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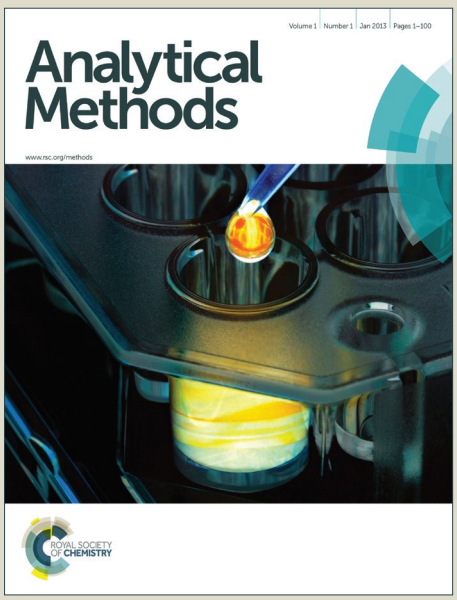

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Application of Mixed-Mode Ultra High Performance Liquid Chromatography to Forensic Drug Analysis

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Methodology is presented for the analysis of drugs of forensic interest employing a single column and the same solutions from two solvent reservoirs at different ratios for orthogonal reversed phase chromatographic and hydrophilic interaction liquidchromatographic separations. For the determination of the basic drugs in the SAMHSA-5 panel in urine, a 2.1 x 150 mm x 2.7 µm superficially porous dimethylpentafluorophenylpropyl (PFP) column was employed using two acetonitrile-water-ammonium fomate solutions as A and B solvents for a binary pumpwith time of flight mass spectrometric (TOF-MS) detection. Applicable to the analysis of seized drugs, the same column and detector was used with acetonitrile-water-ammonium acetate solutions as A and B solvents for the separation of 15 controlled "bath salts". For both applications, employing mixed mode chromatography minimized ion suppression and allowed the unique identification of each analyte. Solid phase extraction (SPE) performed on a mixed mode MM1 column successfully recovered the solutes of interest with good recovery and minimum ion suppression or ion enhancement was observed for the ultra high performance liquid chromatography-TOF-MS analysis of the extracts. For the SPE sample preparation no evaporation and reconstitution step was required, with the elution solvent directly compatible with both the HILIC and RPC analysis on the same stationary phase.

1. Introduction

The SAMHSA-5 drug panel is used for workplace drug testing and includes the following five drug classes: amphetamines (methamphetamine, amphetamine, MDMA, MDA, and MDEA), cocaine (benzoylecgonine (BZE) a metabolite of cocaine), marijuana (carboxy-THC) a metabolite of Δ^9-THC, opiates (codeine, 6-monoacetylmorphine (metabolite of heroin), and morphine), and phencyclidine (PCP) [1].

The analysis of above drugs and metabolites in urine represents an analytical challenge. Traditional techniques such as immunoassays [2] lack specificity which leads to confirmation by other techniques such as gas chromatography mass spectrometry (GC-MS) [2] and liquid chromatography mass spectrometry (LC-MS) [3]. Using GC-MS can be problematic for thermally labile, polar and non-volatile solutes. Therefore, derivatization could be required which increases sample analysis time [4]. For LC-MS, which does not suffer from the above limitations, sample preparation techniques such as liquid liquid extraction and solid phase extraction (SPE) are commonly employed, as in GC-MS, to remove the solutes of interest from the matrix. For LC-MS the use of solid phase extraction minimizes ion suppression and ion enhancement effects, extends column life and minimize contaminants in the MS source [5, 6].

Reversed phase chromatography (RPC) has been employed for most or all of the basic drug classes of the SAMHSA-5 panel [7, 3]. Hydrophilic interaction liquid chromatography (HILIC), a complementary technique to the widely employed reversed phase chromatography,

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offers certain advantages for the analysis of polar solutes, including drugs in urine [8]. This includes the ability to provide an orthogonal separation mechanism which could resolve solutes not separated under reversed phase conditions or provide an additional identification. In addition, the use of the high organic content in the HILIC mobile phases is expected to produces lower limits of MS detection for electrospray ionization (ESI) detection [8]. Using HILIC also can mitigate the need for an evaporation and reconstitution step during solid phase extraction (SPE) sample preparation [9]. Morphine and codeine have been analyzed using HILIC with solid phase extraction [9, 10], with one of the studies [10] not employing an evaporation and reconstitution step. Amphetamine, methamphetamine, MDA, MDMA and MDEA have been analyzed by HILIC, with column switching used for sample preparation [11].

"Bath salts" refers to synthetic drugs related to cathinone, an amphetamine-like stimulant drug naturally occurring in the khat plant. These synthetic cathinones can produce euphoria and increased sex drive, but adverse side effects include paranoia, agitation, and hallucinatory delirium, psychotic and violent behavior [12]. These drugs marketed as "bath salts" to evade detection by authorities typically take the form of a white or brown crystalline powder and are sold in small packages labeled "not for human consumption" [12]. Compounds structurally similar to cathinone are synthesized to circumvent the controlled substances laws. Law enforcement has countered by placing over 15 "bath salts" under temporary or permanent federal control in the United States [13].

Since new structurally similar compounds are created by slightly modifying the chemical structure of a controlled substance, methodology to analyze these solutes should have the ability to distinguish between similar solutes (analogs including positional isomers). Currently GC is widely used for the analysis of synthetic cathinones [14]. However, this technique can be problematic for highly polar cathinone derivatives which could require basic extraction and/or derivatization in order to obtain satisfactory chromatographic performance [14-16]. Additionally, many cathinone derivatives undergo extensive fragmentation under EI (electron impact ionization), and their molecular weight information is either missing or difficult to determine [17]. In contrast liquid phase separation techniques, such as capillary electrophoresis (CE), ultra high performance liquid chromatography (UHPLC) or high performance liquid chromatography (HPLC) do not suffer from the above limitations, and therefore are well suited for the analysis of "bath salts" [14, 18-22]. In addition, CE-MS and UHPLC-MS which can provide either low or high resolution molecular weight information due to its soft molecular fragmentation (electrospray ionization (ESI)) is well suited for the screening of the synthetic cathinones [14, 17, 19-21, 23 Significant overlap in retention times existed for the separation of "bath salts" using RPC [14]. For a more definitive compound identification based on retention time, including minimizing the possibility that the retention time of a target solute matches the retention time of a non controlled isomeric compound, and to facilitate quantitation, the use of a complementary technique such as HILIC could help mitigate this situation [24].

Mixed-mode chromatography, whereby a stationary phase exhibits both RPC and HILIC properties is utilized, has been reported; e.g pentafluorophenyl (PFP) [25, 26], cystine and cysteine bonded silica [27], steviol glycoside modified- silica [28], and a C18-Diol [29]. Solutes examined using these stationary phases exhibited "U-shaped" retention behavior (retention decreases and then increases with organic modifier concentration), which results in changes in separation selectivity. For the PFP stationary phase, the selectivity changes are a result of both ion exchange with surface silanol groups and simultaneous interaction with the bonded phase

ligands via dispersion and polar interactions [25]. PFP columns have been used for both the RP of abused drugs in urine [30] and

The use of 2.7 µm superficially porous (SPP) columns for ultra high performance liquid chromatography (UHPLC), which allows for faster and/ or higher peak capacity separations than conventional HPLC, is well suited for the analysis of drugs in urine and for the screening of "bath salts" in seized drugs.

TOF MS detection with a good dynamic range, the capability for accurate mass measurement over an entire selected mass range is a suitable alternative to traditional quadrupole MS techniques, which provides unit resolution and lacks the ability to easily identify untargeted compounds. This detection scheme was employed for HILIC separation of opiates [9] and the RPC separation of synthetic cathinones [17, 21]..

UHPLC separation conditions for the basic drugs classes in the SAMHSA-5 drug panel and synthetic cathinones, using both RPLC and HILIC, is investigated. This leads to creation of methodology for both the analysis of the SAMHSA drugs in urine employing solid phase extraction and the confirmation and/ or screening of bath salts via retention time. The analysis of carboxy-THC which requires a separate solid phase extraction procedure is beyond the scope of this manuscript.

2. Experimental

2.1 Chemicals and reagents

The reference drug and metabolite standards and synthetic urine were obtained from Cerilliant (Round Rock, TX, USA), while the synthetic cathinones were acquired from Cayman Chemical (Ann Arbor, MI, USA). Agilent ESI electrospray ionization tune mix was acquired from PerkinElmer (Shelton, CT, USA). LC/MS grade water, formic acid, acetonitrile and certified ACS plus ammonium hydroxide were obtained from Fisher Scientific (Fairlawn, NJ, USA).

Buffers were prepared from stock solutions for sample preparation during the solid phase extraction process and/or for use for buffer mixtures used as components of mobile phases. Three stock buffers were prepared during this study including 200 mM ammonium formate (pH~ 3), 200 mM ammonium acetate (pH~9), and 100 mM ammonium acetate (pH~6.4). The 200 mM ammonium formate buffer was prepared by weighing out approximately 12.6 g of ammonium formate into a 1 L volumetric flask, adding 900 mL of water and 25 mL of formic acid, and filling to the mark with water to a total volume of 1 L [31]. The 100 mM ammonium acetate stock solution was prepared by weighing out approximately 7.71 g of ammonium acetate into a 1 L volumetric flask, adding water to the mark of 1 L and 10 drops of concentrated acetic acid [32]. The 200 mM ammonium acetate buffer stock was prepared by weighing out 15.4 g of ammonium acetate and adding it to 900 mL of water in a 1 L volumetric flask, 8 mL of ammonium hydroxide was added to the flask, and the solution was mixed thoroughly. Water was then added to the 1 L mark of the flask [32].

2.2 Instrumentation and Data Analysis

A UHPLC-TOF MS system consisted of a PerkinElmer Flexar 15 liquid chromatograph coupled with a PerkinElmer Axion 2 time of flight-mass spectrometer (Shelton, CT, USA) were used for the analysis of all extracted and neat samples. PerkinElmer Chromera version 3.4.1 and TOF MS

driver version 6.1 was used for the overall instrument control, data acquisition, and processing. The preliminary UHPLC chromatographic separations for the basic drugs in SAMHSA-5 panel were performed using a PerkinElmer Brownlee SPP C18 and a Perkin Elmer Brownlee HILIC column (150 mm x 2.1 mm, 2.7 µm) . , A PerkinElmer PFP column (150 mm x 2.1 mm, 2.7 µm) was used for subsequent separations of both the SAMHSA analytes and the "bath salts" using a dual chromatographic mode approach. The following parameters were used for ESI^+ TOF-MS detection: the dry gas heater for nitrogen was set at 325°C with a gas flow of 14.0 l/min; capillary exit voltage 90V; MS data was acquired in the full scan mode from 100-1000 m/z at three spectra per second.. External calibration was carried out using the ESI tuning mix. Mass spectrometric parameters for the various drug examined are given in Table 1.

A Perkin Elmer Rocker 115 V vacuum pump was used for the manifold set-up for the SPE procedure that was developed. Several columns were experimented with in order to develop the proper SPE method, including: Perkin Elmer Precise-Bed Technology® Supra Clean® mixed mode (MM1) (N9306542) and MM2 (N9306549)), C18 (N9306478), weak cation exchange (WCX) (N9306545), strong cation exchange (SCX) (N9306432), and Supra Poly® HLB polymeric columns (N9306656 and N9306650)(Shelton, CT, USA)

2.3 Chromatographic conditions for RPC and HILIC separations of basic drugs in the SAMHSA-5 panel on a C18 and HILIC column

RPC conditions; column SPP C18; injection volume 1 µL; solvent A: 0.1% formic acid in water and solvent B 0.1% formic acid in acetonitrile; flow rate 0.30 mL/min, and temperature 25 °C. Mobile phase, initial conditions 5%B, 95% A and final conditions 42.2% B, 58.8% B. 6.5 minute linear gradient to final conditions.

HILIC conditions; column SPP HILIC; injection volume 1 µL; solvent 10 mM ammonium formate in acetonitrile: water (9:1); flow rate 0.50 mL/min, and temperature 25 °C. Mobile phase 100% B.

2.4 Chromatographic conditions for RPC and HILIC separations of basic drugs in the SAMHSA-5 panel and "bath salts" on a PFP column

SAMHSA-5 basic drug panel RPC conditions; injection volume 1 µL; solvent A 10 mM ammonium formate in acetonitrile:water (1:9) and solvent B 10 mM ammonium formate in acetonitrile:water (9.5:0.5); flow rate and temperature 0.3 mL/min. and temperature 25°C. Mobile phase, initial conditions 100% A and final conditions 34% A and 66% B. 12 minute linear gradient to final conditions.

SAMHSA-5 basic drug panel HILIC conditions; injection volume 2 µL; solvent A 10 mM ammonium formate in acetonitrile:water (1:9) and solvent B 10 mM ammonium formate in acetonitrile:water (9.5:0.5); flow rate and temperature 0.3 mL/min. and temperature 25°C. Mobile phase 5% A and 95% B run for 6 minutes.

"Bath salts" RPC conditions; injection volume 1 µL; solvent A 5 mM ammonium acetate in acetonitrile:water (1:9) and solvent B 5 mM ammonium acetate in acetonitrile:water (9.5:0.5); flow rate and temperature 0.3 mL/min. and temperature 25°C. Mobile phase, 15% A and 85% B run for 11 minutes.

"Bath salts" HILIC conditions; injection volume 1 µL; solvent A 5 mM ammonium acetate in acetonitrile:water (1:9) and solvent B 5 mM ammonium acetate in acetonitrile:water

(9.5:0.5); flow rate and temperature 0.3 mL/min. and temperature 25°C. Mobile phase, 100% B run for 11 minutes.

2.5 Procedures

2.5.1 Sample preparation "bath salts" and retention factor (k) determination. The standard mixture was prepared by adding 5 µL each of the individual standards (1.0 mg/mL in methanol) into a solution containing 925 mL of a 1:9 mixture of the solvents A and B used for the chromatographic separation. The For retention factor k determinations, the time of the void volume was calculated from the first disturbance of the baseline after injection.

2.5.2 Solid phase extraction procedure for SAMHSA panel in urine. Fresh urine samples were prepared for each extraction. Samples were prepared using 500 µL of synthetic urine, spiking a given amount of drugs and bringing the volume to 1 mL with ammonium formate buffer (200 mM) or acetonitrile.

As reported later in the manuscript, an MM1 performed best for the solutes of interest. The optimized SPE procedure for the extraction of target solutes within the SAMHSA-5 drug panel was as follows. An MM1 mixed mode column with a 1 mL void volume was conditioned with methanol (1 mL), followed by two consecutive washes of ammonium acetate buffer (100 mM, pH~6) (1 mL). The spiked synthetic urine sample (1 mL) was then added to the column and allowed to elute through. The column was washed with water (1 mL), followed by ammonium acetate buffer (100 mM, pH~6) (1 mL), and concentrated acetic acid (diluted 1:10) (1 mL). The sample was then eluted from the column using an elution solvent composed of ammonium hydroxide and acetonitrile (20:80, v/v), in two 1 mL washes. All washes were allowed to elute through the column under positive pressure (between 5 and 15 psi).

2.5.3 UHPLC-TOF-MS assay validation procedures for the determination of the SAMHSA panel in urine. Ion suppression, extraction recovery, and linearity were assessed as previously reported [10, 33]. Two different samples were prepared in order to assess the amount of ion suppression seen under both HILIC and RPC conditions. The first sample, considered the "spiked urine sample" was made by spiking 1 µL of each drug (1.0 mg/mL in MeOH of amphetamine, methamphetamine, MDMA, MDEA, MDA, BZE, PCP, codeine, and morphine) and 1 µL of O6-monoacetylmorphine (1.0 mg/mL in acetonitrile) into a solution containing 500 µL of synthetic urine and 490 µL of acetonitrile. The second sample, or "neat sample" was made by spiking the same amount of each drug as was used in the urine sample preparation into 990 µL of acetonitrile. Both the urine and neat samples were taken through the entire extraction process as noted in Section 2.5. These samples were analyzed under HILIC and RPC conditions. Each of the two samples was injected four times (1 µL injection) and the amount of ion suppression was calculated by dividing the average peak area of the urine sample by the average peak area of the neat sample.

The recovery for the individual analytes from solid phase extraction was determined using HILIC. Two different samples were made in order to perform the recovery study. The first was a "pre-spiked" urine sample, composed of 1 µL of each drug (amphetamine, methamphetamine, MDMA, MDEA, MDA, BZE, PCP, codeine, 6-monoacetylmorphine, and morphine) in a solution of 500 µL of synthetic urine and 490 µL of ammonium formate buffer (100 mM). A "post-spiked" urine sample was composed of 500 µL of synthetic urine, 490 µL of ammonium formate buffer (100 mM), and 10 µL of methanol. Both of these samples were extracted using the solid phase extraction method described in Section 2.5. The elution solvent (NH4OH/ACN 20:80 v/v) resulting from the extraction of the post-spiked urine sample was spiked with 1 µL of each drug (amphetamine, methamphetamine, MDMA, MDEA, MDA, BZE, PCP, codeine, 6-monoacetylmorphine, and morphine). These two samples were analyzed under HILIC conditions and injected four times each (1 µL injection). The percent extraction recovery was determined by dividing with the peak area of the pre-spiked urine sample by the peak area of the post-spiked urine sample.

The linearity of the assay was determined by spiking a post-extracted blank urine sample with differing concentrations of each drug and analyzing them under RPC and HILIC conditions. The "blank urine sample" was composed of 500 µL of urine, 400 µL of ammonium formate buffer (100 mM), and 100 µL of methanol. This sample was extracted using the solid phase extraction method described in Section 2.42, and the resulting elution solvent (NH4OH/ACN 20:80 v/v) was collected and used for the creation of a 10 µg/mL standard stock solution. This standard stock solution was created by spiking 10 µL of each drug (amphetamine, methamphetamine, MDMA, MDEA, MDA, BZE, PCP, codeine, 6-monoacetylmorphine, and morphine) into 900 µL of the elution solvent. Serial dilutions from the solid phase extraction solvent were performed on this 10 µg/mL stock solution in order to generate samples at drug concentrations of 10, 000, 2,000, 400, 200, 80, 40, 16, 8 and 3.3 ng/mL, respectively. These samples were analyzed in triplicate under HILIC and RPC conditions (2 µL and 1 µL injections, respectively).

Limit of detection (LOD) was estimated by measuring the respective signal-to-noise ratio (S/N>3).

3. Results and discussion

3.1 UHPLC-TOF MS separation of SAMHSA basic drug panel solutes of interest

A reversed phase UHPLC-TOF MS separation of a neat standard solution of the target solutes for the SAMHSA basic drug panel is shown in Figure 1. For this separation using a SPP C18 column and a 0.1% formic acid-acetonitrile gradient, co-elution exists between MDA, methamphetamine and O6-monoacetylmorphine. Therefore depending on the relative concentration of these solutes in urine ion suppression could exist and lead to a measured reduced concentration of a target solute. The use of a complementary separation technique such as HILIC UHPLC-TOF MS could resolve the problematic solutes.

A hydrophilic interaction UHPLC-TOF MS separation of a neat standard solution of the above solutes is shown in Figure 2. Although for an isocratic separation using an acetonitrile-ammonium formate mobile phase with a SPP HILIC column there is extensive overlap between several of the solutes, MDA, methamphetamine and O6-monoacetylmorphine are now resolved.

An easier approach would be to use a column with a PFP stationary phase that could be used in both the RP and HILIC mode with the same solutions from the binary solvent reservoir. For A and B solvents consisting of acetonitrile: water with ammonium formate at low and high organic solvent concentration, it was possible to perform separations for the solutes of interest in both the RP and HILIC mode. As shown in Figure 3, complementary separations are obtained as evidenced by the significantly different retention order for the solutes of interest. All solutes are fully resolved by a combination of both techniques.

3.2 Solid phase extraction of analytes of interest

In order to minimize ion suppression, ion enhancement and to preserve the cleanliness of the column and the TOF source, it is necessary to remove components from urine such as creatine, creatinine and urea from the sample matrix. For this purpose solid phase extraction was utilized. Various columns were tested for analyte recovery including the MM1 mixed mode (silica, reversed phase/ strong cation exchange), the MM2 mixed mode (silica, reversed phase/weak cation exchange), WCX (slica, weak cation exchange), C-18 (silica, reversed phase), SCX (silica, strong cation exchange), HLB (polymer, reversed phase), and the Supra-Poly HLB 30 UM (polymer, reversed phase) [34]. Preliminary recovery experiments indicated that the HLB and MM1 columns provided the best extraction efficiency. However the MM1 column, as indicated by TOF MS detection, was the best at removing the natural products found in, and thus it was the column of choice for the developed SPE methodology.

The extraction solvent composed of ammonium hydroxide and acetonitrile (20:80, v/v) was chosen not only to provide good recovery of the analytes of interest, but also to allow direct injection of the solid phase extract into the PFP column under both RPC and HILIC conditions. The presence of a high concentration of acetonitrile will maximize recovery by minimizing hydrophobic interactions of the analytes of interest with the C18 moiety on the solid phase extraction column. The presence of ammonia to maximize recovery is necessary to minimize ion exchange interactions of the basic analytes with the strong cation exchange sites of the solid phase extraction material by increasing the basicity of the solutes and/or if ammonium ion is present (depending on the apparent pH of the extraction solvent) acting as a competing ion. The direct injection into a UHPLC system of the extract containing the analytes of interest would avoid for the solid phase extraction an evaporation and reconstitution step, which would occur when the extraction solvent contains a non-aqueous compatible organic solvent (e.g., methylene chloride) is used. A combination of the mismatch of the solvent strength and apparent pH of solid phase extraction solvent with the starting mobile phase for RPC and the isocratic conditions for HILIC limited the injection volume to 1 and 2 µL, respectively.

3.3 Method validation

Recovery, ion suppression and ion enhancement data for solutes of interest after solid phase extraction is shown in Table 2. Good recovery after solid phase extraction is obtained for the drugs and metabolites (78.1≤%recovery≤102.0). For most solutes recovery losses (or gains) could be explained in part by the %RSD of the peak areas of the pre and post extraction spiked urine samples. For the most part minimal ion suppression and ion enhancement is obtained for RPC and HILIC after solid phase extraction (87.6% ≤ ion suppression/ ion enhancement≤111.1%). For MDMA HILIC and O6-MAM RPC ion enhancement and ion suppression of 120% and ion suppression of 80.2 % respectively is obtained.

Linearity and limit of detection (LOD) data for the drugs and metabolites of interest is shown in Table 3 for both RPC and HILIC. For most solutes, using both chromatographic systems linearity is obtained over two orders of magnitude ($R^2 \geq 0.99$). Amphetamine, which exhibited a poor detection response using HILIC, gave measurable peaks for linearity only at 2000 and 10000 ng/L. For all of the solutes of interest, except for HILIC amphetamine, the LOD was within the SAMHSA guidelines for initial test and confirmatory test cutoff concentrations [35].

Since the higher organic mobile phases in HILIC versus RPC would favor MS ionization for the former technique it was of interest to compare LOQ for both chromatographic modes. Taking in account different peak widths for both techniques and different injection sizes (see Figure 3 and Table 3) only MDMA exhibits the a slightly lower limit of quantitation using HILIC vs RP (~2X), while all other solutes. All other solutes give higher limits of detection using the former technique (~2-15X).

3.4 UHPLC-TOF MS separation of synthetic cathinones

RPC and HILIC separations of 15 controlled synthetic cathinones using a PFP column with the same solutions from the binary solvent reservoir are shown in Figure 4. As per the SAMHSA basic drug panel, complementary separations are obtained as evidenced by the significantly different retention order obtained for the bath salts on both retention modes. As shown in Figure 4, most solutes are fully resolved using a combination of RPC and HILIC. Pentylone and buphedrone which co-elute using RPC are resolved with a resolution of 0.9 using HILIC, which would make the mixed mode approach in tandem with TOF-MS detection suitable for confirmation and/or screening of bath salts. Besides qualitative analysis, the mixed-mode chromatographic approach would be useful for quantitative analysis of seized drugs where co-elution of either ‘bath salts” or target solutes with adulterants could occur.

Since isocratic conditions for RPC and HILIC of synthetic cathinones were relatively close in % Solvent B, it was of interest whether both separation mechanisms were indeed operative for the mobile phases employed. A plot of k versus % Solvent B for “bath salts” exhibits “U shaped” retention behavior with a minimum at 95% Solvent B (see Figure 5). For % Solvent B lower than this minimum value, k increases with decrease in % Solvent B, indicative of reverse phase behavior; while the opposite occurs for % Solvent B above the minimum value, indicative of a HILIC mechanism.

3.5 Orthogonality of RPC and HILIC separations

Although the RPC and HILIC separations of the SAMHSA basic drug panel and the synthetic cannabinoids respectively appeared complimentary, it was of interest to measure the orthogonality of both pairs of separations. RPC versus HILIC regression plots for both the SAMHSA basic drug panel and the "bath salts" are shown in Figures 6 and 7, respectively. Both pairs of chromatographic conditions for each of the applications are orthogonal as indicated by the low R^2 values of 0.0839 and 0.3948, for the SAMHSA basic drug panel and the "bath salts" respectively. The reason for significantly lower R^2 values of the SAMHSA solutes versus the synthetic cathinones is very complex. Solute type, buffer type and concentration, apparent pH, and % acetonitrile can play a role in the differences in separation between RPC and HILIC.

4. Conclusion

A novel method is presented for the analysis of the SAMHSA basic drug panel in urine, and bath salts in seized drugs, which increases accuracy of solute identification, and minimizes sample preparation and or decreases sample analysis time. This rapid approach for enhanced separation selectivity, which uses a single column and the same elution solvents for both RPC and HILIC, could be applicable to other classes of illicit drugs as long as they are amenable to the mixed mode approach.

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Figures

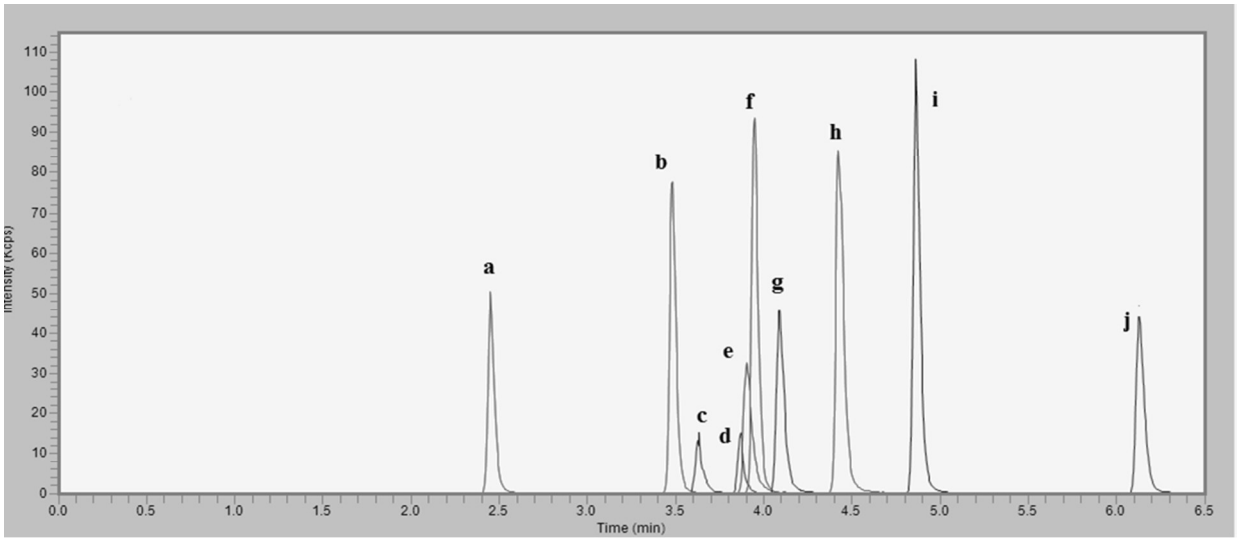

Figure 1. Extracted ion chromatogram (EIC) of a standard mixture of 2000 ng/mL each of (a) morphine, (b) codeine, (c) amphetamine, (d) MDA, (e) methamphetamine, (f) O6-MAM, (g) MDMA, (h) MDEA, (i) BZE, (j) PCP. RPC separation on a SPP C18 column. See experimental section for UHPLC conditions. A and final conditions 42.2% B, 58.8% B. 6.5 minute linear gradient of final conditions.

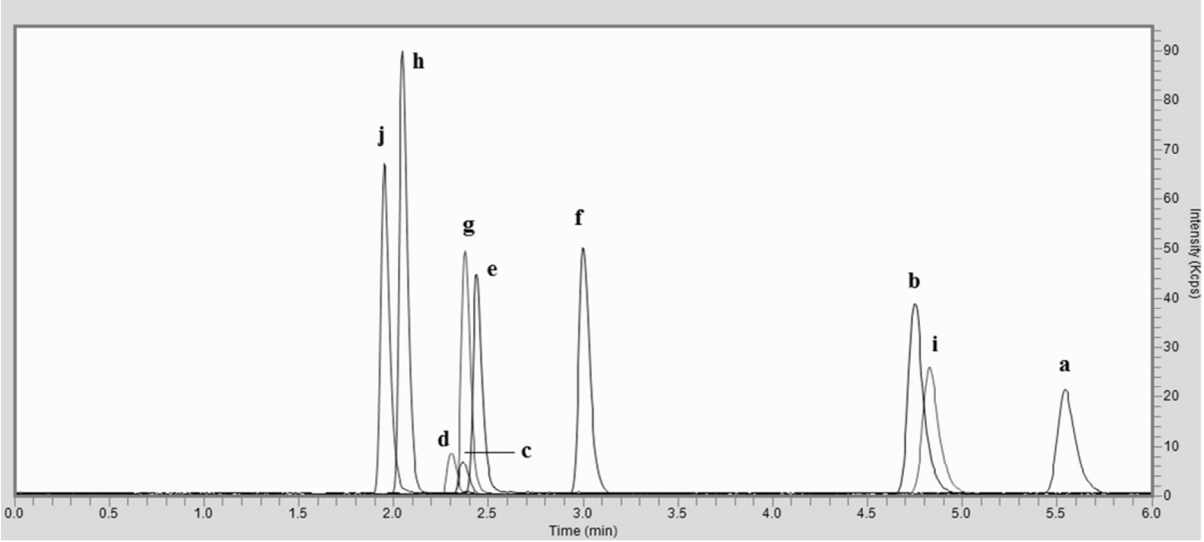

Figure 2. EIC of a standard mixture of 2000 ng/mL each of the solutes a-j, whose identity is shown in figure 1. HILIC separation on a SPP HILIC column. See experimental section for UHPLC conditions.

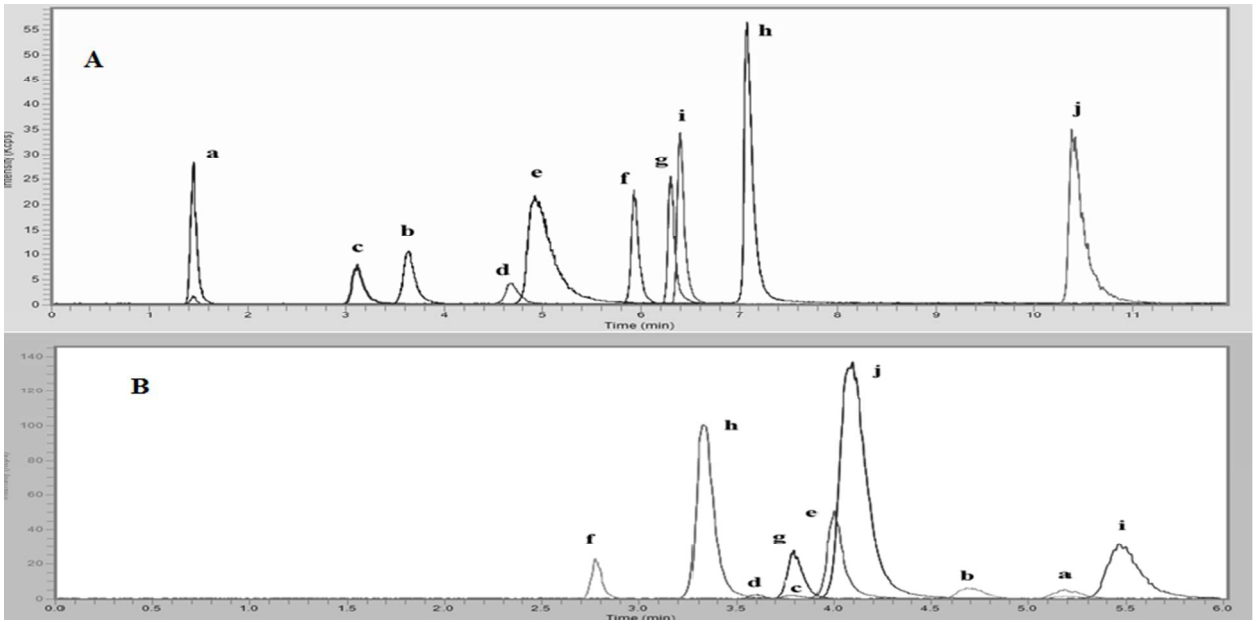

Figure 3. EIC's of a direct SPE extract of synthetic urine containing 2000 ng/mL each of the solutes a-j, whose identity is shown in Figure 1. RPC separation (A) and HILIC separation (B) on a SPP PFP column. See experimental section for SPE and UHPLC conditions.

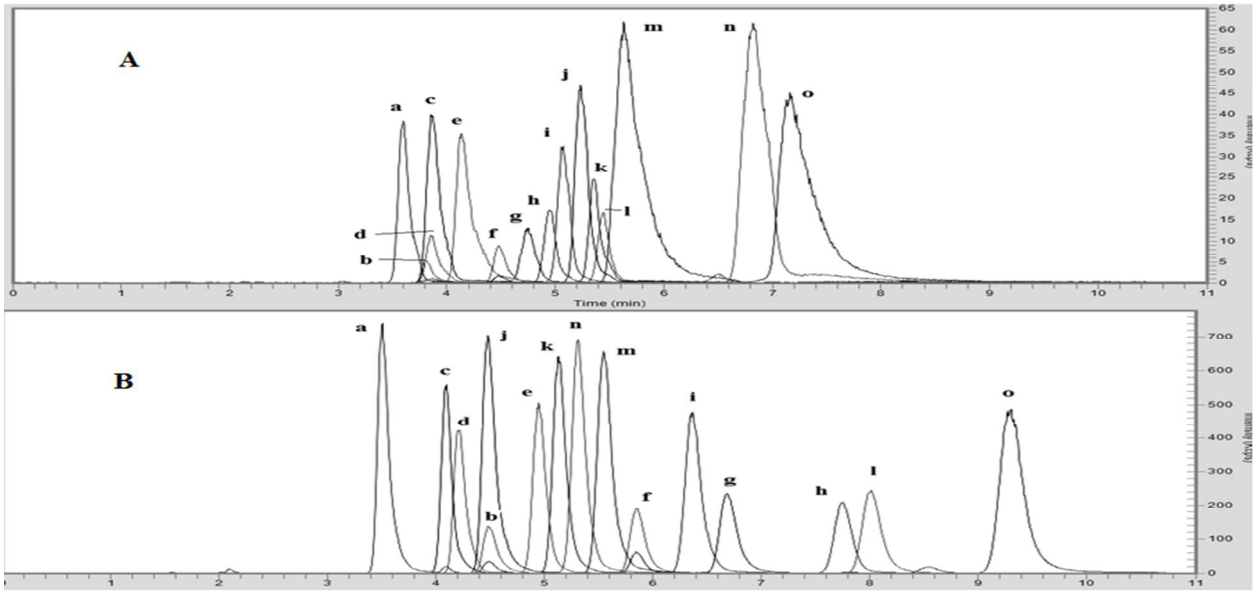

Figure 4. EIC's of a standard mixture of 5000 ng/mL each of (a) pentedrone, (b) 3-fluoromethcathinone, (c) pentylone, (d) buphedrone, (e) butylone, (f) 4-fluoromethathinone, (g) methcathinone, (h) methylone, (i) 4-methylethcathinone, (j) α-PVP, (k) α-PBP, (l) mephedrone, (m) MDPV, (n) naphyrone, (o) 4-MePPP. RPC separation (A) and HILIC separation (B) on a SPP PFP column. See experimental section for UHPLC conditions. The standard mixture was prepared by adding 5 µL each of the individual standards (1.0 mg/mL in methanol) into a solution containing 925 mL of a 1:9 mixture of the solvents A and B used for the chromatographic separation.

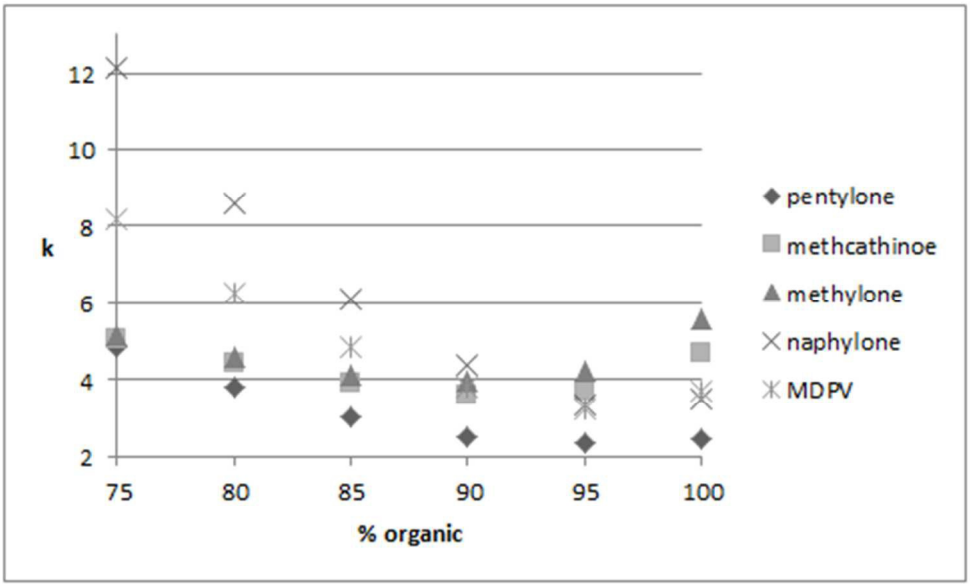

Figure 5. Plot of retention factor k of selected synthetic cathinones versus % solvent B (for a mixture of Solvent A and Solvent B employed in separation of "bath salts"). See experimental section for UHPLC conditions.

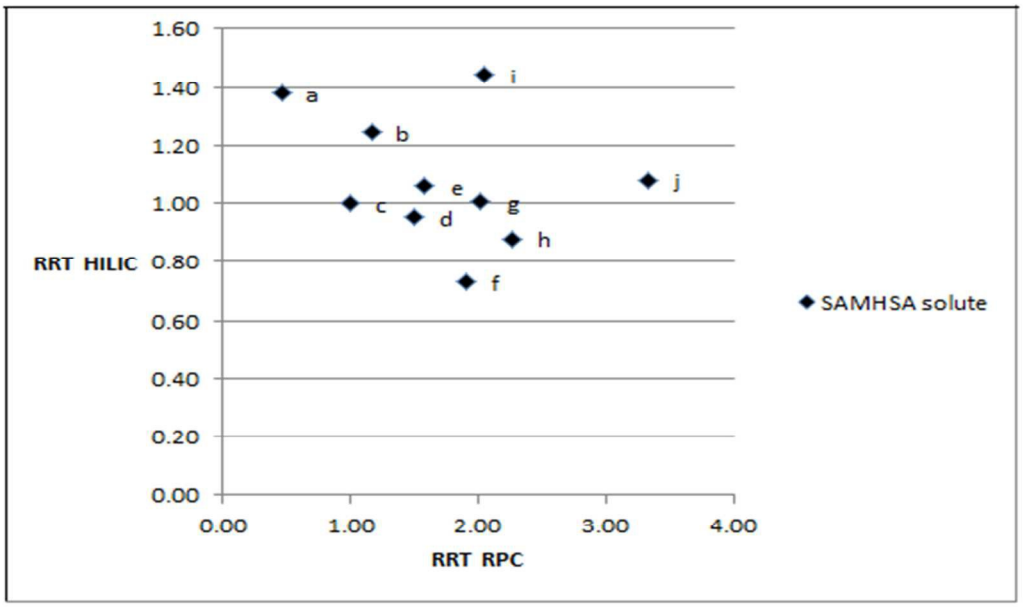

Figure 6. Scatter plot of relative retention (RRT) HILIC versus RRT RPC for SAMHSA mix. Retention times relative to amphetamine. See experimental section for UHPLC conditions.

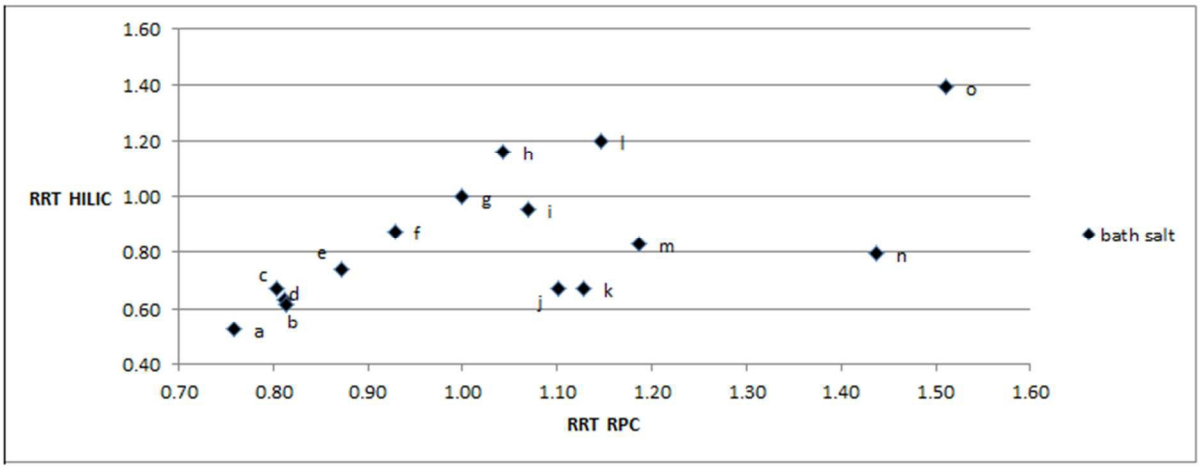

Figure 7. Scatter plot of relative retention (RRT) HILIC versus RRT RPC for controlled bath salts. Retention times relative to methcathinone. See experimental section for UHPLC conditions.

Table 1- Mass spectrometric parameters

Solute	Application	Formula	Mass MH^+
amphetamine	SAMHSA panel	C9H13N	136.1126
methamphetamine	SAMHSA panel	C10H15N	150.1283
MDA	SAMHSA panel	C10H13NO2	180.1025
MDMA	SAMHSA panel	C11H15N02	194.1181
MDEA	SAMHSA panel	C12H17N02	208.1340
PCP	SAMHSA panel	C17H25N	244.2065
morphine	SAMHSA panel	C17H19N03	286.1443
benzoylecgonine	SAMHSA panel	C16H19N04	290.1392
codeine	SAMHSA panel	C18H21N03	300.1600
O6-monoacetylmorphine	SAMHSA panel	C19H21N04	328.1550
methcathinone	"bath salt"	C10H13NO	164.1075
mephedrone	"bath salt"	C11H15NO	178.1232
buphedrone	"bath salt"	C11H15NO	178.1232
4-fluoromethcathinone	"bath salt"	C10H12FNO	182.0981
3-fluoromethcathinone	"bath salt"	C10H12FNO	182.0981
pentedrone	"bath salt"	C12H17NO	192.1388
4-methylethcathinone	"bath salt"	C12H17NO	192.1388
methylone	"bath salt"	C11H13NO3	208.0974
4'-methyl PPP	"bath salt"	C14H19NO	218.1545
α-PBP	"bath salt"	C14H19NO	218.1545
butylone	"bath salt"	C12H15N03	222.1130
α-PVP	"bath salt"	C15H21NO	232.1701
pentylone	"bath salt"	C13H17N03	236.1287
MDPV	"bath salt"	C16H21NO3	276.1600
naphyrone	"bath salt"	C19H23NO	282.1858

Table 2- Recovery, ion suppression and ion enhancement data for UHPLC RP TOF MS and HILIC TOF MS separations of drugs and metabolites described in the experimental section

Drug	Mode	% Recovery %RSD area pre-extraction spiked, post extraction spiked	% Ion Suppression/ Ion Enhancement %RSD urine drug sample, neat drug sample
amphetamine	RPC		109.8 (9.1, 9.3)
amphetamine	HILIC	92.2 (11.7, 9.1)	97.8 (4.5, 11.1)
methamphetamine	RPC		101.8 (16.0, 3.8)
methamphetamine	HILIC	96.1 (4.5, 6.5)	102.1 (3.3, 5.2)
MDA	RPC		95.4 (3.9, 10.9)
MDA	HILIC	83.9 (13.6, 6.8)	110.5 (5.3, 11.4)
MDMA	RPC		98.2 (8.8, 9.8)
MDMA.	HILIC	95.0 (5.8, 7.2)	120.0 (3.6, 6.2)
MDEA	RPC		88.6 (6.2, 8.1)
MDEA	HILIC	94.1 (3.7, 4.5)	104.1 (2.4, 3.0)
PCP	RPC		104.7 (16.8, 14.9)
PCP	HILIC	78.1 (5.7, 4.1)	101.4 (3.1, 7.7)
BZE	RPC		87.6 (7.9, 0.4)
BZE	HILIC	102.0 (8.5, 7.5)	105.4 (7.0, 15.3)
morphine	RPC		105.8 (3.5, 4.5)
morphine	HILIC	97.8 (9.3, 10.4)	111.6 (7.9, 10.0)
codeine	RPC		91.5 (10.8, 6.2)
codeine	HILIC	97.8 (8.4, 7.0)	110.8 (1.9, 11.0)
O6-MAM	RPC		80.2 (26.6, 19.4)
O6-MAM	HILIC	88.7 (7.3, 6.8)	100.3 (3.8, 9.3)

Table 3- Linearity data for UHPLC RP TOF MS and HILIC TOF MS separations of drugs and metabolites described in the experimental section

Drug	Mode	Linearity range (ng/mL)	R^2	LOD (ng/mL)	Initial Test Cutoff Concentration	Confirmatory Test Cutoff Concentration
amphetamine	RPC	40-10000	0.9984	13	500	250
amphetamine	HILIC				500	250
methamphetamine	RPC	16-10000	0.9976	5	500	250
methamphetamine	HILIC	8-2000	0.9999	3	500	250
MDA	RPC	80-2000	0.9990	27	500	250
MDA	HILIC	200-10000	0.9999	67	500	250
MDMA	RPC	40-10000	0.9992	13	500	250
MDMA	HILIC	8-2000	0.9991	3	500	250
MDEA	RPC	16-10000	0.9993	5	500	250
MDEA	HILIC	8-2000	0.9951	3	500	250
PCP	RPC	16-10000	0.9957	5	25	25
PCP	HILIC	3.2-2000	0.9936	1	25	25
BZE	RPC	16-10000	1.0000	5	150	100
BZE	HILIC	40-10000	0.9997	13	150	100
morphine	RPC	16-10000	0.9921	5	2000	2000
morphine	HILIC	80-10000	0.9991	27	2000	2000
codeine	RPC	40-10000	0.9989	13	2000	2000
codeine	HILIC	80-10000	0.9966	27	2000	2000
O6-MAM	RPC	16-10000	0.9996	5	10	10
O6-MAM	HILIC	16-10000	0.9997	5	10	10

Methodology is presented for the rapid analysis of important drugs of abuse using a single extraction procedure and a single UHPLC column with orthogonal methods using different combinations of the same solutions in the solvent reservoir.