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SENSITIVITY STUDY WITH RESPECT TO MATRIX EFFECT IN BIO-ANALTICAL STUDIES -A CRITICAL REVIEW

Adiba Sahiya[1]*, Imam Pasha Syed[1], Anupama Koneru[2] and M. Mushraff Ali Khan[3]

[1]Dept. of Pharmaceutical Quality Assurance, Sultan-ul-Uloom College of Pharmacy, Mount Pleasant, #8-2-249, Road No.3, Banjara hills, Hyderabad-500034, Telangana, India.
[2]Dept. of Pharmacology, Sultan-ul-Uloom College of Pharmacy, Mount Pleasant, #8-2-249, Road No.3, Banjara hills, Hyderabad-500034, Telangana, India.
[3]Dept. of Pharmaceutics, Sultan-ul-Uloom College of Pharmacy, Mount Pleasant, #8-2-249, Road No.3, Banjara hills, Hyderabad-500034, Telangana, India.

***Corresponding Author: Adiba Sahiya**
Dept. of Pharmaceutical Quality Assurance, Sultan-ul-Uloom College of Pharmacy, Mount Pleasant, #8-2-249, Road No.3, Banjara hills, Hyderabad-500034, Telangana, India.

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ABSTRACT
In this critical review authors presented effect of matrix effect in sensitivity ad reproducibility of analytical methods for quantitative estimation of biological samples. In bio analytical method effect of different matrix can cause decrease in sensitivity and affect the assay result and reproducibility. For procedures using matrix in method development it is mandatory to evaluate and quantify matrix effect. According to regulatory guidelines, in validation determination of matrix effect is essential. Matrix effect is defined as the effect of co-eluting residual matrix component of biological sample on the ionization of target. There are two types of the matrix effect absolute matrix effect and relative matrix effect .Two types of absolute matrix effect are ion suppression and ion enhancement. Matrix effect may cause variation in sensitivity with respect to time, inaccuracy of result and chromatographic peak variation. Protocol specifications of matrix reduction should be compatible with analysis. Methods for sample clean up include liquid extraction and solid phase extraction. Matrix effect can be caused by both organic and inorganic substances. Matrix effect is often determined by post column infusion and post extraction spiking methods.

KEYWORDS: Matrix effect, Biological sample, Analysis, LC-MS/MS, Sensitivity.

1. INTRODUCTION
Blood:-Whole blood is comprised of complex matrices. Components in these matrices cause interference in determination .The major source for matrix effect is endogenous phospholipids.[4] The aim of matrix management is the removal of the blood cells and it requires centrifugation and filteration.[1]

Saliva:-Saliva analysis allows non-invasive sampling procedure. Assay for saliva is less reliable because of difficulty in management of saliva. Immunoassay of saliva is also for matrix effect, saliva also shows some suppression in the antibody-binding enzyme in immunoassay .Amount of drug is less compare to other matrices so requires stimulation of drug to obtain the volume required for analysis in method.

Urine:-Urine contains organic molecules, proteins, crystals that cause ion suppression in the LC-MS/MS leading to matrix effect. Other properties like pH, density, ionic strength and fluctuation in composition cause influence in analysis. In analysis estimation of strength of matrix should be mandatory to determine suppression coefficient and enhancement factor for matrix effect.

Sources of matrix effect[2]
Matrix effects is subdivided into 2 groups 1) endogenous matrix effects. 2) Exogenous matrix effects.

- Component of matrix like phospholipids, proteins etc.
- Component introduce during analysis like "excipients, analyte stabilizer", Etc.
- Impurities and salts present in drug and ISTDs
- Solvent and additives in LC
- Degradation result of analyte and other component.
- Xenobiotic and its metabolites present in samples.

During the bioanalytical method development and validation for lab base study, the raw materials must be evaluated for the exogenous materials that can cause matrix effect. Some matrix effect are difficult to identify and prevent during initial stage of analysis.

2. Description

Matrix (chemical analysis)

In chemicalHYPERLINK "https://en.m.wikipedia.org/wiki/Chemical_analysis" HYPERLINK "https://en.m.wikipedia.org/wiki/Chemical_analysis"analysis, matrix refers to the components of a sample other than the analyte of interest. The matrix may have profuse influence on the analysis stratagem, quality and standard of the results obtained; such effects are called matrix effects[3]. The most prevailing method for accounting matrix effects is to build a calibrationHYPERLINK "https://en.m.wikipedia.org/wiki/Calibration_curve" HYPERLINK "https://en.m.wikipedia.org/wiki/Calibration_curve"curve using standard samples of known analyte concentration and which try to imprecise the matrix effect of the sample at feasible extent. This is important for solid samples due to strong matrix influence.

Selection of appropriate methodology employs mainly 2 ways for determination of matrix effect ie qualitative and quantitative way.

Qualitative determination of matrix effect

Generally, post column infusion of analyte is applied which is fast and facile technique. In this method extracted sample matrix is injected into column, while steady flow of analyte is infused in the effluent flow between column and MS source, with it blank solution such as water or buffer must also be injected to analyze baseline for analysis. The regions for "ion suppression" can be recognized via chromatography by comparing base line obtained by blank. Concentration of the analyte injected influence the effect. If concentration injected is high, matrix effect could be concealed. Suppression region can be compared with retention time of analyte. Internal standard use is required for determination of matrix effect.

Quantitative determination of matrix effect

In Quantitative determination technique, the method describes extracting two sets of samples, in one set add analyte to extracted matrix and in other analyte in mobile phase, both sets are prepared with equal concentration of analyte. And processed them equitably, matrix effect can be determined by following equation,

Matrix effect (%) =B/A*100

Recovery (%) =C/B*100

Extraction Efficiency (%) =C/A*100

A=external solution peak area, B=post-extraction sample peak area, C=extracted matrix peak area.

Different approach to minimize matrix effect[4]

For minimizing matrix effect accurate and appropriate sample preparation technique is determined by molecular analysis and specimen type. In sample preparation separation of soluble component from insoluble component is achieved. Depending on type of matrix, the method for detection and characteristic of analyte is used. Technique for system like simple dilution is sufficient to minimize matrix effect and systematically isolation and extraction are also used. Protein precipitation is an active and nonspecific method for sample clean-up. Supplementary method used are LLE and SPE. Liquid-liquid extraction (LLE) deploys partitioning of an analyte into two separate liquids. Differential solubility of an analyte in two immiscible liquids is the precedence of this technique. One phase is water or a buffer, while the other is an organic solvent as toluene. Organic solvent is selected in accordance to obtain maximum recovery of analyte and is influenced based on the analytes solubility in the particular solvent. Yet, analyte recovery using LLE methods strikingly subsided compared to cation exchange SPE with basified methanol. Hence, when used basified MTBE with a two-step extraction procedure, both the cleanliness and analyte recovery (average of 87%) increased (Chambers et al., 2007). Despite LLE provides chaste extracts and chaste analyte recovery, it is more labor meticulous. Solid phase extraction (SPE) method reckon on the affinity of analyte for a stationary phase and are often used to sequester analyte(s) of interest from a outstretched range of matrices including urine, blood, etc. Depending on the properties of the analyte and the solid phase, either the analyte of interest is retained while the unwanted matrix components elute with the solvent wash or vice versa. If analyte is retained then subsequently eluted with different solvent. The obtainable SPE stationary phases available, including normal phase, reversed phase, and ion exchange (Chambers et al., 2007; Supelco, 1998). Further, more solid supports such as mixed-mode resins, and zirconium coated particles for phospholipid removal are specialized one. To effectuate the optimal sample clean-up and analyte recovery, calibrate optimum proportions of stationary phase and eluent. SPE prerequisite extensive method development as conditions depend upon physicochemical properties of analytes and matrix components in the sample. SPE is widely used for clinical sample analysis rather than for high-throughput analysis due to diverse compounds being encountered in drug discovery. Chromatographic condition optimization is widely use for the reduction of matrix effects. Almost invariably, ion-suppression is caused by the co-elution of the matrix components with the analyte of interest.[5]

Therefore, fewer matrix effects are likely to be triggered with heightened chromatographic separation between the analyte and matrix components. Modification of chromatographic parameters results in optimization of chromatography like, such as initial and final eluent strength and gradient duration, then the samples are monitored through both LC-UV and LC- MS/MS, and found that signal suppression was likely caused by the co-elution of the matrix components with the analytes. To reduce the ion suppression, techniques applied include: 1) L Linear gradient duration extension. 2) Initial mobile phase organic content is lowered. 3) Final mobile phase composition adjustment. Matrix effects

were greatly reduced as chromatographic peaks of analytes were completely resolved from matrix components. UPLC due to its excellence in speed, sensitivity and resolution of analyte encounters fewer matrix effects and affords a more robust analytical method than HPLC. Under acidic conditions, basic compounds may encounter matrix effects from salts and highly polar, poorly retained matrix components. Conversely, at basic pH, basic compounds stay neutral, are better retained, elute with high organic content mobile phase, and generate stronger MS signals. ESI and APCI are generally used in LCMS/MS.

3. Regulatory view

According to guidelines in validation of LC MS/MS it provides guidance for determining the source of the discrepancy in the data, though the parameters lead to a robust validated method, there are certain situations where matrix effects may persist. In such cases, sample reanalysis generally leads to widely different results from original one which are often unique to a particular patient's sample(s). Thus a universal LC-MS/MS method will not necessarily remunerate matrix effects interfering with analyte quantitation in clinical trial samples due to their distinctive composition. Further, method validation across patients' samples is impractical due to this inherent uniqueness and hence are handled on a case by case basis. Typically, as specified by Standard Operating Procedures (SOPs) that are written to handle such situations[6] an investigation is conducted and documented.Challenge to the analytical chemist is the unknown mechanism of ion suppression. Sample preparation methods have been used for preparation by using SPE and LLE method.[7]

4. Selectivity, Recovery and Matrix Effects

The selectivity is sophisticated by the absence of interference-free matrix. In Chromatography, peak purity is a part of method validation by analyzing matrices obtained from several donors using detection system (e.g., tandem mass spectrometry (MS/MS)).

For the Standard Addition and Background Subtraction Approaches, as the same biological matrix and analyte are used for study samples and calibration standards, the same recovery and matrix effect occurring. For the Surrogate Matrix and Surrogate Analyte Approaches, the matrix effect and the extraction recovery may differ between calibration standards and study samples.

If the Surrogate Matrix Approach is used, demonstration of similar matrix effect and extraction recovery in both the surrogate and original matrix and if surrogate analyte approach is used then demonstration between the authentic endogenous analytes and surrogate is required. This should be investigated in an experiment using QCs spiked with analyte in the matrix against the surrogate calibration curve and should be within 15%. In the Standard Addition Approach, each sample is analyzed with its own calibration curve.[8]

5. Evaluation of matrix effect and chromatography efficiency: New parameters for validation of method development:

Matrix effects are method validated only if they influence reproducibility or assay linearity or strongly cause ion suppression reducing sensitivity, hence they is a need to evaluate prior to method qualification. This information becomes essential for method assessment, optimization and transfer to other mass spectrometers, and a part of routine LC/MS method validation.

A single quadruple instrument is used that provides higher analyte signal in SIM mode compared to MRM, where signal is limited by fragmentation and transmission efficiencies for routine analysis. We increase S/N while maintaining high throughput performance by implementing the SRM mode for analysis of LC/MS in case of low purity biological samples as they cause signal suppression and negative matrix effect but the method implies expensive cost of initial investment in sophisticated triple quadruple instruments. Decreasing matrix effects and use of comparatively less expensive single quadruple-pole instruments and accomplishing high analytical performance is done by improving quality of chromatography purification. Enhancement of chromatography by implementation of two-dimensional liquid chromatography methods or even off-line purification of analyte with reduction of matrix effect can improve the sensitivity of mass spectrometers by increasing ionization efficiency of a purer analyte. From the viewpoint of method development and optimization, information about the strength of matrix effects and chromatographic efficiency is important if the goal is to achieve optimum sensitivity. According to regulatory viewpoint, method validation review parameters like: precision, recovery, specificity, linearity, limit of detection (LOD) and limit of quantitation (LOQ), ruggedness, and robustness. Critical technical aspect of MS based assay development is estimation of matrix effect strength. The gain in sensitivity attributable to chromatographic efficiency might be evaluated as the S/N ratio of a real sample injected onto the column (LC/MS) Compared to the S/N injected directly to source (FIA) but FIA method is employed only if the sample is pure. A more precise approach is to compare the relative response of analyte spiked into an actual blank sample compared to spike into pure solvent, then analyzed by Q1 SIM. Pure analyte can be added to pure solvent to the same concentration if analyte concentration in complex is known. Another way to estimate effect is to compare an isotope labelled internal standard spiked into the actual sample to being spiked into pure solvent. If ionization efficiency and back-ground are similar for both the analyte and internal standard then the above is applied. If the ratio of these two S/Ns is close to 1, the matrix effect for the assayed analyte is minimal and indicates that the LC part of LC/MS method is optimized and maximum and even theoretical S/N for this particular instrumental configuration has been achieved.

Suppression coefficientvalue, actually depends upon two factors: initial sample matrix complexity and chromatographic separation efficiency. Defining the matrix suppression coefficient relative to the measured sensitivity of tandem mass spectrometry involves considerations. This value is actually composed of the superposition of two factors: (1) efficiency of matrix effect reduction by LC purification and (2) MS/MS enhancement. Evaluation of the degree of S/N enhancement of an MS/MS method requires separate Q3 MRM (SRM)and (its precursor) Q1 SIM tests of the same sample in addition to measurements described above of the S/N ratios of pure standard in solvent compared to standard in a complex matrix sample at Q1 SIM mode. The ratio between S/N for the same complex sample measured at Q3 MRM (SRM) and (precursor) Q1 SIM tests is a criterion of overall MS/MS enhancement. Mass spectrometers, including those relying on MS/MS for isolation of analytes from contaminants in the sample can be done by minimizing matrix effect and increasing the sample purification and sensitivity of spectrometers. Critical information about the quality of LC/MS method development is evaluation of matrix effect and chromatography/mass spectrometry efficiency and further optimization. LC/MS method development must include the estimation of matrix effect strength and tandem enhancement factor. Even the two parameters the suppression coefficient and enhancement factor are considered that inherent to each LC/MS method, as for optimization they are quality control variables. Knowledge of the suppression coefficient and enhancement factor will facilitate subsequent assay validation.[9-12]

6. Investigation of matrix effects in bioanalytical high-performance liquid chromatography/tandem mass spectrometric assays

Effect of matrix in bioanalytical high-performance liquid chromatography/tandem mass spectrometry (HPLC/MS/MS) assays is studied. Exemplification of studies report: (1) Exogenous materials, such as polymers example plastic tubes etc. can cause matrix effect; (2) matrix effects are both ionization mode (APCI or ESI) and source design (Scitex, Finnegan, Micro mass) dependent; and (3) for at least one vendor's design, we found the APCI mode to be more sensitive to matrix effects than the ESI mode. Simple strategies to avoid matrix effects: (1) select the same brand of plastic tubes for processing and storing plasma samples and spiked plasma standards; and (2) try switching the ionization mode or switching to different mass spectrometers when matrix effects are encountered. These two strategies have allowed us to use protein precipitation and generic fast LC techniques to generate reliable LC/MS/MS data for the support of pharmacokinetic studies at the early drug discovery stage.[17-20]

Study of matrix effects in laser plasma spectroscopy by shock wave propagation.

Exploration studies in laser plasma spectroscopy are engrossed on matrix effects in sand/soil mixtures and attempted to explain the increase in the spectral response of trace elements (at constant concentration) with sand percentage. Matrix effect is characterized by propagation of laser induced shock wave caused due to the energy coupled in plasma. Hence main matrix effects were attributed to the depth of the laser-induced crater, which was correlated to a portion of the laser energy that penetrates into sand particulates and does not cause direct ablation.[21-25]

Matrix effect (for LCMS-MS)

Matrix effect is done in LCMS-MS to comprehend ion suppression or enhancement effect in interest of the matrix. The prevailing method of choice for the quantitative determination of drug in biological matrix is chromatography accompanying with atmospheric pressure ionization tandem mass spectrometry. This technique comprise high specificity and sensitivity. However, chromatogram fails to observe the matrix components possessing effect on analysis.[26-27]

8. CONCLUSION

Proper Management of matrix effect for sensitive and reliable bioanalytical method. Focusing on different matrices with its complication while using and advantage of using urine, saliva and blood for bioanalytical method. Majorly focused on causes of matrix effect and method for minimize it.

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