High Throughput Automated Sample Preparation and Analysis of Drugs of Abuse in Human Urine Using LC-MS-MS

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BACKGROUND

Due to the widespread abuse of illicit drugs, toxicology/clinical laboratories are facing a significant increase in patient samples. There is a need to develop a high throughput analytical method that can process large numbers of samples in a limited time frame while being sensitive enough to analyze drugs/metabolites at appropriate cut-off levels. Hence, we developed a high throughput LC-MS/MS method for the analysis of 34 drugs of abuse in human urine with automated sample preparation using MicroLab NIMBUS.

METHOD

Using an 8 channel Microlab NIMBUS, 100 µL of urine sample and 100 µL of master solution containing ISTDs, IMCSzyme® and buffer, were transferred into a 96 deep well plate (2.2mL) and mixed. The plate was then transferred to a heater/shaker for hydrolysis at 60 °C for 30 min. 600 µL of methanol was then added into each well for protein precipitation. 80 µL of the hydrolysate mixture and 420 µL of 0.1% acetic acid were transferred into an Isolute® filter plate (Biotage). Filtration was conducted by MPE (Positive Pressure Module). The filtered samples were analyzed on an Agilent 1290 HPLC coupled with a 6460 QQQ operated in positive dynamic MRM mode. Separation was achieved on an Agilent Poroshell (120 EC-C18, 100X2.1mm, 2.7µm) column using gradient elution of 0.1% acetic acid and acetonitrile at 400 µl/ min. 5 µL was injected.

RESULTS

Table 1 listed the drugs and/or their metabolites analyzed in the method and their reporting cut-offs. Fig. 1 depicted the deck layout of the 8 channel Microlab NIMBUS. Pipetting techniques and parameters for different liquid classes were optimized on NIMBUS for accurate and reproducible pipetting and thorough mixing. Filtration is critical for removing particulates and minimizing matrix effects. The method has been fully validated. Compared to the manual method previously used in our lab, the new method produces comparable or better results in terms of precision (CV%: 1.2—12%), accuracy (97%--114%), recovery (88-112%), matrix effect (88%--110%) and sensitivity (LOD: 0.4-10 ng/mL), while reducing reagent and labor usage, and human variation/error. It is now used routinely for the analysis of patient samples in the opioid dependency program.

Fig. 1 Microlab NIMBUS deck layout

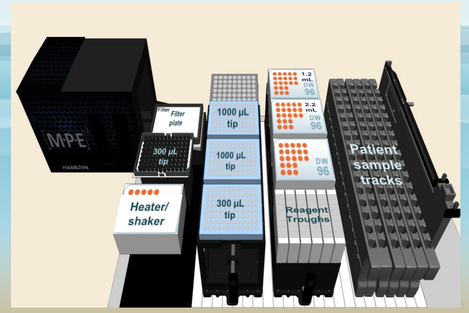

Table 1 List of the drugs and/or metabolites

Morphine	Hydromorphone	Oxazepam
Codeine	Hydrocodone	Nordiazepam
6-MAM	Buprenorphine	Temazepam
Oxymorphone	Norbuprenorphine	Diazepam
Oxycodone	7-aminonitrazepam	Midazolam
Carfentanil	7-aminoclonazepam	Clobazam
Norcarfentanil	Etizolam	Lorazepam
Fentanyl	α-OH-etizolam	Demoxepam
Norfentanyl	Alprazolam	BEG
Tramadol	α-OH-alprazolam	Zopiclone
O-desmethyl-cis-tramadol	α-OH-triazolam	N-dME-zopiclone
	Bromazepam	

Table 2 Cut-offs, LODs/LOQs and linearity (*R^2≥0.99)

Analytes (cut-off, ng/mL)	LOD /LOQ (ng/mL)	Linear up to*
Benzodiazepines (50)	10	1000 - 2000
Opiates (50)	10	500 - 2000
6-MAM (10)	2	200
Fentanyl/ Norfentanyl (5)	1	200
Carfentanil /Norcarfentanil (2)	0.4	200
BEG (50)	10	4000
Buprenorphine /Norpurenorphine (5)	1	200
Zopiclone/N-dME-Zopiclone (50)	2	2000

Fig. 1 Chromatogram of 34 drugs of abuse

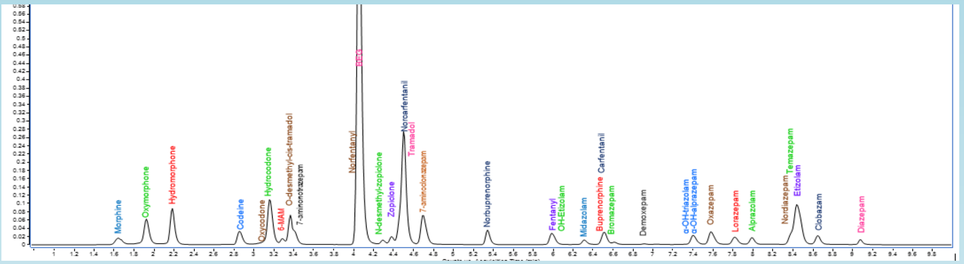

Table 3 Patient sample results (ng/mL)

Sample ID	Olympus screening	LC/MS/MS confirmation (ng/mL)	
Patient A	Buprenorphine	Buprenorphine	6.1
		Norbuprenorphine	761
	Fentanyl	Fentanyl	5.4
		Norfentanyl	84
	Cocaine	BEG	243
	Opiates	Codeine	1515
		Morphine	56330
		Hydromorphone	112
		6-MAM	299
Patient B	Buprenorphine	Buprenorphine	178
		Norbuprenorphine	860
	Benzodiazepines	Oxazepam	4334
		Temazepam	11343
		Nordiazepam	670
		Lorazepam	1117
	Opiates	Codeine	3271
		Hydrocodone	123
		Morphine	1782
		Hydromorphone	30
	-	Zopiclone	38
Patient C	Buprenorphine	Buprenorphine	18
		Norbuprenorphine	106
	Fentanyl	Fentanyl	n.d.
		Norfentanyl	6.8
	-	Carfentanil	7
		Norcarfentanil	42
	Benzodiazepines	Oxazepam	618
		Temazepam	245
		Nordiazepam	176

Table 4 CAP UDC* survey results (ng/mL)

Sample ID	Analyte Name	Results	Ref. value
UDC-11	Morphine	3129	3251
	Hydromorphone	293	258
	6-MAM	16.4	16.5
	Nordiazepam	965	915
UDC-15	Oxazepam	670	711
	temazepam	717	714
	α-OH-alprazolam	207	196
UDC-17	Morphine	3219	3386
	Codeine	2391	2616
UDC-18	Lorazepam	3387	3266
UDC-19	BEG	67.0	73.5
UDC-20	Fentanyl	5.2	4.9
	Norfentanyl	29.0	25.7

* Urine toxicology program by College of American Pathology(CAP).

CONCLUSION

- An automated, high throughput and sensitive LC-MS-MS method for the confirmation of 34 drugs of abuse was developed and validated.
- The method is reliable, cost effective and suitable for large-scale routine analysis.

Acknowledgement: This work was supported by the Opioid Dependency Program from Alberta Health Services.