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Stability Indicating Method Development And Validation For The Estimation Of Antihypertensive Drug Using Rp-Hplc

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ABSTRACT

A simple, reproducible and efficient reverse phase high performance liquid chromatographic method was developed for simultaneous estimation of olmesartan medoxomil (OLM) and hydrochlorothiazide (HCTZ) in combined tablet dosage form. Formulation containing OLM with HCTZ are used as antihypertensive angiotensin II receptor blocker. Chromatography was performed on a 250 mm x 4.6 mm, 5-µm particle size, C8 Qualisil BDS column with a 50:50 (v/v) mixture of buffer and acetonitrile as a mobile phase and the pH was adjusted to

4.7 by adding dilute phosphoric acid. The detection of the combined dosage form was carried out at 225 nm and a flow rate employed was 1 ml min-1. The retention times were 5.074 &

7.242 min for olmesartan medoxomil and hydrochlorothiazide, respectively. Linearity was obtained in the concentration range 20 to 100 µg mL-1 for olmesartan medoxomil and in the range 12.5 to 62.5 µg mL-1 for hydrochlorothiazide, with a correlation coefficient of 0.9956 and 0.989. The result of the analysis were validated statistically and recovery studies confirmed the accuracy and precision of the proposed method

Keywords: *Olmesartan Medoxomil, Hydrochlorothiazide, RP-HPLC, Simultaneous Estimation, UV- Spectroscopy....*

1. INTRODUCTION

Reversed phase HPLC (RP-HPLC) consists of a non-polar stationary phase and a moderately polar mobile phase. One common stationary phase is silica which has been treated with RMe_2SiCl, where R is a straight chain alkyl group such as $C_{18}H_{37}$ or C_8H_{17}. The retention time is therefore longer for molecules which are more non-polar in nature, allowing polar molecules to elute more readily. Retention time is increased by the addition of polar solvent to the mobile phase and decreased by the addition of more hydrophobic solvent. Reversed phase chromatography is so commonly used that it is not uncommon for it to be incorrectly referred to as "HPLC" without further specification.[1,2]

RP-HPLC operates on the principle of hydrophobic interactions which result from repulsive forces between a relatively polar solvent, the relatively non-polar analyte, and the non-polar stationary phase. The driving force in the binding of the analyte to the stationary phase is the decrease in the area of the non-polar segment of the analyte molecule exposed to the solvent.[3]

Mechanism: Retention by interaction of the stationary phase's nonpolar hydrocarbon chain with nonpolar parts of sample molecules.

Stationary phase: It is bonded siloxane with nonpolar functional groups Like n- octadecyl (C-18) or n- octyl (C-8), ethyl, phenyl, -(CH2) n-diol, -(CH2) n-CN.

Mobile Phase: Polar solvents like methanol, acetonitrile, water or buffer (Sometimes with additives of THF or dioxane).4

Applications: Separation of nonionic and ion forming nonpolar to medium polar substances (carboxylic acids hydrocarbons).

4. Sample elution order: Most polar components are eluted first. 5

STEPS INVOLVED IN DEVELOPMENT OF HPLC METHOD 6

Literature Survey:

Here a detailed account of all analytical methods developed for the drug is collected to avoid duplication of the method developed. Details about the structure of the drugs and their physicochemical properties are also collected.

Selection of chromatographic Method:

First, reversed phase should be tried. If not successful, normal phase should be taken into consideration. For ion exchange or ion pair chromatography, first ion suppression by pH control and then reversed phase chromatography should be tried

Selection of stationary phase:

By studying the polarity of sample and mobile phase use of a stationary phase of different polarity should be done to achieve successful separation.

Selection of Column:

The HPLC column is the heart of the method, critical for the separation. The column must possess the selectivity, efficiency and reproducibility to provide good separation. Commonly used reversed phases are C_{18} (octadecyl silane, $USPL_1$), C_8 (octyl silane, $USPL_7$) phenyl ($USPL_{11}$) and cyano (USP L_{18}). They are chemically different bonded phases and demonstrate significant changes in the selectivity using the same mobile phase. For example, a C_8 phase (reversed phase) can prove to be time saving over a C_{18} as it does not retain analytes as strongly as the C_{18} phase. For normal phase applications cyano phases are the most versatile. C_{18} (250 x 4.6mm) column are more often used in the laboratory. These columns are able to resolve a wide variety of compounds due to their selectivity and high plate counts.

Selection of mobile phase:

If the sample contains ionic or ionizable compounds, then use of a buffered mobile phase is recommended to ensure the reproducible results. Under unfavorable circumstances, pH changes as little as 0.1 pH units can have a significant effect on the separation. On the other hand properly used buffer allows controlling the pH easily.

Buffer works best at the pKa of its acid. At this pH, the concentration of the acidic form and the basic form of the buffering species is equal, and the buffering capacity is maximum. Phosphate has two pKa values in the range of interest for silica-based- chromatography. One at pH-2 and the other at pH-7. The pKa of the acidic buffer is 4.75. Citrate has three-pKa value: 3.08, 4.77 and 6.40. Between citrate and phosphate buffers, the entire pH range useful for silica chromatography can be covered. In many cases, silanophillic interactions cause tailing, mostly due to ion-exchange interaction. This can usually be reduced or suppressed by the use of amine-based buffers or by using acidic mobile phases, or a combination there of Whenever buffers or other mobile phase activities are used, the solubility of the mobile phase is check. This is especially true for gradient applications. Acetonitrile is the preferred organic modifier in reverse-phase- chromatography. [7]

Sample preparation:

The sample prepared should be homogeneous. It should be completely soluble in the selected solvent; usually the solvent used to dissolve the sample should be the mobile phase itself or any solvent miscible with the mobile phase.

Chromatographic separation:

After achieving a resolution with a pre-optimized solvent system, to obtain reproducible results following criteria must be satisfied.

Monitoring flow rate.

Keeping the solvent composition intact.

Solvent system must be covered before storage.

Monitoring column temperature.

VALIDATION OF ANALYTICAL TECHNIQUES [8, 9]

Validation is a concept that has been evolving continuously since its first formal appearance in United States in 1978. The concept of validation has expanded through the years to encompass a wide range of activities from analytical methods used for the quality control of drug substances and products to computerized system for clinical trial, labeling or process control. Validation is the overall expression for a sequence of activities in order to demonstrate and document that a specific product can be reliably manufactured by the designed processes, usually, depending on the complexity of today's pharmaceutical products, the manufacturer must ensure.

Validation is a proof that a process works and this must be done using scientific and statistical principles. This is done to establish process capability and to confirm product acceptability. Validation determines process variables and the acceptable limits for these variables and accordingly sets up appropriate in process controls, which specifies alert and action levels. [10]

MATERIAL AND METHOD

Preparation of solutions

Standard solutions:

80 mg Olmesartan and 50 mg Hydrochlorthiazide were weight accurately. Transferred it into 100 ml volumetric flask and 10 ml of methanol were added sonicate it to dissolve and make up the volume with diluents. 5 ml of this solution was transferred to 50 ml volumetric flask and make up the volume with diluents

Sample solution:

Crush & transferred 20 tablets and weight powder equivalent to average weight of tablet. Transferred it to 250 ml volumetric flask, added 10 mL water and sonic ate for 20 min. Made up the volume with diluents. The resulting solution was filtered through 0.45 u membrane filter.

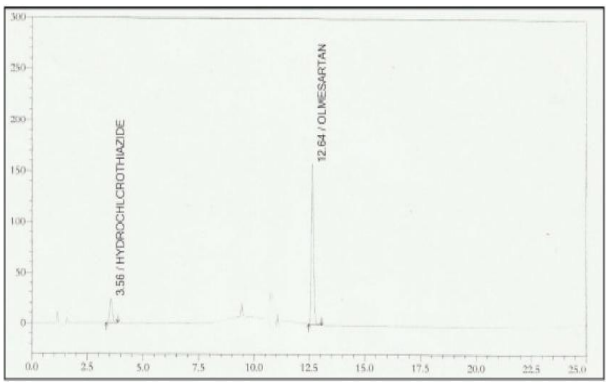
Preparation of mobile phase:

6.8 g of potassium dihydrogen ortho phosphate (KH2PO4) and 5 ml of triethyamine were added in 2000 ml water, solution pH was adjusted to 3.0 with ortho phosphoric acid. The solution was then filtered through 0.45 µ membrane filter and degassed. The mobile phase was prepared by mixing buffer and acetonitrile.

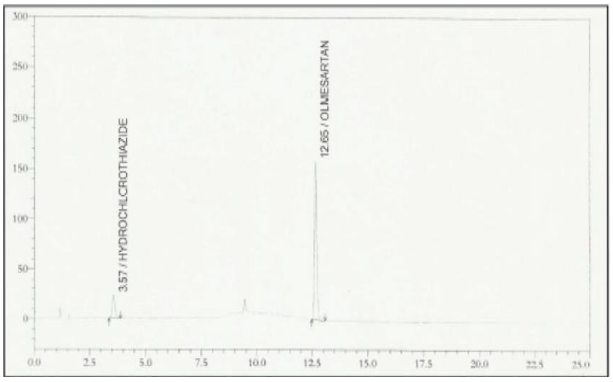
System suitability Test:

System suitability is pharmacopeia requirement and it is used to verify, whether the resolution and reproducibility of chromatographic system are adequate for analysis to be done. The tests were performed by collecting data from six replicate injection of standard drug solution.

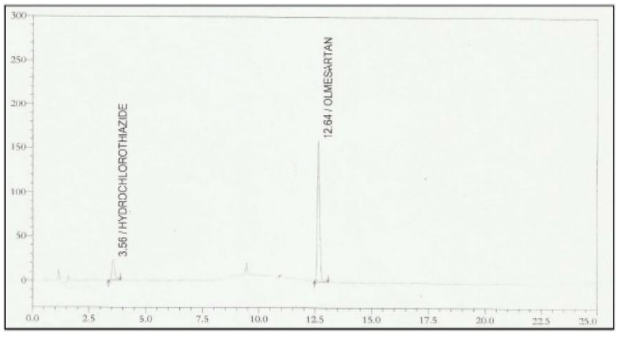
Chromatogram No.1



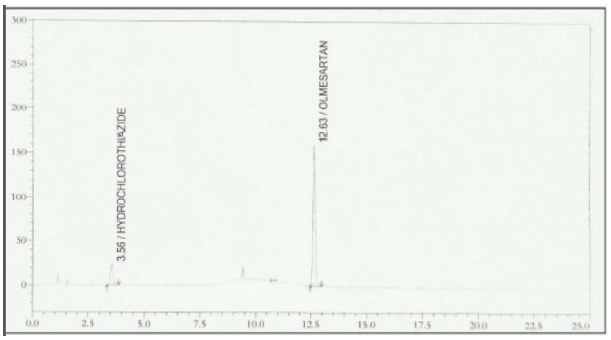
Chromatogram No.2



Chromatogram No. 3



Chromatogram No. 4



Acceptance criteria:

RSD should not be more than 1.0 % for five replicate injections of standard.

USP Tailing factor is not more than 2.0.

The column efficiency as determined for plate count should be more than 2000.

Precision

Preparation of standard solution

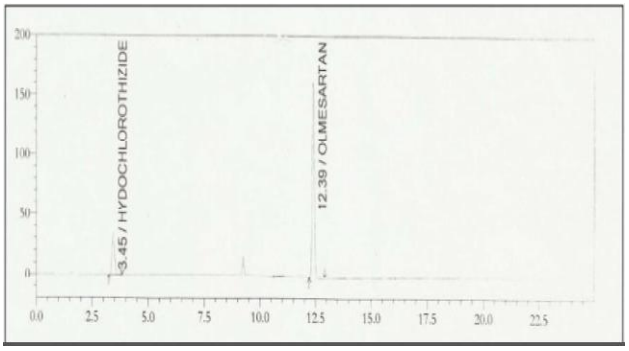
Standard solutions:

80.43 mg olmesartan and 50.38 mg Hydrochlorthazide were weight accurately transferred it in to 100 ml volumetric flask and 10 ml of methanol were added. Sonicate it to dissolve and make up the volume with. Transferred it in to 100 ml volumetric flask and 10 ml of methanol were added Sonicate it to dissolve and make up the volume with diluents. 5 ml of this solution was transferred to 50 ml volumetric flask and made up the volume with diluents.

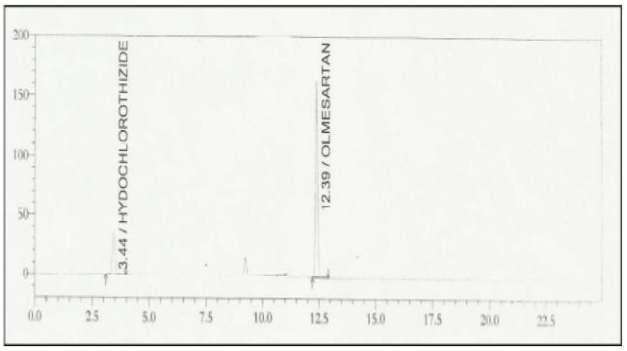
Sample solution:

Crush and powdered 20 tablets and weight powder equivalent to average weight of tablet. Transferred it to 250 ml volumetric flask, added 10 ml water and sonic ate for 20 min. made up the volume with diluents. The resulting solution was filtered through 0.45 µ membrane filter.

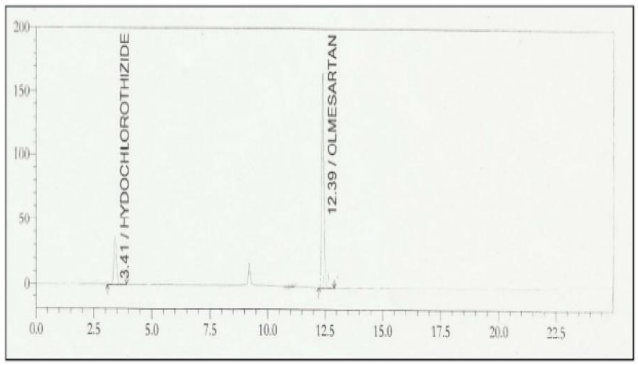
Chromatogram No. 5



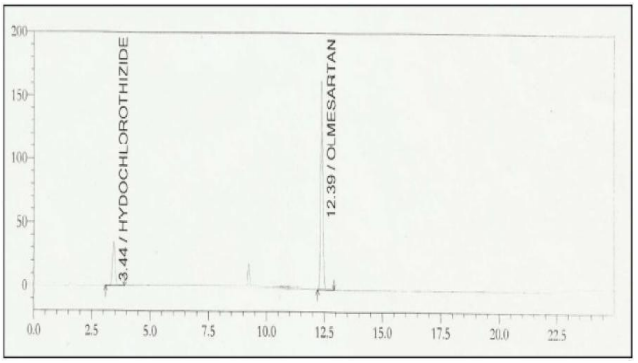
Chromatogram No. 6



Chromatogram No. 7



Chromatogram No. 8



Accuracy:

Preparation of standard solution:

80.80 mg of olmesartan and 50.15 mg Hydrochlothazide were weight accurately. Transferred it into 100 ml volumetric flask and 10ml of methanol were added. Sonicate it to dissolve and made up the volume with diluents. 5 ml of this solution was transferred to 50 ml volumetric flask and made the volume with diluents.

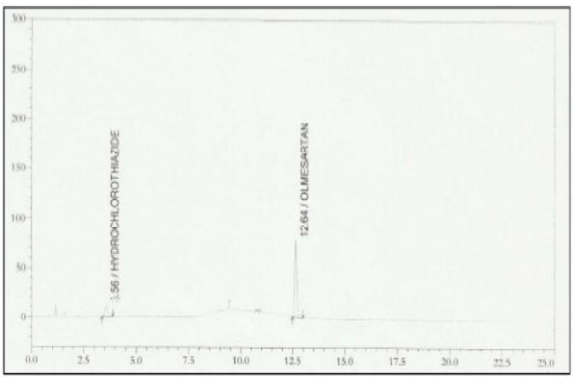
Standard stock solution (Solution A)

200.5 mg of olmesartan and 125.13 mg Hydrochlothazide weight accurately and transferred it into 50 ml volumetric flask, sonic ate to dissolve. Finally made up the volume with diluents

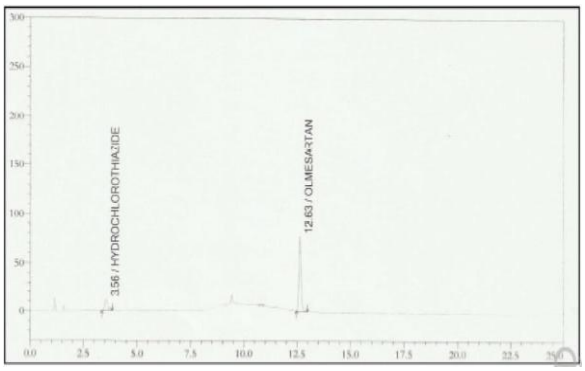
Preparation of sample solution:

50% level: Add 2.5 ml of solution A in 250 ml volumetric flask and made up the volume with diluents. 100 % Level: Add 5 ml of solution A in 250 ml volumetric flask and made up the volume with diluents. 150 % Level: add 7.5 ml of solution A in 250 ml volumetric flask.

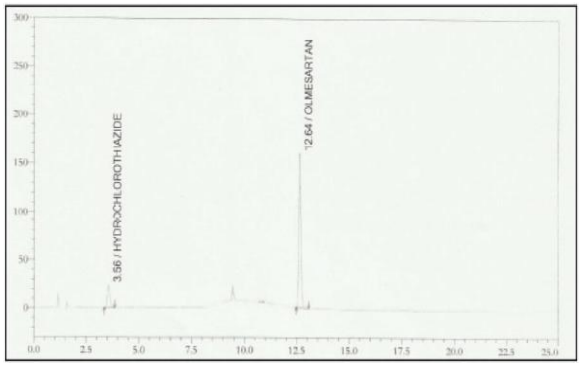
Chromatogram No. 9: 50%- I



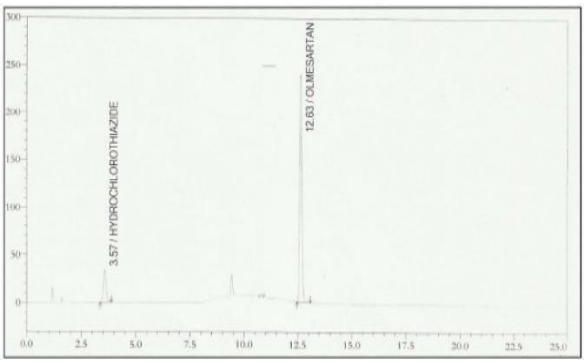
Chromatogram No. 10: 50%- II



Chromatogram No.11: 100%- II



Chromatogram No.12: 150%- II



Linearity:

Standard stock solution (solution A)

200.5 mg olmesartan and 125.13 mg Hydrochlorthiazide were weight accurately and transferred it into 50 ml volumetric flask, with dilute, sonic ate to dissolve. Finally made up volume.

Preparation of standard solution:

50% Level: add 2.5 ml of Solution A in 250 ml volumetric flask and made up the volume with diluents.

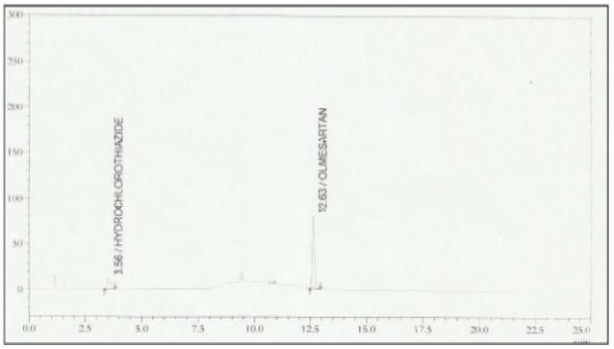
80 % Level: Add 4.0 ml of solution A in 250 ml volumetric flask and made up the volume with diluents.

100% Level: Add 5.0 ml of solution A in 250 ml volumetric flask and made up the volume with diluents.

120% level: Add 6.0 ml of solution a in 250 ml volumetric flask and made up the volume with diluents.

150 % Level: Add 7.5 ml of solution a in 250 ml volumetric flask and made up the volume with diluents.

Chromatogram No. 13: 50%



Specificity:

Placebo preparation:

Powdered placebo 265 mg was weight and treated as per methodology.

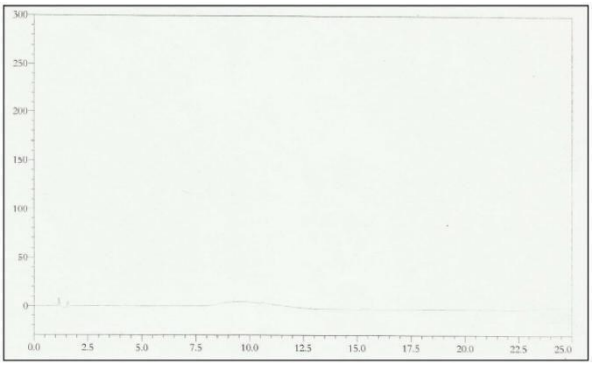
Preparation of standard solution:

80.80 mg olmesartan and 50.15 mg Hydrochlothazide were weight accurately. Transferred it into 100 ml volumetric flask and 10 ml methanol were added. Sonicate it to dissolve and made up the volume with diluents. 5 ml of this solution was transferred to 50 ml volumetric flask and made up the volume with diluents.

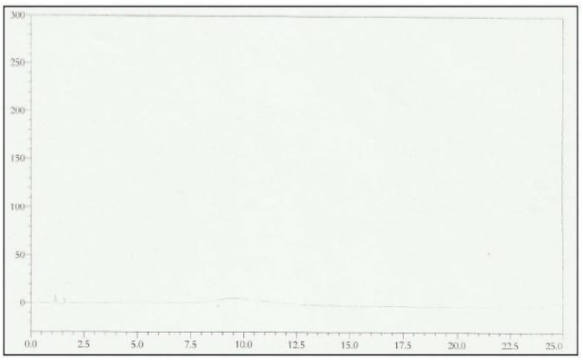
Preparation of sample solution:

Crush and powdered 20 tablets and weight powder equivalent to average weight of tablet. Transferred it to 250 ml volumetric flask, added 10 ml water and sonic ate for 20 min. made up the volume with diluents. The resulting solution was filtered 0.45µ membranes filter.

Chromatogram No.14 Blank



Chromatogram No.15 Placebo



Limit of Detection and Limit of Quantization:

Using linearity data LOD was calculated by Styx Method.

Ruggedness:

Ruggedness of the method was studied by using Chromatogram Shimandzu system.

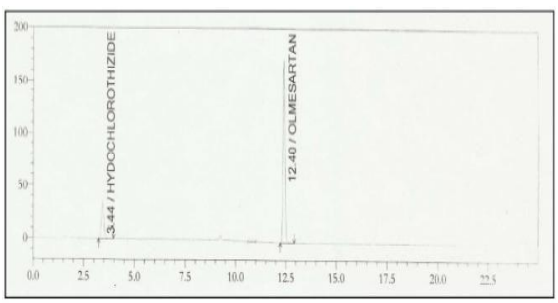
Preparation of standard solution:

80 mg olmesartan and 50 mg Hydrochlothazide wee weight accurately. Transferred it into 100ml volumetric flask and 10 ml of methanol were added. Sonicate it to dissolve and made up volume with diluents. 5 ml of this solution was transferred to 50 ml volumetric flask and made up the volume with diluents.

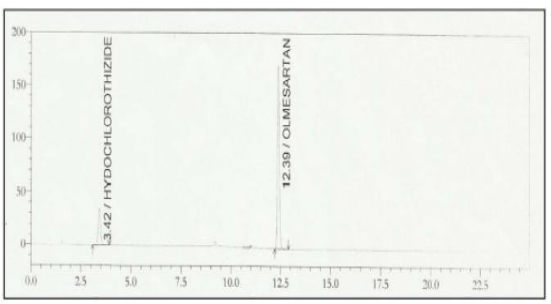
Preparation of sample solution:

Crush and powdered 20 tablets and weight powder equivalent to average weight of tablet. Transferred it to 250 ml volumetric flask, added 10 ml water and sonicate for 20 min .made up the volume with diluents. The resulting solution was filtered throught0.45 u membranes filter.

Chromatogram No.16



Chromatogram No.17



Solution state stability:

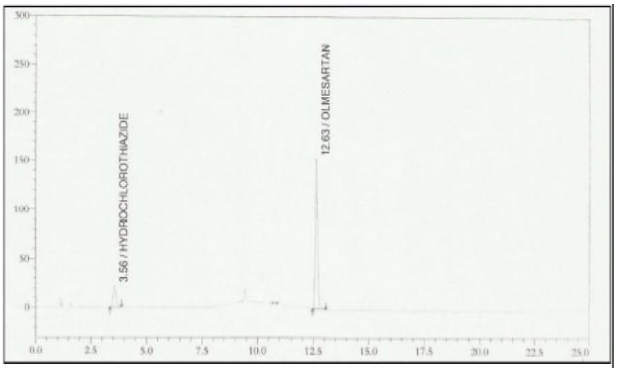
Preparation of standard solution:

80.80 mg olmesartan and 50.15 mg Hydrochlothazide were weight accurately. Transfer it into 100 ml volumetric flask and 10 ml methanol was added. Sonic ate it to dissolve and made up the volume with diluents. 5 ml of this solution was transferred to 50 ml volumetric flask and made up the volume with diluents.

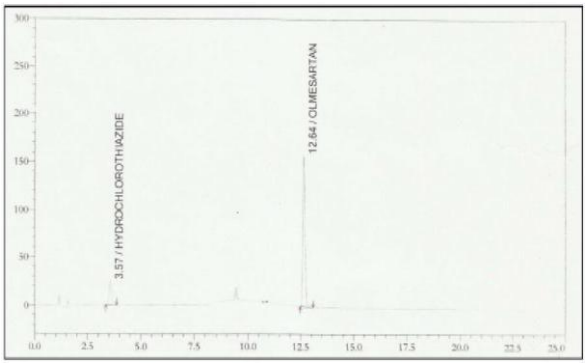
Preparation of sample solution:

Crush and powdered 20 tablets and weight powder equivalent to average weight of tablet. Transferred it to 250 ml volumetric flask, added 10 ml water and sonic ate for 20 min made up the volume with diluents. The resulting solution was filtered through 0.45 u membranes filter.

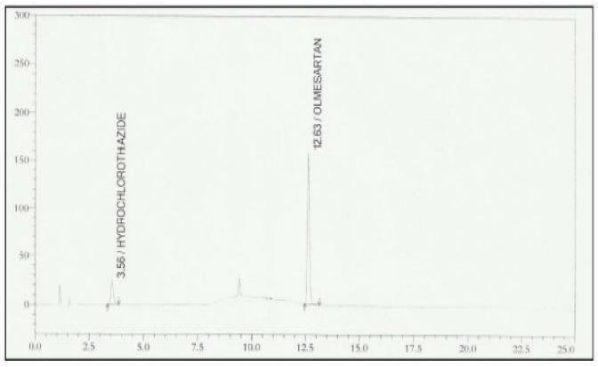
Chromatogram No.18: Initial Standard



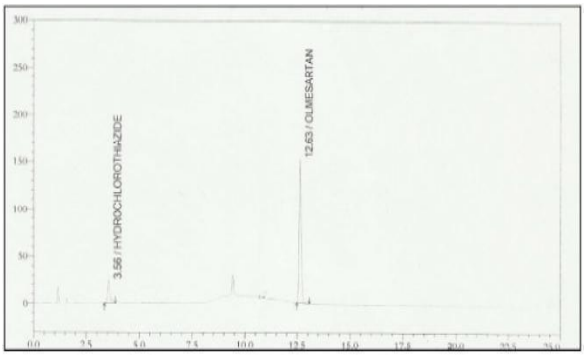
Chromatogram No.19: Initial Sample



Chromatogram No.20: 12 Hours Standard



Chromatogram No.21: 12 Hours Sample



RESULTS AND DISSCUSSION

The present work comprised of development and validation of an analytical method for the simultaneous estimation of Olmisertane and Hydrochlorthiazide in solid dosage form by HPLC & UV spectrophotometry. The commercially available tablet dosage form selected for the estimation are shown in Table.

Marketed formulation

Brand name	Company	Drug content
Product X	Mylan Pharm. Ltd	Olmisertane and Hydrochlorothiazide

The calibration curve was plotted between concentration (2-20 µg/mL) and AUC measured at the selected wavelength of 240 nm and 254 nm for above combination. The concentration of drugs in the tablet was found by using slope and 'Y' intercept of linear curve. Validation challenges showed reproducibility when carried out by different persons, in the same or different laboratories using different reagents, etc.

High Performance Liquid Chromatography

Considering the solubility, solvent triangle optimization, column performance, peak performance, the best wavelength and the mobile phase chosen were:

For Olmisertane and Hydrochlorthiazide (Benicar) - Buffer (ph-3.5): Acetonitrile which flow rate was 1.5 mL/min. pH was adjusted to 3.5 by 0.1% orthophosphoric acid (freshly prepared). The wavelength selected for analysis was 240 nm.

Developed equations for estimation by HPLC are Analysis of Olmisertane and Hydrochlorthiazide (Benicar)

For Olmisertane- AUC =12453 Conc. + 21145 For Hydrochlorthiazide- AUC =36681 Conc. + 34251

Validation of the HPLC Method - The results of validation are summarized in Table

Validation data for the developed HPLC method

Validation parameters	**Benicar**	
	Olmisertane	**Hydrochlorthaizide**
Linearity (r^2)	0.9941	0.9932
Precision (%SD)		
Analyst variation	0.36	0.14
Inter day Variation	0.51	0.02
Accuracy (%SD)	0.13	0.25
Range (µg/ml)	2-10	2-10
Tablet analysis		
(%found)	96.55	94.63

UV Spectrophotometry

For UV Spectrophotometry, the solvent selected on the basis of solubility and stability is Methanol. The wavelength selected was:

By Vierodt's method- For Olmisertane and Amlodipine bisilate the wavelength of **240 nm** and **254 nm** were selected, respectively.

Validation of UV Spectrophotometric method

The results of validation are summarized in Table.

Table: Validation data for the developed UV spectroscopic method

Validation Parameters	**Benicar**	
	Olmi	**Hydro**
Linearity (r^2)	0.9978	0.9986
Analyst variation	0.007	0.04
Inter day Variation	0.16	0.03
Range (µg/ml)	2-20	2-20
(%found)	94.65	4.75

CONCLUSION

All following suitability parameters were optimized to obtain the best resolution between multi-component formulations. The optimized parameters are-

Table: System suitability parameters for HPLC

Variable Column	**Condition**
Dimension.	250mm x 4.60mm
Particle Size	5 □
Bonded Phase	Octadecylsilane (C_{18})
Diluent	Mobile phase
Flow rate	1.5 ml/min
Temperature	Ambient
Sample volume	20 □l

The proposed analytical UV spectrophotometric methods and HPLC spectrometry method were developed and validated thoroughly for quantitative determination of Olmesertane and hydrochlorothiazide in tablets. The linearity range, limit of detection and quantification, precision and accuracy, were performed to determine the suitability of the method. The developed methods were found to be simple, rapid, accurate, precise, and economical and give an acceptable recovery of the analytes, which can be directly and easily applied to the analysis of Olmesertane and hydrochlorothiazide in pharmaceutical tablet formulations.

These full validation assays have been concluded that the developed UV and HPLC methods are linear, sensitive, accurate and precise for the determination of Olmesertane and hydrochlorothiazide in tablets. The developed UV spectrophotometric and HPLC methods are cheaper, simpler and faster than HPLC methods for analysis of Olmesertane and hydrochlorothiazide in the pharmaceutical preparations. These advantages encourage the application of these methods in routine analysis of Olmesertane and hydrochlorothiazide.

Future Scope

This method can be conveniently adopted for the simultaneous estimation of Olmesartan and Hydrochlorothiazide in a combined pharmaceutical dosage form.

The methods developed are precise, accurate and reproducible. The HPLC method can be effectively adopted by a pharmaceutical company involved in commercial manufacturing of dosage form. In this case, the large no. of samples can be handled at a time and the results can be obtained with in short period of time. This has shown the commercial viability and wider acceptability of the method.

In future the other method such as HPTLC can be developed and validated for the simultaneous estimation of Olmesartan and Hydrochlothazide in combined dosage form

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