Unreliable results can always occur unexpectedly, even if an extensive validated method on LC/MS/MS is used

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AIMS: The availability of an affordable LC/MS/MS apparatus is probably the most important breakthrough in clinical and forensic bio-analyses, since the introduction of gas and liquid chromatography, ICP, and immunoassays.
Apart from ion suppression, the unique combination of a simple extraction, selection on masses of mother compounds and metabolites and the unique masses of the MS-fragments, make these analytical methods very selective, flexible and useful.
The objective of this article is to warn for unexpected pitfalls and not to rely implicitly on the reproducibility of the separation material and the unique qualities and reliability of fully validated LC/MS/MS methods.

METHODS: We developed a very useful and reliable method for the analysis of atropine and the cocaine metabolite benzoylecgonine. To 0.1 ml of patient's serum 0.75 ml of 50 µg cyanoimipramine / L of a mixture of methanol: acetonitril (1:5 v/v; pH 6), is added as internal standard, for protein precipitation and for extraction purposes. After vortexing this mixture it is kept at -20oC for 30 minutes and then centrifuged for 5 min. at 11,000 rpm. Of the clear upper layer 5 µL is injected onto a certified 50 × 2.1 mm (ID) C18 HyPurity Aquastar Javelin Express (Thermo Electron, San Jose CA). After extensive validation this method is presently being used for the analysis of serum of six victims of atropinized cocaine.

RESULTS: Concentrations of atropine were found between 27ug/L and 80ug/L. After half a year we again received some serum samples of possible victims of atropinized cocaine. However, it was not possible to detect atropine in these samples or even in the spiked serum controls. Usually the metabolite benzoylecgonine can be detected and not the mother compound cocaine. Atropine has virtually the same mass spectrum as benzoylecgonine. (2) Atropine disappeared under the benzoylecgonine peak because of the minute difference between both MS/MS spectra. The concentrations of atropine were also much smaller than the concentrations of benzoylecgonine. Later we found out that the new analytical column was not exactly identical to the first one, although we did order exactly the same one (same order number, name and supplier). The former LC-column separated both compounds just enough unlike to the new one.
Nowadays we are using two of those analytical columns in series and as mobile phase 5% buffer (ammonium acetate 10g/L, acetic acid 15 mL/L and trifluoracetic anhydride 2 mL/L water) and 95% water to 95% acetonitrile in 5 minutes; flow 0.2 mL/min.
The new mobile phase is using of the difference in acidity of cocaine and of benzoylecgonine, by means of extending the aqueous eluent time of the gradient.

IN CONCLUSION:

First: LC/MS/MS is an extremely selective method, based on the molecular weight of the analytes. Nevertheless, even if compounds differ in molecular weight, a metabolite may have the same MS/MS-spectrum.

Second: Even the most comprehensive validated and published methods are not always robust and reproducible. Small, but crucial details may vary unexpectedly and influence the outcome. Forensic and clinical toxicological analyses always require sufficiently educated and well-experienced analytical specialists.

REFERENCES:

Moffat AC, Osselton MD, Widdop B. Clarke's Analysis of Drugs and Poidons, 2004; Ed 3. 657-658 and 842-845

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