An IDA mediated LC-MS/MS screening procedure with semi-quantitative potential

T. Decaestecker[1], P. Wallemacq[2], C. Van Peteghem[3] and J. Van Bocxlaer[1]

[1]Lab of Medical Biochemistry and Clinical Analysis, Ghent University, Harelbekestraat 72, B-9000 Ghent,
[2]Lab of Toxicology and Special Chemistry, Clinical Hospital St.-Luc, Hippocrate Avenue, B-1200 Brussels,
[3]Lab of Toxicology, Ghent University, Harelbekestraat 72, B-9000 Ghent,
BELGIUM

Tineke.Decaestecker@UGent.be

1. Introduction

This study evaluated an IDA-mediated LC-MS/MS screening methodology to the full, qualitatively as well as quantitatively, and shows its scope and limitations in a forensic setting. IDA is an artificial intelligence based product-ion scan mode providing automatic "on-the-fly" MS to MS/MS switching. Since former studies revealed that the IDA intensity threshold, unequivocally related to the background noise, seemed to be a critical parameter, the solid phase extraction procedure, the liquid chromatographic conditions and the mass spectrometric parameters all were optimized to the advantage of IDA. The as such developed screening procedure was benchmarked against immunochemical techniques as well as chromatographic methods of established forensic laboratories.

2. Materials

Liquid chromatography:
- HPLC: Waters Alliance 2790 separation module integrated with Q-TOF
- Column: Xterra MS C_{18}, 3.5µm, 100x2.1mm
- Flow rate 0.3mL/min
- Column temperature: 40°C
- Mobile phase: 5mM NH_4Ac in H_2O/MeOH/AcCN (80/10/10 (A) & 20/40/40 (B))

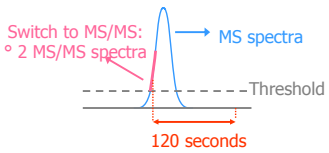
Mass spectrometry:
- Micromass Q-TOF MS equipped with a ES source, in ESI^+ mode

SPE: Isolute C_8 SPE columns

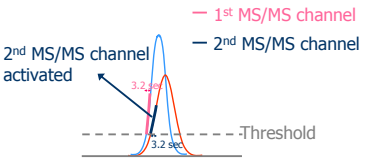
Blood samples: delivered by
- Jellinekcentrum, Amsterdam, The Netherlands
- Laboratory of Toxicology, Ghent University, Belgium

3. Methods

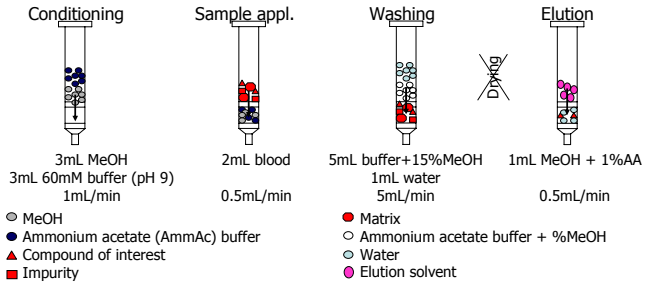
IDA can be visualized as folllows:



The quadrupole is initially set to transmit all masses until (an) ion(s) reach(es) a certain set threshold. Thereupon, the quad automatically switches to the SIR mode, selecting those ion(s), which are next fragmented, thus generating product-ions that are further mass analyzed in the TOF. By performing information dependent scanning at two different fragmentation energies, 2 collision-induced dissociation (CID) spectra for each of the detected compounds are generated. In addition, limitation of the MS/MS acquisition time to an acceptable minimum resulted in an almost instantly switch back to the MS mode. As such, this approach provided MS chromatograms that still could be of use for semi-quantitative purposes. A refractory period of 120sec was set for the last ions detected. The fast analysis capabilities of the system allow 3 simultaneous MS/MS traces of overlapping chromatographic peaks. A complete separation of the drugs was not deemed necessary; optimization of LC parameters was therefore kept to a minimum. Details on the optimization of the MS parameters are given in Ref. 1. A major criterion which governs the applicability of IDA in Systematic Toxicological Analysis (STA) is the lack of interferents which initiate and thus temporarily "occupy" the MS/MS-channels, effectively blinding the method to the compounds of real toxicological interest. Consequently, the applicability of such an IDA method largely depends on the quality of the extraction procedure for a biological sample. To that end, a SPE procedure was optimized in advantage of IDA.



Scheme of the final SPE procedure[2,3]:



4. Results

In a first qualitative phase a series of blood samples was analyzed in 2 ways. Results obtained by traditional STA screening procedures (EMIT, HPLC-DAD, GC-MS, and GC-NPD) were compared to those obtained following the IDA strategy. The results of 1 of these samples are depicted below:

	STA	IDA
Cocaine	✓	✓
Cafeine	✓	✓
Cotinine	✓	✓
MDMA	×	✓
Benzoylecgonine	×	✓
Methadone	×	✓
Bromazepam	×	✓

✓detected; × not detected
MDMA:methylenedioxymethamphetamine

Almost all of the drugs detected by the conventional techniques were identified by IDA, as well as additional drugs that were not by any of the other participants reported, e.g. ranitidine, propanolol, papaverine, etc.
In a next phase the quantitative capabilities of the IDA method have been benchmarked against quantitiative LC-MS/MS methods (target compound analysis)

	IDA	LC-MS/MS
Cocaine	0.545 µg/mL	0.173 µg/mL
MDMA	1.532 µg/mL	1.479 µg/mL
Benzoylecgonine	1.795 µg/mL	2.706 µg/mL
Methadone	99 ng/mL	70 ng/mL

A distinct difference is observed for cocaine and bezoylecgonine, which is attributed to the difference in time on which the IDA and MRM analyses were performed.

5. Conclusion

By assessing the potential and pitfalls of the IDA mediated LC-MS/MS screening approach, its feasibility within a forensic toxicological setting was demonstrated. Regarding its qualitative characteristics, it can be stated that the strategy performed very well and that it can be considered as a valuable alternative for traditional GUS procedures of drugs and toxic compounds. Its semi-quantitative potential proved to be more than suitable within the application field of toxicological analysis.

6. Acknowledgements

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7. References

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