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Urine Multi-drug Screening with GC-MS or LC–MS-MS Using SALLE-hybrid PPT/SPE

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Abstract

To intoxicated patients in the emergency room, toxicological analysis can be considerably helpful for identifying the involved toxicants. In order to develop a urine multi-drug screening (UmDS) method, gas chromatography–mass spectrometry (GC-MS) and liquid chromatography–tandem mass spectrometry (LC–MS-MS) were used to determine targeted and unknown toxicants in urine. A GC-MS method in scan mode was validated for selectivity, limit of detection (LOD) and recovery. An LC–MS-MS multiple reaction monitoring (MRM) method was validated for lower LOD, recovery and matrix effect. The results of the screening analysis were compared with patient medical records to check the reliability of the screen. Urine samples collected from an emergency room were extracted through a combination of salting-out assisted liquid–liquid extraction (SALLE) and hybrid protein precipitation/solid phase extraction (hybrid PPT/SPE) plates and examined by GC-MS and LC–MS-MS. GC-MS analysis was performed as unknown drug screen and LC–MS-MS analysis was conducted as targeted drug screen. After analysis by GC-MS, a library search was conducted using an in-house library established with the automated mass spectral deconvolution and identification system (AMDIS™). LC–MS-MS used Cliquid®2.0 software for data processing and acquisition in MRM mode. An UmDS method by GC-MS and LC–MS-MS was developed by using a SALLE-hybrid PPT/SPE and in-house library. The results of UmDS by GC-MS and LC–MS-MS showed that toxicants could be identified from 185 emergency room patient samples containing unknown toxicants. Zolpidem, acetaminophen and citalopram were the most frequently encountered drugs in emergency room patients. The UmDS analysis developed in this study can be used effectively to detect toxic substances in a short time. Hence, it could be utilized in clinical and forensic toxicology practices.

Introduction

Identifying the source of patient intoxication is extremely important for providing proper treatment. Of the 10,887 poisoning cases in Korea in 2013, 5,414 (49.7%) were related to drug intoxication. In addition, the number of poisoning cases from an unknown was 1,996 cases (18.3%) (1). The urine drug screening (UDS) can be helpful to identify for unknown substances. Immunoassay and gas chromatography–mass spectrometry (GC-MS) have been commonly used in UDS. UDS based on mass spectrometry can simultaneously screen for hundreds of drugs and is considered the gold standard for comprehensive drug screening. Urine multi-drug screening (UmDS) suggested in this study facilitates a more comprehensive drug screen than a general UDS by using both GC-MS for unknown drug screening and LC–MS-MS for targeted drug screening.

Today drug screening using GC-MS and liquid chromatography–tandem mass spectrometry (LC–MS-MS) plays an important

role in clinical toxicology, forensic toxicology, workplace drug testing and doping testing because it is possible to selectively detect target compounds from a complex biological specimen. Nevertheless, the time required for toxicological analysis is not fast enough to be helpful for the proper treatment of patients in emergency room settings. This is because toxicological screening is a multi-step process, including laborious sample preparation, instrumental analysis, peak identification and result interpretation (2–8).

When analyzing toxic substances in biological samples, pretreatment processes such as protein precipitation (PPT), liquid–liquid extraction (LLE) and solid phase extraction (SPE) are required to minimize the interference from matrices. Even if LLE and PPT are effective sample preparation methods, they have limitations such as low sample throughput. While SPE is the most effective separation process at minimizing the influence of interfering substances, it involves higher costs and is more labor intensive than PPT or LLE (6, 9–11).

The hybrid technique that consists of simultaneous PPT and phospholipids removal (hybrid PPT/SPE) method has been used in the past few years to selectively remove phospholipids, which are the main cause of the matrix effect, and then to perform PPT. Commercially available hybrid PPT/SPE products are the Hybrid SPE^{TM} (Sigma-Aldrich), the Ostro^{TM} (Waters) and the CaptivaND^{TM} (Agilent) (10, 12, 13).

Salting-out assisted liquid–liquid extraction (SALLE) can effectively separate analytes from multiple matrices like urine, blood and plasma with water-miscible organic solvents such as acetonitrile (14, 15). In this study, urine was extracted using SALLE-hybrid PPT/SPE, which has not been commonly used in the field of clinical toxicology compared to LLE and SPE. To evaluate the efficiency of the SALLE-hybrid PPT/SPE plate method, the limit of detection (LOD) and the recovery of 144 standard drugs were evaluated using routine GC-MS methods in the laboratory. An LC–MS-MS analysis method of 148 frequently encountered drugs was developed by referring to methods described in the scientific literature (16–18). Lower LOD, recovery and matrix effects were examined by spiking 148 standard drugs into blank urine. In order to reduce the time required for library search and interpretation, the automated mass spectral deconvolution and identification system (AMDIS^{TM}) for GC-MS from the National Institute of Standards and Technology (NIST) was utilized, which has proven to be a powerful and reliable tool for daily routine and emergency toxicology (5, 19, 20). For LC–MS-MS screening, the commercially available Cliquid® software from Sciex® was applied.

The purpose of this study was to establish an effective UmDS procedure, and to identify causative toxicants from urine samples of emergency room patients. Finally, the developed UmDS was applied to analyze the urine samples of 185 drug-intoxicated patients from the emergency room in Chungnam National University Hospital.

Experimental

Chemicals and reagents

Drug standards were purchased from Sigma-Aldrich® (St. Louis, MO, USA) and Cerilliant® (Round Rock, TX, USA). Trimipramine-D_3 and doxepine-D_3, used as internal standard (IS), were purchased from Cerilliant® (Round Rock, TX, USA). Methanol (MeOH), acetonitrile (ACN) and ultrapure water were all HPLC grade, purchased from Honeywell Burdick and Jackson (B&J)^{TM} (Harvey St, Muskegon, MI, USA). Formic acid was purchased from EMSURE® (Darmstadt, Germany), and ammonium formate was obtained from Sigma-Aldrich® (St. Louis, MO, USA). Magnesium sulfate was purchased from Junsei Chemical® (Tokyo, Japan) and sodium sulfate and sodium chloride were purchased from Daejung® (Jeongwang-dong, Shiheung-city, Gyeonggi-do, South Korea). The Ostro^{TM} 96-well plate 25 mg used for the hybrid PPT/SPE was purchased from Waters® (Wexford, Ireland). Ethyl acetate for the LLE was purchased from Honeywell Burdick and Jackson® (Harvey St, Muskegon, MI, USA). Bond Elut Certify ^{TM} cartridges for the SPE were purchased from Agilent® (Waldbronn, Germany).

Preparation of stock solutions

All standard solutions were dissolved in methanol, diluted to 10 μg/mL, and stored at −20°C. Drug-free human urine samples (blank urine samples) used as negative controls were obtained from healthy volunteers, stored at 3°C, and discarded within 2 weeks.

Instrumentation

GC-MS

The GC-MS system was composed of an Agilent 7890B GC combined with an Agilent 5977 A inert MSD. The sample was injected by an auto-sampler (Agilent 7893 A) and splitless injection mode was selected. The column used was an HP-5MS fused-silica capillary column (30 m × 0.25 mm id × 0.25 μm film thickness, Agilent) coated with 5% phenylmethylsiloxane stationary phase and the carrier gas was helium gas (flow rate: 1 mL/min). The oven temperature was maintained at 80°C for 1 min and increased 20°C/min to 300°C and held for 15 min, with a total run time of 30 min. The inlet and transfer line temperatures for sample injection were 250°C and 280°C, respectively. Drug screening was performed using electron ionization (EI) employed at 70 eV in full-scan mode, and the mass range was 50–550 amu.

LC–MS-MS

The LC system used was an Agilent 1200 series HPLC (Agilent, Waldbronn, Germany). The columns used were Restek Allure PFPP columns (50 mm × 2.1 mm, particle size 5 μm, USA) and the guard columns were made with Restek Allure PFPP guard columns (10 mm × 2.1 mm, particle size 5 μm, USA). A 3200 Qtrap (AB Sciex, Darmstadt, Germany) equipped with a TurboIonSpray interface was used for detection in positive ion mode. LC separation was performed as follows. The separation column was stabilized at 30°C. The mobile phases were 2.5 mM ammonium formate + 0.2% formic acid in water (pH 2.6, solvent A) and 2.5 mM ammonium formate + 0.2% formic acid in ACN (solvent B). The flow rate was maintained at 0.6 mL/min. The gradient began with solvent B at 10% for 3.5 min, increased to 90% B until 10 min, and maintained for up to 16 min. Then, it was dropped to 10% B until 16.50 min and maintained until 18 min. In multiple reaction monitoring (MRM) mode, the duration time was 20.025 min and the total cycle time was 2.9521 s. The collision energy was set using compound optimization mode for MRM. The declustering potential was 40–80 V, the entrance potential was 10 V, the cell exit potential was 4 V, and the ion spray voltage was 4,000 V. Q_1 and Q_3 were used in unit resolution. The gas settings were as follows: curtain gas was 206,842 Pa, collision gas was in high mode, ion source gas 1 was 275,789 Pa, and ion source gas 2 was 482,631 Pa. The source temperature was 500°C.

Methods

Extraction for UmDS

A drug mixture composed of nine drugs (amitriptyline, benztropine, doxylamine, carbamazepine, chlorpromazine, citalopram, diltiazem, tramadol and zolpidem) and one IS (trimipramine-D_3) was added to blank urine samples. The blank urine samples and the urine samples collected from drug-intoxicated patients were extracted with SALLE-hybrid PPT/SPE protocol. They were then analyzed by GC-MS and LC–MS-MS as shown in Supplementary Figure S1.

Spiked urine mixture and patient urine samples (190 µL each) and 10 µL of IS were placed into Eppendorff tubes (EP tubes). ACN (600 µL, 1% formic acid) and 75 mg sodium chloride (NaCl) were added for salting-out effect. This mixture was vortexed for 2 min and centrifuged for 5 min at 40,248 × g. To clean up, supernatants were loaded into a hybrid PPT/SPE 96-well plate. After 2,000 Pa pressure was applied to the manifold for 5 min, the eluted solution in a 96-well 2 mL collection plate was transferred to a glass tube and subsequently concentrated under nitrogen gas stream at 50°C for 10 min. The residue was reconstituted with 100 µL methanol and 1 µL of the sample was injected into the GC-MS. For LC–MS-MS analysis, the residue was reconstituted with 300 µL mobile phase (A:B, 90:10) and 5 µL of the sample was injected into LC–MS-MS. SALLE-hybrid PPT/SPE method was optimized through selectivity obtained from extraction methods using ammonium formate (NH_4HCO_2), NaCl, sodium sulfate (Na_2SO_4), and magnesium sulfate ($MgSO_4$) and validated for LOD, specificity, recovery and matrix effects. The optimized SALLE-hybrid PPT/SPE was compared with LLE and SPE through selectivity and recovery.

To compare selectivity and matrix effects among the SPE (21), LLE (9) and SALLE-hybrid PPT/SPE methods, blank urine was spiked at a concentration of 10 µg/mL with 10 standard drugs (acetaminophen, amitriptyline, benztropine, citalopram, diltiazem, diphenhydramine, doxylamine, imipramine, levetiracetam and tramadol) and one IS (trimipramine-D_3). The spiked urine sample was extracted by LLE, SPE and SALLE-hybrid PPT/SPE, and identified with GC-MS. The total ion chromatograms (TICs) obtained after LLE, SPE and SALLE-hybrid PPT/SPE were compared with the intensity of detected signals of matrix. To compare the extraction efficiency among SPE, LLE and SALLE-hybrid PPT/SPE, four replicates per each extraction method were analyzed.

Establishment of an AMDIS™ library for GC-MS UmDS

An in-house AMDIS™ library was established using AMDIS™ (ver. 2.6, NIST). In order to evaluate the practicality of the in-house AMDIS™ library searches, the results of the searches were compared with the results of a Chemstation™ (ver. F.01.00.1903, Agilent) analysis, the data-processing software of Agilent GC-MS Systems. The Wiley 10th/NIST 14 combined library (W11N14.L) was used as a library file of Chemstation™. The AMDIS™ library database was constructed according to the experimental methods of AMDIS™ related literature (2, 5, 19, 20). To collect the GC-MS mass spectra for 144 drugs selected from the drugs listed in the Toxicity information supporting system (Ministry of Food and Drug Safety, Korea) and frequently encountered toxic drugs in Chungnam National University Hospital, all the drugs were divided into 15 groups with similar intensities at the same concentration without retention time (RT) overlapping. Each group was composed of fewer than 10 drugs. All grouped drugs were diluted to 10 µg/mL, and then, the in-house library was established from the mass spectra obtained by GC-MS analysis. In addition, the AMDIS™ library database was supplemented by registering the mass spectra for the drugs found in the urine samples of the drug-intoxicated patients by library searching using Chemstation™. In order to test the AMDIS™, a blank urine sample was spiked with a standard mixture consisting of 33 random drugs at 1–3 µg/mL and were confirmed by in-house AMDIS™ library searching. The AMDIS™ evaluation was conducted in simple mode. The AMDIS™ settings for UmDS were as follows: Component width, 15; sensitivity, very high; resolution, high; shape requirement, medium. The minimum match factor (MMF) was set to 55.

Development of targeted drug screening by LC–MS-MS for an UmDS

The MRM transition method was established using 3200 Qtrap LC–MS-MS and AB Sciex Analyst (22, 23). Two MRM transitions were collected for each drug from the Cliquid® MRM catalog, and the MRM transitions of non-registered drugs in the MRM catalog were obtained separately by infusion. A mixture of 148 standard drugs listed in Table S4 and two IS (trimipramine-D_3) was analyzed by the MRM method to confirm RT and selectivity. The sMRM-information dependent acquisition-enhanced production ion (sMRM-IDA-EPI) scan method has been developed to increase sensitivity and selectivity and to establish an automated method. The MTS (multi-targeted screening) method consists of a survey scan and an IDA-triggered dependent scan. The IDA intensity threshold was set to 500 counts per second. To obtain a dependent EPI scan, the two MRM transitions with the largest intensity exceeding the threshold, per cycle, were selected and EPI scans were performed after a survey scan. EPI scans were performed in scan ranges of 50–1,000 amu. Each blank urine sample was spiked with a standard solution of 18 frequently encountered drugs to prepare a 100 ng/mL concentration. RT, lower LOD, matrix effects and recovery were measured in four replicates.

Application to emergency room urine samples

A total of 185 urine samples related to drug intoxication were received from the Department of Emergency Medicine, Chungnam National University Hospital, over three years from February 2015 to March 2017, with informed consent. The study was approved by the Institutional Review Board of Chungnam National University (201706-BR-034-01). The sample number, color, pH and volume of the samples were recorded. All 185 urine samples were extracted, screened via UmDS method described using both GC and LC techniques, and library search results were analyzed.

Results and Discussion

Demographic analysis of intoxicated patients

There was a total of 298 poisoning cases in the emergency room of Chungnam National University Hospital between February 2015 and March 2017. Supplementary Table S1 shows that most intoxicated patients were in their 40 s and 50 s, with these age groups accounting for 57 (43.5%) and 56 (33.5%) patients, respectively. Males and females accounted for 131 (44.0%) and 167 (56.0%) samples, respectively. Of all intoxicated patients, 185 (62.1%) were drug intoxications and 59 (19.8%) were pesticide intoxications according to medical records. The remaining 48 patients (16.1%) were intoxicated by other substances such as rodenticide, detergent,

and tire wheel cleaner. *Phytolacca americana* (American pokeweed) was found in 6 (2%) cases. In this study, only the urine samples collected from the 185 drug-intoxicated patients were screened. Of all intoxicated patients, 133 (71.9%) informed the toxicants what they consumed to family or doctors whereas no information was available in remaining 52 (28.1%).

Determination of sample preparation methods for UmDS

To determine the appropriate extraction method for UmDS, blank urine samples were spiked with 10 drugs selected from those most frequently encountered. The prepared urine mixture was extracted by LLE, SPE and SALLE-hybrid PPT/SPE and analyzed by GC-MS. The TICs obtained by GC-MS full-scan mode after extraction with LLE, SPE and SALLE-hybrid PPT/SPE were overlaid to compare the residual matrix after extraction. The three types of extraction methods were further compared by calculating the extraction efficiency. The salting-out effect of the SALLE-hybrid PPT/SPE method was optimized by comparing four different inorganic salts; NH_4HCO_2, NaCl, Na_2SO_4 and $MgSO_4$ were tested. The SALLE method using Na_2SO_4 was not analyzed because the urine and solvent mixture was not separated into two phases. As shown in Figure 1A, the SALLE method using NaCl showed the cleanest baseline. Overall, the chromatography of SALLE using NaCl showed better peak separation between the analyte and the matrix than others. Therefore, NaCl was selected as optimal for SALLE-hybrid PPT/SPE.

As shown in Figure 1B, fatty acid amides, which cause matrix effects such as hexadecanamide (RT: 10.60 min) and octadecanamide (RT: 11.50 min) which cause matrix effects, decreased more significantly in the SALLE-hybrid PPT/SPE method than in the SPE or LLE methods. The chromatogram obtained after SALLE-hybrid

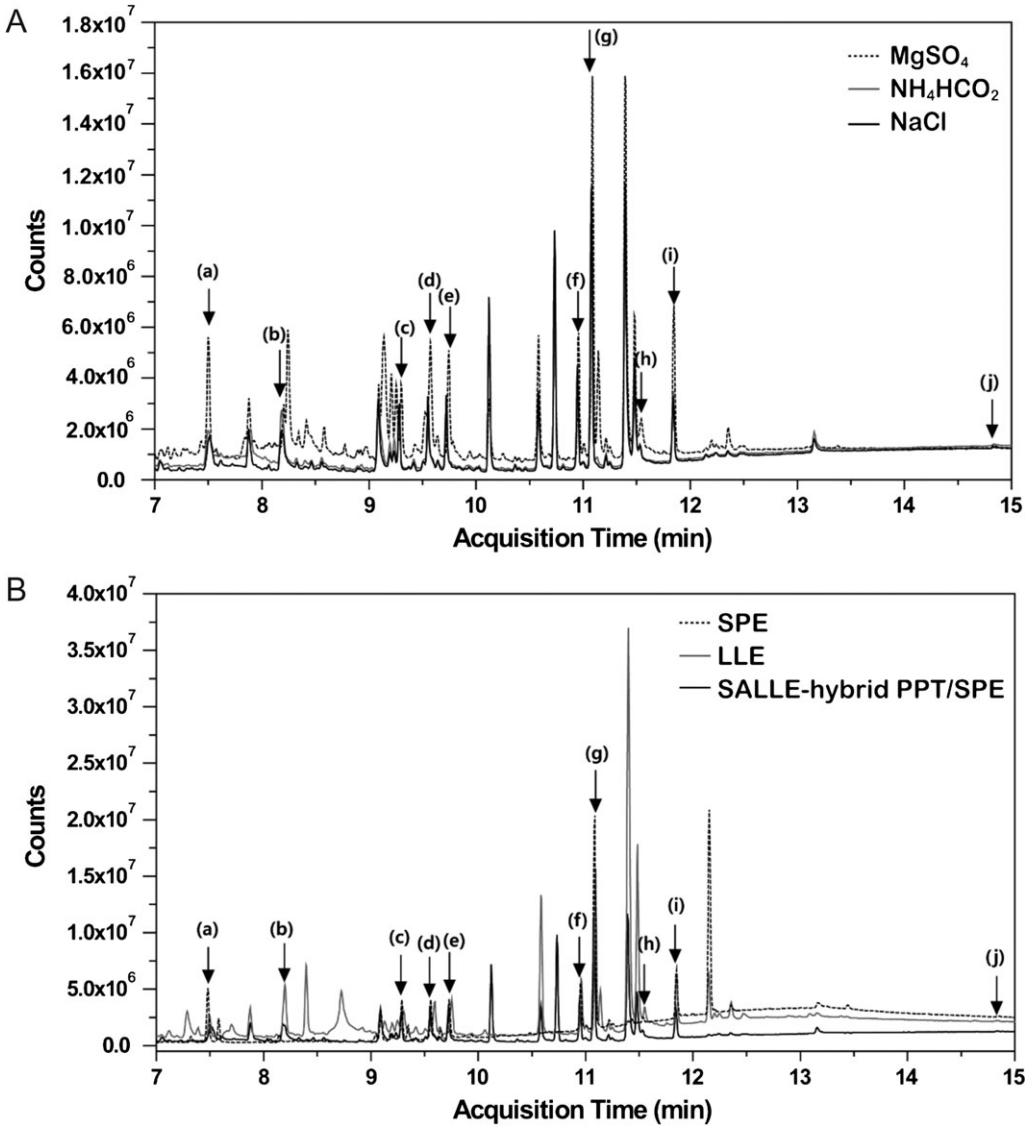

Figure 1. TICs obtained by GC-MS of a mixture of 10 drug standards. (A) by SALLE- hybrid PPT/SPE using MgSO4 (dotted line), NH4HCO2 (gray line) and NaCl (black line) (B) by SALLE-hybrid PPT/SPE using NaCl (black line), SPE (dotted line) and LLE (gray line); [(a) levetiracetam (7.48 min), (b) acetaminophen (8.14 min), (c) diphenhydramine (9.29 min), (d) doxylamine (9.56 min), (e) tramadol (9.73 min), (f) amitriptyline (10.95 min), (g) imipramine (11.08 min), (h) benztropine (11.54 min), (i) citalopram (11.84 min) and (j) diltiazem (14.85 min)].

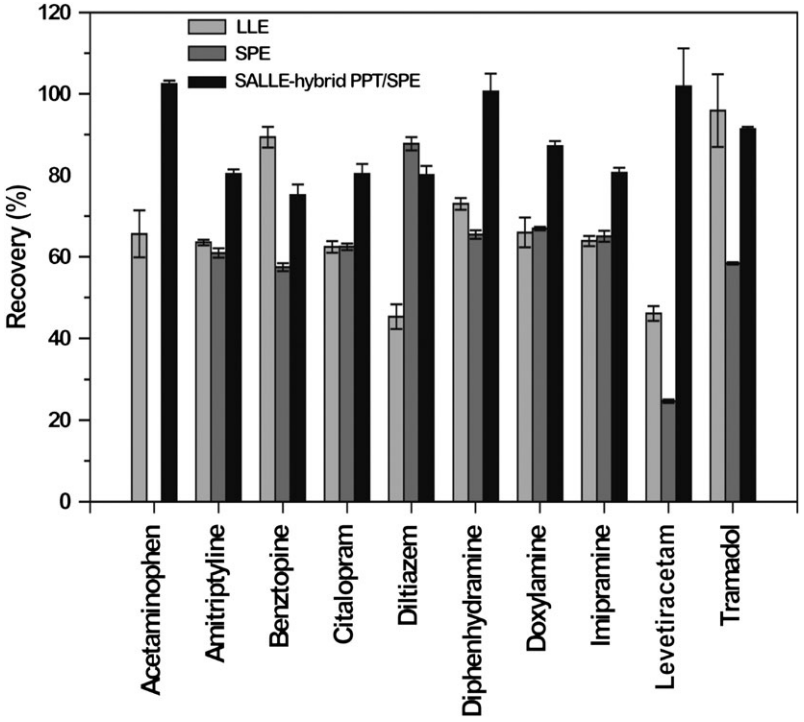

Figure 2. Recovery of 10 drugs in urine at 1 μg/mL by GC-MS using LLE, SPE and SALLE-hybrid PPT/SPE methods. Mean and standard deviation error bars for recovery were taken from four replicates.

PPT/SPE was much cleaner and showed lower background levels than those after LLE and SPE.

As shown in Figure 2, diltiazem and levetiracetam showed a low extraction efficiency of less than 60% in LLE, and in SPE levetiracetam also showed low extraction efficiency and acetaminophen was absent. In contrast, using the SALLE-hybrid PPT/SPE, all drugs exhibited more than 70% extraction efficiency. Supplementary Table S2 shows that most of the drugs extracted using the SALLE-hybrid PPT/SPE method showed 59.5–118% recovery, except for mifepristone (53.5 ± 2.4%), yohimbine (53.4 ± 2.4%), propiverine (52.3 ± 1.4%), and temazepam (41.9 ± 1.6%). The overall mean recovery was satisfactory at 86.7 ± 3.7%. SALLE-hybrid PPT/SPE had a great advantage over conventional LLE and SPE in that the extraction procedure was simple and the time consumed in extraction was shorter than that in the other two methods. For these reasons, the developed method might be a suitable extraction method to quickly identify the intoxicating substances in emergency patients.

Development of an AMDIS™ in-house library

Supplementary Table S3 shows the names and classifications of 144 drugs, mass spectra information (one quantifier ion and three qualifier ions), LOD values and RTs of the standard drugs used for AMDIS™. The values in parenthesis in the ion columns represent the relative ratio to the quantifier ion.

In the study, 10 or more standard drug mixtures were grouped and mixed, and then identified with AMDIS™ after GC-MS analysis. Among drugs that had similar RTs, the drugs that eluted later were affected by the drugs that eluted earlier; hence, the deconvolution did not operate correctly. As a result, some drugs with similar RTs were not detected. These results coincided well with the reports by Stahnke *et al.* and Schlittenbauer *et al.*, in which matrix effects depended on RT rather than on compound-specificity (16, 24). Fifty one out of 144 (35.42%) drugs were detected in between 10 and 12 min in the GC-MS screening method. Thus, 15 sets of standard drug mixtures, which consisted of 8–10 drugs each, were prepared without RT overlap. With these sets of standard drug mixtures, mass spectra were obtained and LODs were measured.

Toxic substances were detected more accurately in AMDIS™ than in Chemstation™. When the peaks overlapped, the library search performed by Chemstation™ resulted in lower library matching values. However, as AMDIS™ used a deconvolution process to compare the spectra with the library spectra, it showed higher performance in library matching. One example was the intensity of zolpidem identified in poisoned patients. Zolpidem showed relatively lower intensity than those of other peaks and was likely to be buried by noise. In 16 cases, zolpidem was missed by Chemstation™, but it could be found by AMDIS™.

Method optimization of LC–MS-MS

In order to survey scan for 148 drugs and two IS in positive mode, an MRM method consisting of 294 MRM transitions was established. RTs of drugs were separated and confirmed using an LC system and MRM method. The RT, MRM transitions and collision energy (CE) of 148 drugs are shown in Supplementary Table S4. A scheduled MRM (sMRM) method was developed from the confirmed RT, and the peak of all drugs was identified through the sMRM-IDA-EPI method in order to increase the sensitivity and specificity of each chromatographic peak. Table I shows the details of the validation results of the developed LC–MS-MS MTS method.

To determine the efficiency of the developed method, the most frequently encountered 18 drugs in the emergency department of Chungnam University Hospital were selected, and the lower LOD,

Table I. LC–MS-MS MTS method development for 18 intoxicating drugs in the Department of Emergency, Chungnam National University Hospital ($n = 5$)

Analyte	Precursor ion (m/z)	Production (m/z)	RT (min)	lower limit of detection (ng/mL)	Recovery (%)	Matrix effect (%)	Analyte	Precursor ion (m/z)	Product ion (m/z)	RT (min)	lower limit of detection (ng/mL)	Recovery (%)	Matrix effect (%)
Acetaminophen	152.1	110.1	1.02	<2.5	83.9	100.8	Propranolol	260.2	183.2	8.84	5.0	70.7	92.9
	152.1	65.1	1.02	5.0	99.7	102.2		260.2	116.1	8.84	5.0	68.2	84.4
Alprazolam	309.1	281.1	6.40	5.0	66.5	130.8	Quetiapine	384.2	253.2	8.81	5.0	67.6	81.6
	309.1	205.1	6.40	5.0	66.4	124.0		384.2	221.2	8.81	5.0	103.5	81.9
Amitriptylin	278.2	233.2	12.34	<2.5	72.6	97.6	Tramadol	264.2	58.1	6.66	2.5	71.6	89.5
	278.2	105.1	12.34	<2.5	72.8	84.9		264.2	115.1	6.66	12.5	76.9	71.5
Chlorpromazine	319.1	214.1	13.20	5.0	63.7	93.9	Venlafaxine	278.2	58.1	7.57	5.0	75.4	93.9
	319.1	86.1	13.20	<2.5	68.7	100.2		278.2	260.3	7.57	5.0	77.1	79.1
Citalopram	325.2	109.1	10.17	<2.5	70.4	90.4	Zolpidem	308.2	235.1	7.79	<2.5	73.9	93.0
	325.2	262.2	10.17	5.0	69.8	102.1		308.2	236.1	7.79	<2.5	76.5	94.1
Clonazepam	316.1	270.1	6.46	12.5	98.4	89.1	Triazolam	343.1	239.1	6.39	5.0	108.9	78.4
	316.1	214.2	6.46	12.5	102.6	92.3		343.1	315.1	6.39	5.0	106.1	80.0
Doxylamine	271.2	167.2	8.16	<2.5	75.0	76.5	Flunitrazepam	314.1	268.1	6.71	5.0	71.9	73.8
	271.2	182.2	8.16	<2.5	78.4	73.2		314.1	239.2	6.71	5.0	101.0	76.4
Imipramine	281.2	86.1	11.78	<2.5	68.2	83.6	Diphenhydramine	256.2	167.1	10.09	<2.5	77.9	81.8
	281.2	58.1	11.78	<2.5	66.7	78.2		256.2	165.1	10.09	<2.5	72.9	98.5
Lorazepam	321.1	275.1	6.06	12.5	101.7	152.3	Benztropine	308.314	167.2	12.23	<2.5	71.2	81.6
	321.1	229	6.06	12.5	107.9	113.3		308.314	165.2	12.23	<2.5	67.5	81.9

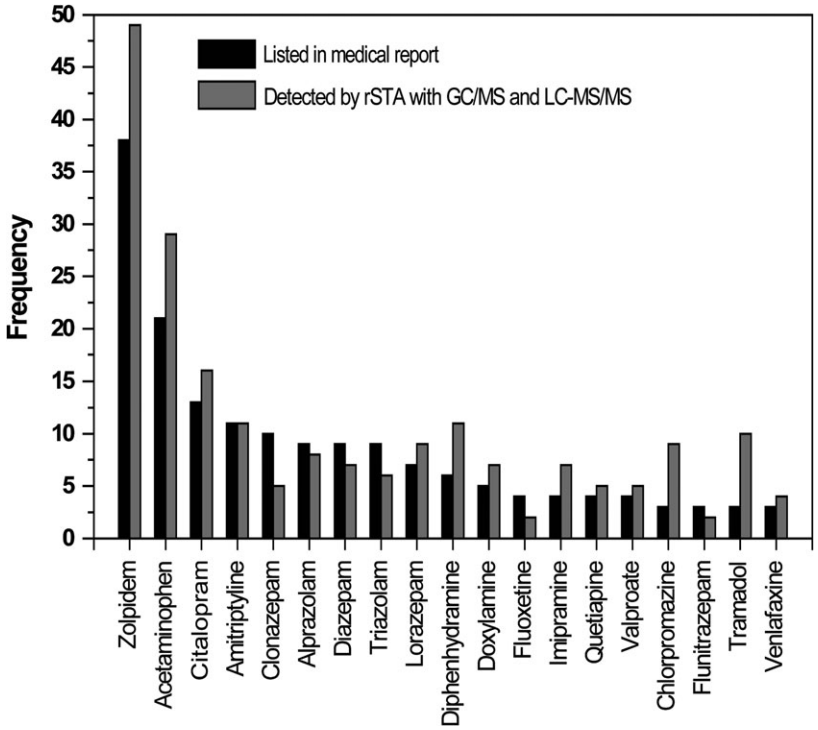

Figure 3. The frequency of toxic substances listed in medical reports collected from intoxicated patients in Chungnam National University Hospital from February 2015 to March 2017 and the screening results of urine samples by GC-MS, LC–MS-MS ($n > 4$).

recovery and matrix effects of these drugs were measured using post-extraction addition. The lower LOD of lorazepam, clonazepam and tramadol were all 12.5 ng/mL, which were higher than those of other toxic drugs. The lowest recovery value was 63.7% (chlorpromazine) and the average recovery value of the 18 drugs was higher than 79.8%. Overall, the matrix effects of benzodiazepine drugs such as alprazolam, lorazepam, triazolam, and flunitrazepam were higher than those of other drugs (Table I).

Analysis of specimen from emergency patients

Figure 3 shows the frequency of suspected toxicants in the medical reports of 185 drug-poisoned patients and the frequency of detected toxicants in the urine samples using the developed GC-MS and LC–MS-MS screening methods. The most frequently suspected toxicants in the medical reports were, in order of frequency: zolpidem, 38; acetaminophen, 21; citalopram, 13; amitriptyline, 11, while the most frequently detected toxicants, in order of frequency were: zolpidem, 49; acetaminophen, 29; citalopram, 16; diphenhydramine, 11; amitriptyline, 11. Zolpidem, acetaminophen and citalopram were the most frequently suspected as well as detected toxicants by GC-MS in 185 drug-poisoned patients. Zolpidem and diphenhydramine were detected more often in the patient samples that did not have detailed medical records. Tramadol was also detected more often because it was frequently administered as an opioid analgesic used to alleviate the patient's acute pain in the emergency room. the patient samples that did not have detailed medical records.

Conclusion

This study was conducted to propose new analytical methods to analyze intoxicated drugs within an hour, which are the main issues of UDS based on GC-MS and LC–MS-MS in the field of emergency toxicology. The applicability of this UmDS method was evaluated using the urine samples of 185 patients from an emergency room. The SALLE-hybrid PPT/SPE method could minimize the sample amount, time, and organic solvent consumed in the extraction processes. With the in-house library database developed using AMDIS^{TM}, the GC-MS data processing and library searching time were reduced. Further, this in-house library was quicker, simpler, and more sensitive than the commercial Chemstation^{TM} library because the in-house library was only targeted for frequently encountered intoxicating drugs. An MTS of LC–MS-MS was able to detect the toxic substances not amenable to GC-MS analysis without derivatization. Using a sMRM-IDA-EPI method, 148 standard drugs in blank urine were effectively analyzed using Cliquid® 2.0 software.

Through the developed analytical method, it was possible to effectively identify drugs in the 185 patient samples. The UmDS was applied to urine samples from emergency room patients after validated for selectivity, LOD, LOQ, recovery matrix effect. The UmDS using GC-MS and LC–MS-MS has the advantage of taking less time to perform which adds to its utility in an emergency room setting.

Supplementary Data

Supplementary data are available at the *Journal of Analytical Toxicology* online.

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