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(Review Article)

Matrix Effect in Bioanalysis: An Overview

Yogesh Modhave

Principal K.M. Kundnani College of Pharmacy, Mumbai, Maharashtra, India

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ABSTRACT

LC-MS/MS can provide superior sensitivity and selectivity, rapid analysis, maximized development efficiencies, and improved turnaround times — its challenges are in large scale application. LC-MS/MS remains one of the most useful tools available for bioanalysis. A rational, strategic approach of developing robust, large-scale, and automated LC-MS/MS methods can reduce slowdowns and bottlenecks in drug development and contribute to synergistic, consistent, long term performance. Despite their enormous utility and diffusion, atmospheric pressure ionization mass spectrometry techniques are subjected to relevant drawbacks called matrix effects (ME).These effects could be summarized in matrix-dependent signal suppression or enhancement that could lead to erroneous quantitative results.Matrix effects can be reduced or eliminated by the optimization of chromatographic conditions, improving sample clean-up and/or by changing the type of ionization employed. The present article focuses mainly on matrix effects with special reference to LC-MS/MS in biological matrix.

Key Words: LC-MS/MS, Matrix effects, Quantitative analysis, Ion suppression.

INTRODUCTON

Liquid chromatography-tandem mass spectrometry (LC-MS/MS, or alternatively HPLCMS/MS) is an analytical technique that combines the physical separation capabilities of liquid chromatography (or HPLC) with the mass analysis capabilities of mass spectrometry. LC-MS/MS is a powerful technique used for many applications which has very high sensitivity and selectivity. LC-MS/MS is very commonly used in pharmacokinetic studies of pharmaceuticals and is thus the most frequently used technique in the field of bioanalysis. These studies give information about how quickly a drug will be cleared from the hepatic blood flow, and organs of the body. MS is used for this due to high sensitivity and exceptional specificity compared to UV (as long as the analyte can be suitably ionised), and short analysis time.

The major advantage MS has is the use of tandem MS-MS. The detector may be programmed to select certain ions to fragment. The process is essentially a selection technique, but is in fact more complex. The measured quantity is the sum of molecule fragments chosen by the operator. As long as there are no interferences or ion suppression, the LC separation can be quite quick. It is common now to have analysis times of 1 minute or less by MS-MS detection, compared to over 10 mins with UV detection[1, 2, 3]. Generally its application is oriented towards the specific detection and potential identification of chemicals in the presence of other chemicals (in a complex mixture). LC-MS/MS is also used in the study of proteomics where again components of a complex mixture must be detected and identified in some manner.

LC-MS/MS is frequently used in drug development at many different stages including Peptide Mapping, Glycoprotein Mapping, Natural Products Dereplication, Bioaffinity Screening, In Vivo Drug Screening, Metabolic Stability Screening, Metabolite Identification, Impurity Identification, Degradant Identification, Quantitative Bioanalysis, and Quality Control[4]. LC-MS/MS is now successfully applied to routine analysis in many areas, including therapeutic drug monitoring (TDM), clinical and forensic toxicology as well as doping control.

CONCEPT OF MATRIX AND MATRIX EFFECTS

In chemical analysis, matrix refers to the components of a sample other than the analyte. The matrix can have a considerable effect on the way the analysis is conducted and the quality of the results obtained; such effects are called matrix effects. The matrix effect may be defined as the change in HPLC-MS response of an analyte, either positive or negative, caused by coeluting matrix compounds, relative to an injection of a pure standard. For example, the ionic strength of the solution can have an effect on the activity coefficients of the analytes. The most common approach for accounting for matrix effects is to build a calibration curve using standard samples with known analyte concentration and which try to approximate the matrix of the sample as much as possible. This is especially important for solid samples where there is a strong matrix influence. In cases with complex or unknown matrices, the standard can be

used. In this technique, the response of the sample is measured and recorded, for example, using an electrode selective for the analyte. Then, a small volume of standard solution is added and the response is measured again. Ideally, the standard addition should increase the analyte concentration by a factor of 1.5 to 3, and several additions should be averaged. The volume of standard solution should be small enough to disturb the matrix as little as possible. In analytical chemistry, matrix effect may be defined as the combined effect of all components of the sample other than the analyte on the measurement of the quantity. Matrix effects have been observed for the analysis of a wide range of analytes including drugs, pesticides, and biomolecules, over many diverse matrices including blood, soft animal tissue, plant tissue, and effluent. The quantitative measure of matrix effects is the matrix factor (MF) and is defined by the following equation:

$$\text{Matrix Factor (MF)} = \frac{\text{(Peak response in presence of matrix ions)}}{\text{(Peak response in aqueous samples)}}$$

If MF=1 indicates no matrix effects
MF<1 indicates ion suppression
MF>1 indicates ion enhancement

LC-MS/MS AND MATRIX EFFECT

LC/MS/MS is a powerful analytical technique for quantitative bioanalysis due to its inherent high sensitivity and selectivity. However, the LC-MS/MS technique is not free from drawbacks. One of the main disadvantage 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. Thus LC/MS/MS is susceptible, however, to matrix effects. The impact of matrix effects on the accuracy, precision and robustness of bioanalytical methods is of growing concern in the pharmaceutical industry[5, 6.] Residual matrix components, endogenous phospholipids in particular, are a significant source of imprecision in quantitative analyses commonly conducted by LC/MS/MS. Matrix effects, originally discussed by Kebarle and Tang[6] in the early 1990s, can be described as the difference between the mass spectrometric response for an analyte in standard solution and the response for the same analyte in a biological matrix, such as plasma. Matrix effects result from co-eluting matrix components that affect the ionization of the target analyte, resulting either in ion suppression, or, in some cases, or, in some cases, ion enhancement. Matrix effects can be highly variable and can be difficult to control or predict. They are caused by numerous factors, including, but not limited to endogenous phospholipids, dosing media, formulation agents and mobile phase modifiers. Matrix effects can be compounded by co-eluting metabolites, impurities or degradation products. Furthermore, matrix effects are analyte specific. All of the above factors can cause significant errors in the accuracy and precision of bioanalytical methods. Current FDA guidance documents now require that these effects be evaluated as a part of quantitative LC/MS/MS method development, validation and routine use[7]. LC-MS/MS analysis of low purity biological samples can cause signal suppression, elevated background, and other negative matrix effects. In this situation, it is only possible to increase S/N while simultaneously maintaining high throughput performance by implementing the SRM mode. This, of course, implies a much higher cost of initial investment in sophisticated triple quadrupole instruments. An alternative approach to increasing S/N is to improve the quality of chromatography purification. This decreases matrix effects and makes possible the use of comparatively less expensive single quadrupole instruments while still achieving high analytical performance. Implementation of assays on single quadrupole mass spectrometers can be a good alternative for non-time intensive or budget limited projects.Therefore, enhancement of chromatography by implementation of two-dimensional LC methods or even off-line purification of analyte (especially from complex biological matrices) with resultant reduction of matrix effect can significantly improve the sensitivity of mass spectrometers by increasing ionization efficiency of a pure analyte.

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) [8]. 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-isotopelabelled) 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, urine, and oral fluid. 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. 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[9].

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. Sample dilution appears to be another way of reducing matrix effects. 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-labeled IS is not possible. From a regulatory point of view, method validation procedures review the following parameters: precision, recovery, specificity, linearity, limit of detection (LOD) limit of quantitation (LOQ), ruggedness, and robustness[10]. Phospholipids are extremely abundant in biological membranes and are formed from glycerol (phosphoglycerides) or sphingosine (sphingomyelins). Phosphoglycerides are composed of glycerol, one or two fatty acid ester chains, and a phosphorylated alcohol; whereas sphingomyelin is composed of sphingosine, an amide linked fatty acid, and a phosphatidyl choline. The glycerophosphocholines (GPCho's) constitute the major phospholipids in plasma[11,12] and are known to cause significant LCMS/MS matrix ionization effects in the positive ion electro spray mode. The glycerine group in these GPCho's can be either 1-mono (2-lyso) or 1, 2-disubstituted (diradyl). The 1, 2-disubstituted GPCho (phosphatidylcholine) is commonly referred to as lecithin. Matrix coextractives are compounds from the matrix that are extracted along with the analyte and are present in the final extract that is analyzed by HPLC-MS. Examples of such compounds include proteins, carbohydrates and lipids in the analysis of food, and organic matter in the analysis of soil.

CONCLUSION

It may be concluded that Matrix effects, i.e. ion suppression or ion enhancement, are well known phenomena in liquid chromatography tandem mass spectrometry (LC-MS/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 i.e. since matrix effects can strongly suppress ionization efficiency and therefore reduce sensitivity, they must be evaluated. The severity of matrix effects is directly dependent upon chromatographic performance 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. Approaches to addressing matrix effects include reducing matrix coextractives by optimizing extraction, cleanup and chromatography methods.

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****Corresponding Author:***
Yogesh Modhave,
Principal K.M.Kundnani College of Pharmacy,
Mumbai, Maharashtra, India
Email: *ysmodhave1@yahoo.co.in*