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ESTIMATION OF XIPAMIDE BY USING HPLC IN PURE AND PHARMACEUTICAL DOSAGE FORM

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Abstract:

A rapid and precise Reverse Phase High Performance Liquid Chromatographic method has been developed for the validated of Xipamide in its pure form as well as in tablet dosage form. Chromatography was carried out on Symmetry C18 (4.6×150mm, 5µ) column using a mixture of Acetonitrile:Water(50:50v/v)as the mobile phase at a flow rate of 1.0ml/min, the detection was carried out at 245nm. The retention time of the Xipamide was 2.4 ±0.02min. The method produce linear responses in the concentration range of 10-50µg/ml of Xipamide. The method precision for the determination of assay was below 2.0%RSD. The method is useful in the quality control of bulk and pharmaceutical formulations.

Keywords: *Xipamide, RP-HPLC, validation.*

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INTRODUCTION:

Analysis may be defined as the science and art of determining the composition of materials in terms of the elements or compounds contained in them. In fact, analytical chemistry is the science of chemical identification and determination of the composition (atomic, molecular) of substances, materials and their chemical structure.

Every country has legislation on bulk drugs and their pharmaceutical formulations that sets standards and obligatory quality indices for them. These regulations are presented in separate articles relating to individual drugs and are published in the form of book called "Pharmacopoeia" (e.g. IP, USP, and BP) Quantitative chemical analysis is an important tool to assure that the raw material used and the intermediate products meet the required specifications [1-6]. Every year number of drugs is introduced into the market. Also quality is important in every product or service, So, it becomes necessary to develop new analytical methods for such drugs (Dr. Kealey and P.J Haines)

CLASSIFICATION OF ANALYTICAL METHODS

The following techniques are available for separation and analysis of components of interest.

Spectral methods

The spectral techniques are used to measure electromagnetic radiation which is either absorbed or emitted (or) transmitted etc.

E.g.UV-Visible spectroscopy, IR spectroscopy, NMR, ESR spectroscopy, Flame photometry, Fluorimetry (A.braithwait and F.J.Smith)

Electro analytical methods

Electro analytical methods involved in the measurement of current voltage or resistance as a property of concentration of the component in solution mixture.

E.g. Potentiometry, Conductometry, Amperometry.

Chromatographic methods

Chromatography is a qualitative and quantitative analytical technique where in a sample mixture is subjected to preferential separation into different components under the influence of a moving phase (mobile phase) over a stationary phase. These separated components are quantified then by suitable parameter.

Chromatography techniques are E.g. Paper chromatography, thin layer chromatography (TLC), High performance thin layer chromatography (HPTLC), High performance liquid chromatography (HPLC), Gas chromatography (GC).

Miscellaneous Techniques

Thermal Analysis DSC, DTA, Gravimetry etc.

Hyphenated Techniques

GC-MS (Gas Chromatography – Mass Spectrometry), LC-MS (Liquid Chromatography – Mass Spectrometry), ICP-MS (Inductivity Coupled Plasma- Mass Spectrometry), GC-IR (Gas Chromatography – Infrared Spectroscopy), MS-MS (Mass Spectrometry – Mass Spectrometry).

Analytical techniques that are generally used for drug analysis also include biological and microbiological methods, radioactive methods and physical methods etc (A.braithwait and F.J.Smith)

INTRODUCTION TO HPLC [7-10]

HPLC is also called as high pressure liquid chromatography since high pressure is used to increase the flow rate and efficient separation by forcing the mobile phase through at much higher rate. The pressure is applied using a pumping system. The development of HPLC from classical column chromatography can be attributed to the development of smaller particle sizes. Smaller particle size is important since they offer more surface area over the conventional large particle sizes. The HPLC is the method of choice in the field of analytical chemistry, since this method is specific, robust, linear, precise and accurate and the limit of detection is low and also it offers the following advantages.

1. Improved resolution of separated substances
2. column packing with very small (3, 5 and 10 µm) particles
3. Faster separation times (minutes)
4. Sensitivity
5. Reproducibility
6. continuous flow detectors capable of handling small flow rates
7. Easy sample recovery, handling and maintenance

Types of HPLC Techniques [11-14]

Based on Modes of Chromatography

These distinctions are based on relative polarities of stationary and mobile phases

Reverse phase chromatography: In this the stationary phase is non-polar and mobile phase is polar. In this technique, the polar compounds are eluted first and non polar compounds are retained in the column and eluted slowly. Therefore it is widely used technique.

Normal phase chromatography: In this the stationary phase is polar and mobile phase is non-polar. In this technique least polar compounds travel faster and are eluted first where as the polar

compounds are retained in the column for longer time and eluted**.**

Based on Principle of Separation

Liquid/solid chromatography (Adsorption): LSC, also called adsorption chromatography, the principle involved in this technique is adsorption of the components onto stationary phase separation of the components occurs by affinity of components toward stationary phase. when the sample solution is dissolved in mobile phase and passed through a column of stationary phase. The basis for separation is the selective adsorption of polar compounds; analytes that are more polar will be attracted more strongly to the active silica gel sites. The solvent strength of the mobile phase determines the rate at which adsorbed analytes are desorbed and elute.It is widely used for separation of isomers and classes of compounds differing in polarity and number of functional groups. It works best with compounds that have relatively low or intermediate polarity.

Liquid/Liquid chromatography (Partition Chromatography): LLC, also called partition chromatography. In liquid-liquid chromatography two phases are liquids. The two liquids must be immiscible. The stationary liquid is present as thin film on an inert solid support and it is usually the more polar liquid (aqueous phase). Separation accurse due to the difference in partition coefficient of solute between the two liquids

Ion exchange: In this the components are separated by exchange of ions between an ion exchange resin stationary phase and a mobile electrolyte phase. A cation exchange resin is used for the separation of cations and anion exchange resin is used to separate a mixture of anions (Andrea Weston and Phyllisr).

Size exclusion: In this type, the components of sample are separated according to their molecular sizes by using different gels (polyvinyl acetate gel, agarose gel). ex: separation of proteins, polysaccharides, enzymes and synthetic polymers.

Chiral chromatography: In this type of chromatography, optical isomers are separated by using chiral stationary phase.

Affinity chromatography: In this type, the components are separated by an equilibrium between a macromolecular and a small molecule for which it has a high biological specificity and hence affinity (Andrea Weston and Phyllisr)

Based on elution technique

Isocratic separation: In this technique, the same mobile phase combination is used throughout the process of separation. The same polarity or elution strength is maintained throughout the process.

Gradient separation: In this technique, a mobile phase combination of lower polarity or elution strength is followed by gradually increasing polarity or elution strength (Andrea Weston and Phyllisr).

Based on the scale of operation

Analytical HPLC: Where only analysis of samples are done. Recovery of samples for reusing is normally not done, since the sample used is very low. Ex: µg quantities.

Preparative HPLC: Where the individual fractions of pure compounds can be collected using fraction collector. The collected samples are reused. Ex: separation of few grams of mixtures by HPLC(Yuri Kazakevich and Rosario Lobrutto).

Based on type of analysis

Qualitative analysis: Which is used to identify the compound, detect the presence of impurities to find out the number of components. This is done by using retention time values.

Quantitative analysis: This is done to determine the quantity of individual or several components of mixture. This is done by comparing the peak area of the standard and sample (Andrea Weston and Phyllisr).

INSTRUMENTATION OF HPLC

The basic liquid chromatography consists of six basic units. The mobile phase supply system, the pump and programmer, the sample valve, the column, the detector and finally a means of presenting and processing the results.

Mobile phase (solvent) reservoirs and solvent degassing: The mobile phase supply system consists of number of reservoirs (200 mL to 1,000 mL in capacity). They are usually constructed of glass or stainless steel materials which are chemically resistant to mobile phase**.**

Mobile phase

Mobile phases in HPLC are usually mixtures of two or more individual solvents. The two most critical parameters for nonionic mobile phases are strength and selectivity.

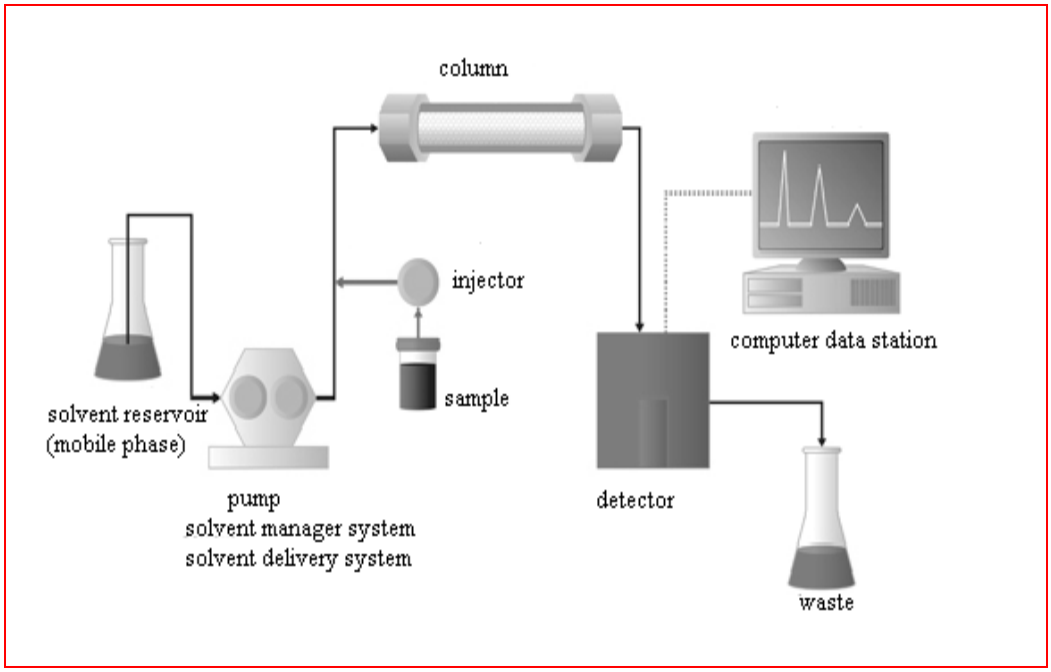

Fig.1: Components of HPLC instrument block diagram.

Mobile phase preparation
Mobile phases must be prepared from high purity solvents, including water that must be highly purified. Mobile phases must be filtered through ≤1 µm pore size filters and be degassed before use.

Degassing of solvents
Many solvents and solvent mixtures (particularly aqueous mixtures) contain significant amounts of dissolved nitrogen and oxygen from the air. These gasses can form bubbles in the chromatographic system that cause both serious detector noise and loss of column efficiency. These dissolved gases in solvent can be removed by the process of degassing. Every solvent must be degassed before introduction into pump as it alter the resolution of column and interfere with monitoring of the column effluent.

Xipamide 4-chloro-N-(2,6-dimethylphenyl)-2-hydroxy-5-sulfamoylbenzamide Like the structurally related thiazide diuretics, xipamide acts on the kidneys to reduce sodium reabsorption in the distal convoluted tubule. This increases the osmolarity in the lumen, causing less water to be reabsorbed by the collecting ducts. This leads to increased urinary output. Unlike the thiazides, xipamide reaches its target from the peritubular side (blood side). Additionally, it increases the secretion of potassium in the distal tubule and collecting ducts. In high doses it also inhibits the enzyme carbonic anhydrase which leads to increased secretion of bicarbonate and alkalizes the urin.

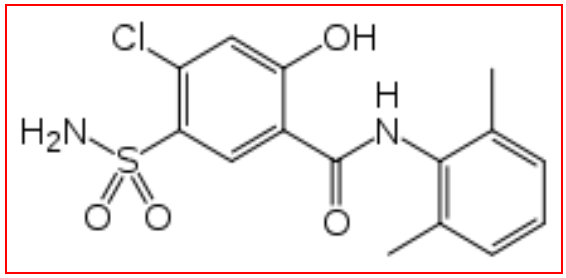

Fig. 2: chemical structure of Xipamide

MATERIALS AND METHODS:

Accurately measured 500ml (50%) of HPLC Acetonitrile and 500ml of Water (50%) were mixed and degassed in a digital ultrasonicator for 10 minutes and then filtered through 0.45 µ filter under vacuum filtration.

Instrumentation and Chromatographic conditions
The analysis was performed by using Symmetry C18 column, 4.6×250mm internal diameter with 5 micron particle size column and UV detector set at 245nm nm, in conjunction with a mobile phase of Acetonitrile:: Water in the ratio of 50:50v/v (pH 5 adjusted with OPA) at a flow rate of 1 ml/min. The retention time of Xipamide was found to be 2.635 minute. The 10µl of sample solution was injected into the system

Preparation of standard solution:
Accurately weighed and transferred 10mg of Xipamide working standard into a 10ml of clean dry volumetric flasks, added about 7ml of Diluents and sonicated to dissolve it completely and made volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.3ml of the above Xipamide stock solutions into a 10ml volumetric flask and diluted up to the mark with diluents.

Mobile Phase Optimization:
Initially the mobile phase tried was Methanol: Water, Acetonitrile: Water with varying proportions. Finally, the mobile phase was optimized to Acetonitrile and Water in proportion 50:50 v/v respectively.

Optimization of Column:

The method was performed with column like Symmetry C18 (4.6×250mm, 5µm) was found to be ideal as it gave good peak shape and resolution at 1ml/min flow

(Optimized chromatogram):

Column : Symmetry C18 (4.6×250mm) 5µ

Column temperature : Ambient

Wavelength : 245nm

Mobile phase ratio : Acetonitrile:Water(50:50v/v)

Flow rate : 1ml/min

Injection volume : 10µl

Run time : 5minutes

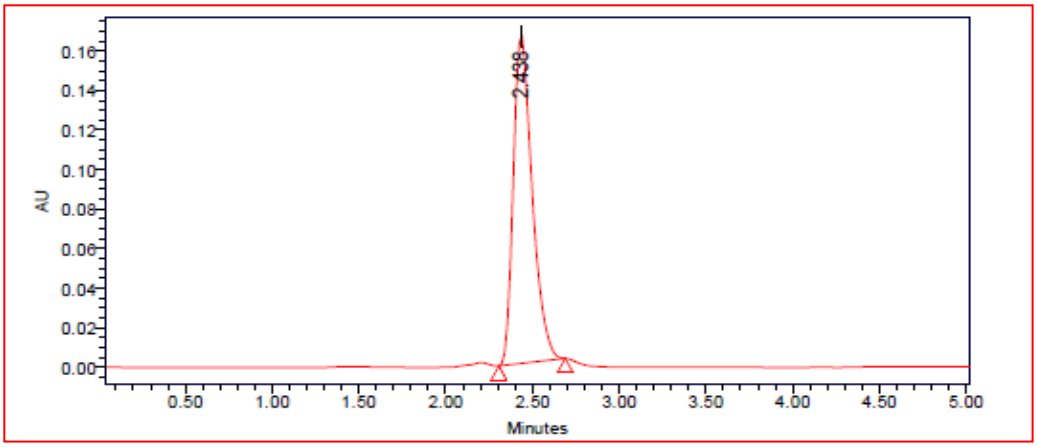

Fig. 3 Typical chromatogram of mixture of Standard solution

VALIDATION

PREPARATION OF MOBILE PHASE:
Preparation of mobile phase:
Accurately measured 500ml (50%) of HPLC Acetonitrile and 500ml of Water (50%) were mixed and degassed in a digital ultrasonicator for 10 minutes and then filtered through 0.45 µ filter under vacuum filtration.

Diluent Preparation:
The Mobile phase was used as the diluent.

Linearity
The linearity of was obtained in the concentration ranges from 10- 50 µg/ml

Table 1: Linearity data of Xipamide

Concentration Level (%)	Concentration µg/ml
60	10
80	20
100	30
120	40
60	50

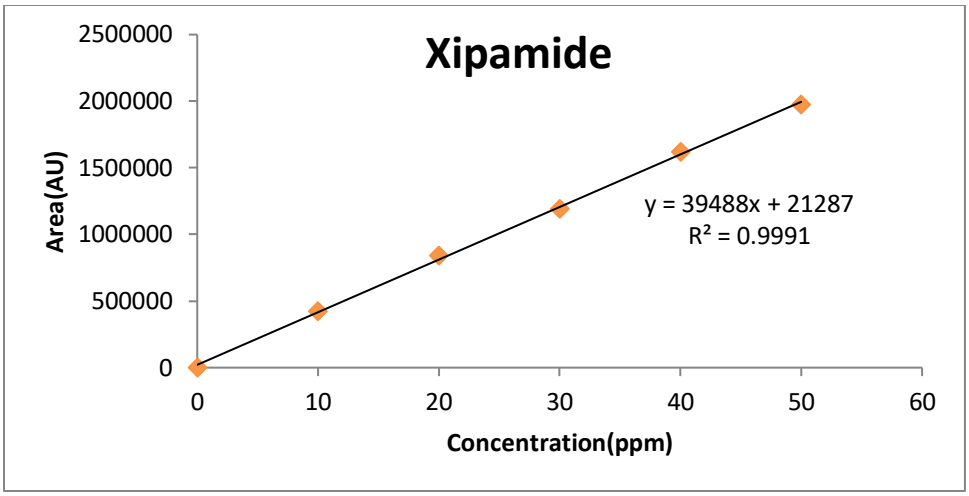

Fig.4. calibration graph of Xipamide

LINEARITY PLOT

Linearity of detector response of assay method was found by injecting seven standard solutions with concentration ranging from 10-50 µg/mL for Xipamide. The graph was plotted for concentration versus peak area. The results were shown in Table-1 and fig 4.

Precision

Repeatability

The precision of test method was determined by preparing six test preparations using the product blend and by mixing the active ingredient with excipients as per manufacturing formula. And the relative standard deviation of assay results was calculated. The results were shown in Table 2

Table 2: Results of repeatability for Xipamide

S. No	Peak name	Retention time	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Xipamide	2.436	1262901	164274	7295	138
2	Xipamide	2.437	1265397	165899	5307	1.36
3	Xipamide	2.438	1262726	166600	4347	1.40
4	Xipamide	2.437	1261429	165280	7288	1.36
5	Xipamide	2.436	1263264	166925	8324	1.35
Mean			1263143			
Std.dev			1437.481			
%RSD			0.113802			

Accuracy

Xipamide tablets content were taken at various concentrations ranging from 50 % to 150 % (50 %, 75 %, 100 %, 125 %, and 150 %) to accurately quantify and to validate the accuracy. The assay was performed in triplicate. The results were shown in Table-4

Table 4: The accuracy results for Xipamide

%Concentration (at specification Level)	Peak area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	613351	15	14.9	99.9	99.9%
100%	1228859	30	29.9	99.8	
150%	1793477	45	44.9	99.9	

LIMIT OF DETECTION (LOD)

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value.The LOD value for Xipamide 2.7µg µg/ml.

Quantitation limit (LOQ)

The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined. The LOQ value for Xipamide 8.25µg/ml

ROBUSTNESS

The robustness was performed for the flow rate of 1ml/min and mobile phase ratio variation from more organic phase to less organic phase ratio for Xipamide. The method is robust only in less flow condition and the method is robust even by change in the Mobile phase ±5%. The standard sample of Xipamide were injected by changing the conditions of chromatography. There was no significant change in the parameters like resolution, tailing factor and plate count. Table 5

Table 5: Results for Robustness of Xipamide

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1 ml/min	1193622	2.438	7564	1.1
Less Flow rate of 0.9mL/min	1197453	2.885	8365	1.2
More Flow rate of 1.2mL/min	1193735	2.065	6574	1.14
Less organic phase (about 5 % decrease in organic phase)	1195867	2.921	9275	1.2
More organic phase (about 5 % Increase in organic phase)	1194756	2.156	6555	1.1

SUMMARY AND CONCLUSION:

RP-HPLC method was developed for estimation of Xipamide in bulk and Pharmaceutical dosage form. Chromatographic separation was, carried out on Symmetry C18 (4.6×250mm) 5µ column with mobile phase comprising mixture of Acetonitrile: Water (50:50%) at a flow rate of 1.0ml/min, the detection was carried out at 245nm The proposed RP-HPLC method was found to be precise, specific, accurate, rapid and economical for estimation of Xipamide in bulk and in its Pharmaceutical dosage form. The sample recoveries in all formulations were in good agreement with their respective Label Claims and the % RSD values were with in 2 and the method was found to be precise. This method can be used for routine determination of Xipamide in bulk and in Pharmaceutical dosage forms.

Table 6.Summary data for Xipamide

Parameters	Xipamide
Retention Time (min.)	2.438
Linearity (µg/ml)	10-50
Correlation Coefficient (r^2)	0.999
Slope	39488
Y - intercept	21287
LOD (µg/ml)	2.7
LOQ (µg/ml)	8.25
Repeatability (% RSD) n=6	0.113802
Intraday Precision (% RSD) n=6	0.11474
Interday Precision (% RSD) n=6	0.485776
Accuracy (%)	99.9%

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