

YOUNG SCIENTISTS INTEREST GROUP INITIATES 'SCIENTIFIC ISSUES' SERIES

An Introduction to the Series by Frank T. Peters (Chairman) & Denise A. McKeown (Secretary)

Dear IATDMCT Members,

As most of you have probably recognized, a Young Scientist Special Interest group has recently been established within IATDMCT. One aim of this group is to promote the interests of the Young Scientist members and to encourage young people to join IATDMCT. Another aim is for active participation of Young Scientists in the association's activities. This shall include regular contributions to IATDMCT News by providing short overviews on topics of interest in Therapeutic Drug Monitoring and Clinical Toxicology or presenting interesting cases that stand out from daily routine work. The following article will start the series of Young Scientist contributions, and all Young Scientist members are invited to write future articles for this section. Proposals for topics and titles of articles can be sent to either of us (frank.peters@uniklinikum-saarland.de, dmckeown@sgul.ac.uk). Young Scientist contributors are also welcome to introduce themselves to all IATDMCT members in the New Members section of the Newsletter. We hope many Young Scientists will take advantage of this opportunity for active participation and that established members will encourage all their young co-workers to become Young Scientist members of IATDMCT.

The First Installment in the Scientific Issues Series from the IATDMCT Young Scientists

MATRIX EFFECTS in LC-MS: IMPORTANT ASPECTS & PRACTICAL EXPERIENCES

by Frank Peters & Denise McKeown

Abstract: Matrix effects, i.e. ion suppression or ion enhancement, are well known phenomena in liquid chromatography-mass spectrometry (LC-MS). They can be caused by compounds of various origins. Since matrix effects may exert a negative effect on important method performance parameters, they have to be tested for and evaluated during method development/validation. This can either be done by the method of post-column infusion or by comparison of signals from neat standards and blank

sample extracts spiked with analyte. Wherever possible, matrix effects should be reduced or eliminated by the optimization of chromatographic conditions, improving sample clean-up and/or by changing the type of ionization employed.

Introduction: During the last decade, liquid chromatography-(tandem) mass spectrometry [LC-MS(-MS)] has left the developmental stage and is now successfully applied to routine analysis in many areas, including therapeutic drug monitoring (TDM), clinical and forensic toxicology as well as doping control. LC-MS(-MS) combines the advantages of classical high performance liquid chromatography (HPLC) with those of MS detection. As a result a wide spectrum of analytes can be analyzed with high selectivity. Significant advantages of LC-MS(-MS) over the well established gas chromatography-mass spectrometry (GC-MS) are that LC-MS(-MS) covers analytes with rather high polarity, high molecular weights, and/or thermolabile properties. However, the LC-MS(-MS) technique is not free from drawbacks. One of the main disadvantages is the phenomenon that co-eluting compounds may suppress or enhance the ionization of the analytes of interest, referred to as matrix effects. If such effects occur, they may considerably affect method performance parameters such as limit of detection (LOD), limit of quantification (LOQ), linearity, accuracy and precision. Clearly the evaluation of possible matrix effects must be an integral part in any LC-MS(-MS) method development and validation. Not surprisingly, the important guidance document on bioanalytical method validation published by Shah *et al.*¹ explicitly recommends studies on possible matrix effects for LC-MS(-MS) procedures "to ensure that precision, selectivity and sensitivity will not be compromised". The following gives a short overview on when matrix effects may be expected, how they can be evaluated, and how they may be prevented. More detailed accounts on this topic and other aspects of method validation using LC-MS(-MS) can be found elsewhere.²⁻⁴

Causes of matrix effects: The phenomenon of ion suppression or enhancement in LC-MS(-MS) depends mainly on the sample matrix, sample preparation procedure, quality of

chromatographic separation, mobile phase additives and ionization type. Electrospray ionization (ESI) is more prone to such effects than atmospheric pressure chemical ionisation (APCI).^{3,5-9} These effects may occur principally when other compounds co-elute with the analyte of interest. In bioanalysis, important sources of such co-eluting compounds are the (biological) sample matrix, exogenous compounds such as drugs and/or their metabolites, (stable-isotope-labelled) internal standards (IS), or mobile phase additives such as trifluoroacetic acid (TFA).

Ion suppression/enhancement effects from endogenous compounds have been reported for various biological matrices used in TDM or toxicology such as blood, plasma or serum,^{5-8,10,11} urine,^{6,12} and oral fluid.^{6,10} They are generally most pronounced for analytes with short retention times. Ion suppression/enhancement is not uncommon at the void volume, hence the notable affect it has on analytes that elute early. However, caution has to be taken as matrix effects can also affect analytes that elute later in a chromatographic run. McKeown *et al* reported a case of this when the analyte, norbuprenorphine from a plasma extract, eluted at a retention time of 2.86 minutes under the chromatographic conditions employed.¹³ A build up of matrix interferences occurred at 10 to 11 minutes, this was not identified initially as it did not affect the first analysis but did affect subsequent injections.

Ion suppression/enhancement from exogenous compounds and/or metabolites present in the sample may also occur. It is very difficult to assess the likelihood of such effects, since a variety of different drugs/drug classes other than the analyte may be present in authentic samples. However, it must be considered in all multi-analyte procedures, in which not all analytes are completely separated from each other by chromatography. In such cases, overlapping analytes may suppress the ionization of each other as described by Egge-Jacobson *et al.* who reported mutual suppression of the co-eluting analytes saquinavir and nelfinavir by up to 30%.¹⁴ A very similar situation may occur when stable-isotope-labelled analogues of the analytes are used as internal standards, such analytes/IS are never fully separated from each other. This has been studied rather extensively owing to the importance

of stable-isotope-labelled IS in modern MS-based analytical techniques. Sojo *et al.* demonstrated that mutual suppression of analytes and their stable-isotope-labelled IS in the ESI mode should not affect quantification, however, they were concerned about a negative influence on the LOD if too high a concentration of IS was used.¹⁵ These findings concerning ESI were confirmed by Liang *et al.*⁹ For APCI mode, the latter reported mutual ionization enhancement for some of the analytes and their IS, possibly improving LOD and LOQ data. From these two studies, one might conclude that while the limits might be affected by ion suppression/enhancement, quantification would not as long as a stable-isotope-labelled IS was used. However, this is not always true as demonstrated by Jemal *et al.*¹² who reported for particular batches of urine, matrix effects that lead to changes in the response ratio (analyte vs. IS), which could have a negative influence on quantification. When no stable-isotope-labelled IS is available more serious problems may be encountered.

Finally, mobile phase additives can considerably suppress/enhance analyte ionization. Such additives not only elute as peaks from the chromatographic systems, but influence the entire chromatographic run, which may vary depending on the type of system used, i.e., isocratic or gradient. For this reason, their potential for ion suppression/enhancement may affect analytes over a much wider range of retention times. Mallet *et al.* studied the potential for ion suppression/enhancement in positive and negative ESI mode for various mobile phase additives such as TFA and ammonium bisphosphate.⁵ The results ranged from severe signal suppression (e.g., up to 87% in the case of TFA, negative mode) to considerable enhancement (e.g., 38.5% for ammonium formate, positive mode).

Evaluation of matrix effects: As mentioned above, studies on matrix effects should be an integral part of method development and validation for any LC-MS(-MS)-based procedure. In the literature, two approaches have been used extensively to study ion suppression/enhancements. In the first approach, a solution of the analyte is infused constantly, using a syringe pump, into the eluent from the column via a post-column tee connection. Continuous post column infusion leads to a constant signal in the detector, unless compounds that elute from the column suppress or

enhance ionisation, which leads to a decreased or increased detector response respectively. Monitoring the detector response after injection of blank matrix extracts can be used to check for possible ion suppression/enhancement from blank matrix compounds and for their retention times. Applications of this approach can be found in the literature.^{5-7,9} Strategies for the second approach were recently published by Matuszewski *et al.*⁸ This paper provides excellent guidance on how to perform and evaluate studies on matrix effects in LC-MS(-MS) analysis. The principal approach involves determination of peak areas for the analyte in three different sets of samples, one consisting of neat standards (set 1), one prepared in blank matrix extracts from different sources and spiked after extraction (set 2), and one prepared in blank matrix from the same sources but spiked before extraction (set 3). From these data, matrix effect (ion suppression/enhancement), extraction recovery, and the so-called process efficiency (combined matrix effect and recovery) can be calculated. The calculations are based on comparison of signal intensities from set 2 and set 1 (matrix effect), set 3 and set 2 (recovery), and set 3 and set 1 (process efficiency). The equations can be found in references.^{2,8} Other examples using the same or very similar approaches for the evaluation of matrix effects can be found in the literature.^{8,12,16,17} With two well established procedures for studying matrix effects, the question arises which one of them is better suited for validation studies. In the authors' opinion, the post-column infusion experiment is very useful during method development, providing information on the retention times where ion suppression/enhancement can be expected. For a validation study, the alternative approach seems to be more suitable, yielding a quantitative estimation of matrix effects and their variability, hence being more objective. However, regardless of the approach utilized in validation studies, it is essential to evaluate several sources of blank matrix.^{3,8} Otherwise, matrix effects caused by less frequently occurring matrix compounds may be overlooked.

Preventing matrix effects: With regard to the detrimental effects ion suppression/enhancement may have on important method performance parameters, they must be prevented wherever possible. Matrix effects that may arise from the endogenous

compounds extracted from the sample matrix can usually be eliminated in two ways. The chromatographic conditions can be optimized to separate the analyte peak from the matrix peak causing the ion suppression/enhancement, and/or sample clean-up can be improved. For example, matrix effects could be eliminated by changing the sample preparation method from direct injection or protein precipitation to solid-phase extraction.^{5,6} Sample dilution appears to be another way of reducing matrix effects.^{6,11} Ion suppression from exogenous compounds or other analytes in multi-analyte procedures usually requires modification of the chromatographic system or at least separate calibration standards for the two mutual suppressants. Chromatographic separation of analytes and their respective stable-isotope-labelled IS is not possible. However, considering the findings of Sojo *et al.*¹⁵ and of Liang *et al.*⁹ mutual suppression of analyte and stable-isotope-labelled IS should not affect quantification, as long as the concentration ratios of analyte and IS remain in a certain range.

Conclusion: Matrix effects caused by co-eluting compounds can negatively affect method performance. Therefore, the evaluation of possible matrix effects is an essential part of method development/validation for any LC-MS(-MS) method. If relevant matrix effects are found, they should be reduced or eliminated by the optimization of chromatographic conditions, improving the sample clean-up and/or by changing the type of ionization employed.

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About Frank Peters and Denise McKeown

Frank T. Peters was born in Trier, Germany. He studied pharmacy at the Johann-Wolfgang-Goethe University in Frankfurt/Main, Germany, and, after practical training at a pharmacy and at a pharmaceutical company, obtained the licence to practice as a pharmacist in 1998. Thereafter, he started working as a research assistant and PhD student of Prof. Hans H. Maurer at the Department of Experimental and Clinical Toxicology of Saarland University in Homburg, Germany, where he finished his PhD thesis in June 2003. Since then, he has been working in the same department as a post-doc fellow and as deputy of Prof. Dr. Hans H. Maurer. The main research interests of Frank are metabolism of drugs and poisons, enantioselective analysis of amphetamines and amphetamine-like designer drugs, analysis of new designer drugs in blood samples, determination of sedating drugs in the context of declaration of brain death, and experimental designs and statistical procedures for analytical method validation. Frank is author or co-author of 27 peer-reviewed publications, one book and six book chapters and he was an invited speaker at several international meetings including the IATDMCT meeting in Basel 2003 (workshop speaker) and the Hong Kong Clinical Chemistry Society joint meeting with IATDMCT in Hong Kong 2005. Besides IATDMCT, he is a member of *The International Association of Forensic Toxicologists (TIAFT)*, the *Society of Toxicological and Forensic Chemistry (GTFCh)*, and the *German Pharmaceutical Society (DPhG)*. Frank is the chairman of IATDMCT's recently established Young Scientist Committee and since 2005 also a member of TIAFT's Young Scientist Committee. In 2003, he received the TIAFT Young Scientist Award for Best Paper Published in 2002. Frank's hobbies are travelling with his wife, good food and drink, running, tending to his garden, reading, and just having a good time with friends.

Denise McKeown was introduced in the *Spotlight on New Members* column (IATDMCT News, September 2005).