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BIOANALYTICAL METHOD DEVELOMENT AND VALIDATION BY LIQUID CHROMATOGRAPHY- AN UPDATED OVERVIEW

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ABSTRACT

Measurement of drug concentrations in biological matrices (such as serum, plasma, blood, urine, and saliva) is an important aspect of medicinal product development. Such data may be required to support applications for new actives substances and generics as well as variations to authorised drug products. The results of animal toxicokinetic studies and of clinical trials, including bioequivalence studies are used to make critical decisions supporting the safety and efficacy of a medicinal drug substance or product. Highly sensitive, selective and validated bioanalytical methods are required for quantitative estimation of drugs in biological matrices. Various analytical techniques like RP-HPLC with UV/PDA detector and FLUORESCENT detector. In today's drug development hyphenated mass spectral methods like LC-MS, GC-MS and tandem mass spectrometry like LC-MS/MS and GC-MS, CE-MS are mostly used. Out of these LC-MS/MS technique using triple quadrapole mass analyser with atmospheric pressure chemical or electrospay ionisation has been widely accepted. Hence an attempt is made in order to collect the information regarding bioanalytical method development by liquid chromatography, Sample preparation methods, Optimisation of chromatographic conditions, Validation of developed bioanalytical method, Principles of bioanalytical method development,Implications for validation of bioanalytical method,Use of validated method,Data analysis of validated method, Reportting of validated method, Incurred sample reanalysis.

INTRODUCTION:

Bio analysis is employed for the quantitative determination of drugs and their metabolites in biological fluids. Studies involving measurement of the drugs or metabolites in the biological fluids such as blood plasma, serum, urine, bile, CSF, etc. Measurement of drug concentrations in biological matrices (such as serum, plasma, blood, urine, and saliva) is an important aspect of medicinal product development. Such data may be required to support applications for new actives substances and

generics as well as variations to authorised drug products. The results of animal toxicokinetic studies and of clinical trials, including bioequivalence studies are used to make critical decisions supporting the safety and efficacy of a medicinal drug substance or product. It is therefore paramount that the applied bioanalytical methods used are well characterised, fully validated and documented to a satisfactory standard in order to yield reliable results. The validation of bioanalytical methods and the analysis of study samples for clinical trials in humans should be performed following the principles of Good Clinical Practice (GCP).[1]

2. NEED FOR BIOANALYSIS

Drug-drug interaction is a situation in which one drug influences the activity of another drug i.e. the effects are increased or decreased, or they produce a new effect that neither produces on its own. Typically, interaction between drugs come existence on poly drug therapy. These may occur out of accidental misuse or due to lack of knowledge about the active ingredients involved in the relevant substances. Pharmacokinetics plays main role for pharmacokinetic drug-drug interaction study for which bioanalysis is required. The side effects produced by the drugs may be due to unexcreted metabolite of drug. To determine the metabolite of the drug causing side effect, pharmacokinetic studies must be done for which bioanalysis is required. Hence for the safety use of drugs, bioanalysis is required which includes bioequivalence studies and pharmacokinetic studies of drug.The focus of bioanalysis is to provide a quantitative measure of the active drug and/or its metabolite(s) for the purpose of pharmacokinetics ,toxicokinetics, bioequivalence and exposure response (pharmacokinetics/pharmacodynamics studies). Bio analysis also applies to drugs used for illicit purposes, forensic investigations; anti-doping testing in sports and environmental concerns [2]

3. BIOANALYTICAL METHOD DEVELOPEMENT:

Highly sensitive, selective and validated bioanalytical methods are required for quantitative estimation of drugs in biological matrices. Various analytical techniques like RP-HPLC with UV/PDA detector and Fluorescent detector. In today's drug development hyphenated mass spectral methods like LC-MS, GC-MS and tandem mass spectrometry like LC-MS/MS and GC-MS, CE-MS are mostly used. Out of these LC-MS/MS technique using triple quadrapole mass analyser with atmospheric pressure chemical or electrospay ionisation has been widely accepted. A bioanalytical method development consists of two main components like Sample preparation and Optimization of chromatographic conditions

3. SAMLE PREPARATION:

Analyte must be removed from the matrix selectively choosing suitable extraction solvent and buffering if required. Selective extraction of analyte in liquid solvent through immiscible solvent can be done in following methods like Protein precipitation, Liquid-Liquid extraction, solid Phase extraction and Hybrid extraction

3.1.1 Ideal characteristics of solvent used for extraction:

1. Extracting solvent must be able to solubilise the analyte**.**

2. A low viscous solvent facilitates mixing with sample matrix

3. Solvent with low boiling point facilitates its removal at the end of the extraction[3].

3.1.2 Selection of extraction solvent: Selection of extraction solvent depends on analyte characteristics like Structure of analyte**,** P^{ka}**,** Solubility. Selection of extraction solvent depends on solubility characteristics like Miscibility, Purity, Efficiency, Polarity, Inertness, Volatility, Viscosity and Density. In General, selectivity of solvent can be improved by choosing least polar solvent in which analyte is soluble. Commonly used solvents for extraction of analyte are Tertiary-Butyl Methyl Ether, Dichloromethane, Ethylacetate, Diethylether

An extraction solvent may be used individually or with any other solvent in combination.

3.1.3 LIQUID- LIQUID EXTRACTION:
Liquid-Liquid extraction is mostly used to extract the analyte because the analyst can remove the drug or metabolite from larger concentration of endogenous material that might interfere with the final analyte determination. Extraction of analyte is performed with water immiscible solvent from the aqueous matrix which is generally pH modified in such a way that analyte will remain totally in unionised form, so it can be distributed mostly to the organic solvent phase. Organic solvent is collected as supernatant after ultra centrifugation and then it is evaporated to dryness to get solid analyte compound as residue[4].

3.2 OPTIMIZATION OF CHROMATOGRAPHIC CONDITIONS:

Developing a new method is aimed towards optimization of method for estimation of drug or its metabolite in biological matrices.

STEP-1

Optimization of instrument conditions: If the drug is analysed by LC-MS, various instrumental parameters are to be optimized like Ion spray voltage, Temperature, Curtain gas, Collision gas, Focussing gas, Nebulising potential, Declustering potential, Collision cell, Collision cell exit potential, Entrance potential. Where as in case of RP-HPLC with UV or PDA detector, instrumental optimised conditions are very less like flow rate, sample cooler temperature, injection volume etc

STEP-2

Optimization of chromatographic conditions:

Optimization of liquid chromatography parameters in bioanalytical method is mainly objected towards Improvement of resolution, Peak shape, Removal of interferences at analytes Rt·5, Various steps involved in optimisation of chromatographic conditions are Selection of mobile phase, Use of organic modifiers, Mobile phase buffering, Selection of column

STEP-III

Selection of internal standard:

Many comparative study trails should be made with selecting IS in order to reduce overlapping. An internal standard at a known concentration is normally added to plasma samples and is utilized to diagnose several potential variations that can occur during sample preparation and ongoing analysis. The structure of the internal standard should be similar to the drug of interest. If this is not possible other standards can be used[6].

VALIDATION OF DEVELOPED BIOANALYTICAL METHOD:

The following experimental design is drawn in order to prove the test method is capable to yield consistent, reliable and reproducible results within the pre-determined acceptance limits. Acceptance criteria for the validation parameters are specified in individual experimental design. Observations and results were recorded in individual method validation data sheets.

The validative parameters are.

Carryover test, Selectivity, Sensitivity, matrix effect, Linearity, Precision and Accuracy, Recovery, Dilution integrity, Ruggedness, Stabilities, Room temperature stability, Refrigerator stock solution stability, Bench top stability, Auto sampler stability, Long term stability, Freeze thaw stability, Wet Extract Stability, Re-injection stability, Concomitant Drug Effect

4.1 Carryover Test

For carryover test, six replicates of matrix blank (COT BLANK), six replicates of extracted high concentrations of analyte(s) of the Calibration Curve range (COT ULOQ) and six extracted LLOQ concentrations of analyte(s) of the Calibration Curve range (COT LLOQ) was prepared.

Calculation:

% of drug carry over = Drug response in COT BLANK/ Average drug response in COT LLOQX100

4.2.1 Matrix Selectivity:

Biological matrix (plasma) from a minimum of eight individual's lots including one hemolytic and one lipemic plasma were procured. An aqueous mixture of drug and internal standard in appropriate concentration were injected to check the retention time. Blank matrix (samples containing K_2EDTA) from 8 batches along with six LLOQ samples spiked in blank matrix (samples containing K_2EDTA) were processed and injected. The interference at the RT of a drug by comparing the response in the blank matrix against the mean response of the extracted LLOQ will be evaluated and the interference at the RT of internal standard by comparing the response in the blank matrix against the mean response of the extracted internal standard in LLOQ samples will also be evaluated.

4.2.2 Analyte Selectivity: Interference at Analyte retention time caused due to internal standard were evaluated by injecting six replicates of matrix blank with internal standard. Area of analyte, if any obtained, will be compared with the mean area of analyte obtained with LLOQ concentration injected. Interference at internal standard retention time caused due to drug will be evaluated by injecting six replicates of matrix blank with drug at ULOQ level. Area of internal standard, if any obtained, will be compared with the mean area of internal standard in LLOQ concentration injected.

4.3-Sensitivity: Sensitivity will be determined in terms of LLOQ, 'Lower Limit of Quantification' where the response of LLOQ must be at least five times greater than the response of interference in blank matrix at the retention time or mass transitions of the analyte(s). Also the S/N ratio must be ≥ 5: 1. Calibration curve (CC) and six samples of LLOQ from the same stock solutions will be prepared. The CC samples and the LLOQ samples were processed and analysed. LLOQ concentrations were back calculated using the calibration curve data and the CV. % and % Nominal was calculated for the same.

Matrix Effect:

The matrix effect was determined at two concentration levels (LQC and HQC concentrations) in eight replicates each for analyte along with internal standard. Two replicates of blank plasma samples were processed from eight individual screened plasma lots (which includes one haemolytic and one lipemic plasma lots). One set each of eight different blank matrices was used to spike the LQC concentration along with ISTD and another set was used for spiking the HQC concentration along with ISTD. One set of aqueous samples equivalent to final LQC and HQC concentrations were prepared by spiked analyte(s) along with ISTD to reconstitution solution and perform the analysis by injecting 6 times each. IS-normalized MF was calculated using the below formula for both LQC and HQC levels: IS normalized Matrix Factor = Peak response area ratio in presence of matrix ion Mean peak response area ratio in absence of matrix ions

4.5 Linearity: Ten concentration levels were prepared in the biological matrix by spiking with known concentration of analyte. Concentration of standards will be chosen on the basis of the concentration range expected in the study and was plotted in the calibration curve. A linear equation weighting factor of $1/X^2$ will be determined to produce the best fit for the concentration/response relationship.

4.6-Precision and Accuracy: Between and Within Batch Precision and Accuracy was calculated by analysing a minimum of three bioanalytical batches. A precision and accuracy batch must contain the following samples with internal standard wherever required: A medium standard (MQC2) dilution with internal standard**,**Blank matrix**,** Blank matrix with internal standard**,** Spiked calibration standards, RS (Reconstitution solution)**,** Six LLOQ quality control samples, Six low quality control samples-Six middle (MQC1 & MQC2) quality control samples, Six high quality control samples, Concentrations of quality control samples from the respective calibration curve will be calculated.Within Batch Precision and Accuracy was determined by

calculating CV % and % Nominal respectively, at each QC concentration level of a bioanalytical batch. Between Batch / Inter Day Precision and Accuracy will be determined by calculating CV % and % Nominal respectively, at each QC concentration level of all bioanalytical batches run on different days. Between Batch / Intra Day Precision and Accuracy will be determined by calculating CV % and % Nominal respectively, at each QC concentration level of all bioanalytical batches run in a same day.

Calculations:

Accuracy (% Nominal) and precision (CV %) for: Within Batch Precision and Accuracy

Accuracy (% Nominal) = Mean of each QC concentration level within a batch x 100
Nominal Value

Precision (CV %) = STDEV of each QC concentration level within a batch x 100
Average of each QC concentration level within a batch,

Between Batch / Intra Day Precision and Accuracy

Accuracy(% Nominal) = Mean of each QC conc. level of all batches run in a day x100
Nominal

Value Between Batch / Inter Day Precision and Accuracy

Accuracy (% Nominal) = Mean of each QC conc. level of all batches x 100
Nominal Value

Precision (CV %) = STDEV of each QC concentration level of all batches x 100
AVERAGE of each QC concentration level of all batches

4.7 Recovery

- Six sets each of quality control samples (LQC, MQC2 and HQC) was withdrawn from deep freezer and thawed or prepare freshly. These quality control samples (extracted samples) were processed by adding the internal standard and injected.
- Eighteen blank matrix samples (screened matrix from single lot) were processed and spiked with six sets of each QC dilutions at low, middle and high concentration along with internal standard into the processed blank matrix samples which represents 100 % extraction of analyte(s) (non-extracted samples).
- All replicates of non-extracted samples were injected.

Calculate mean response, S.D. and CV %,mean absolute % recovery of analyte and internal standard at each QC concentration as follows:

- Mean absolute % recovery of drug =

Mean Peak Area Response of analyte in extracted samples at LQC,MQC2andHQC X100

Mean Peak Area Response of analyte in non-extracted samples at LQC, MQC2 and HQC

Mean absolute % recovery of internal standard = Mean Peak Area Response of internal standard in extracted samples x 100
Mean Peak Area Response of internal standard in non-extracted samples

Finally, calculate the overall recovery for the analyte. Absolute % Recovery = Average of absolute % recoveries of LQC, MQC2 and HQC

4.8 Dilution Integrity

Twelve sets of QCs spiked with about 1.5 to 1.8 times the concentration of the highest standard (ULOQ) were prepared and stored in the deep freezer along with bulk spiked samples. On the day of evaluation, six sets of the QC samples were processed by diluting two-times and remaining six sets of QC samples were processed by diluting four-times prior to extraction by addition of screened blank matrix.

These QC samples were injected along with calibration curve standards processed without dilution and the QC concentrations will be calculated using multiplication factor as 2 (for two times diluted samples) and 4 (for four times diluted samples).

4.9 Ruggedness

A precision and accuracy batch was processed and analyzed with different analyst, with different column of same make and with different sets of reagents.

4.10. tabilities

4.10.1 Room Temperature Stock Solution Stability: A standard stock

solution of analyte and internal standard was prepared. Sufficient aliquots of stock solutions was stored in the refrigerator at temperature at 2°C to 8°C for refrigerated stock solution stability. Room temperature stock solutions stability was performed using remaining volume of the stock solution for a period of at least 6 hours, to assess stability of solution left on the bench while preparing the stock dilution. Fresh standard stock solutions of analyte and internal standard were prepared after a minimum of 6 hours. Final dilutions of both remaining stock solutions (stability samples) and fresh stock solutions (comparison samples) equivalent to final middle quality control sample concentration of analyte along with final concentration of internal standard in reconstitution solution was prepared. Six replicates of the prepared comparison and stability samples were injected immediately after preparation. % Stability of the samples was calculated using the following formulae:

Calculations:

% Stability=

Mean response of stability samples x Comparison stock concentration X 100

Mean response of comparison stock samples x Stability stock concentration

4.10.2 Refrigerated Stock Solution Stability

Refrigerated stock solution stability was carried out to assess the stability of stored stock over a period of time (e.g. 7, 14 and 21 days), during which it can be used. On the day of stability evaluation, fresh standard stock solutions of analyte and internal standard were prepared. Final dilutions of both refrigerated stock (stability samples) and fresh stock solutions (comparison samples) equivalent to final middle quality control sample concentration of analyte along with final concentration of internal standard was prepared in reconstitution solution. Six replicates, of prepared comparison and stability samples were injected immediately after preparation.

Calculations:

% Stability =

Mean response of stability samples x Comparison stock concentration X100

Mean response of comparison stock samples x Stability stock concentration

4.10.3 Freeze Thaw Stability

- Eighteen replicates of quality control samples at each level (i.e. LQC and HQC) was identified during bulk spiking and labelled each six replicate as FT-6, FT-5 and FT-4. The labelled samples were stored in the deep freezer maintained at -70^{o}C.
- After a minimum of 24 hours of freezing, the samples labelled as FT-6 were withdrawn from the deep freezer, thawed them at room temperature and refreeze again.
- After a minimum of 12 hours, the samples labelled as FT-6 and FT-5 were withdrawn from the deep freezer, thawed them at room temperature and refreeze again.
- After a minimum of 12 hours, the samples labelled as FT-6, FT-5 and FT-4 were withdrawn from the deep freezer, thawed them at room temperature and refreeze again.
- On the day of stability evaluation FT-6 samples were withdrawn from the deep freezer (after a minimum of 12 hours of freezing FT-6 samples) and thawed them at room temperature.
- The quality control samples of FT-6 were processed and analysed along with freshly spiked calibration curve standards and quality control samples (Low, Middle and High QC samples). Quality control concentrations were back calculated using the calibration curve data.

Calculations:

Mean % Nominal Concentration of Quality Control Samples =

Mean concentration of LQC or HQC having undergone 6 freeze thaw cycles x 100

Respective nominal concentration of LQC or HQC

4.10.4 Bench top Stability

- Six sets of LQC and HQC was withdrawn from the deep freezer and were kept as unprocessed under

appropriate conditions for a period of 6 hours.

- The bench top stability samples were processed and analysed along with freshly spiked calibration curve standards and quality control samples (Low, Middle and High QC samples). Quality control concentrations were back calculated using the calibration curve data.
- Bench top stability duration was calculated as the time of sample removed from the deep freezer, less than the time of their extraction starting time.

Calculations:

Mean % Nominal Concentration of Quality Control Samples = Mean concentration of LQC or HQC (stability samples) x 100
Respective nominal concentration of LQC or HOQ

Auto Sampler Stability

- Six sets of LQC and HQC samples was processed to facilitate injection at proposed stability period. Processed samples were kept in auto sampler at the specified temperature.
- On the day of stability, the stability QC samples were injected along with freshly spiked calibration curve standards and quality control samples (Low, Middle and High QC samples).
- Autosampler stability durations was calculated as the time of injection of first QC, less the time of their placement in autosampler.
- Quality control concentration was back calculated using the calibration curve data obtained from the freshly spiked calibration standards.

Calculations:

Mean % Nominal Concentration of Quality Control Samples =
Mean concentration of LQC or HQC samples at proposed stability period (in hours) x 100
Respective nominal concentration of LQC or HQC

4.10.6 Long Term Stability at –70 °C

- On the day of evaluation, six sets of long term stored quality control samples (LQC and HQC) stored at -70°C was withdrawn from deep freezer and processed them along with freshly spiked calibration curve standards and quality control samples (Low, Middle and High QC samples).
- Long-term stability duration was calculated as the date of analysis of stability QC samples, less the date of storage of stability QC samples (in days).
- Quality control concentration was back calculated using the calibration curve data obtained from the freshly spiked calibration standards. Mean concentration of the stability samples were compared with the mean of the back-calculated values freshly prepared QC samples.

Calculation:

Mean % Nominal Concentration =
Mean conc. of stability LQC or HQC samples x nominal conc.of freshly prepared LQC or HQC x 100
Mean conc. of freshly prepared LQC or HQC samples x nominal conc. of stability LQC or HQC

4.10.7 Wet Extract Stability

- Six replicates of LQC and HQC samples will be processed and injected to facilitate injection at proposed stability periods. The processed samples will be kept in refrigerator (2-8 °C) over a period of time.
- On the day of stability, the stability QC samples (six replicates of LQC and HQC samples) will be injected along with freshly spiked calibration curve standards and quality control samples (Low, Middle and High QC samples).
- Wet extract stability duration will be calculated as the time of their

placement in auto-sampler, less the sample preparation completion time.

- Quality control concentration will be back calculated using the calibration curve data obtained from the freshly spiked calibration standards.[7-9]

Calculations:

Mean % Nominal Concentration of Quality Control Samples =

Mean concentration of LQC or HQC samples x 100

Respective nominal concentration of LQC or HQC

5. IMPLICATIONS FOR VALIDATION OF BIOANALYTICAL METHOD:

- The matrix-based standard curve should consist of a minimum of six standard points, excluding blanks, using single or replicate samples. The standard curve should cover the entire range of expected concentrations.
- Standard curve fitting is determined by applying the simplest model that adequately describes the concentration-response relationship using appropriate weighting and statistical tests for *goodness of fit.*
- LLOQ is the lowest concentration of the standard curve that can be measured with acceptable accuracy and precision. The LLOQ should be established using at least five samples independent of standards. The highest standard will define the upper limit of quantification (ULOQ) of an analytical method.
- For validation of the bioanalytical method, accuracy and precision should be determined using a minimum of five determinations per concentration level (excluding blank samples).The mean value should be within ±15% of the theoretical value, except at LLOQ, where itshould not deviate by more than ±20%. The precision around the mean value should notexceed 15% of the CV, except for LLOQ, where it should not exceed 20% of the CV.
- The accuracy and precision with which known concentrations of analyte in biological matrix can be determined should be demonstrated. This can be accomplished by analysis of replicate sets of analyte samples of known concentrations C QC samples C from an equivalent biological matrix. At a minimum, three concentrations representing the entire range of the standard curve should be studied: one within 3x the lower limit of

quantification (LLOQ) (low QC sample), one near the center (middleQC), and one near the upper boundary of the standard curve (high QC

Reported method validation data and the determination of accuracy and precision should include all outliers; however, calculations of accuracy and precision excluding values that are statistically determined as outliers can also be reported.

- The stability of the analyte in biological matrix at intended storage temperatures should be established. The influence of freeze-thaw cycles (a minimum of three cycles at two concentrations in triplicate) should be studied.
- The stability of the analyte in matrix at ambient temperature should be evaluated over a time period equal to the typical sample preparation, sample handling, and analytical run times.
- Reinjection reproducibility should be evaluated to determine if an analytical run could be reanalyzed in the case of instrument failure.
- Acceptance/rejection criteria for spiked, matrix-based calibration standards and validation QC samples should be based on the nominal (theoretical) concentration of analytes. Specific criteria can be set up in advance and achieved for

accuracy and precision over the range of the standards, if so desired[10]

8. DATA ANALYSIS OF VALIDATED METHOD:

- In a standard curve, samples with high concentrations should be diluted or reanalysed.
- Concentrations below the LLOQ should be reported as zeroes
- If the calibration standards or QCs fall outside the acceptance criteria, runs are rejected
- The minimum number of QCs to ensure proper controlof the assay should be atleast 5% of the number of unknown samples.
- If carryover occurs, it should be mitigated or reduced
- Samples involving multiple analytes should not be rejected based on the data from one analyte failing the acceptance criteria.
- The additional QCs added in the sample range should be validated for its accuracy and precision before continuing with the analysis

REPORTING OF VALIDATED METHOD

The data from rejected runs should be documented but need not be reported; however, the fact that a run was rejected and the reason for failure should be reported. Reported method validation data and the determination of accuracy and precision should include all outliers; however, calculations of accuracy and precision excluding values that are determined as outliers should also be reported. Sample Data Reintegration: An SOP or guideline for sample data reintegration should be established *a priori.* This SOP or guideline should define the criteria for reintegration and how the reintegration is to be performed. The rationale for the reintegration should be clearly described and documented. Audit trails should be maintained. Original and reintegration data should be reported.

10. INCURRED SAMPLE REANALYSIS:

To verify the reliability of the reported subject sample analyte concentrations, Incurred sample reanalysis is intended. It is conducted by repeating the analysis of subset of subject samples from a given study in separate runs on different days. It critically supports the precision and accuracy measurement established with spiked QCs. Both original and repeat analysis must be conducted using same bioanalytical procedures. ISR should be conducted for all in vivo human BE studies and pivotal PK or PD studies.

RINCIPLES OF ISR:

The total number of ISR samples should be 7% of the study sample size. In selecting samples for reanalysis, adequate coverage of the PK profile in its entirety should be provided and should include assessments around Cmax and in the elimination phase for all study subjects. Two-thirds (67%) of the repeated sample results should be within 20% for small molecules and 30% for large molecules. The percentage difference of the results is determined with equation:

(Repeat – Original)/ Mean x 100

Written procedures should be in place to guide an investigation in the event of ISR failure for the purpose of resolving the lack of reproducibility. All aspects of ISR evaluations should be documented to reconstruct the study conduct as well as any investigations thereof. ISR results should be included in the final report of the respective study[11].

CONCLUSION:

Now-a-days, PolyTherapy is given to treat the diseases. Possibility of Drug-Drug Interaction,side effects become more when such therapy is given. To decrease the rate of possibility of such effects, bioanalysis of drugs is required. Validated bioanalytical method developed according to the guidelines provides methods to estimate the amount of drugs in the biological matrices. Bioavailability and bioequivalence studies can be done using this method.

REFERENCES:

1. Bioanalysis by LC–MS/MS, J. Pharm. Biomed. Anal, 2007, 44(2), 342-55.
2. Evans G. Hand book of bioanalysis,1st ed.; CRC press, 2004
3. Van, Bioanalytical Separations, Wilson I.D., Ed.; Hand Book of Analytical Separations series; Elsevier, Amsterdam, 2003.
4. Howard Hill., Development of bioanalysis: a short history. Bioanalysis, 2009, 1(1), 3- D. A. Wells, High throughput Bioanalytical Sample Preparation Methods and Automation Strategies, 1st ed., Elsevier, New York, 2006..
5. Snyder; Kirkland J.; Glajch J., Practical HPLC Method Development, 2nd Edition, John Wiley & sons, New York, 1997
6. Richard, "*Principles And Practice Of Bioanalysis*" ,Taylor& Francis, (2008) 1-18.
7. Rogatsky, Evaluation of Matrix Effect and Chromatography Efficiency New Parameters for Validation of Method Development, J. Am. Soc. Mass. Spectrum, 2005, 16(11), 1757-9.
8. FDA guidance for industry: Bioanalytical method development and validation. http://www.fda.gov/download/Drugs/Guidance Compliance Regulatory Information/ Guidances/ucm070107.
9. Causon., Validation of Chromatographic Methods in Biomedical Analysis Viewpoint and Discussion, J. Chromatogr. B Biomed. Sci. Appl.1997, 689(1), 175-80.
10. FDA guidance for industry: Bioanalytical method validation *Drug Information Branch (HFD-210),Center for Drug Evaluation and Research (CDER)http://www.fda.gov/cder/guidance/index.htm*
11. FDA guidance for industry: *Division of Drug Information, WO51, Room 2201 Center for Drug Evaluation and Research ,Food and Drug Administration.*