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

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Abstract

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

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

Introduction

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

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

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

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

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

The aim of this work was to develop a rapid, direct injection LC-MS/MS methodology for simultaneous quantification of over one hundred selected CECs, including pharmaceuticals, pesticides, illicit drugs and their metabolites at ng L^{-1} concentrations in influent wastewater. Challenges relating to the consolidation of methods using ESI polarity switching, run time, data quality, injection volume and sensitivity were all addressed as a priority. Furthermore, the use of SPE for matrix removal was assessed to determine any sensitivity enhancement. The performance of the method was evaluated with respect to precision, accuracy, matrix effects, linearity, range, limits of detection and quantitation. Lastly, wastewater samples from 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 MΩ
133 cm) was generated from a Millipore Milli-Q water purification system (Millipore,
134 Bedford, MA, USA). Calcium chloride diydrate (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^{-1}, 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^{-1} or 0.1 mg mL^{-1} 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^{TM} X2 ultra-high
145 pressure LC (Shimadzu Corporation, Kyoto, Japan) on a short 5.0 x 3.0 mm, 2.7 µm

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

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

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

2.3 Method validation

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

coefficient of variation (%CV) difference between the target and QC concentrations, with %CV ≤ ±25% considered acceptable. ME were determined at 100 and 1000 ng L^{-1} (*n*=6 at each level) and expressed as a percentage of the peak areas obtained for background subtracted matrix-matched standards relative to those obtained for a standard of all analytes at the same concentrations prepared with ultrapure water.

2.4 Sample collection and preparation

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

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

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

2.5 Quantification procedures for CECs in influent wastewater

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

3. Results and Discussion

3.1 Direct LC-MS/MS method development

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

extensive matrix removal procedures or use of large injection volumes to achieve ng L^{-1} sensitivity for real samples were investigated.

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

The LCMS-8060 instrument has a maximum scan speed of 30,000 u/sec and a polarity switching speed of 5 ms with a capability to acquire 555 MRMs per second. According to the manufacturer, the ion signal response for each MRM is not influenced by the number of other MRM transitions in the same time window. This enabled monitoring of 292 MRM transitions in one run using rapid polarity switching. With a 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^{-1} 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^{-1} 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

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

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

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

342 1000 ng L^{-1} 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^{-1} (for carfentrazone-ethyl). The median LLOD
346 and LLOQ were determined at 9 and 31 ng L^{-1}, respectively (average LLOD =29 ng L^{-1}
347). 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^{-1} 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^{-1} spiking concentration and for clodinafop-
361 propargyl (-60 %) and spiramycin (+188%) at 1000 ng L^{-1}. 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

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

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

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

3.3.1 London, UK

For London wastewater samples, 40-42 compounds were detected each day and quantified concentrations agreed in the main with previous screening work in 2014 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 1st 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 (±5) ng L^{-1} (Figure 4b).
399 Therefore, given the LLOQ for this compound in influent (50 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 (±34), 592 (±72)
406 and 355 (±31) 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 (±18) ng L^{-1} and 31 (±10) 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

method developed herein for the 2019 campaign, which passed with a threshold Z-Score of <2 [50]. In previous data, BZE loads in wastewater were seen to rise by approximately two-fold between 2011-2015 to ~1100 mg/1000 people/day at weekends. Both cocaine and BZE concentrations were measurable in wastewater here for 2019 samples (Figure 5a), but were slightly lower than those in 2016 (maximum weekend concentrations for cocaine and BZE were 1434 and 3533 ng L^{-1}, respectively, in 2016). Taking into account the population served by the WWTP, the daily flow and exfiltration [51], weekend BZE loads for the catchment corresponded to a mean (± standard deviation) of 1015 (±38) mg/1000 people/day, which was similar to weekend BZE loads measured in 2016 (999 mg/1000 people/day). Therefore, this work provides some preliminary evidence that cocaine consumption in London may have plateaued. Conversion of BZE loads to actual cocaine consumed in the catchment using a conversion factor of 3.59 (to take into account the urinary excretion rate of cocaine for different dosages and administration routes [51]) resulted in a mean weekend (Saturday-Monday) cocaine consumption of 3640 (±140 mg)/1000 people/day (all consumption data from here onward are rounded to nearest ten). It is important to note that population estimates are likely to be one of the largest sources of uncertainty for WBE [52]. For example, the population of Greater London was 8,173,941 people as of the 2011 census. London's population is expected to be larger now and the movement of people is also not accounted for (e.g., commuting to/from the city for work, tourism and large scheduled events). However, by removing the population from the equation and by multiplying the daily BZE wastewater load by the correction factor for cocaine, a generalised estimate for this catchment was calculated at 12.4 (±0.5) kg/day consumed over this weekend in 2019. This catchment represents 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 (±13) ng L^{-1}). 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^{-1} 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 (±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 (±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 (±243) and 2201 (±768) ng L^{-1}, respectively. Azithromycin was detected every day

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

In comparison to London, concentrations of illicit drugs and of BZE in Monterrey wastewater in particular were less than half on average at 1154 (±390) ng L^{-1}.

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 (±0.08) (London) and 0.36 (±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 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 (±3.1) and 1.2 (±1.9) 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 ±170 ng L^{-1} in
512 Monterrey wastewater with only a marginal (~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.

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

3.3.3. WWTP Site in Southwestern USA

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

methamphetamine was present in all samples at two to three-fold the concentrations of Monterrey (weekly average: 4512 ±644 ng L^{-1}). Like Monterrey, chronic occurrence was observed, but with increased concentrations on weekdays instead. The advantage of composite sampling used at this site allowed more reliable back-calculation to determine community consumption trends across the week. In terms of wastewater loading, methamphetamine was estimated at 1331 ±167 mg/1000/people per day which exceeds the highest load determined during the 2018 SCORE EU monitoring campaign (Erfurt, Germany, at 211 mg/1000 people/day) [54]. However, such estimates may need to be treated with caution as sources of methamphetamine in wastewater can also derive from manufacturing activity, which could be significant depending on its scale within a catchment [71]. Enantiomeric profiling for chiral drugs like methamphetamine has been used to differentiate drug manufacturing effluent from consumption behaviour [72, 73], but unfortunately this was not possible to determine here using this method and lay beyond the scope of this work. Nevertheless, the USA has reported significant methamphetamine misuse for many years with >14.5 M people above the age of 12 (>5% of total population) reported in 2016 as having tried the drug at least once in their lifetime [74]. Moreover, ~1.4 M reported using the drug in the year preceding this survey. Using a back-calculation correction factor of 2.44 [55], this yielded an average methamphetamine consumption of 3250 ± 410 mg/1000 people/day in this wastewater catchment (equivalent to ~65 doses/1000 people/day [62]). Cocaine and BZE concentrations on the other hand were much lower (328 ± 402 and 908 ± 387 ng L^{-1} on average, respectively) than London and Monterrey, but peaked at the weekend, as expected. However when using 24-h composite samples, the increased concentration observed on this particular Saturday is also likely to 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 ±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 ±
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 (±14) ng L^{-1}), 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^{-1}. 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^{-1}
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
613 **Acknowledgements**

This work was funded from the Biotechnology and Biological Sciences Research Council (BB/P005187/1), the Global Consortium for Sustainability Outcomes (GCSO) and Environmental Protection Agency (EPA) Ireland (funded under the EPA Research Programme 2014-2020, Ref. 2017-W-MS). The EPA Research Programme is a Government of Ireland initiative funded by the Department of Communications, Climate Action and Environment. Thanks are extended to staff at Thames Water Utilities Ltd. and Servicios de Agua y Drenaje de Monterrey, Laboratorio Central de Calidad de Aguas and Dulces Nombres WWTP for access and collection of wastewater samples. Thanks are also extended to Anthony Sullivan, Chris Titman and Neil Loftus from Shimadzu UK Ltd. for their support on the LC-MS/MS instrument. The authors declare no financial conflict of interest.

Appendix A. Supplementary information (SI)

The SI document provides a list of reference materials, MRM transitions and scheduling, SPE matrix removal data, full method validation data for each analyte, a van Deemter curve for the biphenyl column, flow rate/injection volume optimisation data, source contamination details and lidocaine, cocaine and BZE correlations in 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 *N*≥5 (max *N*=12)	Peak Area Precision RSD%, *n*=6		Matrix Effect CV%, *n*=6		Inaccuracy CV%[a]			Sensitivity	
									LLOD[b]	LLOQ[c]
	R^2	at 100 ng L^{-1}	1000 ng L^{-1}	at 100 ng L^{-1}	1000 ng L^{-1}	250 ng L^{-1}	750 ng L^{-1}	1000 ng L^{-1}	ng L^{-1}	ng L^{-1}
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 (± standard deviation)	0.998 (±0.0037)	11 (±10)	8 (±6)	20 (±34)	14 (±22)	16 (±14)	9 (±7)	-6 (±10)	29 (±59)	95 (±197)

[a] for each of 250 and 750 ng L^{-1} levels, accuracy represents the mean of two replicate matrix-matched standards, for 1000 ng L^{-1} it represents the mean of n=6 replicates.
[b] Lower limit of detection
[c] 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 (5-7th April, 2019) WWTP Population: 3.4 M (as 24-h composite samples)			Monterrey, Mexico (19th-25th Feb, 2019) WWTP Population: 1,708,190 (as grab samples)							WWTP in Southwestern USA (9th-15th 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	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	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	<LLOQ	<LLOQ	<LLOQ	<LLOQ	4375 ±136	<LLOQ	-	-	<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
Benzoylecgonine	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	716 ±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[b]	<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	<LLOQ	-	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	<LLOQ	<LLOQ	<LLOQ	-	<LLOQ	<LLOQ	<LLOQ	-	-	-	-	-	-	-
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	<LLOQ	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	<LLOQ	<LLOQ	<LLOQ	296 ±11	<LLOQ	<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	-	-	-	-	-	-	-	-	-	-	-	-	-	-

[a] Concentration determined by extrapolation of the upper range of the matrix matched calibration curve 0-5,000 ng L^{-1} (*n*=13)
[b] 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)

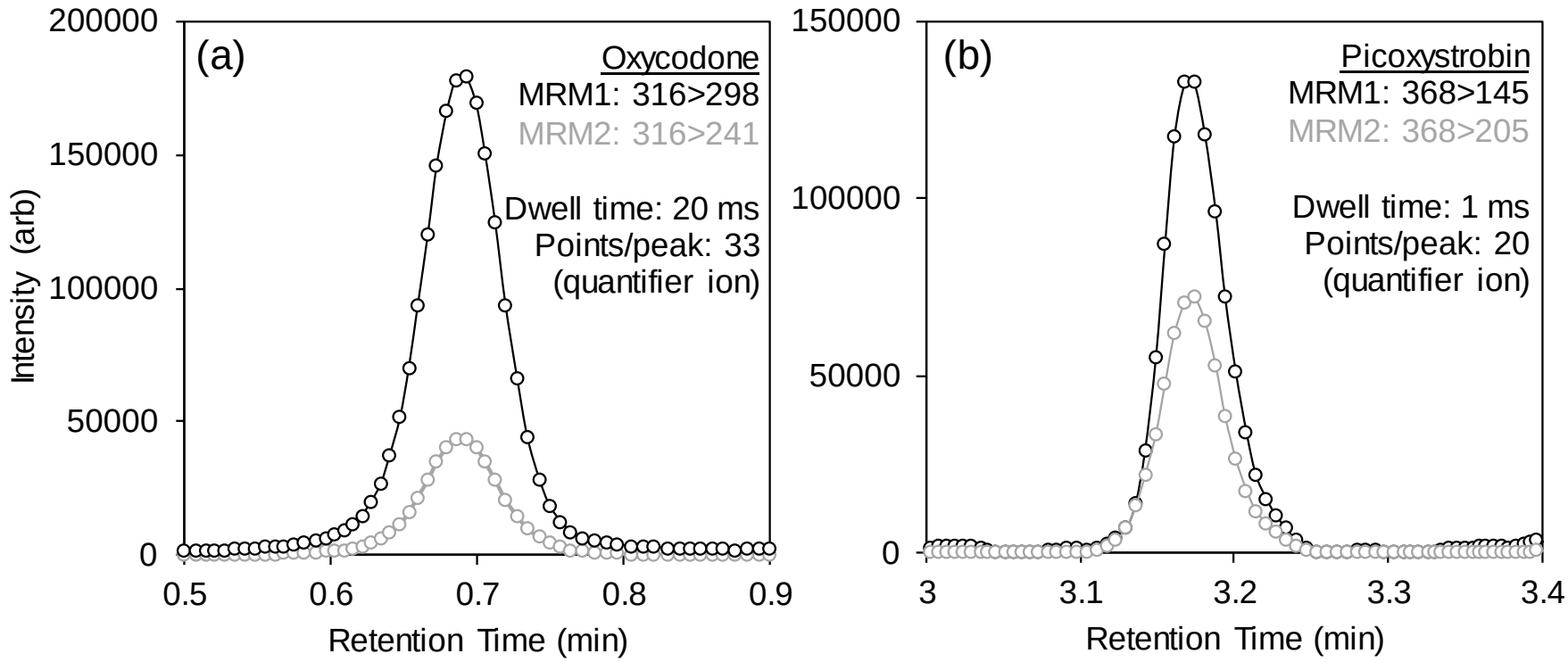

Figure 1. MRM data acquisition frequency and chromatographic peak definition for wastewater spiked with 500 ng L^{-1} of (a) oxycodone (an opioid pharmaceutical) and (b) picoxystrobin (a broad-spectrum fungicide) representing sharper eluting bands of all compounds and measured using two different dwell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

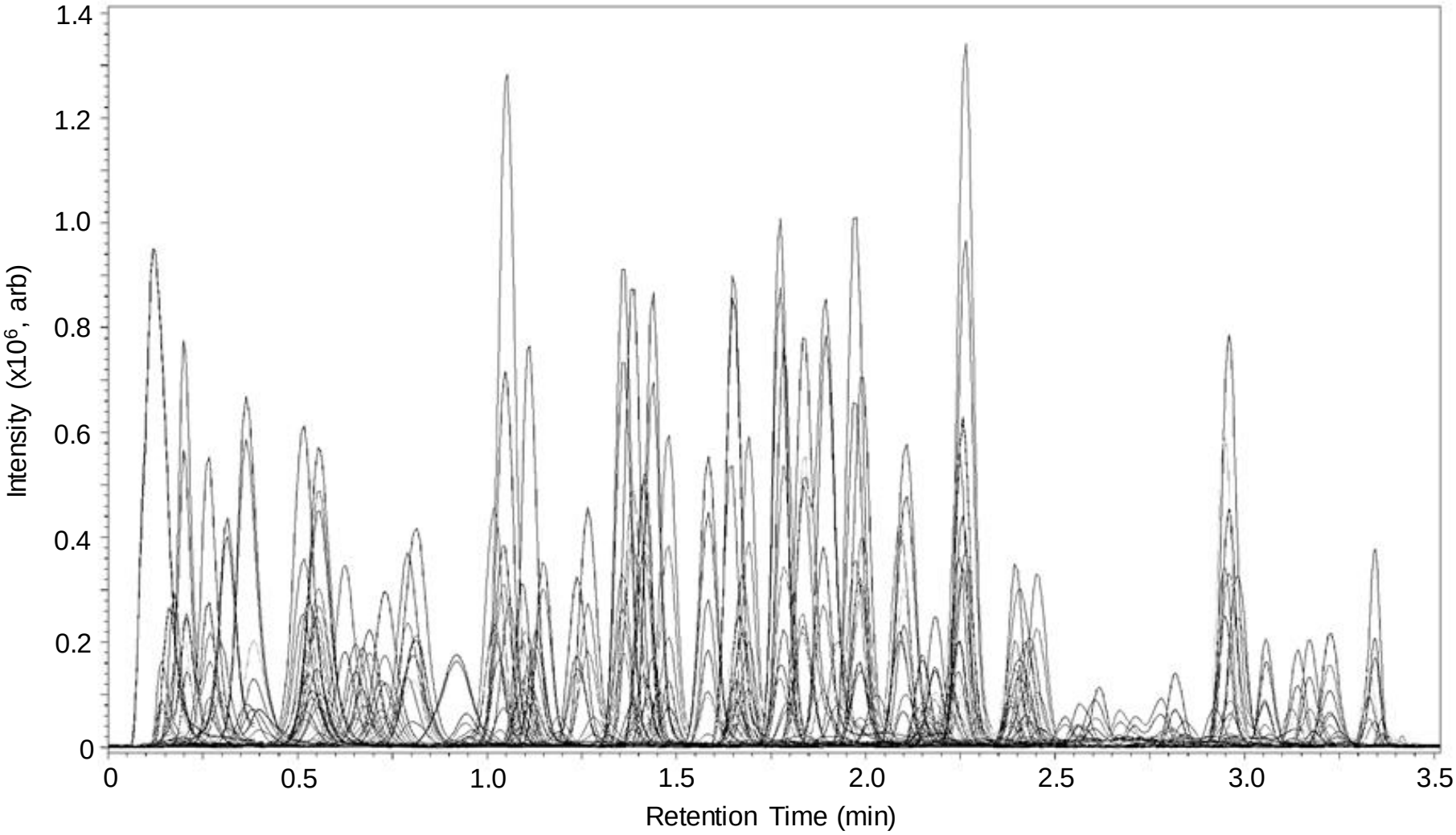

Figure 2. LC-MS/MS chromatogram of a standard mixture containing 135 pharmaceuticals, illicit substances, metabolites, pesticides and 27 SIL-IS.

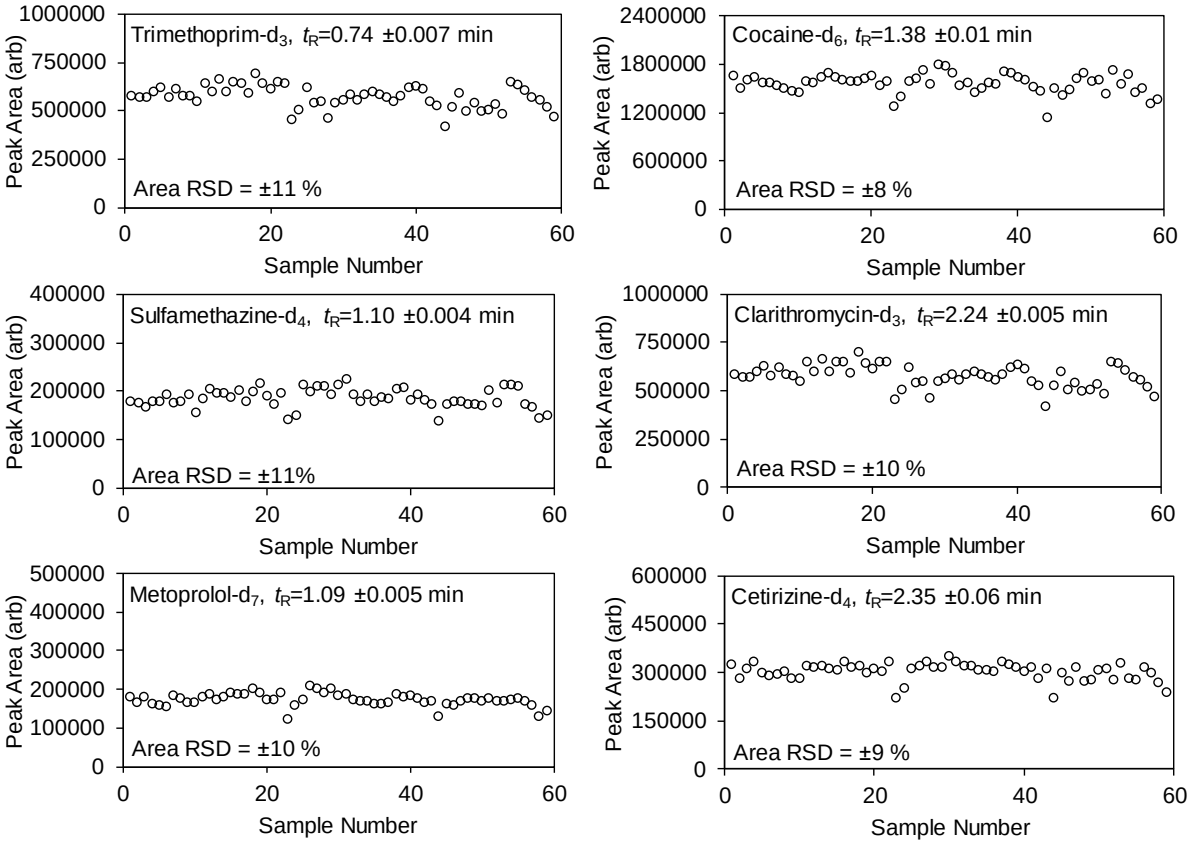

Figure 3. Peak area and retention time stability for selected SIL-IS over a sequence of *n*=59 spiked London wastewater samples (500 ng L^{-1}) and measured using direct LC-MS/MS analysis over a total batch analysis time of 6.4 h.

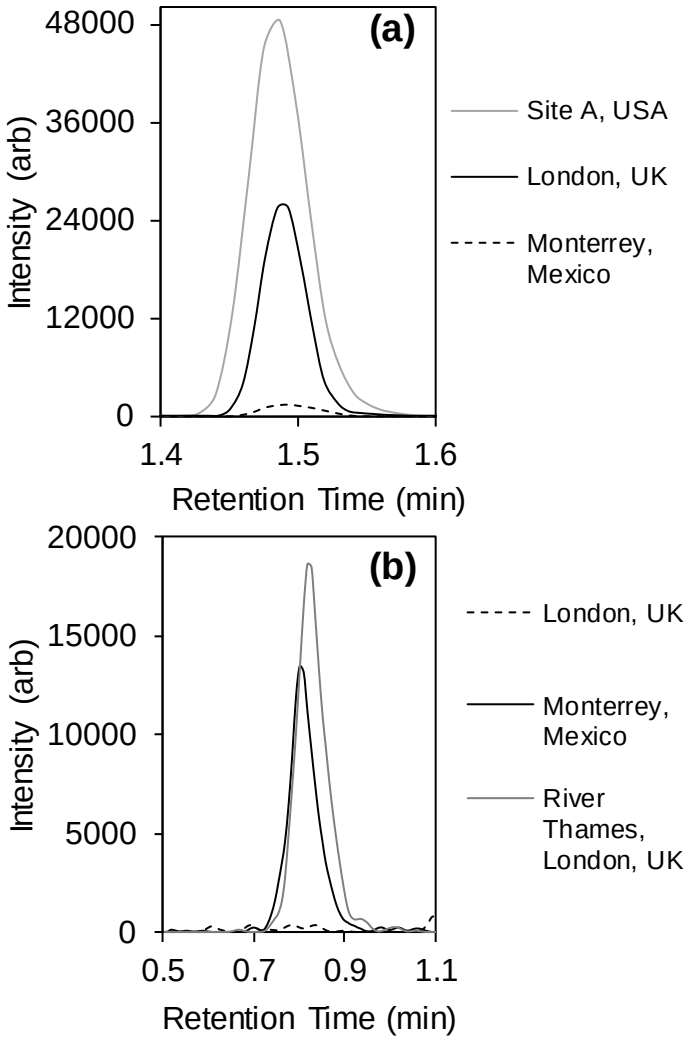

Figure 4. Example SRM transitions using direct LC-MS/MS analysis of influent wastewater from the UK (London), Mexico (Monterrey) and USA showing contamination with (a) azithromycin (London =324 ng L^{-1}, USA =499 ng L^{-1} and Mexico =<LLOQ) and (b) fenuron (Mexico= 123 ng L^{-1}; London = not detected;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

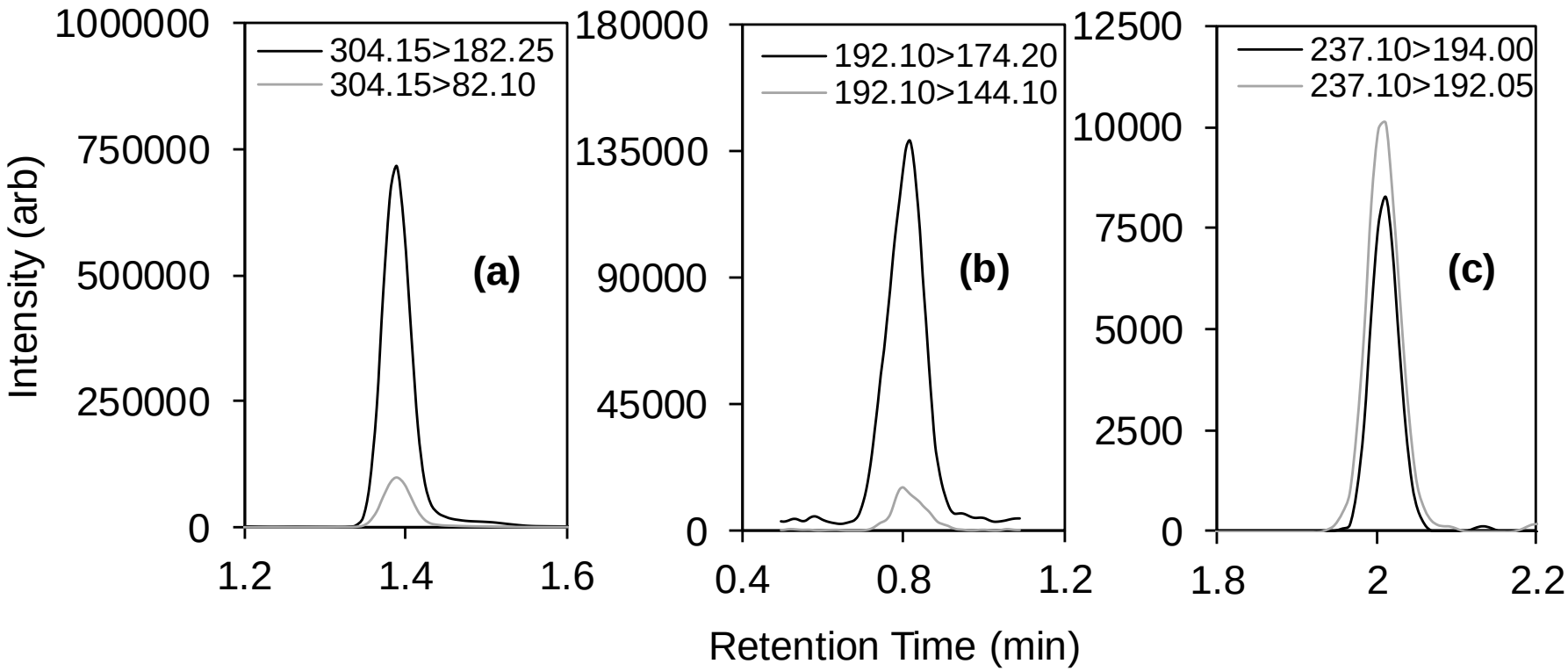

Figure 5. Example MRM chromatograms for selected analytes detected in wastewater samples from June 2019 including (a) cocaine (801 ng L^{-1}, London), (b) 4-MEC (913 ±12 ng L^{-1}, Mexico) and (c) carbamazepine (30 ng L^{-1}, London).