Practical considerations for reliable quantification in postmortem specimens

Birgit Reiter, Kurt Jiran, Thomas Stimpfl

Medizinische Universität Wien, Klinisches Institut für Labormedizin, Forensische Toxikologie, A-1090 Wien, Austria

Abstract

Aim: The aim of this presentation is to critically discuss the practical aspects that have to be considered in the formation of "Guidelines" for a reliable and reproducible quantitative analysis in postmortem specimens.

Methods: Quantitative determinations of target analytes with isotope-labeled analogues and the method of standard addition were performed. An Excel template was created to make the standard addition method more practical.

Results: Both quantitative procedures produced comparable results. The application of an Excel template to plan the experiments for the method of standard addition was very helpful in routine work.

Conclusion: For reliable quantification in postmortem samples, isotope-labeled internal standards should be used. If no isotope-labeled analogue of the target compound is available, the method of standard addition should be applied.

1. Introduction

In postmortem forensic toxicology the detection and unambiguous identification of drugs and poisons is the first essential step, but in many cases, then quantitative results are needed to estimate possible toxic or lethal effects of the identified substance. Reliable quantification is difficult in postmortem forensic toxicology because the complex matrices demand complex sample preparation- and extraction-steps. Moreover, the lack of certified reference materials makes a "classical" validation of the analytical procedure impossible. Reliable reference values from well-documented cases would be very helpful in the interpretation of results. Although high performance analytical techniques are available today, a sufficient amount of reliable reference values for postmortem specimens does not yet exist. Guidelines for reliable quantification in postmortem specimens would be needed in order to create comparable results in different laboratories.

2. Material and Methods

In a prior study, brain samples were homogenized, aliquoted and spiked with different concentrations of isotope-labeled internal standard or certified reference standard, respectively. The homogenized samples were extracted and analyzed by GC-MS [1]. An Excel-sheet including all relevant information for the method of standard addition (such as estimated concentration, concentration of reference solution, amount of sample, sample matrix, number of levels, protocol of added concentrations, calculation of results…) was created. The Excel-sheet is routinely applied in our laboratory and can also be used to archive the data in our LIMS [2]. All quantitative calculations were based on a MS Office Excel scoring sheet from Funk et al. [3].

3. Results and Discussion

Reliable quantitative results in postmortem specimens can only be achieved if the sample preparation and the extraction procedure are optimized for the complex matrix. Moreover, automation of the whole procedure is necessary to produce sufficiently reproducible results in such cases. Homogeneity has to be achieved in the investigated specimens so that representative aliquots can be analyzed.

If isotope-labeled internal standards are used for quantification, a possible protein precipitation via the solvent of the internal standard must be prevented. A homogenous distribution of the internal standard in the sample has to be guaranteed (especially difficult with tissue samples). A further problem is the limited availability of isotope-labeled substances. A concentration difference between the isotope-labeled internal standard and the target analyte (morphine) – from 10 times lower to 10 timers higher – had no significant influence on the results [1].

If no isotope-labeled analogue of the target compound is available, the method of standard addition has to be applied. Representative aliquots, protein precipitation, and homogeneity (see above) aside, additional prerequisites are essential for the successful application of this method. Measurement of weight and volume must be precise, and the analytical method has to be linear in the measured concentration range. The application of the method of standard addition can lead to practical problems (e.g. estimation of target concentration, calculating added concentrations, calculating results...). These can be avoided by the use of an Excel template, which proved very helpful in the routine work.

The quantitative results with the method of standard addition were comparable to the results achieved with an isotope-labeled internal standard (41.7ng versus 44.7ng morphine/g). With regard to accuracy and confidence intervals, most reliable results with the standard addition method were obtained in our routine work over the last two years, when three spiked samples with close to the original concentration were analyzed in addition to the original sample.

4. Conclusions

To keep the effort for reliable quantitative analyses in postmortem specimens as low as possible, isotope-labeled analogues should be applied whenever available. In all other cases, the labor-intensive method of standard addition would be required. The use of an Excel template to plan and document the experiment is recommended.

Sample preparation and extraction should be optimized for the complex matrices of postmortem specimens and automation of the whole procedure proved favorable. For the creation of reference values the background information, PM-interval, diseases, variability in the genetic setup, and the exact definition of the specimen (region of brain) should be reported.

5. References

[1] Reiter B, Stimpfl T. Quantitative determination in complex biological matrices - how much effort do we need? In: F. Pragst F, Arndt T (Hrsg.). XVI. GTFCh-Symposium - Toxikologie psychisch aktiver Substanzen. Verlag GTFCh, 2009; 327-330.
[2] MS Office Excel-sheet can be received from the authors.
[3] Funk W, Dammann V, Donnevert G. Qualitätssicherung in der Analytischen Chemie. 2. Auflage, Wiley-VCH, Weinheim, 2005.