**INTRODUCTION:**

Liquid chromatography/tandem mass spectrometry (LC-MS/MS) with electrospray ionization is a highly specific and sensitive analytical technique that has become the industry standard for quantifying drugs, metabolites, and endogenous compounds in biological matrices. The technique is widely used because of its ability to accurately quantitate analytes of interest with minimal sample clean-up and rapid LC separation. Despite these advantages, LC-MS/MS methodology occasionally encounters problems, some of which are caused by matrix effects. The “matrix” refers to all components in the sample other than analyte(s) of interest (Terence G et al). Matrix effects are defined as “interference from matrix components that are unrelated to the analyte”.

Matrix effects can arise from a number of matrix components including, but not limited to:

- Endogenous biological components such as phospholipids, carbohydrates, and endogenous metabolites.
- Residual formulation components used in the preparation of formulations
- An interaction between the analyte of interest and the matrix, such as covalent binding to plasma proteins or the enzymatic degradation of a prodrug
- Co-eluting drug metabolites
- Concomitant medications
- Mobile phase additives

Matrix effect affects the reproducibility, sensitivity and reliability of the analytical techniques.

Apart from the much spoken endogenous components causing matrix effects, exogenous components (formulation vehicles) used in the preparation of formulations were also concern as they could potentially cause suppression or enhancement of the analyte and internal standard which in turn will have impact on the accuracy of measured concentrations. A lot variety of formulation vehicles ranging from cosolvents, complexing agents, lipid based vehicles and surfactants will be used in the preparation of formulations at preclinical level.
A review of commercially available oral and injectable solution formulations presented by Robert Strickley revealed that the solubilizing excipients used in preclinical formulations include **water soluble organic solvents** (polyethylene glycol 300, polyethylene glycol 400, ethanol, propylene glycol, glycerin, N - methyl - 2 - pyrrolidone, dimethylacetamide, and dimethylsulfoxide), **nonionic surfactants** (Cremophor EL, Cremophor RH 40, Cremophor RH 60, d - α - tocopherol polyethylene glycol 1000 succinate, polysorbate 20, polysorbate 80, Solutol HS 15, sorbitan monooleate, poloxamer 407, Labrafi l 1 M - 1944CS, Labrafi l 1 M - 2125CS, Labrasol, Gellucire 44/14, Softigen 767, and mono - and di - fatty acid esters of PEG 300, 400, or 1750, vitamin E TPGS), **water insoluble lipids** (castor oil, corn oil, cottonseed oil, olive oil, peanut oil, peppermint oil, safflower oil, sesame oil, soybean oil, hydrogenated vegetable oils, hydrogenated soybean oil, and medium - chain triglycerides of coconut oil and palm seed oil), **organic liquids/semisolids** (beeswax, d - α - tocopherol, oleic acid, medium - chain mono - and diglycerides), **various cyclodextrins** ( α - cyclodextrin, β - cyclodextrin, hydroxypropyl - β - cyclodextrin, and sulfobutylether -β - cyclodextrin), and **phospholipids** (hydrogenated soy phosphatidylcholine, distearoylphosphatidyglycerol, 1 - α - dimyristoylphosphatidylcholine, 1 -α - dimyristoylphosphatidyglycerol).

The chemical techniques to solubilize water - insoluble drugs for oral and intravenous administration include pH adjustment, cosolvents, complexation, microemulsions, self - emulsifying drug delivery systems, micelles, liposomes, and emulsions.

The exact mechanisms by which matrix components cause matrix effects are not known. Matrix effects arise at the interface between the LC system and the MS system (King et al., 2000)

Various mechanisms by which the matrix components cause matrix effects are as follows:

- Charge competition between analyte and matrix components (Bennett and Liang, 2004; Chambers et al., 2007)
- Change in droplet surface tension leading to formation of large droplets and insufficient desolvation (Bonfiglio et al., 1999; King et al., 2000)
- Preferential ion evaporation due to matrix components gathering at droplet surface
- Change in mass of analyte ion due to ion pairing and adduct formation
- Co precipitation with nonvolatile matrix components (van Hout et al., 2003)
- Gas phase deprotonation

**Electrospray Ionisation (ESI)** is more prone to matrix effects than the other sources. In the ESI source, analytes must acquire a charge in solution and then successfully transition to gas phase while maintaining their charge. The acquisition of charge in the solution phase and successful transitioning to the gas phase makes the ESI source the most vulnerable to matrix effects when compared to either APCI or APPI (Jessome and Volmer, 2006; King et al., 2000; Trufelli et al., 2011).

**Atmospheric pressure chemical ionization (APCI)** is an alternate method of analyte ionization to ESI. Although less susceptible to matrix effects, APCI can have significantly lower ionization efficiencies for some analytes. In such cases, the loss of analyte signal sensitivity must be weighed against the reduction of matrix effects (Trufelli et al., 2011). APCI, unlike ESI, does not rely on solution phase analyte charging; therefore many of the matrix effects due to droplet formation and phase transformation are eliminated. Instead, desolvation of the analyte occurs almost instantly in a heated ceramic vaporizing collar at the inlet from the LC. However, the high temperature required for desolvation makes APCI unsuitable for the quantitative analysis of thermally labile molecules. APCI is immune to matrix effects happening in the solution phase but prone to matrix effects happening in gas phase. (Gosetti et al., 2010)

**Atmospheric pressure photo ionization (APPI)** is a recently introduced ionization source that achieves ionization by channeling the uncharged gas phase sample molecules through a charged photon beam. APPI has been demonstrated to be less susceptible to matrix effects than ESI and APCI. APPI, like APCI, ionizes the analyte in the gas phase, eliminating potential issues that arise from solvent phase ionization. Additionally, APPI produces higher energy protons that can overcome potential charge competition between the analyte and solvent or extraneous materials, which sometimes occurs in APCI sources (Gosetti et al., 2010).

In general, the consensus is that APCI and APPI systems are less prone to suffer from matrix effects than ESI; however, each method has its advantages and disadvantages (King et al., 2000). In certain matrices and for certain analytes, no significant improvement over ESI
sources was observed, emphasizing that matrix effects should be assessed on a compound by-compound and matrix-by-matrix basis (Lien et al., 2009).

Although it may be possible to resolve an observed matrix effect by changing sources, this is not always an option. For example, analytes often exhibit different ionization efficiencies depending on the mode of ionization. Moreover, changing ion sources does not guarantee elimination of matrix effects. A better option is to understand the specific cause of the matrix effect and then address it directly.

**Sample preparation techniques:**

**Protein precipitation (PPT)** is a rapid, nonspecific method that can be utilized for sample clean-up in a high-throughput, automated manner. PPT-based purification relies on reduced solubility of proteins and highly polar matrix components in aqueous-organic solvent solutions. Acetonitrile, methanol or acetonitrile-methanol mixtures are most commonly used for PPT. More than 90% of plasma proteins can be removed from samples using PPT when the plasma to organic solvent ratio is at least 1 to 2.5 (Chambers et al., 2007; Polson et al., 2003). Unfortunately, many matrix components such as lipids, formulation agents, and other substances remain in the supernatant following centrifugation. These components often cause matrix effects, leading to increased variability between samples.

Alternative sample preparation techniques include LLE and SPE. However these extraction methods are much labor intensive and are not typically used in early drug discovery.

**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 or biological matrix, while the other is an organic solvent. 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., 2007). LLE alone as an extraction technique might not be suitable enough for getting rid of the diversified matrix effects as some of the matrix components are even soluble in the organic solvents used for extraction.

**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. There are numerous SPE stationary phases available, including normal phase, reversed phase, and ion exchange (Chambers et al., 2007; Supelco, 1998). In some cases, where the physicochemical properties of the test article and formulation vehicle are similar, SPE might not be a good extraction technique as the final extracts will have both test articles of interest and matrix effect causing components. 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 (Terence G. Hall et al).

Finally it can be concluded saying that not a single extraction technique will be helpful for eliminating the matrix effects caused by formulation vehicles. Rather than working on extraction techniques directly, a better understanding of the formulation vehicles used, their physicochemical properties, their biological concentrations after dosing, better extraction techniques, their chromatographic retention should be studied first and probable solution to be worked out.