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A STUDY OF GROWTH OF WILLPOWER OF PHARMACEUTICALMEDICINES IN HUMAN PLASMA

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ABSTRACT

The developed bioanalytical methods for estimation of novel pharmaceutical medicines like febuxostat, clebopride, darifenacin, cycloserine and carbocisteine from human plasma employing protein precipitation, solid phase extraction and liquid liquid extraction as extraction technique. In these methods sample preparation is faster, simple and easy for the determination of all these novel medicines in human plasma. These methods developed are accurate and precise validated as per regulatory guideline. These methods are rugged and the interferences from the plasma did not affect the peak response at the retention time of internal standard. These methods has resulted all validation parameter as per regulatory guidelines and thus can be of use in the clinical studies. Stability of all medicines determined at room temperature and at freezing temperature. The validated method for febuxostat allows determination in the linearity range 49.896 to 10060.888 ng/ml. Lansoprazole was used as internal standard. The m/z of febuxostat was 317.1>261.0 and for lansoprazole was 370.1>251.9. The protein precipitation was used as extraction method. The Hypurity C18 (100x4.6mm), 5 µ column were used for chromatographic separation. The run time was 3.3 minutes and volume ofr injection was set 2 µl. The validated method for clebopride allows determination in the linearity range 0.051 to 10.352 ng/ml. Cinitapride was used as internal standard. The m/z of febuxostat was 374.2>184.1 and for cinitapride was 403.3>209.2. The extraction method was solid phase extraction. The injection volume was 15 µl and runtime was 2.2 minutes. The Hypersil Gold C18 (50 x 4.6mm), 5 µ column were used for chromatographic separation. The validated method for darifenacin allows determination in the linearity range 018 to 10025.334 pg/ml. Darifenacin-d4 was used as internal standard. The m/z of febuxostat was 427.400>431.500 and for darifenacin-d4 was 147.100>151.100. The liquid- liquid extraction was used as extraction method. The volume foe injection was set 15 µl and runtime was 2.6 minutes. The Zorbax XDB-C18 (50 x 4.6 mm), 5 µ column was used for chromatographic separation.

KEYWORDS: Growth, Willpower, Pharmaceutical Medicines, Human Plasma, bioanalytical methods

INTRODUCTION

Bioanalytical chemistry is the determination of medicines substances in biological fluids like plasma, serum in terms of qualitative and quantitative analysis. It plays important role in evaluation of pharmacokinetic parameters which required for bioavailability and bioequivalence studies. The validation of developed method required to assure common level of quality therefore use of validated methods increased in current practice.

Analytical methods plays important role in research and development of new product. The quality assurance and quality control are the

main control parameters used in method development and validation. The chromatographic and spectrometric or spectroscopic methods are selected on the basis of their characteristics, features along with deficiencies. The selected method for determination must be gone through every stage of all validation parameters. Investigation of each stage must be determine in same procedural variables, same environment and same matrix which can affect analyte evaluation in biological matrix started from clinical sample collection to sample analysis. Method validation will be start after confirmation of complete method development. Full method validation will be start after promising results of method development because the method developer doesn't have idea about the actual method conditions during method validation. Method development mainly involves evaluation of different conditions as per analyte nature and optimization of those conditions accordingly. The important stages in method development are preparation of sample, separation of prepared sample by using chromatography, detection of separated sample by using suitable detection method. To start method development of newer analyte extensive literature survey required. After literature survey the primary importance given to summarized import points and determines the concept of future work. Literature survey information will be used to select instrument that is comfortable for analysis. This includes analyticalcolumn, high performance liquid chromatography system and detector system like Mass spectrometry. Another parameter is internal standard, select suitable internal standard as per analyte parameters. Also select suitable extraction method which will give high recovery, accuracy and precision along with economical as industrial prospective.

LITERATURE REVIEW

Grabowski B. et al (2006) explained the pharmacokinetic and pharmacodynamics results along with safety of medicines in a dose escalation study in healthy subjects. The research article concludes that medicines showing linear kinetics in arrange 10-120 mg and it is well tolerated in single dose 10 to 240 mg on daily basis.

Joseph R. N. et al (2008) explained about the pharmacokinetic and pharmacodynamics results along with safety in healthy subjects by considering age and gender effect. In the comparison of male and female study data the area under the concentration time curve and unbound peak concentration were higher in Female than male (31.5 vs 23.6 ng/mL and 62.8 vs 53.9 ng/mL).

Dafang Z. et al (2013) developed a protein precipitation extraction method by using acetonitrile. The chromatographic analysis done by using a Capcell PAK C18 column (4.6×100 mm, 5mm). Mobile phase was used as acetonitrile to 5mM ammonium acetate to formic acid (85 : 15 : 0.015, v/v/v) with chromatography flow rate of 0.6 mL/min. The run time was 3 min. Agilent 6460 electrospray tandem mass spectrometer where used by using MRM mode. The quantitation done by using ion transitions m/z 317 > 261 (febuxsotat) and m/z 324 > (261 + 262) (d7-febuxostat, IS). The linearity range was used 10.0–5000 ng/ml.

Hande A. et al (2012) described liquid-liquid extraction technique for medicines from human plasma. The internal standard was used trandolapril. The column BEH C18, 50 mm X 2.1 mm, 1.7 µm was used for analysis and mobile phase was consisting of 0.1% formic acid: acetonitrile in 25:75 ratios. The method run time was 1.5 min. The method resulted LLOQ was 0.075 µg/ml.

Abdul M. A. et al (2011) described that the medicines was highly soluble in methanol so it was selected as the solvent system for the medicines. The linearity range for medicines at its wavelength of detection of 315 nm was obtained as 0.2–15 µg/ml. The LLOQ was resulted 0.5281 µg /ml.

Bhalekar. M. R. et al (2011) has developed a RP-HPLC method for medicines in bulk and pharmaceutical dosage forms. The column used as C18 column 250 x 4.6 mm, 5µm and mobile phase consisting of methanol: ortho-phosphoric acid in the ratio of 90:10 v/v. The flow rate was finalized 1ml per minute and detected by UV detector at 316nm. The run time was 5.28 min. The quantification range was 10-100 µg/ml.

Chitaranjan M. et al (2012) described separation of medicines by using C18 column. The mobile phase was consisted with sodium acetate buffer (pH 4.0): acetonitrile (40:60, v/v). The rate of flow was 1.2 ml per minute and ultraviolet detection at 254 nm. Linearity range was 0.1–200 µg/ml. The LLOQ was 0.0783 µg/ml.

Rama R. N. et al (2012) conducted chromatographic separation on Nucleosil C18 (250 x 4.6mm, 5µm) column by using 10 mM ammonium acetate buffer (pH 4.0 adjusted with 0.2% triethyl amine) and acetonitrile in the ratio (15: 85, v/v) as a mobile phase. The rate of flow was 1.2 ml per minute and it detected by UV at 275nm. The run time was 3.45 ± 0.05 min. The linearity range was 50.0 – 400.0 µg/ml. The LLOQ was 30.23 µg/ml.

Elphine P. A. et al (2009) has developed UV method for determination of medicines in pure and in pharmaceutical formulation. The absorption detected at 263nm in the linearity range 20-100 ug/m.

Grandmaison C. et al (2002) has developed method for cisapride and noncisapride in human urine by API 3000 LC/MS. Cisapride and norcisapride were assayed from a concentration of 1.00 to 150.00 ng/mL and 0.87 to 130.00 ng/mL respectively using Clebopride as internal standard. The extraction was performed using Varian C8 1cc (100 mg) cartridges. A 0.1 M ammonium acetate solution was added to the cartridges, followed by the ISWS and the sample urine. Cartridges were washed with buffer, a solution of Acetonitrile in water and water. Cisapride and norcisapride were eluted using 1% NH3 in methanol. Chromatography was achieved on a Phenomenex Luna C18 analytical column. Run time was 2.50 minute. Positive ion mass spectrometry was used monitoring 466.2 > 184.1 m/z for Cisapride and 314.3 > 184.1 m/z for norcisapride.

Dongsheng O. et al (2010) explain method for medicines determination in plasma using by using itopride as an internal standard. The liquid–liquid extraction used as sample separation method. The chromatographic isocratic gradient mode was used. CAPCELL MG-III C18 (150 mm × 2.1 mm, 5 µm) column was used. The electrospray ionization was used along with MRM interface using the respective [M+H]+ ions, mass to charge ratio 373.9 →184.0 for clebopride, 359.9 → 71.5 for itopride. The linearity range was 69.530–4450.0 pg/ml.

HPLC METHOD FOR ANALYSIS OF MEDICINESS

HPLC method having several advantages like easy automation, rapidity, specificity, accuracy and precision therefore HPLC selected for analysis of most of the medicines. HPLC method involves good extraction as well as isolation procedures. Following are the advantages of HPLC:

- Speed
- Greater sensitivity by using different instruments
- Improved resolution by using different stationary phases
- Reusable columns for different medicines analysis.
- Ideally use for low volatility substances
- Easy to handling and maintenance
- Good sample recovery
- Less time and less labor requires.
- Precise and reproducible
- Integrator calculations are done by instrument itself.
- On a larger scale suitable for preparative liquid chromatography

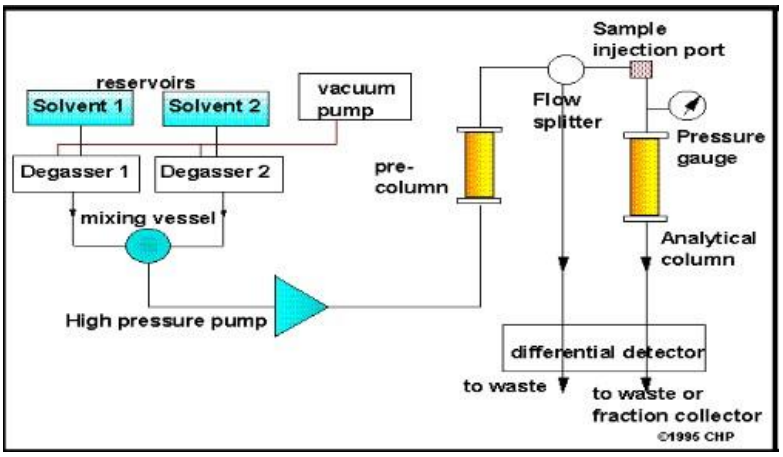

Figure 1: A schematic diagram of HPLC equipment

METHOD DEVELOPMENT AND DESIGN OF SEPARATION METHOD ON HPLC

Analytical method development requires knowledge about the nature of medicines, molecular weight, polarity, solubility ionic character of medicines. On the basis of all this parameters we can develop method for particular medicines into multi component dosage form. Method development never provide exact results at the initiation of development, it is a trial and error basis activity. The most problematic phase is selection of mobile phase and selection of analytical column. The most preferable type is reverse phase chromatography because of most of the compounds are hydrophilic, polar groups and water soluble in nature. Method development and design of separation method depends on selection of best mobile phase, detector, column length and diameter, buffer, pH of buffer, type of stationary phase etc.

a) The Best Mobile Phase

The basic principle of reverse-phase chromatography is mobile phase is polar in nature and stationary phase is non-polar in nature. Mobile phase generally made up of two or more solvents with additives and buffers. The initial approach is selection appropriate analytical column with proper mobile phase. The separation molecule andoptimize the retention of molecule is the second stage after selection of mobile phase. Separation is depending on different degrees of hydrophobicity of solute. The rate of elution of molecule in the mobile phase is control by

the organic modifier. The rate of elution is increased by reducing the polarity. Small change into the mobile phase composition changes different parameters like elution rate, flow rate. The simple alteration of mobile phase composition or rate of flow allows the rate of the elution of solutes to be adjusted to an optimum value and permits the wide scope separation of the chemical types. First isocratic run followed by gradient run is preferred.

b) The Best Detector

The next consideration should be the choice of detector. There is little use in running a separation if detector one uses cannot "see" all the components of interest, or conversely, if it "sees" too much. UV-visible detectors are the most popular as they can detect a broad range of compounds and have a fair degree of selectivity for some analytes. Unfortunately UV-visible detectors are not universal detectors so it is worthwhile to see the chemical nature of the molecule, if it has suitable chromophores, such as aromatic rings, for UV-visible detection.

c) The Best Column Length

Many chromatographers make the mistake of simply using what is available. Often this is a 250 × 4.6 mm C18 column. Due high plate count and wide selectivity of this column, it resolves many of the molecules therefore commonly used in most of the laboratories. While many reverse phase separations can be carried out on such column, its high resolving capabilities are often unnecessary, as illustrated in Figure 2. Method development can be started with shorter columns; 150, 100 or even 50 mm long. This is simply because they have proportionally shorter run times.

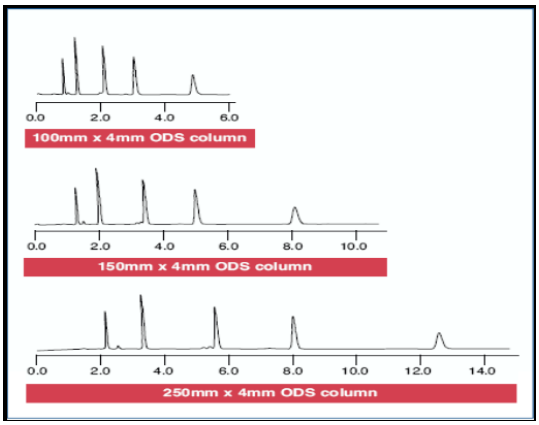

Figure 2: Effect of Column length

d) The Best Stationary Phase

The efficiency of development in method can be help by selecting appropriate stationary phase. For example, reverse phase chromatography the C_8 phase column can be provide better time than C_{18}, because it not retain molecules as strongly as theC_{18} phase. Normal phase chromatography mainly prefers

cyano (nitrile) phases columns because of the versatile nature.

e) The Best Internal Diameter

The optimization of mobile phase and elution rate can be minimized if run time is minimized and for minimization of run time selection of shorter column with appropriate phase necessary. Consideration of column internal diameter may be advantageous for method development. Most preferable column internal diameter is 4.6 mm in analytical laboratories because this require 75% of the solvent flow, this results 25% solvent saving analytical column life and it is more significant if a routine method.

f) Gradient Programming

Gradient programming of mobile phase is considered as fastest and easiest way for method development. In it firstly start with weak solvent and end with suitable solvent strength. Gradient programming start with very fast gradient and after it optimize as per proper separation with adjusting mobile phase combination. Different solvent and buffers may be used for optimization of mobile phase during method development. In HPLC systems different results expected in different system with same gradient therefore method requires validating into different laboratories, isocratic method recommended. The optimization of mobile phase is directly proportional to improvement of separation of molecule. In it different factors consider for solvent selection.

g) Retention

The solvent strength increases which can strongly impact on retention of analyte. The reverse phase chromatography point of view a higher percentage of organic solvent requires in mobile phase. The organic solvent helps to retain the molecule with fast rate.

h) Poor Separation

Generally analyte elute with its impurities and metabolites. To separate all impurities and metabolites along with molecules, different solvent strength uses from lower strength to higher strength so far best separation may be determined. During method development different types of organic solvent is used for better separation e.g. select acetonitrile instead of methanol. By changing pH of buffers may impact on separation of molecule with considering column recommended pH. After achieving optimum condition of separation, improve resolution by changing column length, particle size to increases the analytical column efficiency e.g, in reversed phase analysis started with C8 100mm column will changes with C18 for better resolution may be observe. Along with this select shorter column in this stage for saving time of analysis.

i) Peak Shape

The peak shape is most important factor in chromatographic separation in method development. In this mainly problem creates for basic nature compounds which analyzed by reverse phase chromatography. The high purity silica phase like Wakosil II is preferred for minimization of potential problems. Due to use of modern phase secondary interactions expected minimal. Generally buffers with proper pH selected for sharp peak shape. After getting proper sharp peak any problem remain continue then use organic modifier such as triethylamine but this type of modifier not necessary in modern silica phases such as Wakosil. The auto sampler temperature impact on peak shape is most important consideration during method development. The auto sampler constant temperature maximizes the reproducibility of method. The recommended temperature for better peak shape and separation is 35 to 40°C.

j) Buffer selection

In reverse phase HPLC, according to hydrophobicity of molecules the retention takes place. If hydrophobicity is longer, retention of molecule is longer. When ionization of molecule takes place proportionally hydrophobicity reduces and it result decreasing retention of molecule. In reverse phase HPLC the mobile phase contains acid and bases therefore controlling pH of mobile phase is necessary for better reproducible results.When separating acids and bases a buffered mobile phase is recommended to maintain consistent retention and selectivity. A buffered mobile phase, by definition, resists changes in pH so that the analytes and silica will be consistently ionized, resulting in reproducible chromatography. In case of neutral samples, buffers or additives are not required in mobile phase. Generally addition of buffer in mobile phase requires for acids and bases samples. Less acidic reverse phase columns and amine additives in mobile phase are beneficial for basic or cationic samples. When pKa of the buffer is equal to pH of molecule it results optimum buffering capacity of molecule.

Table 1: HPLC buffers

Buffer	P^{Ka} (25°C)	Maximum Buffer Range	UV Cutoff (nm)
Phosphate,p^{K}_1 H_2PO_4	2.1	1.1-3.1	< 200
Phosphate,p^{K}_2 HPO_4^{2-}	7.2	6.2-8.2	< 200
Phosphate,p^{K}_3 PO_4^{3-}	12.3	11.3-13.3	< 200
Citrate, p^{K}_1 $C_3H_5O(COOH)_2(COO)^{1-}$	3.1	2.1-4.1	230
Citrate, p^{K}_2 $C_3H_5O(COOH)_1(COO)^{2-}$	4.7	3.7-5.7	230
Citrate, p^{K}_3 $C_3H_5O(COO)^{3-}$	6.4	4.4-6.4	230
Carbonate, p^{K}_1 HCO_3^{1-}	6.1	5.1-7.1	< 200
Carbonate, p^{K}_2 CO_3^{2-}	10.3	9.3-11.3	> 200
Formate	3.8	2.8-4.8	210
Acetate	4.8	3.8-5.8	210
Ammonia	9.3	8.3-10.3	200
Borate	9.2	8.2-10.2	N/A

k) Selection of pH

The pH range 1 to 8 mostly used in reversed-phase chromatography can be divided into low pH (1 to 4) and intermediate pH (4 to 8) ranges. Each range has a number of advantages. Low pH has the advantage of creating an environment in which method ruggedness is increases with decreases of peak tailing. For this reason, operating at low pH is recommended. If pH of mobile phase is more than 7 then dissolution of silica takes place which directly shorten lifetime of columns containing silica-based stationary phases. The pKa value [acid dissociation (ionization) constant] for a compound is the pH at which equal concentrations of the acidic and basic forms of the molecule are presentin aqueous solutions. Analytes may sometimes appear as broad or tailing peaks when the mobile phase pH is at, or near, their pKa values. The difference between pH and pka of analyte is 1, it consider as more rugged mobile phase. This shifts the equilibrium so that 99% of the sample will be in one form. The result is consistent chromatography.

CONCLUSION:

The present study is confined to verify the status of the bioanalytical method defines the determination or quantification of analytic or medicines and their metabolites in biological matrices such as plasma, urine, and serum. For reliability it must be validated. Bio analytical method overall procedures involved collection of sample at clinical phase, processing of collected sample, storage of collected sample, and analysis of collected biological matrix for determination of medicines. Bio analytical method validation involves following major activities such as selectivity, accuracy, precision, recovery, sensitivity, and stability. The selective and sensitive bio analytical methods require for the creation of accurate pharmacokinetics, bioavailability, and bioequivalence of medicines data. Validated Bio analytical method is used for quantitative determination of medicines and their metabolites in biological fluids. The difference between different makes of instrument is not surprising and should be considering for method development. Limit of detection and calibration curve are the most important parameters and it's necessary to determined prior to method development as it is platform for future method development work. The signal is measured in blank plasma and measured from base line to peak of apex and divided by peak to peak. The calibration curve is determined between lowest concentration of analytic to the highest required concentration as per reported Cmax concentration.

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