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Rapid gas chromatography–mass spectrometry screening method for human pharmaceuticals, hormones, antioxidants and plasticizers in water

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Abstract

A rapid gas chromatography–mass spectrometry (GC–MS) method was developed and validated allowing quantification at the ng/l level of 19 analytes in water including human pharmaceuticals, hormones, antioxidants and a plasticizer. On-line continuous liquid–liquid extraction with dichloromethane of 10–40 l unfiltered water samples was used to achieve a 10 000–40 000–fold concentration factor. No sample cleanup or derivatization was required. Recoveries ranged from 57 to 120%. Application of the method to water recycling plant effluent demonstrated the presence of nearly all targeted compounds at ng/l to μg/l levels. Screening for nontarget compounds in the treated effluent samples indicated the method could be readily extended to include additional analytes.

Keywords: Water analysis; Extraction methods; Cosmetics; Drugs; Endocrine disruptors; Hormones; Antioxidants; Plasticizers

1. Introduction

Recent investigations in Europe, North America and Brazil have demonstrated the presence of a variety of endocrine disrupting compounds (EDCs), pharmaceutically active compounds (PhACs) and personal care product ingredients (PCPIs) in municipal wastewater and surface waters receiving treated effluent [1–27]. These compounds included analgesics, antibiotics, antiepileptics, hypolipidemics (blood lipid regulators), antineoplastics, antiseptics, β-adrenergic antagonists (β-blockers), β_2-selective adrenergic agonists, fragrance compounds, oral contraceptives, preservatives and psychiatric drugs. Reported concentrations were typically in the ng/l to low μg/l range.

Large quantities of prescription and non-prescription drugs are produced and used in the United States. For example, approximately 11 000 000 kg of antibiotics are used in human therapy annually [28]. Natural and synthetic steroidal hormones are administered as oral contraceptives and in hormone replacement therapy. PCPIs are similarly produced in large quantities and are present in myriad consumer products. Pharmaceuticals may be subject to metabolism in the dosed individual and are excreted as the unaltered parent compound, conjugates and/or other metabolites. Endogenous hormones are also excreted as part of normal human physiology. Some PhACs and PCPIs enter sewage treatment works when they are excreted, improperly disposed of or washed from skin surfaces. In wastewater treatment plants, some PhACs and PCPIs are subject to microbial transformation during biological treatment. However, many of these compounds are not efficiently removed by conventional wastewater treatment processes [29] and survive to enter receiving waters. Current ecotoxicity tests are insufficiently comprehensive to accommodate the large range of potential toxicants and broad spectrum of possibly subtle effects elicited by human pharmaceuticals, hormones and personal care product ingredients. Chemical characterization is therefore required to assess the ecological risks associated with PhACs and PCPIs in wastewater treatment plant effluent and surfaces waters [30].

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Methods examining the occurrence of PhACs and PCPIs in the environment have primarily relied on liquid chromatography with detection by electrospray ionization mass spectrometry (LC–ESI-MS) [21,23] or tandem mass spectrometry (LC–ESI-MS–MS) [13,22,25,27]. Analytes were typically enriched by solid-phase extraction (SPE) [13,21–23,25,27]; freeze-drying has also been used [13]. While LC–MS holds great promise in advancing understanding of the occurrence of potentially toxic anthropogenic compounds in the environment, the technology is expensive for routine analysis and is not readily available to many researchers at present.

Several groups have developed gas chromatography-mass spectrometry (GC-MS), methods to investigate the occurrence of PhACs and PCPIs in wastewater, surface waters and groundwater, typically extracting 1–2 l samples [6–12,14–19,20,24,26]. Nearly all GC–MS methods described in the literature use SPE followed by derivatization before analysis by GC–MS [6–12,15,18–20,24,26] and/or GC–MS–MS [8,9,11,12,17,20]. Other extraction methods employed with GC–MS methods include liquid–liquid extraction (LLE) [14], continuous 1 l recycling liquid–liquid extraction [16,18] and solid-phase microextraction (SPME) [14,19].

The US Geological Society recently conducted a national reconnaissance of the occurrence in USA streams of 43 pharmaceuticals, 12 steroidal hormones and 40 organic wastewater derived contaminants [5]. These target compounds were determined using five separate analytical methods: human and veterinary pharmaceuticals were measured using three tandem SPE and LC–ESI-MS methods; steroidal hormones were isolated by 1 l recycling continuous liquid–liquid extraction (R-CLLE), derivatized and analyzed by GC–MS; other wastewater related compounds were isolated by R-CLLE and determined by GC–MS [5]. The important results reported by Kolpin et al. [5] represented a significant analytical effort. However, the routine screening of samples for a broad spectrum of PhACs, PCPIs and EDCs using multiple analytical methods would be quite time- and resource-intensive. In addition, derivatization to decrease analyte polarity, enhance volatility and increase thermal stability may result in lower analyte recovery and analyst exposure to toxic derivatization reagents. The need exists for relatively simple methods allowing rapid, efficient, simultaneous quantitative analysis of numerous PhACs, PCPIs and EDCs without the need for derivatization.

In the above methods employing SPE and SPME, the effect on recovery of competition between dissolved organic matter and the solid-phase extractant for target analytes was not investigated. Recent theoretical treatment of SPME for complex environmental matrices indicated such competition may significantly impact quantitative analyses [31]. An advantage of LLE, on the other hand, is that analytes present at ng/l concentrations are sufficiently dilute so as to have thermodynamically independent partition coefficients. Thus, the analytes do not interfere with each other during the extraction as is possible with SPE and SPME.

An alternative approach to SPE and SPME to achieve high concentration factors is to extract large volume water samples. A 2 l/h on-line PTFE coil recycling high volume continuous liquid–liquid extraction method used to extract 10–40 l water samples has been described in the literature [32]. Recoveries of USEPA priority pollutants by this method were shown to be approximately 10% less than 1 l separatory funnel extraction [i.e., US Environmental Protection Agency (EPA) Method 625]. We employed a similar on-line continuous liquid–liquid extraction (O-CLLE) method in this study on a large volume 10 l/h continuous liquid–liquid extractor [33].

In this contribution, we describe the development of a rapid GC–MS screening method appropriate for routine measurement of multiple human pharmaceuticals, hormones, plasticizers and preservatives at ng/l levels in water samples. A short (12 m) column and steep temperature programming ramp (18 °C/min) allowed rapid GC separation followed by sensitive detection by MS in the selected ion monitoring (SIM) mode. Method detection limits between 8 and 85 ng/l were achieved without sample cleanup or derivatization. A 40 000-fold concentration factor was achieved by O-CLLE of water samples with dichloromethane (DCM). Obviously, the GC–MS method could be applied to SPE and SPME isolation procedures.

Targeted analytes included seven human pharmaceuticals, two drug metabolites, seven natural and synthetic steroidal hormones, two antioxidants and a plasticizer (Table 1). With one exception (the antiepileptic drug carbamazepine), the targeted human pharmaceuticals were among the 200 most prescribed drugs in the USA during 2000–2001, as were the hormones 17β-estradiol and 17α-ethinylestradiol [34]. Carbamazepine was one of the most widely used anti-seizure agents used at the time of this study [35]. The two antioxidants butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) are widely used in foods and food packaging, even though BHA is a possible human carcinogen [36]. Natural and synthetic steroidal hormones were included on the analyte list because such compounds have a demonstrated ability to modulate endocrine function in non-target organisms [30]. The need for efficient evaluation of these compounds in the environment provides incentive for developing a sensitive and rapid method to screen for their presence. Application of the method to water recycling plant effluent demonstrated the presence of nearly all targeted compounds. Results of non-target screening indicate the method can be readily extended for the analysis of further PhACs, EDCs and PCPIs.

2. Experimental

2.1. Chemicals

Analytical grade reference standards were purchased from Sigma–Aldrich (St. Louis, MO, USA). Standard stock

solutions of BHT, *N*-butylbenzenesulfonamide (*N*-BBSA), caffeine, carisoprodol, estrone, 17α-ethinylestradiol, gemfibrozil, ibuprofen, stanolone and testosterone were prepared in DCM. Diazepam, fenofibrate, 17β-estradiol and estriol stock solutions were prepared in acetone. Stock solutions for carbamazepine, clofibric acid and *p*-toluenesulfonamide (*p*-TSA) were prepared in methanol; those for BHA were in ethanol. Hormone stock solution concentrations were 2000 μg/ml; those for all other standards were 5000 μg/ml. Working solutions of the standard mixture were 200 μg/ml in estriol, 20 μg/ml in BHT and all other hormones, and 40 μg/ml in the remaining compounds. Stock and working standard solutions were prepared fresh monthly and were stored in at −20 and 4 °C, respectively.

DCM was GC pesticide grade (EM Science, Gibbstown, NJ, USA). Methanol and acetone were HPLC grade (Burdick and Jackson, Muskegon, MI, USA). Absolute ethanol was purchased from Quantum Chemical Corp. (USI Division, Tuscola, IL, USA).

2.2. On-line continuous liquid–liquid extraction (O-CLLE)

A 10 l/h PTFE coil recycling on-line continuous liquid–liquid extraction at pH 3 was used to achieve high (up to 40 000-fold) concentration factors. The 10 l/h O-CLLE was developed based upon a 2 l/h CLLE developed by Wu and Suffet [37] and upgraded by Baker et al. [32]. The 10 l/h CLLE is comprised of water and solvent (e.g., methylene chloride) reservoirs and is pumped (via RHiCKC, Fluid Metering, Oyster Bay, NY, USA) at a water:solvent ratio of 10:1 at a rate of 10/(l/h) into two equivalent 9.75 m PTFE coil extraction columns coils (i.d. = 0.32 cm) rolled around a 10.2 cm plastic pipe, where the liquid–liquid extraction occurs. The mixture is continually pumped to a phase separation chamber where the water is drained off and the solvent is directed to an automated Kuderna–Danish EVACS-type continuous solvent evaporator system [38]. EVACS solvent evaporators use a level sensor to balance the amount of solvent entering the system and being recycled. The solvent is heated by two 240 W, 115 V, mica-band heating elements (Power Modules, Cheltenham, PA, USA). The final concentrated 10 ml extract is evaporated off-line in a micro-Kuderna–Danish after drying through a sodium sulfate Pasteur pipette column. Side-by-side comparison of the 2 and 10 l/h, CLLEs demonstrated equivalent recovery, but greater precision with the 2 l/h extractor at the trade-off of 5 times the speed of extraction. Both extractors are within the efficiency range of EPA method 625.

In the present study, large volume (40 l) water samples were acidified to pH 3 with phosphoric acid. Ascorbic acid (10 mg/l) and sodium sulfate (75 mg/l) were added to minimize oxidation, remove any residual chlorine and to salt out analytes. A 10:1 water-to-DCM ratio was maintained during the extraction process. The 40–60 ml volume of the final DCM extract was reduced to 1 ml using a Kuderna–Danish evaporative concentrator. Analyte recovery efficiency was determined by extracting triplicate 40 l water samples from a Milli-Q Plus water system (18 MΩ cm resistivity; Millipore, Bedford, MA, USA) spiked with 1 ml of the standard mixture (19 analytes and a surrogate standard) in DCM.

2.3. Liquid–liquid extraction

To provide a basis for comparison of O-CLLE extraction efficiencies, analyte recovery by separatory funnel liquid–liquid extraction of 1 l water samples was also determined [39]. A 500 ml Milli-Q water samples was adjusted to pH 3 with H_3PO_4 then spiked with 5 ml of the standard mixture in DCM and thoroughly mixed. Three 100 ml aliquots were removed, and to each aliquot 7.5 mg sodium sulfate and 1.0 mg ascorbic acid were added. Each aliquot was extracted in a separatory funnel with DCM using a 10:1 water to solvent ratio. DCM extracts were subsequently concentrated to 1 ml using a Kuderna–Danish evaporative concentrator.

2.4. GC separation and MS analysis

Gas chromatographic and mass spectrometric conditions are presented in Table 2. Fig. 1 shows the total ion chromatogram (TIC) for a recovery evaluation. A mass spectral library was prepared from the standard compounds and compared to published spectra (NIST98.L and PMW-TOX3.L drug library purchased from Agilent Technologies, Wilmington, DE, USA). An Agilent ChemStation G-1701DA was used for data acquisition and handling.

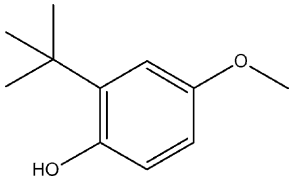
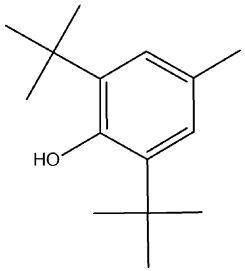
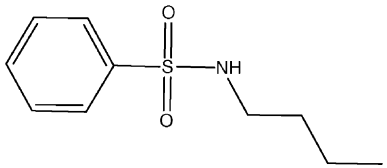
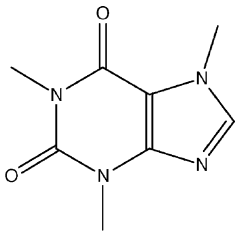
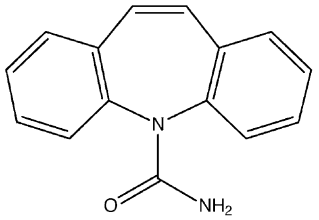
2.5. Limit of quantification and calibration

Method detection limits (MDLs) were determined according to [40] using 8 replicates. Calibration curves (6–10 points) were generated using the standard mixture over a concentration range of 0.2 to 40 μg/ml. [$^2H_{12}$]Chrysene (Chrysene-d_{12}) (10 μg/ml) was used as an internal standard, and [$^2H_{10}$]acenaphthene (acenaphthene-d_{10}) (20 μg/ml) was employed as a surrogate standard.

2.6. Sampling procedure

Large volume (40 l) treated effluent samples were collected from three water reuse plants in southern California. These plants were designated P1–3. In the state of California, treatment requirements for nonpotable reuse are contained in Title-22 (T-22), and wastewater that has undergone such treatment is referred to as T-22 water. These plants employed treatment trains that conformed to the requirements of T-22 and consisted of conventional primary and secondary treatment followed by flocculation, filtration and chlorine disinfection. T-22 effluent samples were collected prior to chlorine disinfection in solvent-rinsed amber glass bottles with PTFE-lined caps and represented 24 h composites. Samples were acidified to pH 3, stored on ice during

Table 1
Targeted analytes

Analyte	Chemical structure	Primary functional group(s)	Function
BHA		Hydroxy group para to methoxy group	Antioxidant
BHT		Phenolic group	Antioxidant
N-Butylbenzenesulfonamide		Secondary sulfonamide group	Plasticizer
Caffeine		Alkaloid, dioxo purine groups	Stimulant
Carbamazepine		Primary amide group	Antiepileptic

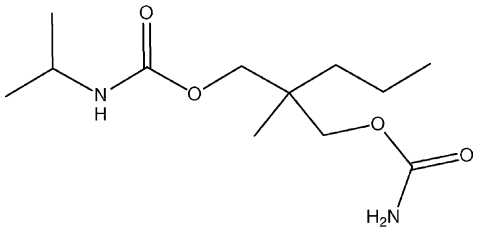
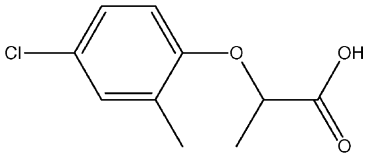
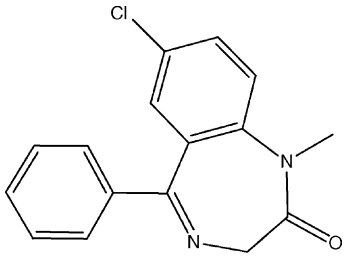
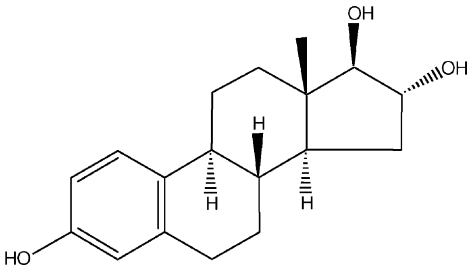
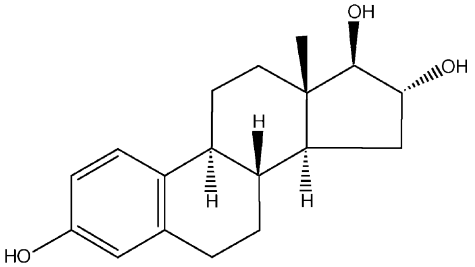
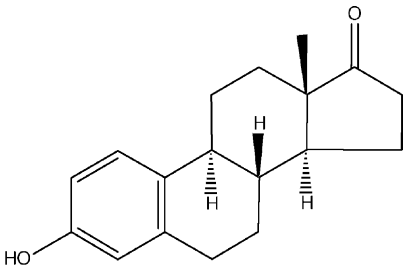
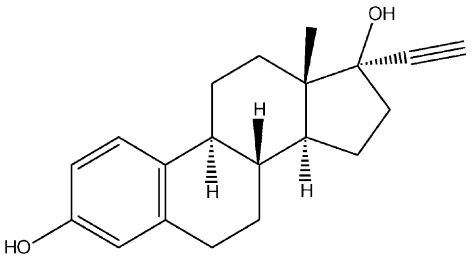
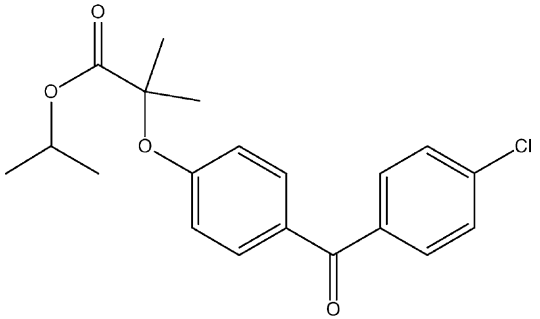
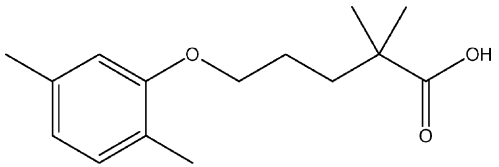
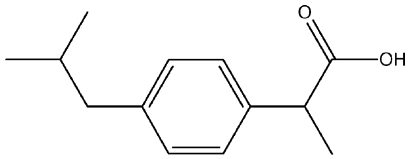
Carisoprodol		Dicarbamate	Skeletal muscle relaxant
Clofibric acid		Carboxylic, ether and chlorine groups	Blood lipid regulator
Diazepam		Lactam, phenyl and chlorophenyl groups	Psychiatric drug
17β-Estradiol		Hydroxy and phenolic groups	Hormone
Estriol		Hydroxy and phenolic groups	Hormone

Table 1 (*Continued*)

Analyte	Chemical structure	Primary functional group(s)	Function
Estrone		Phenolic and keto groups	Hormone
17α-Ethinylestradiol		Hydroxy, phenol and acetynyl groups	Synthetic hormone, contraceptive
Fenofibrate		Phenoxy, ester and para-chloro benzoyl groups	Blood lipid regulator
Gemfibrozil		Phenoxy group and carboxylic group adjacent to quaternary carbon	Blood lipid regulator
Ibuprofen		Carboxylic group	Analgesic/anti-inflammatory

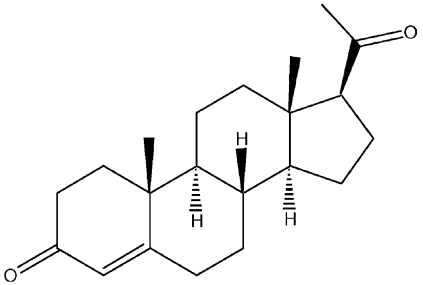
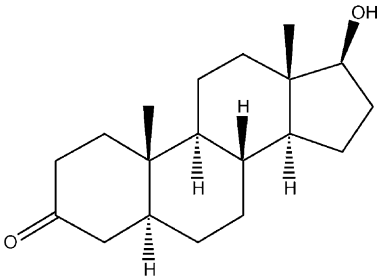
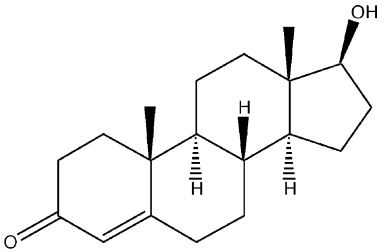
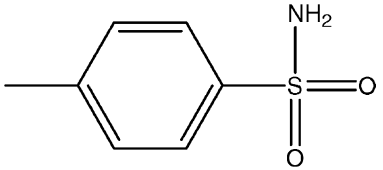
Progesterone		2 keto groups, C=C bond	Hormone
Stanolone[4-dihydro-testosterone]		Keto and hydroxy groups	Hormone
Testosterone		Keto and hydroxy groups, C=C bond	Hormone
p-Toluenesulfonamide		Primary sulfonamide group	Hypoglycemic metabolite

Table 2
GC–MS parameters

Gas chromatography	
Gas chromatograph	Hewlett-Packard 6890 GC system
Column	12 m × 0.20 mm i.d. HP-1 capillary column, 0.33 μm film thickness (J&W, Agilent Technologies, Wilmington, DE, USA)
Carrier gas	Helium (research grade, 99.99% purity), 0.312 bar
Temperature program	50 °C hold for 1 min; 18 °C/min to 285 °C; 8 min hold at 285 °C
Injection port	2 μl, splitless, 280 °C; after 75 s split valve opened and injection port purged at 45 ml/min
Mass spectrometry	
Mass spectrometer	Hewlett-Packard 5973 quadrupole mass-selective detector
Inlet system	Direct on-line, transfer line 285 °C
Source	EI, 70 eV, 230 °C
Mass analyzer	50–500 *m/z* scan at 3 scan/s, 1 mass unit resolution

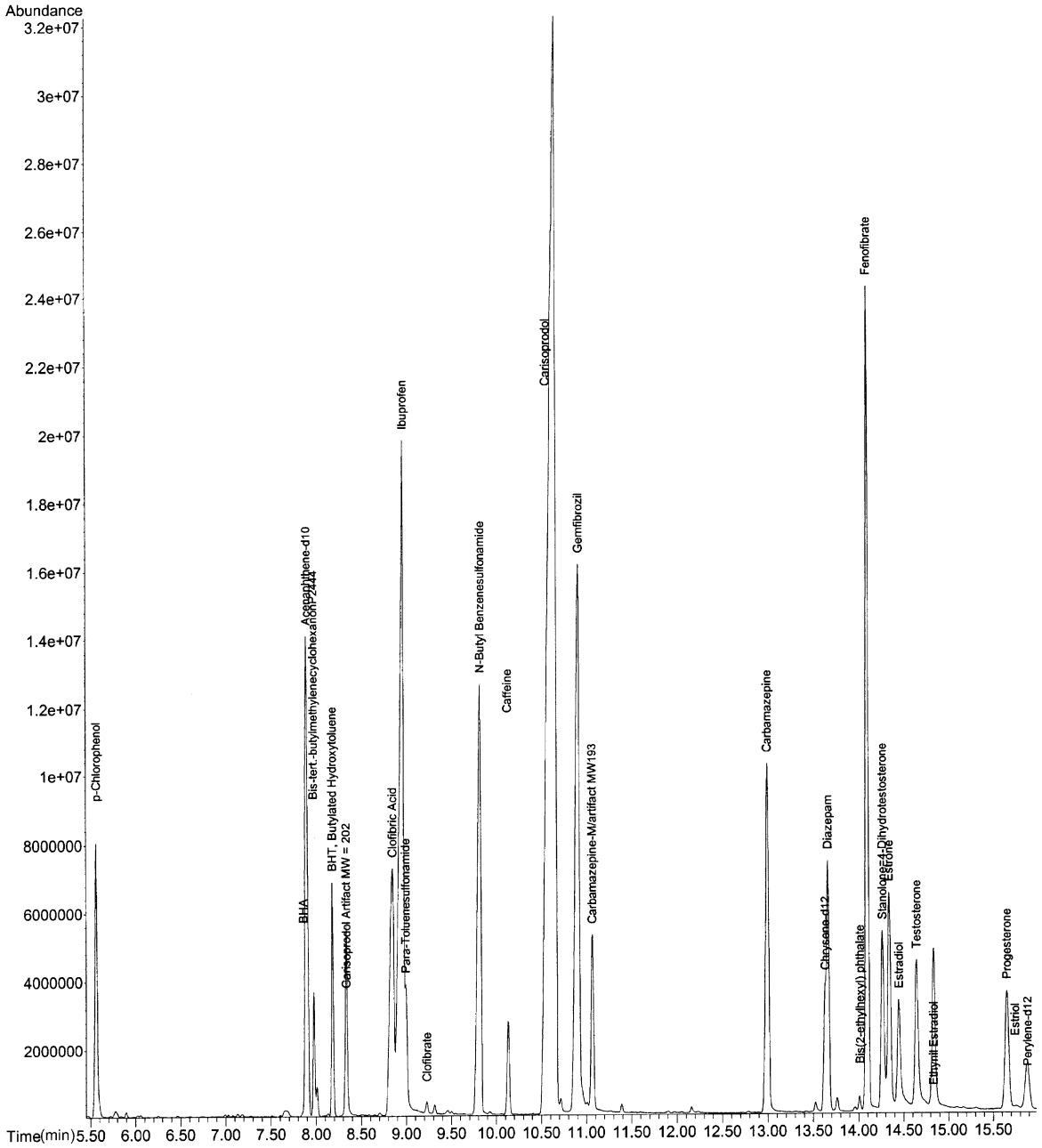


Fig. 1. Total ion chromatogram for the recovery analysis of targeted human pharmaceuticals and metabolites, hormones, antioxidants and plasticizers.

transport and refrigerated at 4 °C until processed. Effluent samples were extracted into DCM by O-CLLE as described above. Solvent was recycled, and the resulting ~40 ml extract concentrated to 1 ml by Kuderna–Danish evaporation achieving a concentration factor of 40 000.

2.7. Stability during storage

Treated effluent samples were collected, extracted into DCM and concentrated in 1999–2000, and analyzed by a broad spectrum GC–MS technique [41]. DCM extracts were stored frozen and analyzed in 2002 by the method described in this paper. Due to the time lag between sample extraction and analysis (in 2002) we examined the stability of the 19 target analytes during a 15 month storage period at 4 °C. Standard mixtures were prepared in DCM in 40 ml amber glass vials, stored at 4 °C and analyzed after 9, 10, 12 and 15 months. It is argued that if analytes proved stable at 4 °C for 9–15 months, they would also be stable at −20 °C for the same time period.

3. Results and discussion

3.1. LLE and O-CLLE recoveries

Analyte recovery efficiency at pH 3 was determined by O-CLLE (40 l) [33] and batch separatory funnel LLE (100 ml) [39]. Recoveries (mean ± standard deviation) for the 19 targeted compounds are presented in Table 3. For LLE, recoveries ranged from 43.8 to 132.4% with standard deviations between 3.4 and 21.4%. O-CLLE recoveries were between 15 and 120% with standard deviations ranging from 6.2 to 40.2%. For most analytes, recoveries did not differ significantly between LLE and O-CLLE ($P > 0.05$). The difference in mean extraction efficiency for estriol between LLE (60.5%) and O-CLLE (15.0%) was especially large. This difference is in part attributable to difficulties in quantitation as this compound eluted last, and its peak was typically broad. As expected, variance in O-CLLE recovery efficiency of 40 l samples at high ng/l to low μg/l levels was significantly higher ($P < 0.05$) than that for LLE of 100 ml samples at high μg/l levels for some analytes (viz., BHT, *p*-TSA, clofibric acid, estrone, 17α-ethinylestradiol, stanolone, testosterone and progesterone). Only for carbamazepine did LLE recovery variance significantly exceed that of O-CLLE ($P < 0.05$). When expressed as relative standard deviation, recovery variability for fenofibrate, gemfibrozil and progesterone were also higher in O-CLLE than LLE. R.S.D. for caffeine recovery by LLE was substantially higher than that for O-CLLE (37.2% versus 10.4%). The highest and lowest recoveries by O-CLLE were 17α-ethinylestradiol (120%) and estriol (15%); for LLE the highest and lowest recoveries were carisoprodol (132.4%) and caffeine (43.8%). The low recovery for estriol reflects its higher water solubility compared to the other steroidal hormones. The low recovery for caffeine at pH 3 (59.5 ± 6.2% and 43.8 ± 5.4% for O-CLLE and LLE) reflects its basic character ($pK_a = 14.0$ at 25 °C) [42].

Table 3
Mean absolute recoveries, standard deviations and relative standard deviations (%) for target analytes from triplicate spiked Milli-Q water samples at pH 3[a]

Analyte	O-CLLE			LLE		
	C_0 (μg/l)	Mean ± S.D. (%)	R.S.D. (%)	C_0 (μg/l)	Mean ± S.D. (%)	R.S.D. (%)
BHA	1	85.1 ± 16.8	19.7	400	80.1 ± 15.8	9.9
Acenaphthene-d_{10}	0.5	104 ± 17.7	17.0	200	96.9 ± 4.9	16.3
BHT	0.5	70.0 ± 24.0	34.3	200	91.3 ± 7.9	5.9
Clofibric acid	1	77.2 ± 24.7	32.0	400	90.6 ± 3.2	3.5
Ibuprofen	1	82.1 ± 18.9	23.0	400	94.8 ± 21.4	18.8
p-Toluenesulfonamide	1	77.8 ± 40.2	51.7	400	91.3 ± 10.2	8.0
N-Butylbenzenesulfonamide	1	113 ± 9.7	8.6	400	100.9 ± 17.5	10.0
Caffeine	1	59.5 ± 6.2	10.4	400	43.8 ± 5.4	37.2
Carisoprodol	1	113 ± 17.9	15.9	400	132.4 ± 16.5	12.5
Gemfibrozil	1	56.5 ± 10.9	19.3	400	89.1[b] ± 10.7	7.4
Carbamazepine	1	107 ± 3.8	3.6	400	114.6 ± 16.3	14.4
Diazepam	1	80.4 ± 17.8	22.1	400	114.3 ± 18.8	14.8
Fenofibrate	1	120 ± 30	25.0	400	78.6 ± 12.5	11.0
Stanolone	0.5	117 ± 21.7	18.6	200	110.1 ± 10.1	2.9
Estrone	0.5	81.2 ± 12.9	15.9	200	116.7[b] ± 16.1	2.3
17β-Estradiol	0.5	73.3 ± 17	23.2	200	113.9 ± 16.9	14.8
Testosterone	0.5	101 ± 20.5	20.4	200	89.2 ± 13.1	4.5
17α-Ethinylestradiol	0.5	120 ± 24.3	20.2	200	120.1 ± 15.1	3.0
Progesterone	0.5	89.1 ± 18	20.2	200	103.9 ± 5.0	4.8
Estriol	5	15 ± 3.4	22.7	2000	60.5[b] ± 10.0	16.5

[a] Abbreviations: C_0, initial aqueous concentration; O-CLLE, (40 l) on-line continuous liquid–liquid extraction; LLE, (100 ml) liquid–liquid extraction.
[b] Mean recovery for LLE significantly higher than for O-CLLE ($P < 0.05$).

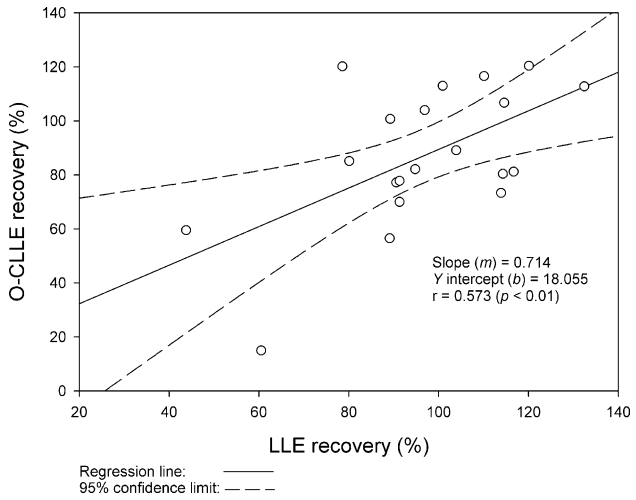


Fig. 2. O-CLLE vs. LLE recovery (%) of target analytes at pH 3. Data points represent mean recovery values.

O-CLLE recovery is plotted against LLE recovery in Fig. 2. LLE recovery was weakly, but significantly correlated with O-CLLE recovery ($r = 0.573$, $r^2 = 0.328$, $P < 0.01$). The slope of the regression line was 0.714. The figure also displays the 95% confidence interval of the regression line. Three compounds appear to be recovered quite differently by O-CLLE and LLE: estriol, gemfibrozil and fenofibrate. Estriol was the last compound to elute from the GC column. Peak tailing due to its longer retention time made quantification difficult and affected reproducibility (Table 4).

Extraction under acidic conditions maintained weak acids and phenols in their neutral, protonated state. Partial breakdown of pharmaceuticals containing amide functionalities (i.e., carisoprodol, carbamazepine) was observed under our gas chromatographic conditions as evidenced by the appearance of artifactual peaks. Degradation may have been due to thermal decomposition during injection or reaction at active sites on the column stationary phase. Although amides are generally more resistant to hydrolysis than esters [43], charge delocalization in these compounds affected their reactivity. Carisoprodol is a straight chain compound; the lone electron pairs of the carbonyl oxygen and the adjacent nitrogen stabilize the primary amide group. The secondary amide bond is weaker. The parent compound (molecular mass = 260 *u*) looses a 58 *u* fragment leading to a breakdown product peak at *m*/*z* 202. For carbamazepine (molecular mass = 236 *u*), electron delocalization in the ring system stabilizes the structure and allows the primary amide ring substituent to cleave under the column conditions employed, with the formation of the breakdown product peak at *m*/*z* 193. For both carbamazepine and carisoprodol, the concentration of parent compounds and breakdown products were quantified using the same quantification ion: total concentrations of carbamazepine and carisoprodol are reported based upon single ion monitoring at *m*/*z* 158 and 193, respectively.

Table 4
Relative retention times, method detection limits, monitored ions (SIM) and linearity for target analytes[a]

Analyte	RRT	TIC			SIM				
		MDL[b] (ng/l)	r^2	Linear range[c] (ng/l	Quantification ion *m*/*z*	Dwell time (s)	MDL[b] (ng/l)	r^2	Linear range[c] (ng/l)
Acenaphthene-d_{10}	0.573	30	0.986	12.5–1000 (10)	164	30	25	0.996	12.5–500 (10)
BHA	0.576	15	0.985	25–1000 (9)	165	10	10	0.994	25–1000 (10)
BHT	0.595	15	0.985	12.5–500 (10)	205	10	8	0.993	12.5–500 (10)
Clofibric acid	0.643	70	0.921	150–1000 (6)	128	20	25	0.978	25–1000 (9)
Ibuprofen	0.648	80	0.990	100–750 (7)	163	10	20	0.992	50–750 (8)
p-TSA	0.686	50	0.988	100–750 (7)	91	10	20	0.985	25–1000 (9)
N-BBSA	0.718	15	0.984	25–1000 (10)	170	15	10	0.995	25–1000 (10)
Caffeine	0.743	20	0.988	25–1000 (10)	194	10	15	0.996	25–1000 (10)
Carisoprodol	0.772	20	0.970	25–1000 (10)	158	25	10	0.993	25–1000 (10)
Gemfibrozil	0.796	55	0.984	100–1000 (5)	122	15	10	0.996	50–750 (8)
Carbamazepine	0.960	25	0.982	100–1000 (8)	193	10	10	0.992	25–750 (9)
Chrysene-d_{12}	1.000[d]				240	20			
Diazepam	1.005	35	0.997	25–1000 (10)	283	20	25	0.999	25–1000 (10)
Fenofibrate	1.037	25	0.997	25–1000 (10)	121	10	20	0.995	25–1000 (10)
Stanolone	1.058	25	0.999	12.5–500 (9)	231	10	10	0.992	12.5–500 (10)
Estrone	1.064	15	0.999	25–500 (8)	270	20	10	0.996	12.5–500 (10)
17β-Estradiol	1.084	40	0.999	50–500 (7)	272	25	20	0.985	25–500 (8)
Testosterone	1.087	15	0.979	50–375 (6)	124	10	10	0.987	12.5–500 (10)
17α-Ethinyl estradiol	1.094	25	0.998	25–500 (8)	213	10	20	0.994	12.5–500 (10)
Progesterone	1.146	25	0.999	75–500 (7)	314	20	10	0.994	12.5–500 (10)
Estriol	1.174	250	0.972	500–5000 (7)	288	30	85	0.975	500–5000 (7)

[a] Abbreviations: MDL, method detection limit; RRT, relative retention time; SIM, selected ion monitoring; TIC, total ion chromatogram.
[b] Based on a 40 l water sample.
[c] Number of points in calibration curve given in parentheses.
[d] Retention time for chrysene 13.38–13.40 min.

3.2. Gas chromatography–mass spectrometry

Table 4 lists targeted compounds in order of elution with their respective relative retention times, method detection limits and linearities. All analytes eluted in the Kováts retention index range of 1500–2600. For each extract analyzed, data were collected in both the TIC and SIM modes. The SIM mode was used to identify and quantify target analytes present, while TIC was used for confirmation. SIM was used with a single quantitation ion and two or three confirmation ions in most cases. (In a minority of the samples, only one confirmation ion was used.) Analytes were considered positively identified when the ratios of quantitation to confirmation ions were within ±10% relative to standards. Linear calibration curves were generated and method detection limits were determined for both modes. MDLs for the TIC ranged from 15 to 250 ng/l; those for SIM were between 8 and 85 ng/l using 40 l samples and isolation by O-CLLE. As expected, MDLs obtained by SIM were lower than those in the TIC mode, sometimes appreciably so (e.g., clofibric acid, *p*-TSA, gemfibrozil, estriol). Detection limits were lower for SIM by a factor of 1.3 to 2.8 for the majority of the analytes. The lowest MDL achieved in the SIM mode was for BHT (8 ng/l); the highest was for estriol (85 ng/l). For most compounds the detection limits achieved by this method compare favorably with

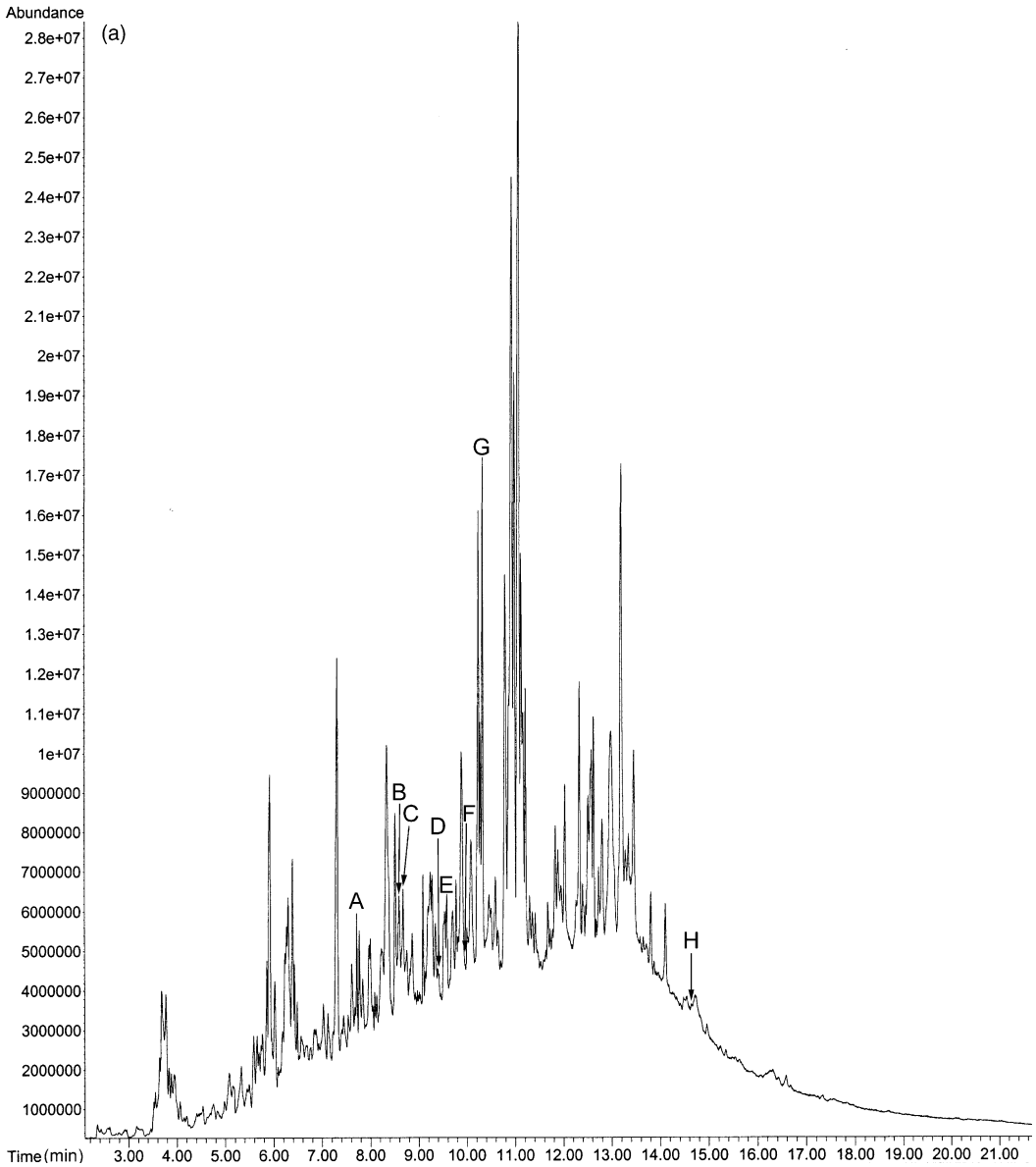


Fig. 3. Chromatograms from plant 3 Title-22 effluent obtained in (a) total ion current and (b) selected ion monitoring modes. Abbreviations: A, butylated hydroxyanisole; B, clofibric acid; C, ibuprofen; D, *p*-toluenesulfonamide; E, *N*-butyl benzenesulfonamide; F, caffeine; G, carisoprodol; H, 17α-ethinylestradiol.

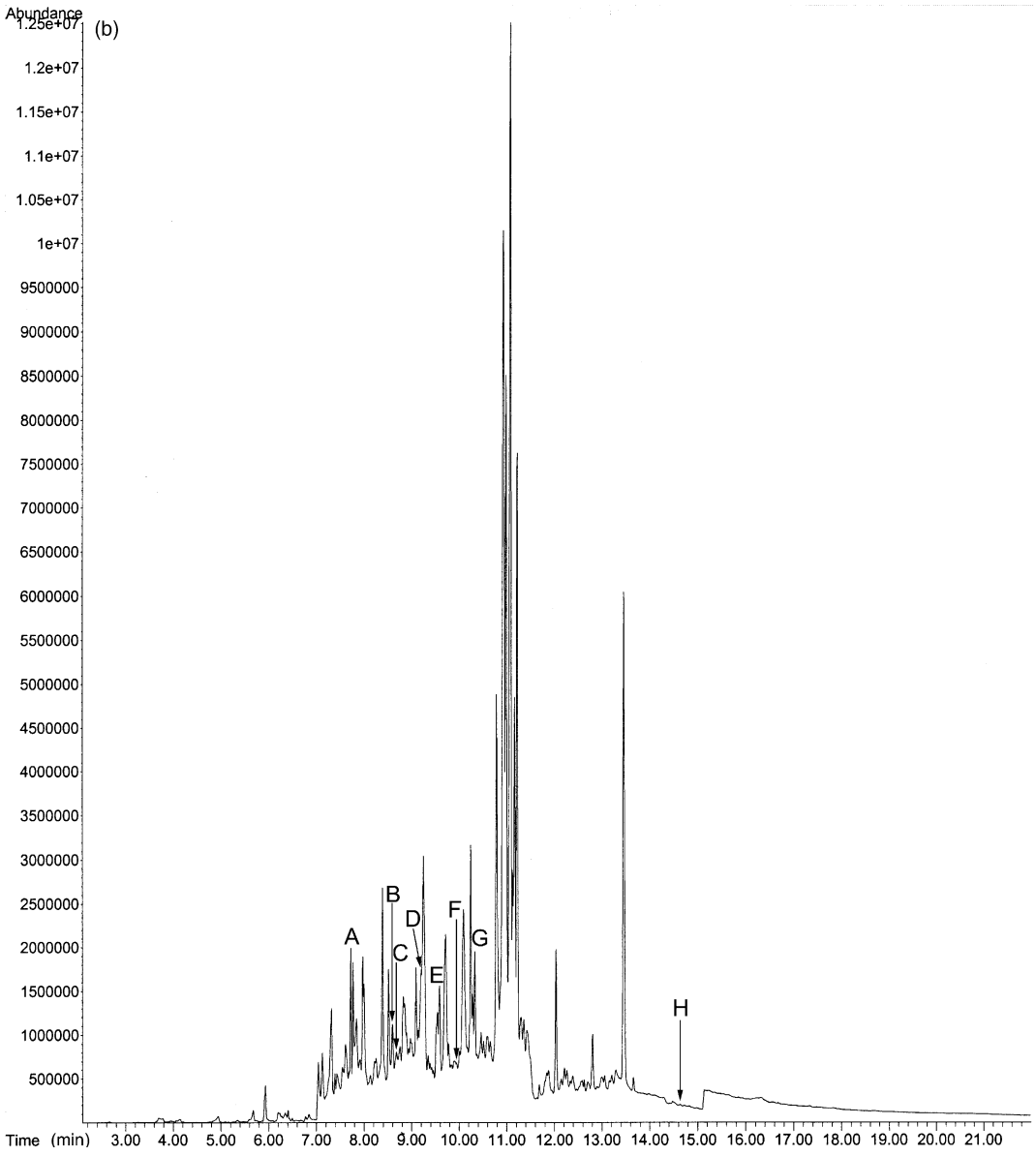


Fig. 3. (*Continued*).

those reported by others for GC–MS [5,15] and LC–MS [5,15,22] methods.

The utility of employing the TIC mode was apparent when additional EDCs, PhACs and PCPIs were tentatively identified in environmental samples extracts; e.g., synthetic musks, phthalates, alkylphenols, bacteriostatic agents, pesticides (cf. Section 3.4 below). The identity of HHCB was confirmed by comparison of the retention time and mass spectrum with that of the pure standard. Reported concentrations were not corrected for recovery.

Calibration curves were obtained for the 19 target compounds by linear regression of average total ion peak or selected ion peak versus concentration. Mean coefficients of determination (r^2) for calibration curves are presented in Table 4. In all cases, r^2 values for SIM mode were greater than 0.975 indicating good linearity. For TIC mode, r^2 values exceeded 0.970 except for clofibric acid ($r^2 = 0.921$).

3.3. Stability of analytes during extract storage

With the exception of estriol, all target analytes were stable in DCM under refrigeration. Estriol standard in DCM was stable for ten months with amounts recovered being 100% of its initial standard concentration. However, it degraded to 61 and 34% of initial concentration after 12 and 15 months, respectively. Due to matrix complexities in actual samples, these stability results cannot be directly extrapolated to environmental samples. Sample matrix effects may have resulted in differing, likely lower recoveries. These stability results however, gave an indication of the stability of the target analytes during storage of the environmental

Table 5
Human pharmaceuticals, hormones, antioxidants and plasticizers in Title-22 effluents from three water reclamation plants[a]

Compound	Concentration (ng/l)		
	P1	P2	P3
BHA	41	127	16
BHT	20	45	ND
Clofibric acid	ND	105	258
Ibuprofen	101	80	55
p-TSA	184	116	115
N-BBSA	910	351	520
Caffeine	ND	59	280
Carisoprodol	ND	ND	129
Gemfibrozil	43	ND	ND
Carbamazepine	48	3	ND
Diazepam	ND	ND	ND
Fenofibrate	1	ND	ND
Stanolone	ND	ND	ND
Estrone	4	42	ND
17β-Estradiol	ND	ND	ND
Testosterone	ND	ND	ND
17α-Ethinylestradiol	43	31	40
Progesterone	ND	ND	0.5
Estriol	ND	ND	ND

[a] P1, P2 and P3 represent water reclamation facilities in southern California.

samples DCM extracts between 1999 and 2000 (collection time) and June 2002 (GC–MS analysis time).

3.4. Application to water reuse plant effluent samples

Application of the rapid GC–MS screening method to T-22 effluent samples from the three water reuse plants in southern California revealed the presence of numerous target analytes (Table 5). Fig. 3 displays chromatograms acquired in the TIC and SIM modes for one T-22 effluent. Except for diazepam, 17β-estradiol, estriol, stanolone and testosterone, target analytes were detected in the effluent from one or more water reuse plant. The BHA, *N*-BBSA, ibuprofen, and *p*-TSA were detected in all samples. *N*-BBSA concentrations (351–910 ng/l) were high compared to most other target analytes. Plants 1–3 effluents contained 10, 10 and 9 target analytes, respectively. Five analytes (viz., BHA, ibuprofen, *p*-TSA, *N*-BBSA and 17α-ethinylestradiol) were present in effluent from all three plants. The concentration of 17α-ethinylestradiol was approximately the same in all effluent samples.

Although only those analytes listed in Table 2 were targeted, identified and quantified, additional chemicals were identified in the TIC mode. A variety of non-target organic wastewater contaminants were identified by spectral

Table 6
Non-target organic compounds tentatively identified in water recycling plant effluent

Compound	RRT[a]	SI value[b]	Note[c]	Compound	RRT[a]	SI value[b]	Note[c]
Human pharmaceuticals							
Naproxen	0.865	0.93	B				
Personal care product ingredients							
1-Acetonaphthone	0.640	0.95	B	Chlorophene	0.776	0.92	B
AHTN[d]	0.769	0.95	B	HHCB[e]	0.763	0.92	A
Benzophenone	0.646	0.96	B	Isobornyl propionate	0.524	0.90	B
Chloroxylenol	0.516	0.81	C	Vitamin E acetate	1.367	0.93	B
4-Chorobenzoic acid	0.492	0.90	B				
Flame retardant chemicals							
Tris(2-butoxyethyl) phosphate	0.975	0.91	B				
Fecal sterols							
Cholest-en-3-one	1.422	0.90	B	Cholesterol	1.316	0.99	B
Cholestan-3-ol	1.291	0.93	B				
Miscellaneous organic wastewater contaminants							
Benzyl alcohol	0.648	0.93	B	Erucylamide	1.108	0.91	B
Benzoic acid	0.391	0.90	B	2-(Methylthio)-benzothiazole	0.633	0.99	B
Benzothiazole	0.431	0.90	B	Ninhydrin	0.457	0.83	B
(1H)benzotriazole-5-methyl	0.591	0.96	B	Phthalic anhydride	0.457	0.91	B
Butoxyethoxy ethyl acetate	0.503	0.91	B	Triphenyl phosphine sulfide	1.049	0.92	C
2,4-Dichlorophenol	0.405	0.96	B				

[a] Retention time relative to the chrysene-d_{12} internal standard.
[b] Correlation of mass spectra with that in the US National Institute of Standards and Technology (NIST) library.
[c] Explanation of notes: (A) compound identity confirmed by comparison of mass spectrum and/or retention time to that of an authentic standard. (B) Confidence in structure high. Excellent match between mass spectrum and that of EI library (SI ≥ 0.90); no other close matches. (C) Confidence in structure moderately high. Good match between mass spectrum and that of EI library (SI = 0.75–0.89), no other close matches. Criteria applied to designate the level of confidence for identification and were derived from literature references [39,42].
[d] Likely identity is the synthetic musk AHTN (7-acetyl-1,1,3,4,4,6-hexamethyltetrahydro-naphthalene, Tonalide) not contained in the NIST library. The library search routine found a high correlation with the mass spectrum of the structurally similar, discontinued synthetic musk ATTN (7-acetyl-6-ethyl-1,1,4,4-tetramethyltetralin, Versalide).
[e] HHCB is the synthetic musk 1,3,4,6,7,8-hexahydro-4,6,6,7,8,8-hexamethyl-cyclopenta[g]-2-benzopyran or Galaxolide.

matching against the NIST/EPA/NIH 98 mass spectral library and visual comparison of unknown and matched mass spectra. Compounds with their library search purity values and level of confidence associated with each tentative identification are reported in Table 6. Criteria applied to designate the level of confidence for identification are presented at the bottom of the table and were derived from literature references [41,44]. A total of 44 compounds (19 target and 25 non-target compounds) were present or tentatively identified in water recycling plant effluent. Once their identities have been confirmed, the method presented here could be employed for quantification of the non-target compounds using appropriate calibration curves provided O-CLLE recovery was acceptable and degradation did not occur during chromatographic separation. Non-target compounds included the antiseptic agent chloroxylenol; the disinfectant chlorophene; the anti-inflammatory drug naproxen; the preservative *p*-chlorobenzoic acid; the synthetic musks AHTN and HHCB; the fragrance isobornyl propionate; the perfume fixative benzophenone; the flame retardant chemical tris (2-butoxyethyl) phosphate; the polymer additive erucylamide; and the fecal sterols cholesten-3-one, cholestan-3-ol and cholesterol. The recovery study demonstrated the presence of *p*-chlorophenol, dibutyl phthalate and bis (2-ethylhexyl) phthalate as laboratory contaminants likely from the DCM extraction and concentration steps. We therefore cannot attribute the occurrence of these compounds in sample extracts to their presence in treated effluent.

4. Conclusions

Potential ecological and human health risks associated with the presence of human pharmaceuticals, hormones and PCPIs in surface waters and drinking water necessitate the development of rapid, sensitive and direct analytical methods to support investigations of their occurrence and environmental behavior. A rapid GC–MS method was developed to quantitatively analyze 19 organic wastewater contaminants in less than 23 min with detection limits in the ng/l range. The GC–MS method required no derivatization or cleanup. This analytical method can be used in conjunction with a variety of extraction methods. When extraction was performed at pH 3, recoveries ranged from 15 to 120% for 40 l O-CLLE and 44–132% for 100 ml LLE. MDLs were between 15 and 250 ng/l in the TIC and between 8 and 85 ng/l in the SIM data acquisition modes. Identification of non-target compounds using the TIC data acquisition mode suggested that the method can be readily extended for the analysis of further PhACs, hormones and PCPIs.

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