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Trace determination of pharmaceuticals and other wastewater-derived micropollutants by solid phase extraction and gas chromatography/mass spectrometry

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ABSTRACT

The presence of pharmaceuticals and other wastewater-derived micropollutants in surface and groundwaters is receiving intense public and scientific attention. Yet simple GC/MS methods that would enable measurement of a wide range of such compounds are scarce. This paper describes a GC/MS method for the simultaneous determination of 13 pharmaceuticals (acetaminophen, albuterol, allopurinol, amitriptyline, brompheniramine, carbamazepine, carisoprodol, ciclopirox, diazepam, fenofibrate, metoprolol, primidone, and terbinafine) and 5 wastewater-derived contaminants (caffeine, diethyltoluamide, *n*-butylbenzene sulfonamide, *n*-nonylphenol, and *n*-octylphenol) by solid phase extraction (SPE) and derivatization with BSTFA. The method was applied to the analysis of raw and treated sewage samples obtained from a wastewater treatment plant located in the mid-Atlantic United States. All analytes were detected in untreated sewage, and 14 of the 18 analytes were detected in treated sewage.

1. Introduction

Widespread aquatic contamination by pharmaceuticals and other wastewater-derived organic micropollutants has been named as one of the key environmental challenges of the new millennium [1]. Exposure to such chemicals at environmentally relevant concentrations has been associated with a range of deleterious effects, among them are endocrine disruption [2] and reduced survival or reproductive success [3–5]. Such ecotoxic effects are of particular concern when contaminants are present as mixtures [6], as is often the case for waters receiving municipal wastewater discharges. While more than 100 pharmaceuticals have been detected in ground, surface, and sewage waters [7–11], this number represents only a tiny fraction of roughly 10,000 different drugs currently available as human therapeutic agents [12].

To date, most multi-residue methods for the trace determination of pharmaceuticals and other wastewater-derived micropollutants have utilized liquid chromatography coupled with detection by tandem mass spectrometry (LC/MS/MS) [13]. While such methods are quite powerful, the high cost of LC/MS/MS instrumentation places them out of the reach of many environmental researchers. In contrast, benchtop gas chromatography/mass spectrometry (GC/MS) systems are far more common in environmental laboratories, although relatively few methods have been published that utilize GC/MS for the routine analysis of pharmaceuticals in environmental matrices [14]. The main challenge in the analysis of pharmaceuticals via GC/MS is the presence of functional groups with "active" but poorly reactive hydrogens (e.g., amines, amides, aliphatic –OH groups, and some phenols), for which aggressive derivatization reagents are required [15]. The focus of the present paper is to present a GC/MS method capable of simultaneously analyzing a wide array of such compounds (often termed "basic" pharmaceuticals), along with some important neutral pharmaceuticals that do not require derivatization.

Several techniques have been successfully employed in the analysis of basic pharmaceuticals by GC/MS. Among the most popular of these is silylation (most commonly using MSTFA, BSTFA, or MTBSTFA), an approach successfully employed by numerous researchers to analyze pharmaceuticals of widely varying reactivity, including many with amine and phenolic functional groups [16–23]. Acylation and, to a lesser extent, alkylation have also been successfully used to derivatize basic pharmaceuticals, and these

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methods are frequently employed in a dual-step procedure in conjunction with silylation [17,18,24,25]. Although many previously developed methods have proven sufficiently sensitive for the analysis of environmental samples, with few exceptions [17,19] they have only been validated for a small number of analytes that are often closely structurally related (e.g., β-blockers [26] or steroid hormones [27]). A need still exists for multi-residue GC/MS methods that can be utilized for the routine analysis of a large and diverse array of basic pharmaceuticals, particularly in matrices as complex as municipal sewage.

This paper describes efforts to develop and validate a GC/MS method for the routine measurement of "base–neutral" pharmaceuticals and other wastewater-derived contaminants in aqueous milieu by SPE followed by derivatization and analysis via GC/MS. Six different silylating and acylating reagents were investigated, and experiments were conducted to optimize reaction time, temperature, and reaction solvent identity. SPE sorbent media identity, elution solvent identity, and extraction pH were also systematically investigated to optimize analyte recoveries. The optimized method was applied to the simultaneous analysis of 18 analytes (13 pharmaceuticals representing 12 therapeutic classes, and 5 other wastewater-derived contaminants) in untreated and treated municipal sewage. The analytes, shown in Fig. 1, were selected based upon their estimated usage, as well as their potential ecotoxicty [28]. Of these analytes, 6 (allopurinol, brompheniramine, ciclopirox, fenofibrate, primidone, and terbinafine) do not appear to have been sought by previous researchers in the United States.

2. Experimental

2.1. Chemicals and materials

Unless stated otherwise, all standards and reagents were of the highest available purity, and were used without modification. Details on the procurement, storage, and handling of analyte and isotopically labeled surrogate standards, as well as on the preparation of spiking and calibration solutions, are presented in Supplementary Materials.

2.2. Derivatization procedure

Reaction solvent identity, reaction time, reaction temperature and derivatizing agent identity were systematically varied to determine optimum derivatization conditions (Fig. 2). The optimization experiments were performed on 16 basic analytes (albuterol, atenolol, carbamazepine, carisoprodol, ciclopirox, furosemide, hydrochlorothiazide, hydroxychloroquine, labetalol, metoprolol, primidone, propranolol, trimethoprim, lamotrigine, oxcarbazepine, and triclocarban) selected for preliminary study, and the optimized method was subsequently tested on the final suite of analytes. Optimization of the derivatization reaction was performed using target analytes at concentrations ranging from 500 μg/L to 5 mg/L. Criteria used in assessing the success of derivatization included monitoring the background interference in GC/MS total ion chromatograms, the symmetry and sharpness of chromatographic peaks, completeness of derivatization (when this could be determined for neutral analytes), and the apparent derivatization yield for the target analytes under different experimental conditions.

MSTFA (33% TMCS, v/v), BSTFA (33% TMCS, v/v), MTBSTFA (33% t-BDMCS, v/v), MBTFA, HMDS (33% TMCS, v/v), and TMSI (33% TMCS, v/v) were investigated as derivatizing agents in neat form. For each of the five silylation reagents, 100 μL of derivatizing agent was added to analyte residue (evaporated to dryness from an acetone stock solution of target analytes), and the samples were allowed to react at room temperature, 60, 80, 100, and 120 °C (room temperature, 90 and 130 °C were explored for MTBSTFA) for 60 min. Experiments were similar for MBTFA, except that reactions were performed at 60, 80, and 100 °C for 20 min. Experiments were performed in duplicate or triplicate.

As BSTFA (containing 33% TMCS, v/v) appeared to perform best in initial experiments, additional tests were conducted to determine the reaction solvent identity that would optimize derivatization of the target analytes. Reactions were performed at 60 °C for 60 min using equal volumes of BSTFA (33% TMCS, v/v) and untreated acetonitrile (ACN), purified ACN (dried using an activated alumina column), pyridine, pyridine (dried as described above), ethyl acetate, dichloromethane (DCM), isooctane, tetrahydrofuran (THF), *N*,*N*-dimethylformamide, toluene, and ACN:THF:DCM (1:1:1, v/v). The effect of TMCS on the derivatization efficiency was investigated by varying its concentration (1, 10, 15, 20, 30, 33, 40%, v/v) in neat BSTFA, and allowing the solution to react at 60 °C for 60 min. The effect of subsequent reaction with MBTFA on the derivatization efficiency was also investigated. In these experiments, samples that had been derivatized with BSTFA (33% TMCS, v/v) at 60 °C for 60 min were then allowed to react with MBTFA at 50 and 60 °C for 20 min. The reaction in BSTFA (33% TMCS, v/v) was investigated at 60 °C for 5, 10, 20, 30, 40, 60, 80, and 120 min to determine the optimal reaction time under these conditions. Finally, the stability of the BSTFA-derivatized analytes was investigated by analyzing samples at 24, 48, and 72 h after derivatization. These experiments were performed in neat BSTFA (30% TMCS, v/v), and BSTFA (30% TMCS, v/v) with equal volumes of ACN, purified ACN, and ACN:THF:DCM (1:1:1, v/v). Again, all tests were performed in duplicate.

Method validation, as well as the analysis of environmental samples, was conducted using our optimized derivatization approach, in which 100 μL of BSTFA (33% TMCS, v/v) was added to 100 μL of ACN:THF:DCM (1:1:1, v/v) containing the target analytes at or near method detection limits (5–50 ng/L). This solution was heated at 60 °C for 60 min, followed by cooling to −20 °C for 10 min in a freezer, spiking with 50 ng of 4,4′-di-*tert*-butylbiphenyl-d_5 (used as an injection standard) in ACN, and analysis via GC/MS.

2.3. Solid phase extraction (SPE) procedure

In developing an SPE procedure, sorbent identity, pH, and elution solvent were selected as relevant parameters, and were systematically varied (Fig. 2). Performance was assessed by comparing post-SPE peak intensities of samples initially spiked at identical concentrations. As with derivatization, optimization of the SPE procedure was performed using target analytes at concentrations ranging from 500 μg/L to 5 mg/L. Oasis® HLB solid phase extraction (SPE) cartridges (200 mg, *N*-vinylpyrrolidone and divinylbenzene mixture, 30 μm particle size; Waters, Milford, MA), C18-U (un-end-capped silica), MCAX (both 200 mg; from Supelco, St. Louis, MO), Oasis® MCX (200 mg; Waters), Strata X, and Strata XC (both 500 mg; from Phenomenex, Torrance, CA) were tested as the sorbent phases. As with derivatization, the SPE procedure was specifically optimized for 16 basic analytes (see above), and then was subsequently applied to the other analytes.

To evaluate the recoveries of the different sorbents, all cartridges were conditioned with 10 mL of MeOH, followed by 10 mL of Milli-Q water adjusted to the sample pH. Aliquots (500 mL) of Milli-Q water spiked with analytes (at 1.0 μg/L) were passed through each sorbent material at 3–6 mL/min using a vacuum manifold. The Strata X, Oasis HLB, Oasis MCX, and Supelco C18-U cartridges were eluted with methanol. The Strata XC and Supelco MCAX cartridges were eluted with methanol (5% ammonium hydroxide, v/v). Care was taken to avoid allowing the cartridges to run dry during the loading and elution steps.

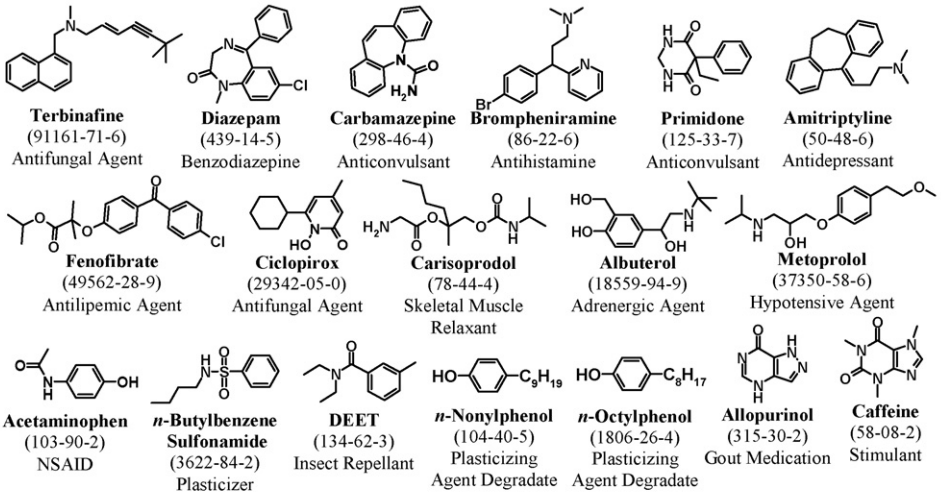

Fig. 1. Structures, CAS numbers, and usage classifications for the target analytes.

Additional tests were performed on Oasis® HLB cartridges to determine optimal sample pH and elution solvent. The optimal sample pH was determined under the conditions described above, using Milli-Q water/target analyte solutions that had been adjusted to pH 8–11. These same SPE conditions were used to determine the optimal elution solvent at pH 10. In each experiment, 7–10 mL of methanol, acetone, ACN, THF, ethyl acetate, acetone:ACN (1:1, v/v), ACN:THF (1:1, v/v), ACN:DCM (1:1, v/v), acetone:THF (1:1, v/v), diethyl ether:THF (1:1, v/v), DCM:THF (1:1, v/v), ACN:THF:DCM (1:1:1, v/v), ACN:THF:DCM (1:1:2, v/v), and ACN:THF:DCM (1:1:3, v/v) were evaluated as elution solvents.

Final optimized conditions adopted for method validation and sample analysis were as follows: filtered water samples were adjusted to pH 10 using 1.0 M NaOH, and were spiked with isotopically labeled surrogate standards (dissolved in acetone) at a final concentration of 500 ng/L for each surrogate. Oasis® HLB cartridges were conditioned by passing 10 mL of methanol and 10 mL of Milli-Q water (pH 10) through each cartridge by gravity. Water samples were then loaded onto the cartridges at a flow rate of 3–6 mL/min under vacuum. After extraction, the cartridges were dried under positive pressure with N_2 gas, and were stored (for convenience) overnight at −20 °C. Analytes were eluted under gravity using 7 mL of methanol. After elution, extracts were evaporated at ambient temperature under N_2 gas to apparent dryness, were reconstituted in 100 μL of ACN:THF:DCM (1:1:1, v/v), and were derivatized according to the optimized procedure described above.

2.4. Collection and analysis of wastewater samples

Grab samples of influent (7.4 L) and effluent (8.0 L) were collected in solvent-rinsed amber glass bottles from the Back River Wastewater Treatment Plant (BRWWTP) in Baltimore, MD on January 19th, 2008. Influent samples (250 mL) and effluent samples (500 mL) used for method validation were filtered through 1.2 μm Millipore GF/C filters (Bedford, MA) to remove suspended solids, and were extracted within 2 h of collection using the optimized SPE procedure.

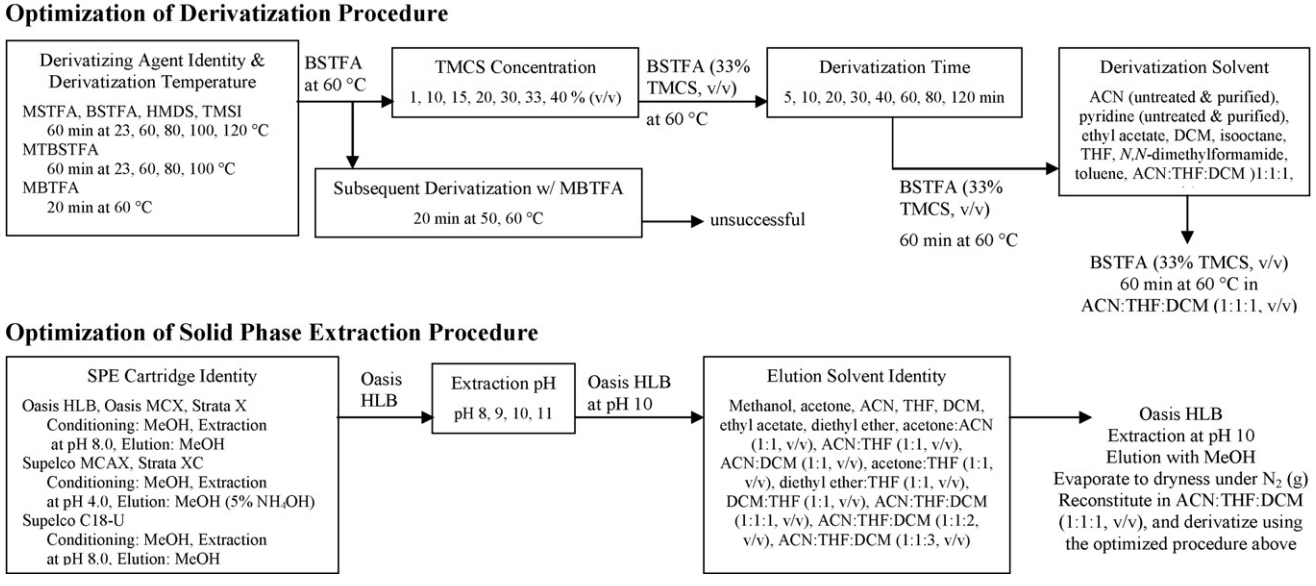

Fig. 2. Flowchart describing steps taken in optimizing the derivatization and solid phase extraction procedures.

Table 1
Retention times, analyte number, and monitoring and quantitation ions for target analytes and surrogate standards. Quantitation ions are shown in bold and monitoring ions in italics.

Target analyte [analyte number]	Retention time (min)	Monitoring and quantitation ions (m/z)	Target analyte [analyte number]	Retention time (min)	Monitoring and quantitation ions (m/z)
DEET [1]	10.52	*119*, **190**, *191*	*n*-Nonylphenol [10]	15.41	**179**, *277*, *292*
Carisoprodol [2]	10.76	**160**, *176*, *189*	*n*-Nonylphenol-d_3	15.41	**182**, *280*, *295*
Allopurinol [3]	11.02	**265**, *279*, *280*	Albuterol [11]	16.51	*350*, **369**, *440*
Ciclopirox [4]	12.14	**192**, *211*, *224*	Metoprolol [12]	17.39	**223**, *239*, *324*
Acetaminophen [5]	13.08	*166*, *181*, **223**	4,4′-Di-*tert*-butylbiphenyl-d_4 [IS]	17.63	**255**, *269*
Acetaminophen-d_3	13.14	*169*, *184*, **226**	Brompheniramine [13]	18.19	*167*, **247**, *249*
Primidone [6]	13.71	**146**, *232*, *334*	Amitriptyline [14]	19.28	*189*, **202**, *215*
n-Octylphenol [7]	14.04	**179**, *180*, *278*	Carbamazepine [15]	19.72	*165*, **193**, *250*, *293*
n-Octylphenol-d_3	14.04	**182**, *183*, *281*	Terbinafine [16]	19.86	*234*, *248*, **276**
Caffeine [8]	14.45	*109*, *165*, **194**	Diazepam [17]	22.15	**256**, *284*, *286*
Caffeine-d_3	14.45	*112*, *168*, **197**	Diazepam-d_5	22.15	**261**, *289*, *291*
n-Butylbenzene-sulfonamide [9]	14.60	*214*, **242**, *270*	Fenofibrate [18]	22.94	*232*, **273**, *360*

2.5. Quality assurance/quality control

The validation of the optimized method, as well as the determination of recoveries and occurrence in the wastewater samples, was conducted according to the guidelines laid out in EPA Method 526 for the determination of semivolatile organic compounds by SPE and GC/MS [29]. Briefly, method detection limits (MDLs) were determined in Milli-Q water by the extraction, derivatization, and analysis of 7 replicate laboratory-fortified blanks (LFBs) spiked at estimated detection limits (5–50 ng/L) for each target analyte. EPA Method 526 calculates the MDL as MDL = $St_{(n-1,1-\alpha=0.99)}$, where S is the standard deviation of the 7 replicates, and t is the Student's t-value for a 99% confidence interval. Method reporting limits (MRLs) were computed as three times the MDL, or the concentration of the lowest calibration standard, depending on which was higher. Analysis of each wastewater sample was performed in triplicate, and was accompanied by the analysis of two laboratory-fortified sample matrices (LFMs, spiked with surrogate and standard compounds, and used to compute analyte recoveries after correcting for background concentrations), two laboratory fortified blanks (LFBs, also spiked with surrogates and standard compounds), and one laboratory reagent blank (LRB), all treated identically. Five-point calibration curves were run before and after sample analysis, and a continuing calibration check (standard treated as sample) was performed every 3–5 samples. 4,4′-Di-*tert*-butylbiphenyl-d_5 was used as an internal standard, and was added before instrumental analysis.

2.6. GC/MS analysis

Analyses were conducted using a Fisons (currently Thermo Scientific; Waltham, MA) 8000Top gas chromatograph coupled to a Fisons MD800 quadrupole mass spectrometer. Aliquots (2 μL) of derivatized sample were injected in splitless mode (240 °C) onto a DB-5MS (Agilent; Wilmington, DE) 30 m length × 0.25 mm ID × 0.25 μm phase thickness column. The GC temperature program was: 105 °C for 1 min, ramp at 8 °C/min to 285 °C, with a final 10 min hold at 285 °C. Mass spectra were obtained in electron ionization mode (70 eV) with selected ion monitoring (SIM) and a filament/multiplier delay time of 8 min. Quantitation ions and monitoring ions are provided in Table 1. Additional analyses were conducted in full scan mode (m/z 100–614 with 0.35 s/scan) to acquire mass spectra for each derivative. Data were processed using *Xcalibur* software.

3. Results and discussion

3.1. Optimization of derivatization

3.1.1. Derivatizing agent identity

Five silylation reagents (MSTFA, BSTFA, HMDS, TMSI and MTBSTFA) and one acylation reagent (MBTFA) were investigated for the derivatization of the analyte suite. Greater emphasis was placed on silylation because it is less selective [15], and therefore was likely to be applicable to a wider array of analytes.

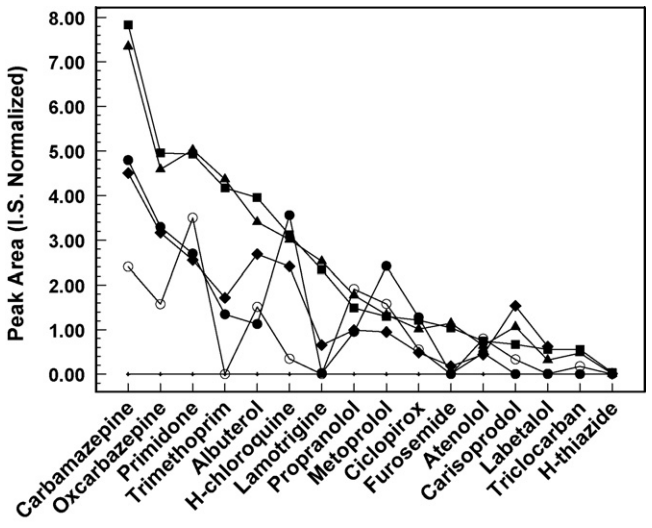

Fig. 3. Effect of derivatizing agent identity on peak area (internal standard-normalized) for 16 target analytes. Results are displayed for BSTFA (■; 33% TMCS, v/v), MSTFA (▲; 33% TMCS, v/v), MTBSTFA (●; 33% t-BDMCS, v/v), HMDS (♦; 33% TMCS, v/v), MBTFA (○), and TMSI (+; 33% TMCS, v/v). Experiments were conducted at 60 °C.

In identifying the optimal derivatizing agent, peak areas (normalized to the internal standard) were compared for a subset of 16 basic analytes after reaction with each derivatizing agent according to the conditions summarized in Section 2. TMCS (or in the case of MTBSTFA, t-BDMCS) was added at a concentration of 33% (v/v) to all of the silylation reagents. The results (Fig. 3) indicated that BSTFA (33% TMCS, v/v) and MSTFA (33% TMCS, v/v) were the most useful derivatizing agents tested, providing the greatest or second greatest peak areas for 14 and 13 of the 16 analytes investigated, respectively. In fact, the performance of these reagents was largely identical, although the BSTFA (33% TMCS, v/v) derivatives appeared to be slightly less stable at higher temperatures. MTBSTFA (33% t-BDMCS, v/v) performed less favorably, providing peak areas only 60–70% of those provided by BSTFA (33% TMCS, v/v) for most analytes. Further, MTBSTFA did not produce measurable derivatives for approximately one-fourth of the analytes tested. Both of these observations are likely attributable to steric hindrances encountered by the larger t-BDMCS group, though in some cases the thermal stability of the derivatives may be an issue. HMDS (33% TMCS, v/v) also performed more poorly than BSTFA or MSTFA, providing peak areas that were typically only 50% as large. As HMDS is generally regarded as a weaker silylating reagent [15],

this is not surprising. Interestingly, TMSI (33% TMCS, v/v) failed to produce measurable derivatives for most of the analytes (and for none of those provided in Fig. 3) under the conditions tested. This may be due to the paucity of readily ionizable hydroxyl groups among the target analytes. Finally, MBTFA performed somewhat poorly under the conditions tested, with only 60% of the tested analytes yielding derivatives that could be identified, despite the presence of numerous primary and secondary amines in the analyte suite. Multiple peaks were observed in the chromatograms from the MBTFA-treated samples, and analyses of mass spectra indicated that many of the analytes underwent decomposition during the derivatization reaction or subsequent analysis.

As many of the analytes targeted in this study possess both hydroxyl/phenolic and amine/amide functional groups, sequential derivatization with BSTFA (33% TMCS, v/v) and MBTFA was also investigated in an attempt to further increase the peak area of derivatives. In these experiments, the subset of basic analytes was allowed to react with BSTFA at 60 °C for 60 min and then with MBTFA at 50 and 60 °C for 20 min. This approach generally increased peak areas when only one primary derivative was formed (as was the case for atenolol, carisoprodol, and hydrochlorothiazide; data not shown), but led to the formation of multiple (in some cases as many as four) derivatives for a substantial number of compounds. This approach was, therefore, abandoned.

3.1.2. Other reaction conditions

By virtue of its performance in the screening tests and its relatively low cost, BSTFA was selected as a derivatizing agent. To further optimize the derivatization procedure, a series of experiments was conducted to determine the effects of TMCS concentration, time, temperature, and solvent identity on the BSTFA reaction. Data from these experiments are presented in Supplementary Materials. Briefly, the TMCS concentration had a minimal effect on the derivatization of most compounds, but increased peak areas for those compounds that possess deactivated amines (Table S1). A TMCS concentration of 33% (v/v) proved sufficiently concentrated to achieve optimal results for the target analytes. The effect of time on the derivatization reaction was significant for the first 60 min and less so at longer intervals (Table S2). A 1-h reaction period was selected for method validation. Peak areas were found to increase markedly from room temperature to 60 °C, above which a slight decrease was observed for most analytes (Table S3). As mass spectra indicated that analyte decomposition may have occurred at elevated temperatures, 60 °C was chosen for all further experiments.

Thirteen aprotic solvents and solvent combinations were investigated as reaction media. In general, reaction solvent identity had a minimal effect on peak area (Table S4), and an equal-volume mixture of ACN:THF:DCM (1:1:1, v/v) was chosen because it favorably reconstituted analyte residues. During preliminary investigations, we found that all derivatized analytes were degraded by at least 50% after 3 days. Neutral analytes were stable during that period. Efforts were made to improve the temporal stability of the derivatized analytes (including storing at −20 °C, sealing in glass ampules, and adding pyridine as a proton scavenger), but these proved largely unsuccessful. To compensate for the rapid decomposition of derivatives, sample runs were kept below 18 h, and full calibrations were run at the beginning and end of each run along with continuous calibration checks every four samples.

3.2. Optimization of solid phase extraction

3.2.1. Sorbent media identity and sample pH

Six solid phase extraction media were investigated during the optimization procedure. Emphasis was placed on the newer polymeric sorbents, as they are stable in basic pH regimes and have been used successfully by other researchers [13,14]. Oasis® HLB was selected for further analysis, in part because it was the sorbent capable of achieving measurable recoveries for the largest number of compounds tested.

As the analyte suite possesses both ionizable oxygen and nitrogen substituents, the pH at which the extraction is performed is likely to be very important. Results of experiments in which sample pH was varied are provided in Supplementary Materials (Table S5). Unfortunately, none of pH values investigated was found to provide optimal recoveries for all target analytes. This conclusion is supported by the observation that the pK_a values of the analytes span a range such that no pH exists where all of the compounds are predominantly neutral. A pH value of 10 was selected for further study, as it performed slightly better than others tested, and coincided with the upper operating range for the HLB sorbent media.

3.2.2. Elution solvent identity

Substantial effort was devoted to optimizing conditions for the elution of sorbed analytes. The broad spectrum of polarity encompassed by the analyte suite, and the need for a solvent capable of easy blow-down, were the dominant factors in selecting solvents and solvent mixtures for testing. Initial emphasis was placed on aprotic solvents, as protic solvents are unsuitable for conducting silylation reactions. In total, more than 16 solvents and solvent mixtures were explored, many of which are rarely utilized in SPE. Results are provided in Supplementary Materials. Among the more volatile solvents, recoveries were generally greatest in ACN:THF:DCM (1:1:1, v/v) when extracting microgram quantities (Table S6). In trace-level analyses, however, its use led to

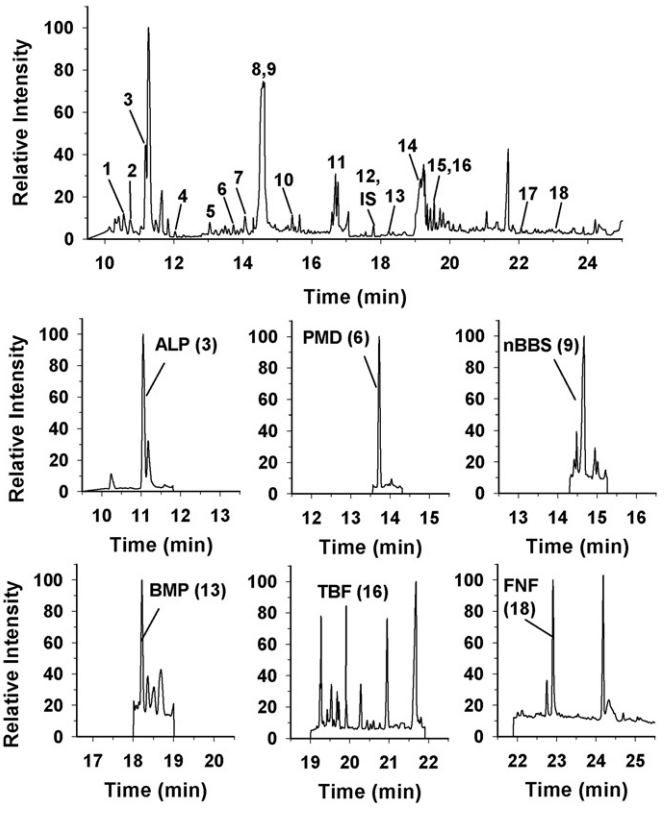

Fig. 4. Multiple ion chromatograms of 500 ng/L of target analytes in wastewater influent. Selected ion monitoring chromatograms for allopurinol (ALP), primidone (PMD), *n*-butylbenzene sulfonamide (nBBS), brompheniramine (BMP), terbinafine (TBF), and fenofibrate (FNF) are also presented for this sample. The sample was analyzed after concentration via SPE and derivatization using the optimized procedure. Refer to Table 1 for chemical identification.

interferences that precluded accurate quantitation. Further investigations indicated that this interference is caused by leaching of interferents from the SPE sorbent media. Methanol was subsequently selected as the elution solvent, and all extracts were evaporated to apparent dryness under N_2 (g) prior to derivatization. Experiments were also conducted to determine the minimum volume of elution solvent required for extraction; 7 mL was found to be sufficient.

3.3. Chromatographic resolution

The optimized method is capable of derivatizing all of the target basic analytes shown in Fig. 1, with the partial exception of carbamazepine, for which approximately 40% of the compound remained underivatized (as determined by comparing peak areas, and assuming identical molar response factors). The derivatization procedure does not appear to affect the stability or chromatography of the neutral analytes in the suite. Several pharmaceuticals, not shown in Fig. 1, proved poorly suited to analysis by this method. Cyclobenzaprine was found to be thermally unstable. Acyclovir, alprazolam, atenolol, ciclopirox, diltiazem, doxylamine, estazolam, furosemide, hexachlorophene, hydrochlorothiazide, hydroxychloroquine, labetalol, oxcarbazepine, phenazone, propranolol, trazodone, triamterene, triazolam, triclocarban, and trimethoprim were excluded because their peak intensities were extremely low under the optimized derivatization conditions.

Elution times, quantitation and monitoring ions for each analyte are listed in Table 1. A typical multiple ion chromatogram for 125 ng/L of each target analyte in Milli-Q water, after SPE and derivatization, is presented in Supplementary Materials (Fig. S1). The differences in peak intensity primarily arise from differences in molar response factors. Only caffeine and *n*-butylbenzene sulfonamide, and carbamazepine and terbinafine, were observed to co-elute. A stable baseline and minimal column bleed were observed. Fig. 4 shows a multiple ion chromatogram, as well as example selected ion monitoring chromatograms, for the base–neutral analytes in a lab-fortified (500 ng/L) grab sample of sewage treatment plant (STP) influent. Although the wastewater influent chromatogram is more complex than the Milli-Q water chromatogram, it has relatively few interfering peaks, and none that prevented accurate quantitation of target analytes.

3.4. Validation of proposed method

Method detection limit (MDL) values and method reporting limit (MRL) values were calculated using EPA Method 526 guidelines [29]. MDL and MRL values for the 18 target analytes are provided in Table 2. MDLs were in the range of 1–10 ng/L for all analytes except carbamazepine, whose incomplete derivatization may hinder quantitation. Analyte recoveries (determined using LFM samples) in Milli-Q water, wastewater influent and wastewater effluent are also presented in Table 2. In the case of wastewater samples, recoveries were computed after correcting for background concentrations of target analytes, as determined from the analysis of unspiked samples. Analyte recoveries varied widely within each matrix, though recoveries for analytes computed from laboratory-fortified field samples were generally in good agreement with those determined from isotopically labeled surrogates (when available). The wide variation in recovery among analytes demonstrates the importance of using isotopically labeled surrogates and/or laboratory-fortified field matrices in the analysis of field samples. Recoveries were below 50% for 7 of the 18 analytes under the optimized SPE conditions, though standard deviations were generally within 5–10% of the measured recoveries on these analytes, even in wastewater influent.

3.5. Application of methods to wastewater samples

In total, 18 out of the 18 analytes were detected in BRWWTP influent, and 14 were detected in BRWWTP effluent (Table 3). Acetaminophen, amitriptyline, caffeine, and ciclopirox could all be detected in full scan mode. When corrected for SPE recoveries,

Table 2
Analyte recoveries in Milli-Q water, wastewater influent and effluent. Also shown are method detection limits (MDLs) and minimum reporting limits (MRLs) for each analyte obtained from spiked Milli-Q water samples.

Compound	MDL[a] (ng/L)	MRL[a] (ng/L)	Recovery (%)[b]		
			Milli-Q water	Influent	Effluent
DEET	1	3	72 (2)	43 (0)	59 (1)
Carisoprodol	4	12	127 (6)	89 (11)	121 (5)
Allopurinol	1	3	92 (5)	78 (13)	74 (3)
Ciclopirox	3	9	111 (4)	53 (2)	119 (4)
Acetaminophen	2	10	22 (3)	9 (3)	13 (1)
Acetaminophen-d_3	–	–	18 (2)	15 (1)	18 (1)
Primidone	1	3	101 (7)	88 (4)	84 (0)
n-Octylphenol	1	3	56 (2)	41 (2)	41 (2)
n-Octylphenol-d_3	–	–	49 (1)	45 (3)	40 (0)
Caffeine	3	25	93 (4)	Unk[c]	69 (0)
Caffeine-d_3	–	–	100 (2)	39 (2)	75 (1)
n-Butylbenzene-sulfonamide	1	3	89 (1)	34 (2)	60 (1)
n-Nonylphenol	1	6	27 (2)	34 (1)	20 (0)
n-Nonylphenol-d_3	–	–	28 (1)	44 (3)	22 (1)
Albuterol	2	6	51 (1)	24 (4)	28 (3)
Metoprolol	4	20	46 (16)	46 (2)	64 (6)
Brompheniramine	–	–	77 (2)	109 (5)	93 (12)
Amitriptyline	6	18	95 (7)	93 (53)	79 (5)
Carbamazepine	30	90	106 (6)	30 (3)	97 (17)
Terbinafine	10	30	21 (1)	41 (1)	25 (1)
Diazepam	1	3	85 (6)	73 (4)	62 (2)
Diazepam-d_5	2	6	86 (10)	65 (2)	65 (1)
Fenofibrate	–	–	29 (1)	38 (0)	73 (3)

[a] Calculated using EPA Method 526 (see text for details).
[b] Calculated from LFM samples spiked at 125 ng/L (Milli-Q water) or 500 ng/L (wastewater influent and effluent), after correcting (in the case of wastewater samples) for concentrations in unfortified samples; numbers in parentheses represent one standard deviation for the analysis of two replicate samples.
[c] Unk means recovery could not be computed from the LFM because background concentrations in the sample were high relative to spiked concentrations.

Table 3
Concentrations (ng/L) of target analytes in Back River Wastewater Treatment Plant influent and effluent. Values in parentheses represent standard deviations for the analysis of three replicate samples. Note that reported concentrations have been corrected for SPE recoveries.

Target Analyte	Influent	Effluent	Target analyte	Influent	Effluent
DEET	450 (8)	120 (5)	*n*-Nonylphenol	130 (10)	103 (4)
Carisoprodol	410 (33)	141 (6)	Albuterol	780 (40)	110 (10)
Allopurinol	10 (4)	ND[a]	Metoprolol	770 (55)	130 (8)
Ciclopirox	1410 (38)	321 (25)	Brompheniramine	40 (11)	ND
Acetaminophen	>2000[b]	130 (8)	Amitriptyline	>2000[b]	1490 (58)
Primidone	130 (6)	100 (18)	Carbamazepine	600 (17)	140 (12)
n-Octylphenol	100 (10)	ND	Terbinafine	510 (18)	120 (31)
Caffeine	>2000[b]	60 (4)	Diazepam	40 (2)	40 (3)
n-Butylbenzene-sulfonamide	694 (55)	120 (5)	Fenofibrate	250 (16)	ND

[a] ND: not detected.
[b] Concentration is above the highest calibration standard.

concentrations of many analytes were roughly similar to those reported for other wastewater treatment plants [14,30], though influent concentrations of acetaminophen, amitriptyline, and caffeine were notably higher. The concentration of each of these compounds in BRWWTP influent was above the highest calibration standard (2000 ng/L); their concentrations could not, therefore, be accurately determined. In the case of acetaminophen, its poor SPE recovery may also have contributed to errors in accurate quantitation. If so, this highlights the caution that must be exerted when correcting for the recovery of poorly retained analytes, as substantial magnification of errors can occur. In total, 11 of the 14 target compounds detected in the effluent were present at concentrations greater than 100 ng/L. To our knowledge, 6 of the analytes detected in this study (allopurinol, brompheniramine, ciclopirox, fenofibrate, primidone, and terbinafine) have not been reported previously in the United States as environmental micropollutants.

4. Conclusions

A simple and reliable method has been developed for the analysis of so-called "base–neutral" pharmaceuticals and other wastewater-derived micropollutants in environmental samples. The method uses SPE, derivatization with BSTFA (33% TMCS, v/v), and analysis via GC/MS to achieve detection limits comparable to those obtained with LC/MS/MS. In so doing, the method aids in satisfying the environmental community's need for robust, multi-residue GC/MS methods for the trace analysis of an important class of aquatic pollutants. It has been successfully applied in this study to detect 6 of these pollutants (allopurinol, brompheniramine, ciclopirox, fenofibrate, primidone, and terbinafine) for the first time in the United States.

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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.chroma.2009.11.062.

References

[1] R.P. Schwarzenbach, B.I. Escher, K. Fenner, T.B. Hofstetter, C.A. Johnson, U. von Gunten, B. Wehrli, Science 313 (2006) 1072.
[2] S. Jobling, C.R. Tyler, Environ. Health Perspect. 114 (Suppl. 1) (2006) 7.
[3] J.L. Oaks, M. Gilbert, M.Z. Virani, R.T. Watson, C.U. Meteyer, B.A. Rideout, H.L. Shivaprasad, S. Ahmed, M.J.I. Chaudhry, M. Arshad, S. Mahmood, A. Ali, A.A. Khan, Nature 427 (2004) 630.
[4] T.B. Henry, J.W. Kwon, K.L. Armbrust, M.C. Black, Environ. Toxicol. Chem. 23 (2004) 2229.
[5] M. Crane, C. Watts, T. Boucard, Sci. Total Environ. 367 (2006) 23.
[6] F. Pomati, S. Castiglioni, E. Zuccato, R. Fanelli, D. Vigetti, C. Rossetti, D. Calamari, Environ. Sci. Technol. 40 (2006) 2442.
[7] D.W. Kolpin, E.T. Furlong, M.T. Meyer, E.M. Thurman, S.D. Zaugg, L.B. Barber, H.T. Buxton, Environ. Sci. Technol. 36 (2002) 1202.
[8] A. Nikolaou, S. Meric, D. Fatta, Anal. Bioanal. Chem. 387 (2007) 1225.
[9] K.K. Barnes, D.W. Kolpin, E.T. Furlong, S.D. Zaugg, M.T. Meyer, L.B. Barber, Sci. Total Environ. 402 (2008) 192.
[10] S.A. Snyder, J. Int. Ozone Assoc. 30 (2008) 65.
[11] C. Stamm, A.C. Alder, K. Fenner, J. Hollender, M. Krauss, C.S. McArdell, C. Ort, M.K. Schneider, Geogr. Comp. 2 (2008) 920.
[12] G.W.A. Milne (Ed.), Drugs: Synonyms and Properties, 2nd edition, Wiley, Hoboken, NJ, 2002.
[13] S.D. Richardson, Anal. Chem. 79 (2007) 4295.
[14] S.D. Richardson, T.A. Ternes, Anal. Chem. 77 (2005) 3807.
[15] K. Blau, J. Halket (Eds.), Handbook of Derivatives for Chromatography, 2nd edition, Wiley, Chichester, 1993.
[16] T.A. Ternes, R. Hirsch, J. Mueller, K. Haberer, Fresenius, J. Anal. Chem. 362 (1998) 329.
[17] J. Segura, R. Ventura, C. Jurado, J. Chromatogr. B 713 (1998) 61.
[18] M. Meisenheimer, T.A. Ternes, Vom Wasser 94 (2000) 203.
[19] K. Reddersen, T. Heberer, J. Sep. Sci. 26 (2003) 1443.
[20] S. Zuhlke, U. Dunnbier, T. Heberer, J. Chromatogr. A 1050 (2004) 201.
[21] J.B. Quintana, J. Carpinteiro, I. Rodriguez, R.A. Lorenzo, A.M. Carro, R. Cela, J. Chromatogr. A 1024 (2004) 177.
[22] J.B. Quintana, R. Rodil, S. Muniategui-Lorenzo, P. Lopez-Mahia, D. Prada-Rodriguez, J. Chromatogr. A 1174 (2007) 27.
[23] D.L. Lin, S.M. Wang, C.H. Wu, B.G. Chen, R.H. Lu, J. Food Drug. Anal. 16 (2008) 1.
[24] S. Paterson, R. Cordero, S. McCulloch, P. Houldsworth, Ann. Clin. Biochem. 37 (2000) 690.
[25] A. Solans, M. Carnicero, R. de la Torre, J. Segura, J. Anal. Toxicol. 19 (1995) 104.
[26] D.B. Huggett, I.A. Khan, C.M. Forana, D. Schlenka, Environ. Pollut. 121 (2003) 199.
[27] T.A. Ternes, M. Stumpf, J. Mueller, K. Haberer, R.D. Wilken, M. Servos, Sci. Total Environ. 225 (1999) 81.
[28] M.S. Kostich, J.M. Lazorchak, Sci. Total Environ. 389 (2008) 329.
[29] S.D. Winslow, B. Prakash, M.M. Domino, B.V. Pepich, D.J. Munch, Method 526 Determination of Selected Semivolatile Organic Compounds in Drinking Water by Solid Phase Extraction and Capillary Colomn Gas Chromatography/Mass Spectrometry (GC/MS)., National Exposure Research Laboratory Office of Research and Development, U.S. Environmental Protection Agency, Cincinnati, OH, 2000.
[30] J. Debska, A. Kot-Wasik, J. Namiesnik, Crit. Rev. Anal. Chem. 34 (2004) 51.