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# Review Article on Matrix Effect in Bioanalytical Method Development

**Ganda V*, Patel R**

**Depertment of Quality Assurance, Parul Institute of Pharmacy, Vadodara, Gujarat, India**

**Abstract :** In bioanalytical method effect of different matrix can cause decrease in sensitivity and affect the assay result and reproducibility.matrix effect and selectivity issues have long been associated with bioanalytical technique.main reason for matrix effect is ion suppression.it is not always possible to remove matrix effect but in those cases it is mandatory to evaluate and quantify matrix effect.it is important to give attention while using matrix during method development.to minimize effect of matrix many method like dilution,reducing iinjection volume,stable isotop labeled internal standard,and others.matrix effect removal is important in automation system.for the viewpoint of regulatory guidelines,in validation determination of matrix effect is essential.the severity of matrix effect is directly dependent on chromatographic performance.it is essential information for method assessment.

**Key Words:** Matrix effect, Process for quantification, Determination of matrix effect

## Introduction[1]:

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 e.g. absolute matrix effect and relative matrix effect. Absolute matrix effect is defined as difference between response of equally concentrated analyte in solvent and in matrix extracts. two types of absolute matrix effect one is ion suppression which is more often observed and other is ion enhancement. Relative matrix effect is defined as the variation of absolute matrix effect between several lots of same matrix.[1]

Matrix effect may cause decrease or increase in sensitivity over the time, increased baseline,imprecision of result,retention time change and chromatographic peak tailing.[2]

Some sample preparation procedures have been of tremendous significance in reducing matrix effect.as demand for assay sensitivity rises because of evaluation increase in potent drug and which can be detected in low concentration.[2]

Selection of sample preparation to minimize matrix effect is heavily determined by molecular analysis and specimen type.Volume of reagent, flow rate, and various protocol parameters of matrix reduction should be compatible with analysis[3]

In the number of cases,matrix components which might cause ionization enhancement or suppression are removed during sample cleaning process. Many method used for sample clean up most simple method is protein precipitation method. Other method used is liquid liquid extraction and solid phase extraction method.

Matrix effect can be caused by both organic and inorganic substances present, amines, urea, carbohydrates etc.

Other possible theory about matrix effect is interfering compounds increase the droplet's viscosity and surface tension, thereby decreasing solvent evaporation rate.so less amount of analyte reach to the gas phase.

Sometime non volatile material in the matrix can also decreases the rate of droplet formation so coprecipitation of analyte and therefore prevent the droplet to reach gas phase ions.

Matrix effect are often determined by postcolumn infusion and postextraction spiking methods.

**Challenge in selection of biological matrices**

Generally in bioanalytical method urine,blood, and saliva used as a biological matrices,each matrices have its own property of reaction.

**Blood**

Whole blood is a complex matrices which contains number of components.many components present in matrices cause interference in the determination.endogenous phospholipids have been identified as a major source for matrix effect.[4]

Serum protein and anticoagulant which is used in blood,has been found source for the matrix effect.

Main aim of matrix management is the removal of the blood cells and hemolysed material is necessory in analysis.it requires centrifugation and filteration.[5,6]

**Saliva**

Saliva is easy for analysis it aloows noninvesive sampling procedure.assay for salive is used from long time.[7]

Result of saliva assay is less reliable because of its difficulty in management of saliva.immunoassay of saliva is also for matrix effect,saliva also shows some supression in the antibody-binding enzymein immunoassay.[8]

Amount of drug is less compare to other matrices so requires stimulation of drug to obtaiin volume is required for analysis in mathod.[9]

**Urine**

Management of urine marices is difficult due to present in many component.urine contains organic molecules,proteins,crystals,cells.many of this molecules causse ion supression in the LC-MS/MS which leads to matrix effect.[4]

Different in variation in composition in urine cause different in the analysis.and other properties of urine pH,density,viscosity,ionic strength cause effect in analysis**.**fluctuation in composition cause influence in analysis.

At first step of analysis estimation of strength of matrix shold be mandatory to determine.supression coefficient and enhancement factor are two parameter for matrixeffect.[1]

**Sources of matrix effect[11,12]**

Matrix effect are subdivided into 2 groups 1)endogenous matrix effects caused by compounds naturally occuring in sample. 2)exogenous matrix effects caused by component introduced during analysis.

1. Component of matrix like lipids,phospholipids,proteins etc.
2. Component introduce during analysis like excipients,anticoagulant,analyte stabilizer,reagent used. Etc.
3. Impurities and salts present in drug and ISTDs.
4. Solvent and additives in LC.
5. Degradation product of analyte and other component.
6. Xenobiotic and its metabolites present in samples.

During the bioanalytical method development and validation for lab base study many exogenous materials effects which should be carefully evaluated of the reagents and supplies used for sample preparation.

Some matrix effect are dificult to identify and prevent during early stage of analysis.

**Detection of matrix effect[13]**

Several methods available for detection and quantification of matrix effect.but selection of appropriate methodology employed typically depends on method which used mainly 2 ways of determination qualitative and quanitative way for determination.

**Qualitative determination of matrix effect**

Generally,post column infusion of analyte is used.it is fast and easy technique for determination of matrix effect.

In this method extracted sample matrix is injected into column using the method,while steady flow of analyte is infused in the effluent flow between column and MS source,with it blank solution such as water,buffer must also be injected to analyze baseline for analysis.so reagion for ion suppresion can be seen in the chromatography by comparing base line obtained by blank.

Effect depends on the concentration of the analyte injected.concentration injected is high,matrix effect could be masked.supression 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 this method,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 identically,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[17-24]**

**Various methods used for minimizing matrix effect are**

**sample preparation technique[17-20]**

Accurate and appropriate sample preparation technique helps in minimizing matrix effect.different methods like optical,magnetic,mechanical,electrical,have been demonstrated to be effective.

Depending on type of matrix,method for detection,and charecteristic of analyte is used.for different system different technique used like for some system simple dilution is sufficient for minimize matrix effect.

Regularly isolation and extraction are used for removal of matrix effect.in sample preparation seperation of soluble component from insoluble component is achieved.for that seperation is used.

Protein precipitation is rapid and non specific method used for sample cleanup.other method used is LLE and SPE.

Liquid-liquid extraction (LLE) is based on the partitioning of an analyte into two separate liquids. The technique works by taking advantage of the differential solubility of an analyte in two immiscible liquids. One of the phases usually is water or a buffer solution, while the other is an organic solvent such as toluene, diethyl ether, hexane, dichloromethane, or MTBE. Selection of the proper organic solvent to obtain maximum recovery should be based on the analyte's solubility in the particular solvent. Chambers et al. compared LLE methods to several SPE and PPT methods in terms of each technique's overall cleanliness, matrix effects, and analyte

recovery. They found that using MTBE and basified MTBE with a single extraction technique resulted in clean extracts that were similar to those obtained from cation exchange SPE and were better than PPT. However, analyte recovery using these two LLE methods significantly decreased compared to cation exchange SPE with basified methanol - average % recovery values for MTBE LLE, basified MTBE LLE, and cation exchange SPE with basified methanol were 43%, 38%, and 94%, respectively. Yet, when they used basified MTBE with a two step extraction procedure, both the cleanliness and analyte recovery (average of 87%) increased (Chambers et al., 2007). While LLE provides clean extracts and decent analyte recovery, it is much more labor intensive than PPT. Furthermore, as evidenced by the Chambers' investigation, multiple extractions may be necessary to obtain a sufficient quantity of analyte, decreasing overall efficiency that is essential for high-throughput sample clean-up and analysis in early drug discovery.

Solid phase extraction (SPE) methods rely on the affinity of an analyte for a stationary phase and are often used to isolate analyte(s) of interest from a wide range of matrices including urine, blood, tissue homogenates, 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 the unwanted matrix components are retained and the analyte elutes with the solvent wash. In the first case, the retained analyte is subsequently eluted with a different solvent. There are numerous SPE stationary phases available, including normal phase, reversed phase, and ion exchange (Chambers et al., 2007; Supelco, 1998). In addition, more specialized solid supports such as HILIC, mixed-mode resins, and zirconium coated particles for phospholipid removal are also commercially available. Table 5 shows typical analyte, matrix, and stationary phase/sorbent examples. The stationary phase and eluent can be adjusted to achieve the optimal sample clean-up and analyte recovery. For example, extraction of primary, secondary, and tertiary amines from biological fluids would be best accomplished by using strong cation exchange (SCX stationary phase); whereas extraction of large, hydrophobic molecules from biological matrices or water should be performed via reversed phase SPE with a C18-T (wide pore) stationary phase (Phenomenex, 2009). Optimal SPE conditions depend upon physicochemical properties of analytes and matrix components in the samples and require extensive method development. Therefore, SPE is less useful for a high-throughput analysis of a diverse set of compounds encountered in the early stages of the drug discovery but is widely used for clinical sample analysis. A more thorough discussion on LLE and SPE can be found elsewhere.

**Chromatographic condition optimization**[21-22]

Widely use for the reduction of matrix effects is the optimization of chromatographic conditions. In most cases, ion-suppression is caused by the co-elution of the matrix components with the analyte of interest. Therefore, with increased chromatographic separation between the analyte and the matrix components, fewer matrix effects are likely to be determined.

Optimization of chromatography involves modification of chromatographic parameters, such as initial and final eluent strength and gradient duration,. They then monitored the samples 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 ionization suppression, several strategies were successfully applied: 1) extension of the linear gradient duration. 2) lowering of the initial mobile phase organic content. 3) adjustment of the final mobile phase composition. The chromatographic peaks of the analytes were almost completely resolved from the matrix components and the matrix effects were greatly reduced .

Ultra Performance Liquid Chromatography (UPLC) provides advantages over traditional HPLC in speed, sensitivity, and resolution of analytes. When analyzing nine different drugs in three surface water samples, Van De Steene and Lambert observed severe matrix effects using HPLC. After implementing analogue ISTDs, significant matrix effects were still observed. However, through UPLC implementation, these matrix effects were significantly reduced, and accurate quantitation of all nine compounds using analogue ISTDs became feasible. With improved sensitivity and resolution, , UPLC generally encounters fewer matrix effects and affords a more robust analytical method than HPLC.

Mobile phase pH can influence the retention times of ionizable analytes (basic or acidic) by changing the ionization equilibrium. Under HPLC conditions with an acidified mobile phase, basic compounds are present as charged species. As a result, they are poorly retained on the column and elute early with a highly aqueous mobile phase. 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.several studies found that APCI to be less susceptible to matrix effect.however some matrixeffect persist while using APCI.

In ESI flow rate found to be influence the degree of ion supression.decrease in matrix effect as decrease in flow rate.

**Calibration with internal standards**[23-24]

For correcting matrix effect use appropriate internal standard,internal standard and analyte need to co-eluted to ensure that they analyzed in same condition.stable isotop labelled internal standard used.it believed that isotop labelled ISTD decreases matrix effect.

For an ISTD to effectively compensate for matrix effects it should have a retention time similar to the analyte of interest. The analyte response can be normalized to the ISTD peak simply by dividing the analyte peak area by the ISTD peak area. Since the ISTD concentration is equal across all of the samples, comparing the analyte's peak area to the ISTD's peak area serves to normalize the data and compensate for signal response variability caused by matrix effects. Ideally, a stable-isotope-labeled analyte analog can be used as an ISTD. Since this SIL-ISTD would have nearly identical chemical and structural properties as the analyte of interest, the two compounds should behave similarly during sample preparation and LC-MS analysis

Careful assesment should be made during method development ,SIL internal standard is costly,because for each analyte internal standard required.

In ligand binding assay buffer composition,pH and ionic strength are critical in reducing interference.

Displacing agent are used sometime to minimize matrix effect of binding between serum protein and analyte.

Adequate washing is important in removing non specifically adsorbed component in surface,insufficient washing leads to high contamination.

**Regulatory view**

According to guidelines in validation of LCMS/MS provides guidence for determine the source of the discrepancy in the data. Although the parameters described above almost always 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 what was originally reported. These cases are frequently associated with unknown matrix effects, which are often unique to a particular patient's sample(s). As mentioned previously, clinical trial samples are distinctive in their composition, thus a universal LC-MS/MS method will not always remove or compensate for the components that may interfere with analyte quantitation. Further, method validation across different patients' samples is impractical due to this inherent uniqueness. These situations, when they occur, are handled on a case by case basis. Typically, an investigation into the discrepancy is conducted and documented and the reporting of the data is specified by Standard Operating Procedures (SOPs) that are written to handle such situations.

## Conclusion

Proper management of matrix effect is essential for sensitive and reliable bioanalytical method.this review article focused on different matrices used and its complication while using it and advantage of using urine,saliva and blood for bioanalytical method.main causes of matrix effect and different method for minimize matrix effect.

The mechanisms by which matrix components cause ionization suppression (or enhancement) are still not well-understood. This serves as a testament to the challenge they provide to the analytical chemist. In the years since the first published articles describing matrix effects, analytical chemists have recognized the importance of understanding and mitigating matrix effects.

Sample preparation method have been used for prepartion by using SPE and LLE method.different steps taken for minimize matrix effect are taken.

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