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1 **HIGH-THROUGHPUT MULTI-RESIDUE QUANTIFICATION OF CONTAMINANTS**  
2 **OF EMERGING CONCERN IN WASTEWATERS ENABLED USING DIRECT**  
3 **INJECTION LIQUID CHROMATOGRAPHY-TANDEM MASS SPECTROMETRY**

4  
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26 **Abstract**

27 A rapid quantitative method for 135 contaminants of emerging concern (CECs) in  
28 untreated wastewater enabled with direct injection liquid chromatography-tandem  
29 mass spectrometry is presented. All compounds were analysed within 5 min on a short  
30 biphenyl cartridge using only 10  $\mu\text{L}$  of filtered sample per injection. Up to 76  
31 compounds were monitored simultaneously during the gradient (including mostly two  
32 transitions per compound and stable isotope-labelled analogues) while yielding >10  
33 data points per peak. Evaluation of seven solid phase extraction sorbents showed no  
34 advantage for wastewater matrix removal. Excellent linearity, range, accuracy and  
35 precision was achieved for most compounds. Matrix effects were <11% and detection  
36 limits were <30  $\text{ng L}^{-1}$  on average. Application to untreated wastewater samples from  
37 three wastewater treatment works in the UK, USA and Mexico, enabled quantification  
38 of 56 compounds. Banned and EU 'watch-list' substances are critically discussed,  
39 including pesticides, macrolide antibiotics, diclofenac, illicit drugs as well as multiple  
40 pharmaceuticals and biocides. This high-throughput method sets a new standard for  
41 the speedy and confident determination of over a hundred CECs in wastewater at the  
42 part-per-trillion level, as demonstrated by performing over 260 injections per day.

43

44 **Keywords:** wastewater, direct injection LC-MS/MS, pharmaceuticals, illicit drugs,  
45 pesticides

46

## 47 **Introduction**

48           Contaminants of emerging concern (CECs), such as pharmaceuticals, illicit  
49 drugs, pesticides, herbicides, personal care products and each of their  
50 metabolites/transformation products are being ubiquitously found in a variety of  
51 environmental compartments at parts per billion/trillion concentrations given their  
52 widespread usage in healthcare, recreational/illicit drug use, and agriculture.  
53 Monitoring population-level consumption behaviour and/or exposure to such  
54 substances through wastewater-based epidemiology (WBE) has become a viable  
55 means to gather near real-time information on temporal and spatial trends across  
56 towns and major cities globally for a number of years [1, 2]. Regarding environmental  
57 exposure to CECs, wastewater has been identified as a primary source of  
58 contamination in receiving waters and soils [3, 4]. This has led to a large body of  
59 research focussing on their occurrence, fate and effects in biota and ecology [5-7]  
60 including establishment of an EU ‘watch list’ for CECs [8].

61           Most analytical techniques for targeted CEC determinations have used liquid  
62 chromatography-tandem mass spectrometry (LC-MS/MS) for pharmaceuticals [10,  
63 11], illicit drugs [12-14] and pesticides [15-17] in wastewaters. LC-MS/MS using triple  
64 quadrupole mass analysers has dominated targeted CEC analysis due to their  
65 sensitivity, quantitative precision and selectivity via multiple reaction monitoring  
66 (MRM) [18]. However, for large numbers of compounds, triple quadrupoles can often  
67 be limited by a maximum number of simultaneous MRM transitions which, for  
68 hundreds of CECs, can be further constrained by the requirement for multiple  
69 transitions per compound for confirmation. This has been generally overcome by  
70 scheduling MRMs within defined retention time windows to maximise coverage as well  
71 as peak definition and sensitivity, but chromatographic efficiency and resolution also

72 remains important. Therefore, fast-scanning mass analysers are desirable to increase  
73 throughput. Analysis of large numbers of compounds using liquid chromatography-  
74 high resolution mass spectrometry (LC-HR-MS) has also proved effective including  
75 the potential flexibility for discovery of new compounds, metabolites and  
76 transformation products along with simultaneously performed targeted analysis [19-  
77 21]. For a number of reasons, HR-MS detectors are still not achieving the sensitivity  
78 of quadrupole-type instruments by comparison [22, 23]. Faster HR-MS scan speeds  
79 may be required using sub-maximal resolution settings to adequately define narrow  
80 chromatographic bands for quantitative applications at ng L<sup>-1</sup> sensitivity [21, 26]. Aside  
81 from LC-MS analysis speed, sample pre-treatment involving solid-phase extraction  
82 (SPE) is widely applied in environmental analysis of CECs to achieve sufficient  
83 sensitivity at low to mid ng L<sup>-1</sup> levels [27-29]. However, SPE method development for  
84 so many compounds is often very complex to optimise and time-consuming, costly  
85 and impractical for application in high-volume monitoring campaigns. The large array  
86 of chemically diverse compounds and their metabolites makes the availability and  
87 selection of suitable sorbents a challenge [30]. Thus, a need for making compromises  
88 arises and the SPE process can limit the analytical coverage for complex mixtures.

89 In comparison to those methods employing SPE, few 'direct injection' LC-  
90 MS/MS-based methods exist for CECs. Of those that have been developed, most have  
91 been developed for small numbers of compounds [32-34]. Among these methods for  
92 >20 compounds, for example, large sample injection volumes of 80-400 µL [35-37]  
93 have been used along with relatively long gradients [38, 39], or separate runs for each  
94 electrospray ionisation (ESI) source polarity to confidently achieve the robust ng L<sup>-1</sup>  
95 sensitivity required [40, 41]. The fastest reported analysis time for larger numbers of  
96 CECs in wastewater was reported in 2017 by Campos Mañas et al. as 31 min [42],

97 using two separate methods and 10  $\mu\text{L}$  injection volumes onto an LC-quadrupole-  
98 linear ion trap MS instrument enabling  $\sim 46$  injections per day. In many cases CECs  
99 are relatively polar molecules and most studies have used  $\text{C}_{18}$  stationary phases for  
100 LC separations. More recently, biphenyl stationary phases have emerged as a  
101 potential alternative [43]. Couchman et al. recently configured a short 5 x 3 mm  
102 biphenyl guard column directly to the ESI source to perform rapid separations of 20  
103 drugs and metabolites in blood in 36 seconds using a high mobile phase flow rate of  
104 2  $\text{mL min}^{-1}$  [44]. The method was then applied to the quantification of clozapine and  
105 norclozapine in 76 plasma samples within 3 days (including data processing and  
106 interpretation) and lower limits of quantification (LLOQs) lay at 10  $\text{ng mL}^{-1}$  in matrix for  
107 both analytes. This approach potentially offers several advantages for high-throughput  
108 monitoring of mid-polarity CECs in wastewaters. Therefore, even though direct  
109 injection-type methods remain rare, the current challenge lies in the speed of LC-  
110 MS/MS analysis to improve throughput for large monitoring campaigns at reduced cost  
111 while maintaining analytical quality.

112 The aim of this work was to develop a rapid, direct injection LC-MS/MS  
113 methodology for simultaneous quantification of over one hundred selected CECs,  
114 including pharmaceuticals, pesticides, illicit drugs and their metabolites at  $\text{ng L}^{-1}$   
115 concentrations in influent wastewater. Challenges relating to the consolidation of  
116 methods using ESI polarity switching, run time, data quality, injection volume and  
117 sensitivity were all addressed as a priority. Furthermore, the use of SPE for matrix  
118 removal was assessed to determine any sensitivity enhancement. The performance  
119 of the method was evaluated with respect to precision, accuracy, matrix effects,  
120 linearity, range, limits of detection and quantitation. Lastly, wastewater samples from  
121 selected wastewater treatment plants (WWTPs) from the UK, USA and Mexico were

122 analysed using the developed high throughput method. The novelty of this work lies in  
123 the improved simplicity and convenience for sample preparation and the successful  
124 application of ultra-fast LC-MS/MS transition scanning to enable the determination of  
125 135 compounds for application in WBE, with up to 261 injections performed in any 24-  
126 hour time period.

127

## 128 **2. Materials and Methods**

### 129 *2.1 Reagents, chemicals and consumables*

130 LC-MS grade methanol (Dorset, UK), LC-MS grade acetonitrile (Rehovot,  
131 Israel), hydrochloric acid (37 %, v/v) (Steinheim, Germany), formic acid (Steinheim,  
132 Germany) were acquired from Sigma-Aldrich. Ultrapure water (resistance of 18.3 M $\Omega$   
133 cm) was generated from a Millipore Milli-Q water purification system (Millipore,  
134 Bedford, MA, USA). Calcium chloride dihydrate (Acros Organics, Loughborough, UK),  
135 magnesium sulfate (Sigma-Aldrich, Steinheim, Germany), potassium chloride (Alfa  
136 Aesar, Heysham, UK) and sodium hydrogen carbonate (Fisher Scientific,  
137 Loughborough, UK) were used to prepare artificial freshwater at concentrations of at  
138 80, 12, 3 and 17 mg L<sup>-1</sup>, respectively. A list of all 135 reference standard materials and  
139 27 stable isotope labelled internal standards (SIL-IS) is given in the supplementary  
140 information. Working standards (either using 1.0 mg mL<sup>-1</sup> or 0.1 mg mL<sup>-1</sup> reference  
141 standards and as the free base form for HCl salts) were prepared in methanol or  
142 acetonitrile and stored in silanised amber vials (20 mL) at -20 °C.

### 143 *2.2 Instrumentation*

144 Liquid chromatography was performed using a Shimadzu Nexera<sup>TM</sup> X2 ultra-high  
145 pressure LC (Shimadzu Corporation, Kyoto, Japan) on a short 5.0 x 3.0 mm, 2.7  $\mu$ m

146 particle size Raptor™ biphenyl cartridge (Thames Restek, Saunderton, UK) housed  
147 within an EXP® Direct Connect Holder. Mass spectrometry was performed using an  
148 LCMS-8060 (Shimadzu Corporation, Kyoto, Japan). As the electrospray ionisation  
149 (ESI) source was not electrically grounded, the column was configured via a short  
150 piece of narrow bore polyether ether ketone (PEEK) tubing. A sample injection volume  
151 of 10 µL was used at an optimised flow rate of 0.5 mL min<sup>-1</sup>. Mobile phases were 0.1  
152 % (v/v) formic acid in ultrapure water (A) and 0.1 % (v/v) formic acid in  
153 acetonitrile:methanol (1:1, v/v) (B). Optimised gradient elution conditions were as  
154 follows: 10 % mobile phase B for 0.2 min; a linear ramp from 10-60 % from 0.2-3.0  
155 min; a step gradient from 60-100 % at 3.0 min; and held at 100 % B for a further 1.0  
156 min before re-equilibration time for 1.0 min, resulting in total run time of 5.0 min.  
157 Between runs, a 30 s period was also necessary for needle washing (acetonitrile) and  
158 autosampler cycling for the next sample.

159 For LC-MS/MS, Pureshield argon was used as a collision-induced dissociation  
160 (CID) gas (BOC Gases, Guildford, UK). Nitrogen and dry air were generated using  
161 Genius 1051 gas generator (Peak Scientific, Inchinnan, UK). Multiple reaction  
162 monitoring (MRM) was performed with positive-negative ionisation polarity switching.  
163 The quadrupoles Q1 and Q3 were set to unit resolution. Chromatographic data were  
164 acquired by LabSolutions™ (version 5.93, Shimadzu) and processed using  
165 LabSolutions Insight (version 3.2, Shimadzu, Kyoto, Japan). Automated MRM  
166 optimisation of each precursor was performed using LabSolutions software (version  
167 5.93, Shimadzu). All MRM parameters, including product ion m/z, collision energy  
168 (CE), dwell time, pause time, Q1 and Q3 pre-bias voltages were determined and  
169 optimised via 10 µL flow injection LC-MS at ambient temperature without an analytical  
170 column. Sample was delivered under isocratic conditions at 70 % mobile phase B and



171 a flow rate of 0.5 mL min<sup>-1</sup>. MRM parameters were optimised using individual analyte  
172 standards in methanol at 1.0 µg mL<sup>-1</sup>. Two MRM transitions were used where possible  
173 for confirmation of analytes, and the most intense transition used for quantification.  
174 For SIL-IS, only one transition was used for quantification purposes. The MS  
175 conditions and optimised MRM transitions are summarised in Tables S1 and S2 in the  
176 Supplementary Information.

### 177 2.3 Method validation

178 The method was validated for the analysis of wastewater samples with direct injection  
179 LC-MS/MS according to guidelines published by the International Council for  
180 Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH)  
181 [45]. Raw wastewater from London was used for analytical performance testing using  
182 a pooled mixture of wastewater taken over seven days. Linearity, range, lower limit of  
183 detection (LLOD), LLOQ, precision and matrix effects (ME) were assessed as per the  
184 guidance. Background subtraction was performed for any analyte already present in  
185 the sample as required. Briefly, acceptable linearity and range were defined based on  
186 a minimum of N≥5 calibrants yielding coefficients of determination (R<sup>2</sup>) ≥0.99 from a  
187 set range of matrix-matched standards tested covering N=11 concentration levels from  
188 5-5000 ng L<sup>-1</sup>. The LLOD was calculated as three times the standard deviation of the  
189 response at the lowest calibrant in the defined range. LLOQ was determined as ten  
190 times this standard deviation. Precision was performed at 100 and 1000 ng L<sup>-1</sup> (n=6 at  
191 each concentration) in matrix and expressed as percentage relative standard deviation  
192 (%RSD). Accuracy of the method was performed at three concentrations levels, i.e.,  
193 250 and 750 ng L<sup>-1</sup> (each in duplicate) and 1000 ng L<sup>-1</sup> (for n=6). Fortified wastewater  
194 was prepared as a quality control (QC) and analyte concentration was determined  
195 from the matrix-matched calibration curve and reported as the percentage of

196 coefficient of variation (%CV) difference between the target and QC concentrations,  
197 with  $\%CV \leq \pm 25\%$  considered acceptable. ME were determined at 100 and 1000 ng  
198 L<sup>-1</sup> ( $n=6$  at each level) and expressed as a percentage of the peak areas obtained for  
199 background subtracted matrix-matched standards relative to those obtained for a  
200 standard of all analytes at the same concentrations prepared with ultrapure water.

#### 201 2.4 *Sample collection and preparation*

202 A total of 17 samples were taken in three different WWTPs in Monterrey  
203 (Mexico), London (UK), and a third city in the USA to demonstrate the feasibility of the  
204 approach for large scale international monitoring campaigns in the future. Sites were  
205 not selected based on priority, but based on access to samples by the collaborating  
206 academic institutions. However, no CEC occurrence data currently exists in the  
207 literature for the Monterrey site. Different standard procedures were employed for  
208 sample collection at each location. In the UK and the USA, 24-hour 30-min time-  
209 proportional composite influent wastewater samples were collected from major  
210 metropolitan areas. In London, samples were taken at a major WWTP over a weekend  
211 from 5-7 April 2019 (population served by WWTP: 3,400,000 or ~40 % of Greater  
212 London). Each day, 6 x 500 mL sub-samples of the full composite wastewater sample  
213 were transferred to Nalgene bottles, which were pre-rinsed with methanol and  
214 ultrapure water to avoid potential contamination and shipped at 4°C to the laboratory.  
215 A single 500 mL grab sample of river water (River Thames, UK) was taken in a  
216 Nalgene bottle in the same way on 01/07/2019 from Gabriel's Pier in Central London  
217 (51°30'30.3"N; 0°06'36.7"W). UK river and wastewater samples were then filtered  
218 using Whatman® 47 mm diameter, 0.67 mm thickness, 2.7 µm pore size GF/D glass  
219 microfibre filters (Fisher Scientific Ltd., Loughborough, UK) under vacuum and stored  
220 at -20 °C until analysis. These filters were used to minimise analyte losses via sorption.

221 Samples from the USA were collected from the 9-15 September 2019 at an  
222 anonymised WWTP in the south-west of the country (population served: 60,888, ~33  
223 % of the immediate surrounding city area population of 180,000). Upon collection,  
224 samples were immediately put on ice and transported to the partnering laboratory in  
225 the USA within 24 h. Following this, frozen 5-10 mL aliquots were sent in amber glass  
226 vials to London over 24-48 h and stored in the freezer (-20°C) until analysis within one  
227 week. Finally, grab samples of influent wastewater were taken from Mexico for a full  
228 week (19-25 February 2019) from Dulces Nombres WWTP (Monterrey) using Nalgene  
229 bottles and were acidified to pH 2 using HCl and again 10 mL aliquots were shipped  
230 frozen in glass containers to the London laboratory within 24-48 h where they were  
231 kept frozen until analysis. This WWTP serves a population of 1,708,190 (~44% of the  
232 population of the surrounding metropolitan area including the municipalities of San  
233 Pedro, Guadalupe, Dulces Nombres, Santa Catarina, Apodaca and part of Monterrey  
234 city itself). Given the smaller volumes available for USA and Mexico samples,  
235 particulates were removed using single-use 0.2 µm Teflon membrane filters  
236 configured to BD Plastipak™ syringes. All samples shipped from overseas were still  
237 ice cold upon receipt, which minimised the possibility of analyte loss from degradation  
238 [46]. As Monterrey samples were also acidified, this has previously been shown to  
239 further improve the stability of pharmaceuticals and illicit drugs in wastewaters [47].  
240 However, to simulate the 48-hour transit period, relative analyte stability was also  
241 confirmed. For this, six spiked aliquots of wastewater were prepared at 500 ng L<sup>-1</sup>  
242 (including SIL-IS), not acidified and frozen. Three aliquots were removed and left to  
243 thaw on the bench over 48 hours with no added cold insulation or ice storage, and  
244 then analysed by LC-MS/MS. The relative % instability was calculated using a ratio of

245 the mean peak areas measured in the thawed and frozen wastewater samples,  
246 respectively.

### 247 *2.5 Quantification procedures for CECs in influent wastewater*

248 To maintain dilution factors and to prepare matrix-matched calibrants and for  
249 fortification with SIL-IS, a fixed volume of 100  $\mu\text{L}$  of standard/SIL-IS standard solutions  
250 in methanol was added to 900  $\mu\text{L}$  of filtered wastewater. For quantification of CECs,  
251 matrix-matched, background-subtracted calibrations were performed for each WWTP  
252 separately via fortification with all analytes over a range of 0-5,000  $\text{ng L}^{-1}$  ( $N=13$ ) along  
253 with all 27 SIL-IS at a fixed concentration of 500  $\text{ng L}^{-1}$  into a pooled mixture of all  
254 samples. For analytes where corresponding SIL-IS were available, quantification was  
255 performed using sample peak area ratios relative to those within the background  
256 subtracted, matrix-matched calibration curve. For quantification of compounds where  
257 no SIL-IS were available, standard addition calibration was performed using their peak  
258 areas directly. All statistical analysis was performed in Microsoft® Office Excel (WA,  
259 USA).

## 260 **3. Results and Discussion**

### 261 *3.1 Direct LC-MS/MS method development*

262 For development of a direct LC-MS/MS method for routine wastewater monitoring,  
263 several critical issues needed to be considered and resolved first. A relatively rapid  
264 separation time was preferred to enable high-throughput and to assess any gains in  
265 sensitivity. Secondly, careful scheduling of MRM transitions and MS loop times were  
266 necessary to ensure sufficient data acquisition frequency for reliable quantification,  
267 ideally as a single run and to include ESI polarity switching. Finally, circumvention of

268 extensive matrix removal procedures or use of large injection volumes to achieve ng  
269 L<sup>-1</sup> sensitivity for real samples were investigated.

270 The rapid LC-MS/MS approach by Couchman et al. [44] was adapted and  
271 further optimised. Initial mobile phase conditions of 10 % B enabled better resolution  
272 of more compounds and with better and linear distribution across the runtime. The  
273 ratio between mobile phase (via flow rate) and injection volumes was investigated.  
274 Gradient events were kept proportional over incremental runtime lengths using 0.1-2  
275 mL min<sup>-1</sup> flow rates (using a constant 10 µL injection volume). Peak intensities of 27  
276 SIL-IS in influent wastewater from London reached a maximum at 0.5 mL min<sup>-1</sup> (Figure  
277 S1). For some compounds, a two- to three-fold intensity improvement was achieved  
278 (e.g., benzoylecgonine, risperidone and tramadol). At lower flow rates, matrix  
279 suppression was most likely the cause of lower intensity (despite a smaller sample  
280 dilution factor) rather than excessive band broadening. On the other hand, reduced  
281 intensity at higher flow rates were most likely due to excessive dilution of sample.  
282 Chromatographic efficiency was also four-fold better at 0.5 mL min<sup>-1</sup> in comparison to  
283 the original 2.0 mL min<sup>-1</sup> flow used by Couchman et al. (i.e., plate height (HETP) ≈7  
284 µm and number (*n*) ≈135,000 plates/m (Figure S2) [44]. 'Dilute-and-shoot' methods  
285 have become popular in recent years, but an offline dilution step was successfully  
286 removed as a result of this approach.

287 The LCMS-8060 instrument has a maximum scan speed of 30,000 u/sec and  
288 a polarity switching speed of 5 ms with a capability to acquire 555 MRMs per second.  
289 According to the manufacturer, the ion signal response for each MRM is not influenced  
290 by the number of other MRM transitions in the same time window. This enabled  
291 monitoring of 292 MRM transitions in one run using rapid polarity switching. With a  
292 typical peak width of 10-20 s and with dwell times between 1-20 ms, more than 10

293 data points per peak could be generated (e.g., see Figure 1 for oxycodone and  
294 picoxystrobin). Overall, this level of definition was maintained for up to 76 compounds  
295 monitored simultaneously with mostly two MRM transitions per compound in addition  
296 to any SIL-IS SRM transitions. With an injection-to-injection time of 5.5 min, up to 261  
297 injections could be performed in a 24-hour period which, to our knowledge, represents  
298 the highest throughput in this field for monitoring so many CECs in wastewater in a  
299 single run with polarity switching enabled.

300         Using a 500 ng L<sup>-1</sup> SIL-IS spiked wastewater sample and injection volumes of  
301 0.5-20 µL, it was found that signal intensity deviated from linearity above 10 µL (Figure  
302 S3(a)) and for several compounds peak shape deteriorated. Secondly, and as perhaps  
303 expected, the variance in replicate measurements decreased as injection volume  
304 increased and %RSDs lay below 5 % on average for 10 and 15 µL injection volumes  
305 (Figure S3b). The optimised separation of all compounds and SIL-IS spiked into a  
306 London wastewater sample is shown in Figure 2. The sensitivity of the method was  
307 considered suitable for direct analysis at this point, but obviously could be improved  
308 using analyte-selective SPE for enrichment, but would add considerable time.  
309 Alternatively, SPE was considered here for active matrix removal as a more practically  
310 convenient way to improve sensitivity and increase throughput (i.e., by minimising any  
311 extra time, as analytes were collected in the SPE eluate after loading). Single or  
312 combinations of sorbents with little/no analyte recovery could prove beneficial to  
313 minimise ME, as employed recently for trace explosives determination in wastewater  
314 [31]. This was evaluated using two matrices, filtered artificial freshwater and raw  
315 wastewater (each spiked at 500 ng L<sup>-1</sup> with a selection of 105 analytes that were in  
316 stock at the time). Both types of sample were analysed directly by the optimised LC-  
317 MS/MS method and compared to extracts of corresponding samples that were subject

318 to SPE with no prior pH adjustment. The resulting peak areas were expressed as a  
319 percentage and shown in Table S3. In general, peak areas were much lower for most  
320 compounds in samples subjected to SPE and some were not detected at all. It was  
321 concluded that samples should be analysed directly following filtration only.

### 322 3.2 *Direct LC-MS/MS method performance for CECs in influent wastewater*

323 A summary of method performance for all 135 CECs determined in London influent  
324 wastewater is shown in Table 1 (full data for each compound in Table S4). Linearity  
325 was excellent for most compounds with coefficients of determination of  $R^2 \geq 0.99$  for  
326 127 (94%) compounds. Limited sensitivity was the general cause for poorer  
327 performance for the eight remaining compounds and especially for cymoxanil,  
328 norethisterone, prodiamine and indomethacin where  $R^2$  was  $\geq 0.99$ , but for  $n < 5$   
329 calibrants at the higher concentration range. Overall, the imprecision in peak area  
330 (expressed as mean ( $\pm$ standard deviation)) was excellent at 11 ( $\pm 10$ ) % and 8 ( $\pm 6$ ) %  
331 on average at 100 and 1000 ng L<sup>-1</sup>, respectively. Over 82% of compounds displayed  
332 %CV  $\leq 15\%$  at both concentrations. The highest variance was noted for diflubenzuron  
333 and prodiamine at both concentration levels (52 and 32 % RSD, respectively).  
334 Precision over a sequence of  $n=59$  spiked wastewater samples was also assessed  
335 using SIL-IS internal standards at 500 ng L<sup>-1</sup> in wastewater (see Figure 3 for a  
336 selection). In general, there were no major drifts or deviations in either retention time  
337 or peak area. It is highly likely that the low injection volume contributed to high stability  
338 in chromatographic performance and mass spectrometry response though some  
339 evidence of matrix deposition within the ion source at the end of long batch sequences  
340 was observed (Figure S4). No reduction in LC-MS/MS performance was evident  
341 throughout this study. Lastly, mean ( $\pm$ standard deviation) accuracy at 250, 750 and

342 1000 ng L<sup>-1</sup> lay at -13 (±17) %, -8 (±9) % and -6 (±10) % respectively, which was also  
343 considered acceptable.

344 Sensitivity was excellent for such a simple analytical method. LLODs varied  
345 from 0.05 (for memantine) to 533 ng L<sup>-1</sup> (for carfentrazone-ethyl). The median LLOD  
346 and LLOQ were determined at 9 and 31 ng L<sup>-1</sup>, respectively (average LLOD =29 ng L<sup>-1</sup>  
347 <sup>1</sup>). In comparison to other direct LC-MS/MS methods for influent wastewater, this  
348 method displayed largely similar or better sensitivity in some cases though there were  
349 relatively few common compounds for a full comparison (and especially when injected  
350 analyte mass on column is considered). However, for at least two previously published  
351 methods [40, 41], this method used five to ten-fold smaller injection volumes which  
352 could reduce the amount of matrix contamination of the ESI source over longer batch  
353 analyses. The remaining method by Campos-Mañas et al. also used 10 µL injection  
354 volumes [42], but with two separate longer gradient runs (total analysis time 31 min).  
355 On average, MEs for all 135 compounds spiked at 100 and 1000 ng L<sup>-1</sup> in wastewater  
356 were -3 (±40) % and 0 (±26) %. However, by taking the absolute value of %  
357 suppression (-) or enhancement (+) data, the calculated overall median was 11 % ME  
358 for all compounds, again showing excellent performance. It was noted that the highest  
359 MEs were observed for antipyrine (-84%, indicating enhancement) and spiramycin  
360 (+337%, indicating suppression) at 100 ng L<sup>-1</sup> spiking concentration and for clodinafop-  
361 propargyl (-60 %) and spiramycin (+188%) at 1000 ng L<sup>-1</sup>. The relative absolute mean  
362 instability of analytes in spiked wastewater samples measured after thawing frozen  
363 spiked samples over 48 hours was 7 (±12) % (n=3, Table S5) and not considered  
364 significant for most analytes. However, instability was particularly high for azelnidipine,  
365 ketoconazole and fenoxaprop-ethyl with +85, +73 and +58 % loss, respectively, which  
366 indicated either that the change in matrix led to a suppression in signal, or that these



367 compounds transformed rapidly over this time, For compounds with increased signal  
368 in thawing samples, transformation of other related substances present in the sample  
369 could have led to this result (e.g., cleavage of conjugated metabolites) or the variance  
370 across replicate samples was higher. As quantification for all sites was performed  
371 using matrix-matched standards prepared at the same time, much of the suppression  
372 component of this apparent difference was likely to have been accounted for.  
373 However, reported concentrations of these compounds in wastewater samples should  
374 be treated with caution, as it was impossible to accurately account for stability in every  
375 sample received.

### 376 3.3. *Analysis of wastewater samples from the UK, USA and Mexico*

377 A total of 58 individual compounds were detected across all samples and, of these, 56  
378 were quantifiable (Table 2). No carryover was observed between matrix-matched  
379 calibrants, standards, blanks and/or samples. The approximate percentage of the  
380 national population covered by these works in each country was UK = 5 %, Mexico =  
381 2 % and USA <1 %. Therefore, extrapolation to perform international comparisons on  
382 this level was not appropriate. Our primary focus was therefore placed on a catchment  
383 level comparison in this preliminary study using the new direct analysis method, which  
384 conveniently enabled shipment of several small samples internationally to be analysed  
385 in one laboratory under the same conditions.

386

#### 387 3.3.1 London, UK

388 For London wastewater samples, 40-42 compounds were detected each day  
389 and quantified concentrations agreed in the main with previous screening work in 2014  
390 using SPE and LC coupled to high resolution accurate mass spectrometry (LC-HR-

391 MS) [3, 21]. However, this direct LC-MS/MS method included several new compounds,  
392 most notably biocides, of which only terbutryn was found in London wastewater  
393 samples. Recently, fenuron was determined at high frequency in biota and river water  
394 in Suffolk, UK by our group, even though it has been removed from use in the UK [48].  
395 Fenuron was not detected in London wastewater on this occasion. However, following  
396 a preliminary analysis of a Thames River water grab sample taken on the 1<sup>st</sup> July 2019,  
397 fenuron occurrence was again confirmed and following quantification using standard  
398 addition calibration ( $n=12$ ,  $R^2=0.994$ ), it was quantified at  $169 (\pm 5) \text{ ng L}^{-1}$  (Figure 4b).  
399 Therefore, given the LLOQ for this compound in influent ( $50 \text{ ng L}^{-1}$ ), treated  
400 wastewater discharged by the London WWTP may not represent a continuous primary  
401 source of fenuron to the receiving aquatic environment, but more spatial and temporal  
402 monitoring is required to locate its source(s). Aside from pesticides, relatively little  
403 recent occurrence data exist for EU 'watch-list' compounds present in influent  
404 wastewater from Central London including diclofenac, clarithromycin and azithromycin  
405 (Figure 4a) which were all determined at mean concentrations of  $482 (\pm 34)$ ,  $592 (\pm 72)$   
406 and  $355 (\pm 31) \text{ ng L}^{-1}$ , respectively, across all three days. This represented  
407 approximately 1.5-fold the average concentrations determined for each compound in  
408 influent at five WWTPs upstream from London which also discharge into the Thames  
409 River and as reported recently by Nakada et al. [49]. In the Thames River grab sample,  
410  $117 (\pm 18) \text{ ng L}^{-1}$  and  $31 (\pm 10) \text{ ng L}^{-1}$  were determined for diclofenac and clarithromycin,  
411 respectively (no azithromycin was detected).

412 In addition to pharmaceutical compounds, our group has also contributed illicit  
413 drug monitoring data for London wastewater from 2011-2019 as part of several  
414 international WBE studies. Validated methods at each laboratory are normally subject  
415 to annual international laboratory scrutiny via blind testing exercises, including the

416 method developed herein for the 2019 campaign, which passed with a threshold Z-  
417 Score of  $<2$  [50]. In previous data, BZE loads in wastewater were seen to rise by  
418 approximately two-fold between 2011-2015 to  $\sim 1100$  mg/1000 people/day at  
419 weekends. Both cocaine and BZE concentrations were measurable in wastewater  
420 here for 2019 samples (Figure 5a), but were slightly lower than those in 2016  
421 (maximum weekend concentrations for cocaine and BZE were 1434 and 3533 ng L<sup>-1</sup>,  
422 respectively, in 2016). Taking into account the population served by the WWTP, the  
423 daily flow and exfiltration [51], weekend BZE loads for the catchment corresponded to  
424 a mean ( $\pm$  standard deviation) of 1015 ( $\pm 38$ ) mg/1000 people/day, which was similar  
425 to weekend BZE loads measured in 2016 (999 mg/1000 people/day). Therefore, this  
426 work provides some preliminary evidence that cocaine consumption in London may  
427 have plateaued. Conversion of BZE loads to actual cocaine consumed in the  
428 catchment using a conversion factor of 3.59 (to take into account the urinary excretion  
429 rate of cocaine for different dosages and administration routes [51]) resulted in a mean  
430 weekend (Saturday-Monday) cocaine consumption of 3640 ( $\pm 140$  mg)/1000  
431 people/day (all consumption data from here onward are rounded to nearest ten). It is  
432 important to note that population estimates are likely to be one of the largest sources  
433 of uncertainty for WBE [52]. For example, the population of Greater London was  
434 8,173,941 people as of the 2011 census. London's population is expected to be larger  
435 now and the movement of people is also not accounted for (e.g., commuting to/from  
436 the city for work, tourism and large scheduled events). However, by removing the  
437 population from the equation and by multiplying the daily BZE wastewater load by the  
438 correction factor for cocaine, a generalised estimate for this catchment was calculated  
439 at 12.4 ( $\pm 0.5$ ) kg/day consumed over this weekend in 2019. This catchment represents  
440 only 43% of the total population of Greater London and therefore the combined

441 consumption in kg/day is likely to be much larger for the whole city. Furthermore, these  
442 estimates represent consumption of pure cocaine only and street-level cocaine is likely  
443 to be mixed with adulterants and diluents to varying degrees (such as lidocaine, which  
444 was also determined here at an average concentration of 177 ( $\pm 13$ ) ng L<sup>-1</sup>). Therefore,  
445 this approach may be useful for government and law enforcement agencies to monitor  
446 illicit drug markets in near real-time by covering large numbers of catchments  
447 simultaneously. For example, a national wastewater programme has been in effect in  
448 Australia since 2016, and such activities may benefit from higher throughput and more  
449 comprehensive analytical methods like the one developed herein [53].

450         Other illicit drugs unique to wastewater samples from London in comparison to  
451 the other two sites studied were ketamine, MDMA and mephedrone, the latter of which  
452 was only quantifiable near the LLOQ on the Sunday (which likely represents  
453 occurrence due to excretion following Saturday night activity). Mephedrone was last  
454 determined by our group in London wastewater in March 2014 between 42 and 160  
455 ng L<sup>-1</sup> across the week and this indicated significant reduction in population-level  
456 consumption following its legal restriction [21]. For MDMA, the average weekend  
457 wastewater load was 88 ( $\pm 35$ ) mg/1000 people/day. Following this, and by using a  
458 correction factor of 4.4 to back-calculate to consumed quantities [55], MDMA  
459 consumption was estimated at 390 ( $\pm 160$ ) mg/1000 people/day over these three days.

460

### 461 3.3.2 Monterrey, Mexico

462         Between 24 and 35 compounds were detected each day across the week in  
463 Monterrey wastewater. The highest concentrations and occurrence frequency were  
464 observed on average for two antibiotics, trimethoprim and sulfamethoxazole at 1499  
465 ( $\pm 243$ ) and 2201 ( $\pm 768$ ) ng L<sup>-1</sup>, respectively. Azithromycin was detected every day

466 (Figure 4(a)), but <LLOQ and lower than either London or USA samples. No  
467 clarithromycin was detected. In addition to these antibiotics, lincomycin, sulfapyridine  
468 were also quantifiable every day. Very little occurrence data exists for pharmaceuticals  
469 in untreated wastewaters from Mexico for comparison and this represents one of the  
470 most comprehensive analyses to date. That said, using a LC-MS/MS method for 35  
471 pharmaceuticals, Rivera-Jaimesa et al., quantified 11 compounds in wastewater from  
472 Cuernavaca, including the same two antibiotics albeit at lower concentrations of 125-  
473 790 ng L<sup>-1</sup> for trimethoprim and 775-2010 ng L<sup>-1</sup> for sulfamethoxazole [56]. However,  
474 four to five-fold higher concentrations of diclofenac on average were observed in  
475 Cuernavaca wastewater in comparison to those measured in this study. With respect  
476 to the capital, Mexico City, concentrations of up to 320, 450, 2600, 500 and 100 ng L<sup>-1</sup>  
477 <sup>1</sup> for trimethoprim, clarithromycin, metoprolol, diclofenac and bezafibrate, respectively,  
478 were recently reported by Siemens et al. [57]. Fenuron was also determined in  
479 Monterrey wastewater here at consistent concentrations on average across the week  
480 at 170 (±36) ng L<sup>-1</sup>. However, wastewater entering this particular WWTP derives  
481 mainly from households and a single defined source of fenuron is unclear. It could  
482 arise from exposed fruits and vegetables consumed by the population [58] either by  
483 direct application of pesticides to crops or indirectly via wastewater irrigation, both of  
484 which are common practices in Mexico [59, 60]. According to the European Chemicals  
485 Agency, there may also be a contribution from other sources as it is widely used in a  
486 number of materials including adhesives, sealants, coating products, polymers, and  
487 paints, and for building purposes in fabricated metal products, plastics and electronic  
488 goods [61].

489 In comparison to London, concentrations of illicit drugs and of BZE in Monterrey  
490 wastewater in particular were less than half on average at 1154 (±390) ng L<sup>-1</sup>.

491 Recreational usage was evident with a two-fold increase in its concentration observed  
492 at the weekend. A similar pattern was observed for cocaine across the week and the  
493 ratio between both compounds at both sites were also relatively consistent at 0.31  
494 ( $\pm 0.08$ ) (London) and 0.36 ( $\pm 0.06$ ) (Monterrey). Unfortunately, however, as composite  
495 samplers were not available at this site, reliable back-calculation to determine daily  
496 BZE loads from grab samples was not possible for Monterrey to compare per capita  
497 usage. In addition to cocaine, other substances were determined including  
498 methamphetamine and methedrone. A single water-loss transition (192>174) peak  
499 was also observed for 4-methylethcathinone (4-MEC) in six out of seven samples.  
500 However, as isomers of 4-MEC exist (e.g., 2- and 3-MEC, 3-,4-methylbuphedrone and  
501 2-,3-,4-ethyl methcathinone), its identity could not be confirmed in these samples with  
502 a second transition, and especially in the absence of reference material  
503 measurements for these other isomers. This single transition for 4-MEC was also  
504 detected in all London and USA wastewater samples. One sample from Monterrey  
505 yielded two transitions for 4-MEC and its concentration was then determined at 913  
506  $\text{ng L}^{-1}$  (Figure 5(b)). Very few occurrences of 4-MEC have been reported except for  
507 Gonzalez-Marino et al. who reported 4-MEC in wastewater from Milan and south  
508 western UK at 0.9 ( $\pm 3.1$ ) and 1.2 ( $\pm 1.9$ )  $\text{ng L}^{-1}$  which was significantly lower than that  
509 measured in this study [62]. Methedrone was determined at comparatively higher  
510 concentrations on the Saturday in Monterrey samples. In contrast to London,  
511 methamphetamine was determined with consistency every day at  $1762 \pm 170 \text{ ng L}^{-1}$  in  
512 Monterrey wastewater with only a marginal ( $\sim 15\%$ ) rise in concentration at the  
513 weekend potentially, indicating sustained use by the population. Interestingly, MDMA  
514 was not detected in Monterrey or any USA samples, again in contrast to London.

515 Other compounds detected that are worthy of note were clozapine,  
516 carbamazepine (CBZ) (Figure 5(c)) and its metabolite carbamazepine-10,11-epoxide  
517 which could each be quantified in Monterrey wastewater every day at higher  
518 concentrations than observed in London. Carbamazepine is widely used in the  
519 treatment of epilepsy, psychiatric conditions, bipolar disorder and is used to treat  
520 chronic neuropathic pain [63]. Cytochrome P-450 3A4 is primarily responsible for  
521 transformation into its epoxide metabolite and only ~1% is excreted as CBZ itself in  
522 urine [64, 65]. At therapeutic doses, the epoxide concentration is generally about 20%  
523 of CBZ. Over 90% of the epoxide is further hydrated to trans-10,11-dihydroxy-10,11-  
524 dihydro-carbamazepine before excretion in urine [66, 67] and this metabolite has been  
525 detected at higher concentrations than CBZ in wastewater previously [68]. However,  
526 the ratio of CBZ to the epoxide in wastewater was higher than expected at ~35 ( $\pm 11$ )  
527 % across all samples. Clozapine is used to treat antisocial personality disorder in  
528 adults, and it is a gold standard to treat resistant schizophrenia and bipolar disorder.  
529 In Mexico, the prevalence of psychiatric disorders has been reported as 6-16% for  
530 males and 2-9% for females. In children and adolescents, the prevalence is 2-10%  
531 [69, 70]. Clozapine is also an antipsychotic drug and was introduced in Mexico in 1994.  
532 In general, however, fewer antipsychotic and antidepressant-type residues were  
533 detected in Monterrey wastewater in comparison to London.

534

### 535 3.3.3. WWTP Site in Southwestern USA

536 Comparatively fewer compounds ( $n=25-27$ ) were detected in wastewater samples  
537 from this site. This WWTP serves a smaller and more suburban population in  
538 comparison to the other two sites, but still derives from a major metropolitan area. A  
539 few occurrences are worthy of discussion. With respect to illicit drugs,

540 methamphetamine was present in all samples at two to three-fold the concentrations  
541 of Monterrey (weekly average:  $4512 \pm 644 \text{ ng L}^{-1}$ ). Like Monterrey, chronic occurrence  
542 was observed, but with increased concentrations on weekdays instead. The  
543 advantage of composite sampling used at this site allowed more reliable back-  
544 calculation to determine community consumption trends across the week. In terms of  
545 wastewater loading, methamphetamine was estimated at  $1331 \pm 167 \text{ mg/1000/people}$   
546 per day which exceeds the highest load determined during the 2018 SCORE EU  
547 monitoring campaign (Erfurt, Germany, at  $211 \text{ mg/1000 people/day}$ ) [54]. However,  
548 such estimates may need to be treated with caution as sources of methamphetamine  
549 in wastewater can also derive from manufacturing activity, which could be significant  
550 depending on its scale within a catchment [71]. Enantiomeric profiling for chiral drugs  
551 like methamphetamine has been used to differentiate drug manufacturing effluent from  
552 consumption behaviour [72, 73], but unfortunately this was not possible to determine  
553 here using this method and lay beyond the scope of this work. Nevertheless, the USA  
554 has reported significant methamphetamine misuse for many years with  $>14.5 \text{ M}$   
555 people above the age of 12 ( $>5\%$  of total population) reported in 2016 as having tried  
556 the drug at least once in their lifetime [74]. Moreover,  $\sim 1.4 \text{ M}$  reported using the drug  
557 in the year preceding this survey. Using a back-calculation correction factor of 2.44  
558 [55], this yielded an average methamphetamine consumption of  $3250 \pm 410 \text{ mg/1000}$   
559 people/day in this wastewater catchment (equivalent to  $\sim 65 \text{ doses/1000 people/day}$   
560 [62]). Cocaine and BZE concentrations on the other hand were much lower ( $328 \pm 402$   
561 and  $908 \pm 387 \text{ ng L}^{-1}$  on average, respectively) than London and Monterrey, but  
562 peaked at the weekend, as expected. However when using 24-h composite samples,  
563 the increased concentration observed on this particular Saturday is also likely to  
564 include contributions from excretion of unmetabolised drug taken on the previous day



565 in the first urinary morning void. Wastewater loads for BZE were of the order of 265  
566  $\pm 106$  mg/1000 people/day and using the correction factor of 3.59 [75], this  
567 corresponded to a cocaine consumption estimate in this smaller catchment at  $950 \pm$   
568  $380$  mg/1000 people/day. Interestingly, a high Spearman correlation ( $r=0.90$ ) was  
569 observed between daily lidocaine and benzoylecgonine concentrations across the  
570 week for this particular site and indicated that lidocaine occurrence may have been  
571 driven by its use as a diluent in cocaine powder (Figure S5). Consistent, low  
572 concentrations of the opioid, oxycodone, were also observed in USA samples  
573 (average =  $49 (\pm 14)$  ng L<sup>-1</sup>), which was not present in either London or Monterrey  
574 wastewaters. It was not possible to differentiate between medicinal use and misuse of  
575 this compound using wastewater analysis. Unfortunately, more opioid standards for  
576 fentanyl, morphine, heroin, methadone and codeine were not available at the time of  
577 method development, but the speed of the MS instrument used in this method would  
578 be able cope with more MRM transitions if needed, though stability for reliable WBE  
579 back-calculations for some of these compounds is often limited.

580         Aside from illicit and misused drugs, several antibiotics were determined in USA  
581 wastewater samples. With respect to macrolide antibiotics, occurrence of azithromycin  
582 largely mirrored that of London, but concentrations of clarithromycin were lower on  
583 average. Lincomycin, like in London, was not detected. Trimethoprim occurrence was  
584 lower than Monterrey by three-fold on average, and roughly double that measured in  
585 London wastewater. Other notable higher occurrences of pharmaceutical residues for  
586 this site included diphenhydramine and oxazepam. Interestingly, and despite its  
587 widespread reported occurrence in the literature on a global level, carbamazepine was  
588 only detected on two days at this site and at  $<40$  ng L<sup>-1</sup>. Pesticide occurrence was also

589 measured and three were unique to this site including prometon, azoxystrobin and  
590 bupropion.

591

## 592 **Conclusion**

593 A rapid, direct injection LC-MS/MS method was successfully developed and  
594 validated for the quantitative determination of 135 CECs in wastewater at the ng L<sup>-1</sup>  
595 concentration level. With a total analysis time of 5.0 min including re-equilibration, this  
596 enabled ~261 injections in 24 hours. With only a 10 µL injection volume, it also aided  
597 convenient and cost-effective international shipment of smaller samples and reduced  
598 the space required for archiving. Success of this method depended heavily on the use  
599 of a short, high-efficiency biphenyl LC column, the flow rate, injection volume:mobile  
600 phase ratio, MS dwell times/acquisition speed and MS detector sensitivity. The use of  
601 SPE for matrix interference removal (rather than analyte concentration) was found to  
602 be of no advantage to further enhance sensitivity. Excellent method performance was  
603 achieved over ranges of up to three orders of magnitude. When applied to influent  
604 wastewater samples from three WWTPs in London (UK), Monterrey (Mexico) and a  
605 third site in the South West USA, 56 compounds could be determined directly including  
606 pesticides, pharmaceuticals, illicit drugs and their metabolites. To our knowledge, this  
607 represents the fastest single LC-MS/MS method for direct analysis of wastewater for  
608 quantitative determinations of so many compounds at this sensitivity level. Direct  
609 analysis methods like this will likely enable rapid characterisation of CEC occurrence  
610 to monitor community-level consumption patterns and ultimately environmental risk  
611 assessment.

612

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625

## 626 **Appendix A. Supplementary information (SI)**

627 The SI document provides a list of reference materials, MRM transitions and  
628 scheduling, SPE matrix removal data, full method validation data for each analyte, a  
629 van Deemter curve for the biphenyl column, flow rate/injection volume optimisation  
630 data, source contamination details and lidocaine, cocaine and BZE correlations in  
631 wastewater samples.

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**Table 1.** Summary of analytical performance characteristics for all 135 CECs using direct injection LC-MS/MS. For full individual analyte data, please refer to Table S4.

	Linearity	Peak Area Precision		Matrix Effect		Inaccuracy			Sensitivity	
	$N \geq 5$ (max $N=12$ )	RSD%, $n=6$		CV%, $n=6$		CV% <sup>a</sup>			LLOD <sup>b</sup>	LLOQ <sup>c</sup>
	R <sup>2</sup>	at 100 ng L <sup>-1</sup>	1000 ng L <sup>-1</sup>	at 100 ng L <sup>-1</sup>	1000 ng L <sup>-1</sup>	250 ng L <sup>-1</sup>	750 ng L <sup>-1</sup>	1000 ng L <sup>-1</sup>	ng L <sup>-1</sup>	ng L <sup>-1</sup>
Maximum	0.999	55	32	+337	+188	+66	+13	+9	533	1777
Minimum	0.967	2	1	-84	-60	-97	-54	-44	0.06	0.21
Absolute Median	0.999	8	6	11	9	12	8	-4	9	31
Absolute Mean ( $\pm$ standard deviation)	0.998 ( $\pm 0.0037$ )	11 ( $\pm 10$ )	8 ( $\pm 6$ )	20 ( $\pm 34$ )	14 ( $\pm 22$ )	16 ( $\pm 14$ )	9 ( $\pm 7$ )	-6 ( $\pm 10$ )	29 ( $\pm 59$ )	95 ( $\pm 197$ )

<sup>a</sup> for each of 250 and 750 ng L<sup>-1</sup> levels, accuracy represents the mean of two replicate matrix-matched standards, for 1000 ng L<sup>-1</sup> it represents the mean of  $n=6$  replicates.

<sup>b</sup> Lower limit of detection

<sup>c</sup> Lower limit of quantitation

**Table 2.** Occurrence of CECs in influent wastewater samples from three WWTPs from the UK, Mexico and the USA measured using direct LC-MS/MS analysis (average of n=3 replicates ± standard deviation).

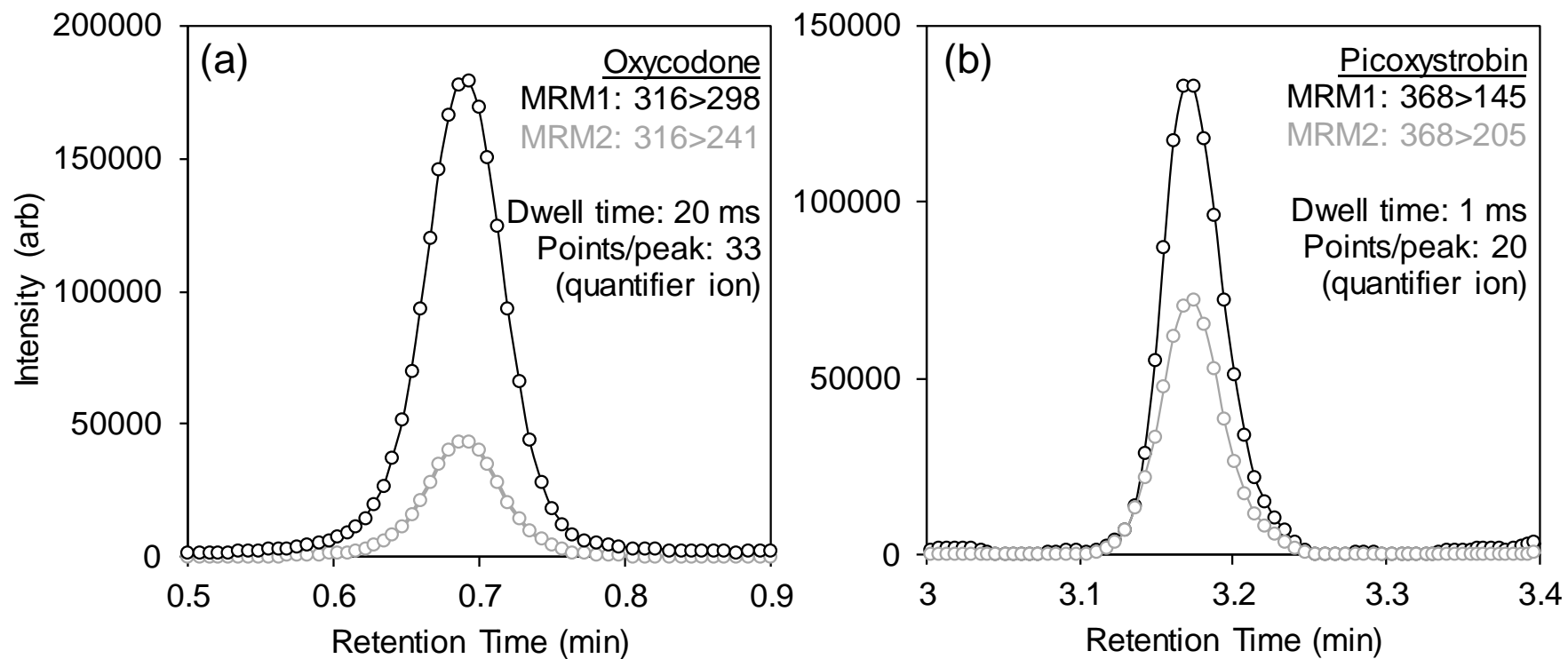
Analyte	London, UK (5-7 <sup>th</sup> April, 2019) WWTP Population: 3.4 M (as 24-h composite samples)			Monterrey, Mexico (19 <sup>th</sup> -25 <sup>th</sup> Feb, 2019) WWTP Population: 1,708,190 (as grab samples)							WWTP in Southwestern USA (9 <sup>th</sup> -15 <sup>th</sup> Sept., 2019) WWTP Population: 60,888 (as 24-h composite samples)						
	Sat	Sun	Mon	Tues	Wed	Thu	Fri	Sat	Sun	Mon	Mon	Tue	Wed	Thu	Fri	Sat	Sun
4-Methyl-ethcathinone	-	-	-	-	-	-	913 ±12	-	-	-	-	-	-	-	-	-	-
Acetamiprid	-	-	-	-	-	34 ±6	-	-	-	-	-	-	-	-	-	-	-
Ametryn	-	-	-	-	-	-	-	99 ±4	-	-	-	-	-	-	-	-	-
Amitriptyline	95 ±9	72 ±4	79 ±11	-	-	-	-	-	-	-	72 ±5	77 ±4	82 ±5	87 ±7	81 ±4	78 ±2	75 ±4
Amlodipine	30 ±12	10 ±14	12 ±10	113 ±11	-	-	-	112 ±21	-	-	-	-	-	-	-	-	-
Antipyrine	<LLOQ	-	<LLOQ	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Atorvastatin	446 ±25	414 ±27	485 ±10	-	-	-	-	-	-	-	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	-
Atrazine	-	-	-	-	-	-	-	48 ±3	35 ±5	26 ±1	-	-	-	-	-	-	-
Azithromycin	324 ±71	356 ±99	386 ±26	<LLOQ Q	<LLOQ Q	<LLOQ Q	<LLOQ Q	<LLOQ Q	<LLOQ Q	<LLOQ Q	391 ±35	410 ±49	545 ±27	865 ±79	721 ±105	499 ±53	403 ±47
Azoxystrobin	-	-	-	-	-	-	-	-	-	-	321 ±5	127 ±3	189 ±8	207 ±10	212 ±12	169 ±9	137 ±4
Bezafibrate	263 ±20	290 ±24	307 ±24	<LLOQ Q	<LLOQ Q	<LLOQ Q	<LLOQ Q	<LLOQ Q	4375 ±136	<LLOQ Q	-	-	<LLOQ	<LLOQ	<LLOQ	-	-
Bisoprolol	77 ±7	83 ±2	83 ±5	10 ±1	9 ±1	8 ±2	11 ±2	12 ±2	9 ±2	8 ±1	-	-	-	-	-	-	-
Bupropion	-	-	-	-	-	-	-	-	-	-	<LLOQ	<LLOQ	23 ±7	40 ±7	162 ±7	160 ±13	71 ±12
Benzoylcegonine	2635 ±376	2786 ±7	2931 ±88	998 ±36	784 ±21	791 ±34	949 ±30	1768 ±42	1597 ±119	1196 ±42	341 ±9	613 ±13	754 ±19	915 ±42	1263 ±39	1485 ±41	988 ±24
Carbamazepine	30 ±9	195 ±14	310 ±14	290 ±20	244 ±17	229 ±14	276 ±37	274 ±39	261 ±5	223 ±16	-	-	-	33 ±4	24 ±7	-	-
Carbamazepine epoxide	<LLOQ	<LLOQ	<LLOQ	97 ±3	98 ±6	95 ±5	74 ±8	119 ±12	-	81 ±9	-	-	-	-	-	-	-
Citalopram	325 ±22	303 ±16	327 ±11	-	-	-	-	-	-	-	179 ±14	259 ±22	270 ±14	294 ±6	257 ±15	216 ±5	191 ±1
Clarithromycin	673 ±68	568 ±14	536 ±28	-	-	-	-	-	-	-	244 ±26	-	-	<LLOQ	<LLOQ	-	-
Clopidogrel	<LLOQ	<LLOQ	<LLOQ	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Clozapine	29 ±6	24 ±4	27 ±2	22 ±6	6 ±2	8 ±3	11 ±1	10 ±3	4 ±2	10 ±5	-	-	-	-	-	-	-
Cocaine	801 ±92	660 ±33	1138 ±71	296 ±18	301 ±19	334 ±8	358 ±11	501 ±8	701 ±45	402 ±26	32 ±2	31 ±4	36 ±5	32 ±6	719 ±53	1003 ±87	443 ±41
Diazepam	69 ±5	68 ±3	65 ±8	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Diclofenac	458 ±23	521 ±92	467 ±46	412 ±17	341 ±6	355 ±39	632 ±72	453 ±35	542 ±42	338 ±24	139 ±9	106 ±11	140 ±12	105 ±7	144 ±21	104 ±9	143 ±11
Diphenhydramine	86 ±15	98 ±15	139 ±16	119 ±4	59 ±1	54 ±3	72 ±4	97 ±5	72 ±2	59 ±10	647 ±40	713 ±48	844 ±8	873 ±64	682 ±56	588 ±34	451 ±16
Fenuron	-	-	-	190 ±6	174 ±4	237 ±27	123 ±6	172 ±14	144 ±4	156 ±6	-	-	-	-	-	-	-
Fluoxetine	56 ±4	50 ±4	58 ±7	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Hydrochlorothiazide	133 ±20	144 ±22	154 ±19	826 ±83	589 ±42	581 ±198	581 ±113	580 ±32	546 ±103	597 ±167	634 ±58	491 ±31	645 ±140	650 ±111	641 ±63	719 ±119	370 ±81
Ketamine	150 ±28	160 ±8	173 ±7	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Ketoconazole <sup>b</sup>	<LLOQ	<LLOQ	213 ±26	692 ±101	399 ±49	359 ±53	326 ±43	361 ±30	-	236 ±71	-	-	-	-	-	-	-

Levamisole	-	-	-	-	135 ±18	-	171 ±7	207 ±13	176 ±32	-	-	-	-	-	-	-	
Lidocaine	191 ±25	173 ±6	167 ±5	415 ±15	268 ±17	385 ±12	275 ±19	563 ±7	300 ±11	236 ±12	170 ±6	318 ±3	359 ±4	399 ±6	560 ±12	552 ±4	359 ±3
Lincomycin	-	-	-	669 ±32	510 ±43	533 ±17	775 ±48	331 ±94	488 ±6	487 ±37	-	-	-	-	-	-	
MDMA	140 ±19	245 ±12	342 ±22	-	-	-	-	-	-	-	-	-	-	-	-	-	
Meclizine	32 ±3	33 ±3	33 ±1	26 ±11	12 ±2	16 ±5	13 ±5	<LLO Q	-	27 ±6	-	-	-	-	-	-	
Mefenamic acid	137 ±23	162 ±16	166 ±28	-	-	-	-	-	-	-	-	-	-	-	-	-	
Mephedrone	-	-	4 ±3	-	-	-	-	-	-	-	-	-	-	-	-	-	
Methamphetamine	-	-	-	1549 ±16	1714 ±6	1676 ±19	1619 ±20	1713 ±34	2094 ±43	1969 ±21	3405 ±79	3995 ±58	5023 ±33	4845 ±32	5173 ±122	4873 ±81	4269 ±30
Methedrone	-	-	-	-	-	-	-	127 ±36	-	-	-	-	-	-	-	-	
Methylphenidate	-	50 ±2	48 ±1	13 ±0.2	13 ±1	12 ±1	15 ±1	16 ±1	17 ±1	13 ±2	-	-	-	-	-	-	
Metoprolol	60 ±1	57 ±1	60 ±4	275 ±8	213 ±9	226 ±13	226 ±9	259 ±29	209 ±10	221 ±11	7 ±1	-	83 ±19	47 ±7	43 ±17	6 ±12	-
Nortriptyline	65 ±2	64 ±1	67 ±4	-	-	-	-	-	-	-	-	-	-	-	-	-	
Orphenadrine	-	-	-	27 ±1	-	-	-	27 ±2	-	-	-	-	-	-	-	-	
Oxazepam	-	-	-	-	-	-	-	-	-	-	75 ±24	82 ±18	75 ±4	<LLOQ	76 ±8	99 ±10	<LLOQ
Oxycodone	-	-	-	-	-	-	-	-	-	-	42 ±3	39 ±5	50 ±3	56 ±4	64 ±2	67 ±4	33 ±3
Prometryn	-	-	-	-	-	-	-	36 ±2	-	-	-	-	-	-	-	-	
Prometon	-	-	-	-	-	-	-	-	-	-	5 ±2	4 ±0.5	3 ±1	4 ±3	3 ±2	4 ±0.4	3 ±1
Propranolol	100 ±5	71 ±8	72 ±14	<LLO Q	<LLO Q	<LLO Q	-	<LLO Q	<LLO Q	<LLO Q	-	-	-	-	-	-	
Sertraline	93 ±18	74 ±5	92 ±5	93 ±8	69 ±7	65 ±5	71 ±6	64 ±8	-	-	-	-	-	-	-	-	
Sulfamethoxazole	317 ±31	318 ±107	235 ±74	2802 ±107	2938 ±52	2254 ±155	882 ±58	1781 ±87	<LLO Q	2550 ±14	446 ±24	576 ±49	841 ±24	769 ±46	717 ±15	541 ±82	496 ±15
Sulfapyridine	458 ±47	513 ±20	449 ±71	342 ±15	422 ±19	539 ±32	<LLO Q	<LLO Q	<LLO Q	296 ±11	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	
Sulfathiazole	-	-	-	63 ±7	-	-	-	-	-	-	-	-	-	-	-	-	
Temazepam	80 ±3	75 ±3	88 ±4	-	-	-	-	-	-	-	-	-	-	-	-	-	
Terbutryn	25 ±1	23 ±1	19 ±2	-	-	-	-	-	-	-	-	-	-	-	-	-	
Tramadol	512 ±87	428 ±7	431 ±11	309 ±5	271 ±14	232 ±7	272 ±16	502 ±11	273 ±17	248 ±11	2731 ±64	243 ±14	202 ±13	147 ±15	183 ±6	112 ±6	98 ±8
Trimethoprim	193 ±11	147 ±4	185 ±15	1741 ±236	1500 ±68	1353 ±77	1579 ±150	1841 ±101	1145 ±5	1337 ±134	223 ±13	361 ±4	580 ±30	417 ±5	569 ±13	401 ±31	354 ±8
Valsartan	<LLOQ	341 ±46	389 ±24	827 ±36	428 ±94	457 ±87	663 ±39	1032 ±25	-	469 ±61	642 ±78	411 ±18	609 ±67	576 ±72	477 ±49	351 ±33	<LLOQ
Venlafaxine	289 ±30	256 ±9	282 ±23	113 ±2	96 ±3	102 ±4	100 ±3	120 ±5	103 ±5	104 ±1	52 ±7	144 ±16	162 ±17	208 ±39	193 ±16	161 ±11	69 ±9
Verapamil	51 ±3	50 ±1	51 ±1	-	-	-	-	-	-	-	-	-	-	-	-	-	

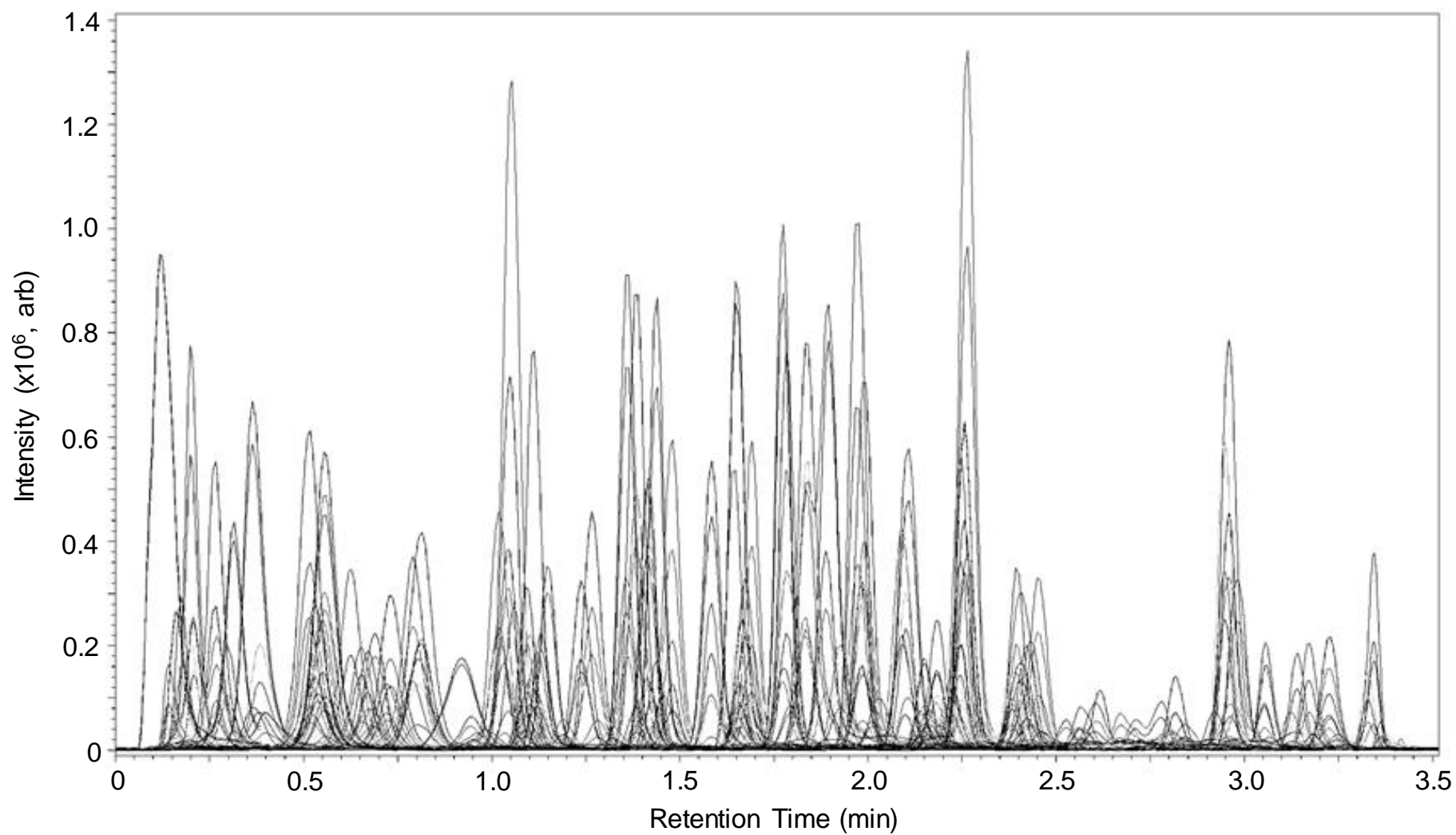
<sup>a</sup> Concentration determined by extrapolation of the upper range of the matrix matched calibration curve 0-5,000 ng L<sup>-1</sup> (n=13)

<sup>b</sup> Relative instability of this compound was higher at 73 % on average during stability testing and the reported concentrations here have not taken this into account.

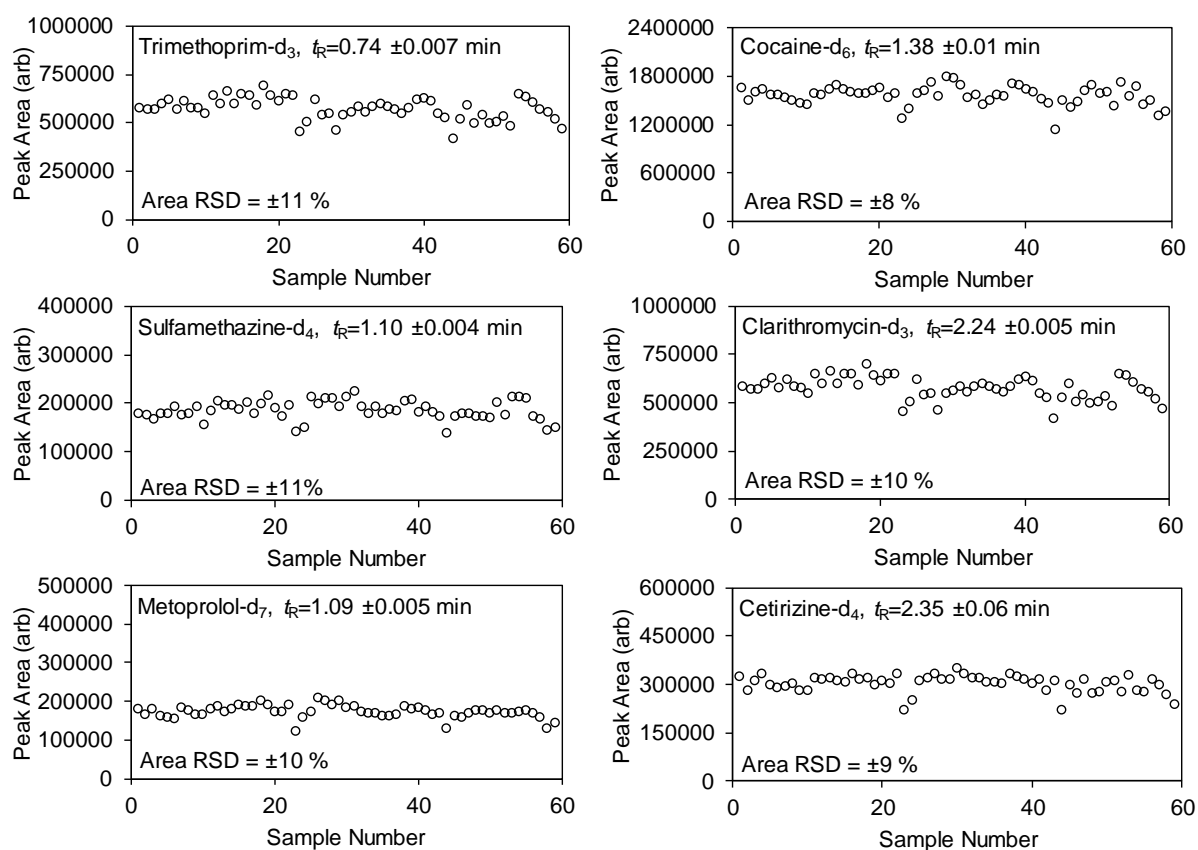
- Denotes not detected (<LLOD)



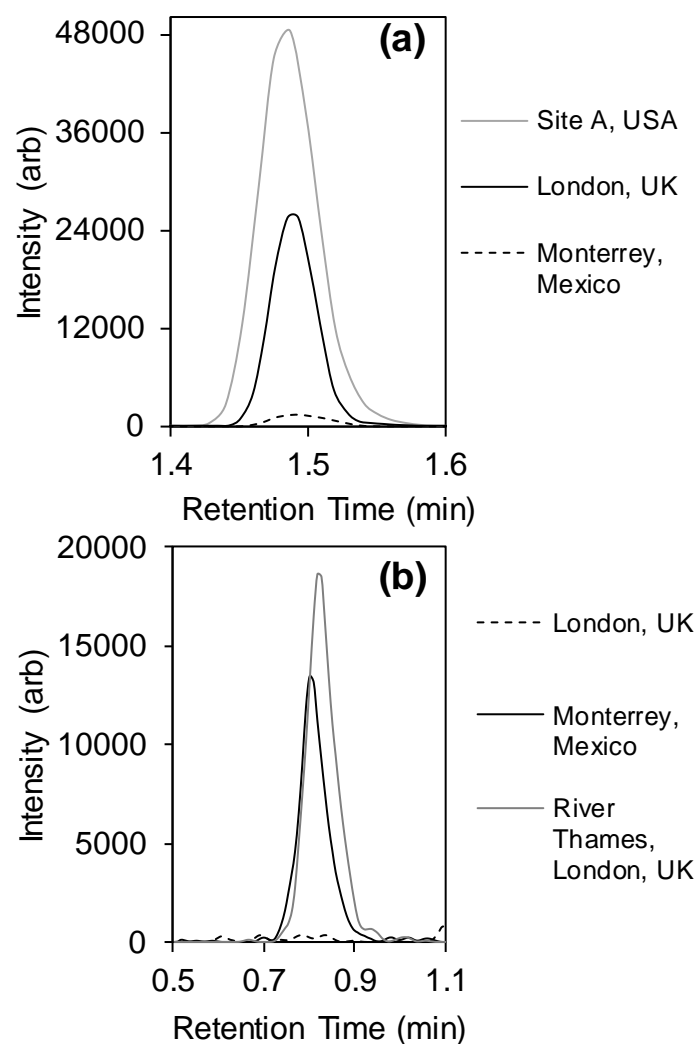
**Figure 1.** MRM data acquisition frequency and chromatographic peak definition for wastewater spiked with 500 ng L<sup>-1</sup> of (a) oxycodone (an opioid pharmaceutical) and (b) picoxystrobin (a broad-spectrum fungicide) representing sharper eluting bands of all compounds and measured using two different dwell times of 1 and 20 ms.



**Figure 2.** LC-MS/MS chromatogram of a standard mixture containing 135 pharmaceuticals, illicit substances, metabolites, pesticides and 27 SIL-IS.



**Figure 3.** Peak area and retention time stability for selected SIL-IS over a sequence of  $n=59$  spiked London wastewater samples ( $500 \text{ ng L}^{-1}$ ) and measured using direct LC-MS/MS analysis over a total batch analysis time of 6.4 h.

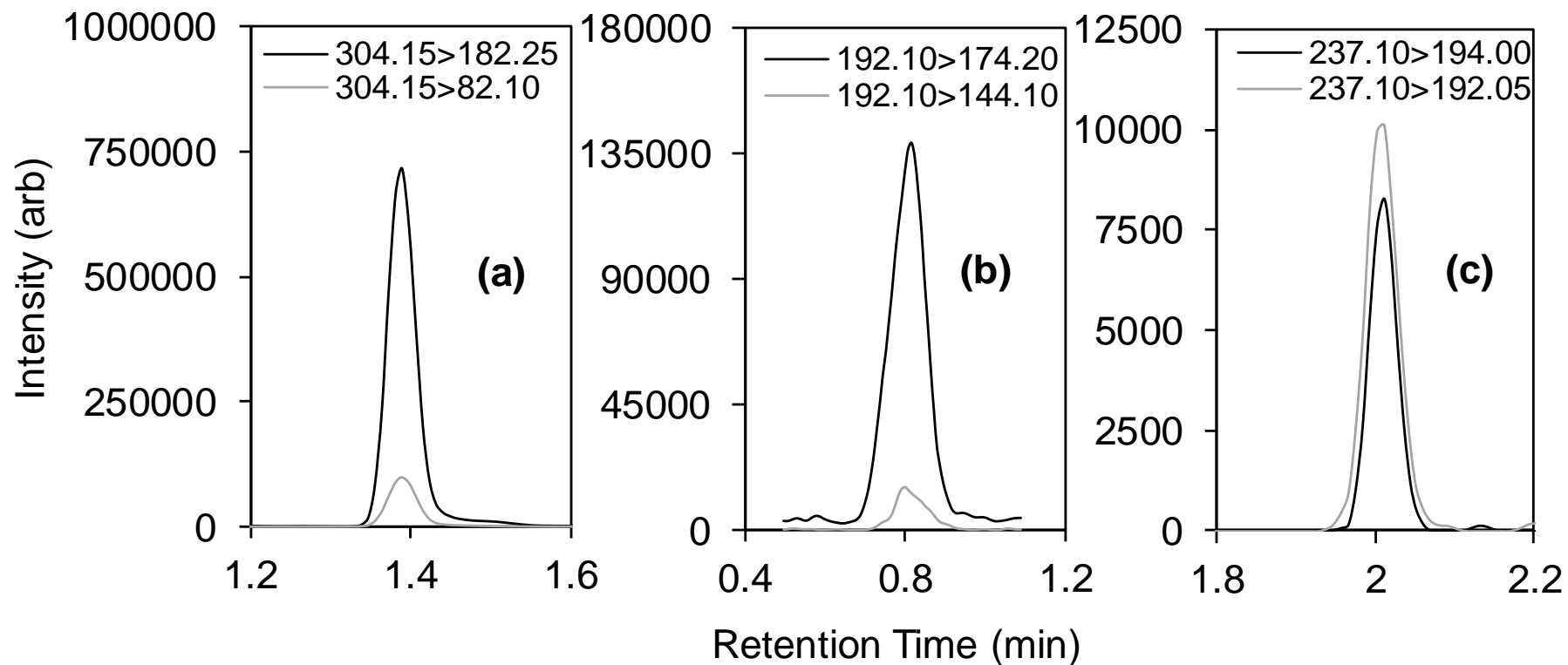


1

2 **Figure 4.** Example SRM transitions using direct LC-MS/MS analysis of influent  
 3 wastewater from the UK (London), Mexico (Monterrey) and USA showing  
 4 contamination with (a) azithromycin (London = 324 ng L<sup>-1</sup>, USA = 499 ng L<sup>-1</sup> and  
 5 Mexico = <LLOQ) and (b) fenuron (Mexico = 123 ng L<sup>-1</sup>; London = not detected; and  
 6 Thames river water from Central London = 169 ng L<sup>-1</sup>).



7



8  
9 **Figure 5.** Example MRM chromatograms for selected analytes detected in wastewater samples from June 2019 including (a)  
10 cocaine (801 ng L<sup>-1</sup>, London), (b) 4-MEC (913 ±12 ng L<sup>-1</sup>, Mexico) and (c) carbamazepine (30 ng L<sup>-1</sup>, London).