Literature Review:

- **Thomas M. Annesley (2007)** had tested the effect of methanol from 9 different sources for ionisation suppression on immunosuppressants. The decrease in signal for the immunosuppressant drugs was shown to result from differential ionization associated with the selected methanols. Product ion intensity varied by 10-fold among the methanols tested. For sirolimus, tacrolimus, and mycophenolic acid, the percentage change in ionization was the same for the drug and its corresponding internal standard. Post column sirolimus infusion evaluation revealed that a 1000-fold analyte concentration difference did not affect ionization. The proportions of ammonium, sodium, and potassium adducts of sirolimus precursor ions differed in relation to the source of methanol. Organic solvents used in mobile phases and extract preparation of biological samples may be associated with ion suppression, affecting adduct formation and assay sensitivity.

- **Craig Aurand et al., (2009)** had evaluated the performance of recently developed sample prep Hybrid SPE precipitation for the purpose of matrix removal and analyte recovery from spiked samples. His findings were that by using Hybrid SPE PPT platform will be helpful for the total removal of phospholipids. Chromatographic run times can be brought down to a few minutes, instead of longer run times which in turn increase the throughput of analysis. Good recoveries were obtained by using this precipitation technique.

- **Tom Benjits et al., (2004)** had evaluated the matrix effects of environmental water samples on 35 Endocrine Disrupting Chemicals (EDCs) in negative and positive ESI LC-MS/MS. It was shown that mobile-phase additives could significantly influence matrix effects. Addition of acids resulted in a severe signal suppression (average ME%: <65%), and 1mM ammonium formate increased the average ME% to 84%. The importance of an efficient sample clean-up and internal standardization also was demonstrated. Cleaner extracts resulted in reduced matrix effects (average ME%: 89%) and labeled internal standards proved to have a beneficial effect especially on signal reproducibility (average CV% 4.2% versus 2.6%). The results from the present work indicate that evaluation of matrix effects should become an integrated part of quantitative LC–ESI-MS/MS method development and validation.
Elizabeth J. Want et al., (2006) had used cerium modified column and LLE as extraction procedure to remove phospholipids from serum and obtain a more comprehensive metabolite profile. XCMS, an in-house developed data analysis software platform, showed that the intensity of existing endogenous metabolites increased, and that new metabolites were observed. This application of phospholipids capture in combination with XCMS non-linear data processing has enormous potential in metabolite profiling, for biomarker detection and quantitation.

Bennett P et al., (2004) had worked on selective extractions in quantitative LC-MS/MS for removing phospholipids and reduce the matrix effects. A major source of matrix effects in positive ESI mode was due to the phospholipids. Acidic pH organic LLE obtained higher removal of phospholipids than at other pHs. SPE types tested (optimized for analyte recovery) resulted in significantly more phospholipids than LLE. LLE method with MTBE, hexane or a mixture of these two at pH 2.0 has removed 200 times more phospholipids than commonly used SPE.

Tianyi Zhang et al., (2004) stated that phospholipids are ionized efficiently under positive ion mode due to the presence of a quaternary nitrogen atom. However, phospholipids also generate negative ions through a demethylation product. This can result from the formation of a cluster with a corresponding counter ion such as acetate or chloride. The cluster ions can be activated to remove a methyl group as well as the positive charge from the choline residue. The negative ionization of phospholipids is less pronounced compared to positive ion mode. However, because of the ionic nature, the abundance, and the hydrophobicity of phospholipids in biological matrix, it makes them logical candidates to influence ionization in negative ion mode electrospray MS sources.

Bonfiglio et al., (1999) had tested Methyl-t-butyl ether (MTBE) liquid-liquid, Oasis and Empore solid-phase, and acetonitrile (ACN) protein precipitation sample preparation methods using the post-column infusion system. In all cases, ACN protein precipitation samples showed the greatest amount of ESI response suppression while liquid-liquid extracts demonstrated the least. In addition, the three test compounds, phenacetin, caffeine, and a representative Merck compound, demonstrated that ESI response suppression is compound dependent. Suppression
was greatest with caffeine, the most polar analyte, and the smallest for the Merck compound, the least polar analyte.

- **Chambers E et al., (2007)** had made comparisons among various sample preparation methods including protein precipitation (PPT), liquid-liquid extraction (LLE), pure cation exchange solid-phase extraction (SPE), reversed-phase SPE and mixed-mode SPE. The combination of polymeric mixed-mode SPE, the appropriate mobile phase pH and UPLC technology provides significant advantages for reducing matrix effects resulting from plasma matrix components and in improving the ruggedness and sensitivity of bioanalytical methods.

- **Muller C et al., (2002)** had studied ion suppression effects during electrospray-ionisation mass spectrometry (ESI-MS) caused by different sample preparation procedures for serum was investigated. With continuous post column infusion of two test compounds-codeine and glafenine-the ion suppression effects of extracted biological matrix obtained after a standard liquid-liquid extraction, a mixed-mode solid-phase extraction (SPE) method, a protein precipitation method and a combination of precipitation with polymer-based mixed-mode SPE have been investigated. It could be demonstrated, that ion suppression is not generally present at any retention time when using reversed-phase HPLC with rather long gradient programs, but may play an important role in case of high-throughput LC-MS analysis, when the analyte is not separated from the LC-front, or in flow injection analysis without chromatographic separation.

- **Riet Dams et al., (2003)** had evaluated the synergistic effect of ionization type, sample preparation technique, and bio-fluid on the presence of matrix effect in quantitative liquid chromatography (LC)-MS/MS analysis of illicit drugs by post-column infusion experiments with morphine (10-µg/mL solution). Three bio-fluids (urine, oral fluid, and plasma) were pretreated with four sample preparation procedures [direct injection, dilution, protein precipitation, solid-phase extraction (SPE)] and analyzed by both LC-electrospray ionization (ESI)-MS/MS and LC-atmospheric pressure chemical ionization (APCI)-MS/MS. Our results indicated that both ionization types showed matrix effect, but ESI was more susceptible than APCI. Sample preparation could reduce (clean up) or magnify (pre-concentrate) matrix
effect. Residual matrix components were specific to each bio-fluid and interfered at different time points in the chromatogram. Simple dilution of urine was sufficient to allow for the analysis of the analytes of interest by LC-APCI-MS/MS. Acetonitrile protein precipitation provided both sample clean up and concentration for oral fluid analysis, while SPE was necessary for extensive clean up of plasma prior to LC-APCI-MS/MS.

- Richard King et al., (2000) had showed results from experiments designed to determine the relative importance of gas phase processes and solution phase processes into ionization suppression observed in biological sample extracts. The data indicate that gas phase reactions leading to the loss of net charge on the analyte is not likely to be the most important process involved in ionization suppression. The results point to changes in the droplet solution properties caused by the presence of nonvolatile solutes as the main cause of ionization suppression in electrospray ionization of biological extracts.

- Mei H et al., (2003) had performed series of studies to investigate some of the causes for matrix effects ('ion suppression' or 'ion enhancement') in bioanalytical high-performance liquid chromatography/tandem mass spectrometry (HPLC/MS/MS) assays. Based on the findings, they had proposed the following simple strategies to avoid matrix effects: (1) select the same brand of plastic tubes for processing and storing plasma samples and spiked plasma standards; (2) avoid using Li-heparin as the anticoagulant; and (3) try switching the ionization mode or switching to different mass spectrometers when matrix effects are encountered. These three 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.

- Xu X et al., (2005) had studied the matrix effects caused by commonly dosing vehicles such as Hydroxyproyl-beta-cyclodextrin (HPBCD), methyl cellulose (MC), Tween 80 and PEG400 in pharmacokinetic (PK) studies during the early drug discovery stage. Five test compounds with CLog P values ranging from 0.9 to 5.4 were spiked into the collected rat plasma. After protein precipitation, these samples were analyzed using a generic fast-gradient HPLC/MS/MS method. Results indicated
that there was no observed matrix effect for all five compounds when 20% HPBCD or 0.4% MC was used as the vehicle in either the IV or the PO route, respectively. In addition, 0.1% Tween 80 dosed either IV or PO caused significant ion suppression (50-80%, compared to results obtained from plasma samples free from vehicles) for compounds that eluted at the beginning of the chromatogram. Also, PEG400 when used in an oral formulation caused significant ion suppression (30-50%) for early eluting compounds. Overall, the APCI mode proved to be less vulnerable to matrix effects than the ESI mode.

- Shou WZ et al., (2003) stated that dosing vehicles are typically employed in high concentrations to dissolve the test compounds in dose formulations which can pose significant problems for the liquid chromatography/tandem mass spectrometric (LC/MS/MS) analysis of incurred samples due to potential signal suppression of the analytes caused by the vehicles. Commonly used dosing vehicles, including polyethylene glycol 400 (PEG 400), polysorbate 80 (Tween 80), hydroxypropyl beta-cyclodextrin, and N,N-dimethylacetamide, were fortified into rat plasma at 5 mg/mL before extraction. Results thus obtained indicated that polymeric vehicles such as PEG 400 and Tween 80 caused significant suppression (> 50%, compared with results obtained from plasma samples free from vehicles) to certain analytes, when minimum sample cleanup was used and the analytes happened to co-elute with the vehicles. Effective means to minimize this 'dosing vehicle effect' included better chromatographic separations, better sample cleanup, and alternative ionization methods.

- Tong XS et al., (2002) had used PEG400 as a probe compound and exploited the concentration-time profile of the excipient in plasma from rats dosed both orally and intravenously is determined. These excipient plasma concentrations can result in a 2-5-fold increase in calculated plasma clearance values when the excipient interferes with the quantitation of the dosed compound. This can result in false rejection of a compound in a drug discovery screen. Several plasma purification methods and enhanced chromatographic selectivity are examined as ways to minimize or avoid excipient effects, particularly for very polar compounds. The combination of efficient sample purification and selective chromatography provides an effective way to
diminish the significant interference effects of PEG400 and Tween 80. When appropriate, using negative ion mode MS or changing a dosing vehicle excipient, such as substituting propylene glycol for PEG400, provides an alternative approach for eliminating signal interference.

- **Weaver R et al., (2006)** stated that at best, ion suppression leads to decreased sensitivity but at worst could lead to incorrectly determined pharmacokinetic (PK) parameters. Polyethylene glycol (PEG 400), an excipient often used in pre-clinical dosing vehicles. PEG was also found to be present in large quantities in the blood collection tubes used for pre-clinical PK studies. Ion suppression was observed for many analytes, either due to the use of PEG in the dosing vehicle or in blood collection tubes. The elimination of large ion suppression effects was attained by simple chromatographic gradient changes and the use of alternative blood collection tubes. The effect of the above was to increase the detected plasma concentration levels, which resulted in a change in key PK parameters.

- **Souveran et al., (2004)** had investigated matrix effect on mass spectrometry response with commercially available electrospray ionisation (ESI) and atmospheric pressure chemical ionisation (APCI) sources coupled with a single quadruple mass spectrometer. A post-column infusion system was used to observe the MS signal alterations of methadone, selected as the model compound, in plasma. For this purpose, two sample preparation procedures were tested: (1) conventional off-line sample preparations with liquid-liquid extraction (LLE), solid-phase extraction (SPE) and protein precipitation (PP) with perchloric acid (PA) and acetonitrile (ACN) and (2) on-line SPE with two different extraction columns packed with a large particle support (LPS) and with restricted access material (RAM), respectively. Whatever the sample preparation procedures, APCI source appeared to be less liable to matrix effect than ESI source. Among the different off-line sample preparations, LLE was the most efficient extraction procedure.

- **Smeraglia J et al., (2002)** had proposed a number of approaches to improve reproducibility and robustness of LC-MS-MS methods that are subjected to matrix effect. The modifications described are related to instrumentation and methodological issues and include modified ionisation, ionisation switching, extraction modification.
and gradient high pressure liquid chromatography (HPLC) techniques and have demonstrated significantly improved robustness of complex bioanalytical methods to avoid matrix-related issues.

- **Eva saar et al., (2009)** had compared extraction efficiency and matrix effects using common liquid–liquid and solid-phase extraction procedures in both ante-mortem and post-mortem specimen using LC–MS–MS for analysis of antipsychotics. Extraction efficiencies and matrix effects were determined in five different blank blood specimens of each blood type. The samples were extracted using a number of different liquid–liquid extraction methods and compared with a standard mixed-mode solid-phase extraction method. Matrix effects were determined using a post-extraction addition approach. The extraction comparison of ante-mortem and post-mortem blood showed considerable differences, in particular the extraction efficiency was quite different between ante-mortem and post-mortem blood.

- **Yunsheng Hsieh et al., (2001)** had developed a higher-throughput bioanalytical method based on fast-gradient (1 min run time) high-performance liquid chromatography (HPLC) coupled with tandem mass spectrometry (MS/MS) for screen-type analyses of plasma samples from early drug discovery studies in support of exploratory pharmacodynamic studies. The HPLC system equipped with minibore column was interfaced with either atmospheric pressure chemical ionization (APCI) or electrospray (ESI) ionization techniques. The matrix ion suppression effect of both quantitative HPLC/MS/MS analyses was compared using the post-column infusion system. The use of the described methods provided advantages such as a shorter chromatographic region of ion suppression, less solvent consumption and shorter run times in comparison with standard analytical column HPLC/MS/MS methods.

- **Bernard K Choi et al., (2000)** had investigated the effect of liquid chromatography separation on liquid chromatography–tandem mass spectrometry (LC–MS–MS) signal response for the characterization of low-molecular-mass compounds in a complex matrix. Matrix induced signal suppression appears throughout the entire LC–MS–MS analysis of wheat forage extract, with greatest suppression occurring at early retention times. Experimental results show that co-elution of matrix components and analytes from the LC column may be most strongly attributed to column
overloading rather than similar analyte and matrix retention behavior. As a result, two-dimensional (LC–LC) separation can be a highly effective approach to address signal suppression effects for the quantitative LC–MS–MS analysis of complex matrix samples.

- **Claudio De Nardi et al., (2006)** described the steps taken to move from a fast to a ballistic gradient in routine liquid chromatography/tandem mass spectrometry (LC/MS/MS) analysis of plasma samples from pharmacokinetic (PK) profiling of new chemical entities. The reduction of column dimensions from $50 \times 4.6\,\text{mm}$ to $30 \times 2.1\,\text{mm}$ followed by optimization of chromatographic separation led to a decrease in the typical runtime from 5 (fast) to 2 min (ballistic) using an API4000 tandem mass spectrometer in Turbo Ionspray mode for detection. Two different approaches were used to evaluate matrix effect: post-column infusion and comparison of the peak areas of neat standards and standards spiked after extraction into different pools of plasma. Peak shape, width and height, selectivity and sensitivity of the method were taken into account for chromatographic evaluation. The ballistic method was successfully cross-validated with the conventional fast gradient chromatographic assay.

- **Fabio Gosetti et al., (2009)** discussed the pitfalls of the matrix effect in mass spectrometry detection hyphenated to liquid chromatography separation. Matrix effect heavily influences both qualitative and quantitative analyses, giving rise to suppression or enhancement of the signal. All data generated together lead to conclusion that the chemical properties of the target analyte, the kind of matrix, the matrix to analyte concentration ratio, the extraction process, the chromatographic conditions as well as the kind of the mass spectrometry instrumentation and the ionisation conditions can play a role. Depending on an unpredictable combination of conditions, signal suppression or enhancement can be observed.

- **Xinghua Guo et al., (2006)** investigated the structures and origins of typical chemical background noise ions in positive atmospheric pressure ionization liquid chromatography/mass spectrometry (API LC/MS). This was done by classifying chemical background ions using precursor and product ion scans on most abundant background ions to draw a family tree of the commonly occurring chemical
background ions. A significant contribution from the contaminants (airborne, from tubing and/or solvents) from plasticizer additives (phthalates, phenyl phosphates, sebacates and adipates, etc.) and silicones is concluded. These ions of contaminants can also serve as nuclei for the clustering of HPLC solvent or additives, such as water and acetic acid, thereby leading to a second family of background ions. One of the other interesting conclusions is that there is a clear difference in structures between the chemical background ions and the protonated analytes generated under atmospheric pressure ionization.

- **Sven Ake Gustavsson et al., (2001)** used trifluoroacetic acid, heptafluorobutanoic acid and perfluoroheptanoic acid as ion-pairing reagents. The signal intensities of eight amine analytes were measured in the presence of these fluorinated carboxylic acids and compared with the signal intensity when using an ion-pair free formic acid–ammonium formate buffer. It was shown that the ESI signal from most of the studied analytes decreased about 30–80% when the fluorinated carboxylic acids were added to the mobile phase at useful concentrations. The use of these acids in ion-pair chromatography was also compared to the more conventional sodium heptane sulphonate additive. It was found that the chromatographic performance was comparable. Finally, the long-term performance of the ESI interface and the chemical background caused by these fluorinated reagents were examined. No degradation of the ESI interface performance could be seen for over 24 h of continuous infusion.

- **Ismaiel OA et al., (2010)** had stated that glycerophosphocholines are the major phospholipids in plasma that have been widely shown to cause significant matrix effects on electrospray ionization efficiencies for target analytes. Thirteen compounds were selected which were representatives of eight phospholipids classes, mono, di, triacylglycerols, cholesterol and cholesterol esters. Post-column infusion experiments were carried out to compare relative ion suppression effects of these compounds. Chlorpheniramine and loratadine were selected as model test analytes. A simple LC/MS/MS method was developed to monitor these endogenous components in sample extracts and their extraction recoveries from a plasma pool were compared using protein precipitation, liquid-liquid extraction, supported-liquid extraction, solid phase extraction and Hybrid SPE-precipitation methods. Endogenous lipid
components other than GPChos, such as cholesterol and triacylglycerols, may result in significant matrix effects and should be monitored during method development. Use of the results presented here, along with a consideration of analyte chemical structure, the type of matrix and the type of sample preparation procedure, may help a bioanalytical scientist to better anticipate and minimize matrix effects in developing LC/MS/MS-based methods.

- Jessome Lori Lee et al., (2006) presented two commonly used techniques to detect the presence of the matrix effect. Modifying instrumental components and parameters, chromatographic separation, and sample preparation are all considered as means of reducing or possibly eliminating ion suppression.

- Risto Kostiainen et al., (2009) stated that selection of eluent composition requires particular attention since a solvent that is optimal for analyte ionization often does not provide acceptable retention and resolution in LC. Compromises must then be made between ionization and chromatographic separation efficiencies. They presented an overview of studies concerning the effect of eluent composition on the ionization efficiency of ESI, APCI and APPI in LC-MS.

- Larger PJ et al., (2005) observed in a preliminary pharmacokinetic study strong ion-suppression from a polysorbate co-solvent, which, if undetected, would have given highly erroneous pharmacokinetic results and possibly could have led to the inappropriate elimination of a promising drug candidate. Different chromatographic methods were tested indicating that the separation step was essential in controlling these effects. A method based on matrix dilution is proposed to check for these effects during the use of discovery support methods, where full validation is not practical.

- Liang HR et al., (2003) had investigated the phenomena of ionization suppression in electrospray ionization (ESI) and enhancement in atmospheric pressure chemical ionization (APCI) in selected-ion monitoring and selected-reaction monitoring modes for nine drugs and their corresponding stable-isotope-labeled internal standards (IS). It was found that these nine drugs had mutual suppression effect in ESI and enhancement effect in APCI mode of analysis. The mutual ionization suppression or enhancement between drugs and their isotope-labeled IS could possibly influence
assay sensitivity, reproducibility, accuracy and linearity in quantitative liquid chromatography/mass spectrometry (LC/MS) and liquid chromatography/tandem mass spectrometry (LC/MS/MS). However, calibration curves were linear if an appropriate IS concentration was selected for a desired calibration range to keep the response factors constant.

- **Matuszewski B K et al., (2003)** stated that determination of the matrix effect allows the assessment of the reliability and selectivity of an existing HPLC–MS/MS method. If the results of these studies are not satisfactory, the parameters determined may provide a guide to what changes in the method need to be made to improve assay selectivity. In addition, a direct comparison of the extent of the matrix effect using two different interfaces (a heated nebulizer, HN, and ion spray, ISP) under otherwise the same sample preparation and chromatographic conditions was made. It was demonstrated that, for the investigational drug under study, the matrix effect was clearly observed when ISP interface was utilized but it was absent when the HN interface was employed.