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Review on Sample Preparation for Drug Discovery

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ABSTRACT

This article includes various methods of sample preparation of mammalian plasma by using different approaches. In each step of sample preparation the necessary measures should be investigated to determine the extent to which environmental, matrix, material, or procedural variables can affect the estimation of analyte in the matrix from the time of collection of the material up to and including the time of analysis. The main object of these measures is to remove matrix component present in plasma and make sample more efficient for analysis. Here five methods of sample preparation were described according to its utility towards sample preparation and efficacy of analysis

Keywords : Matrix, Analyte, Serum, Biological fluids and Plasma

1. INTRODUCTION

Sample preparation for drug discovery applications represents various methods according to present demands and available techniques. In case of biological and in vitro samples, the main object is to remove the dissolved components from the plasma (sample) to make mammalian plasma more appropriate for analysis. The dissolved components like lipids, salts, protein and cellular components may interfere in analysis of samples. The presence of these components on biological samples may occult the smooth run of samples in HPLC. To get desirable effects and to use sample more efficiently it's necessary to remove matrix components present in biological samples. While considering sample preparation methods for an LC/MS/MS drug discovery phase we should be familiar of difficulties arises from the starting of preparation to final injection of sample to the system. Every discovery or methods have set of standards and requirement for analysis and to facilitate the experimental requirements the major step is to remove the desired matrix component completely to accomplish the experiment or discovery. The other factors that influence the sample preparation are the chemical structure of analytes and duration of time to develop a procedure for sample preparation. The next steps are the automation of procedure and how the procedure proceeds. After considering all these points carefully and dealing with difficulties effectively the sample preparation can be quickly developed.

There are following methods for sample preparation

a. **Direct injection**
b. **Protein precipitation**
c. **Liquid liquid extraction**
d. **Ultrafiltration**
e. **Solid phase extraction**

2. DIRECT INJECTION

This method is simple and efficient way of analysis and mostly used for clean matrices. In this method the sample is diluted and injected onto the chromatographic column. According to method both sample and matrix component are injected and from this feature the problems may arise during analysis because samples

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containing appropriate amount of salts, protein and cellular components. The biological samples containing high amount of contamination can be reduced by using following effective approach. The sample containing high amount of salt content can be installed an automated divert valve between the HPLC column and the mass spectrometer ion source. The second method developed to deal with special problems related with direct injection is that the use of restricted access media (RAM) and turbulent flow chromatography.

3. PROTEIN PRECIPITATION

Protein precipitation is simple and useful tool for sample preparation. This is most powerful approach to remove proteins present in mammalian plasma. To facilitate this process simple organic solvent such as methanol and acetonitrile in ratio of (2:1) has been reported to remove 98% of proteins present in plasma. In protein precipitation method an exact volume of denaturing solvent is added to sample containing protein and other cellular components. Protein precipitation is facilitated by vortexing, centrifugation and allowing collection of denatured protein into a pellet at the bottom of the vessel. This process is known as phase separation. The denaturation process causes an inability of protein's to bind a analyte molecules, analytes are released from proteins and remain in the supernatant liquid, although some analytes are occluded with the protein pellet. The samples are centrifuged and phases are separated by siphoning off the clear supernatant. The supernatant is injected directly without prior phase separation.

4. LIQUID- LIQUID EXTRACTION

Liquid-liquid extraction method has good sample cleanup technique for nonpolar or moderately polar analytes that can be deionized in solution using pH adjustment. Liquid-liquid extraction has been used extensively for an analytical sample pretreatment to remove unwanted matrix component. It's based on the principle of differential solubility and partitioning equilibrium of analyte molecules between aqueous and organic phase. In this method initially pH adjustment of sample has been done with the help of buffer. The next step includes addition of immiscible organic extraction solvent, followed by vortexing to facilitate equilibrium portioning of analyte molecules between phases. The phases are separated and the aqueous component is discarded. The organic phase is evaporated to dryness and resuspended with mobile phases or a similar solvent system and then injected onto column.

5. ULTRAFILTRATION

This method allows separation based on molecular size. This method is useful to quantify free drug fraction present plasma, serum and other biological fluids. The central component is a molecular weight cut off membrane, positioned in the bottom of a small cup containing the sample (donor). Upon sample introduction and centrifugation, this membrane allows molecules smaller than the molecular cut off to pass a receiver container, while retaining larger molecules species. Small drug molecules that are not protein bound are permitted pass through and larger molecules are retained in donor compartment. This ultrafiltrate fluid in the receiver compartment is directly injected into the LC / MS system.

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6. SOLID PHASE EXTRACTION

Solid phase extraction is a miniature version of the liquid chromatography. The initial step is conditioning of sorbent bed. The sample is buffered so that ionization of analytes is minimized. The sample is loaded onto the sorbent and the sample solvent is pulled through by vaccum, positive pressure or centrifugal force. Analyte and many matrix components partition with sorbent and are retained. Some salts and matrix components pulled through with the sample solvent. A series of sorbent wash step are done. These wash steps use weak solvent to remove matrix components and leave analyte unaffected. Finally, an elution step uses a strong solvent to remove analyte molecules from sorbent .This elute containing analytes molecules from elution is collected and injected directly.

7. TESTING PARAMETERS OF SAMPLE PREPARATION

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The prepared samples for drug discovery or analysis can be tested and judged by various parameters available for it. These parameters give validity and build confidence for data quality and analytical results by using parameters. Routinely evaluated parameters are dynamic range, quantitation limit, linearity, assay precision, assay selectivity, assay accuracy and extraction recovery. The method development and establishment phase defines the chemical assay. The fundamental parameters for a bioanalytical method validation are accuracy, precision, selectivity, sensitivity, reproducibility, and stability. Measurements for each analyte in the biological matrix should be validated. In addition, the stability of the analyte in spiked samples should be determined.

8. SUMMARY

Sample preparation is a major part of analytical or bioanalytical method development. The selection of sample preparation based on various parameters like matrix component, analyte structure and appropriate procedure for further processing. The integration of sample handling with sample preparation has potential to improve the efficacy of the discovery of bioanalytical process. It may be important to consider the variability of the matrix due to the physiological nature of the sample. In the case of LC-MS-MS-based procedures, appropriate steps should be taken to ensure the lack of matrix effects throughout the application of the method, especially if the nature of the matrix changes from the matrix used during method validation. The objective of sample preparation of biological samples (mammalian plasma) by using analytical procedure is to demonstrate that it is suitable for the intended purpose and is essential to employ well characterized and fully validated analytical methods to yield reliable results which can be satisfactorily interpreted.

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