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A review on bioanalytical method development and pre-method validation concepts using SPE and LLE process by LC-MS/MS method

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Abstract

The review article provides guidance for the development of a Bio-Analytical Method intended for the estimation of drugs in biological fluids. The development of a suitable analytical method for the identification, isolation and quantification of different drugs and or their metabolites from biological fluids is an essential and challenging component of pharmacokinetic studies. The uses of detection techniques that are highly sensitive and specific for the quantification of drugs and or metabolites in biological fluids are preferred. To collect the information on physiochemical, pharmacokinetic, chromatographic and extraction procedures of the drug/Metabolites from available sources, e.g. Medline, Journals, Analytical abstract, Physician Desk Reference, Library etc. Summarize the physiochemical, pharmacokinetic properties, chromatographic and extraction procedures. Select the equipment according to the availability and sensitivity. Select the initial chromatographic conditions and also identify the extraction. The selection of Internal standard should be similar to the analyte. Prepare the required solutions and stock dilutions at the required concentration. Tune the instrument with suitable solutions and optimize parameters for Q1/Q3 ions. Condition the system and column with the selected mobile phase. Perform pre-method validation experiments. Select the best weighing factors for the standards. Compile all the chromatograms and raw data and archive since the development of a Bio-Analytical method for a given drug is so essential to start with, this guidance for approaching and conclude for a suitable method that later can be validated. It is important to note that this is intended to identify minimal criteria for producing consistent and comparable data.

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INTRODUCTION

Bio-analysis plays an important vital role on new drug development and validation. At present, bio-analysis provides an essential note on toxicological evaluation with pharmacokinetic & pharmacodynamics parameters during drug development. Bio-analytical is one of the top-notch method developments for drug development (Badola *et al.*, 2018). It is an excellent quantitative determination way for the identification of analytes in biological matrices.

The bioanalysis protocol includes Sampling, Sample preparation, Analysis, Calibration, Data eval-

uation and Reporting. The current bioanalysis field is based on good GLP and proper sample preparation and well instrument handling skill are required (Gelpí, 1987).

At the pharmaceutical research industry, the development of bioanalytical methods and GLP plays a major role than novel drug development and discovery. Bioanalysis is a valid process for derive and defines the number of analytes present in biological samples (blood, plasma, serum saliva, urine, feces, skin, hair, organ tissue).

It is not just a method to measure small (Højskov *et al.*, 2010) molecules such as drugs and metabolite, but it also helps us to identify large molecule like proteins. Bioanalysis has a vital role to find the toxicokinetic (TK), pharmacokinetic (PK) and pharmacodynamics (PD) studies of new drugs. High throughput screening of sample preparation and hyphenated ways for handling analytical instruments are much needed in modern bioanalysis platform (Meesters and Voswinkel, 2018). Liquid chromatography (LC) combined with tandem mass spectrometry (MS/MS) have been found for a long period in drug bioanalysis. Method validation is a major part of bioanalysis study (Drummer and Gerostamoulos, 2002).

This present review will give an idea on bioanalytical method development and pre-method development validations in accordance with GLP guidelines that have been presented.

METHOD DEVELOPMENT

There are some major things to consider in bioanalytical method development (Chai *et al.*, 2013).

They are Analyte and IS chemical structure, Stability both short and long term, Solubility properties, pKa value, Adsorption or interaction of plastic or glass.

Literature survey

Collect the information on physiochemical, pharmacokinetic, chromatographic and extraction procedures of the drug/Metabolites from available sources, e.g. Medline, Journals, Analytical abstract, Physician Desk Reference, Library etc. Summarize the physiochemical, pharmacokinetic properties, chromatographic and extraction procedures.

Procurement of drug standards, chemicals, reagents, columns, biological matrix and others

Procure the drug standards, chemicals, reagents, columns, biological matrix and any other relevant materials from possible sources.

Choice of initial conditions

Identify the different drugs for their suitability as internal standard. Select the equipment according to the availability and required sensitivity. Select initial chromatographic conditions, with column, buffer, mobile phase, flow rate, detector, and others, if any. Identify the extraction procedure like liquid phase extraction or solid-phase extraction, or any other technique considering the required sensitivity (Chen *et al.*, 2013).

Selection of Internal Standard

Select the internal standard similar to the physical and chemical nature (structure) of Analyte.

Preparation of Stock Solution

Prepare a stock solution of the appropriate concentration of the reference/working standard and internal standard in a suitable solvent. It is recommended to weigh more than or equal to 2 mg of the standard for the preparation of the stock solution (Ashri and Abdel-Rehim, 2011). Consider the % assay (on an is basis, unless otherwise specified), molecular weight or any other relevant information after weighing the reference/working standard and internal standard to obtain the actual concentration of the stock solution.

Preparation of aqueous Stock Dilutions

Select the range for the calibration curve standards based on the expected in-vivo concentration profile derived from literature. Keep the highest concentration on the calibration curve at least 2 times to that of the expected maximum drug concentration in the matrix (Goeringer *et al.*, 2000). Keep the lowest concentration on the calibration curve (LLOQ) as at least 5% of the expected maximum drug concentration in the matrix. Use at least six non-zero standards to construct a calibration curve. Prepare the aqueous stock dilutions to cover the expected entire range of CC standards and QC samples. Prepare the aqueous stock dilutions of CC standards and QC samples from the different stock solution in case of method validation, whenever possible (Ahnoff *et al.*, 2015) and peptides. The difference between the stock concentration of CC and QC should be within ±2%. In the case of Clinical sample analysis, the single stock may be used for the preparation of CC standard and QC sample.CC and QC can be prepared from the single stock solution for method validation in case of compounds/metabolites difficult to synthesize.

Make different concentrations as multiples of LLOQ or an approximate percentage of the highest CC standards. The suggestive concentrations for standard above LLOQ and the following standard are given below:

1. LLOQ (lowest calibration curve standard) - at least 5% of the expected Cmax.
2. The first standard after LLOQ should be below 2 to 3 times the LLOQ
3. Standard before ULOQ should be between 70-85% of ULOQ.
4. ULOQ (upper calibration curve standard) — at least 2 times the Cmax

It is advisable to have a calibration curve with 3 points at the lower end and 3 points close to the highest concentration in the CC range (Pragst *et al.*, 2004). Two or more standards in the intermediate range should be selected depending on the actual CC range. The CC range determines the number of standards in the CC, and there is no need to restrict the number of standards to an eight-point calibration.

QC level for method validation

LOQQC- must be equivalent to LLOQ, i.e. STDA (between 100 and 105% of LLOQ)

LQC - 2.5-3.0 times of LLOQ

INTQC - 5-30 % of the highest calibration curve standard (optional)

MQC - 30-45 % of the highest calibration curve standard

HQC - 70-85% of the highest calibration curve standard

DQC - 2 times of HQC concentration

QC level for Clinical sample analysis

LQC - 2.5-3.0 times of LLOQ

INTQC - 5-30 % of the highest calibration curve standard (optional)

MQC - 30-45 % of the highest calibration curve standard

HQC - 70-85% of the highest calibration curve standard

DQC - 2 times of HQC concentration

Use HPLC grade to prepare stock dilutions

Inject aqueous CC/QC samples and check for their acceptance.

In case any sample (CC standard/QC sample) is out of range, take the necessary corrective action. In such case, prepare a fresh final dilution sample and confirm for its acceptance, if required (Paterson *et al.*, 2004). If more than three calibration curve standard / QC samples failed to meet acceptance criteria, then prepare a fresh AQS dilution solution and confirm its acceptance criteria after rectifying excel calculation and dilution procedure (Hoizey *et al.*, 2005).

Bulk Spiking

Calculate the number of samples and volume of matrix required for the method validation or study sample analysis. Special population bioequivalence studies shall be conducted by use of calibration standards/quality control samples prepared in the same population matrix to avoid endogenous matrix related variability (Quintela *et al.*, 2005). Use chromatographically screened biological matrix for the bulk spiking of CC standards and QC samples. Prepare documents for specificity/selectivity experiment or blank screening record to record this event. Spike appropriate volume of aqueous dilutions to the pooled screened biological matrix to obtain the desired concentration of the CC standards and QC samples.

Guidance for the mixing, aliquoting/distribution and storage of CC/QC sample

Place the stopper on the volumetric flasks and mix the contents while taking care of no-leak during the mixing process. Aliquot equal volumes of each spiked CC standards and QC samples into respective pre-labelled polypropylene storage vials. Cap the vials and arrange them in pre-labelled zip locks bags, and store them at the intended storage temperature.

Acceptance Criteria for CC Standards and QC Sample

Standard curve fitting is determined by applying the simplest model that adequately describes the concentration-response relationship using appropriate weighting factor for the goodness of fit (Liu *et al.*, 2010).

A significant interference at the retention time of the internal standard is defined as a peak area of greater than 5% of the mean internal standard peak area in the accepted calibration standards used to calculate the calibration curve. A significant interference of this type results in the rejection of the batch. A significant interference at a retention time of analyte in the standard blank and/or standard zero (blank+IS) is defined as a peak with a peak response (or area) of more than 20% of the analyte in the lowest standard used to calculate the calibration curve. A significant interference of this type results in the rejection of the batch.

The lowest and highest standard should fall within the acceptance criteria for Method Validation experiments. During clinical sample analysis, if the lowest/highest standard does not pass the criteria, consider the next accepted lowest/highest standard.

In case of failure of one calibration curve standard, exclude the standard from the calibration curve with reason and if the analytical batch met acceptance criteria, accept the analytical batch.

If more than one calibration curve standards failed in a batch, first exclude the most deviating calibration curve standard (either positive or negative deviation) and then the least deviating calibration curve standard with reason. Accept the analytical batch after excluding two calibration standards.

In the case of Analyte or Internal standard poor chromatography exclude the calibration standard and accept the analytical batch (Altun *et al.*, 2010).

Exclude the calibration standard if no peak response observed in either Analyte or Internal Standard or in both exclude the standard form calibration curve and accept the analytical batch.

In all the above cases, ensure that two consecutive calibration curve standard should not fail within a batch (Ben-Hander *et al.*, 2013). Use blank biological samples only for assessing potential interference but not for calculation in regression analysis. The correlation coefficient (r^2) should be more than 0.98.

Quality Control Samples Acceptance Criteria

For MV, accept the batch if the accuracy of at least 70% of quality control samples at each level fall within 15% of their respective nominal concentration except for the LOQQC. The precision of the six replicates should be A 5%.

Accept the batch if the accuracy of LOQQC samples falls within +20% of the nominal value. The precision of the six replicates should be 20%.

For clinical sample analysis, accept the batch if at least 67% of quality control samples and at least 50% at each level (LQC, INTQC, MQC, HQC and DQC) fall within 15% of their respective nominal concentration.

Reporting of Small Differences in Concentrations of Calibration Standards and QC Samples

For a given Bio-analytical study, a sufficient number of aliquots of each calibration standard and QCs must be prepared to enable an analysis of all the study samples.

A new set of calibration standards and/or QCs must be prepared using freshly prepared drug stock (if original stock is within the stability period, then that also can be used) in cases where there is an insufficient number of calibration standards and/or QCs available to complete the study.

A maximum amount of care must be exercised to ensure that the concentrations of the new standards and QCs match those, which have been used previously when assaying the study samples (Javanbakht *et al.*, 2012). However, there is the possibility that the concentrations of the "old" and "new" standards and QCs differ slightly. Use the following criteria for acceptance of new CCs and/or QCs.

For the acceptance of new CCs - Run a batch of old QCs (6 sets) against the new calibration curve standards (Kamaruzaman *et al.*, 2013).

Use the nominal concentrations (actual concentrations) of the "new" standards to calculate the concentrations of the samples during further study sample analysis.

Preparation of Buffer, Mobile Phase, Reagents and Solutions

Solution Preparation

To Ensure the cleanliness of the area, apparatus and glassware before preparing any solution. Weigh accurately or measure the substance/solvent/chemical and carefully transfer the content in a suitable container that is prelabeled (Keevil, 2016). Dissolve them in the required solution/reagent in suitable solvent and vortex/sonicate for few minutes if required.

Mobile Phase Preparation

To Measure the required organic, aqueous solvent phase separately in different measuring cylinders and transfer them into a suitable container. Mix the contents well by mild shaking (Goff *et al.*, 2020). Fix the Buchner funnel with flask and connect with vacuum pump. Filter the solutions using membranes if required. Fill the solvent in the Buchner funnel and switch on the pump. Wait until the operation is completed. After filtration, sonicate the mixture for degasification.

Chromatography method

To tune the instrument (LC-MS/MS) with suitable solutions. Dilute the analyte and internal standard at the required concentration based on the sensitivity of the instrument. Infuse the solution and tune the literature reported Q1/Q3 masses. Optimize the compound parameters for both Ql and Q3 Ions. In case of unavailability of reported literature ions, search for stable (base peak) of Q1 and Q3 Ions, optimize the instrument parameters for the stable response. Condition the system and column with the selected mobile phase. Check for the baseline stability by injecting a mobile phase and or solvent. Find out the retention time of the drug by injecting its dilution on to the chromatographic device (Liang *et al.*, 2013). Modify the chromatographic conditions so as to obtain satisfactory chromatography.

Follow the above step for drug and or metabolites, internal standards and a mixture of them. Modify the chromatographic conditions for the isolation, identification and resolution between the drugs. Search for the (authenticated) literature Cmax proof for the same dosage form/strength/route of administration for which bioequivalence to be determined. Keep at least 2-3 times of Cmax concentration as ULOQ and LLOQ. Extract the blank biological matrix as per the selected extraction procedure. Check the interferences at the retention time of the drugs (Lindegårdh *et al.*, 2005). Spike the drug solutions in the biological matrix and extract the drug with the extraction procedure and optimize the extraction procedure and chromatographic conditions. Ensure that the matrix effect meets the acceptance criteria. Summarize the above chromatographic conditions (Qi *et al.*, 2015). Prepare a set of unextracted and extracted samples in the expected range of the calibration curve based on the pharmacokinetic data available in the literature and inject on into the chromatographic device. Check the preliminary comfort on chromatography, linearity and recovery. Repeat these experiments until they are acceptable.

PRE-METHOD VALIDATION

Perform the pre-method validation after deriving the analytical method as designed. The system suitability carried over as per procedure and system suitability must provide the same response for multiple injections at the same sample. The test should not carry a sample through a needle from one sample to another. To use the following procedure to set the LLOQ and ULOQ. The values are arranged in increasing order. The determination of the difference between the highest and the lowest value of the series (range). The determination of the difference between the lowest value of the series and the nearest result (module) (Rappold, 2018). Divide this difference (module) by the range, obtaining a Q value. Q > Q95 %, the lowest value is rejected and if Q < Q95% the lowest value, is accepted. If the lowest value is rejected, determine the new range and test the highest value of the series (If the lowest value is rejected, using the same range, test the highest value of the series).

Repeat the process until the lowest and the highest values are accepted, i.e., if the lowest value is accepted, then the highest value is accepted until the lowest, and the highest values are accepted (Posada and Crandall, 2001).

Perform the calculation for linearity, precision, accuracy, and reproducibility by preparing and injecting the following samples

1. Un extracted standard curve (Aqueous CC)
2. One precision and accuracy batch
3. Recovery

Selection of weighing factor for the calibration curve

The calibration curve plots the concentration of the standard against its peak area or height. When the points for the standards plotted must determine the best weighing factor for the best fit.

CONCLUSION

The development of a Bio-Analytical Method intended for the estimation of drugs in biological fluids plays an important role in the Quantitative Analysis. The development of a suitable analytical method for the identification, isolation and quantification of different drugs and or their metabolites from biological fluids is an essential and challenging component of pharmacokinetic studies. The article covers the procedure involved in the extraction of drugs from biological fluids and its importance in the analytical method development.

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Conflict of Interest

The authors declare that they have no conflict of interest for this study.

REFERENCES

Ahnoff, M., Nyström, A.-C., Schweikart, F., Ekdahl, A. 2015. Matrix effect explained by unexpected formation of peptide in acidified plasma. *Bioanalysis*, 7(3):295–306.

Altun, Z., Skoglund, C., Abdel-Rehim, M. 2010. Monolithic methacrylate packed 96-tips for high throughput bioanalysis. *Journal of Chromatography A*, 1217(16):2581–2588.

Ashri, N. Y., Abdel-Rehim, M. 2011. Sample treatment based on extraction techniques in biological matrices. *Bioanalysis*, 3(17):2003–2018.

Badola, A., Joshi, P., Kothiyal, P. 2018. A General Review on Bioanalytical Method Development and

Validation for LC-MSMS. *International Journal of Trend in Scientific Research and Development*, 2(4):1340–1347.

Ben-Hander, G. M., Makahleh, A., Saad, B., Saleh, M. I. 2013. Hollow fiber liquid phase microextraction with in situ derivatization for the determination of trace amounts of metformin hydrochloride (anti-diabetic drug) in biological fluids. *Journal of Chromatography B*, 941:123–130.

Chai, D., Wang, R., Bai, N., Cai, Y., Liang, B. 2013. Development and validation of a highly sensitive LC-MS/MS method for quantitation of bivalirudin in human plasma: application to a human pharmacokinetic study. *Biomedical Chromatography*, 27(12):1788–1793.

Chen, Y., Zhu, J., Yu, Z., Zhang, Y., Liu, Y. 2013. Determination of three drugs and their metabolites in saliva by ultra-performance liquid chromatography-tandem mass spectrometry. *Chinese Journal of Chromatography*, 30(11):1148–1152.

Drummer, O. H., Gerostamoulos, J. 2002. Postmortem Drug Analysis: Analytical and Toxicological Aspects. *Therapeutic Drug Monitoring*, 24(2):199–209.

Gelpí, E. 1987. Bioanalytical aspects on method validation. *Life Sciences*, 41(7):849–852.

Goeringer, K. E., Raymon, L., Christian, G. D., Logan, B. K. 2000. Postmortem Forensic Toxicology of Selective Serotonin Reuptake Inhibitors: A Review of Pharmacology and Report of 168 Cases. *Journal of Forensic Sciences*, 45(3):633–648.

Goff, C. L., Farre-Segura, J., Stojkovic, V., Dufour, P., Peeters, S., Courtois, J., Nizet, A., De Vos, N., Cavalier, E. 2020. The pathway through LC-MS method development: in-house or ready-to-use kit-based methods? *Clinical Chemistry and Laboratory Medicine (CCLM)*, 58(6):1002–1009.

Hoizey, G., Lamiable, D., Trenque, T., Robinet, A., Binet, L., Kaltenbach, M. L., Havet, S., Millart, H. 2005. Identification and Quantification of 8 Sulfonylureas with Clinical Toxicology Interest by Liquid Chromatography–Ion-Trap Tandem Mass Spectrometry and Library Searching. *Clinical Chemistry*, 51(9):1666–1672.

Højskov, C. S., Heickendorff, L., Møller, H. J. 2010. High-throughput liquid–liquid extraction and LCMSMS assay for determination of circulating 25(OH) vitamin D3 and D2 in the routine clinical laboratory. *Clinica Chimica Acta*, 411(1-2):114–116.

Javanbakht, M., Moein, M. M., Akbari-adergani, B. 2012. On-line clean-up and determination of tramadol in human plasma and urine samples using molecularly imprinted monolithic column coupling with HPLC. *Journal of Chromatography B*, 911:49–54.

Kamaruzaman, S., Sanagi, M. M., Endud, S., Ibrahim, W. A. W., Yahaya, N. 2013. MCM-41 solid phase membrane tip extraction combined with liquid chromatography for the determination of non-steroidal anti-inflammatory drugs in human urine. *Journal of Chromatography B*, 940:59–65.

Keevil, B. G. 2016. LC–MS/MS analysis of steroids in the clinical laboratory. *Clinical Biochemistry*, 49(13-14):989–997.

Liang, S., Bian, X., Ma, J., Arogunjo, M., Deorukhkar, A. A., Krishnan, S., Xie, H. 2013. Development and validation of a sensitive LC/MS/MS method for the determination ofγ-tocotrienol in rat plasma: application to pharmacokinetic studies. *Biomedical Chromatography*, 27(1):58–66.

Lindegårdh, N., Annerberg, A., Blessborn, D., Bergqvist, Y., Day, N., White, N. J. 2005. Development and validation of a bioanalytical method using automated solid-phase extraction and LC-UV for the simultaneous determination of lumefantrine and its desbutyl metabolite in plasma. *Journal of Pharmaceutical and Biomedical Analysis*, 37(5):1081–1088.

Liu, H.-C., Liu, R. H., Lin, D.-L., Ho, H.-O. 2010. Rapid screening and confirmation of drugs and toxic compounds in biological specimens using liquid chromatography/ion trap tandem mass spectrometry and automated library search. *Rapid Communications in Mass Spectrometry*, 24(1):75–84.

Meesters, R. J., Voswinkel, S. 2018. Bioanalytical Method Development and Validation: from the USFDA 2001 to the USFDA 2018 Guidance for Industry. *Journal of Applied Bioanalysis*, 4(3):67–73.

Paterson, S., Cordero, R., Burlinson, S. 2004. Screening and semi-quantitative analysis of post mortem blood for basic drugs using gas chromatography/ion trap mass spectrometry. *Journal of Chromatography B*, 813(1-2):323–330.

Posada, D., Crandall, K. 2001. Selecting the Best-Fit Model of Nucleotide Substitution. *Systematic Biology*, 50(4):580–601.

Pragst, F., Herzler, M., Erxleben, B.-T. 2004. Systematic toxicological analysis by high-performance liquid chromatography with diode array detection (HPLC-DAD). *Clinical Chemistry and Laboratory Medicine (CCLM)*, 42(11):1325–1340.

Qi, Y., Geib, T., Schorr, P., Meier, F., Volmer, D. A. 2015. On the isobaric space of 25-hydroxyvitamin

D in human serum: potential for interferences in liquid chromatography/tandem mass spectrometry, systematic errors and accuracy issues. *Rapid Communications in Mass Spectrometry*, 29(1):1–9.

Quintela, O., Cruz, A., de Castro, A., Concheiro, M., López-Rivadulla, M. 2005. Liquid chromatography–electrospray ionisation mass spectrometry for the determination of nine selected benzodiazepines in human plasma and oral fluid. *Journal of Chromatography B*, 825(1):63–71.

Rappold, B. A. 2018. Special Considerations for Liquid Chromatography–Tandem Mass Spectrometry Method Development. *Clinics in Laboratory Medicine*, 38(3):539–551.