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Journal of Clinical and Experimental Pharmacology

Commentary

A Note on Liquid Chromatography and Mass Spectrometry

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ABOUT THE STUDY

Bioanalytical liquid chromatography-mass spectrometry is a technique that uses liquid chromatography with the mass spectrometry. LC-MS is commonly used in laboratories for the quantitative and qualitative analysis of drug substances, drug products and biological samples. LC-MS has played a significant role in evaluation and interpretation of bioavailability, bioequivalence and pharmacokinetic data. Through LC-MS biological samples are determined throughout all phases of method development of a drug in research and quality control.

Method Development: Method of analysis are being routinely developed, improved, validated, collaboratively studied and applied. Chromatographic separations are mainly required which depend on the samples to be analyzed. The chromatographic procedure is important for the systemic approach to LC-MS/MS method development. In most cases as desired separation can be achieved easily with only a few experiments. In other cases a considerable amount of experimentation may be needed.

Procedure for Method Development: Collect the physicochemical properties of drug molecules from the literature.

- Determine solubility profile
- MS scanning and optimization
- Mobile phase selection
- Selection of extraction method and optimization
- Selection of chromatographic method (based on solubility study, retention of compound)

Reversed Phase Chromatography: Reversed phase packing's such as C18, C8 are the most popular and most widely used for reversed phase. In addition to these C4, C2 and phenyl bonded are also available. Reversed phase sorbents generally involves conditioning with an organic solvent followed by an aqueous solvent.

Normal Phase Chromatography: Normal phase packing's include silica, amino and alumina. Normal phase packing generally requires conditioning with a non-polar solvent and elution is carried with polar solvents. Compounds which are with basic pH functional groups are retained by silica. However, polar compounds are irreversibly retained on a silica surface and in this case amino may be used.

Steps in LC-MS/MS method development

Proper knowledge about the sample is necessary for an effective method development. Some information regarding the analyte is necessary, they are:

- Number of compounds present
- Molecular weights of compound
- Sample Solubility
- Drug Stability
- Concentration range of compounds in samples of interest

Method optimization

During the optimization stage, the initial sets of conditions that were evolved during the method development are improved and maximized in terms of resolution and peak shape, plate counts asymmetry, capacity, elution time, detection limits, limit of quantization, and overall ability to quantify the specific analyte of interest. Optimization of a method can follow either of two general approaches such as manual or computer driven. The manual approach includes varying one experimental variable at a time, while holding all others constant, and recording the changes in response .The variables might include flow rates, mobile or stationary phase composition, temperature etc.

Mode of separation technique

Since most of the pharmaceutical compounds are polar in nature so reverse phase chromatography is normally tried first in which a non-polar stationary phase is used. The mobile phase consists of water or buffer and organic phase (acetonitrile or methanol). Hence polar compounds get eluted first and non-polar compounds are retained for a longer time. The stationary phases used in reverse phase chromatography are n-octadecyl (RP-18), n-octyl (RP-8), ethyl (RP-2), phenyl, cyano, diol and hydrophobic polymers. It is the first choice for most samples; especially neutral or un-ionized compounds that dissolve in water-organic mixtures. Normal phase is tried if reverse phase

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Received: May 26, 2021; **Accepted**: June 09, 2021; **Published**: June 16, 2021

Citation: Nandez S (2021) A Note on Liquid Chromatography and Mass Spectrometry. J Clin Exp Pharmacol. 11:284.

fails where the sample may be strongly retained with 100% acetonitrile as mobile phase.

Selection of stationary phase

Prior to selection of column it is necessary to understand the properties of column packing material. Silica tends to dissolve above pH 8 and cross-linked polymeric particles, for example, polystyrene or poly methacrylates are used for separation of bases, which can withstand strongly basic mobile phase. Silica particles have surface silanol groups, -SiOH which are used for chemical bonding of stationary phases by silanization reactions with chlorosilanes. About half of the silanol groups are chemically bonded and the rest are capped with tri methyl silyl groups to render them inert. The most commonly used non-polar bonded phases (for reversed phase chromatography) are C18 and C8 with C18 being the most popular (known as octadecylsilane); C8 is intermediate in hydrophobicity, where C18 is non-polar. The main criterion in selection and optimization of mobile phase is to achieve optimum separation of all the individual impurities and degradants from each other and from the analyte peak. The parameters which need to be considered while selecting and optimizing the mobile phase are buffer, pH of the buffer and mobile phase composition.

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