RESEARCH ARTICLE

Comprehensive method for the detection and quantification of drugs of abuse in urine by liquid chromatography mass spectrometry in a drug rehabilitation clinical setting

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Abstract

Within the clinical setting of drug rehabilitation, it is important to be able to monitor for the use of drugs using sensitive and selective techniques whilst accounting for high throughput and numbers of patients to provide rapid results to clinicians. To meet this need, a comprehensive LC-MS-MS method for the confirmation and quantitation of a wide variety of drugs of abuse relevant to drug rehabilitation in the United Arab Emirates has been developed, validated and applied to patient urine samples. Following automated solid phase extraction, detection and quantitation involved multiple reaction monitoring with electrospray ionization. With few exceptions, within and between-batch accuracy and precision performance was shown to be within 20% across all drug types including amphetamines and related stimulants, benzodiazepines, opiates/opioids, cocaine and metabolites, cannabinoids, hallucinogens and ketamine (including metabolites) in urine. Results for 280 drug positive patient specimens showed good agreement with the previous in-house GC–MS approach. The LC-MS-MS replaces the existing GC-MS approach and can be expanded easily with the introduction of additional MRM transitions as and when required (e.g. if new or other drugs of abuse are to be considered) to support the work of the clinical team in this special area of clinical toxicology and medicine.

Keywords: LC-MS; Urine; Clinical toxicology; Drugs of Abuse; UAE

1. Introduction

The National Rehabilitation Center (NRC) is a national response centre in the United Arab Emirates (UAE) for drug addiction prevention, treatment and rehabilitation of both inpatients and outpatients.[1] Medical care is supported by clinical laboratory services, including toxicology for the detection of drugs in patients' samples (primarily urine). The results of these tests have allowed the NRC to detect the drugs within the substance-using patient population and monitor trends in those detections in order to provide an evidence-based assessment of drugs within the UAE area applicable to the wider Middle East region.[2] As a result of continual increasing numbers of patients and cases of drug abuse, the need for fast, sensitive, selective and accurate comprehensive methods has become paramount to support rapid, evidence-based medicine. For many years within the laboratory, such analysis has involved a traditional approach of immunoassay for high throughput initial drug screening, with gas chromatography with mass spectrometry (GC-MS) for confirmation.[3] As immunoassay provides rapid analysis with no sample pre-preparation covering a broad range of drugs, it remains an important tool in initial screening within a drugs of abuse context. However, given the methodology can lack sensitivity and specificity, confirmation tests by chromatography and mass spectrometry are required to provide greater sensitivity and specificity especially targeted towards drugs of abuse.[4-5] Within the United Arab Emirates region, the most important and relevant drugs of abuse include amphetamines and related stimulants, benzodiazepines, opiates/opioids, cannabinoids (Δ^9-tetrahydrocannabinol (THC) and metabolites), common hallucinogens such as PCP and LSD and the dissociative anaesthetic ketamine.[2] Within a drug rehabilitation setting, clinicians often require the analysis of large panels of drugs and metabolites that can be used to ensure compliance with prescribed pain medication regimens and to detect abuse or diversion of medications. With prescription drug abuse reaching epidemic levels, demand has grown for analytical methods that can ensure accurate results for comprehensive drug lists with reasonable analysis times for provision of results to aid clinical interpretation. Although many drugs are amenable to gas chromatography, there are challenges associated with those that are thermo labile and/or chemically polar and derivatisation is also often needed, none of which is an issue with liquid chromatography. Coupling liquid chromatography with tandem mass spectrometry (LC-MS-MS) for enhanced sensitivity has therefore become a widely used analytical technique within many toxicology applications involving numerous matrices.[6-8]

Within clinical toxicology and drugs of abuse monitoring, urine is the matrix of choice due to the wide detection window to cover many days of drug history. Although the matrix presents few direct sample preparation challenges, it is necessary to ensure a wide range of drugs are extracted and the use of solid phase extraction (SPE) allows the exploitation of various chemical processes to assist.[9-10] The selection of sorbent and the solvents/reagents for conditioning, washing and elution of desired analytes are critical. In the following described method, mixed modes (non-polar plus cation exchange mechanism) with polymer based SPE columns have been used. For acidic or basic analytes containing ionizable functional groups, mixed-mode sorbents combining non-polar and ion exchange retention mechanisms can provide additional selectivity and clean up. This approach is

particularly useful for extraction of analytes from urine, as the dual retention mechanism allows strong interference elution solvents to be used, which eliminate phospholipids and other unwanted co-extracted species from the final extract.[10]

The following paper describes the development and validation of a LC-MS-MS method for urine with solid phase extraction for the identification and quantitation of 67 drugs and metabolites from a wide variety of different groups relevant to the NRC clinical requirements and replaces the existing GC-MS methodology.

2. Materials and Methods

2.1 Reagents and Standards

All calibration, quality control and internal standards were prepared from reference materials of 99% purity purchased from Cerilliant (Sigma-Aldrich, St. Louis, USA). All solvents and reagent were of HPLC or LCMS grade and purchased from Fisher Scientific (Loughborough, UK), Merck (Darmstadt, Germany) or Sigma-Aldrich (St. Louis, USA). Drug-free urine tested by Abbott Architect Immunoassay and GC–MS was used to prepare calibrators, controls, and fortified samples.

2.2 Equipment

Extraction was performed using an automated Biotage Extrahera™ system (Biotage, Sweden) in 28 position configuration. Biotage EVOLUTE® EXPRESS CX 150 mg/6mL were used for solid phase extraction. Samples were eluted to glass test tubes and dried under nitrogen flow using Biotage TurboVap LV evaporator.

LC–MS–MS analysis was performed using a Shimadzu Nexera LCMS-8040 system (Shimadzu Corporation, Kyoto, Japan) with Lab solution software. The HPLC system included a mobile phase pump, autosampler and column oven. The instrument was operated in multiple reaction monitoring (MRM) mode with an electrospray ionization (ESI) probe in positive ESI mode.

2.3 Sample preparation

From 1 mg/ml methanolic reference material, stock solutions (10 µg/ml) of 14 amphetamines and related stimulants (amphetamine, methamphetamine, (S)-cathinone, butylone, dibutylone, methylone, dimethylone, methylphenidate, mephedrone, methaqualone, MDA, MDEA, MDMA, PMA), 24 benzodiazepines and metabolites (7-aminoclonazepam, 7-aminonitrazepam, alpha-hydroxytriazolam, alpha-hydroxyalprazolam, alpha-hydroxymidazolam, alprazolam, bromazepam, chlordiazepoxide, clobazam, clonazepam, desalkylflurazepam, diazepam, estazolam, flunitrazepam, flurazepam, lorazepam, midazolam, nitrazepam, nordiazepam, oxazepam, phenazepam, temazepam and triazolam), opiates/opioids (norbuprenorphine, buprenorphine, 6-monoacetylmorphine (6-MAM), fentanyl, hydromorphone, codeine, hydrocodone, morphine, oxymorphone, cis-tramadol, EDDP, methadone, o-desmethyl-cis-tramadol, oxycodone, propoxyphene and naloxone), cannabinoids (11-hydroxy-Δ^9-THC, 11-nor-9-carboxy-Δ^9-THC, and Δ^9-THC), cocaines (hydroxycocaine, cocaine, cocaethylene, benzoylecgonine and norcocaine), hallucinogens (PCP and LSD), ketamine and norketamine were used for preparing spiked standards.

From 0.1 mg/ml methanolic reference material, deuterated internal standard stock solutions (1000 ng/mL) of amphetamine-D8, buprenorphine-D4, propoxyphene-D5, LSD-D3, ketamine-D4, benzoleccgonine-D8 and cocaine-D3

were prepared in methanol and stored in the freezer until required.
Calibrator standards of between 5 and 200 ng/mL (depending on the analyte) were prepared in urine. Matrix-matched positive controls containing all 67 analytes at 5 or 10 ng/mL, 20 or 40 ng/mL and 100 or 200 ng/mL (depending on the analyte) in addition to negative controls containing only internal standard at 16.6 ng/mL were prepared in urine for extraction.

2.4 Hydrolysis and Solid Phase Extraction

2.4.1 Hydrolysis

To 3mL of urine, 2mL of pH 5.0 100mM acetate buffer, 50µL or 100µL internal standard solution (depending on the analyte to be quantified) and 100µL ß-glucuronidase (100,000 units/mL) were added. The samples were briefly vortex mixed, then incubated at 60°C for 2 hours. After cool down at room temperature, 500µL of 10% H_3PO_4 was added and centrifuged at 2000 rpm for 3 minutes. The samples were then extracted by automated Biotage Extrahera system with pre-conditioned EVOLUTE® EXPRESS CX 150 mg/6 mL columns.

2.4.2 Solid Phase Extraction

5mL of each hydrolysed urine sample was loaded onto an EVOLUTE EXPRESS CX column. Gradient positive pressure was applied initially at 0.7bar for 120 seconds, then 1bar for 120 seconds followed by up to 1.4bar for 60 seconds and 5 minutes plate dry. Each column was washed with 6mL of 4% phosphoric acid and 6mL of methanol: water (1:1 v/v) with positive pressure applied at 2.5 bar for 100 seconds then plate dry for 200 seconds. Finally, analytes were eluted by elution solvent (dichloromethane: isopropanol:ammonium hydroxide solution 78:20:2) with gradient positive pressure applied initially at 0.5bar for 250 seconds, then 2.0bar for 60 seconds to complete elution followed by 30 seconds plate dry.
After extraction, 100µL of methanolic HCl was added and vortex mixed prior to evaporation to dryness under nitrogen flow using a Biotage Turbovap evaporator at 40°C for approximately 15 minutes. The residue was reconstituted in 500µL 1:1 methanol:water, vortex mixed and analyzed by LC–MS–MS.

2.5 LC-MS-MS Analysis

Analytes were eluted from a Raptor Biphenyl 2.7µm 100x2.1mm (Restek, Bellefonte, USA) analytical column at a 30°C column oven temperature and flow rate of 0.3ml/min. The mobile phases consisted of Mobile Phase-A (2mM ammonium formate with 0.002% formic acid in water) and Mobile Phase-B (2mM ammonium formate with 0.002% formic acid in methanol) and the gradient programme shown in Table 1.

Table 1. Mobile-Phase Gradient Used for LC separation

Mobile Phase A (%)	Mobile Phase B (%)	Total Flow (ml/min)	Time (min)
95	5	0.3	1
60	40	0.3	2
0	100	0.3	10.5
0	100	0.5	11
95	5	0.5	13
95	5	0.3	14

For mass spectrometry detection, the MS instrument was operated with an ESI probe in positive and negative modes depending on the analyte. The interface conditions were kept as interface temperature 350^{o}C, DL temperature 250^{o}C, nitrogen as nebulizer gas at 3L/min and drying gas flow at 15L/min, heater temperature 400^{o}C and a loop time of 0.6 seconds. Each analyte was optimised for appropriate collision energy and associated parameters for multiple reaction monitoring (MRM) of precursor and product ions for sensitivity and selectivity. Tables 2a-d shows the analytes and respective MRM transitions monitored by each drug group with the first listed transition being the quantifier and the second being the qualifier.

Table 2a. Retention Times (min), MRM Transitions Monitored (m/z), for Amphetamines

Analyte	RT	MRM	Analyte	RT	MRM
(S)-Cathinone	3.745	150.1 > 132.1 150.1 > 105.1	Mephedrone	4.845	178.1>160.2 178.1>145.1
Amphetamine	3.948	136.1 > 91.1 136.1 > 65.1	Methamphetamine	4.272	150.1>91.1 150.1>119.1
Butylone	5.008	222.0 > 174.1 222.0 > 131.1	Methaqualone	8.141	251.1>132.1 251.1>91.1
Dibutylone	5.266	236.1 > 161.1 236.1 > 191.1	Methylone	4.478	208.1>160.1 208.1>132.1
MDA	4.314	180.1 > 163.1 180.1 > 105.1	Methylphenidate	5.712	234.1>84.1 234.1>56.1
MDEA	5.009	208.0 > 163.1 208.0 > 105.1	PMA	4.355	166.1>121.1 166.1>149.1
MDMA	4.640	194.1 > 163.1 194.1 > 135.1	Amphetamine-D8	3.906	144.1>97.1 144.1>127.2
Dimethylone	4.682	222.0 > 72.2 222.0 > 147.1			

Table 2b. Retention Times (min), MRM Transitions Monitored (m/z), for Benzodiazepines

Analyte	RT	MRM	Analyte	RT	MRM
7-Aminoclonazepam	5.702	285.9>121.1 285.9>250.1	Flunitrazepam	8.696	313.9>268.1 313.9>239.1
7-Aminonitrazepam	5.686	252.1>121.1 252.1> 94.1	Flurazepam	7.346	388.1>315.1 388.1>183.1
Alpha-Hydroxytriazolam	8.061	359.0>331.0 359.0>176.0	Lorazepam	7.646	321.0>275.0 321.0>229.0
Alpha-hydroxyalprazolam	9.397	325.6>296.9 325.6>216.7	Lormetazepam	8.69	335.0>289.0 335.0>227.1
Alpha-Hydroxymidazolam	8.493	341.9>324.0 341.9>203.0	Midazolam	9.329	325.9>291.1 325.9>249.1
Alprazolam	8.924	308.9>281.0 308.9>205.0	Nitrazepam	7.944	281.9>236.0 281.9>180.1
Chlordiazepoxide	8.388	300.1>227.0 300.1>282.0	Nordiazepam	8.506	270.9>140.1 270.9>208.0
Clobazam	8.5	300.9>259.1 300.9>224.1	Oxazepam	7.812	286.9>241.0 286.9>269.0
Clonazepam	7.929	316.0>270.0 316.0>214.0	Phenazepam	8.451	348.8>206.1 348.8>179.1
Desalkyl Flurazepam	8.024	288.9>140.1 288.9>104.1	Temazepam	8.77	300.9>255.0 300.9>283.0
Diazepam	9.435	284.9>193.0 284.9>154.0	Triazolam	8.687	343.0>308.0 343.0>315.0
Estazolam	8.731	294.9>267.0 294.9>205.1	Diazepam-D5	9.418	289.9>198.1 289.9>262.1

Table 2c. Retention Times (min), MRM Transitions Monitored (m/z), for Opiates and Opioids

Analyte	RT	MRM	Analyte	RT	MRM
Buprenorphine	7.767	468.3> 55.1 468.3>414.2	EDDP	7.489	278.2>234.3 278.2>249.2
Norbuprenorphine	6.257	414.3>152.2 414.3>165.2	Methadone	8.102	310.2>105.1 310.2>264.9
6-Acetylmorphine	3.955	328.2>165.0 328.2>211.0	Norpropoxyphene	7.033	326.4>91.1 326.4>252.0
Fentanyl	6.815	337.3>105.1 337.3>188.0	O-Desmethyl-Cis-Tramadol	3.896	250.1>58.0 250.1>42.0
Hydromorphone	3.425	286.2>184.9 286.2>156.9	Oxycodone	4.053	316.2>298.0 316.2>169.0
Norfentanyl	4.705	233.1> 55.0 233.1> 84.1	Propoxyphene	7.139	340.3>58.1 340.3>265.9
Codeine	3.887	300.2>165.1 300.2>152.0	Naloxone	3.9	328.0>212.3 328.3>310.1
Hydrocodone	4.299	300.1>199.0 300.1>128.0	Cis-Tramadol	4.959	264.2>58.0 264.2>77.1
Meperidine	5.262	248.1>174.1 248.1>220.1	Propoxyphene-D5	7.253	345.1>58.1 345.1>271.2
Morphine	3.263	286.2>165.0 286.2>152.1	Buprenorphine-D4	7.592	472.3>59.1 472.3>400.3
Oxymorphone	3.34	302.1>227.2 302.1>198.2			

Table 2d. Retention Times (min), MRM Transitions Monitored (m/z), for Cocaine, Cannabinoids and Hallucinogens

Analyte	RT	MRM	Analyte	RT	MRM
M-hydroxycocaine	4.617	320.2>182.1 320.2>121.1	11 Hydroxy-Δ9-THC	9.552	331.2>313.1 331.2>193.1
Cocaine	5.718	304.1>182.1 304.1> 77.1	11-Nor-9-Carboxy-Δ9-THC	9.847	345.2>327.1 345.2>299.1
Cocaethylene	6.313	318.2>196.1 318.2> 82.1	Δ9-THC	10.406	315.3>193.1 315.3>123.1
Benzoylecgonine	5.905	290.0>168.1 290.0> 77.1	Δ9-THC-D3	10.398	318.3>196.1 318.3>126.3 318.3>248.1
Norcocaine	5.907	290.0>168.1 290.0>136.1	PCP	7.345	244.2> 86.1 244.2>159.2
Cocaine-D3	5.703	307.2>185.2 307.2> 85.1	2-Oxo-3-Hydroxy-LSD	4.621	356.2>237.0 356.3>222.0
Benzoylecgonine-D8	4.868	298.2>171.1 298.2>110.1	LSD	6.384	324.3>223.1 324.3>208.0
Norketamine	5.245	224.1>125.1 224.1> 89.1	LSD-D3	6.345	327.3>226.1 327.3>208.0
Ketamine	5.523	238.0>125.1 238.0> 89.1	Ketamine-D4	5.483	242.2>129.1 242.2>224.0

2.6 Method Validation

The method was validated for specificity, calibration range and linearity, bias/accuracy, (im)precision and method uncertainty. Precision data were assessed using drug-free urine spiked at low (e.g. 10 ng/mL), medium (e.g. 40 ng/mL) and high (e.g. 100 ng/mL) concentration control levels for each analyte. Control samples were analysed in triplicate across eight different days. Precision was expressed as the percentage coefficient of variation about the mean value (%CV). Accuracy (bias) was calculated using the same processed samples as in the precision study. Quantitation values were achieved by calculating the peak area ratios for the precursor to product ion transition of the analyte to its relative internal standard. A 6 point calibration curve covered a concentration range of 5-100 or 10-200 ng/mL for each analyte and was assessed for linearity using regression analysis. The limit of detection (LOD) was determined by analysing drug-free (blank) urine samples with internal standard on multiple days to determine a mean measured concentration and standard deviation of each analyte. From this the LOD was calculated based on the mean plus 3 stand deviations. The lower and upper limit of quantification (LLOQ and ULOQ) for each analyte was determined from accuracy/precision results (n=6).

Positive patient specimen results from this method were compared with the previous validated GC–MS method or immunoassay method. Correlation of results was determined using 441 residual patient specimens (which were de-identified prior to use to protect personal health information), many of which contained more than one drug.

3. Results and Discussion

Linear calibrations of R^2 equal or greater than 0.99 were achieved for all analytes. Limit of detections are shown in Table 3 with LLOQs of either 5, 10 or 20 ng/mL (depending on the analyte) with precision and accuracy within 20% and upper limits of quantitation (ULOQ) determined as being the highest calibrator (i.e. 100 or 200 ng/mL) demonstrated by high concentration control sample analysis with precision and accuracy within 20%.

Table 3. Limit of Detection (ng/mL)

Analyte	LOD	Analyte	LOD	Analyte	LOD	Analyte	LOD
Amphetamine	6.9	7-aminoclonazepam	0.189	Temazepam	0.143	11-OH-THC	5.34
Butylone	0.03	7-aminonitrazepam	0.153	Triazolam	0.850	THC-COOH	8.27
(S)-Cathinone	0.15	α-hydroxytriazolam	0.318	6-MAM	1.36	THC	3.4
Dibutylone	0.19	α-hydroxymidazolam	0.251	Buprenorphine	0.81	2-Oxo-3-hydroxy-LSD	1.98
Dimethylone	0.16	Alprazolam	0.032	Codeine	0.18	PCP	3.07
MDA	0.24	Chlordiazepoxide	0.054	Hydrocodone	1.39	LSD	2.53
MDEA	0.04	Clobazam	0.080	EDDP	0.42	Ketamine	2.45
2,3-MDMA	0.37	Clonazepam	0.327	Fentanyl	1.16	Norketamine	2.91
3,4-MDMA	0.05	Desalkylflurazepam	0.061	Hydromorphone	2.52		
Mephedrone	0.62	Diazeapm	0.109	Meperidine	1.53		
Methamphetamine	1.92	Estazolam	0.033	Methadone	0.18		
Methaqualone	0.07	Flunitrazepam	0.151	Morphine	1.63		
Methylone	0.03	Flurazepam	0.011	Naloxone	0.11		
Methylphenidate	0.05	Lorazepam	0.531	Norfentanyl	1.02		
PMA	0.18	Lormetazepam	0.098	Normeperidine	2.69		
m-Hydroxycocaine	3.65	Midazolam	0.020	ODT	1.84		
Benzoylecgonine	2.36	Nitrazepam	0.191	Oxycodone	2.57		
Cocaethylene	2.05	Nordiazepam	0.152	Propoxyphene	2.53		
Cocaine	3.12	Oxazepam	0.608	Tramadol	1.83		
Norcocaine	4.31	Phenazepam	0.248				

Within-batch and between-batch accuracy and precision data are shown in Tables 4a-e and Tables 5a-e with few exceptions, accuracy and precision was within typical acceptance criteria of 20% and 20%CV for all drug/analyte types.[11] Specifically, for amphetamines and related stimulants based on 10, 40 and 100 ng/mL controls, within-batch accuracy and precision was -15.5 to 16.6% and 2.2 to 17.1%CV, respectively with between-batch accuracy and precision of -12.0 to 12.7% and 3.2 to 12.0%CV, respectively. For benzodiazepines based on 10, 40 and 100 ng/mL controls, within-batch accuracy and precision was -20.3 to 18.8% and 3.2 to 15.5%CV, respectively with between-batch accuracy and precision of -15.5 to 19.8% and 2.4 to 25.3%CV, respectively. For opiates/opioids based on 5/10, 20/40 and 100/200 ng/mL controls, within-batch accuracy and precision was -15.8 to 12.7% and 1.5 to 26.2%CV, respectively with between-batch accuracy and precision of -13.4 to 24.5% and 1.8 to 13.2%CV, respectively. For cocaine and metabolites based on 10, 40 and 100 ng/mL controls, within-batch accuracy and precision was -16.8 to 15.6% and 2.3 to 7.5%CV, respectively with between-batch accuracy and precision of -12.3 to 12.4% and 4.1 to 10.7%CV, respectively. For cannabinoids, hallucinogens and ketamine (and metabolites) based on 10, 40 and 100 ng/mL controls, within-batch accuracy and precision was -16.0 to 1.9% and 2.2 to 13.7%CV, respectively with between-batch accuracy and precision of -16.3 to 2.1% and 2.1 to 10.3%CV, respectively.

Table 4a. Accuracy/Bias (%), for Amphetamines

	Within Batch			Between Batch		
Analyte	**10 ng/mL**	**40 ng/mL**	**100 ng/mL**	**10 ng/mL**	**40 ng/mL**	**100 ng/mL**
Amphetamine	16.6	-6.6	-8.3	12.7	-7.5	-12.0
Butylone	-5.5	-1.8	0.8	<0.1	1.7	0.8
(S)-Cathinone	0.8	3.3	5.2	7.2	10.6	6.1
Dibutylone	-2.6	-8.4	-5.9	-1.4	-3.2	-3.3
Dimethylone	-8.4	-5.8	-1.6	-5.6	-3.9	-1.3
MDA	9.3	0.1	-7.0	12.2	3.7	-4.3
MDEA	-15.0	-10.1	-2.0	-5.8	-0.9	1.6
2,3-MDMA	-10.3	-11.3	-6.3	-2.9	-0.4	2.6
3,4-MDMA	-12.2	-11.8	-7.1	-5.0	-1.0	2.2
Mephedrone	-1.0	-1.0	-2.6	-3.7	2.6	-4.1
Methamphetamine	0.3	-10.3	-10.8	9.4	-3.5	-8.0
Methaqualone	-3.8	-3.9	1.9	4.1	4.5	6.5
Methylone	-8.3	-3.5	0.9	3.4	5.7	5.8
Methylphenidate	-8.0	-9.9	-5.2	-6.0	-4.7	-1.7
PMA	-2.3	-7.5	-7.4	6.6	2.9	0.4

Table 4b. Accuracy/Bias (%), for Benzodiazepines

	Within Batch			Between Batch		
Analyte	**10 ng/mL**	**40 ng/mL**	**100 ng/mL**	**10 ng/mL**	**40 ng/mL**	**100 ng/mL**
7-aminoclonazepam	0.46	11.20	7.57	8.27	18.00	10.13
7-aminonitrazepam	1.46	14.49	10.26	6.41	19.78	14.04
α-hydroxytriazolam	-5.44	18.25	13.86	-1.91	15.29	12.45
α-hydroxymidazolam	-19.60	-12.63	-17.66	-13.71	-5.26	-7.34
Alprazolam	-14.06	-6.38	-10.24	-6.40	1.68	-1.50
Chlordiazepoxide	0.98	13.48	10.07	3.92	14.61	12.23
Clobazam	-15.24	-8.81	-19.49	3.70	17.42	6.07
Clonazepam	1.45	11.56	7.45	5.23	10.62	4.80
Desalkylflurazepam	-8.04	-8.62	-18.17	-9.32	-0.25	-8.10
Diazeapm	-13.23	-5.64	-12.41	-9.34	0.64	-5.17
Estazolam	-13.39	-6.71	-12.56	-6.89	3.36	-2.37
Flunitrazepam	-10.02	-7.15	-13.97	-3.99	1.62	-6.36
Flurazepam	5.94	18.81	15.24	5.76	11.33	7.04
Lorazepam	13.77	18.30	9.21	5.62	11.12	3.78
Lormetazepam	4.14	18.07	7.37	6.98	12.30	5.44
Midazolam	-5.74	1.30	-2.65	-9.06	-3.84	-6.17
Nitrazepam	-18.73	-15.00	-20.30	-15.51	-8.73	-8.52
Nordiazepam	-19.17	-11.99	-18.46	-7.32	-1.24	-6.45
Oxazepam	-18.87	-2.94	-12.29	-5.13	5.73	0.30
Phenazepam	-4.75	-4.72	-6.82	-3.52	3.81	0.48
Temazepam	-6.58	-1.53	-8.47	-3.03	2.88	-3.56
Triazolam	-7.05	14.02	10.15	-6.92	4.90	1.30

Table 4c. Accuracy/Bias (%), for Opiates and Opioids

	Within Batch			Between Batch		
Analyte	**5 ng/mL**	**20 ng/mL**	**100 ng/mL**	**5 ng/mL**	**20 ng/mL**	**100 ng/mL**
6-MAM	10.6	9.5	-2.4	6.3	0.6	-7.6
Buprenorphine	<0.1	1.0	-0.7	-4.9	-5.3	-4.2
Codeine	6.6	<0.1	-10.5	10.3	3.4	-7.1
Fentanyl	-1.5	-0.5	-9.6	1.1	-3.3	-12.2
Hydrocodone	-9.4	-2.9	-10.9	18.4	2.4	-5.5
Hydromorphone	-7.5	-0.2	-11.8	17.3	-5.7	-11.7
Meperidine	12.7	-4.0	-15.8	11.9	3.3	-9.0
Morphine	12.3	-7.7	-12.9	13.2	-4.7	-10.4
Norfentanyl	11.2	6.2	-9.0	12.8	-1.4	-13.4
Normeperidine	7.6	6.3	-15.7	24.5	10.0	-11.3
Analyte	**10 ng/mL**	**40 ng/mL**	**200 ng/mL**	**10 ng/mL**	**40 ng/mL**	**200 ng/mL**
EDDP	9.1	8.4	-2.5	5.6	10.0	1.1
Methadone	2.1	-1.6	-8.2	-0.7	0.4	-3.2
Naloxone	-10.7	4.0	-10.5	-8.4	1.9	-9.2
ODT	6.7	-9.1	-13.2	9.6	-4.5	-5.6
Oxycodone	4.2	-0.5	-8.3	12.9	4.0	-1.7
Propoxyphene	6.2	-9.3	-11.6	11.0	1.6	1.3
Tramadol	7.2	-4.5	-5.9	7.5	-3.3	-0.4

Table 4d. Accuracy/Bias (%), for Cocaine

	Within Batch			Between Batch		
Analyte	**10 ng/mL**	**40 ng/mL**	**100 ng/mL**	**10 ng/mL**	**40 ng/mL**	**100 ng/mL**
m-Hydroxycocaine	-6.44	-4.39	-8.23	-8.97	-1.83	-4.19
Benzoylecgonine	-16.75	-10.18	-8.17	-12.30	-3.14	-0.26
Cocaethylene	-6.96	-4.08	-2.51	-2.77	2.46	4.46
Cocaine	8.29	13.68	15.55	3.09	7.91	12.39
Norcocaine	-7.06	-3.70	-5.13	1.19	6.83	6.80

Table 4e. Accuracy/Bias (%), for Cannabinoids and Hallucinogens

	Within Batch			Between Batch		
Analyte	**10 ng/mL**	**40 ng/mL**	**100 ng/mL**	**10 ng/mL**	**40 ng/mL**	**100 ng/mL**
11-OH-THC	1.86	-6.22	-8.59	0.35	-2.15	-5.18
THC-COOH	--	-6.29	-16.04	--	-3.28	-15.72
THC	-0.14	-3.51	-5.3	-2.21	-4.74	-6.63
PCP	-9.93	-8.98	-9.24	-16.32	-11.16	-4.34
2-Oxo-3-hydroxy-LSD	-7.74	-4.11	-11.31	-9.31	-4.16	-6.6
LSD	-3.42	0.13	-0.96	-4.99	-0.9	0.45
Norketamine	-12.55	-11.36	-13.22	-6.71	-3.95	-4.65
Ketamine	-4.91	-2.38	-4.26	-2.27	0.96	2.14

Table 5a. Precision (%CV), for Amphetamines

	Within Batch			Between Batch		
Analyte	**10 ng/mL**	**40 ng/mL**	**100 ng/mL**	**10 ng/mL**	**40 n g/mL**	**100 ng/mL**
Amphetamine	6.6	6.3	2.2	9.9	3.2	4.7
Butylone	7.5	8.0	4.9	7.7	8.2	3.8
(S)-Cathinone	5.7	6.9	8.7	5.8	7.8	10.6
Dibutylone	4.9	7.4	5.7	6.3	6.8	3.2
Dimethylone	5.2	7.8	6.1	6.7	6.0	10.5
MDA	2.8	6.1	3.4	9.3	7.8	5.2
MDEA	5.5	9.6	5.0	11.7	7.8	4.4
2,3-MDMA	4.9	8.3	3.7	5.8	8.6	6.9
3,4-MDMA	5.2	8.7	4.1	7.0	8.4	7.5
Mephedrone	12.0	8.7	10.3	11.1	8.4	9.3
Methamphetamine	4.9	17.1	4.2	9.1	12.0	3.6
Methaqualone	4.6	6.4	2.6	10.8	10.0	3.9
Methylone	13.3	8.7	7.1	9.6	9.1	10.5
Methylphenidate	6.5	8.2	4.3	8.0	7.9	9.9
PMA	3.4	6.6	2.7	7.5	9.1	6.4

Table 5b. Precision (%CV), for Benzodiazepines

	Within Batch			Between Batch		
Analyte	**10 ng/mL**	**40 ng/mL**	**100 ng/mL**	**10 ng/mL**	**40 n g/mL**	**100 ng/mL**
7-aminoclonazepam	12.91	5.60	11.34	10.03	2.40	5.53
7-aminonitrazepam	11.37	4.63	10.50	9.28	2.94	4.83
α-hydroxytriazolam	9.58	6.69	3.60	7.00	8.16	4.32
α-hydroxymidazolam	10.24	7.66	5.11	16.93	16.83	16.62
Alprazolam	8.60	7.60	5.07	11.43	10.84	8.79
Chlordiazepoxide	6.24	7.46	4.00	16.98	11.10	12.92
Clobazam	4.10	8.67	5.32	25.31	19.75	19.78
Clonazepam	10.39	10.65	3.76	8.90	8.03	8.82
Desalkylflurazepam	14.48	6.78	4.63	13.52	11.85	10.16
Diazeapm	7.15	6.91	3.83	8.88	12.54	12.64
Estazolam	6.57	7.62	4.16	12.22	12.40	11.15
Flunitrazepam	5.81	8.84	4.26	13.03	15.22	11.56
Flurazepam	7.29	8.94	5.50	6.53	8.03	6.19
Lorazepam	15.50	6.67	4.74	14.28	7.80	6.45
Lormetazepam	9.30	6.48	3.88	10.40	10.47	6.41
Midazolam	8.44	7.74	4.49	4.61	7.15	4.21
Nitrazepam	8.36	8.59	3.23	11.73	14.32	13.04
Nordiazepam	5.25	7.11	4.25	16.59	11.72	12.14
Oxazepam	15.01	9.52	4.30	18.40	7.47	12.64
Phenazepam	8.26	8.69	6.81	8.81	11.82	9.09
Temazepam	10.74	9.57	3.83	9.55	7.33	6.02
Triazolam	10.52	8.37	4.63	6.46	9.80	6.62

Table 5c. Precision (%CV), for Opiates and Opioids

	Within Batch			Between Batch		
Analyte	**5 ng/mL**	**20 ng/mL**	**100 ng/mL**	**5 ng/mL**	**20 n g/mL**	**100 ng/mL**
6-MAM	14.4	2.8	2.7	4.3	5.0	4.2
Buprenorphine	9.3	3.9	1.5	5.9	4.2	4.4
Codeine	14.0	2.9	6.6	5.8	3.4	10.0
Fentanyl	9.4	3.7	1.9	9.7	10.4	9.7
Hydrocodone	16.5	3.6	5.4	12.0	7.5	10.2
Hydromorphone	26.2	12.0	3.0	11.3	7.0	5.4
Meperidine	7.7	3.4	5.4	13.2	8.4	10.8
Morphine	16.1	8.6	5.3	13.1	9.8	9.5
Norfentanyl	15.1	3.8	2.9	5.4	6.7	5.7
Normeperidine	19.5	7.6	4.9	10.2	7.1	10.0
Analyte	**10 ng/mL**	**40 ng/mL**	**200 ng/mL**	**10 ng/mL**	**40 n g/mL**	**200 ng/mL**
EDDP	6.3	7.6	5.5	7.8	6.6	5.2
Methadone	9.8	4.9	5.8	6.0	2.6	5.0
Naloxone	13.3	3.2	6.9	6.5	5.8	4.8
ODT	12.1	4.7	5.7	11.9	7.0	8.7
Oxycodone	8.8	3.3	6.6	6.7	4.7	8.6
Propoxyphene	5.0	4.7	5.9	13.0	14.9	16.7
Tramadol	9.9	1.8	6.3	5.4	1.8	4.2

Table 5d. Precision (%CV), for Cocaine

	Within Batch			Between Batch		
Analyte	**10 ng/mL**	**40 ng/mL**	**100 ng/mL**	**10 ng/mL**	**40 n g/mL**	**100 ng/mL**
m-Hydroxycocaine	4.63	7.47	2.34	6.92	9.25	10.73
Benzoylecgonine	2.48	6.00	2.92	7.18	7.06	7.85
Cocaethylene	3.12	6.27	4.12	4.13	5.41	5.84
Cocaine	2.34	5.38	4.19	7.49	6.68	4.51
Norcocaine	4.32	6.73	3.01	6.15	7.85	8.64

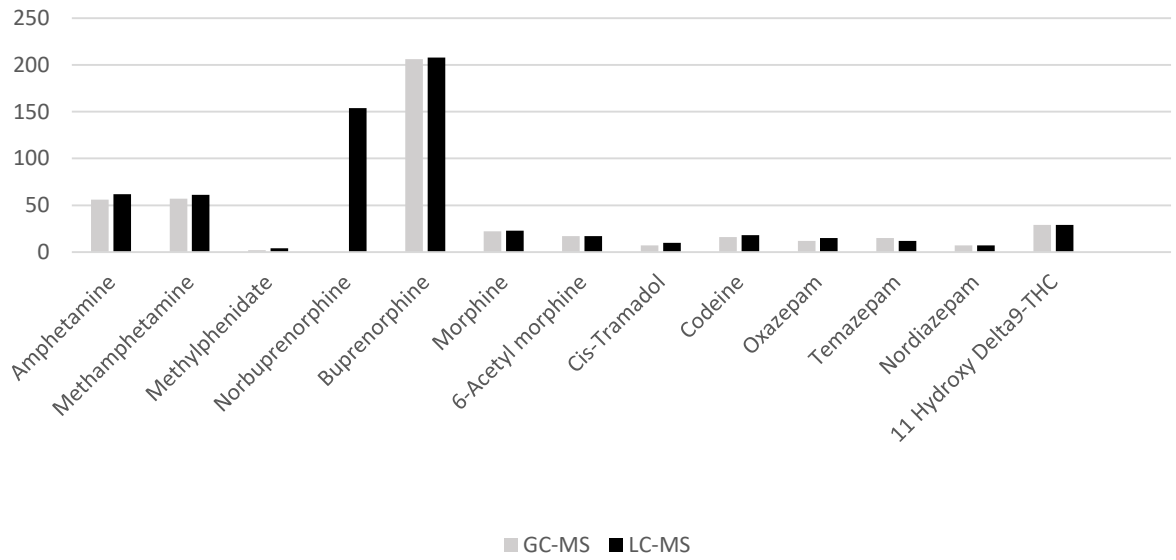
Table 5e. Precision (%CV), for Cannabinoids, Hallucinogens and Ketamines

	Within Batch			Between Batch		
Analyte	**10 ng/mL**	**40 ng/mL**	**100 ng/mL**	**10 ng/mL**	**40 n g/mL**	**100 ng/mL**
11-OH-THC	10.88	13.72	8.12	8.99	9.07	5.99
THC-COOH	--	8.47	5.54	--	8.06	4.40
THC	8.46	10.33	12.83	7.80	8.41	6.93
PCP	4.71	6.01	6.02	6.84	8.56	5.93
2-Oxo-3-hydroxy-LSD	3.74	8.08	4.44	9.65	10.34	7.94
LSD	2.18	6.67	3.14	1.72	4.85	2.45
Norketamine	3.4	6.11	3.83	4.67	6.42	7.77
Ketamine	2.33	5.79	2.94	2.13	4.24	3.93

As urine drug and metabolite concentrations can be affected by many factors (including pH) and may require creatinine correction to account for dehydration, excessive hydration and variations of glomerular filtration rate, concentrations are not always that useful or are appropriate depending on the application. As such, strict adherence to typical accuracy and precision acceptance of within 20% (especially for blood concentrations for medico-legal or other purposes) is not absolutely necessary. Within the clinical context of drug rehabilitation and monitoring of drug use, distinguishing historic/past use (e.g. through the notional determination of a very low concentration - colloquial trace amount) compared to more active or recent use can be useful. Therefore, of greater importance is appropriate LOD and LLOQ levels to ensure drugs and metabolites can be detected and measured at low concentrations, thereby informing clinicians of patient drug use.

A comparative study of results for positive patient specimens are plotted in Figure 1 showing good agreement with the existing and previously validated GC-MS method and immunoassay screening results. The drugs detected represent common drugs of abuse encountered in clinical toxicology in UAE. The LC-MS-MS method described in this paper, was particularly beneficial in detecting stimulants (i.e. amphetamine, methamphetamine and methylphenidate) as well as buprenorphine and its metabolite, norbuprenorphine which did not feature in the GC-MS method.

Figure 1. Patient specimen comparison with GC-MS method



Whilst LC-MS-MS technology is more expensive than single quadrupole GC-MS, the sensitivity and selectivity advantages along with a broader scope of analysis and potential shorter run times offset the cost differential. Furthermore, if relying on its selectivity benefits, LC-MS-MS can also be used within a “screen and confirm” approach without using immunoassay for presumptive screening, which may result in overall cost savings due to the application of a single technique.

Conclusions

Utilising automated solid phase extraction and LC-MS-MS analysis, a comprehensive method for the confirmation of a wide variety of drugs of abuse relevant to drug rehabilitation in the United Arab Emirates has been developed, validated and applied to patient urine samples. Results for 280 drug positive patient specimens showed good agreement with the previous in-house GC–MS method. The LC-MS-MS method provides rapid, sensitive and selective detection and quantitation to replace the existing GC-MS approach which can be expanded easily with the introduction of additional MRM transitions as and when required (e.g. if new or other drugs of abuse are to be considered) to support the work of the clinical team in this special area of clinical toxicology and medicine.

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Declaration of Interest

The authors have no declarations of interest.

References

1. Al Ghaferi HA, Ali AY, Gawad TA, Wanigaratne S. Developing substance misuse services in United Arab Emirates: the National Rehabilitation Centre experience. *BJPsych International.* 2017;14:92-96.

2. Al Ghafri H, Abuelgasim EA, Ali AY, Al Mamari S, Gawad TA, Alawadhi A, Shawky M, Hassan N, Elliott S. A 6-year review of drug trends in the United Arab Emirates from the perspective of the National Rehabilitation Center (NRC), Abu Dhabi. *Current Topics in Toxicology.* 2020;16:151-156.

3. Al Ghafri H, Elrasheed A, Al-Mamari S, Assaf M, Al Jenaibi M, Elarabi H, Alawadhi A. Rashi A, Al Meheiri F, Jawad T, Yousif A, Elliott S. Assessment of the use of point-of-care pregabalin testing for drug monitoring, compared to gas chromatography mass-spectrometry – a pilot study. *J Med Toxicol Clin Forensic Med.* 2018;4:1-3.

4. Krasowski MD, Pizon AF, Siam MG, Giannoutsos S, Iyer M, Ekins S. Using molecular similarity to highlight the challenges of routine immunoassay-based drug of abuse/toxicology screening in emergency medicine. *BMC Emerg Med.* 2009;9:5

5. Saitman A, Park HD, Fitzgerald RL. Using molecular similarity to highlight the challenges of routine immunoassay-based drug of abuse/toxicology screening in emergency medicine. *J. Anal. Toxicol.* 2014;38(7):387-396.

6. Orfanidis A, Gika HG, Theodoridis G, Mastrogianni O, Raikos N. A UHPLC-MS-MS Method for the Determination of 84 Drugs of Abuse and Pharmaceuticals in Blood. *J Anal Toxicol.* 2021;45(1):28-43.

7. Tang MH, Ching CK, Lee CY, Lam YH, Mak TW. Simultaneous detection of 93 conventional and emerging drugs of abuse and their metabolites in urine by UHPLC-MS/MS.
J Chromatogr B Analyt Technol Biomed Life Sci. 2014;969:272-284.

8. Valen A, Leere Øiestad ÅM, Strand DH, Skari R, Berg T. Determination of 21 drugs in oral fluid using fully automated supported liquid extraction and UHPLC-MS/MS. *Drug Test Anal.* 2017;9(5):808-823.

9. Galloway JH, Ashford M, Marsh ID, Holden M, Forrest AR. A method for the confirmation and identification of drugs of misuse in urine using solid phase extraction and gas-liquid chromatography with mass spectrometry. *J Clin Pathol.* 1998;51(4):326-329.

10. Paterson S, Cordero R, McCulloch S, Houldsworth P. Analysis of urine for drugs of abuse using mixed-mode solid-phase extraction and gas chromatography-mass spectrometry. *Ann Clin Biochem.* 2000;37:690-700.

11. Wille SM, Coucke W, De Baere T, Peters FT. Update of standard practices for new method validation in forensic toxicology. *Curr. Pharm. Des.* 2018;23@5442–5454.

