Development of a high-throughput differential mobility separation–tandem mass spectrometry (DMS-MS/MS) method for clinical urine drug testing

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A R T I C L E   I N F O

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A B S T R A C T

Introduction: Differential mobility separation (DMS) is an analytical technique used for rapid separation of ions and isomers based on gas phase mobility prior to entering a mass spectrometer for analysis. The entire DMS process is accomplished in fewer than 20 ms and can be used as a rapid alternative to chromatographic separation.

Objective: The primary objective was to evaluate the utility of DMS-tandem mass spectrometry (DMS-MS/MS) as a replacement for immunoassay-based clinical toxicology testing.

Methods: A sensitive DMS-MS/MS method was developed and validated for simultaneous identification of 33 drugs and metabolites in human urine samples. After DMS optimization, the method was validated and used to screen 56 clinical urine samples. These results were compared to results obtained by immunoassay.

Results: The DMS-MS/MS method achieved limits of detection ranging from 5 to 100 ng/mL. Moreover, the total analysis time was 2 min per sample. For the method performance evaluation, DMS-MS/MS results were compared with previously obtained urine toxicology immunoassay results. DMS-MS/MS showed higher sensitivity and identified 20% more drugs in urine, which were confirmed by LC-MS/MS.

Conclusion: The DMS-MS/MS as applied in our lab demonstrated the capability for rapid drug screening and provided better analytical performance than immunoassay.

Introduction

Urine drug testing in the clinical laboratory is traditionally a two-step process. Samples are first screened by a group of immunoassay-based tests for specific drugs or drug classes. Then, the positive samples are analyzed by a confirmatory test, such as gas chromatography–liquid chromatography–mass spectrometry (LC-MS or LC-MS) [1–3]. Limitations of this approach for urine drug testing are well known. Immunoassay methods are relatively inexpensive, fast, and simple; however, these assays are not available for all drugs of clinical interest and can generate high rates of false positive and false negative results [4]. GC-MS and LC-MS methods are highly sensitive and specific, but they require sample preparation and chromatographic separation, which can be time consuming and labor-intensive; run-time per sample can range from 5 to 30 min and testing is typically performed in a batch limiting its use as a technique for rapid toxicological screening [5–9].

Several direct MS techniques, including desorption electrospray ionization (DESI), atmospheric pressure photo ionization (DAPPI), direct analysis in real time (DART), paper spray, fiber spray ionization (FSI) and wooden-tip electrospray ionization (WT-ESI) have been used...
to develop fast and sensitive screening methods with minimal sample preparation steps [5,9-13]. However, without the use of column chromatography, there is no separation of analytes from background ions, which can result in increased matrix effects, decreased sensitivity and lack of differentiation of isobaric compounds [10,14].

As an alternative to chromatography, ion mobility spectrometry (IMS) is an analytical technique that offers a rapid separation of ions and isomers based on their gas phase mobility prior to MS in the presence of a weak electric field. The mobility of an ion depends on its size, charge, and shape. Ion separation can be accomplished on the millisecond time scale, which makes the technique attractive for high-throughput applications, such as clinical drug screening [15-19].

Differential ion mobility separation (DMS), also known as field asymmetric waveform ion mobility spectrometry (FAIMS), is a type of IMS that operates at atmospheric pressure and separates ions based on the difference between ion mobility in low and high electric fields. The DMS cell is made of two flat electrodes that are parallel to each other and shape a mobility region. The ions are drawn by the transport gas flow towards the MS. Separation Voltage (SV) is a sinusoidal high-voltage radio frequency that is applied across the electrodes, perpendicular to the direction of the transport gas flow. SV generates a separation waveform in the gap between electrodes. Due to the difference between high and low field ion mobility coefficients, ions will migrate toward the electrodes and leave the flight path unless their trajectory is corrected by a direct current (DC) voltage, called the compensation voltage (COV) [20-23]. Conventional IMS records the flight time (drift time) of an ion through the ion transport channel (drift tube). The rate of the process is limited by the drift time and ions can be lost between cycles. However, DMS can be operated continuously by using a certain combination of SV and COV fields to allow filtration of targeted ions without colliding with the electrodes [16,24]. An organic solvent, also called a modifier, can also be added into the DMS cell to increase the separation power and the peak capacity. Clustering and declustering can occur between ions and the modifier molecules in the low and high electric fields, respectively. This phenomenon increases the ion mobility differential between low-field and high-field, resulting in improved separation [25-26].

There are limited reports of the measurement of drugs of abuse in postmortem tissue, urine and serum by DMS-MS [18,24-26]. Drugs including amphetamine, methamphetamine, and cocaine and its metabolites have been separated and quantified in urine and serum by DMS-MS [18,24-25,27]. However, these methods mainly focused on determination of one class of drug in biological specimens. Hall et. al. also reported a DMS-MS method for quantification of five drug metabolites, norfentanyl, noroxazepam, benzoylecgonine, 6-acetylmorphine and morphine glucuronide, in spiked urine samples. Even though this method had good sensitivity and reproducibility, it was still limited to a small number of drugs and metabolites [24]. One study reported a method for quantification of a larger group of analytes (30 drugs and metabolites) using a combination of DMS and liquid extraction surface analysis in postmortem tissues [26]. However, this report mostly focused on the aspects of method development and the proposed method was ultimately applied to only two real samples.

In this study, a DMS-MS/MS method is described for simultaneous screening of 33 drugs and metabolites in urine. The limit of detection (LOD), recovery and matrix effects (ME) for each compound were validated. For the method performance evaluation, patient urine samples were tested with standard routine immunoassays, DMS-MS/MS and an LC-MS/MS method that was used for confirmation. The method was compared with immunoassays for the following drug classes: amphetamines/ecstasy, cocaine metabolite (benzoylcegonine), benzodiazepines, buprenorphine, opiates, methadone metabolite (2-ethyliden-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP)), oxycodone/oxymorphone, 6-Monoacetylmorphine (6-MAM), and fentanyl.

Materials and methods

Reagents and standards

Drug standards were purchased from Cerilliant (Round Rock, TX) and Cayman Chemical (Ann Arbor, MI). Amphetamine-D6, hydrodromphine-D6, Cocaine-D3, Alprazolam-D5, fentanyl-D5 and buprenorphine-D4 were used as internal standards (IS) and were purchased from Cerilliant. Acetone, methanol, water, ammonium formate and formic acid were purchased from Fisher Scientific (Wal-tham, MA). Acetone, ethanol and ethyl acetate were purchased from Sigma-Aldrich (St. Louis, MO). All solvents and reagents were LC-MS or analytical grade. Drug-free urine was obtained from UTAK laboratories (Valencia, CA). BGTurbo® glyceral free high efficiency recombiant β-glucuronidase and instant buffer were acquired from KURA Biotech (Rancho Dominguez, CA).

Compounds were divided into five groups. Each group contained only one isomeric compound, being either morphine, hydromorphone, codeine, hydrocodone or 7-aminoconlazepam. Other analytes were randomly divided between these groups. Five combined stock solutions based on this classification were prepared in methanol at 10 µg/mL. Standard working solutions were obtained by serial dilutions, over the range of 10–10,000 ng/mL. The IS working solution was prepared at 5000 ng/mL. All dilutions were made with methanol. These working solutions were used to make calibrators and quality controls (CQs) in urine. Stock solutions were kept at –20 °C when not in use and replaced every 6 months. Fresh working solutions were prepared for each day of analysis or validation. A volume of 10 µL of working solution was spiked into 100 µL of urine to generate the corresponding calibrators or QC samples. This yielded concentrations of 1, 5, 10, 50, 100 and 1000 ng/mL in urine.

Sample preparation

To release the glucuronidated urinary metabolites, BG Turbo glucuronidase enzyme was chosen to provide fast partial hydrolysis. To each 100 µL of urine, 10 µL of IS working solution (5000 ng/mL), 50 µL BG Turbo glucuronidase, and 40 µL instant buffer were added. Samples were incubated at 55 °C for 10 min. Hydrolysis efficiency was between 20 (opioids) to 96% (benzodiazepines) for different drug classes. Following hydrolysis, 100 µL of the urine samples was transferred to a 96-Well Protein Precipitation Filter Plate (Sigma–Aldrich, St. Louis, MO). Then, 300 µL of acetone was added to each well and thoroughly mixed with the sample for cleanup and removal of the enzyme. A vacuum was applied to filter samples. 20 µL of filtrate was injected for DMS-MS/MS analysis. For LC-MS/MS analysis, the filtrate was diluted 1:1 with 10 mM ammonium formate and 20 µL was injected.

Method development

To develop a sensitive and accurate method for urine drug screening, various factors and parameters were optimized for both the DMS and the tandem mass spectrometry (MS/MS) methods. For the sensitive detection of analytes, the triple quadrupole mass spectrometer was set to operate under unit mass resolution. Since the analytes were weak bases, positive ion mode was used. The detection of analytes and ISs was conducted using MRM, providing high sensitivity and selectivity. Compound and source-related parameters were optimized first in the DMS Off operation mode. Collision energy (CE), declustering potential (DP) and collision cell exit potential (CXP) were optimized with direct infusion of standard solutions by performing automatic MS/MS optimization (10 ng/mL in methanol at a flow rate of 7 µL/min). The most abundant fragment ion for each compound was selected for the monitored ion-transition in MRM mode. Ion source/gas parameters were optimized with manual tuning using T-infusion (flow rate of 0.3 mL/min) to achieve the strongest intensity for the MS/MS signal (Table 1).
The DMS parameters were optimized to obtain the best signal and separation of compounds. The key parameters that influence DMS separation and sensitivity are SV, DR and the chemical modifier. These parameters were optimized in the DMS On operation mode by using T-infusion (flow rate of 0.3 mL/min).

### DMS-MS/MS conditions

The DMS separation was achieved using SelectiON+ (ABSciex, Redwood City, CA) coupled to a Sciex QTRAP 6500 + mass spectrometer. A Shimadzu ExionLC™ AD HPLC system (Kyoto, Japan) was interfaced to the mass spectrometer and used for sample injection without an analytical column. Flow rate was set at 0.3 mL/min and the mobile phase consists of 0.1% formic acid in methanol. The injection volume was 20 μL and the flow rate was 0.7 mL/min. The gradient condition was (in minutes, % mobile phase B): (0,0), (0.7, 40), (4.5, 100), (6, 60) and (7.5, 20). Samples were analyzed by mass spectrometry in positive ion ESI mode. The source parameters were as follows: curtain gas of 30 psi, ion spray voltage of 2500 V, ion source temperature of 650 °C, medium collision gas, ion source gas 1 (GS1) of 50 psi and GS2 of 60 psi. MRM mode was utilized, and MRM parameters were the same as DMS-MS/MS method (Table 1). Target scan time was 0.5 s and all analytes were monitored within a ± 0.5 min retention time window.

### Method validation

The method was verified by evaluating the LOD, recovery and ME. The sensitivity of the method was defined by the LOD, which was considered to be the lowest concentration that could be measured with a signal-to-noise ratio (S/N) of 3. LOD was determined by spiking drug standards into drug-free urine of ten healthy subjects at various concentrations (1, 5, 10, 50, 100 and 1000 ng/mL).

Recovery and ME in urine were calculated from the area ratio of spiked samples, post-preparation spiked samples and standard solutions at 100 and 1000 ng/mL. For each concentration, three spiked samples, three post-preparation spiked samples and three neat solutions were prepared. The post-preparation spiked samples were made by spiking standard solutions into blank urine processed by the same sample preparation. The neat standards for each concentration were prepared by spiking working standard solutions into water.

The recovery was represented by the area ratios between spiked samples and the average of neat standard solutions at the same concentration. ME was represented by the area ratios between post-preparation spiked samples and the average of corresponding neat standard solutions. A ratio over 100% demonstrates an enhancing ME, a ratio below 100% demonstrates a suppressing ME, and the percentage of enhancement or suppression is calculated as the absolute difference from 100%.

### Method comparison

To compare the performance of the DMS-MS/MS and immunosay methods, 56 patient urine samples were analyzed by both methods for the same 9 classes of drugs and metabolites. This study was approved by the University of California–San Francisco Committee on Human Research, which deemed that patient consent was not required.

Urine samples were submitted to the San Francisco General Hospital Clinical Laboratory for routine urine toxicology analysis, which included screening by immunoassays for amphetamines/ecstasy (CEDIA, Thermo Scientific, cut-off 1000 ng/mL), cocaine metabolite benzoylcygon (CEDIA, Thermo Scientific, cut-off 300 ng/mL), benzoisazepines (CEDIA, Thermo Scientific, cut-off 200 ng/mL), buprenorphine (EIA, Lin-Zhi International, cut-off 10 ng/mL), opiates (OPI) (CEDIA, Thermo Scientific, cut-off 300 ng/mL), methadone metabolite (EDDP) (CEDIA, Thermo Scientific, cut-off 100 ng/mL), oxycodone/oxymorphone (OXY) (DRI, Thermo Scientific, cut-off 100 ng/mL), 6-

### Table 1

Optimized ESI (+) mass spectrometric and DMS conditions for multiple reaction monitoring (MRM) of compounds.

| Name                  | Q1   | Q3   | CE   | COV  | CXP  | DP  |
|-----------------------|------|------|------|------|------|-----|
| Amphetamine           | 136.1| 91.0 | 27   | −36  | 8    | 21  |
| Methamphetamine       | 150.1| 91.0 | 29   | −42  | 8    | 51  |
| Pregabalin            | 160.0| 142.3| 15   | −39  | 9    | 46  |
| Gabapentin            | 172.1| 154.2| 20   | −40  | 4    | 40  |
| DDA                   | 180.1| 133.0| 25   | −28  | 3    | 8   |
| MDMA                  | 194.1| 105.1| 33   | −26  | 10   | 46  |
| Norfentanyl           | 233.2| 84.1 | 23   | −34  | 8    | 36  |
| 5-Demethyl Tramadol   | 250.1| 58.0 | 41   | −34  | 8    | 56  |
| Tramadol              | 264.1| 50.1 | 47   | −24  | 6    | 56  |
| Noroxazepam           | 271.0| 140.0| 37   | −24  | 10   | 56  |
| EDDP                  | 278.2| 234.2| 41   | −8   | 10   | 36  |
| Diazepam              | 285.0| 193.2| 43   | −8   | 10   | 76  |
| 7-Methoxonazepam       | 286.1| 222.2| 33   | −19  | 12   | 91  |
| Hydromorphone         | 286.1| 185.1| 41   | −11  | 16   | 126 |
| Morphine              | 286.1| 152.2| 75   | −10  | 4    | 126 |
| Oxazepam              | 287.0| 241.1| 31   | −37  | 8    | 81  |
| Benzoyleucogenine     | 290.1| 168.1| 27   | −32  | 12   | 76  |
| Codeine               | 300.2| 152.1| 83   | −10  | 8    | 76  |
| Hydrocodeine          | 300.2| 199.0| 41   | −10  | 10   | 51  |
| Temazepam             | 301.1| 255.1| 51   | −22  | 6    | 81  |
| Oxymorphone           | 302.1| 227.1| 41   | −29  | 14   | 101 |
| Cocaine               | 304.0| 182.2| 27   | −22  | 10   | 61  |
| Alprazolam            | 309.1| 281.1| 47   | −5   | 22   | 116 |
| Methadone             | 310.0| 77.1 | 77   | −6   | 8    | 41  |
| Oxycodone             | 316.2| 256.2| 33   | −27  | 16   | 91  |
| Lorazepam             | 321.0| 275.0| 41   | −31  | 18   | 91  |
| Hydroxyalprazol        | 325.1| 297.0| 35   | −11  | 10   | 91  |
| Midazolam             | 326.1| 291.1| 53   | −5   | 18   | 111 |
| 5-MAM                 | 328.1| 165.1| 51   | −7   | 8    | 91  |
| Fentanyl              | 337.3| 188.1| 31   | −15  | 10   | 96  |
| Hydroxyimidazolam     | 342.1| 324.1| 31   | −20  | 4    | 96  |
| Norbuprenorphine       | 414.3| 165.2| 111  | −1   | 8    | 116 |
| Buprenorphine         | 468.3| 396.2| 55   | −6   | 10   | 101 |
| Buprenorphine-D4      | 472.3| 400.0| 57   | −6   | 12   | 141 |
| Fentanyl-D5           | 482.3| 188.1| 31   | −15  | 6    | 81  |
| Alprazolam-D5         | 414.0| 286.0| 47   | −5   | 22   | 116 |
| Cocaine-D3            | 307.0| 185.0| 27   | −22  | 10   | 61  |
| Hydroxyalprazol-D6    | 292.3| 185.0| 43   | −11  | 12   | 106 |
| Methamphetamine-D8    | 158.1| 93.0 | 15   | −42  | 6    | 51  |
| Amphetamine-D6        | 142.1| 95.0 | 21   | −36  | 8    | 46  |
| Collision Energy (CE), Compensation Voltage (COV), Declustering Potential (DP), Collision Cell Exit Potential (CXP) |

Table 1. Optimal ESI (+) mass spectrometric and DMS conditions for multiple reaction monitoring (MRM) of compounds.
Monoacetylmorphine (6-MAM) (EMIT II Plus, Siemens, cut-off 10 ng/mL), and fentanyl (DRI, Thermo Scientific, cut-off 2 ng/mL). The urine remaining after routine testing was aliquoted and stored at −20 °C for additional testing by DMS-MS/MS and LC-MS/MS. A drug was confirmed if the parent or metabolite was detected by the LC-MS/MS method.

For comparing DMS-MS/MS with the immunoassay, detected drugs and metabolites by DMS-MS/MS or LC-MS/MS were grouped and reported by their drug class. For example, if benzoylecgonine and/or cocaine was detected in a patient’s sample, it was counted only as cocaine. The sensitivity and specificity for both methods were calculated based on the following formulas: Sensitivity = True positives / (True positives + False negatives) and Specificity = True negatives / (True negatives + False positives).

Data analysis

Analyst 1.7® and Sciex OS quant 1.6® software packages from ABSciex (Redwood city, CA, USA) were utilized for system control, data acquisition and data analysis. Sciex OS quant 1.6® was used in the summation mode for data interpretation.

Results and discussion

Effect of SV on DMS separation

The effect of SV on analyte separation was evaluated in the range of 2000 to 3800 V. Discharge errors occurred at SV greater than 3800 V and no separation was observed at SV less than 2000 V. The best separation for all analytes was observed at the SV of 3800 V, when the COV was ramped between 55 to 15 V. A high SV is desirable since it amplifies a higher mobility coefficient difference between the high and low electric fields, resulting in improved separation. Fig. 1 shows an example of how variations in the SV (2000, 2500, 3500 and 3800 V) can impact the separation of compounds for one class of drugs. Benzodiazepines were chosen as a representative example where COV was ramped from −40 to 10 V while other parameters were held constant.

Effect of chemical modifier on DMS separation

DMS resolution and peak capacity can be increased by adding chemical modifiers to the transport gas. Although using modifiers can increase DMS gas phase separation, decrease in the MS response may also occur. During method development, the effect of five organic modifiers (i.e., methanol, ethanol, acetonitrile, acetone and ethyl acetate) on the separation of analytes was evaluated. DMS separation of benzodiazepines, as an example, with or without modifiers is shown in Fig. 2. Addition of organic modifier increased the separation of the peaks on the COV scale compared to nitrogen alone. While different COV values were observed for each modifier, the best separations were observed with acetone and ethyl acetate. The use of ethyl acetate and ethanol caused a significant decrease in the MS signal compared to nitrogen (intensities of all analytes decreased by 73–100%).

Morphine/hydromorphone, codeine/hydrocodone and morphine/7-aminoclonazepam are well-known structural isomers and accurate identification and quantitation cannot be achieved without separation prior to analysis by mass spectrometry. Even after adding modifier solvents, we were not able to separate opioid isomers. Morphine and 7-aminoclonazepam have the same mass and similar MS/MS spectra. While loss of intensities (up to 50%) were observed with acetone, a complete separation was achieved for morphine (COV: −10 V) and 7-aminoclonazepam (COV: −19 V). Acetone ultimately was chosen as the modifier. The optimal COV values are listed in Table 1. The final separation of all analytes on the COV scale is shown in Fig. 3.

Effect of DMS resolution enhancement (DR) on separation

To maximize the separation power, the DR function can be used during the method development. It adds extra nitrogen to the DMS cell in the opposite direction of the transfer gas, which slows the ion flow through the DMS cell, thereby increasing the resolution. However, use of the DR setting can result in a decrease in signal intensity of more than

Fig. 1. Effect of separation voltage (SV) on the separation of benzodiazepines. A) SV: 2000 V, B) SV: 2500 V, C) SV: 3500 V, D) SV: 3800 V. Benzodiazepines were chosen as a representative example between different classes of drugs. COV was ramped from −40 to 10 V and other parameters held constant: DMS temperature, low; modifier, acetonitrile; modifier composition, Low; DMS offset, −3; DMS resolution enhancement, Open.
The effect of DR on DMS separation and intensities are shown in Fig. 1. Setting DR at 10 improved the separation power, however, the MS signal was completely lost for amphetamine, methamphetamine, pregabalin, gabapentin and MDA, and decreased more than 4-fold for other analytes. Due to the impact of DR on sensitivity, especially for smaller molecules, this function was not used in the final method.

Method validation

Results of the method validation are shown in Table 2. The LODs ranged from 5 to 100 ng/mL, which was significantly lower compared to immunoassay, except for fentanyl (DMS-MS/MS: 5 ng/mL, immunoassay: 2 ng/mL) (Table 2). Recovery and ME in urine were calculated to be 100 and 1000 ng/mL (n = 3). The average values are shown in Table 2. ME ranged between −72% and 53% with both ion suppression.
Table 2  
Validation results for the DMS-MS/MS method for 33 non-FDA drugs and metabolites in urine.

| Name                | LOD (ng/mL) | Recovery (%) | ME (%) | IS Paring |
|---------------------|-------------|--------------|--------|-----------|
| Amphetamine         | 50          | 52 ± 6.61    | –72 ± 5.17 | Amphetamine-d6 |
| Methamphetamine    | 5           | 56 ± 5.08    | –42 ± 4.63 | Methamphetamine-d8 |
| MDA                 | 50          | 40 ± 8.87    | –58 ± 3.36 | Amphetamine-d6 |
| MDMA                | 50          | 99 ± 3.01    | –52 ± 4.60 | Amphetamine-d6 |
| Fentanyl            | 5           | 93 ± 2.11    | 5 ± 1.80  | Fentanyl-D5 |
| Norfentanyl         | 10          | 137 ± 4.80   | –33 ± 5.88 | Fentanyl-D5 |
| Methadone           | 50          | 56 ± 2.66    | –38 ± 8.67 | Fentanyl-D5 |
| EDDP                | 5           | 168 ± 7.05   | 53 ± 2.28  | Fentanyl-D5 |
| Diazepam            | 10          | 4 ± 5.86     | –67 ± 7.53 | Alprazolam-D5 |
| Nordiazepam         | 100         | 46 ± 7.30    | –69 ± 9.62 | Alprazolam-D5 |
| 7-aminoclonazepam   | 50          | 67 ± 4.81    | –64 ± 3.80 | Alprazolam-D5 |
| Oxazepam            | 5           | 71 ± 1.98    | –32 ± 2.59 | Alprazolam-D5 |
| Temazepam           | 50          | 68 ± 6.45    | –39 ± 2.33 | Alprazolam-D5 |
| Alprazolam          | 10          | 38 ± 3.57    | –45 ± 7.02 | Alprazolam-D5 |
| Lorazepam           | 10          | 12 ± 3.83    | –66 ± 1.99 | Alprazolam-D5 |
| Hydroxy alprazolam  | 100         | 30 ± 1.10    | –60 ± 3.44 | Alprazolam-D5 |
| Midazolam           | 10          | 175 ± 10.98  | 4 ± 6.00  | Alprazolam-D5 |
| Hydroxymidazolam    | 10          | 60 ± 0.98    | –35 ± 2.69 | Alprazolam-D5 |
| Hydromorphone       | 50          | 104 ± 0.68   | –33 ± 10.01 | Hydromorphone-D6 |
| Morphine            | 50          | 50 ± 2.67    | –41 ± 8.96 | Hydromorphone-D6 |
| Codeine             | 50          | 116 ± 1.95   | –1 ± 3.93  | Hydroxymorphone-D6 |
| Hydrocodone         | 5           | 112 ± 2.23   | –35 ± 7.22 | Hydroxymorphone-D6 |
| Oxycodone           | 50          | 122 ± 5.85   | 1 ± 7.83   | Hydromorphone-D6 |
| Oxymorphone         | 50          | 135 ± 4.15   | 7 ± 0.99   | Hydromorphone-D6 |
| Cocaine             | 10          | 123 ± 8.06   | –20 ± 6.36 | Cocaine-D3 |
| Benzylecgonine      | 10          | 52 ± 7.75    | –8 ± 7.03  | Cocaine-D3 |
| 6-MAM               | 5           | 96 ± 3.11    | –53 ± 10.12 | Fentanyl-D5 |
| Norbuprenorphine    | 100         | 30 ± 1.66    | –70 ± 5.76 | Fentanyl-D5 |
| Buprenorphine       | 10          | 98 ± 1.78    | –37 ± 2.55 | Buprenorphine-D4 |
| Tramadol            | 50          | 107 ± 0.58   | –11 ± 1.80 | Buprenorphine-D4 |
| O-Demethyl          | 5           | 121 ± 6.70   | –25 ± 1.80 | Fentanyl-D5 |
| Tramadol            | 50          | 29 ± 8.90    | –71 ± 8.22 | Fentanyl-D5 |
| Pregabalin          | 72          | 29 ± 8.90    | –71 ± 8.22 | Fentanyl-D5 |
| Gabapentin          | 50          | 38 ± 10.68   | –70 ± 8.03 | Fentanyl-D5 |

* Absolute recovery (%AR, n = 3), and matrix effect (%ME, n = 3) and enhancement observed; however, the majority of observed ME was suppression. The co-extraction of biological sample components can compete with the analytes of interest during ESI and cause variable suppression or enhancement in the MS response for the analytes. Since DMS separation happens after the ionization process, the high matrix effects observed for amphetamines, benzodiazepines, norbuprenorphine, pregabalin and gabapentin are likely due to the lack of separation between analytes or background ions during the ionization step (Table 2). Lower recovery was also observed for the same groups of compounds.

**Method comparison**

The rapid separation and identification of small molecules is still a challenge for high-throughput analysis. This is especially true for complex samples containing many target analytes, isomeric substances and a variety of unknown matrix components. DMS-MS/MS has the potential to accelerate drug screening due to its robust and reproducible selectivity and separation ability. In this study, the DMS-MS/MS results were compared with immunoassay results in 56 patient urine samples. DMS-MS/MS identified 215 drugs, of which all were confirmed by LC-MS/MS. Immunoassay identified 186, of which 179 were confirmed (Table 3 and Fig. 4). The specificity of the DMS-MS/MS for all drug classes was 100%, while the specificity ranged from 73 to 100% for immunoassay.

The sensitivity of the DMS-MS and immunoassays was in the range of 71–94% and 15–91%, respectively. The lowest sensitivity was observed for fentanyl (71%) for DMS-MS/MS and 6-MAM (15%) for immunoassays. False negatives reported by immunoassay were 50% higher than for DMS-MS/MS. Cocaine, oxycodone and 6-MAM were repeatedly missed by immunoassay.

Overall, the DMS-MS/MS method was more analytically sensitive and performed better than immunoassay in identifying drugs and metabolites in 56 clinical urine samples (Tables 2, 3 and Fig. 4). Moreover, the total analysis time, was 2 min per sample and required simple sample preparation. DMS also allowed for direct and simultaneous identification of drugs and metabolites, not just drug classes (except for opiates). Although immunoassays are widely used for urine drug screening, they are not available for all drugs monitored in clinical laboratories (4). In this study for example, immunoassays were not available for pregabalin, gabapentin, tramadol or its metabolite, demethyl tramadol. GC-MS or LC-MS techniques are essential for analysis of these types of drugs. Here, they were included in the DMS-MS/MS method and the specificity (100%) and sensitivity (75–89%) were also calculated (Table 3). While the DMS-MS/MS method shows great promise for rapid drug screening, further studies are needed to comprehensively explore the potential of DMS-MS/MS for drug screening. The limitations of the method, described here, include the pre-analytical sample preparation time, which includes hydrolysis and a short incubation. Evaluation of the glucuronide metabolites directly may be possible; alternatively utilization of a reagent that performs rapid hydrolysis at room temperature may simplify the sample preparation workflow. Since this DMS-MS/MS method was developed for screening, only one MRM transition was monitored for each analyte in attempt to shorten the MS cycle time providing more data points for each analyte. The addition of a second transition and calculation of the ion ratio may allow for enhanced specificity allowing for differentiation of isomers, such as morphine and hydromorphone. In the current study they are grouped by drug class for comparison with immunoassay screening. Future method modifications may allow for direct comparison to LC-MS/MS confirmatory methods.

**Conclusion**

A sensitive and specific DMS-MS/MS method has been successfully developed and applied to the identification of 33 drugs and metabolites.
in clinical urine samples. With gas phase separation and a 2 min run time, DMS-MS/MS provided robust and reliable results, without the need for extensive sample preparation or time-consuming column chromatography.

Compared with immunoassays, DMS achieved lower LODs and better sensitivity. It also provided a simultaneous identification platform for each drug and metabolite individually, not only the drug classes. The DMS-MS/MS method developed in this study is a compelling alternative to conventional immunoassays, but with more accurate identification and higher throughput – a necessity in the practice of clinical toxicology.

**Data availability**

All of the data underlying this article are available in the article and in its online supplementary material.

**CRediT authorship contribution statement**

Shirin Hooshfar: Writing – original draft, Methodology, Validation, Visualization, Data curation. Simone Tchu: Methodology. Cassandra Yun: Methodology, Software. Kara L Lynch: Writing – review & editing, Visualization, Funding acquisition, Supervision.

**Declaration of Competing Interests**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

**Appendix A. Supplementary data**

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jmsacl.2021.12.008.

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