_7O_3]$; 167.0338 $[C_8H_7O_4]$; 181.0494 $[C_9H_9O_4]$; 205.0858 $[C_{12}H_{13}O_3]$; 279.1589 $[C_{16}H_{22}O_4]$ ▪ Rt plausible and consistent among the analogues series ▪ Similarity 0.79 with MassBank (SM822401) ▪ Good match in MetFrag	▪ Plasticizer	2b	Andalien river Bio-Bio river Lo Galindo lagoon WWTP Trebal
 **Dibutyl adipate** $C_{14}H_{26}O_4$	9.41	$[M + H]^+$	259.1904	0.055	▪ Presence of characteristic fragments *m/z*: 185.1173 $[C_{10}H_{17}O_3]$; 129.0546 $[C_6H_9O_3]$; 111.0440 $[C_6H_7O_2]$ ▪ Rt plausible ▪ Similarity 0.97 with MassBank KW103803 ▪ Best match in MetFrag	▪ Cosmetics ▪ Film forming ▪ Plasticizer	2a	Andalien river Bio-Bio river Lo Galindo lagoon WWTP Trebal

Table 1. continued

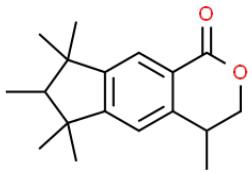
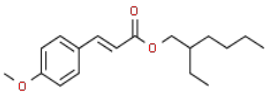
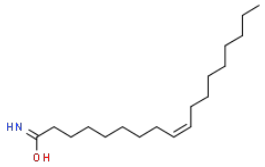
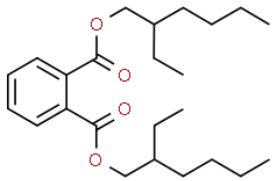
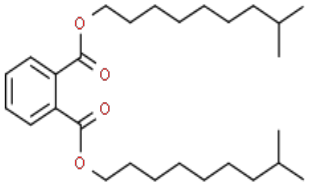
Suspect analyte	t_R (min)	Ion species/ Adducts	Accurate mass (m/z)	Mass error (ppm)	Evidence for identification	Annotation	Level of confidence	Location
 **Galaxolidone** $C_{18}H_{24}O_2$	9.53	$[M + H]^+$ $[M + Na]^+$	273.1848	-0.390	▪ Presence of characteristic fragments *m/z*: 129.0698 [$C_{10}H_9$]; 142.0777; [$C_{11}H_{10}$]; 157.1012 [$C_{12}H_{13}$]; 182.1091 [$C_{14}H_{14}$]; 197.1324 [$C_{15}H_{17}$]; 210.1038 [$C_{15}H_{14}O$]; 211.1116 [$C_{15}H_{15}O$]; 212.1558 [$C_{16}H_{20}$]; 225.1274 [$C_{16}H_{17}O$]; 227.1798 [$C_{17}H_{23}$]; 240.1509; [$C_{17}H_{20}O$]; 255.1743 [$C_{18}H_{23}O$] ▪ Rt plausible ▪ Similarity 0.68 with MassBank (AU205304) ▪ Good match in MetFrag	▪ Metabolite of the synthetic musk galaxolide	2a	Andalien river Bio-Bio river Lo Galindo lagoon WWTP Trebal
 **Octinoxate** $C_{18}H_{26}O_3$	10.48	$[M + H]^+$ $[M + Na]^+$	291.1954	-0.245	▪ Presence of characteristic fragments *m/z:* 133.0648 [C_9H_9O]; 161.0596 [$C_{10}H_9O_2$]; 162.0630 [$C_9{}^{13}CH_9O_2$]; 179.0701 [$C_{10}H_{11}O_3$]; 180.0736 [$C_9{}^{[13]}CH_{11}O$] ▪ Rt plausible ▪ Similarity 0.86 with MassBank (AU250206) ▪ Best match in MetFrag	▪ Organic UV-B filter common in sunscreen and other skin care products	2a	Bio-Bio river Lo Galindo lagoon WWTP Trebal
 **(9Z)-Octadec-9-enamide** $C_{18}H_{35}NO$	10.94	$[M + H]^+$ $[M + Na]^+$ $[M + K]^+$	282.2792	0.208	▪ Presence of characteristic fragments *m/z*: 69.0699 [C_5H_9]; 83.0855 [C_6H_{11}]; 97.10121 [C_7H_{13}]; 247.2419 [$C_{16}H_{33}$]; 265.2523 [$C_{18}H_{33}O$]; 282.2790 [$C_{18}H_{35}NO$] ▪ Rt plausible ▪ Best match in MetFrag	▪ Surface active agent ▪ Adhesives and sealant chemicals ▪ Lubricants and lubricant additives ▪ Processing aids ▪ Surface active agents	3	Bio-Bio river WWTP Trebal

Table 1. continued

Suspect analyte	t_R (min)	Ion species/ Adducts	Accurate mass (m/z)	Mass error (ppm)	Evidence for identification	Annotation	Level of confidence	Location
 **Di(2-ethylhexyl)phthalate** $C_{24}H_{38}O_4$	11.34	$[M + H]^+$ $[M + Na]^+$ $[M + K]^+$	391.2836	-1.754	▪ Presence of characteristic fragments *m/z*: 71.0856 [C_5H_{11}]; 121.0284 [$C_7H_5O_2$]; 149.0233 [$C_8H_5O_3$]; 163.0388 [$C_9H_7O_3$]; 167.0338 [$C_8H_7O_4$]; 181.0493 [$C_9H_9O_4$]; 279.1588 [$C_{16}H_{22}O_4$] ▪ Rt is consistent among the analogues series ▪ Similarity 0.67 with MassBank (SM818501) ▪ Good match in MetFrag	▪ Plasticizer ▪ Adhesive and binding agent in industry	3	Andalien river Bio-Bio river Lo Galindo lagoon WWTP Trebal
 **Diisodecyl Phthalate** $C_{28}H_{46}O_4$	12.03	$[M + H]^+$ $[M + Na]^+$ $[M + K]^+$	447.3463	-1.311	▪ Presence of characteristic fragments *m/z:* 71.0856 [C_5H_{11}]; 85.1012 [C_6H_{13}]; 95.0491 [C_6H_7O]; 121.0284 [$C_7H_5O_2$]; 149.0233 [$C_8H_5O_3$]; 163.0389 [$C_9H_7O_3$]; 167.0339 [$C_8H_7O_4$]; 181.0494 [$C_9H_9O_4$]; 307.1902 [$C_{18}H_{27}O_4$] ▪ Rt is consistent among the analogues series ▪ Best match with MetFrag	▪ Industrial uses such as automobile undercoating, building materials, wires and cables. ▪ General purpose plasticizer for preferred plasticizer for PVC in wire and cable applications.	3	Bio-Bio river Lo Galindo lagoon WWTP Trebal

Analysis of suspects phthalates

The analysis on the tentative identification of suspects is exemplified below for the phthalates family. Phthalates are compounds used as plasticizers, which are incorporated mechanically in plastics (usually PVC) to increase flexibility, workability, or distensibility. In 100 % and 75 % of the samples analyzed, 5 and 6 phthalates were tentatively identified, respectively, including benzyl butyl phthalate (BBP) and di(2-Ethylhexyl) phthalate (DEHP) compounds with endocrine disrupting activity and probable human carcinogenic activity.

According to the supporting evidence on tentative identification provided by the DCT, 5 phthalates were detected at t_R 7.62, 9.32, 9.35, 9.42 and 11.34 (Figure 1A). All of them with plausible t_R accurate molecular ion mass and more than 4 fragments. Therefore, all these identifications are technically considered level 3 initially. The increase in t_R is seen as the carbon chain sizes of the phthalates increase, which was considered during identification.

In the XIC at t_R 7.62 the exact mass of the $[M+H]^+$ ion *m/z* 223.0965 $[C_{12}H_{14}O_4]^+$ (associated error 0.020 ppm), the sodium adduct $[M+Na]^+$ and also the most abundant fragment *m/z* 177.0547 $[C_{10}H_9O_3]$ were confirmed, all characteristics to diethyl phthalate. MS/MS spectra confirmed the presence of the characteristic fragments for these compounds with *m/z*: 93.0336, 111.0441, 121.0285, 149.0234, 163.039, 177.0546 and 181.0496 corresponding to $[C_6H_5O]$, $[C_6H_7O_2]$, $[C_7H_5O_2]$, $[C_8H_5O_3]$, $[C_9H_7O_3]$, $[C_{10}H_9O_3]$, $[C_9H_9O_4]$, respectively. These fragments matched well in MetFrag for diethyl phthalate but provided the best scores for monobutyl phthalate and monoisobutyl phthalate. However, in MassBank, diethyl phthalate (similarity 0.83 to record SM835301) fits better than monoisobutyl phthalate (similarity 0.77 to record SM823305), and consequently diethyl phthalate was assigned identification level 2a.

DSFP does not discriminate between the identification of structural isomers with similar retention time and common fragments (Alygizakis et al., 2019). Therefore, there were certain cases with the same t_R assignment and MS/MS information for more than one suspected compound in the DCT. This is the case of dibutyl and diisobutyl phthalate. In the XIC (Figure 1A) two peaks with very close t_R (9.32 and 9.42) are present, with the molecular ion $[M+H]^+$ *m/z* 279.1590 as the highest intensity, the fragment $[C_8H_5O_3]^+$ with *m/z* 149.0232 as the second highest intensity and the sodium adduct $[M+Na]^+$ with m/z 301.1410 as the third highest intensity. These facts indicate that both could be possible compounds.

In the MS/MS spectra (Figure 1B and 1C), the same characteristic fragments appear at both retention times with *m/z* 92.0256, 111.0441, 121.0284, 149.0233, 163.0388, 167.0338, 181.0494, 205.0857 corresponding to $[C_6H_4O]$, $[C_6H7O2]$, $[C_7H_5O_2]$, $[C_8H_5O_3]$, $[C_9H_7O_3]$, $[C_8H_7O_4]$, $[C_9H_9O_4]$ and $[C_{12}H_{13}O_3]$ respectively. Therefore, MetFrag yields for both compounds the same score. In MassBank, MS/MS spectra at both t_R fit dibutyl phthalate (record SM822401), but at 9.42 min the similarity obtained was higher (0.81) than at 9.32 min (0.74). It was also found that under the same analysis conditions diisobutyl phthalate elutes first than dibutyl phthalate according to the LU118902 and LU082302 records in MassBank. In addition, (Schymanski et al., 2015) reported that diisobutyl phthalate according to the NIST database has an RTI of 1819, while dibutyl has an RTI of 1909. According to all this evidence, the suspect at t_R 9.32 was tentatively identified as diisobutyl phthalate and the one with t_R 9.42 as dibutyl phthalate, and both were assigned level 2b identification.

In turn, according to DCT, a well-defined peak appears in XIC with *m/z* 313.1432 at t_R 9.35 (Figure 1A), corresponding to the molecular ion $[M+H]^+$ $[C_{19}H_{23}O_4]^+$ with an associated error of -0.752, as well as the sodium adduct with *m/z* 335.1249, which fits very well with BBP. In

the MS/MS spectrum, in addition to some characteristic fragments for this compound and other phthalates (Table 1), *m/z* 77.0386 and 91.0542 corresponding to the fragments [C_6H_5] and [C_7H_7], respectively, were found with higher intensity (Figure 1D), which are related to the aromatic ring at the end of the carbon chain of BBP. MetFrag yielded the best match for this compound according to the KEGG and ChEBI databases, and MassBank a similarity of 0.71 concerning to the spectrum SM836901; therefore, this compound was defined as level 2a.

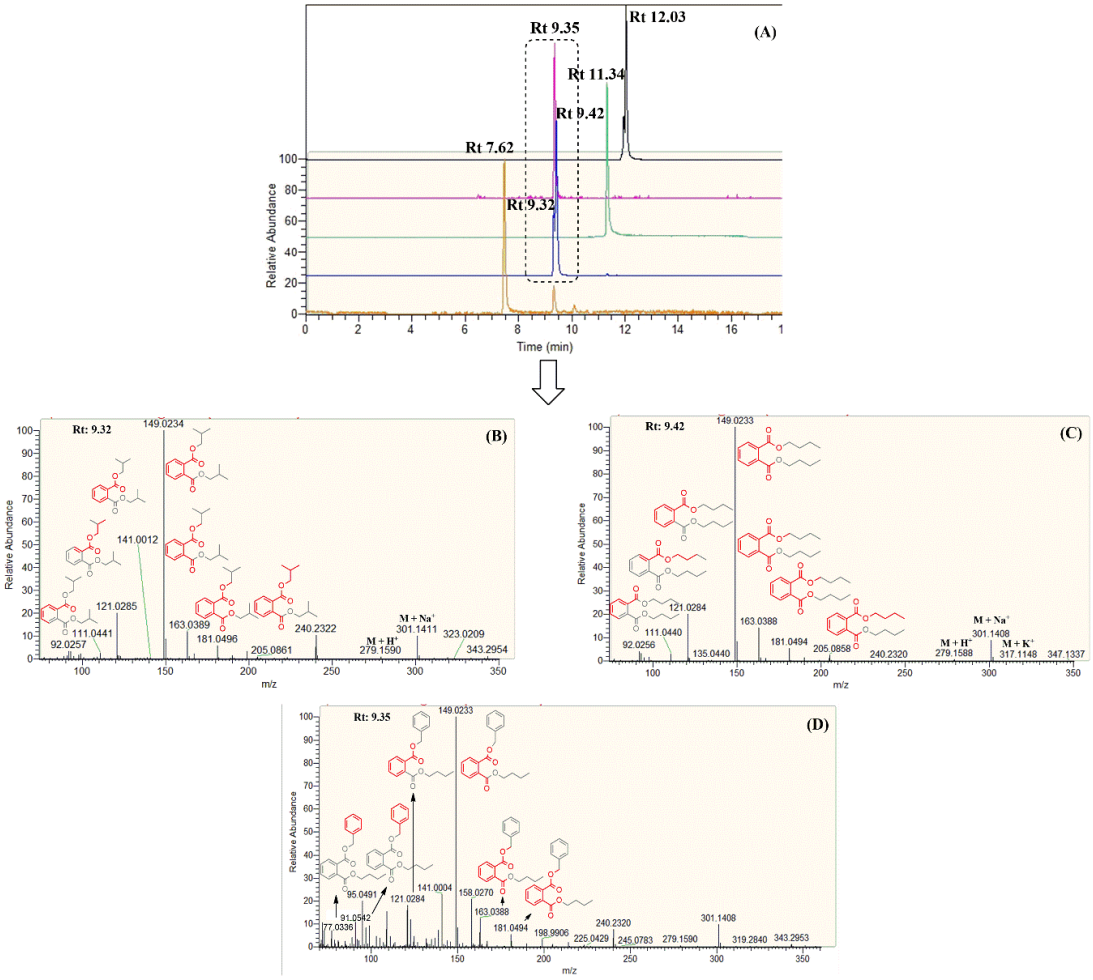


Figure 1. (A) Extracted ion chromatogram of the tentatively identified phthalates. MS/MS spectra of tentatively identified phthalates at retention times 9.32 (B), 9.42 (C) and 9.35 minutes (D).

Furthermore, DCT reports the possible presence of di(2-Ethylhexyl) phthalate and n-octyl phthalate at the same t_R (11.33 min) (Figure 1A), both compounds with molecular formula $C_{24}H_{38}O_4$. In XIC, *m/z* 391.2841 corresponding to the molecular ion $[M+H]^+$ and adducts $[M+Na]^+$ and $[M+ K]^+$ were confirmed. Characteristic fragments in MS/MS spectra matched with both compounds: m/z 121.0284, 149.0233, 163.0388, 167.0338, 181.0493, 279.1588 corresponding to $[C_7H_5O_2]$, $[C_8H_5O_3]$, $[C_9H_7O_3]$, $[C_8H_7O_4]$, $[C_9H_9O_4]$ and $[C_{16}H_{22}O_4]$, respectively. MetFrag gives a higher score for dioctyl phthalate (score 1.00) than for di(2-Ethylhexyl)phthalate (score 0.98). In MassBank, dioctyl phthalate showed a similarity of 0.68 with the record RP019803 and di(2-Ethylhexyl)phthalate 0.67 with record SM818501. However, both spectra were obtained under different analysis conditions (equipment, collision energy, etc.). This information was not considered sufficient to differentiate one or the other compound, although level 3 identification was assigned to di(2-Ethylhexyl)phthalate since the spectral information of record SM818501 was obtained under experimental conditions closer to those used in this study.

In addition to the suspected phthalates tentatively identified above, another phthalate was tentatively identified, which exhibited a high signal in TIC at Rt 12.03 min (Figure 1A) and a strong signal in XIC for the *m/z* 149.0233 fragment characteristic of phthalates. No compound of this family was suggested in the DCT at that t_R, probably because the compound is not included in the list of suspected EXPHRMSMSAVAL employed. In TIC the molecular ion $[M+H]^+$ *m/z* 447.3464 of higher intensity (associated error -1.534 ppm) and $[M+Na]^+$ and $[M+K]^+$ adducts corresponding to diisodecyl phthalate or another isomer were found. Likewise, in MS/MS, the following characteristic fragments of phthalates were detected:121.0284, 149.0233, 163.0389 167.0339, 181.0494 and 307.1902, corresponding to $[C_7H_5O_2]$, $[C_8H_5O_3]$, $[C_9H_7O_3]$, $[C_8H_7O_4]$, $[C_9H_9O_4]$, $[C_{18}H_{27}O_4]$, respectively. MetFrag explains these 6 fragments

and 3 more (Table 1) and assigns a score of 1.00 for diisodecyl phthalate and 3 other isomers (di-n-decyl phthalate, dodecyl isophthalate and diisodecyl isophthalate) according to the PubChem database, although there is no spectral information on these compounds in MassBank to support the identification of any of the four compounds. In addition, t_R is consistent with all the other tentatively identified smaller molecular size phthalates. At this point, commercial importance criteria (the number of references and data sources from ChemSpider and the number of patents from PubChem) were considered to assign an identity based on the possibility of this compound being found in the environment. In this sense, diisodecyl phthalate has 95/236 references/data sources in ChemSpider and 37.220 patents in PubChem, followed by didecyl phthalate (84/122 references/data source in ChemSpider and 19.550 patents in PubChem), diisodecylisophthalate (319 patents in PubChem) and didecyl isophthalate (289 patents in PubChem). Therefore, according to this background, diisodecyl phthalate was assigned identification level 3.

The other compounds tentatively identified and reported in Table 1 were analyzed following the same steps described above for the analysis of phthalates. All those for which no spectral information is available or did not have an exact match in MassBank or another database were kept with identification level 3, such as the pesticide aminocarb, antioxidant 2,5-Di-tert-butylhydroquinone, surfactants 4-Dodecylbenzenesulfonic acid, 4-(decan-4-yl)benzenesulfonic acid, (9Z)-Octadec-9-enamide and the plasticizer tributyl phosphate. For all these compounds, final confirmation with a reference standard is still required.

In the case of caffeine and ciprofloxacin compounds, information was available from reference standards analyzed under the same working conditions; therefore, the identification confirmation of these two suspects was performed by comparing their retention times and fragments with the standards.

4. Conclusions

DSFP proved to be a powerful and useful tool for rapid suspect screening that contributed to reducing working times. However, it is worth noticing the importance of manual scrutiny of mass spectra and chromatograms, and of the comparison with spectral libraries and in silico fragmentation software, to solve problems with the identification of structural isomers and to avoid reporting an inadequate level of confidence. The retrospective suspect screening of samples allowed for the identification and tentative identification of several CECs in Chilean surface waters and wastewater samples, including pesticides, industrial compounds such as phthalates, adipate and phosphates, UV filters, personal care products, pharmaceuticals such as antibiotics, and sweeteners, among others.

Acknowledgments

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SUPPLEMENTARY MATERIAL

Title: Suspect screening of contaminants of emerging concern in Chilean surface and wastewaters

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SM1: Reagents, chemicals and materials

The methanol and acetonitrile employed were HPLC grade from Merck® and Sigma-Aldrich®. Ethyl acetate, formic acid and ammonium acetate was purchase from Merck®. Water HPLC grade were purchased from Fisher Scientific and ultrapure water was obtained from a Millipore Milli-Q system (resistivity > 18 mΩ cm). Nitrogen > 99.9% (Linde Gas). PVDF filters (47mm diameter, 0.45 μm size) were purchased from Axivia (Delhi, India). The rotating disks used in this work were provided by Dr. Pablo Richter of the University of Chile. To carry out the extractions and desorption, a MIX 15 eco multi-position magnetic stirrer from 2 mag AG (Munich, Germany) was used. The pH was adjusted with a pH-meter HANNA Edge® HI2020. Oasis® PRiME HLB phase was supplied by Waters (USA).

Table SM 2. Chromatographic and HRMS conditions for sample analysis

Positive Ionization	
Column	Acquity UPLC BEH-C18 (100 x 2.1 mm, 1.7 µm) column (Waters, Massachusetts, USA)
Mobile phase	(A) Water with formic acid 0.1% (v/v) (B) Methanol with formic acid 0.1% (v/v)
Gradient	95% A at 0 min; 25% A at 7 min; 0% A at 10-15 min; 0% A at 15min; 95% at 17-21min
Flow	0.3 mL min^{-1}
Oven temperature	40 ºC
Injection volume	10 µL
Electrospray Ionization Parameters	
Capillary Voltage	4kV
Capillary temperature	350 º C
Probe heater temperature	250 º C
Sheath gas	40 a.u
Auxiliary gas	10 a.u
Negative Ionization	
Column	Acquity UPLC BEH-C18 (100 x 2.1 mm, 1.7 µm) column (Waters, Massachusetts, USA)
Mobile phase	(A) Water with ammonium acetate 5mM (B) Methanol with ammonium acetate 5mM
Gradient	95% A at 0 min; 50 % A at 3; 10% A at 6 min; 0% A at 13-17 min; 17min; 95% A at 18- 20 min
Flow	0.3 mL min^{-1}
Oven temperature	40ºC
Injection volume	10 µL
	Electrospray Ionization Parameters
Capillary Voltage	3kV
Capillary temperature	350 º C
Probe heater temperature	300 º C
Sheath gas	40 a.u
Auxiliary gas	10 a.u

Table SM 3. Set of standards for the calibration of the chromatographic RTI

Compound	Ionization (ESI)	RIT (min)	Compound	Ionization (ESI)	RIT (min)
Histamine	+	0.81	Amitrole	-	0.96
Guanylurea	+	0.91	Benzoic acid	-	1.72
Amitrole	+	0.91	Acephate	-	2.19
Chlormequat	+	0.94	Salicylic acid	-	2.37
Methamidophos	+	1.99	Simazine 2-Hydroxy	-	3.69
Vancomycin	+	2.95	Tepraloxydim	-	3.75
Cefoperazone	+	4.35	Bromoxynil	-	3.69
Trichlorfon	+	4.73	MCPA	-	4.39
Dichlorvos	+	6.23	Valproic acid	-	4.68
Tylosin	+	7.11	Phenytoin	-	4.96
TCMTB	+	7.69	Flamprop	-	4.99
Rifaximin	+	8.08	Dinoterb	-	5.2
Spinosad A	+	9.48	Benodanil	-	5.35
Avermectin B1a	+	10.34	Inabenfide	-	5.88
Emamectin B1a	+	10.57	Coumaphos	-	6.59
Ivermectin B1a	+	10.91	Triclosan	-	7.02
Nigericin	+	10.94	Salinomycin	-	6.99
			Avermectin B1a	-	7.84

Table SM 4. Suspect compounds corresponding to identification level 4.

These suspected compounds exhibited plausible Rt, accurate mass and more than three characteristic fragments based on DCT results. However, after manual analysis of the chromatograms and mass spectra, several fragments did not match in the spectral libraries or in MetFrag, so a level 4 identification was considered.

Compound Name	InChIKey	Formula	Screened Adduct	Level of confidence	Location
Tetrahydrocannabinol	CYQFCXCEBYINGO-UHFFFAOYSA-N	$C_{21}H_{30}O_2$	$[M + H]^+$	4	Bio Bio river Andalien river Lo galindo lagoon
Cannabidiol	QHMBSVQNZZTUGM-ZENAZSQFSA-N	$C_{21}H_{30}O_2$	$[M + H]^+$	4	Bio Bio river Andalien river Lo galindo lagoon
Cannabichromene	UVOLYTDXHDXWJU-UHFFFAOYSA-N	$C_{21}H_{30}O_2$	$[M + H]^+$	4	Bio Bio river Andalien river Lo galindo lagoon
Delta 8-THC	HCAWPGARWVBULJ-UHFFFAOYSA-N	$C_{21}H_{30}O_2$	$[M + H]^+$	4	Bio Bio river Andalien river Lo galindo lagoon
CAY10429	YWEZXUNAYVCODW-RBUKOAKNSA-N	$C_{21}H_{30}O_2$	$[M + H]^+$	4	Bio Bio river Andalien river Lo galindo lagoon
CP 47,497	ZWWRREXSUJTKNN-AEFFLSMTSA-N	$C_{21}H_{34}O_2$	$[M + H]^+$	4	Trebal WWTP
(Z)-Hexadec-9-enoic acid	SECPZKHBENQXJG-FPLPWBNLSA-N	$C_{16}H_{30}O_2$	$[M + H]^+$	4	Bio Bio river Lo galindo lagoon Trebal WWTP
Ferutinin	CYSHNJQMYORNJI-YUVXSKOASA-N	$C_{22}H_{30}O_4$	$[M + H]^+$	4	Bio Bio river Trebal WWTP
Linoleic acid	OYHQOLUKZRVURQ-HZJYTTRNSA-N	$C_{18}H_{32}O_2$	$[M + H]^+$	4	Andalien river
Thymol	MGSRCZKZVOBKFT-UHFFFAOYSA-N	$C_{10}H_{14}O$	$[M + H]^+$	4	Lo galindo lagoon
Dimethyl decanedioate	ALOUNLDAKADEEB-UHFFFAOYSA-N	$C_{12}H_{22}O_4$	$[M + H]^+$	4	Trebal WWTP
Tetradecanoic acid, 2,3-dihydroxypropyl ester	DCBSHORRWZKAKO-UHFFFAOYSA-N	$C_{17}H_{34}O_4$	$[M + H]^+$	4	Trebal WWTP
Valerophenone (1-Pentanone, 1-phenyl-)	XKGLSKVNOSHTAD-UHFFFAOYSA-N	$C_{11}H_{14}O$	$[M + H]^+$	4	Trebal WWTP
Dinoprost	PXGPLTODNUVGFL-RTYMFESYSA-N	$C_{20}H_{34}O_5$	$[M + H]^+$	4	Trebal WWTP
Carbaprostacyclin	XZFRIPGNUQRGPI-WBQKLGIQSA-N	$C_{21}H_{34}O_4$	$[M + H]^+$	4	Trebal WWTP
U-44069	DJKDIKIDYDXHDD-REGKDVDGSA-N	$C_{21}H_{34}O_4$	$[M + H]^+$	4	Trebal WWTP
Dexchlorpheniramine	SOYKEARSMXGVTM-UHFFFAOYSA-N	$C_{16}H_{19}ClN_2$	$[M + H]^+$	4	Trebal WWTP
Pyrethrin I	ROVGZAWFACYCSP-UHFFFAOYSA-N	$C_{21}H_{28}O_3$	$[M + H]^+$	4	Trebal WWTP
2-Hydroxy-4-methoxybenzophenone	DXGLGDHPHMLXJC-UHFFFAOYSA-N	$C_{14}H_{12}O_3$	$[M + H]^+$	4	Trebal WWTP
3,5-Dimethyl-p-anisic acid	WXVQURJGDUNJCS-UHFFFAOYSA-N	$C_{10}H_{12}O_3$	$[M + H]^+$	4	Trebal WWTP
estr-4-ene-3,17-dione	JRIZOGLBRPZBLQ-QXUSFIETSA-N	$C_{18}H_{24}O_2$	$[M + H]^+$	4	Trebal WWTP

CAPÍTULO II SECCIÓN 3

Title: Simultaneous degradation of 30 pharmaceuticals by anodic oxidation: main intermediaries and by-products

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Abstract

The anodic oxidation (AO) of 30 pharmaceuticals including antibiotics, hormones, antihistaminics, anti-inflammatories, antidepressants, antihypertensives, and antiulcer agents, in solutions containing different supporting electrolytes media (0.05 M Na_2SO_4, 0.05 M NaCl, and 0.05 M Na_2SO_4 + 0.05 M NaCl) at natural pH was studied. A boron-doped diamond (BDD) electrode and a stainless-steel electrode were used as anode and cathode, respectively, and three current densities of 6, 20, and 40 mA cm^{-2} were applied. The results showed high mineralization rates, above 85%, in all the tested electrolytic media. 25 intermediaries produced during the electrooxidation were identified, depending on the supporting electrolyte together with the formation of carboxylic acids, NO_3^-, SO_4^{2-} and NH_4^+ ions. The formation of intermediates in chloride medium produced an increase in absorbance. Finally, a real secondary effluent spiked with the 30 pharmaceuticals was treated by AO applying 6 mA cm^{-2} at natural pH and without addition of supporting electrolyte, reaching c.a. 90% mineralization after 300 min, with an energy consumption of 18.95 kW h m^{-3} equivalent to USD 2.90 m^{-3}. A degradation scheme for the mixture of emerging contaminants in both electrolytic media is proposed. Thus, the application of anodic oxidation generates a high concentration of hydroxyl radicals that favors the mineralization of the pharmaceuticals present in the spiked secondary effluent sample.

Keywords: Anodic oxidation; Boron-doped diamond electrode; Hydroxyl radical; Pharmaceuticals; Secondary Effluent.

1. Introduction

During the last decades, a large amount of many organic micropollutants has been released into the environment as a result of anthropogenic activities (Hernández et al., 2019). Most of these micropollutants have raised particular concern and thus currently they are known as contaminants of emerging concern (CECs). These compounds, are chemical substances not commonly monitored that present potential to enter the environment and cause ecological or human adverse effects, whether known or probable (Geissen et al., 2015; Rodriguez-narvaez et al., 2017).

An important group of CECs are pharmaceutically active substances (Gómez et al., 2010; Petrovic, 2014). Pharmaceuticals are designed to prevent or treat human and animal diseases (Ebele et al., 2017; Gracia-lor et al., 2012) by a specific mode of action (Fent et al., 2006); nevertheless, its extensive use worldwide produces bioaccumulation and toxic undesirables effects in aquatic and terrestrial ecosystems (Ebele et al., 2017), such as antibiotic resistance (Ebele et al., 2017; Grenni et al., 2018; Rabbia et al., 2016) and alteration of plasma levels of certain biomolecules (Ebele et al., 2017). On the other hand, in humans the risk of metabolic disorders, neurological disorders, damage to the immune system, hormone levels disorder and alterations in female and male reproductive system has been reported (Barrios-Estrada et al., 2018).

Although risk assessments indicate that it is very unlikely that the trace concentrations found in the water present risks to human health (acute toxicity) (World Health Organization, 2012), the risks associated with prolonged exposure (associated to chronic toxicity) and the combined effects of mixtures of them are unknown and of concern (Hernández et al., 2019; Noguera-oviedo and Aga, 2016; Vasiljevi and Lau, 2009).

Effluents from wastewater treatment plants (WWTPs) are considered one of the most important entry pathways of pharmaceuticals to the aquatic environment (Babic and Horvat, 2007; Gracia-

lor et al., 2012; Sancho et al., 2012). WWTPs fail to fully remove most pharmaceuticals (Boix et al., 2015; Hernández et al., 2019, 2011), because they are not designed to remove this type of contaminants (Barbosa et al., 2016; Patel et al., 2019). Thus, many of them have been detected in concentrations from ng L^{-1} to mg L^{-1} in urban wastewater (Alygizakis et al., 2020; Česen et al., 2019; Peña-Guzmán et al., 2019; Racar et al., 2020).

To remove pharmaceuticals more efficiently in the WWTPs, electrochemical advanced oxidation processes (EAOPs) have gained increasing attention as a promising advanced oxidation process (AOP) (Moreira et al., 2017) due to their effectivity to oxidize both organic and inorganic compounds (Rivera-Utrilla et al., 2013).

Anodic Oxidation (AO) is the most popular EAOPs owing to its versatility and ease of scalability (Flores et al., 2017; Martínez-Huitle et al., 2015; Özcan et al., 2008). In AO, the pollutants are oxidized by heterogeneous M(•OH) formed from water electrolysis at the anode surface (Eq. (1)) (Martínez-Huitle and Ferro, 2006).

$$M + H_2O \rightarrow M(^{\bullet}OH) + H^+ + e^- \qquad (1)$$

The hydroxyl radical (•OH) is a powerful oxidant (E^0 (•OH/H_2O)= 2.80 V vs SHE) that reacts with organic compounds by abstraction of a hydrogen atom (dehydrogenation), electrophilic addition to an unsaturated bond (hydroxylation), electron transfer (redox) reaction and ipso-substitution of halogen atom (Mousset et al., 2018) until its complete mineralization to CO_2, water and inorganic ions (Flores et al., 2017; Yu et al., 2014). The electrochemical generation and the chemical reactivity of M(•OH) strongly depend on the anode material (Comninellis et al., 2008). The boron-doped diamond (BDD) electrode is a non-active anode (Espinoza et al., 2018; Panizza and Cerisola, 2009) that favors the indirect oxidation of organic compounds. Additionally, depending on the ions presents in the solution, other oxidants can be produced, for example, persulfate ion ($S_2O_8^{2-}$), peroxodicarbonate ($C_2O_6^{2-}$), peroxodiphosphate ($P_2O_8^{2-}$) (Martínez-Huitle and Brillas,

2009) or active chlorine species (Cl_2, HClO/ClO^-) (Contreras et al., 2015a). These oxidants facilitate the oxidation of pollutants present near the anode and/or in the bulk of the solution, as well as water disinfection in case of chlorine species (Candia-Onfray et al., 2018).

Several authors have reported the removal of pharmaceuticals by AO (Dirany et al., 2010; García-Montoya et al., 2015; Sopaj et al., 2015). However, there are few studies applying AO to treat a large group of pollutants in both, synthetic or real wastewater (Garcia-Segura et al., 2015; Lan et al., 2017).

This work aims to study the simultaneous degradation of 30 pharmaceuticals, antibiotics, hormones, antihistaminic, anti-inflammatories, antidepressants, antihypertensive, and antiulcer agents by AO using a BDD anode in synthetic solutions, and in a secondary effluent from a WWTP applying different current densities and electrolytic media. Moreover, an exhaustive study by liquid chromatography coupled to high-resolution mass spectrometry (LC-HRMS) and ion exclusion chromatography was carried out to identify intermediaries and reaction products formed in the process.

2. Materials and methods

2.1 Reagents

Trimebutine maleate, ketorolac trometamol, caffeine, acetaminophen, chlorphenamine maleate, sodium diclofenac, ibuprofen and sodium metamizole (>99% purity) was supplied by Pasteur S.A Laboratory (Santiago de Chile, Chile). Tetracycline hydrochloride (95% purity), norfloxacin, ciprofloxacin, naproxen, β-estradiol, estrone, progesterone, and sulfamethazine (>98% purity) were obtained from Sigma-Aldrich® (Santiago de Chile, Chile). Salicylic acid (>99% purity), analytical grade anhydrous sodium sulfate and sodium chloride used as background electrolyte, were purchased from Merck® Santiago de Chile, Chile). Mefenamic acid, venlafaxine, sertraline,

escitalopram, fluoxetine, azithromycin, amoxicillin, losartan, enalapril, famotidine, omeprazole, loratadine and loperamide in commercial tablets, were acquired from the established trademarks in Chile.

Carboxylic acids were purchased from Sigma-Aldrich®, while maleic, formic and acetic acids were from Merck®.

All the other chemicals employed were HPLC grade or analytical grade from Merck® and Sigma-Aldrich®. All solutions were prepared with ultrapure water obtained from a Millipore Milli-Q system (resistivity > 18 mΩ cm).

2.2 Wastewater samples

Secondary effluent samples were collected in July 2019, from "Aguas Andinas, Mapocho/Trebal" WWTP in Padre Hurtado (33°32'82"S / 70°50'08"W), Santiago de Chile (Chile). Table 1 reports the main parameters determined for the secondary effluent. The spiked of the wastewater was carried out with a mixture of 30 drugs at the concentrations reported in SM1.

2.3. Electrochemical experiments

Electrochemical system. The electrolysis was carried out in a one compartmental 100 cm^3 electrochemical cell with constant stirring at 25 ± 2 °C. A BDD thin-film electrode from Adamant Technologies® was used as anode and a stainless-steel plate was used as the cathode (BDD/SS system), both with 5.0 cm^2 of area and an inter-electrode gap of 1 cm. The experiments were performed applying constant current densities (j) using an EHQ power supply model PS3010. Cyclic voltammetry was carried out in an Autolab Postentiostat/Galvanostat PGSTAT 204 system using a 10 cm^3 glass electrochemical cell. BDD was used as working electrode (0.19 cm^2 area), Ag/AgCl (1M) as reference electrode, and a platinum wire as counter electrode. The voltammograms were recorded from 0 to 3V at 5 mV s^{-1}.

2.4 Instruments and analytical procedures for water analysis

The pH was measured with a pH-meter HANNA HI5222. Conductivity and total dissolved solids were measured using a HI98311 Waterproof EC/TDS/Temperature Tester.

The turbidity of the wastewater was measured with the HANNA instrument HI98703 portable turbidimeter. The chemical oxygen demand (COD) was determined after chemical digestion of the samples during 2 hours at 150ºC in a HANNA multiparameter bench photometer for wastewater treatment HI83214. The concentration of ammonium ion was analyzed using the HANNA AHR test tube HI93764B-25 (0 to 100 mg L^{-1}) and reagent HI93764-0, while nitrate was determined using HI93766-50 test tube (0 to 30 mg L^{-1}) and nitrate reagent sachet HI93766-0. Then, the concentration of both ions was measured in the spectrophotometer HI83214. The presence of chlorite, chloride, chlorate, nitrate and sulfate ions was determined using a Metrohm Ion Chromatograph, 930 Compact IC Flex model. The separation was carried out in a Metrosep A Supp 5 – 250/4 (250 x 4.0 mm (i.d), 5µm) column and mobile phase composed of Na_2CO_3/$NaHCO_3$ (3.2:1 mmol L^{-1}) was used, at a flow rate of 0.7 mL min^{-1}. The injection volume was 20 µL.

The change in the absorbance of the initial wastewater, compounds solution, and secondary effluent during electrolysis was monitored by a spectrophotometer Agilent 8453. The total organic carbon (TOC) variation was monitored by a Shimadzu (TOC-L) analyzer, obtaining an initial average TOC value among the three evaluated electrolytic media of 72.62 ± 0.2 mg L^{-1}. The carboxylic acids generated as intermediaries were monitored and quantified by ionic-exclusion chromatography using an HPLC Prominence 12.770 (Shimadzu) with diode array detector 41.571 model. A Bio-Rad Aminex HPX 87H, 300 × 7.8 mm (i.d.) column at 30 °C was used and the detection was performed at 210 nm. The mobile phase was 4 mM H_2SO_4 with a 0.6 mL min^{-1} flow. The corresponding calibration curves were constructed using pure acid standards.

The energy consumption per volume of electrolyzed solution was obtained from eq. 2:

Energy Consumption (kW hm^{-3}) = $IE_{cell}\, t$ / 1000 V_s (2)

where I is the applied current (A), E_{cell} is the average cell voltage (V), t is the electrolysis time (h), and V_s is the volume of the treated solution (m^3) (Candia-Onfray et al., 2018; Contreras et al., 2015; Salazar et al., 2016b, 2017).

2.5 LC-ESI- Q-Exactive analysis

To identify aromatic intermediates generated during electrooxidation by ultra-high performance liquid chromatography-high resolution mass spectrometry (UHPLC-HRMS), aliquots collected at different electrolysis times were mixed for a total of 10 mL, then they were extracted three times with 30 mL of CH_2Cl_2 and ethyl acetate separately to extract as many intermediates as possible. Each organic fraction collected (90 mL) was dried with anhydrous Na_2SO_4, filtered and evaporated in rotary evaporator. Then, the organic fraction evaporated was reconstituted in 1mL of methanol and injected into the UHPLC-Q-Exactive (Thermo Fisher Scientific, San Jose, CA, USA) system with a hybrid quadrupole–Orbitrap analyzer.

The ionization was carried out with an electrospray ionization (ESI) source working in positive and negative modes under capillary voltages of + 4kV and -3kV, respectively. The capillary temperature was 350 ºC in both modes, while the probe heater temperatures were 250 º C in positive and 300 ºC in negative mode. The sheath gas and the auxiliary gas flows were set at 40 and 10 a.u.

The chromatographic separation was performed using an Acquity BHE-C18 (100 x 2.1 mm, 1.7 µm) column (Waters, Massachusetts, USA), keeping the oven temperature at 40ºC. The mobile phase used in positive mode was: (A) water containing formic acid 0.1% (v/v) and (B) methanol also containing formic acid 0.1% (v/v) at 0.3 mL min^{-1} in gradient mode. The percentage of (A) was changed as follows: 0 min, 95%; 7 min, 25%; 10 min, 0%; 15 min; 0%, 17 min; 95%, 21 min,

95%. In negative mode, the modifications were (A) water containing ammonium acetate 5mM and (B) methanol containing ammonium acetate 5mM at 0.3 mL min^{-1} in gradient mode. The percentage of (A) was modified as follows: 0 min, 95%; 3 min, 50%; 6 min, 10%; 13 min; 0%, 17 min; 0%, 18 min, 95%; 20 min, 95%. The analysis run time was 21 and 20 min, respectively, and sample injection volume was 10 μL.

Spectra were acquired in independent data acquisition (DIA) mode. This acquisition mode allows different scans; the first is a full scan at low collision energy (10 eV) in the range m/z 66.70−1000, where all the compounds mass arriving to the analyzer are determined. Then, a MS/MS second scan at high collision energy (40 eV) is performed to all the compounds that had arrived to the analyzer without differentiating the origin and obtaining a MS/MS spectra of all the ions also in the range m/z 66.70−1000.

2.6 Data processing

All the raw data obtained using XcaliburTM4.1 software (Thermo Scientific), were converted into .mzML files in Msconvert 3.0 software (ProteoWizard) using a threshold of 5000 in ESI (+) and 1000 in ESI (-) and peak picking as filters. These files were exported to the norman-data.eu site. In addition, information was provided on the type of sample, origin, and chromatographic and ionization conditions. A set of standards was introduced for the calibration of the chromatographic retention time (expressed in minutes) obtained under working conditions, in (ESI+) (Guanylurea; 0.91, Amitrol; 0.91, Histamine; 0.81, Chlormequat; 0.94, Methamidophos; 1.99, Vancomycin; 2.95, Cefoperazone; 4.35, Trichlorfon; 4.73, Dichlorvos; 6.23, Tylosin; 7.11, TCMTB; 7.69, Rifaximin; 8.08, Spinosad A; 9.48, Emamectin B1a; 10.57, Avermectin B1a, 10.34, Nigericin; 10.94, Ivermectin B1a; 10.91), and in (ESI-) (Amitrole; 0.96, Benzoic acid; 1.72, Acephate; 2.19, Salicylic acid; 2.37, Simazine 2-Hydroxy; 3.69, Tepraloxydim; 3.75, Bromoxynil; 3.69, MCPA; 4.39, Valproic acid; 4.68, Phenytoin; 4.96, Flamprop; 4.99, Benodanil; 5.35, Dinoterb; 5.2,

Inabenfide; 5.88, Coumaphos; 6.59, Triclosan; 7.02, AvermectinB1a; 7.84, and Salinomycin; 6.99).

Screening to find matches was performed using the EXPHRMSMSAVAL list in positive (6828 compounds) and in negative (3042 compounds) mode, from Norman Suspect List Exchange. Norman Substance Database in https://www.normannetwork.com/nds/SLE/ ("NORMAN Substance Database," n.d.). Finally, an .xlsx file is imported from the site containing the possible compounds identified based on identification proofs.

2.7 Identification of intermediaries by-products

The identification was carried out using the identification criteria, mass accuracy (± 5 ppm), retention time tolerance (± 20%) and MS/MS data. In addition, the mechanisms of interaction of hydroxyl radicals and active chlorine species with pharmaceuticals reported in literature were considered for the selection of possible intermediaries (Cavalcanti et al., 2013; Deborde and von Gunten, 2008; Mousset et al., 2018).

3. Results and discussion

3.1 Effect of the supporting electrolyte and the applied current on the degradation of pharmaceuticals

First, 100 cm^3 of a standard mixture of the 30 pharmaceuticals selected corresponding to 72.62 ± 0.2 mg L^{-1} of TOC in 0.05 M Na_2SO_4 at initial pH 7.80 ± 0.02 were electrolyzed applying 6, 20 and 40 mA cm^{-2} during 300 min. at constant stirring (cell potential 7.06, 10.33 and 13.06 V, respectively). Fig. 1A and 1B show the TOC abatement and absorbance decay, respectively, as a function of the electrolysis time at different current. In all cases, TOC and absorbance decreased as the electrolysis time elapsed due to the attack of hydroxyl radicals generated on the surface of the BDD electrode. At the end of the electrolysis, the %TOC removal depends on the applied

current density (Fig. 1A), being greater when the current density is higher (Brocenschi et al., 2016), with 73.35, 80.25 and 90.56% of TOC decay applying 6, 20 and 40 mA cm^{-2}, respectively. Similarly, the absorbance of the solutions decreased (Fig. 1B), although at minute 300 a very slight increase occurred for the highest current density applied (40 mA cm^{-2}), suggesting the formation of intermediates with opposite absorptive properties.

Additionally, the mineralization of the pharmaceuticals was evaluated in 0.05 M NaCl medium applying the same current densities and at the same initial pH of the previous experiment. The cell potentials were 8.99, 11.67, and 15.52 V for 6, 20 and 40 mA cm^{-2} intensity, respectively. Fig. 1C shows the TOC decay as a function of the current densities applied. Similar percentage of mineralization were obtained in this electrolytic medium at the three applied current densities. When NaCl is used as supporting electrolyte, in addition to the formation of hydroxyl radicals, other oxidizing species may be generated, for example, active chlorine species as Cl_2 and $HClO/ClO^-$ (Rivera-Utrilla et al., 2013). These active chlorine species also react with pharmaceutical compounds, causing their oxidation, and together with the hydroxyl radicals produced on the surface of the BDD anode, they reach mineralization, both of the initial compounds and of the intermediates produced during electrolysis. The active chlorine species not only contribute to the oxidation of organic contaminants but also to the disinfection of water (Candia-Onfray et al., 2018).

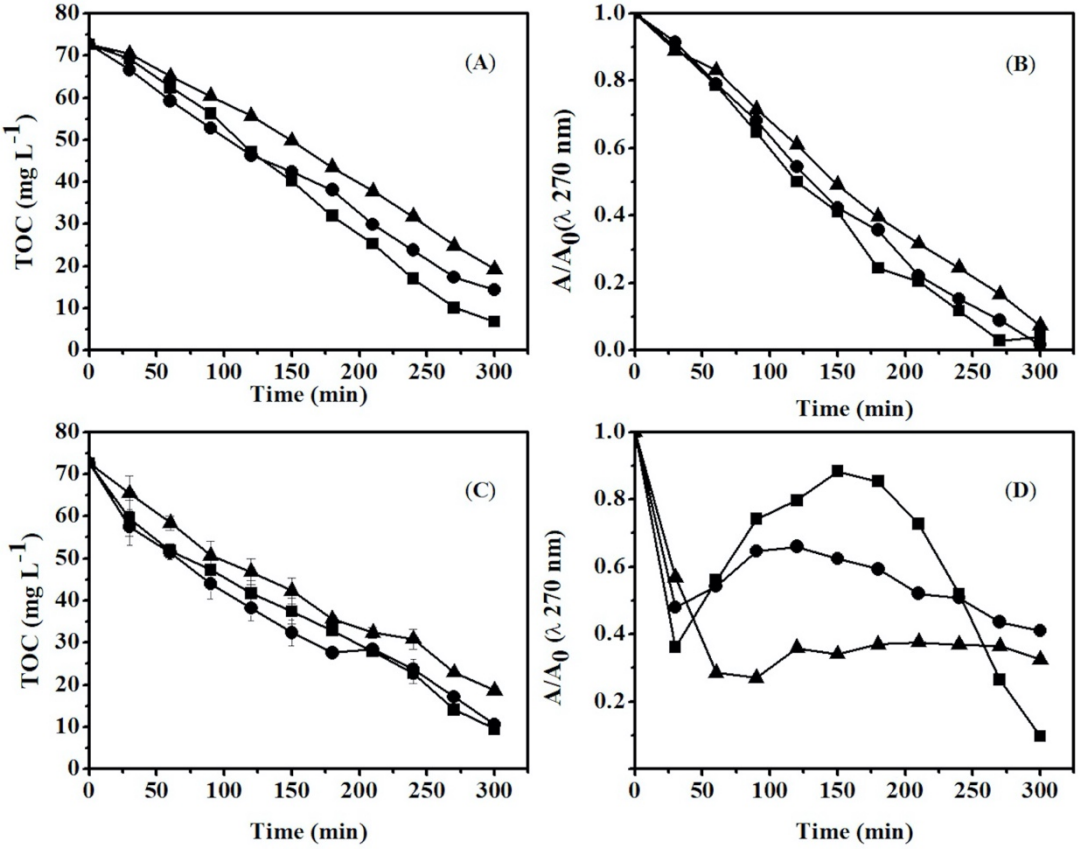

Figure 1. TOC abatement and absorbance decay at 270 nm as a function of the electrolysis time applied to 100 mL the mixture pharmaceuticals solution, corresponding to 72.62 mg L^{-1} TOC in 0.05 M Na_2SO_4 (A and B) and 0.05 M NaCl (C and D), using a stirred BDD/SS cell at 25 ºC and pH 7.8. Intensity density applied was (▲) 6, (●) 20, and (■) 40 mA cm^{-2} .

In addition, other oxidizing species can be produced from the action of •OH in the presence of Cl^- ion, such as ClO_2^-, ClO_3^- and ClO_4^- (Sirés et al., 2014), which would contribute to the oxidation of organic compounds, but less efficiently than $HClO/ClO^-$ (Martínez-Huitle et al., 2015). In Fig. 2, cyclic voltammograms in different electrolytic media and the secondary effluent sample spiked

with pharmaceuticals are displayed. The oxidation of chlorine begins first at potentials close to 2.25 V (curve A), while oxygen oxidation starts at 2.6 V (versus Ag/AgCl sat.) in sulfate media (curve C). This indicates that the oxidation of chlorine is thermodynamically favored concerning the oxidation of oxygen, which would be in agreement with the slightly higher degradation of the contaminants in the presence of sodium chloride.

A large difference in the absorbance change at 270 nm during electrolysis was observed in NaCl medium (Fig. 1B) compared to the obtained in sodium sulfate (Figure 1D). This difference is consistent with that reported by Jalife-Jacobo et al., 2016 in the study of discoloration of the diazo dye Congo Red in these same electrolytic media at the same concentration (Jalife-Jacobo et al., 2016). When current densities of 20 and 40 mA cm^{-2} were applied, the absorbance at 270 nm increased, almost at the same time. This may be due to the formation of oxychlorides and/or organochlorine intermediaries that have the maximum absorption wavelength close to 270 nm. For example, Murugananthan et al., 2010 have reported a $\lambda_{máx}$ displacement up to 292 nm, ascribed to the formation of the ClO^- ion in ketoprofen's electrolysis conducted in 0.1 M chloride medium using a BDD anode (Murugananthan et al., 2010).

To evaluate the influence of both supporting electrolytes in the same solution, electrolysis of the 30 pharmaceuticals containing a mixture of 0.05 M NaCl and 0.05 M Na_2SO_4 (pH 7.43 ± 0.03) was performed. The cell potentials were 7.32, 10.32 and 10.96 for 6, 20 and 40 mA cm^{-2}, respectively. Under these conditions, besides $^{\bullet}OH$, chlorine and sulfate oxidizing species, additional Cl^- and $SO_4^{-\bullet}$ radicals can be formed from the reaction between $Cl^{\bullet}$ and SO_4^{2-} (Eq. (3)) (Lan et al., 2017). Therefore, the presence of both salts could have a greater effect on the degradation of pollutants.

$$Cl^{\bullet} + SO_4^{2-} \rightarrow (SO_4^{-})^{\bullet} + Cl^- + e^- \qquad (3)$$

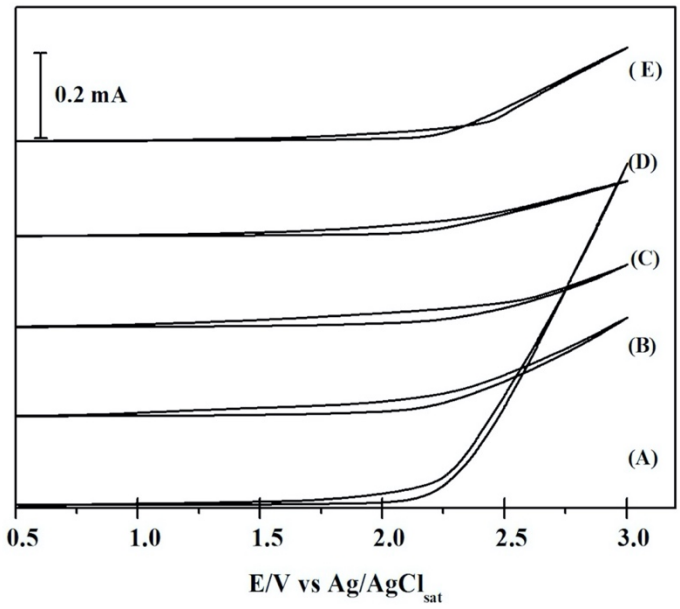

Figure 2. Cyclic voltammograms of (A) 0.05 M NaCl, (B) 0.05 M NaCl + 0.05 M Na_2SO_4, (C) 0.05 M Na_2SO_4, (D) secondary effluent, (E) secondary effluent spiked with 30 pharmaceuticals corresponding to 72.62 mg L^{-1} TOC, using a BDD working electrode, Ag/AgCl as reference electrode, and a platinum wire as the counter electrode. Sweep rate applied 5 mV s^{-1}.

However, the mineralization rates reached at current densities of 6 and 20 mA cm^{-2} for 0.05 M NaCl (74.28 and 85.50%) and 0.05 M Na_2SO_4 (73.35 and 80.25%) separately were slightly higher than those achieved when the electrolytes were mixed (71.04 and 80.20 %) (Fig. 3A). Therefore, the addition of sulfate does not have an additional positive effect on the TOC removal rates achieved at these current densities, but the obtained rates were more similar to that obtained in sulfate alone, even lower, however, the mineralization percentages were similar. This result is very important because in real wastewater, the presence of these two salts is high. In this electrolytic medium, the concentrations of intermediaries are generated in smaller concentrations with respect to the observed in Fig. 1D, since absorbance was lower for each one of the current

densities evaluated compared to those found in chloride electrolysis. Specifically, for the electrolysis in 0.05 M NaCl, maximum absorbance values of 0.8837, 0.6597, and 0.3763 (Fig. 1D) were obtained for current densities of 40, 20, and 6 mA cm^{-2}, respectively, while in 0.05 M NaCl and 0.05 M Na_2SO_4 solution, the maximum absorbance values were 0.4271, 0.3368, and 0.2485 for the same j (Fig. 3B). Besides, the oxidation reaction of chloride begins at potentials close to 2.25V, which is similar to that observed in the presence of sodium chloride (Fig. 2B). Considering that energy consumption is an important factor in an electrochemical treatment, the efficiency of the electro-oxidation of the 30 pharmaceuticals was estimated for each electrolytic media at selected density currents by means of Eq. (2). Considering an electrical energy cost of about CLP$ 110.0 (USD 0,154) (Chilean price, taxes excluded) per kWh consumed (Compañía Chilena de Energía Eléctrica, Chile), the total cost of each electrolysis was calculated in USD. Applying current densities of 6 and 20 mA cm^{-2}, between 70 and 85% of mineralization was reached in all electrolytic media evaluated, with the highest energy consumption corresponding to the electrolysis in chloride of 13.48 and 77.60 kWh^{-3}, which is equivalent to USD 2.10 and USD 12.00, respectively. As expected, the highest energy consumption occurred at the highest current density (40 mA cm^{-2}) for the three electrolytic media; however, the electrolysis in sulfate had the highest energy expenditure (130.60 kWh^{-3}), with a cost of USD 20.00. Consequently, to

achieve a 90% mineralization rate the cost of electrolysis is 12.5-folds higher compared to electrolysis in sulfate media, when applying 6 mA cm^{-2}.

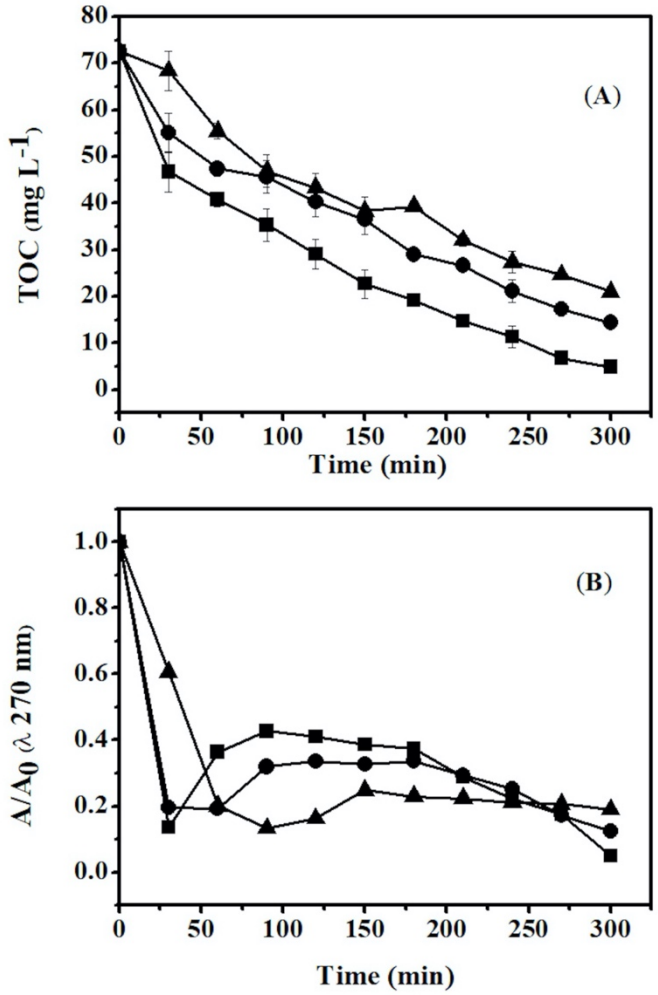

Figure 3. TOC abatement (A) and absorbance decay at 270 nm (B) with respect to electrolysis time for the treatment of 100 cm^3 of solution containing a mixture of 30 pharmaceuticals in 0.05 M NaCl + Na_2SO_4, using a stirred BDD/SS cell at 25 ºC, pH 7.8, applying (▲) 6, (●) 20 and (■) 40 mA cm^{-2}.

3.2 Intermediates and by-products generated during anodic oxidation of pharmaceuticals.

To identify the main intermediaries and by-products formed during the electrooxidation of the 30 pharmaceuticals, the samples electrolyzed in 0.05 M NaCl and 0.05 M Na_2SO_4 background were

analyzed by HRMS. The short chain carboxylic acids generated were monitored and quantified by ionic exclusion chromatography. Moreover, ions generated at the end of electrolysis were identified and quantified by means of ion chromatography and spectrophotometric analysis.

3.2.1 Intermediates in sulfate background solution

The identification of 15 by-products produced during electrolysis in 0.05 M Na_2SO_4 according to the procedure described in *section 2.6* was carried out. Their denominations, molecular formula, retention time, measured exact mass, identifications proofs and suggested chemical structure are reported in SM2.

Intermediaries 1,3,7-trimethyl-9*H*-purine-2,6,8-trione **(1)** and 1,3-diazinane-2,4,5,6-tetrone **(2)** come from caffeine and could be generated from various pathways: (i) hydroxylation of the imidazole ring between N = C followed by oxidation to form **1**; (ii) demethylation of all methyl groups of caffeine and (iii) opening of the imidazole ring followed by decarboxylation at the amino positions and subsequent deamination and oxidation of the hydroxyls that bind to the pyrimidine ring to form intermediate **2**, similar to that reported by (Cavalcanti et al., 2013) for the degradation of omeprazole.

Dehalogenation reactions occur to produce N, *N*-dimethyl-3-phenyl-3-pyridin-2-ylpropan-1-amine **(3)**, 3-phenyl-3-pyridin-2-ylpropanoic acid **(4)**, piperazine-2,5-dione **(5)**, and benzo[g]quinoline **(10)** from chlorpheniramine, ciprofloxacin and loratadine. Furthermore, the abstraction of hydrogen caused by •OH radicals, followed by oxidation at position 1 and 4 in piperazine released from fluoroquinolones must occurs to produce intermediate **5**. On the other hand, further to the dechlorination of loratadine, the contraction of cycloheptane ring can occurs to produce intermediate **10** (Miao and Metcalfe, 2003; Vogna et al., 2004).

The 2-[2-(2,6-dichloro-4-hydroxyanilino) phenyl]acetic acid compound **(6)** could be produced by addition reaction to double bond from diclofenac (hydroxylation). A decarboxylation reaction

occurs to give the compound 2-(2,6-dichloroanilino) benzaldehyde **(8)** and a cyclization reaction to give 1-(2,6-dichlorophenyl)-3*H*-indol-2-one **(7)** from the degradation of diclofenac. This last reaction to give rise to intermediate **7** is in accordance with (Zhao et al., 2009).

Compound (8*R*,9*S*,13*S*,14*S*)-3-hydroxy-2-methoxy-13-methyl-7,8,9,11,12,14,15,16-octahydro-6*H*-cyclopenta[a]phenanthren-17-one **(9)** could be generated from estrone or β-estradiol (after oxidation of the hydroxyl at carbon 17); however, the formation of this intermediate with the introduction of a methoxy group at position 2 could be due to reactions among organic radicals produced during the attack of hydroxyl radicals.

Compound 2-Phenylphenol **(11)** comes from the breaking between N-C bond of biphenyl and the imidazole ring in losartan, together a C-C breakdown between the biphenyl and the tetrazole ring. On the other hand, N-(1,5-dimethyl-3-oxo-2-phenylpyrazol-4-yl) formamide **(12)** could originate due to the attack of hydroxyl radicals to the S position of metamizole, later 1,5-dimethyl-2-phenylpyrazol-3-one **(13)** are formed by the consecutive attack of •OH.

Abstraction of hydrogen at position 11 followed by release of acetyl at position 17 and the subsequent oxidation of the hydroxyl that enters in that position of the progesterone would produce compound (8*S*,9*S*,10*R*,13*S*,14*S*)-10,13-dimethyl-1,2,6,7,8,9,12,14,15,16-decahydrocyclopenta[a]phenanthrene-3,11,17-trione **(14)**.

The 2-(1-hydroxycyclohexyl)-2-(4-methoxyphenyl)-*N*, *N*-dimethylethanamine oxide compound **(15)** could be formed from the interaction between oxygen and the protonation of the N moiety in Venlafaxine.

Finally, since the break of the aromatic rings maleic (**26**) and oxamic (**27**) acids are generated, which continue being attacked by •OH forming acetic (**28**) and formic acid (**29**) until the complete mineralization to CO_2 and the release of ions.

3.2.2 Intermediates in chloride background solution

During the electrolysis in 0.05 M NaCl, 11 possible intermediaries were identified. Their denominations, molecular formula, retention time, measured exact mass, identifications proofs and suggested chemical structure are reported in SM3.

The possible generation pathway of the compound 2-Phenylphenol **(11)** was previously discussed for electrolysis in 0.05 Na_2SO_4 solution. However, in the electrolysis in 0.05 M NaCl medium, additional different compounds were identified.

In this media, in addition to the hydroxyl radicals generated on the surface of the BDD electrode, active chlorine species were produced and reacted with the contaminants (Deborde and von Gunten, 2008).

N-demethylation, dehalogenation reaction and piperazine moiety release caused by attack of the •OH radicals on the structures of ciprofloxacin and norfloxacin could give rise to compound 4-oxo-1H-quinoline-3-carboxylic acid **(16)**. Furthermore, the intermediate 1H-quinolin-4-one **(17)** can be occur from the decarboxylation of **16.**

The 2-(2-chloroanilino) benzaldehyde compound **(18)** could be generated from decarboxylation in phenylacetic moiety and the release of a chlorine in the dichloroaniline moiety from diclofenac.

The quinoline compound **(19)** could originate from the successive attacks of •OH, which led to the opening of the benzene ring in 2-Phenylphenol **(11)** (formed in sulfate background solution) and subsequent decarboxylation.

The addition of an •OH and subsequent oxidation on the pyridine ring of quinoline **(19)** can also produce intermediate 1H-quinolin-4-one **(17)**.

Compounds 2-(3-chloro-2-methylanilino) benzoic acid **(20)** and 2-(2,6-dichloro-3-methylanilino) benzoic acid (**21)** are two possible chlorinated intermediaries from mefenamic acid. Compound **20** could have been formed from the attack of •OH radicals on the methyl group in the *meta*

position with respect to the amino moiety of mefenamic acid to form a carboxyl group. Further, the carboxyl group in this molecule could be released and chlorine added to form a bond due to the positive polarization of HClO ($Cl^{\delta+}$-$OH^{\delta-}$). In addition, it could act by electrophilic substitution taking out an H^+ that could previously enter when the decarboxylation took place. Meanwhile, intermediate **21** could originate from the inclusion of a chlorine in an *ortho* position with respect to the N moiety either by addition or electrophilic substitution as explained previously in the formation of intermediate **20**, and by the entry of other chlorine by substitution also in *ortho* position with respect to the N moiety.

On the other hand, due to the consecutives attacks of hydroxyl radicals to the S position of metamizole could originate 4-amino-1,5-dimethyl-2-phenylpyrazol-3-one **(22)**.

O-dealkylation which divided the main structure of trimebutine and the following O-dealkylation of the methoxy groups of the trimethoxy benzoic acid moiety may originate intermediate (3R,4S,5R)-3,4,5-trihydroxycyclohexene-1-carboxylic acid **(23)**.

Compounds 4-[2-(dimethylamino)ethyl]phenol **(24)** and 4-(2-aminoethyl) phenol **(25)** could be generated from cleavage of the C-C bond between the cyclohexanol group and the rest of the venlafaxine molecule. Subsequently, O-dealkylation must have occurred to form **24**, followed by two demethylation on N-C bond to form **25**.

Finally, the breakdown of the simplest aromatic rings yielded short-chain carboxylic acids.

Ionic exclusion chromatography allowed for the identification and quantification of the maleic, formic, oxalic and acetic acids generated. The maximum levels of acetic, oxamic and formic acid reached were 19.7, 2.27 and 2.12 mg L^{-1}, respectively, while the concentration of maleic acid did not exceed 1.7 mg L^{-1}, due to the result of the direct rupture of the aromatic rings present in the pollutants that can be attacked continuously by hydroxyl radicals, creating simpler acids such as formic, acetic and oxalic. In addition, the formation of oxamic acid occurs as a consequence of

the breakdown of N-aromatics (Vidal et al., 2019, 2018).

The presence of inorganic ions such as ammonium (NH_4^+) and nitrate (NO_3^-) generated during the electrolysis of the pharmaceuticals in sulfate and chloride media, was determined by spectrophotometry and confirmed by ion chromatography for NO_3^- ion. The spectrophotometric method yielded NO_3^- ion concentrations of 11.5 and 180.1 mg L^{-1} before and after electrolysis, respectively, while NH_4^+ concentrations were 12.0 and 0 mg L^{-1}, respectively. These results were similar in both electrolytic media. With all these results, an abbreviated diagram of intermediaries and by-products generated in electrolysis with 0.05 M NaCl and 0.05 M Na_2SO_4 solutions is proposed and showed in Fig. 4.

3.3 Mineralization of 30 pharmaceuticals in a secondary effluent water samples.

From an applicative standpoint, it is necessary to investigate the mineralization of the pharmaceuticals in a real matrix to evaluate the effect of its composition on the performance of the AO process. Some experiments were carried out in a real secondary effluent from a WWTP at natural pH, without the addition of supporting electrolyte, using BDD anode at 6 mA cm^{-2} (11.48 V cell potential). The application of 6 mA cm^{-2} was based on the results shown above and the lowest energy consumption obtained in all the studied media. Table 1 reports the physicochemical characteristics of the secondary effluent used in this study. As seen in Table 1, the secondary effluent contained 22.68 mg L^{-1} of TOC, which increased to more than 93.3 mg L^{-1} when the solution was spiked with the 30 compounds at the concentration reported in SM1.

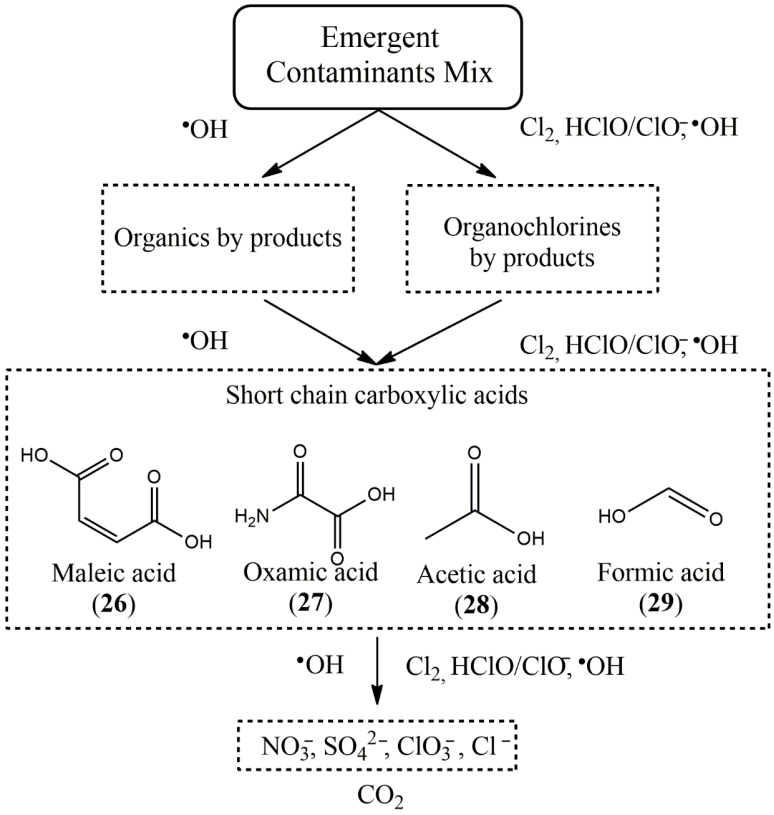

Figure 4. Proposed general pathway of the anodic oxidation of a mix of 30 pharmaceuticals in two different electrolytic media.

These concentrations mostly exceed those found for these pollutants in environmental waters, however they were chosen in order to ensure an adequate detection of by-products by LC-HRMS. Fig. 5 illustrates the time course of TOC removal during the degradation of 30 pharmaceuticals spiked into a secondary effluent water sample under the above described conditions. According to Fig. 5A, almost total mineralization occurred after 360 min, with a residual TOC of 10.64 mg L^{-1}.

Table 1. Characterization of secondary effluent from the WWTP

Parameters	Initial value
Color	Light yellow
Odor	strong
Turbidity (NTU)	7.72
Total Dissolved Solid (mg L^{-1})	1202
Conductivity (μS)	2402
Absorbance at 280 nm (U.A)	0.375
Chemical Oxygen Demand (mg L^{-1})	35.50 ± 0.70
Total Organic Carbon (mg L^{-1})	22.68
Total Chlorine (mg L^{-1})	0.16 ±0.02
Nitrate NO_3^- (mg L^{-1})	7.70 ± 0.14
Sulfate $S_2O_4^-$ (mg L^{-1})	8.17 ± 3.25
Ammonium $NH4^+$ (mg L^{-1})	42.50 ± 2.12

This result shows that through AO, the complete transformation to CO_2 of the pharmaceuticals and the original organic component of the secondary effluent occurs. The remaining TOC in solution could correspond to those short-chain carboxylic acids that were produced in the electrolytic media described above. The percentage of mineralization after 300 min (81.61%) was higher in the secondary effluent than that obtained in the electrolytic media evaluated (0.05 M sulfate, 0.05 M chloride and a mixture of both at 0.05 M) at a current density of 6 mA cm^{-2}, although no support electrolyte was incorporated in this case. The secondary effluent sample was analyzed by ion chromatography, showing levels of sulfate and total chlorine of 0.6 mM and 0.45 mM, respectively. Thus, the composition of the secondary effluent water sample had a positive influence in the mineralization of pharmaceuticals. The highest percentage of mineralization obtained could be mainly due to the action of the hydroxyl radical on the BDD surface, the active chlorine especies, and to a lesser extent, to other oxidants with weak character such as peroxodiphosphate and peroxodicarbonate generated from phosphates and carbonates (in much

lower concentration than the other ions) that coexist in the effluent water, they also react with organic pollutants causing their oxidation (Cotillas et al., 2017).

The Fig. 5B shows the noticeable decay of spectral bands intensity ($\lambda_{máx}$ 270 nm) during the electrooxidation of the 30 pharmaceuticals in the effluent water by applying 6 mA cm^{-2}. Absorbance decreased linearly (results are not shown) over electrolysis time. Therefore, it can be assumed that there was no formation of organochlorine compounds that modified absorption as in the case of electrolysis performed with 0.05 M NaCl and 0.05 M NaCl + Na_2SO_4 electrolyte, due to the low concentration of chloride in the wastewater. A high mineralization rate was achieved without the addition of salts during electrolysis and the pH during electrolysis was close to natural pH (7.66), which is also an advantage for the applicability of AO on a larger scale to treat wastewater.

The electrical consumption in this electrolysis by applying 6 mA cm^{-2} for 330 minutes was 18.95 kW h m^{-3}, which is equivalent to 2.90 USD m^{-3}, corresponding to 88.6% mineralization. Thus, AO is an economic and effective process for the treatment of wastewater that contains organic emerging contaminants such as pharmaceuticals.

A possible coupling with biological treatments could be attractive in order to further reduce the energy costs associated with the electrooxidation time. In this way, AO could be applied until obtaining lower molecular weight intermediates, or intermediates where antimicrobial activity is lost in the case of antibiotics, and then biologically converting them into CO_2 and CH_4 (Vidal et al., 2018).

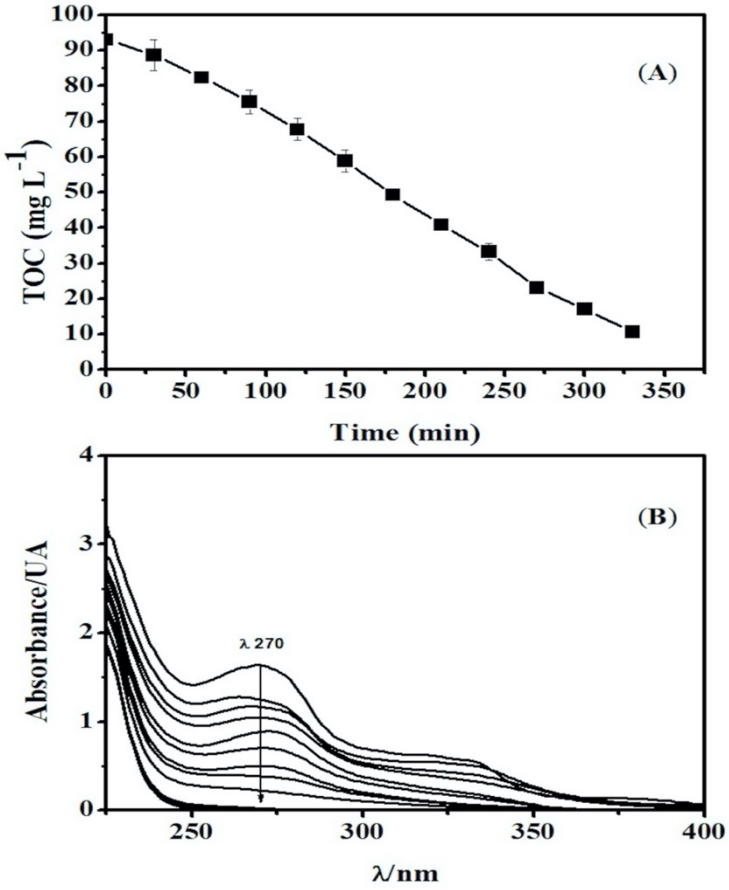

Figure 5. (A) TOC abatement and (B) spectra of real wastewater containing 30 pharmaceuticals (corresponding to 72.62 mg L^{-1} TOC) with respect to electrolysis time for the treatment of 100 cm^3 of solution without the addition of supporting electrolyte, using a stirred BDD/SS cell at 25 ºC, pH 7.66, applying 6 mA cm^{-2}.

Change in concentration of ions after electrolysis was confirmed by ion chromatography and the result is shown in Fig. 6. An increase in NO_3^- concentration close to 16-folds was observed after 300 min. of electrolysis, which can be attributed to the degradation of pharmaceuticals containing atoms of N such as famotidine and losartan, among others. The behavior of other ions such as sulfate, chlorine, and chlorate before and after electrolysis is shown as well in the ion

chromatogram shown in Fig. 6. SO_4^{2-} ion concentration also increased at the end of the electrolysis and it was attributed to breakdown of S-compounds (Cavalcanti et al., 2013) as sulfamethazine, amoxicillin, omeprazole, and famotidine. Moreover, the concentration of chloride (Cl^-) decreased due to the increase of the chlorate (ClO_3^-) concentration (Sirés et al., 2014). These results confirm the positive influence of the matrix components of the secondary WWTP's effluent in the mineralization of pharmaceuticals.

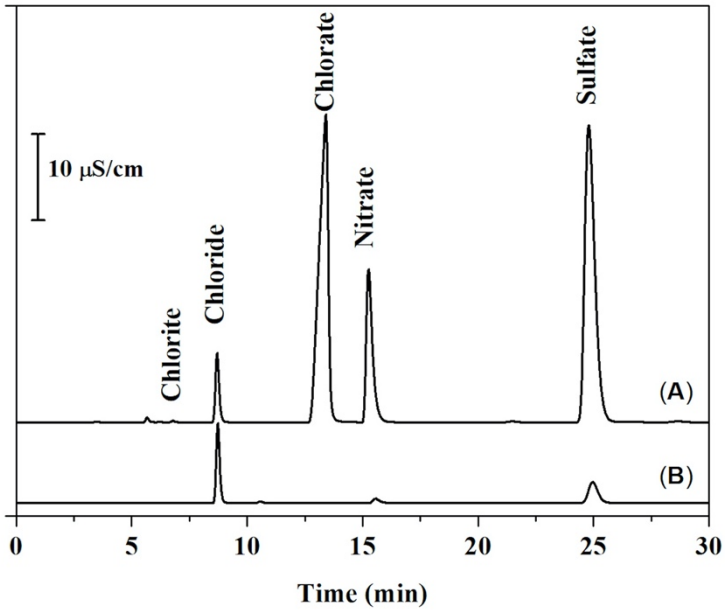

Figure 6. Ion Chromatogram of the wastewater spiked with a mix of 30 pharmaceuticals (A) before and (B) after electrolysis applying 6 mA cm^{-2} during 330 minutes in a stirred BDD/SS cell at 25 ºC, pH 7.66.

4. Conclusions

The mineralization of 30 pharmaceuticals by AO in three different electrolytic media was achieved. A TOC removal >95% was obtained applying 40 mA cm^{-2}, in the presence of 0.05 M

Na_2SO_4 + 0.05 M NaCl, due to the action of hydroxyl radicals and active chlorine species produced on the BDD anode. 25 intermediaries produced during the electrooxidation were identified, obtaining clear differences in the compounds formed when the supporting electrolyte is NaCl or Na_2SO_4. In all electrolytic media, occur the generation of carboxylic acids, NO_3^-, SO_4^{2-} and NH_4^+ ions.

A secondary effluent water sample spiked with 30 pharmaceuticals was treated by AO reaching 88% TOC abatement, applying 6 mA cm^{-2} at natural pH, and without the addition of supporting electrolyte. These results provide an evidence of the suitability AO application for the elimination of organic contaminants of emerging concern, such as pharmaceuticals, because it is not necessary to modify the pH or to add chemicals to the wastewater. Furthermore, the energy consumption for the pharmaceutical's removal was 18.95 kW hm^{-3}, which is equivalent to USD 2.90 m^{-3}. This implies that AO is an economic and effective process for treating wastewater or other water types that contains emergent contaminants such as pharmaceuticals.

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SUPLEMENTARY MATERIAL

Title: Simultaneous degradation of 30 pharmaceuticals by anodic oxidation: main intermediaries and by-products

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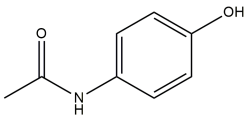
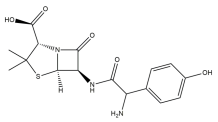
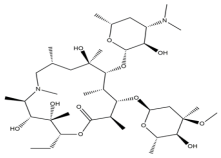
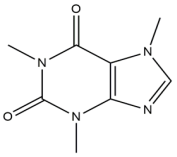
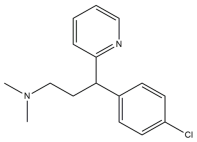
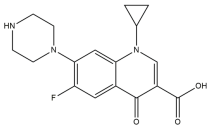
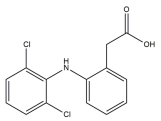
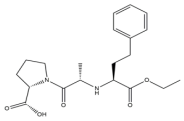
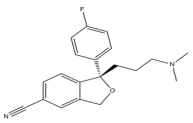
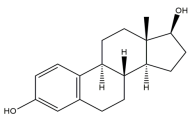
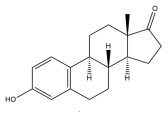
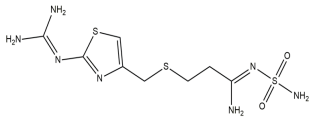
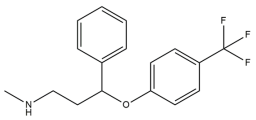
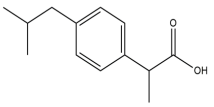
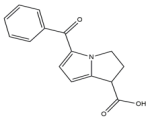
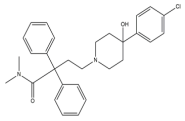
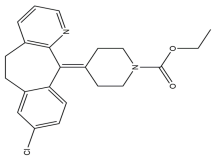
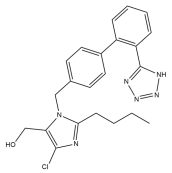
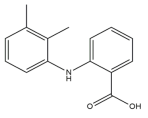
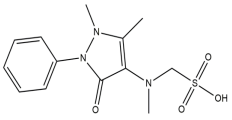
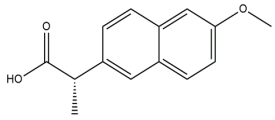
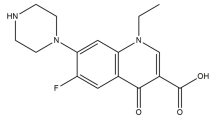
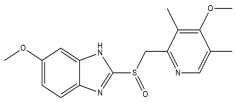
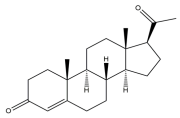
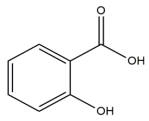
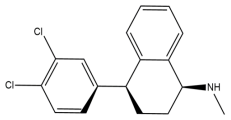
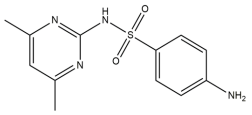
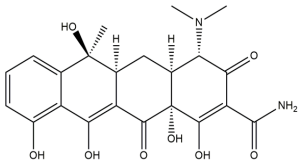
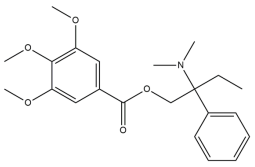
SM1. Pharmaceutical compounds used in this work including molecular formula, chemical structure, exact mass, and concentration.

Compound	*Calculated exact mass*	*Molecular formula*	*Structure*	*Concentration (mg L^{-1})*
Acetaminophen	151.0628	$C_8H_9NO_2$		502.00
Amoxicillin	365.1039	$C_{16}H_{19}N_3O_5S$		15.07
Azithromycin	748.5079	$C_{38}H_{72}N_2O_{12}$		249.50
Caffeine	194.0798	$C_8H_{10}N_4O_2$		500.00
Chlorpheniramine	274.1231	$C_{16}H_{19}ClN_2$		514.00
Ciprofloxacin	331.1327	$C_{17}H_{18}FN_3O_3$		512.00
Diclofenac	295.0161	$C_{14}H_{11}Cl_2NO_2$		14.90
Enalapril	376.1992	$C_{20}H_{28}N_2O_5$		119.20
Escitalopram	324.1632	$C_{20}H_{21}FN_2O$		44.60
β-Estradiol	272.1772	$C_{18}H_{24}O_2$		24.00

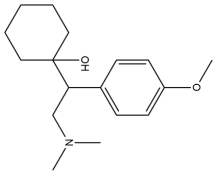
SM1. Continued.

Estrone	270.1614	$C_{18}H_{22}O_2$		18.00
Famotidine	337.0444	$C_8H_{15}N_7O_2S_3$		502.50
Fluoxetine	309.1335	$C_{17}H_{18}F_3NO$		24.80
Ibuprofen	206.1301	$C_{13}H_{18}O_2$		56.00
Ketorolac	255.0889	$C_{15}H_{13}NO_3$		402.00
Loperamide	476.2225	$C_{29}H_{33}ClN_2O_2$		9.95
Loratadine	382.1443	$C_{22}H_{23}ClN_2O_2$		4.88
Losartan	422.1616	$C_{22}H_{23}ClN_6O$		0.08
Mefenamic acid	241.1097	$C_{15}H_{15}NO_2$		19.04
Metamizol	311.0934	$C_{13}H_{17}N_3O_4S$		498.00

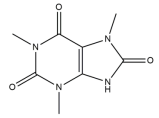
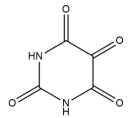
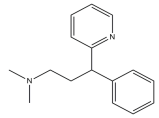
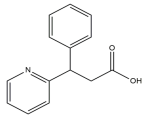
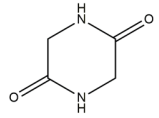
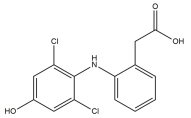
SM1. Continued.

Naproxen	230.0937	$C_{14}H_{14}O_3$		80.00
Norfloxacin	319.1327	$C_{16}H_{18}FN_3O_3$		504.00
Omeprazole	345.1142	$C_{17}H_{19}N_3O_3S$		99.45
Progesterone	314.2240	$C_{21}H_{30}O_2$		0.80
Salicylic acid	138.03115	$C_7H_6O_3$		508.00
Sertraline	305.0733	$C_{17}H_{17}Cl_2N$		3.06
Sulfamethazine	278.0832	$C_{12}H_{14}N_4O_2S$		408.00
Tetracycline	444.1527	$C_{22}H_{24}N_2O_8$		500.00
Trimebutine	387.2040	$C_{22}H_{29}NO_5$		38.00

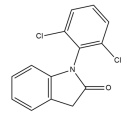
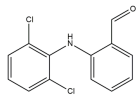
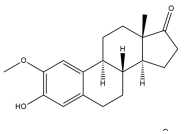
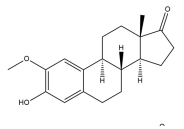
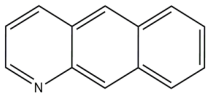
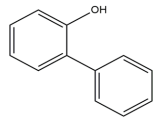
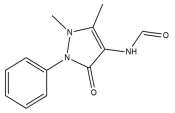
SM1. Continued.

Venlafaxine	277.2036	$C_{17}H_{27}NO_2$		501.20

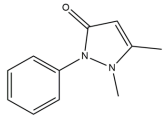
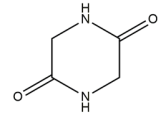
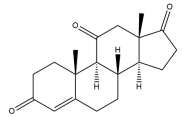
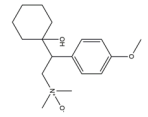
SM2. Identified by-products in 0.05 M Na_2SO_4 background solution

Possible precursor	*Intermediaries*	*Molecular formula*	*RT*	*Ion specie and monoisotopic mass*	*Measured exact mass (m/z)*	*Mass error (ppm)*	*Fragments/ Intensity*	*Identification proof*	*Proposed Structure*	*Number*
Caffeine	1,3,7-trimethyl-9*H*-purine-2,6,8-trione	$C_8H_{10}N_4O_3$	1.56	$[M-H]^-$ 209.0680	209.0679	-0.833	194.0449/38632	Mass accuracy, plausible RT, 1 fragment		1
Caffeine	1,3-diazinane-2,4,5,6-tetrone	$C_4H_2N_2O_4$	1.26	$[M-H]^-$ 140.9941	140.9942	1.414	97.9886/421720	Mass accuracy, plausible RT, 1 fragment		2
Chlorpheniramine	N, *N*-dimethyl-3-phenyl-3-pyridin-2-ylpropan-1-amine	$C_{16}H_{20}N_2$	5.23	$[M+H]^+$ 241.1699	241.1699	0.022	166.0627/59698	Mass accuracy, plausible RT, 1 fragment		3
Chlorpheniramine	3-phenyl-3-pyridin-2-ylpropanoic acid	$C_{14}H_{13}NO_2$	4.00	$[M+H]^+$ 228.1019	228.102	-0.108	210.0913/251450	Mass accuracy, plausible RT, 1 fragment		4
Ciprofloxacin	piperazine-2,5-dione	$C_4H_6N_2O_2$	1.55	$[M-H]^-$ 113.0357	113.0359	2.376	85.0409/161604 83.0253/39863	Mass accuracy, plausible RT, 2 fragments		5
Diclofenac	2-[2-(2,6-dichloro-4-hydroxyanilino) phenyl]acetic acid	$C_{14}H_{11}Cl_2NO_3$	5.37	$[M-H]^-$ 310.0043	310.004	-0.912	115.0556/168918	Mass accuracy, plausible RT, 1 fragment		6

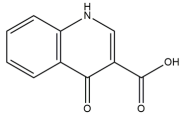
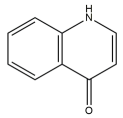
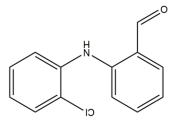
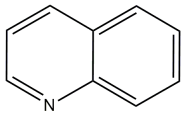
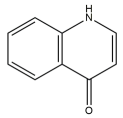
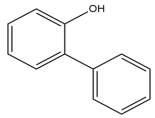
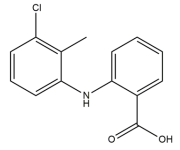
SM2. Continued.

Diclofenac	1-(2,6-dichlorophenyl)-3*H*-indol-2-one	$C_{14}H_9Cl_2NO$	8.86	$[M+H]^+$ 278.0134	278.0135	0.305	95.04917/40142780	Mass accuracy, plausible RT, 1 fragment		**7**
Diclofenac	2-(2,6-dichloroanilino) benzaldehyde	$C_{13}H_9Cl_2NO$	10.03	$[M+H]^+$ 266.0134	266.0134	-0.095	177.0547/5665852	Mass accuracy, plausible RT, 1 fragment		8
Estradiol	(8*R*,9*S*,13*S*,14*S*)-3-hydroxy-2-methoxy-13-methyl-7,8,9,11,12,14,15,16-octahydro-6*H*-cyclopenta[a]phenanthren-17-one	$C_{19}H_{24}O_3$	8.14	$[M+H]^+$ 301.1798	301.1798	0.031	189.0911/300278 137.0598/294631 103.0543/1238705	Mass accuracy, plausible RT, 3 fragments		9
Estrone	(8*R*,9*S*,13*S*,14*S*)-3-hydroxy-2-methoxy-13-methyl-7,8,9,11,12,14,15,16-octahydro-6*H*-cyclopenta[a]phenanthren-17-one	$C_{19}H_{24}O_3$	8.14	$[M+H]^+$ 301.1798	301.1798	0.031	189.0911/300278 137.0598/294631 103.0543/1238705	Mass accuracy, plausible RT, 3 fragments		9
Loratadine	benzo[g]quinoline	$C_{13}H_9N$	7.01	$[M+H]^+$ 180.0808	180.0804	-1.862	179.0731/93097	Mass accuracy, plausible RT, 1 fragment		10
Losartan	2-Phenylphenol	$C_{12}H_{10}O$	5.46	$[M-H]^-$ 169.0659	169.066	0.716	141.0712/953252	Mass accuracy, plausible RT, 1 fragment		11
Metamizol	N-(1,5-dimethyl-3-oxo-2-phenylpyrazol-4-yl) formamide	$C_{12}H_{13}N_3O_2$	4.45	$[M+H]^+$ 232.1081	232.1081	0.118	146.0602/140559	Mass accuracy, plausible RT, 1 fragment		12

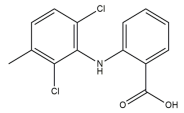
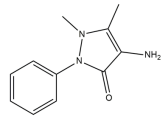
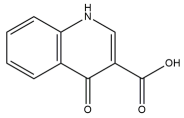
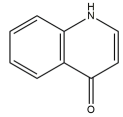
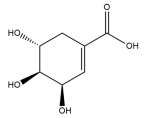
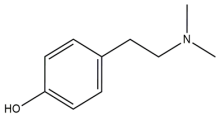
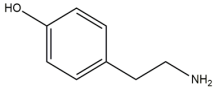
SM2. Continued.

Metamizol	1,5-dimethyl-2-phenylpyrazol-3-one	$C_{11}H_{12}N_2O$	5.31	$[M+H]^+$ 189.1022	189.1023	0.534	149.0710/673174 147.0918/799976 133.0761/439567 106.0652/148623 104.0494/59631 94.0652/2576818 92.0476/73385	Mass accuracy, plausible RT, 7 fragments		13
Norfloxacin	piperazine-2,5-dione	$C_4H_6N_2O_2$	1.55	$[M-H]^-$ 113.0357	113.0359	2.376	85.0409/16160483.0253/39863	Mass accuracy, plausible RT, 2 fragments		5
Progesterone	(8*S*,9*S*,10*R*,13*S*,14*S*)-10,13-dimethyl-1,2,6,7,8,9,12,14,15,16-decahydrocyclopenta[a]phenanthrene-3,11,17-trione	$C_{19}H_{24}O_3$	8.14	$[M+H]^+$ 301.1798	301.1798	0.031	121.0618/559680	Mass accuracy, plausible RT, 1 fragment		14
Venlafaxine	2-(1-hydroxycyclohexyl)-2-(4-methoxyphenyl)-*N*,*N*-dimethylethanamine oxide	$C_{17}H_{27}NO_3$	5.56	$[M+H]^+$ 294.2064	294.2064	0.239	122.0676/163014	Mass accuracy, plausible RT, 1 fragment		15

SM3. Identified by-products in 0.05 M NaCl background solution

Possible precursor	*Intermediaries*	*Molecular formula*	*RT*	*Ion specie and monoisotopic mass*	*Measured exact mass (m/z)*	*Mass error (ppm)*	*Fragments/ Intensity*	*Identification proof*	*Proposed Structure*	*Number*
Ciprofloxacin	4-oxo-1H-quinoline-3-carboxylic acid	$C_{10}H_7NO_3$	4.37	$[M+H]^+$ 190.0499	190.0502	1.952	144.0423/194504 116.0496/36360	Mass accuracy, plausible RT, 2 fragments		16
Ciprofloxacin	1H-quinolin-4-one	C_9H_7NO	3.75	$[M-H]^-$ 144.0455	144.0456	0.709	116.0508/75572	Mass accuracy, plausible RT, 1 fragment		17
Diclofenac	2-(2-chloroanilino) benzaldehyde	$C_{13}H_{10}ClNO$	7.83	$[M+H]^+$ 232.0524	232.0523	-0.378	214.0418/1223128 178.0652/961035	Mass accuracy, plausible RT, 2 fragments		18
Loratadine	quinoline	C9H7N	3.41	$[M+H]^+$ 130.0651	130.0653	1.651	77.0386/1897205	Mass accuracy, plausible RT, 1 fragment		19
Loratadine	1H-quinolin-4-one	C_9H_7NO	3.75	$[M-H]^-$ 144.0455	144.0456	0.709	116.0508/75572	Mass accuracy, plausible RT, 1 fragment		17
Losartan	2-phenylphenol	$C_{12}H_{10}O$	5.37	$[M+H]^+$ 171.0804	171.0813	5.197	153.0685/653001	Mass accuracy, plausible RT, 1 fragment		11
Mefenamic acid	2-(3-chloro-2-methylanilino) benzoic acid (Tolfenamic acid)	$C_{14}H_{12}ClNO_2$	7.54	$[M+H]^+$ 262.0629	262.0631	0.792	246.0491/1046532 245.0558/5006008 244.0522/3076308 229.0289/144770 209.0833/70265	Mass accuracy, plausible RT, 5 fragments		20

SM3. Continued.

Mefenamic acid	2-(2,6-dichloro-3-methylanilino) benzoic acid (Meclofenamic acid)	$C_{14}H_{11}Cl_2NO_2$	9.62	$[M+H]^+$ 296.0239	296.0244	1.385	180.0786/122348	Mass accuracy, plausible RT, 1 fragment		21
Metamizol	4-amino-1,5-dimethyl-2-phenylpyrazol-3-one	$C_{11}H_{13}N_3O$	3.71	$[M+H]^+$ 204.1131	204.1137	2.508	77.0386/2154302	Mass accuracy, plausible RT, 1 fragment		22
Norfloxacin	4-oxo-1H-quinoline-3-carboxylic acid	$C_{10}H_7NO_3$	4.37	$[M+H]^+$ 190.0499	190.0502	1.952	144.0423/194504 116.0496/36360	Mass accuracy, plausible RT, 2 fragments		16
Norfloxacin	1H-quinolin-4-one	C_9H_7NO	3.75	$[M-H]^-$ 144.0455	144.0456	0.709	116.0508/75572	Mass accuracy, plausible RT, 1 fragment		17
Trimebutine	(3R,4S,5R)-3,4,5-trihydroxycyclohexene-1-carboxylic acid	$C_7H_{10}O_5$	1.25	$[M-H]^-$ 173.0455	173.0455	-0.332	73.0294/526412	Mass accuracy, plausible RT, 1 fragment		23
Venlafaxine	4-[2-(dimethylamino)ethyl]phenol	$C_{10}H_{15}NO$	2.9	$[M+H]^+$ 166.1226	166.1228	1.023	77.0386/1789974	Mass accuracy, plausible RT, 1 fragment		24
Venlafaxine	4-(2-aminoethyl) phenol	$C_8H_{11}NO$	2.34	$[M+H]^+$ 138.0913	138.0914	0.724	77.0386/1711873	Mass accuracy, plausible RT, 1 fragment		25

CAPÍTULO III: CONCLUSIONES FINALES

CONCLUSIONES

El trabajo investigativo de esta tesis doctoral tuvo como base el desarrollo de metodologías para el análisis de CECs en matrices ambientales acuosas, así como, estudiar la transformación y/o remoción de los contaminantes inducidas por AO. Considerando los resultados presentados anteriormente, es posible concluir:

I. Se desarrolló un método para la determinación simultánea de 25 productos farmacéuticos con diferentes propiedades químico-físicas, mediante UHPLC-MS/MS. Esto permitió que un mismo análisis, puedan ser determinados a la vez compuestos con ionización positiva y negativa.

II. En un estudio preliminar, la RDSE permite la extracción simultánea de los 25 fármacos a pH 8, utilizando 15 mg de fase Oasis® PriME HLB, aplicando una velocidad de agitación de 1200 rpm y durante un tiempo de extracción de 120 min. La etapa de desorción se realizó con 10 mL de metanol acidificado al 1% durante 30 min, a una velocidad de agitación de 1200 rpm. Sin embargo los FCs no exceden el 40% para la mayor parte de los compuestos extraídos.

III. La prueba t para muestras emparejadas mostró diferencias estadísticamente significativas entre los FCs alcanzados con el procedimiento RDSE y el procedimiento SPE adaptado de la literatura.

IV. Para mejorar los resultados obtenidos por RDSE se propone realizar otros ensayos, aumentando el rango de velocidad de agitación y el volumen de la muestra, así como incorporar estándares internos marcados isotópicamente durante el procedimiento para corregir la respuesta analítica de los compuestos más afectados por la pérdida durante la extracción o por el efecto matriz.

V. El pH influyó en los FCs alcanzados dentro de un mismo procedimientos de extracción, pero las principales diferencias se observaron entre métodos utilizando las mismas condiciones de pH.

VI. La aplicación de la RDSE en conjunto con la HRMS y el uso de la DSFP permitió el *screening* retrospectivo de muestras de aguas superficiales chilenas (río Bio-Bio, río Andalien y laguna Lo

Galindo) y de un efluente secundario proveniente de la PTAR Trebal.

VII. Se identificaron tentativamente 62 CECs sospechosos con nivel de confianza 2a, 2b y 3, mientras que 2 fueron confirmados por comparación con estándares de referencia. Dentro de ellos se incluyen pesticidas, plastificantes como los ftalatos, productos de cuidado personal, productos farmacéuticos, edulcorantes, retardantes de llama, entre otros. Al menos 26 de los 64 se detectaron una vez y 9 se detectaron en todas las muestras, lo que demuestra la omnipresencia de los CECs en el medio ambiente.

VIII. La DSFP demostró ser una herramienta potente y muy útil para el *screening* de sospechosos que contribuyó a reducir los tiempos de trabajo. Sin embargo, cabe destacar la importancia del escrutinio manual de los espectros de masas y los cromatogramas, así como la comparación con las bibliotecas espectrales y el software de fragmentación in silico, para resolver los problemas de identificación de los isómeros estructurales y evitar informar de un nivel de confianza inadecuado.

IX. Se logró la mineralización de 30 productos farmacéuticos mediante AO en tres medios electrolíticos diferentes. Se obtuvo una eliminación de TOC >95% aplicando 40 mA cm^{-2}, en presencia de 0,05 M de Na_2SO_4 + 0,05 M de NaCl, debido a la acción de los radicales hidroxilo y de las especies de cloro activo producidas en el ánodo de BDD. Se identificaron 25 intermediarios producidos durante la electrooxidación, obteniéndose claras diferencias en los compuestos formados cuando el electrolito soporte es NaCl o Na_2SO_4. En todos los medios electrolíticos, se produce la generación de ácidos carboxílicos, iones NO_3^-, SO_4^{2-} y NH_4^+.

X. Se alcanzó un 88% de reducción de TOC en el tratamiento de un agua residual proveniente de un efluente secundario, cargada con 30 productos farmacéuticos, empleando AO a una densidad de corriente de 6 mA cm^{-2}, pH natural y sin adición de electrolito soporte. Estos resultados demuestran la idoneidad de la aplicación del AO para la eliminación de CECs, como los

productos farmacéuticos, ya que no es necesario modificar el pH ni añadir productos químicos al agua residual para obtener buenos resultados. Además, el consumo de energía para la eliminación de los fármacos fue de 18,95 kW hm^{-3}, lo que equivale a 2,90 dólares. Esto implica que el AO es un proceso económico y eficaz para tratar las aguas residuales u otros tipos de agua que contengan CECs como los productos farmacéuticos.