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LC-MS(/MS) based general unknown screening procedure (GUS) for drugs in blood using information dependent acquisition (IDA): pros and cons

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1. Introduction

This work is the sequel of an earlier performed study on the evaluation of IDA for comprehensive drug profiling analyses using a Q-TOF, i.e. a study that aimed to set the scene on the evaluation of IDA for GUS procedures. A major criterion which governs the applicability of IDA in systematic toxicological analysis (STA) is the lack of interferents which initiate, and thus "occupy", the MS-MS channels, effectively blinding the method to the compounds of real toxicological interest. Therefore, a SPE procedure was statistically fully optimised with respect to IDA and incorporated in our LC-MS strategy.

2. Aims

- Evaluation of the qualitative and quantitative performance of the GUS procedure after incorporation of the statistically optimised SPE-procedure

3. Materials & Methods

- Liquid chromatography:
  - HPLC: Waters Alliance 2790 separation module integrated with Q-TOF
  - Column: Xterra MS C18, 3.5µm, 100x2.1mm
  - Flow rate 0.3mL/min
  - 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 C8 SPE columns
- Analytes: 17 neutral and basic compounds + butorphanol (IS)

4. Results

- Qualitative performance:
  - In a preliminary study the qualitative evaluation of IDA has partly been performed [1]; per compound MS & MS/MS spectra are obtained
  - MS to MS/MS-threshold plays an important part; this is the threshold that defines the moment for switching to MS/MS. It influences the detection limit, the quality of the MS/MS spectra and the interpretation of the data set
    - Low threshold: detection limit ↓, quality MS/MS spectra ↓, complicated interpretation (more interfering ions)
    - High threshold: detection limit ↑, quality MS/MS spectra ↑ , simplified interpretation (less interfering ions)

	LOD – Threshold 400	LOD – Threshold 100
XTC	7.5 ng/mL	2 ng/mL
Codeine	20 ng/mL	5 ng/mL
Methadone	5 ng/mL	1 ng/mL
Haloperidol	20 ng/mL	7.5 ng/mL
Methaqualone	5 ng/mL	1 ng/mL

Table 1: Comparison of LOD at 2 different threshold values for XTC, codeine, methadone, haloperidol and methaqualone

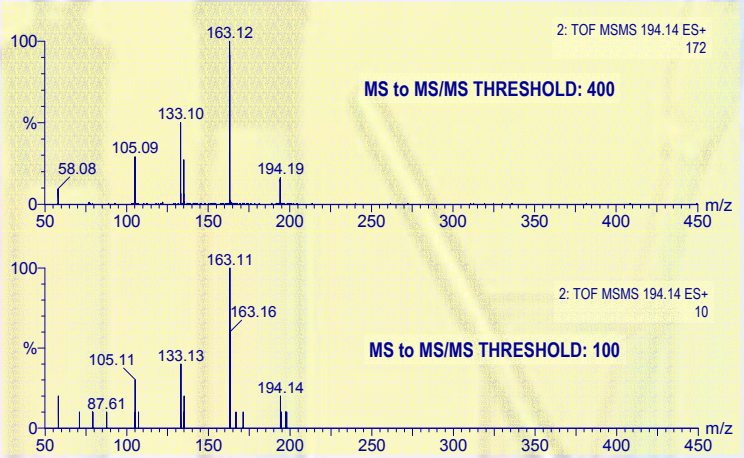

Figure 1: MS/MS spectra of XTC recorded at 2 different MS/MS thresholds

- Quantitative performance:
  - For all of the 17 compounds deviation from linearity was observed. Therefore, quadratic calibration was applied. In some cases, better coefficients of determination were obtained after logarithmic transformation.

	Cocaine	Strychnine	Lidocaine	Codeine
Regression curve				
y=a+b*x	0.8450	0.9094	0.7511	0.9407
y=a.b*logx	0.9605	0.9398	0.9858	0.9067
y=a+b*x+c*x^2	0.9814	0.9934	0.9548	0.9947
y=a+b*logx+c*lox^2	0.9963	0.9984	0.9916	0.9845
logy=a+b*logx+c*lox^2	0.9982	0.9953	0.9990	0.9977

Figure 2: Comparison of coefficients of determination (r^2) for diffenrent types of regression curves for cocaine, strychnine, lidocaine and codeine

4. Conclusion

The high selectivity of LC-MS/MS using IDA and the fact that no foreknowledge is required are in favour of this GUS procedure. Then again, on the one hand a loss of sensitivity was observed by increasing the MS to MS/MS threshold (to simplify the interpretation of the data) and on the other hand, the obtained quantitative information can only be used as an indication, as a result of the deviation from linearity

5. References and acknowledgements

[1] T. Decaestecker et al., Rapid Commun. Mass Spectrom. 14, 1787-1792 (2000).

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