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On-line solid phase extraction LC–MS/MS analysis of pharmaceutical indicators in water: A green alternative to conventional methods

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ABSTRACT

A method using automated on-line solid phase extraction (SPE) directly coupled to liquid chromatography/tandem mass spectrometry (LC–MS/MS) has been developed for the analysis of six pharmaceuticals by isotope dilution. These selected pharmaceuticals were chosen as representative indicator compounds and were used to evaluate the performance of the on-line SPE method in four distinct water matrices. Method reporting limits (MRLs) ranged from 10 to 25 ng/L, based on a 1 mL extraction volume. Matrix spike recoveries ranged from 88 to 118% for all matrices investigated, including finished drinking water, surface water, wastewater effluent and septic tank influent. Precision tests were performed at 50 and 1000 ng/L with relative standard deviations (RSDs) between 1.3 and 5.7%. A variety of samples were also extracted using a traditional off-line automated SPE method for comparison. Results for both extraction methods were in good agreement; however, on-line SPE used approximately 98% less solvent and less time. On-line SPE coupled to LC–MS/MS analysis for selected indicators offers an alternative, more environmentally friendly, method for pharmaceutical analysis in water by saving time and costs while reducing hazardous waste and potential environmental pollution as compared with off-line SPE methods.

1. Introduction

Advances in analytical methods have led to an increase in emerging contaminant detection in water around the world [1–8]. These emerging contaminants include, but are not limited to, pesticides, household chemicals, industrial chemicals, hormones, fire retardants, disinfection by-products, and pharmaceuticals. Many compounds from each of these groups could be classified as potential endocrine disrupting compounds (EDCs) [9–12]. Pharmaceuticals have become particularly important due to the recent media coverage which has reported that pharmaceuticals are being frequently detected in drinking water [5,13]. This has created elevated concern over the safety and quality of drinking water and an increase in the need for water testing by water utilities and regulatory agencies. Although, by design, pharmaceuticals have human health effects at their therapeutic doses, research continues to determine if there is reason for concern at the trace levels found in drinking water.

Pharmaceuticals enter the environment primarily via municipal wastewater effluent [14–17], which can then migrate through water systems and into source water intended for drinking water supplies. Advanced wastewater treatment processes (i.e. ozonation or UV advanced oxidation) have been shown to significantly reduce the concentrations of emerging contaminants in wastewater before they enter the environment [18]. However, not all compounds are completely removed by each of these treatment techniques [18–21] and not all wastewater treatment plants employ the use of advanced treatment processes. With this comes concern over the quality and safety of the finished drinking water that is impacted by wastewater. Therefore, it is important to monitor occurrence and treatment system performance in order to assess potential ecological and human exposure. However, this can result in numerous costly and time-consuming water analyses.

Pharmaceutical detection in the environment is becoming more pronounced partly due to advances in analytical technology. As instrumentation and methodology improve, laboratories can detect compounds at lower levels than previously thought possible. Recent methods can detect pharmaceuticals in water at low to sub-ng/L concentrations with minimal sample clean-up [2,22,23]. However, many emerging methods for trace contaminant analysis require collection and shipment of large sample volumes (>500 mL) along with multiple extractions and analyses which can be labor intensive and time consuming. There are also sizeable costs associated with these analyses due to materials and time needed to prepare, extract, and analyze each sample. Sample analyses typically require substantial volumes of potentially harmful solvents and chemicals used for sample extraction, glassware washing, and instrument cleaning. This generates large amounts of hazardous

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chemical waste as well as exposes laboratory personnel to dangerous chemicals and fumes. In addition to the chemical waste, there is also extensive waste created from the collection bottles (glass and/or plastic) and extraction supplies.

As awareness of more sustainable and environmentally friendly practices continues to grow, laboratories have also begun to look for ways to improve inefficient laboratory procedures and move towards "greener" alternative methods for environmental analysis. On-line solid phase extraction (SPE) is emerging as an effective technique for the analysis of trace contaminants, such as drugs of abuse, pesticides, pharmaceuticals, and hormones, in a variety of matrices (water, urine and plasma). This technique has been coupled to UV, triple quadrupole and ion trap detectors [24–29] with method reporting limits (MRLs) ranging from 0.69 to 10,000 ng/L, with higher MRLs reported in methods using UV detection and in biological fluid matrices. Many of these methods require pre-concentration steps, complex SPE column switching set-ups, multiple software programs, or only apply to a limited amount of matrices. A previous review comparing on-line SPE to off-line SPE reported that while on-line SPE offered many advantages, it also had numerous disadvantages and limitations including the complexity of the valve-switching set-ups and operation, lack of flexibility as compared to off-line SPE, and possible matrix interferences from loading the entire extracted sample [30]. Most of these problems have been resolved with recent advances in automated on-line SPE systems, integrated and flexible software programs, and application of tandem mass spectrometry (MS/MS) detectors for better selectivity and sensitivity. In addition to these, on-line SPE offers further advantages, such as small sample volumes, minimal amount of solvents required for extraction and instrument cleaning, little or no sample preparation, while yielding comparable method reporting limits.

A fully automated method for pharmaceutical analysis using on-line SPE directly coupled to liquid chromatography tandem mass spectrometry (LC–MS/MS) was developed and evaluated in four different water matrices. Six pharmaceutical indicator compounds were used to test the accuracy and precision of the on-line SPE method in various water matrices, such as finished drinking water, surface water, wastewater effluent and septic tank influent. To validate the on-line SPE method, samples were extracted and analyzed alongside a traditional off-line SPE method with a similar LC–MS/MS method for detection and quantification. All compounds, for both extraction methods, were quantified using isotope dilution to correct for SPE inefficiency and matrix suppression. The on-line SPE method was shown to provide a robust, sensitive, and reliable method with results similar to the off-line SPE method. The on-line SPE method offers a more efficient alternative to traditional off-line SPE and a more environmentally friendly procedure for water testing and monitoring.

2. Experimental

2.1. Compound selection

Since it is unfeasible to monitor for every pharmaceutical, a more practical solution is to monitor a subset of selected pharmaceuticals that act as representative compounds which are specifically chosen to give valuable information regarding water quality and treatment process efficacy [31–33]. The six pharmaceuticals selected for this study (Table 1) were chosen based on the following criteria: (1) representation of a variety of physical properties, such as functional groups and polarity, (2) representation of a diversity of pharmaceutical classes, (3) high frequency of environmental occurrence, and (4) removal efficiencies by drinking water and wastewater treatment techniques. For instance, meprobamate has been shown to be resistant to ozonation, chlorination, and UV while carbamazepine and phenytoin are removed by ozonation, but resistant to chlorination and UV. Trimethoprim also is resistant to UV, but can be removed by chlorination and ozonation [18,34]. These selected indicator pharmaceuticals and their corresponding removal efficiencies using disinfection and oxidation treatment processes at typical treatment doses are summarized in Appendix A (Table 1). In addition to these four pharmaceuticals, both atenolol and primidone have also been shown to exhibit a high frequency of occurrence and concentrations [20,35–37] making them ideal indicators in assessing wastewater influence.

2.2. Materials

All standards and reagents used were of the highest purity commercially available. All pharmaceuticals were obtained from Sigma–Aldrich (St. Louis, MO, USA). Meprobamate-d_3 and trimethoprim-d_9 were obtained from Toronto Research Chemicals (Ontario, Canada). Phenytoin-d_{10} and atenolol-d_7 were obtained from C/D/N Isotopes (Pointe-Claire, Canada). Carbamazepine-d_{10} and primidone-d_5 were obtained from Cambridge Isotope Laboratories (Andover, MA, USA). All solvents were trace analysis grade from Burdick and Jackson (Muskegon, MI). Reagent water was obtained using a Milli-Q Ultrapure Water Purification System (Millipore, Bedford, MA, USA). All concentrated stocks were prepared in methanol and stored at −20 °C while mixed spiking solutions were prepared in reagent water and stored at 4 °C.

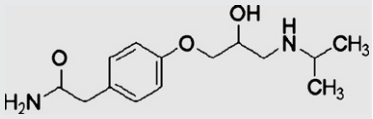
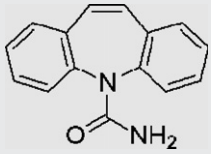
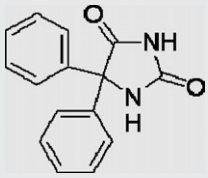
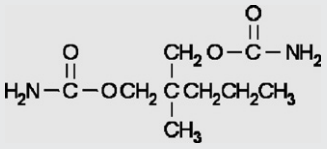
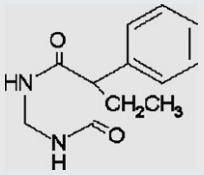
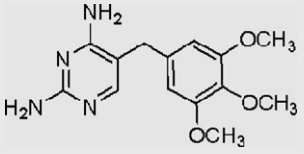
2.3. Sample collection

Samples were collected in 15 or 40 mL amber glass vials (Supelco, St. Louis, MO, USA) for on-line SPE samples and in 1-L amber glass bottles (Eagle-Picher, Miami, OK, USA) in the case of traditional off-line SPE. All sample containers contained 1 g/L sodium azide for preservation and 50 mg/L ascorbic acid to quench any residual oxidant. Samples were immediately refrigerated and stored at 4 °C until extraction. All samples were extracted within 7 days of extraction with the exception of preservation study samples. Septic influent water samples were filtered prior to extraction using a 90 mm, glass fiber filter (GF/F)(Whatman, England).

2.4. On-line solid phase extraction and liquid chromatography

All samples were extracted using a Symbiosis™ Pharma (Spark Holland, Emmen, The Netherlands) automated extraction-liquid chromatography system in the XLC mode operated through Analyst® 1.4.2 (Applied Biosystems, Foster City, CA, USA). The autosampler (Reliance™, Spark Holland) was adapted to hold 48 vial trays. Although only 1 mL of sample is necessary for extraction and analysis, a volume of 10 mL was measured in a volumetric flask before spiking isotopically labeled standards. This volume allowed enough sample for duplicates, matrix spikes, and dilutions if necessary. Each sample was spiked with 20 μL of an isotope dilution solution at 50 μg/L in reagent water, for a final concentration of 100 ng/L. A 1.5 mL aliquot of each sample was then transferred to a 2 mL autosampler vial. Autosampler vials were not completely filled so that a small amount of headspace would remain. This prevented contact between the outer pre-puncturing needle and the sample to minimize carry-over. Although only 1 mL of sample was loaded onto the SPE cartridges, 1.5 mL of sample volume was drawn up to over-fill the sample loop and to ensure a full and reproducible amount of sample was used for each extraction. Extractions were performed using Waters Oasis HLB Prospekt cartridges (30 μm, 2.5 mg, 10 mm × 1 mm, 96 tray) (Milford, MA). Prior to sample loading, each cartridge was sequentially conditioned with 1 mL of dichloromethane (DCM), MTBE, methanol, and reagent

Table 1
Target pharmaceuticals, physical properties, use and structures.

Compound	MW	Use	Log K_{ow} (pK_a)[a]	Isotopically labeled analog	Structure
Atenolol	266	Beta-blocker	0.16 (9.6)	Atenolol-d_7	
Carbamazepine	236	Anti-convulsant	2.45	Carbamazepine-d_{10}	
Phenytoin	252	Anti-epileptic	2.47 (8.33)	Phenytoin-d_{10}	
Meprobamate	218	Anti-anxiety	0.70	Meprobamate-d_3	
Primidone	218	Anti-convulsant	0.91	Primidone-d_5	
Trimethoprim	290	Anti-infective	0.91 (7.12)	Trimethoprim-d_9	

[a] SRC PhysProp Database [40].

water. Samples were loaded onto the SPE cartridge at 1 mL/min after which the cartridge was washed with 1 mL of reagent water. After sample loading, the analytes were eluted from the SPE cartridge to the LC column with 200 μL methanol, using the LC peak focusing mode.

A 5 mM ammonium acetate in reagent water solution (A) and methanol (B) gradient was used for LC mobile phases. Analytes were separated using a 150 mm × 4.6 mm Luna C18(2) with a 5 μm particle size (Phenomenex, Torrance, CA). Full XLC parameters, including the mobile phase gradient and flow rates, are shown in Table 2. For the first 2 min, the initial mobile phase flow rate was set at 700 μL/min, where it combined with the eluting methanol extract (at 100 μL/min), for a combined flow rate of 800 μL/min. After 2 min, when elution was completed, the mobile phase flow rate was increased to 800 μL/min. This ensured a constant flow rate (800 μL/min) throughout the entire extraction and LC program. The actual amount of time for each sample to process was 27 min, with approximately 5 min for SPE and 22 min for LC–MS/MS analysis. However, once the first sample has been eluted and LC–MS/MS analysis has begun, the automated on-line SPE system simultaneously begins extraction of the next sample due to the instruments ability to overlap extraction of one sample and analysis of the previous sample. Because sample extraction and analysis can continue to overlap in this manner, the perceived analysis time is approximately 22 min per sample.

Table 2
XLC parameters for on-line SPE.

Step	Solvent/source	Volume (μL)	Rate (μL/min)
Conditioning	DCM	1000	5000
	MTBE	1000	5000
	MeOH	1000	5000
Equilibration	DI	2000	5000
Sample extraction	Sample	1000	1000
Cartridge wash	DI	1000	2000
Clamp flush	MeOH	1000	5000
	DI	1000	5000
Elution (focusing)	MeOH	200	100
LC gradient (min)	***A* (%)**	***B* (%)**	**Rate (μL/min)**
0:01	100	0	700
2:00	100	0	700
2:01	90	10	800
2:30	90	10	800
2:31	50	50	800
10:00	0	100	800
17:00	0	100	800
17:01	90	10	800
22:00	90	10	800

2.5. Off-line solid phase extraction and liquid chromatography

The complete method description and parameters for automated SPE and LC separation has been published previously [23], along with reporting limits, calibration, and additional method QA/QC information. Briefly, samples were collected in 1 L amber glass, pre-silanized bottles preserved with 1 g/L sodium azide and quenched with 50 mg/L ascorbic acid. A 500 mL sample volume was spiked with isotopically labeled standards prior to extraction and extracted using an Autotrace® system (Caliper Corporation, Hopkington, MA) equipped with 5 mL, 200 mg HLB glass cartridges (Waters Oasis, Millford, MA). The SPE cartridges were first conditioned sequentially with MTBE, methanol, and reagent water. After conditioning, the samples were loaded onto the SPE cartridges. After sample loading the SPE cartridges were rinsed with reagent water and dried with nitrogen for 30 min. Analytes were eluted with a combination of MTBE and methanol into 15 mL calibrated centrifuge tubes. The extract was concentrated with a gentle stream of nitrogen to a final volume of 500 μL and then transferred to an autosampler vial for LC–MS/MS analysis. LC separation was performed using column and mobile phases described in the previous section, although a flow rate of 800 μL was used for the entire gradient and a 10 μL injection volume. Calibration curves ranged from 0.10 to 100 μg/L and were required to have correlation coefficients (R^2) greater than 0.99 with $1/x^2$ weighting.

2.6. Mass spectrometry

All analyses were performed using a hybrid triple quadrupole-linear ion trap mass spectrometer (4000 QTRAP, Applied Biosystems, Foster City, CA) using electrospray ionization (ESI) in positive MRM (multiple reaction monitoring) mode. Mass spectrometer parameters were determined previously for atenolol, trimethoprim, meprobamate, phenytoin and carbamazepine [23]. For primidone and primidone-d_5, parameters were optimized using the quantitative optimization feature available through the software (Analyst 1.4.2, Applied Biosystems) in a similar manner as the previously mentioned five compounds. Briefly, a 100 μg/L stock solution was prepared in methanol and infused into the mass spectrometer at 10 μL/min. Using the manual tuning, both ESI positive and negative modes were tested for precursor ions ($M+H^+$ or $M-H^-$ ions). Once an ionization mode and precursor ion was selected, the automated quantitative optimization feature was used to determine products ions, as well as their corresponding declustering potentials, collision energies, and exit potentials. A quantitation and confirmation transition was monitored for all analytes which are included in Appendix A (Table 2). The quantitation transition was selected to be the precursor to product ion transition that yielded the most abundant instrument response for the best sensitivity, while the confirmation transition was selected as the second most abundant in intensity.

2.7. On-line SPE method reporting limits and calibration

Method detection limits (MDLs) were determined by extracting 12 reagent water samples spiked with unlabeled analytes at 10 ng/L and isotopically labeled standards at 100 ng/L. The MDL was calculated by multiplying the standard deviation of the replicates by the appropriate Student's *T*-value for $n-1$ degrees of freedom at a 99% confidence level. Method reporting limits (MRLs) were chosen at greater than three to five times the MDL for each analyte. Precision tests were performed at 50 and 1000 ng/L in reagent water ($n=12$) to ensure instrument and method performance at low and high concentrations. Extracted calibration curves were performed using eight calibration points at 10, 25, 50, 100, 250, 500, 750 and 1000 ng/L for each analyte generated by appropriate dilutions of a 10 μg/L stock solution containing all six analytes. Calibration curves were prepared fresh every 60 days in reagent water and stored at 4 °C with no observable loss or degradation of analytes. Isotope standards were added at 100 ng/L to all calibration, blanks and samples, prior to extraction. Linear regression with $1/x^2$ weighting was used to generate calibration curves for all analytes with correlation coefficients (R^2) required to be greater than 0.99. A calibration verification standard was analyzed after every batch of eight samples to monitor the instrument's performance. Verifications had to be within ±20% of the original calibration standard or sample extractions and analyses were stopped. All compounds were quantified using isotope dilution and relative response ratios.

2.8. On-line SPE blanks

A series of unspiked reagent water samples ($n=7$) containing isotopically labeled standards were analyzed to ensure that the unlabeled compounds were not present in blank samples and that the isotope standards did not interfere with unlabeled analyte analysis. In addition, unspiked reagent water samples were analyzed at regular intervals to monitor blank contamination and instrument carry-over.

2.9. On-line SPE matrix spikes

Finished drinking water, surface water, tertiary wastewater effluent, and septic tank influent were spiked with unlabeled analytes for matrix recovery tests. For each water matrix, six spiked samples were extracted along with duplicate unspiked samples. Finished drinking and surface water matrices were spiked at 100 ng/L, while the wastewater effluent and septic tank influent were spiked at 500 ng/L due to the higher ambient concentrations of pharmaceuticals in those matrices.

3. Results and discussion

3.1. On-line SPE method development

Based on the calculated MDLs, all analytes had an MRL of 10 ng/L, except for atenolol which was 25 ng/L. Precision tests at both low and high calibration points resulted in low relative standard deviations (RSDs) for all compounds (2.5–5.4% for 50 ng/L and 1.3–5.7% for 1000 ng/L), indicating a high degree of reproducibility at both ends of the calibration range.

None of the pharmaceuticals were detected above their MRLs in the series of blank samples tested (data not shown) or in the blank reagent water samples that were extracted alongside each batch of samples. Blank reagent water samples were also extracted following high concentration standards and wastewater effluent samples using the same SPE cartridge. No carry-over was observed in these tests suggesting that the SPE cartridges were properly cleaned and conditioned after each extraction and could be re-used.

All four water matrices tested resulted in acceptable recoveries and precision for all six pharmaceuticals (Table 3). RSDs were well below 10% for all matrices, with most below 5%. Recoveries ranged between 88 and 118% for all six analytes in all four matrices tested. The only exception was atenolol in the septic influent water samples, where the matrix spike was not observable above the relatively large amount of atenolol already occurring in the septic water samples (38,000 ng/L). In order to correct for ambient concentrations already present in each water matrix, the average of duplicate samples was used for matrix spike adjustments.

Table 3
Method detection limits and reporting limits, average concentrations for unspiked water samples ($n = 2$), average matrix spikes recoveries ($n = 6$), and percent relative standard deviations for target pharmaceuticals using on-line SPE.

	MDL	MRL (ng/L)	Finished drinking water			Surface water			Wastewater effluent			Septic tank influent		
			Unspiked average (ng/L)	Spike recovery (%)	RSD (%)	Unspiked average (ng/L)	Spike recovery (%)	RSD (%)	Unspiked average (ng/L)	Spike recovery (%)	RSD (%)	Unspiked average (ng/L)	Spike recovery (%)	RSD (%)
Atenolol	4.56	25	<	101	3.8	<	115	2.7	810	88	7.6	38,000	na[a]	na[a]
Carbamazepine	2.75	10	<	97	5.1	<	113	3.4	190	117	1.9	<	100	6.0
Phenytoin	1.53	10	<	98	6.5	<	111	4.1	190	99	5.1	<	104	5.2
Meprobamate	2.03	10	<	97	2.7	32	104	2.3	520	100	2.6	11	112	1.5
Primidone	2.93	10	<	99	7.1	<	110	6.1	170	101	5.3	24	107	1.4
Trimethoprim	1.55	10	<	99	3.4	<	107	3.7	28	113	1.3	<	118	1.0

(<) Analyte concentration was below the MRL for on-line SPE method.
[a] Atenolol matrix spikes were not observable due to high ambient concentrations in unspiked samples.

3.2. Extraction methods comparison

To demonstrate the comparability of on-line SPE and traditional off-line SPE, a separate set of water samples were taken from various treatment stages of two drinking water treatment facilities, including raw intake and finished drinking water, and also samples from additional sites including wastewater effluent and Colorado River water. Matrix spikes from this study and previous research [23] have shown that both SPE methods have low method variability for the six pharmaceutical indicators, with RSDs less than 10% and most less than 5%, in a variety of water matrices. Results for all analytes detected by both extraction methods were in relative good agreement (Table 4) suggesting that the performance of the on-line SPE LC–MS/MS method is similar to the traditional SPE method. Two exceptions were carbamazepine in the April 2008 surface water and meprobamate in the May 2008 finished drinking water.

The only significant difference between the two extraction methods was their reporting limits. The traditional SPE method allows for a larger volume of sample, resulting in lower MRLs, which ranged from 0.25 to 1.0 ng/L for target pharmaceuticals. For wastewater and re-use water systems, low reporting limits are usually not as important, as compound levels tend to be larger, making on-line SPE ideal for these types of water systems. In addition, extracting less sample volume helps to minimize matrix suppression that can affect LC–MS/MS analysis [38].

Wastewater and reuse water samples were also extracted and analyzed using on-line SPE LC–MS/MS approximately 7 months after they were previously extracted by off-line SPE to evaluate storage and preservation techniques. Fig. 1(a) and (b) compare results from both extraction methods for two different wastewater treatment plants (WWTP1 and WWTP2) in Western Arizona, while Fig 1(c) compares results of a sample collected from a water recharge basin in Southern California. No significant differences were observed between the two methods suggesting that, for these water matrices, preservation by sodium azide (stored at 4 °C) was adequate in maintaining compound integrity for up to 7 months. This could indicate that shorter holding times are not absolutely necessary, but could possibly be extended without comprising the quality of sample data for these pharmaceuticals. Longer holding times could provide laboratories the flexibility to plan more efficient extraction and analysis schedules. Although the compounds tested here showed a longer holding time is possible, other pharmaceuticals would need to be evaluated before any conclusions could be made as to their stability during sample collection and extended storage.

3.3. Environmental implications

Although both extraction methods produced similar results, the differences in amount of materials, hazardous waste, and costs are profound. Table 5 compares the sample volume, total amount of solvents used for each extraction, and the estimated extraction time for six samples (since the off-line SPE method extracts in batches of six). One obvious advantage with on-line SPE is the elimination of solvents necessary for glassware and instrument cleaning. Table 5 also shows the size and weight of the collection bottles to approximate how shipping costs would significantly decrease with smaller sample volumes and less weight of the shipping contain-

Table 4
Comparison of results of two drinking water treatment facilities (1 and 2) and additional monitoring samples extracted side-by-side using on-line and off-line SPE methods (ng/L).

	Raw intake 1		Finished 1		Raw intake 2		Finished 2		Colorado river		Wastewater effluent	
	On-line SPE	Off-line SPE	On-line SPE	Off-line SPE	On-line SPE	Off-line SPE	On-line SPE	Off-line SPE	On-line SPE	Off-line SPE	On-line SPE	Off-line SPE
April 2008												
Atenolol	<25	***5.6***	<25	<1.0	<25	***5.3***	<25	<1.0	<25	<1.0	***820***	***790***
Carbamazepine	***11***	***5.4***	<10	<0.50	<10	***4.6***	<10	<0.50	<10	<0.50	***180***	***170***
Phenytoin	<10	***5.6***	<10	***1.0***	<10	***4.4***	<10	***2.0***	<10	<1.0	***190***	***170***
Meprobamate	***16***	***15***	<10	***7.6***	***12***	***13***	***10***	***9.4***	***15***	***13***	***500***	***460***
Primidone	<10	***4.1***	<10	***1.0***	<10	***3.6***	<10	***1.3***	<10	<0.50	***160***	***150***
Trimethoprim	<10	***0.9***	<10	<0.25	<10	***0.9***	<10	<0.25	<10	<0.25	***27***	***28***
May 2008												
Atenolol	<25	***2.6***	<25	<1.0	<25	***4.3***	<25	<1.0	<25	***4.0***	***620***	***630***
Carbamazepine	<10	***4.6***	<10	<0.50	<10	***3.5***	<10	<0.50	<10	***5.1***	***190***	***170***
Phenytoin	<10	***5.3***	<10	<1.0	<10	***4.5***	<10	***1.6***	<10	***4.6***	***180***	***190***
Meprobamate	***15***	***15***	<10	***6.3***	***14***	***12***	***11***	***8.4***	***15***	***14***	***600***	***530***
Primidone	<10	***3.7***	<10	***0.7***	<10	***3.0***	<10	***1.1***	<10	***3.8***	***150***	***140***
Trimethoprim	<10	***0.8***	<10	<0.25	<10	***0.8***	<10	<0.25	<10	***1.0***	***24***	***24***

Detections indicated in bold.

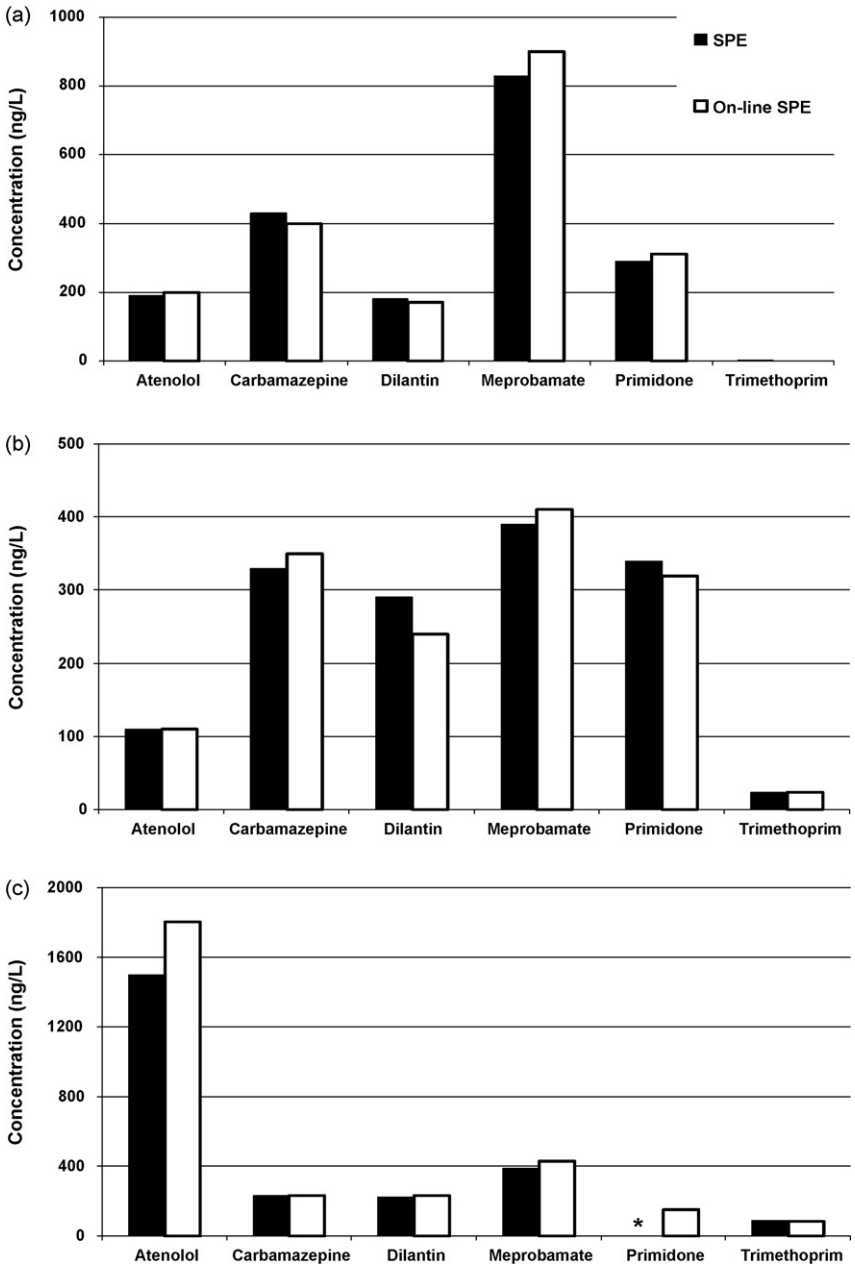


Fig. 1. Comparison of results for extended holding times. SPE (off-line) samples were extracted within 14 days of sample collection, while on-line SPE samples were extracted approximately 7 months later. (a) Wastewater effluent from WWTP1, (b) wastewater effluent from WWTP2 and (c) water recharge basin (*primidone not analyzed).

ers. These estimates use the example of the round trip cost to ship six samples between Southern Nevada (Clark County) to Southern California (Orange County) and are simply shown to depict how sample volumes and weights can affect overall costs. The amount of time it would take to process six samples is calculated from the automated extraction methods as well as time for sample evaporation and instrument analysis. Since the on-line SPE method does not require SPE cartridge drying or nitrogen evaporation of the extract, it significantly reduces the amount of extraction time to only a few minutes per sample.

In addition to source and finished drinking water, on-line SPE would be an ideal method for pharmaceutical analysis in wastewater and water-re-use monitoring programs where compound concentrations are expected to be larger and where trace level (sub-ng/L) reporting limits are not usually necessary. On-line SPE is also a useful tool for laboratory research such as evaluating new treatment technologies, compound removal experiments and laboratory bench scale tests, where large volumes of sample would be difficult to collect. It would also be beneficial for experiments, such as kinetic tests or experiments with many variables, in which numerous data points and faster turn around of results are desirable.

Although on-line SPE results in slightly higher MRLs as compared to the off-line SPE, it has been debated whether or not these lower MRLs are relevant or meaningful when these reporting limits are based purely on technology and advanced methodology and not health effects [39]. Snyder et al. [39] recommends MRLs that are approximately a hundred times less the acceptable daily intake as a drinking water equivalent (ADI-DWEL). Using this approach, health-based MRLs for atenolol, carbamazepine, phenytoin, meprobamate and trimethoprim would range between 0.1 and 67 μg/L (100–67,000 ng/L). Therefore, the on-line SPE report-

Table 5
Extraction method comparisons for sample volumes, volume of solvents used, extraction and analysis time, and estimated shipping costs to collect and process six samples.

	Off-line SPE	On-line SPE
Sample volume (mL)	3000	6
Solvents used during extraction (mL)	300	24
Solvents used to clean SPE instrument	480	0
Solvents used to clean glassware (mL)	510	0
Total amount of solvents used (mL)	1290	24
Extraction time, including evaporation (min)	270	30
Analysis time (min)	102	102
Total time (min)	372	107[a]
Collection bottle volume (mL)	1000	15
Weight of each collection bottle (g)	500	15
Total weight of collection bottles (g)	1500 (3 bottles)	90 (6 bottles)
Price to ship from LV to OC (empty)	$12.50	$0.75
Price to ship from OC to LV (full)	$37.50	$2.25
Total cost for sample collection	$50.00	$3.00

[a] On-line SPE can overlap sample extraction and analysis resulting in less time than combined total.

ing limits (10–25 ng/L) would still be 10–6700 times lower than the recommended MRLs.

On-line SPE coupled to LC–MS/MS analysis has been shown to be a rapid, sensitive, and robust alternative method to traditional off-line SPE. On-line SPE greatly reduces the amount of chemical waste due to the hazardous organic solvents necessary for extraction and glassware cleaning. For example, the on-line SPE method uses approximately 54-fold less solvent than the off-line SPE method, or less than 2% (Table 5). This would reduce the amount of environmental pollution generated by off-line SPE methods by 98%. It does not seem prudent to continue laboratory practices that generate considerable amounts of hazardous chemical waste in order to detect low ng/L levels of environmental contaminants. Laboratory methods need to progress towards more economical and sustainable methods for "greener" environmental analyses.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.talanta.2009.06.006.

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