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Simultaneous Separation of Different Types of Amphetamine and Piperazine Designer Drugs by Capillary Electrophoresis with a Chiral Selector*

ABSTRACT: The recent emergence of a new class of piperazine-type compounds has brought about the need for laboratory screening methods for both seized drugs and toxicological samples. These piperazine compounds, which include 1-benzylpiperazine (BZP) and 1-(3-trifluoromethylphenyl)piperazine (TFMPP), exhibit comparable physiological effects and can be substituted for the classic amphetamine-type drugs. We have optimized a chiral capillary electrophoresis (CE) separation that detects a set of 6 piperazine and 4 chiral amphetamine compounds in under 23 min using a 200 mM phosphate buffer at a pH = 2.8 with 20 mM hydroxypropyl-β-cyclodextrin (HPβCD). In addition to the above compounds, a series of "clandestine" BZP diHCl samples were also analyzed using this method to assess the ruggedness of the procedure. The novel CE separation was tailored to simultaneously detect these piperzine compounds in addition to amphetamine-type drugs. Distinct migration time and UV-spectral data were obtained for all compounds of interest.

KEYWORDS: forensic science, capillary electrophoresis, benzylpiperazines, amphetamines, chiral separation

On September 20, 2002 a new set of piperazine drugs were temporarily placed on the Drug Enforcement Administration's Schedule I of the Controlled Substance Act of "1970 (1)." Two drugs of abuse, 1-benzylpiperazine (BZP) and 1-(3-trifluoromethylphenyl)-piperazine (TFMPP), will remain on the emergency schedule list until further ruling can be made regarding their hazards to public safety. Over the last few years these two compounds, and related analogs, have emerged as "legal" substitutes to the classic amphetamine-type compounds (2–4). At a dose of 125 mg, BZP mimics the physiological effects of d-amphetamine. Additionally, TFMPP has been claimed to exhibit physiological effects similar to 3,4-methylenedioxymethamphetamine (MDMA), or ecstasy. Other documented analogs with a potential for abuse include 1-[4-methoxyphenyl]-piperazine (*p*MeOPP), 1-[2-methoxyphenyl]-piperazine (*o*MeOPP), and 1-[3-chlorophenyl]-piperazine (*m*CPP) shown in Fig. 1 (2).

In 1944, 1-benzylpiperazine was first used as a potential anti-parasitic agent (1). Since then, BZP has not been used for any other legitimate medical purpose and for this reason, the DEA's current scheduling of BZP and TFMPP is as Schedule I. Ingestion of BZP results in euphoria and an increase in heart rate, pulse rate, and systolic blood pressure (5). TFMPP, which produces hallucinogen-like effects similar to d-amphetamine and *m*CPP, is also a serotonin-releasing agent (6,7). The drug *m*CPP is an active metabolite of the non-tricyclic antidepressant drug Trazodone® (Desyrel, Trazon, Trialodine) (8,9). Many neuropharmacological studies have been performed on both rats and humans to determine the extent of *m*CPP's anxiogenic effects as a 5-HT_2 receptor agonist (10,11).

Studies were performed in 2001 and 2003 by Tancer and Johnson (6,7) to compare the effects of MDMA with *m*CPP and d-amphetamines. Their latest study involved giving *m*CPP and d-amphetamine to young individuals with a history of stimulant and MDMA abuse in order to examine drug induced behavior. Although *m*CPP did not exhibit reinforcing effects characteristic of MDMA and d-amphetamine, the response to the drug was generally positive with euphoric feelings and increased drug likeability. The subjective effects of MDMA were similar to that of the *m*CPP profile.

Amphetamine abuse has existed since its inception as a clinical drug during the 1930s in the form of Benzedrine inhalers (12). Throughout World War II, amphetamines were used by soldiers to stay alert, thus in the decades following the war, abuse of this stimulant was widely recognized (13). Once amphetamines were controlled and classified as a Schedule II substance, owing to their easy manufacturing, methamphetamine emerged as a popular substitute. Clandestine methamphetamine laboratories remain a major problem in the United States. Amphetamines and methamphetamines are the starting point for additional designer drugs such as, 3,4-methylenedioxyamphetamine (MDA) and 3,4-methylenedioxymethamphetamine (MDMA). MDMA is currently extremely popular among the club drug scene at raves, which are parties where drugs are openly distributed (14).

Reports of piperazine tablet seizures have appeared frequently in this year's Drug Enforcement Administration's 2003 and 2004 Microgram Bulletins (5,15–17). There have also been accounts

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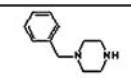
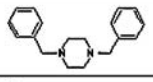
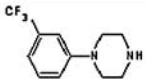
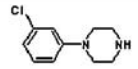
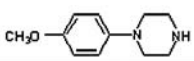
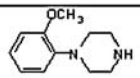
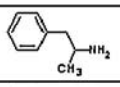
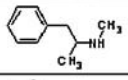
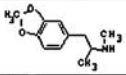
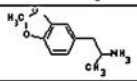
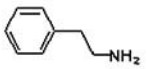
Structure	Name	pK_a
	BZP 1-Benzylpiperazine	9.59 ± 0.2
	DBZP 1,4-dibenzylpiperazine	7.25 ± 0.2
	TFMPP 1-[3-trifluoromethylphenyl]piperazine	8.66 ± 0.2
	*m*CPP 1-[3-chlorophenyl]piperazine	8.72 ± 0.2
	*p*MeOPP or 1,4-MeOPP 1-[4-methoxyphenyl]piperazine	9.00 ± 0.2
	*o*MeOPP or 1,2-MeOPP 1-[2-methoxyphenyl]piperazine	8.73 ± 0.2
	Amphetamine	9.94 ± 0.2
	Methamphetamine	10.38 ± 0.2
	MDMA (Ecstasy) 3,4-methylenedioxymethamphetamine	10.32 ± 0.2
	MDA 3,4-methylenedioxyamphetamine	9.94 ± 0.2
	Phenethylamine (I.S.)	9.90 ± 0.2

FIG. 1—*Structures of piperazine-like and amphetamine-like compounds and their pKa values (32).*

of piperazine tablets being used in combination with gamma-hydroxybutyric acid (GHB) and gamma-butyrolactone (GBL) (3,18). Recently, the owner of Genapharm, Inc. in Austin, Texas pleaded guilty to charges of possessing controlled drugs with the intent to distribute. Over 5,000 tablets of BZP, made to look like ecstasy, were seized by authorities (19). Figure 2 illustrates the U.S. states where BZP and TFMPP encounters have occurred (20).

With the emergency scheduling and its subsequent illicit use, BZP samples encountered in forensic analysis generally originate from clandestine manufacturing sites. As with any clandestine manufactured drug, the purity of the substance may be in question since the synthesis route used to manufacture the drug can vary. The final product may contain unreacted starting materials as well as unwanted side reaction products, which are commonly generated during synthesis. Low yield reactions will contain low levels of the drug substance under investigation and may also contain high concentration(s) of by products and/or starting materials. These unwanted compounds can interfere with the analysis of the substance. Techniques for the analysis of these drugs must also be able to separate analog compounds from the product since the use of analog compounds may be substituted for the product compound to avoid detection.

Because of the novelty of piperazine-like compounds, there is limited information available on the analysis of these drugs using validated forensic techniques. Staack et al. has published work involving toxicological studies and quantitation of piperazine compounds by GC-MS (21,22). de Boer et al. suggested a variety of analytical strategies for detection of BZP in capsules (2), but the potential for a capillary electrophoretic method has not been realized. In the past, capillary electrophoresis (CE) has proven useful in the separation of amphetamine-type compounds (23–28). Because of the possibility of using the piperazine drugs as amphetamine substitutes, we have chosen to optimize a similar chiral CE separation to accommodate these new piperazine related drugs (23). Because the reported effects of these two classes of drugs are strikingly similar, this method will greatly benefit laboratory analysis where the abused or seized substance is in question.

As its name implies, chiral CE involves the separation of enantiomers by electrophoretic means. The most successful approach to chiral CE has been through the use of chiral selectors; molecules that may preferentially interact with one enantiomer of a chiral compound. Cyclodextrins, which in their native state are uncharged and have negligible UV-absorbance above 200 nm, are among the most prevalent chiral selectors used in CE. The structure of cyclodextrins is comprised of repeating glucose molecules that form a truncated cone. Analyte molecules can interact with the cyclodextrins to form inclusion complexes. These interactions include hydrophobic, electrostatic, and hydrogen bonding, and have sufficient specificity to provide chiral separations. The size of the cyclodextrin's hydrophobic cavity, to act as the host, is based on the number of glucose units ($\beta = 7$, most commonly used). Cyclodextrins can be used in their native form or as chemically modified derivatives that can enhance enantioselectivity. A chemically modified hydroxypropyl-β-cyclodextrin (HP-β-CD) will be utilized in this study as an

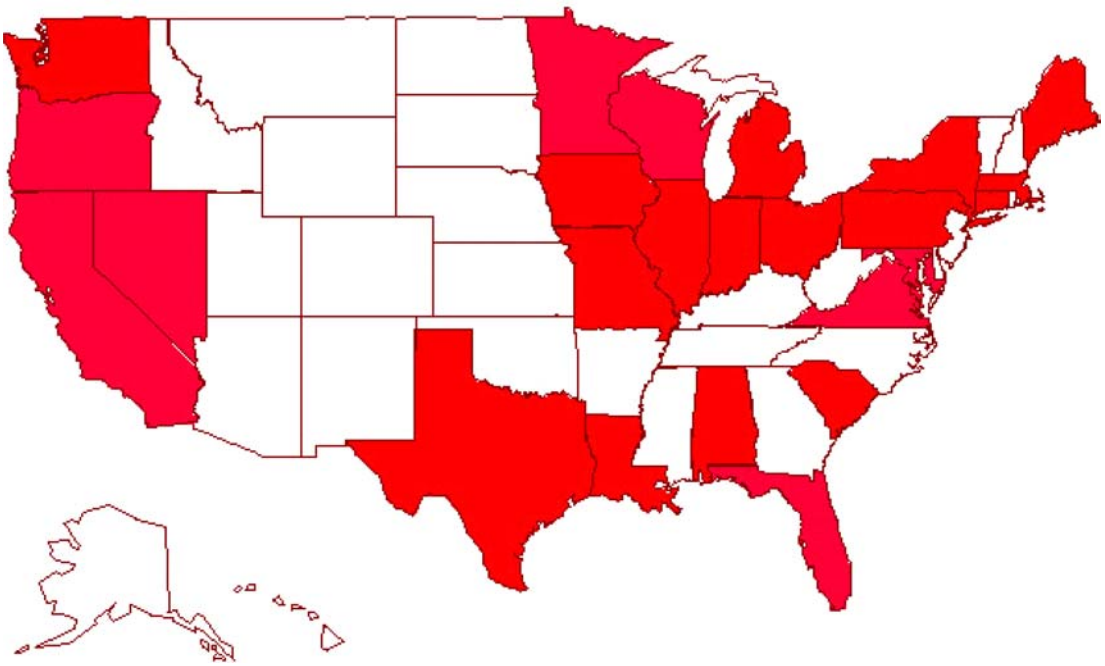


FIG. 2—*BZP and TFMPP Encounters by Law Enforcement Map (as of 10/9/03) prepared by the U.S. Department of Justice Drug Enforcement Administration, Office of Diversion Control (Chemical Operations Section).*

inclusion type chiral selector. By employing the cyclodextrin as a buffer additive in CE, it becomes more cost effective when compared to the larger volumes of buffer required for HPLC analysis.

Chemicals and Reagents

1-Benzylpiperazine was purchased in its liquid form from Sigma-Aldrich (St. Louis, MO) and crystallized at ambient temperature to yield a BZP solid. (confirmed by NMR). All other compounds described herein were used without further purification. Also obtained from Sigma-Aldrich were 1-(4-methoxyphenyl)piperazine, 1-(2-methoxyphenyl)piperazine, 1,4-dibenzylpiperazine, and ± 3,4-methylenedioxymethamphetammine (MDMA). 1(3-trifluoromethylphenyl)piperazine was obtained from Alfa Aesar (Ward Hill, MA). Both 1-(3-Chlorphenyl)piperazine and the internal standard 3-Chloroaniline were purchased from TCI America (Portland, OR). ±Methamphetamine was purchased from Radian (Austin, Tx) while ±Amphetamine and ±MDA were obtained from Cerilliant (Austin, Tx). BZP dihydrochloride (diHCl) salt and 1,4-dibenzylpiperazine (DBZP) were prepared in the laboratory. The starting materials and chemicals used to synthesize these two compounds were obtained from Sigma-Aldrich and were of high purity. Sodium phosphate was obtained from Acros (New Jersey) and 85% Phosphoric Acid was obtained from Spectrum Quality Products Inc. (Gardena, CA). Hydroxypropyl-β-cyclodextrin (HP-β-CD) with a substitution rate: 4.9 was obtained from the eCAP^{TM} Chiral Methods Development Kit (Beckman Instruments, Inc., Fullterton, CA).

The chiral CE buffer was prepared by a modification of the literature procedure (30). Sodium Phosphate and 85% phosphoric acid was combined to yield a 200 mM phosphate buffer. A final pH of 2.8 was reached after adjustment with 1 M NaOH. The chiral selector hydroxypropyl-β-cyclodextrin was added at a concentration of 20 mM. The solid phase extraction eluting solvent was prepared fresh daily and consisted of a ratio of 78/20/2 methylene chloride/isopropyl alcohol (IPA)/ammonium hydroxide (CH_2Cl_2/$CH_3CH(OH)CH_3$/NH_4OH) (31).

Instrumentation

Separations were carried out on an Agilent Capillary Electrophoresis System equipped with a photo diode array detector. The capillary was obtained from Polymicro (Phoenix, Az) with an approximate total length of 64.5 cm, an effective length of 56 cm, and an internal diameter of 50 μm. Typical injections were 40 mbar for 4 s for the optimization studies and 40 mbar for 6 s for the calibration and extracted samples. The capillary temperature was kept at 25°C with a run voltage of 25 kV. Each new capillary was rinsed with 0.1 M NaOH for 15 min and water for 10 min. Prior to each run the capillary was rinsed with 0.1 M NaOH for 2 min, water for 2 min, and then sodium phosphate β-CD buffer for 5 min. The detection wavelength was 210 nm.

The LC-MS instrument used was an Agilent 1100 Series LC-MSD operated in the positive ion mode with a fragmentor voltage of 100 volts. Nitrogen was used as the drying gas at 300°C with a flow rate of 10 L/min. The nebulizer pressure was maintained at 20 psig. The chromatographic method for the MS analysis of piperazines utilized a Zorbax SB-C18 column (150 mm × 2.1 mm) with a mobile phase consisting of acetonitrile and a volatile ion pairing agent (10 mM tridecafluoroheptanoic acid). The mobile phase was delivered at 0.30 mL/min and was run as a 20 min gradient from 37% acetonitrile to 90% acetonitrile.

Liquid-Liquid Extraction

A modified version of Kinberger's procedure (30) was used for the liquid-liquid extraction of standard mixtures and spiked urine. In a 4 mL conical screw cap vial, 1.0 mL of standard sample and 200 μL of 1.0 M sodium hydroxide was added. The specimen was shaken and extracted 3 times with 1.0 mL solvent. The aqueous layer was drawn out with a stainless steel syringe and the organic layers were combined in a clean conical vial. To the organics, 100 μL of 0.10 M hydrochloric acid was added. The layers were mixed and the aqueous portion was transferred to a 100 μL sample vial.

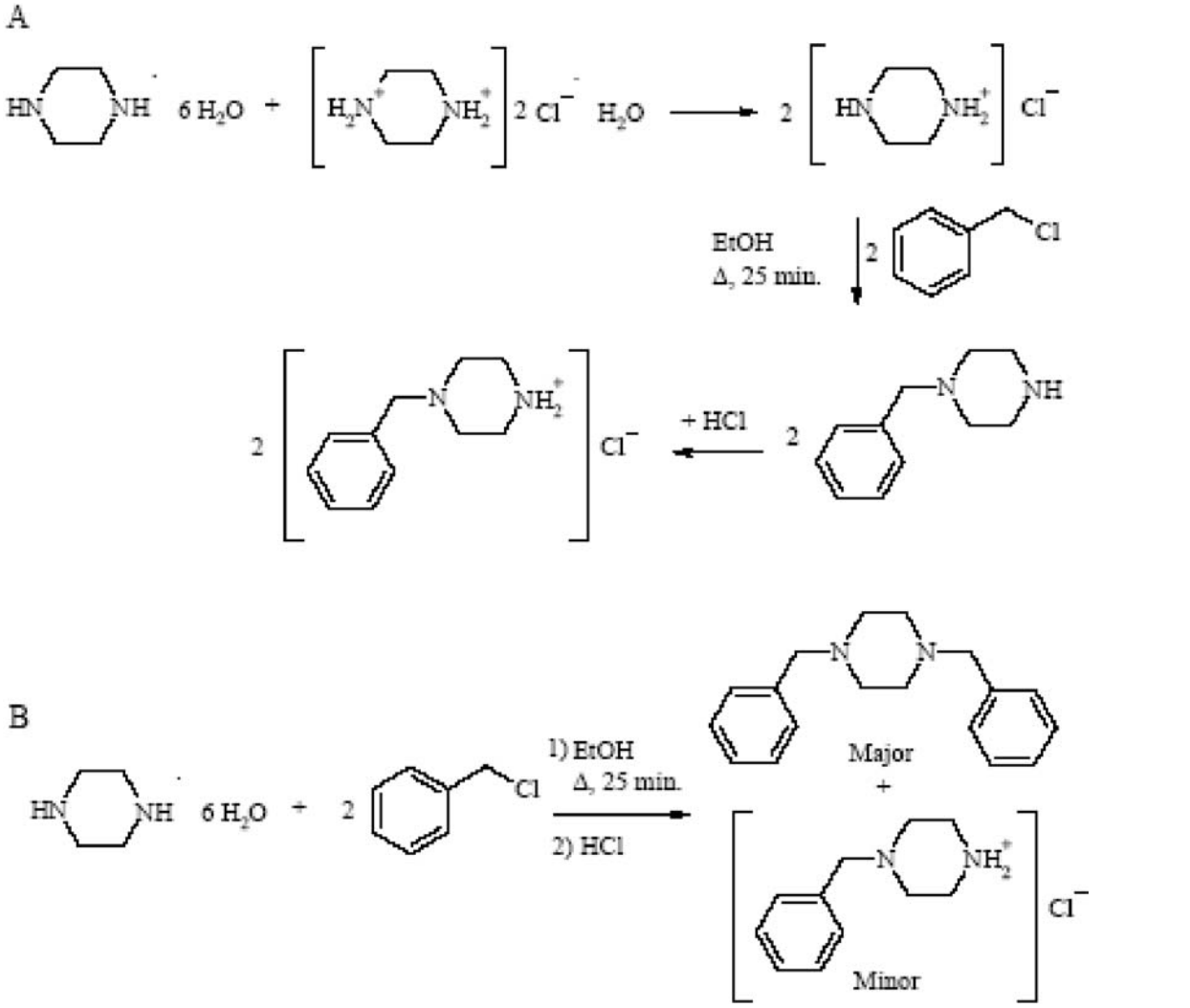


FIG. 3—*Clandestine Synthesis of piperazines. A.) Synthesis #1: equal molar amounts of piperazine hexahydrate, piperazine diHCl monohydrate, and benzyl chloride. B.) Synthesis # 2: equal molar amounts of piperazine hexahydrate and benzyl chloride.*

Solid Phase Extraction

The extraction was performed using an altered version of the Varian Bond Elut Certify Amphetamines in Urine (31) procedure to be used with 1210–2051 cartridges. The SPE columns were conditioned using 2.0 mL of methanol and then 2.0 mL of 100 mM phosphate buffer with a pH = 6.0 drawn through with a low vacuum ($\leq$3 inches Hg). To 1.0 mL of standard or sample, 1.0 mL of the phosphate buffer was added to adjust the pH. After the specimen was slowly loaded onto the column, it was rinsed with 1.0 mL of acetic acid, dried for 5 min under vacuum, rinsed with 3.0 mL of methanol, and then dried for 2 min ($\geq$10 inches Hg). The analytes were eluted using 2.0 mL of fresh CH_2Cl_2/IPA/NH_4OH, evaporated to dryness under a steady stream of nitrogen, and then reconstituted using 100 μL of 1:1 methanol to deionized water.

Synthesis of Benzylpiperazine and Benzylpiperazine Analog Compounds

Because of the increased legal control of BZP and TFMPP, we anticipate that new methods for the clandestine synthesis of piperazine analogs will develop over time. Due to this potential problem, greater focus needs to be placed on the detection of those piperazine analogs that are not yet scheduled such as DBZP, *o*MeOPP, *p*MeOPP, and *m*CPP.

To generate a series of clandestine BZP diHCl samples, synthetic procedures obtained from the Internet were used to manufacture BZP diHCl and DBZP. The synthesis procedures are shown in Fig. 3. The main goal was to determine what types of products would be generated from a synthetic procedure obtained via a non-literature source. The synthetic procedure was also modified to investigate other types of products, which could be generated as a result of the modifications to the synthesis such as 1,4-DBZP.

The products from synthesis 1 and 2 were initially characterized using LC-MS (Fig. 9). Additional characterization of the products from synthesis 1 and 2 was conducted using Fourier transform infrared (FT-IR) spectroscopy, proton NMR (H^1NMR), and gas chromatography with mass spectral detection (GC-MS) and are in agreement with the structures and data shown in Fig. 9. The purities of each synthetic product were determined using LC-MS. The resulting products from two syntheses shown in Fig. 3 were then analyzed using the capillary electrophoresis method described in this paper.

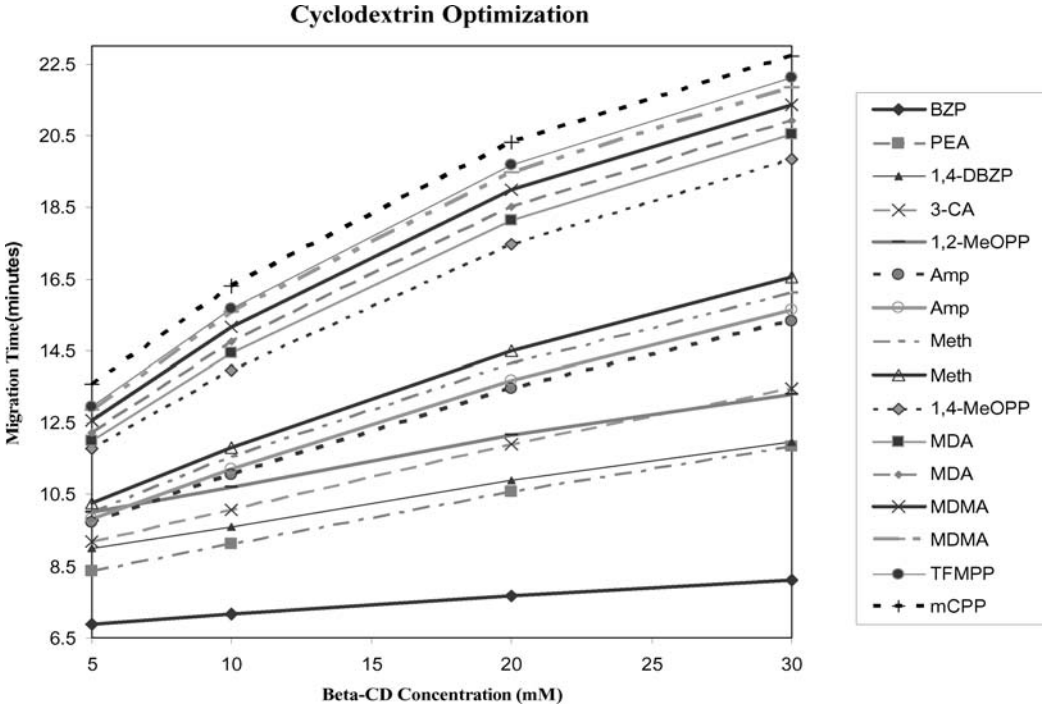


FIG. 4—*Mobility of Piperazines and Amphetamines with respect to the concentration of the chiral selector hydroxypropyl-β-cyclodextrin (HP-β-CD). The capillary had total length of 64.5 cm, an effective length of 56 cm, and an internal diameter of 50 μm. Injections were 40 mbar for 4 seconds, the temperature was kept at 25°C, and the run voltage was 25 kV.*

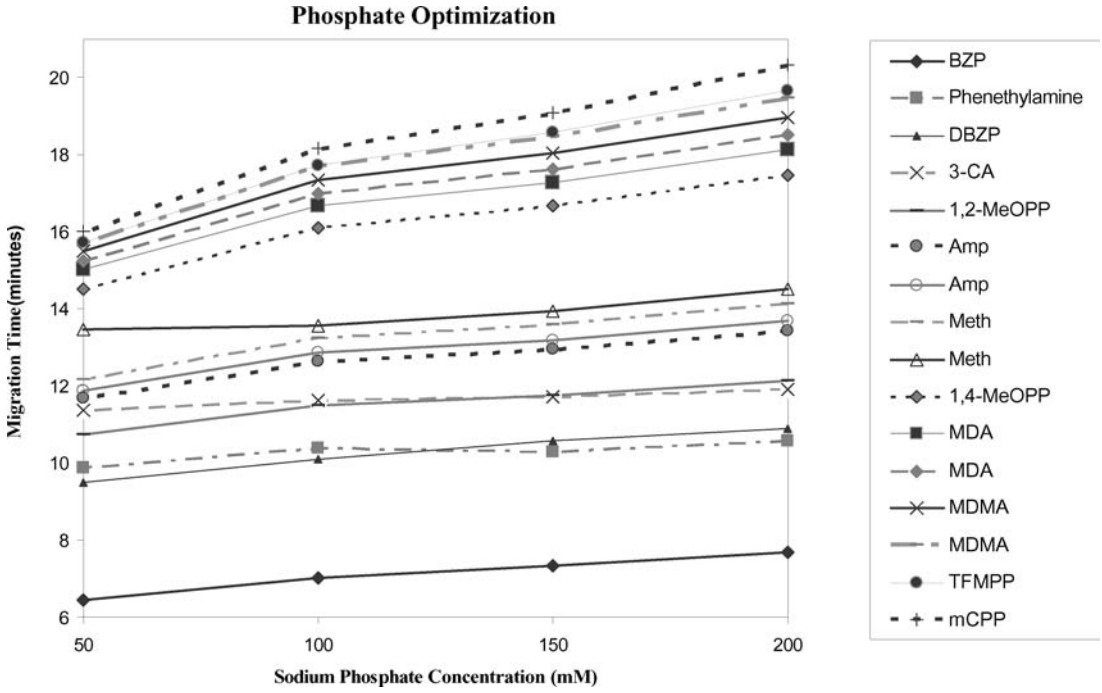


FIG. 5—*Mobility of Piperazines and Amphetamines with respect to the concentration of sodium phosphate in the buffer. Conditions were the same as in Fig. 4.*

Results and Discussion

The ultimate goal of this analytical method was to achieve a screening procedure for both piperazines and amphetamines owing to a high probability of their joint application. Even though piperazines are not chiral compounds, our CE buffer contained a cyclodextrin to achieve baseline separation of the amphetamine-like compounds. This required careful optimization of conditions in order to find a procedure that offers full resolution for all compounds of interest.

The optimization included the determination of the most favorable sodium phosphate concentration, hydroxypropyl-β-cyclodextrin (HP-β-CD) concentration, pH, and temperature. The choice of cyclodextrin type can be difficult and compound specific. Previous studies have also shown that the concentration of the cyclodextrin can affect both the analyte resolution and the migration

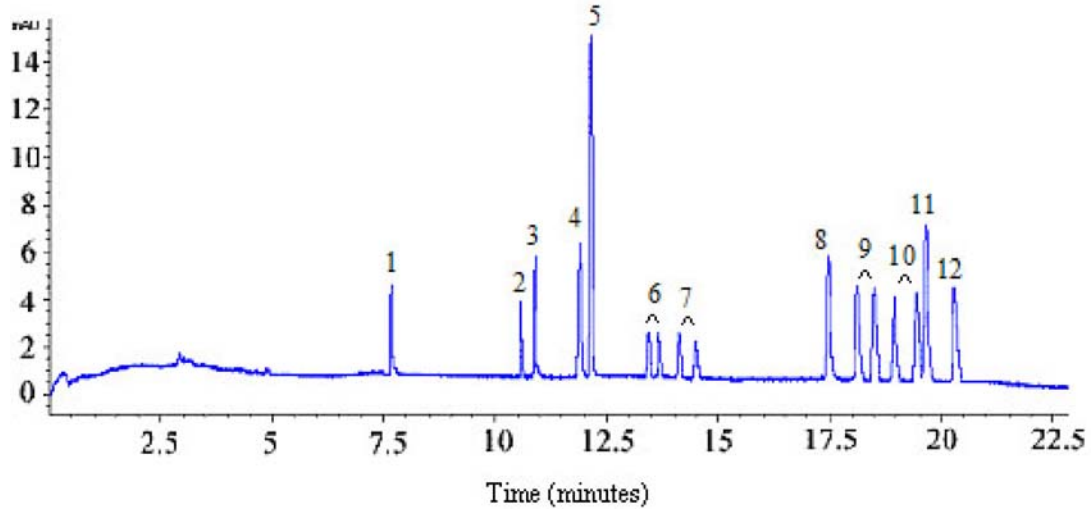


FIG. 6—*The optimized chiral separation of amphetamine and Piperazine compounds using the standard drug mixture at 30 μg/mL. The runs were performed in a 50 mm ID capillary with a total length of 64.5 cm and an effective length of 56 cm. The pressure injection was for 6 seconds at 40 mbar. The capillary temperature was kept at 25°C with a run voltage of 25 kV and a detection wavelength of 210 nm.(1) BZP, (2) Phenethylamine, (3) DBZP, (4) 3-Chloroaniline, (5) oMeOPP, (6) D,L- Amphetamine, (7) D,L-Methamphetamine, (8) pMeOPP, (9) D,L-MDA, (10) D,L-MDMA, (11) TFMPP, (12) mCPP.*

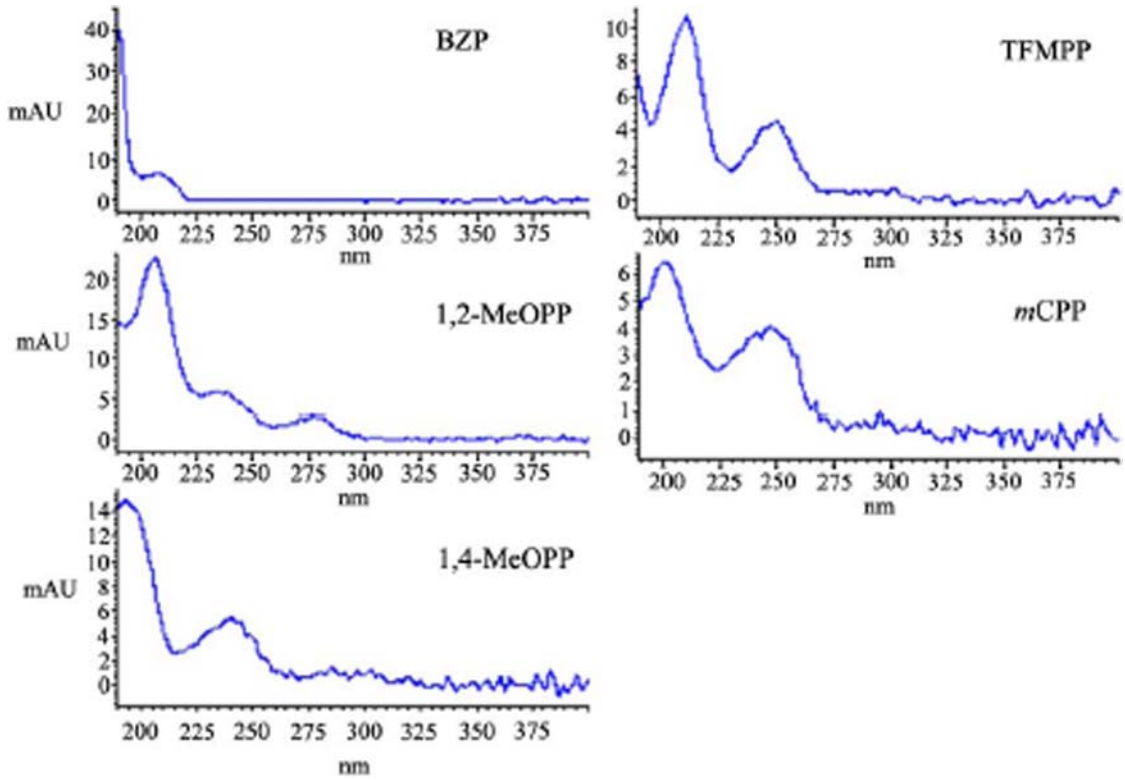


FIG. 7—*UV-spectral data for the Piperazine-like compounds taken from the standard mixture.*

time. Temperature can also have an effect on the selectivity of cyclodextrins. Figure 4 illustrates the result of increased HP-β-CD on migration time and order of the 10 component standard mixture of amphetamines and piperazines. As the amount of cyclodextrin increased, the migration time of the drugs also increased. While there was no major difference in the resolution and order of elution, 20 mM HP-β-CD offered the best selectivity for all of the analytes of interest. Figure 5 shows that the migration time also increases when the concentration of phosphate in the run buffer was increased. The optimized electropherogram for the analysis of both groups of drugs using a 200 mM sodium phosphate at pH = 2.8 with 20 mM HP-β-CD is displayed in Fig. 6. The spectral data generated from the piperazine compounds is shown in Fig. 7.

The run-to-run, day-to-day, and column-to-column reproducibility can be found in Table 1. Calibration curves were generated to verify the linearity of detector response to the piperzine-type compounds. The curve data and limits of detection are displayed in Table 2.

TABLE 1—*Run-to-run reproducibility data from four different runs performed on two different days. Column-to-column data for a comparison with a second column. Conditions were the same as in Fig. 6.*

Compound	Day to Day			Column to Column		
	Migration	std	rsd	Migration	std	rsd
BZP	7.6	0.1	1.1	7.3	0.2	3.0
Phenethylamine	10.5	0.4	4.0	10.1	0.3	2.6
DBZP	10.6	0.0	0.2	11.5	0.4	3.5
*o*MeOPP	11.8	0.1	1.1	11.5	0.4	3.5
Amphetamine	13.4	0.1	0.4	13.1	0.3	2.6
	13.6	0.1	0.4	13.3	0.3	2.6
Methamphetamine	14.1	0.1	0.4	13.8	0.4	2.6
	14.4	0.1	0.4	14.1	0.4	2.7
*p*MeOPP	17.0	0.2	1.3	16.3	0.7	4.3
MDA	18.0	0.1	0.5	17.6	0.5	3.1
	18.4	0.1	0.5	18.0	0.6	3.1
MDMA	18.9	0.1	0.5	18.4	0.6	3.1
	19.4	0.1	0.5	18.9	0.6	3.2
TFMPP	19.6	0.1	0.5	19.1	0.6	3.3
*m*CPP	20.3	0.1	0.5	19.7	0.6	3.2

TABLE 2—*Calibration curve data for 6 piperazine-like compounds. Conditions were the same as in Fig. 6.*

Compound	Slope	R^2	Detection Limit (μg/mL)
1-benzylpiperazine	0.26 ± 0.02	0.9868	0.58
1,4-dimethylphenylpiperazine	0.46 ± 0.03	0.9859	5.2
1-(2-methoxyphenyl)piperazine	1.50 ± 0.09	0.9895	3.5
1-(4-methoxyphenyl)piperazine	0.95 ± 0.07	0.9838	0.037
1-(3-trichlorophenyl)piperazine	1.19 ± 0.09	0.9845	1.3
1-(3-chlorophenyl)piperazine	0.71 ± 0.04	0.9885	4.3

Spiked Urine Samples

Since previous work on the extraction and analysis of piperazines from forensic samples is limited, both liquid-liquid and solid phase extractions were investigated for their usefulness. Extraction methods for piperazine samples were developed using existing methods published for amphetamine samples (30). Due to the polar nature of these compounds, liquid-liquid extractions are often difficult. This problem was overcome by performing a back extraction into 0.10 M hydrochloric acid (HCl). The extraction efficiencies for

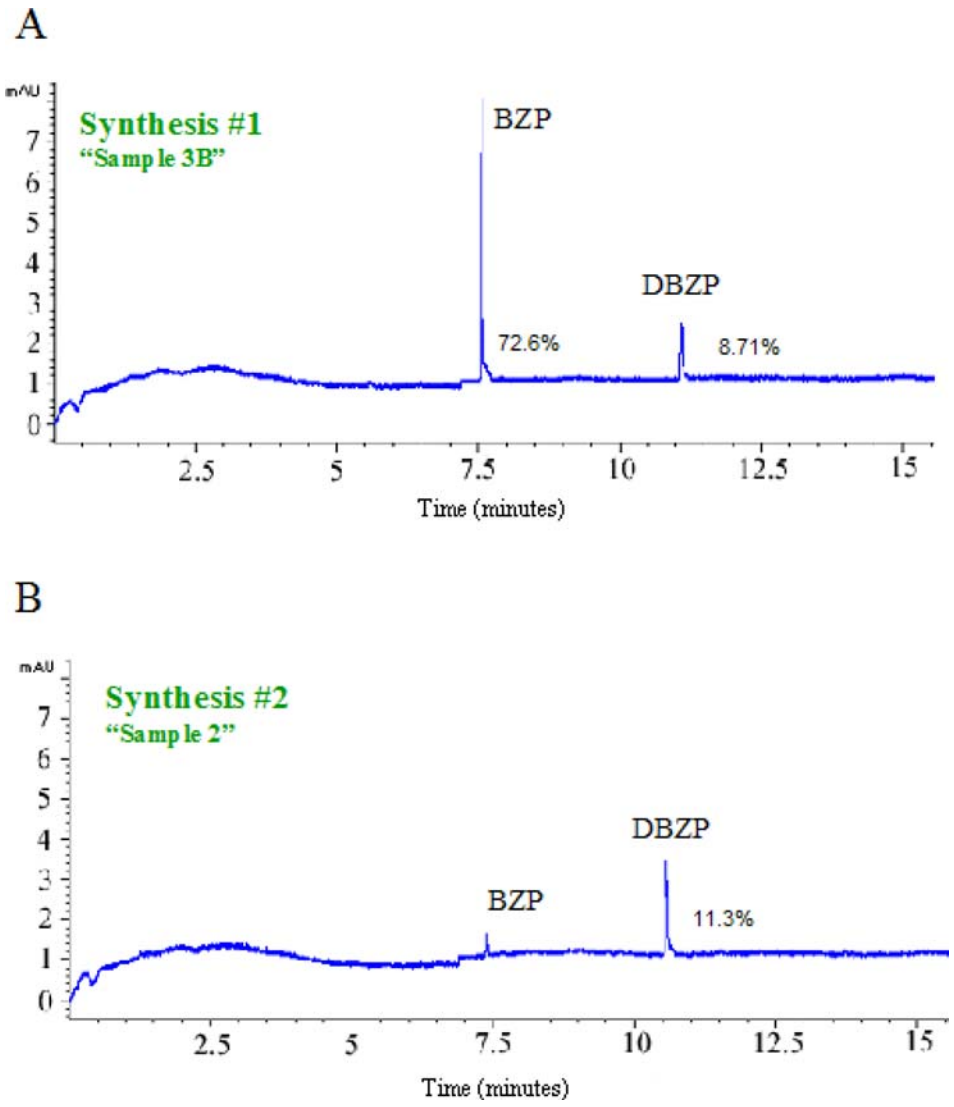


FIG. 8—*CE analysis of synthesized piperazines. Conditions were the same as in Fig. 6. A.) Analysis of synthesis #1 that generated BZP as the primary product with small amounts of the DBZP. B.) Analysis of synthesis #2 that generated DBZP as the primary product with trace amounts of BZP.*

TABLE 3—*Liquid-liquid extraction data for 50 ug/mL samples extracted with two different solvents. In a conical vial, 1-mL of 50 ug/mL water standard and 200 μL of 1.0 M sodium hydroxide was added. The specimen was extracted with 3 × 1.0 mL solvent. The organic layer was back extracted with 100 μL of 0.1 M hydrochloric acid. The aqueous portion was transferred to a sample vial and injected. Conditions were the same as in Fig. 6. Extraction efficiencies were based on n = 2.*

Solvent	BZP	DBZP	1,2-MeOPP	1,4-MeOPP	TFMPP	*m*CPP
Diethyl ether	88%	60%	82%	79%	99%	96%
Ethyl acetate	71%	65%	78%	85%	67%	45%

diethyl ether and ethyl acetate are shown in Table 3. Diethyl ether gave the highest extraction efficiency. The use of phosphoric acid in place of HCl did not improve upon the extraction. Table 4 contains extraction efficiency data for the solid phase extraction for 20 μg/mL and 50 μg/mL standard water samples. Urine samples spiked at 50 μg/mL were also analyzed to demonstrate similar extraction efficiencies.

Synthesized Samples

Examples of the electropherograms from samples of piperazine synthesis #1 and #2 are shown in Fig. 8. In Synthesis 1, BZP diHCl was the predominant product with a small amount DBZP. Synthesis #1 produced BZP at a yield of 72.6% and 8.7% DBZP. In Synthesis 2, DBZP was the predominant product with a small amount BZP. Synthesis #2 produced DBZP at a yield of 14.9% and trace amounts of BZP diHCl . The overall peak ratios for each synthesis that were determined by the CE method were consistent with those obtained by LC-MS (Fig. 9).

Conclusions

A novel CE method for the simultaneous detection of piperazine and amphetamines compounds has been developed for the analysis of these drugs in spiked urine and synthesized samples. The method utilizes hydroxypropyl-β-cyclodextrin for the separation of enantiomers of amphetamine-type drugs. Both a liquid-liquid

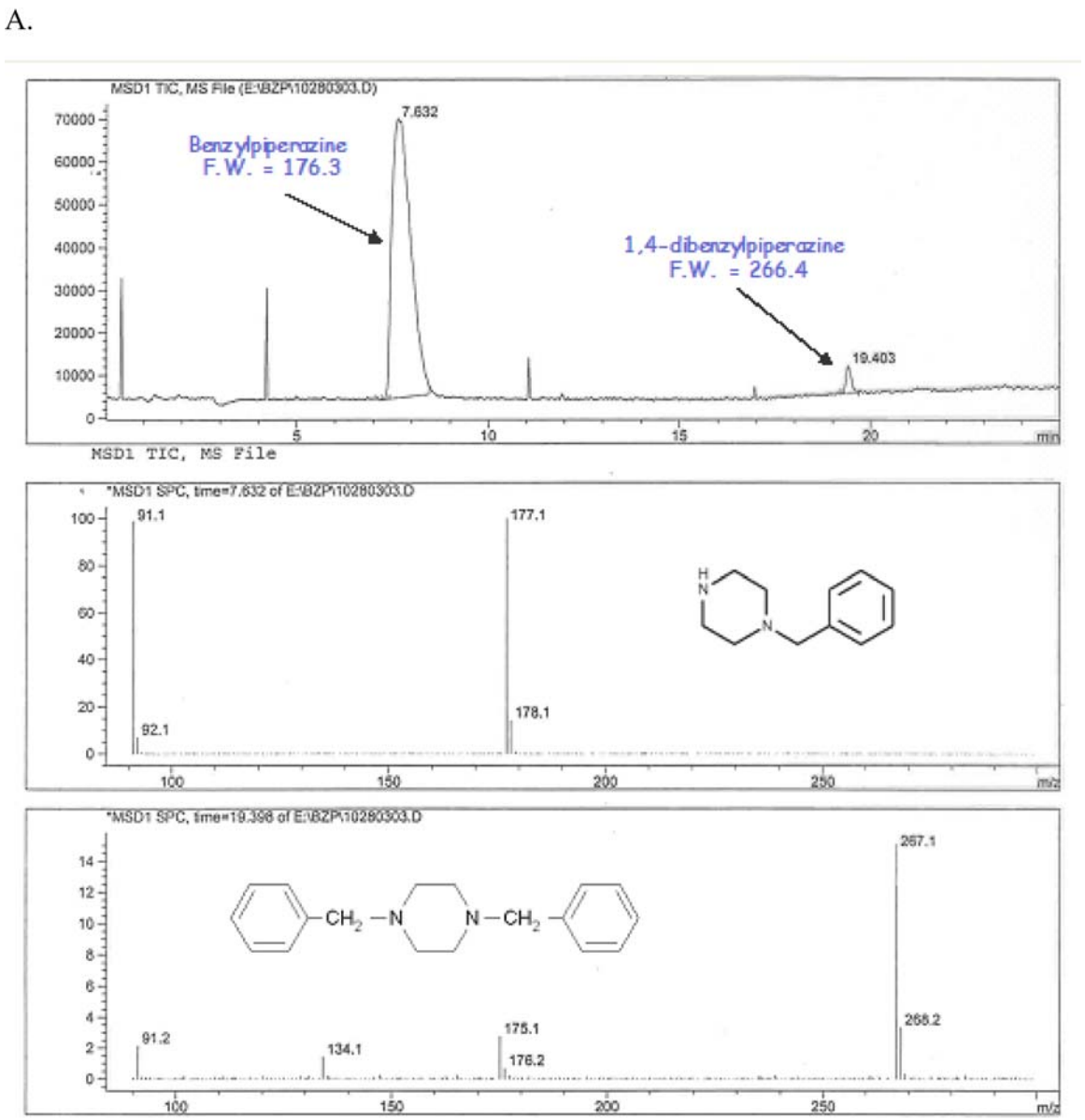


FIG. 9—*A.) LC-MS data of BZP diHCl (white crystals) from BZP Synthesis # 1, B.) LC-MS data of DBZP (white crystals) from BZP Synthesis # 2.*

B.

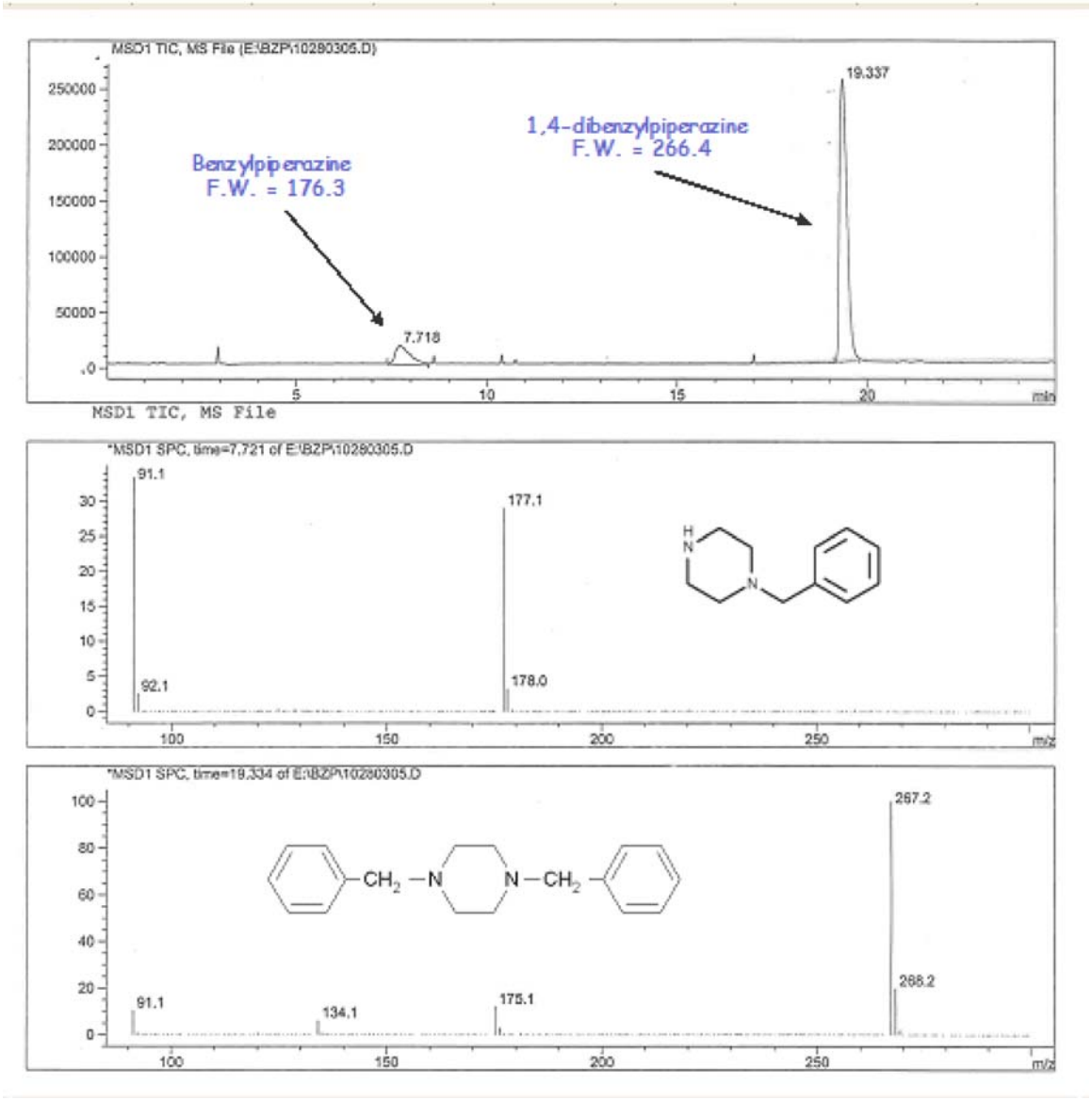


FIG. 9—*Continued*.

TABLE 4—*Solid Phase Extraction data acquired by using an altered version of the Varian Bond Elut Certify Amphetamines in Urine procedure (1210–2051 cartridges). Average % Extraction Efficiency (EE) based on n = 3. A.) Spiked water standards at 50 µg/mL, B.) Spiked water standards at 20 µg/mL, C.) Two different urine samples spiked at 50 µg/mL. Conditions were the same as in Fig. 6.*

A.		BZP	DBZP	*o*MeOPP	D-Amp	*p*MeOPP	TFMPP	*m*CPP
50 µg/mL	Average % EE	85.3	77.4	93.4	64	90.1	85	78.6
	Standard Dev	2.5	8.6	6.9	17	6.1	11	6.6
B.		BZP	DBZP	*o*MeOPP	D-Amp	*p*MeOPP	TFMPP	*m*CPP
20 µg/mL	Average % EE	68.4	62.8	81	34	74.2	80	66.4
	Standard Dev	1.5	5.2	10	16	5.1	11	8.0
C.		BZP	DBZP	*o*MeOPP	D-Amp	*p*MeOPP	TFMPP	*m*CPP
Urine 1	% EE	90.3	87.1	94.6	88.3	91.1	87.9	84.7
Urine 2	% EE	91.4	96.0	96.1	88.9	89.9	88.9	87.7

extraction, using diethyl ether, and a solid phase extraction, using a commercial procedure, were offered for sample pretreatment options. Consistent with the reaction scheme, Synthesis #1 yielded BZP as the primary product, while Synthesis #2 favored DBZP.

Despite the new scheduling of BZP and TFMPP, it is clear by the analysis of our synthetic products that other non-scheduled piperazines can be present when clandestine procedures are used. Future studies are needed to characterize other reactions and develop improved techniques to quantitate reaction products. Additionally, tailoring the CE method to provide lower limits of detection would be helpful for the detection of low-level toxicological samples. This can be done using field amplified sample stacking and other preconcentration techniques.

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