Supplementary Material (ESI) for Journal of Environmental Monitoring

Supplementary information.

Using Environmental Analytical Data to Estimate Levels of Community Consumption of Illicit Drugs and Abused Pharmaceuticals.

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The following is a detailed description of the development of the SPE procedure.

SPE sorbent selection.

The illicit drugs and abused pharmaceuticals chosen for study comprise a set of analytes that are weak to moderately basic, with the exception of Δ^9-THC which is uncharged and also span across a broad range of polarities as can be seen from the pKa and the octanol water partition coefficient data (Log *P* values) in Table S1.

Table S1 pK_a and Log *P*, (as theoretically calculated *X*Log*P*) data for the selected illicit drugs and pharmaceutical analytes.

Analyte	pK_a	Log *P*
Morphine	9.85[1]	0.96[2]
Amphetamine	9.80[3]	1.76[4]
MDMA	9.90[5]	-0.32[6]
Benzoylecgonine	2.25, 11.2[7]	1.29[8]
Ketamine	7.50[9]	2.88[4]
Cocaine	8.60[10]	2.31[4]
Heroin	7.60[11]	1.69[4]
Cocaethylene	-	-
LSD	7.80[12]	2.10[4]
Methadone	9.10[13]	3.92[4]
EDDP	-	4.76[4]
Papaverine	8.07[14]	3.00[4]
Temazepam	-	2.99[4]
Fluoxetine	7.37, 4.69[15]	4.65[4]
Diazepam	3.40[13]	2.92[4]
Δ^9-THC	10.60[16]	6.48[4]

Due to the expectation that the majority of the chosen analytes would be presumed to exist in their protonated cationic form in solution mixed mode cation exchange sorbents, both weak and strong, were investigated along with the previously used hydrophilic lipophilic balanced polymeric sorbent. The sorbents used were from the Phenomenex Strata-X™ family of polymeric functionalised phases for analyte enrichment, the structures of which are shown in Fig.S1.

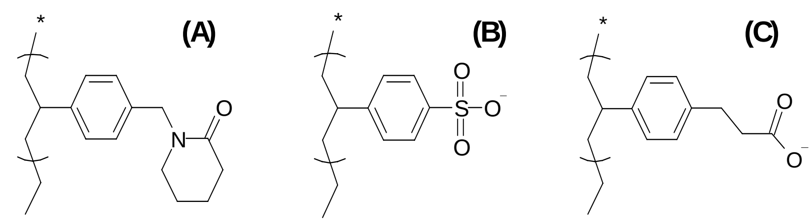

Fig.S1 The structure of (A) the Phenomenex Strata-X™ hydrophilic lipophilic balanced polymeric sorbent, (B) the additional strong cation exchange functionality of the Strata-XC™ sorbent and (C) the weak cation exchange functionality of the Strata-XCW™ sorbent.[17]

In order to ascertain which of the above sorbents provided the highest degree of analyte recovery, a 500 mL aliquot of a 2 μgL^{-1} mixed analyte spiked solution prepared in reagent water was extracted using each of the above sorbents. The solution pH was adjusted to pH 7.0, 2.0 and 5.0 for the Strata-X™, Strata-XC™ and Strata-XCW™ sorbents, respectively. Elution was performed using 10 mL of methanol or in the case of the mixed mode cation exchange sorbents, 10 mL of 5% v/v ammonium hydroxide in methanol. The percentage analyte recovery in each instance was determined by comparison of the resulting peak areas with those of a directly injected 2 mgL^{-1} mixed standard, see Table S2.

From Table S2 it can be seen that the highest degree of analyte recovery was achieved when using the Strata-XC™ mixed mode strong cation exchange sorbent with acceptably high recovery of all analytes with the exception of heroin. Although it cannot be said with certainty, it is assumed that heroin hydrolysed to morphine under the acidic conditions used, hence the excessive recovery of morphine. The sorbent selection study was performed using LC with UV detection at 230 nm and therefore, the cases in which analyte recovery greater then 100% may have arisen due

to co-elution with unknown peaks. High levels of analyte recovery were also achieved with the weak mixed mode Strata-XCW™ sorbent. However, recovery was generally less than the Strata-XC™ sorbent with the exception of LSD where results achieved with the three investigated sorbents were similar. When using the Strata-X™ sorbent, which exploits both reversed-phase and hydrogen bonding as mechanisms of retention, analyte retention can be observed to increase with increasing levels of hydrophobicity. Analytes such as MDMA and methadone, which are expected to exist as cations in solution under the experimental conditions exhibited low levels of retention as expected. In all cases no appreciable recovery of both amphetamine and Δ^9-THC was achieved. Although the reasons for such are not inherently clear, it has previously been noted that amphetamine is readily purged from solution during solvent evaporation using nitrogen and often requires acidification of the elution solvent in order to prevent such effects.[18] Δ^9-THC was expected to be neutral in solution under the experimental conditions, the reported pK_a value corresponds to the dissociation of the phenolic group of the molecule, and therefore, retention was expected when using the Strata-X™ sorbent. Δ^9-THC exhibits high retention during the chromatographic analysis and therefore, the possibility of excessive retention on the extraction sorbent was investigated. However, when the Strata-X™ sorbent was eluted with larger volumes of solvent, still no recovery of Δ^9-THC was noted. As a result of these observations both amphetamine and Δ^9-THC were omitted from further study as their enrichment appeared unfeasible using the SPE approach.

Table S2 Initial sorbent selection investigations. Calculated analyte recovery for a 2 μgL^{-1} mixed spike using the sorbents and conditions mentioned within the text, (values quoted are mean % recovery ± standard deviation, n = 3).

Analyte	Strata-X™	Strata-XC™	Strata-XCW™
Morphine	143 ± 7	124 ± 6	-
Amphetamine	-	-	-
MDMA	44 ± 1	88 ± 1	75 ± 5
Benzoylecgonine	78 ± 2	70 ± 2	58 ± 1
Ketamine	70 ± 3	89 ± 5	66 ± 2

Cocaine	102 ± 1	115 ± 1	94 ± 2
Heroin	22 ± 1	26 ± 1	13 ± 1
Cocaethylene	94 ± 1	106 ± 2	91 ± 2
LSD	56 ± 4	52 ± 1	57 ± 1
Methadone	29 ± 1	97 ± 1	71 ± 1
Temazepam	84 ± 1	80 ± 1	73 ± 1
Fluoxetine	30 ± 1	71 ± 1	61 ± 1
Diazepam	82 ± 1	81 ± 1	70 ± 1
Δ^9-THC	-	-	-

Elution solvent selection.

As the chosen Strata-XC™ sorbent contains the same sorbent 'backbone' as the Strata-X™ sorbent which was previously used for the enrichment of pharmaceutical residues[19], it was decided to investigate whether the elution solvent of 5% v/v ammonium hydroxide in methanol as recommended by the product literature[17] could be replaced with 5% v/v ammonium hydroxide in 1:1 acetone ethyl acetate as used previously due to both the increased solvent strength and ease at which the acetone ethyl acetate mixture can be reduced in volume under nitrogen. To determine which solvent system provided the optimum levels of analyte recovery, 1 μgL^{-1} spiked solutions of chosen analytes prepared in reagent water were adjusted to pH 2.0 using HCl and extracted using the selected Strata-XC™ sorbent. After complete sample introduction and sorbent drying SPE cartridges were individually eluted with 10 mL the aforementioned solvent systems that were then reduced in volume under nitrogen, reconstituted in internal standard solution and analysed using LC-MS/MS. Analyte recovery was determined by comparison with the resulting peak areas of a directly injected 1 mgL^{-1} mixed standard, see Table S3.

In most instances, analyte recovery was again acceptable. However, recoveries obtained when using 5% v/v NH_4OH in methanol were in some instances excessively high, for example, in the case of cocaine, which was almost double that obtained when using the 5% v/v NH_4OH in 1:1 acetone ethyl acetate elution solvent system. Another observation noted was that there was no recovery of heroin when using 5%

v/v NH_4OH in methanol. For all subsequent investigations elution was performed using 5% v/v NH_4OH in 1:1 acetone ethyl acetate.

Table S3 Calculated analyte recovery for the optimisation of the elution solvent.

Analyte	5% v/v NH_4OH in methanol	5% v/v NH_4OH in 1:1 acetone ethyl acetate
Morphine	53	8
MDMA	107	60
Benzoylecgonine	87	122
Ketamine	132	109
Cocaine	164	87
Heroin	0	27
Cocaethylene	100	73
LSD	33	43
EDDP	62	62
Methadone	93	83
Fluoxetine	31	36
Temazepam	81	90
Diazepam	83	77

Having selected the elution solvent, the minimum volume of solvent required for complete analyte elution was then determined. A Strata-XC™ cartridge was successively eluted with 15 x 1 mL portions of 5% v/v NH_4OH in 1:1 acetone ethyl acetate, each of which in turn was reduced in volume under nitrogen, reconstituted and analysed using LC-MS/MS. The relative recovery of each analyte was determined and plotted against the volume of elution solvent as depicted in Fig.S2. For the purposes of clarity Fig.S2 has been simplified and only depicts the resulting elution profiles of cocaine, methadone and temazepam, however, identical traces were recorded in the case of all of the investigated analytes. From Fig.S2 it can be seen that 8 mL of solvent is sufficient to remove all retained analyte from the sorbent bed, however, an elution volume of 10 mL was chosen for all further investigations in order to ensure completeness.

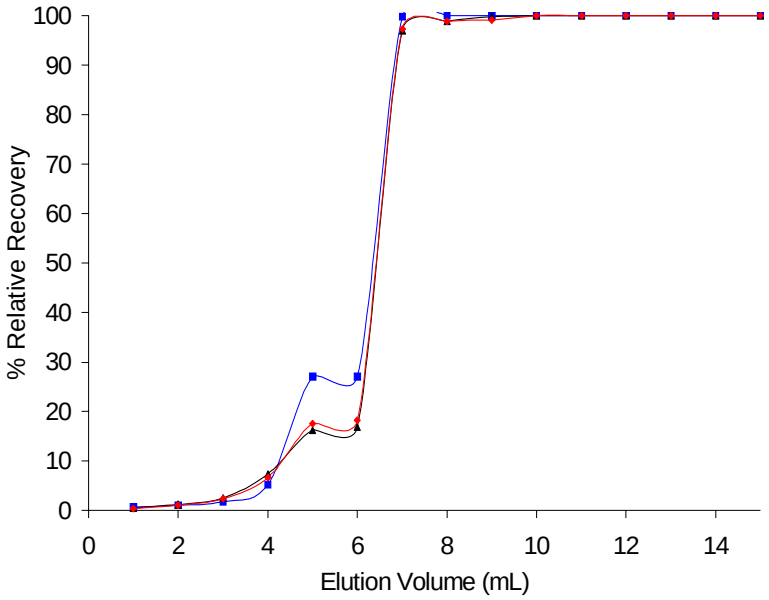

Fig.2 Plot of % relative recovery versus SPE elution volume using Strata-XC SPE cartridges and elution with 5% v/v NH4OH in 1:1 acetone ethyl acetate. *Key:* black trace; methadone, blue trace; cocaine, red trace; temazepam.

Extraction pH optimisation.

The pH of the extraction solution was optimised in order to determine the sample loading pH that provided the highest levels of analyte recovery. 500 mL aliquots of 1 μgL^{-1} spiked solutions were prepared in 10 mM buffer solutions and extracted using the Strata-XC™ sorbent. Extractions were performed at pH 2 using reagent water adjusted with HCl, pH 3 and 4 using ammonium formate buffer, pH 5 using ammonium acetate buffer, pH 6 and 7 using MES and pH 8 using TRIS. After elution with 10 mL of 5% v/v NH_4OH in 1:1 acetone ethyl acetate, solvent removal and reconstitution, the extract was analysed using LC-MS/MS and as before, the levels of analyte recovery were determined by area comparison with a 1 mgL^{-1} standard, the calculated recovery values are inserted as Table S4.

Table S4 Calculated analyte recovery for the optimisation of the elution solvent.

Analyte	% Recovery						
	pH 2 (HCl)	pH 3 (formate)	pH 4 (formate)	pH 5 (acetate)	pH 6 (MES)	pH 7 (MES)	pH 8 (TRIS)
Morphine	5	6	16	2	6	7	14
MDMA	62	45	55	67	62	62	38
Benzoylecgonine	113	90	112	99	81	106	55
Ketamine	70	61	62	71	70	68	73
Cocaine	11	15	7	33	54	49	0
Heroin	22	11	5	33	56	55	0
Cocaethylene	36	38	31	57	69	69	2
LSD	40	46	48	48	60	60	88
EDDP	51	40	39	62	63	40	45
Methadone	60	63	59	74	76	74	43
Fluoxetine	43	25	32	41	47	52	17
Temazepam	79	71	87	81	82	92	61
Diazepam	78	72	85	85	82	94	71

Analyte recovery was observed to vary quite significantly with the pH of the extraction solution and in most instances there appears to be no significant trend between the determined levels of analyte recovery and the pH at which the extraction was performed. It was expected that recovery would increase with deceasing solution pH as all analytes under investigation are weakly basic. Such an effect was expected to be of particular significance for benzoylecgonine, which exists as a zwitterion in neutral solution, whereby performing the extraction at an acidic pH should result in protonation of the acidic functionality of the molecule (pK_a 2.25) and therefore, minimise any possible electrostatic repulsion from the similarly charged sulphonic acid functionality of the extraction sorbent. However, such an effect appears to be absent, with acceptably high recovery of benzoylecgonine determined at investigated pH values in the range of pH 2.0-7.0.

Upon examination of the recovery data in Table S4, it was decided that pH 6.0 appeared to be the optimum pH for sample extraction, as levels of recovery for the majority of the chosen analytes were acceptable.

Matrix removal using selective washing.

From the SPE product literature it was recommended that washing be performed with solutions containing low proportions of organic solvent and also when using mixed mode cation exchangers the wash solution should also be acidic in order to 'lock' retained basic analytes onto the sulphonic acid sorbent.[17] The wash solvent investigated was a solution of 10% v/v Methanol in 0.1 M formic acid. In order to examine the effect this solution on the retained analytes, a 500 mL aliquot of a 5 μgL^{-1} spiked solution, prepared in MES buffer pH 6.0, was extracted using the Strata-XC™ SPE cartridges. The spiking level was deliberately higher than usually used during method development so as any analyte breakthrough during the sorbent washing procedure was clearly detectable upon LC-MS/MS analysis. Without allowing the cartridge run dry, the sorbent was successively washed with 20 x 1 mL portions of the wash solution, each of which was collected and individually determined using LC-MS/MS. After washing the sorbent with the first 20 mL of wash solution, a further 3 x 10 mL washings were performed leading to a total of 50 mL solvent washing altogether. The final mL of wash solution was collected and analysed in each of these instances. Upon complete sorbent washing, 500 µL of glacial acetic acid was then added to the sorbent and allowed to percolate slowly through the packed bed in order to aid with drying. In an attempt to ascertain as to whether or not the addition of the glacial acetic acid had any effect upon analyte retention, the 500 µL portion added was also collected and subsequently determined using LC-MS/MS.

The extracted ion chromatograms for both the protonated molecular ion and the MS/MS product ion transitions were generated for each of the test analytes in all of the determined wash solutions and also the 500 µL addition of glacial acetic acid. However, no traces of any analyte were detected in any of the collected wash solution fractions. Based upon this finding it was suggested that the sorbent could be washed with 50 mL of the 10% v/v methanol in 0.1 M formic acid solution without any significant analyte loss. Knowing the minimum volume of wash solvent that could be applied to the sorbent bed, the effect of washing upon a real sample matrix, in this case river water collected from the River Boyne, near Navan, Co. Meath, Ireland was

then investigated. Fig.S3 depicts the resulting chromatogram for a 200 ngL^{-1} spike in river water adjusted to pH 6.0.

From Fig.S3 it can clearly be seen that the washing procedure appears to be highly effective in removing any retained matrix components as large distinguished peaks, corresponding to the MS/MS product ion transitions, can easily be detected at a low spiking level in a real sample matrix.

Benzoylecognine stability during washing step

An acid catalysed esterification of benzoylecognine might occur during the washing procedure affecting the results observed for cocaine. However, such a procedure is thought not to occur due to the relatively low contact time between retained benzoylecognine and the wash solution of 10% methanol in 0.1 M formic acid. However, in order to test the hypothesis that an acid catalysed esterification may occur, individual 10 μgL^{-1} solutions of benzoylecognine and cocaine were prepared in both methanol and the wash solution. These solutions were allowed stand at room temperature for approximately an hour, after which time they analysed using direct infusion electrospray ionisation tandem mass spectrometry (ESI-MS/MS). In each instance only the protonated molecular ion was observed, (m/z 290 and m/z 304 for benzoylecognine and cocaine, respectively) with product ion transitions to m/z 168 and m/z 182 or benzoylecognine and cocaine. There was no evidence of any formation of cocaine in the solution of benzoylecognine prepared in 10% methanol in formic acid, similarly there was no evidence of hydrolysis of cocaine to yield benzoylecognine in the solution of cocaine prepared in 10% methanol in formic acid. Based upon the performed ESI-MS/MS study, it can be concluded that washing the sorbents with 10% methanol in formic acid does not create conditions wherein cocaine can be formed from retained benzoylecognine.

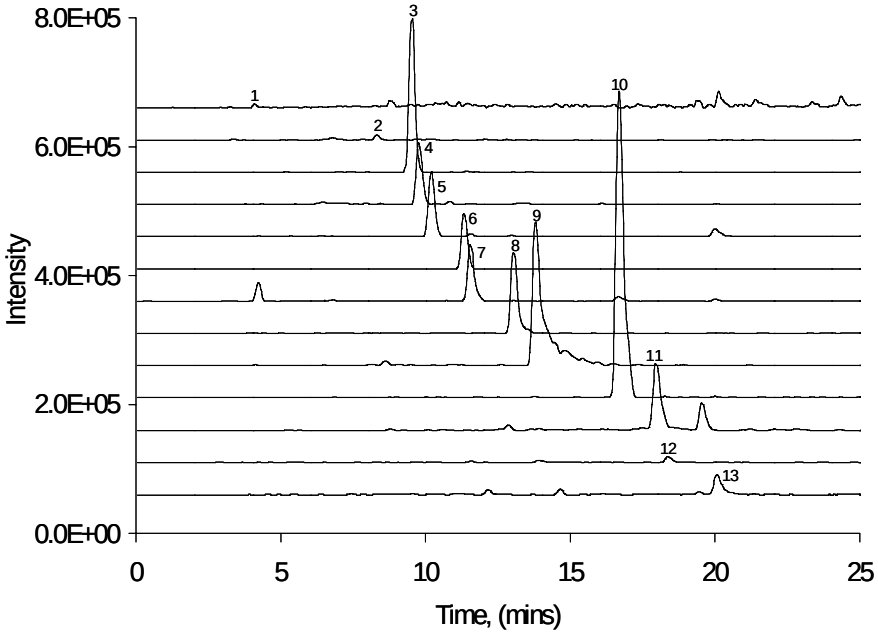

Fig.S3 Extracted ion chromatograms for a 200 ngL^{-1} mixed analyte spike solution prepared in river water collected from the River Boyne. Peak identification: **1** Morphine m/z 286 – m/z 268, T_R 4.1 mins; **2** MDMA m/z 194 – m/z 163, T_R 8.6 mins; **3** Benzoylecgonine m/z 290 – m/z 168, T_R 9.7 mins; **4** Ketamine m/z 238 – m/z 220, T_R 10.0 mins; **5** Cocaine m/z 304 – m/z 182, T_R 10.2 mins; **6** Cocaethylene m/z 318 – m/z 196, T_R 11.5 mins; **7** LSD m/z 324 – m/z 223, T_R 11.8 mins; **8** EDDP m/z 278 – m/z 249, T_R 12.5 mins; **9** Papaverine (Internal Standard) m/z 340 – m/z 202, T_R 13.9 mins; **10** Methadone m/z 310 – m/z 265, T_R 16.8 mins; **11** Temazepam m/z 301 – m/z 283, T_R 17.9 mins; **12** Fluoxetine m/z 301 – m/z 148, TR 18.3 mins; **13** Diazepam m/z 285 – m/z 257, T_R 20.0 mins.

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