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Development and Validation of an LC-MSMS Method for Drug of Abuse Confirmation Analytics including Solid Phase Extraction

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Abstract

A new UPLC-MS/MS system from Waters at the University Hospital of Basel (USB) was evaluated for drug of abuse confirmation analytics. Methods developed for solid phase extraction and analysis of opiates, amphetamines, cocaine and metabolites. The opiate method has been validated and several factors tested: test for interferences (selectivity), linearity and precision of calibrations, limits of quantification and detection, reproducibility, matrix effects in LC-MS/MS analysis and recovery of SPE, comparison of results of patient samples with an LC-MS system and stability of the analytes in the sample matrix.
Other matters investigated: ion suppression between analytes and internal standards, use for detection of contaminants in medicaments and the possibility of analyzing at least 29 analytes in one LC-MS/MS run.

Introduction

Currently, in the toxicology lab of the USB, drug of abuse confirmation analytics is done on an old ThermoFisher Navigator LC-MS for opiates, amphetamines, cocaine, hallucinogens and metabolites of the substances. A new Waters Xevo TQ MS was acquired to replace the old system for these analyses and, additionally, as a possible analysis system for other substances and applications such as therapeutic drug monitoring.

Furthermore, a Gilson Aspec GX-271 liquid handler was purchased, for sample preparation through SPE.
SPE and analysis methods were developed, and verified by validation for one substance group (opiates).
A general SPE method was developed which showed > 50% extraction rates for all tested substances.

Results

The developed new LCMSMS method has a run time for the gradient of 1.69 min. The total run time with washing and equilibration of the column is 4 min.
For the LC method MRM (multiple reaction monitoring) methods on the MSMS were made for opiates, amphetamines and cocaine. With these methods it was possible to analyse up to 15 substances in one run.

The overview of results of the validation for the opiate SPE and LC-MSMS methods is given in Figure 1.

substance	RT	selectivity	LOD	LLOQ	linearity upper limit	reproducibility	matrix effects	SPE recovery	stability	method parity	competative ionisation	other oberservations
	rel			ug/L	ug/L		%	%		%		
D3-morphine	0.73	✓	The LOD will be defined per sample by the through TargetLynx calculated concentraion		800	✓	53	73	✓		**	
D3-codeine	1.19	✓			800	✓	42	73	✓		*	!
norcodeine	1.19	✓		2.0	800	✓	69	68	✓	96	*	
hydromorphone	1.00	✓		0.5	800	✓	57	70	✓	100		
morphine	0.73	✓		0.5	800	✓	52	73	✓	100	**	
hydrocodone	1.41	✓		0.5	800	✓	36	70	✓	100		
codeine	1.23	(✓)		2.0	800	✓	55	72	✓	100	*	
dihydrocodeine	1.19	✓		1.0	800	✓	73	74	✓	100	*	!
6-monoacetylmorphine	1.38	✓		4.0	500	✓	36	78	✓	100		
6-acetylcodeine	1.82	✓		0.5	800	✓	63	67	✓	92	***	
heroin 3,6-diacetylmorphine	1.83	✓		0.5	800	✓	66	64	×	90	***	
morphin-3-glucuronide	0.47	(✓)		2.0	500	(✓)	25	68	✓	94		
morphin-6-glucuronide	0.69	✓		0.5	800	✓	57	68	✓	100		
codein-6-glucuronide	1.15	(✓)		1.0	800	✓	80	72	✓	100		

✓	passed
(✓)	passed, with minor issues
×	instable
* / ** / ***	risk of ion suppression through coeluting analytes
!	disturbance of D3-codeine through C13-dihydrocodeine

Figure 1: Overview of results for opiate method

LLOQ have been found to be between 0.5 and 6.0 µg/L. For most substances the reproducibility from LLOQ to 500 µg/L was given with a CV of < 10% and calibrations showed linearity up to 800 µg/L.

The test for Matrix effects showed that different samples can have varying degrees of ion suppression. Use of a deuterated internal standard of the respective substance is needed for precise quantification.
Recoveries of SPE were between 56 and 87 %.
Stability tests show that heroin has a decrease after one day, even at -20 °C. (Clinically not relevant, half time appr. 3 minutes).
Results showed 98 % parity with comparison methods.

Tests show that ion suppression of D3-morphin happens when large quantities of morphine are present.
As a consequence, for quantification purposes, this has to be taken into account by choice of a different general internal standard when this occurs.

To investigate the possibilities of analysis of a wide range of substances all tested substances (opiates, amphetamines and cocaine) were combined into one LC-MSMS method. The MRM program setup is seen in Figure 2

Testing with an aqueous drug mix of all these substances showed that analysis of such a range of substances is possible.

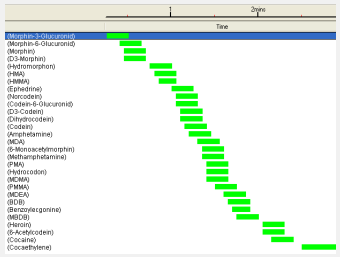

Figure 2: MRM program for 29 substances

Conclusions

The results of the validation show that the methods are suitable for confirmation analytics. Further investigation should be made in the possibility of making sample preparation online (at present the whole process is to long).

It was possible to build an LC-MS/MS MRM method in which 29 substances could be analysed in one run.
Care should be taken when quantifying (choice of internal standards and sample dilution).

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