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Method Development and Validation of simultaneous estimation for Amlodipine besylate and Olmesartan medoxomil by HPTLC method

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ABSTRACT

The present work deals with method development and analytical validation of a novel, precise and accurate HPTLC methods for the simultaneous estimation of Amlodipine besylate and Olmesartan medoxomil. Literature review has shown that High performance liquid chromatography and UV-Visible Spectroscopy methods have been reported for the estimation of these drugs, but no HPTLC method has been done, thus this study had to be done. An analytical method development for the simultaneous estimation of Amlodipine besylate and Olmesartan medoxomil was developed. For simultaneous HPTLC method, the analytical separation was achieved on aluminium plates pre-coated with Silica Gel 60 F254 Acetonitrile: Water: Toluene (6:3:1) v/v/v used as the mobile phase with densitometric scanning at 270 nm. Good and acceptable linearity was obtained for the drug in the range of 5 - 15 µg/mL with $r^2 > 0.999$. All the precision measurements made were well within the acceptable limits. The percentage recovery was found to be 99.59 & 99.61 % HPTLC method for Amlodipine besylate and Olmesartan medoxomil respectively. The parameters of validation were in accordance with the outlined ICH guidelines for method development in the estimation of drugs. Hence in conclusion the method can be applied day today lab practice for the determination of Amlodipine besylate and Olmesartan medoxomil simultaneously using HPTLC instrument.

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INTRODUCTION

The literature review for Amlodipine besylate and Olmesartan medoxomil reveals that methods like HPLC, UV spectrophotometry (for characterization) were reported for the determination of Amlodipine besylate and Olmesartan medoxomil in pharmaceutical formulations and biological fluids. It was found that no HPTLC method have yet been reported for the simultaneous estimation of Amlodipine besylate and Olmesartan medoxomil in pharmaceutical formulation. The objective of the work is to develop a rapid, simple, precise, accurate, sensitive, and reliable HPTLC method for the quantitative determination of Amlodipine besylate and Olmesartan medoxomil in pharmaceutical formulation according to ICH Guidelines. (ICH guideline). High Performance Thin Layer Chromatography a powerful method suitable for qualitative and quantitative analytical tasks. Applications of HPTLC, such as identification and quantitation of constituents, impurities, active substances, process development and optimization, process monitoring, and cleaning validation have been demonstrated.

Amlodipine besylate is a long- chain acting calcium channel blocker used as an anti-hypertensive and

in the treatment of angina. 3-Ethyl 5-methylester, (±)-2-[(2- aminoethoxy) methyl]-4-(o-chlorophenyl)-1, 4- dihydro-6-methyl-3,5-pyridinedicarboxylate is the IUPAC name. (Indian Pharmacopoeia 2010). Amlodipine besylate selectively inhibits calcium influx across cell membranes in cardiac and vascular smooth muscle, with greater effect acting on vascular smooth muscle. Olmesartan medoxomil inhibits the vasoconstrictor effects of angiotensin II by selectively blocking the binding of angiotensin II to the AT1 receptor in vascular smooth muscle. Angiotensin II is a potent vasoconstrictor that increases the blood pressure in the body. It is used for the treatment of high blood pressure. Lowering of high blood pressure helps to prevent strokes, heart attacks, and kidney problems. The estimation for the two drugs using HPTLC was developed and validated according to the ICH guidelines. (S Pournima *et al*., 2011; Pournima *et al*., 2010; D.P Kardile *et al*., 2010; Asmit. Kamble *et al*., 2009; Chintan Patela *et al*., 2007). The chemical structure of Amlodipine besylate and Olmesartan medoxomil are shown in Figure 1 and 2.

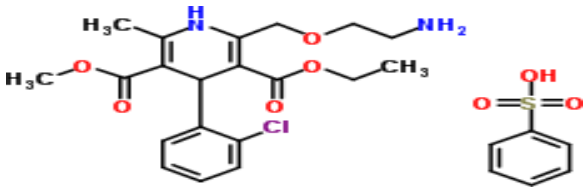

Figure 1: Chemical structures of Amlodipine besylate

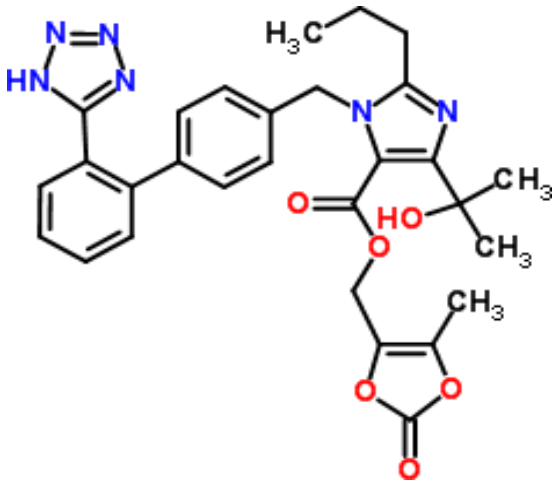

Figure 2: Chemical structures of Olmesartan medoxomil

MATERIALS AND METHODS

Reagents and Chemicals used

Methanol, Acetonitrile, toluene, water was used and pure standard samples Amlodipine besylate and Olmesartan medoxomil obtained as a gift sample. Tablet formulation of brand Azor containing 5mg of amlodipine besylate and 20mg of olmesartan medoxomil was procured from local pharmacy.

Instrumentation and Chromatographic condition

Chromatography performed with a Camag HPTLC sample Applicator-Linomat5, Twin trough Chamber 20x10, Camag TLC scanner, HPTLC plates (Merk) precoated with Silica gel 60 F254 on and Camag HPTLC Document photo. The software employed is WINCAT 5 and MS Excel 2007. Mobile phase: Acetonitrile: Water: Toluene (6:3:1) v/v. Amlodipine besylate and Olmesartan medoxomil both are freely soluble in Methanol and Soluble in water. The development time is 45 minutes and temperature maintained at ambient conditions. The injection volume is 2µl. The Rf value of Amlodipine besylate and Olmesartan medoxomil is 0.57 and 0.77 respectively. A mixed standard solution containing Amlodipine besylate and Olmesartan medoxomil (2000mg/ml) was prepared and used for further analysis. 2µl of the above sample solution was spotted on the HPTLC plate precoated with silica gel 60 GF254 on Aluminium sheets and subjected to method development. The plates after development was dried and scanned at 270nm. The recorded densitogram was shown in Figure 3.

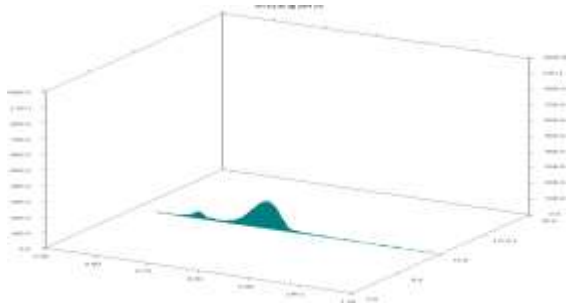

Figure 3: Densitogram of standard solution of Amlodipine Besylate and Olmesartan Medoxomil

Preparation of standard solutions

The standard solution was prepared by weighing 10mg Amlodipine besylate and Olmesartan medoxomil into 10ml standard flask. Half the volume of Methanol was added and made the drug to dissolve completely and then volume was made up to the mark with Methanol to get a concentration of 2mg/ml (2000µg/ml) Amlodipine besylate and Olmesartan medoxomil respectively

Preparation of working solution

The sample was prepared by weighing 10mgAmlodipine besylate and Olmesartan medoxomil was of in 10ml of standard flask. Half the volume of Methanol added and made the drug to dissolve completely and then volume is made up

to the mark with Methanol to obtain a concentration of 2mg/ml (2000µg/ml) Amlodipine besylate and Olmesartan medoxomil was respectively.

Determination of λ max

The stock solution of Amlodipine besylate and Olmesartan medoxomil was diluted to obtain a concentration of 10 µg/ml using methanol. The solution was scanned in the UV region from 400-200nm.UV spectra of Amlodipine besylate and Olmesartan medoxomil and the λ max were found to be 238nm and 257nm respectively. The isobestic point was found to be 307nm in figure 4.

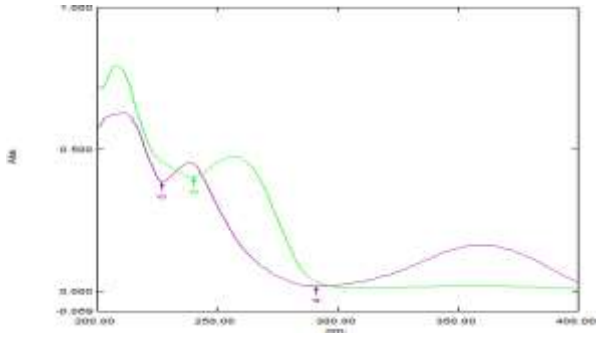

Figure 4: Overlay UV Spectra of Amlodipine Besylate and Olmesartan Medoxomil

RESULTS AND DISCUSION

Method development

Various trails were carried for the optimization of chromatographic conditions for the selection of various mobile phases. Finally a different mixture of Acetonitrile: Water: Toluene in the ratio of (6:3:1) v/v/v were selected which is effective mixture than the other mixture used for the separation.

Validation of the method

The objective of method validation of an analytical procedure is to demonstrate its intended purpose were proposed according to ICH guidelines, the validation parameters were:

Linearity

Appropriate aliquots of Amlodipine besylate and Olmesartan medoxomil standard stock solution were taken into six different 10ml volumetric flasks and diluted up to the mark with methanol to obtain the final concentration ranging from 100-600 µg/ml for Amlodipine besylate and Olmesartan medoxomil. The 2µl of the above solution were developed on the precoated TLC plate. The plate was subjected to development. The plates were dried and scanned at 270nm. The peak area was recorded and calibration curves were constructed. Linearity line was found to obey Beer's Law in the concentration ranges of 1000-3000ng/spot for Amlodipine besylate and Olmesartan medoxomil. The results were entered tabular column in Table-2. The overlay densitogram and linearity plot were shown in figure 5 & 6.

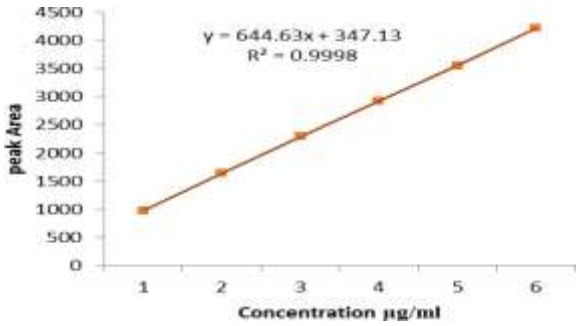

Figure 5: Linearity plot of Amlodipine besylate

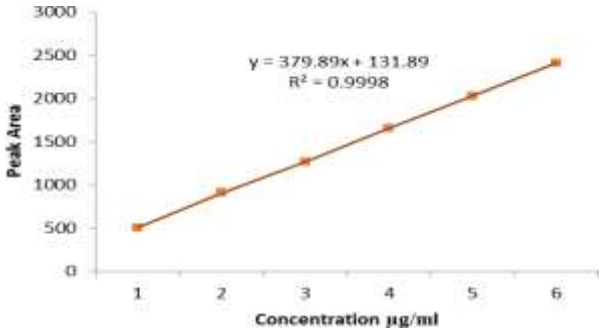

Figure 6: Linearity plot of Olmesartan medoxomil

Precision

The intra-day precision studies within the day (intermediate precision) were carried out by estimation the corresponding responses 6 times on the same day and on three different days at the 100% concentration level (1000 µg/ml of Amlodipine besylate and Olmesartan medoxomil). The 2µl of the above solution were spotted on the precoated TLC plate and subjected to development. The plates after development were dried and scanned at 254nm. The peak areas were recorded. The results were shown in Table-1 and the overlay densitogram showing precisions are shown in Figure 7 respectively.

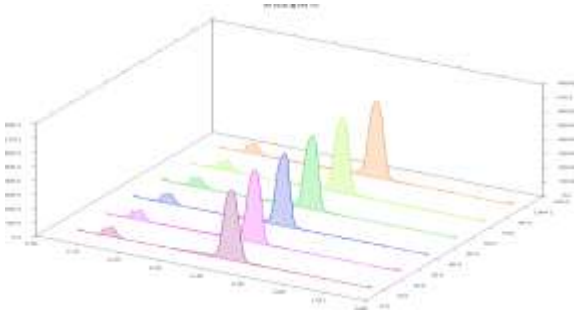

Figure 7: Overlay densitogram showing precision for Amlodipine besylate and Olmesartan medoxomil

Table 1: shows Method characteristic of Amlodipine besylate and Olmesartan medoxomil

Method characteristic	Amlodipine besylate	Olmesartan medoxomil
%RSD	0.45	0.29
R2	0.999	0.999
Slope	644.6	379.8
Intercept	347.1	131.8
LOD	0.59	1.08
LOQ	**1.8**	3.2

Table 2: Accuracy study of Amlodipine besylate and olmesartan medoxomil

Drug	Recovery level (%)	Initial amount added (µg/ml)	Amount added (µg/ml)	Amount recovered (µg/ml)	Recovery (%)
Amlodipine besylate	50	400	100	497.95	99.59
	100	400	600	999.14	99.91
	150	400	1100	1495.58	99.71
Olmesartan medoxomil	50	400	100	498.54	99.65
	100	400	600	998.04	99.16
	150	400	1100	1501.77	100.7

Mean ± SD*=average of three determinations

Detection limit (LOD) and Quantitation limit (LOQ)

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 detection limit is expressed as

$$LOD = 3.3\ \sigma/Slope$$

Where, σ = Relative standard deviation of the response.

The Quantitation limit of an analytical procedure is the lowest amount of analyte in a sample, which can be quantitatively determined with suitable precision and accuracy. Quantitation Limit (LOQ) may be expressed as

$$LOQ = 10\ \sigma/Slope$$

Where, σ = Relative standard deviation of the response.

The result representing LOQ was showing in Table-1.

Accuracy

Accuracy of the method was performed. Accuracy were performed at 50%, 100%, 150% level of target concentration. The percentage recovery was found within limits (98-102%w/w) and results are presented in Table 2.

CONCLUSION

A simple, selective and cost-effective stability indicating HPTLC method for the estimation of Amlodipine besylate and Olmesartan medoxomil was developed and validated. It shows that the validation parameters were found within the limits prescribed by ICH guidelines. The method developed in this study is satisfactorily rapid, sensitive, accurate, precise, robust and reproducible for the simultaneous estimation of Amlodipine besylate and Olmesartan medoxomil in tablet formulation. Therefore, the developed HPTLC method can be recommended for release into routine quality control laboratory for the simultaneous estimation of Amlodipine besylate and Olmesartan medoxomil in pharmaceutical formulations.

REFERENCES

International Conference on Harmonization of Technical Requirement for Registration of Pharmaceutical for Human Use ICH Guideline Validation of Analytical procedures text and methodology Q2(R1).

Indian Pharmacopeia (2010), Volume II, pg no : 1045.

British Pharmacopeia (2011),volume II, pg no:143

S Pournima Patil, D Pramodini Kulkarni, S Mallappa Burkul, N Harinath More, A Sachin Pishawikar. Rphplc- method for simultaneous estimation of Amlodipine besylate and Olmesartan medoxomil from tablet. International journal of pharmacy and pharmaceutical sciences 2011; 146-149.

Pournima patil, Vaishali barge, Harinath more, Sachin piswikar. Simple, sensitive and specific spectrophotometric method were developed and validated for quantitation of olmesartan medoxomil (olm) and amlodipine besylate (aml) in tablet dosage form. International journal of current pharmaceutical research 2010; 77-79.

D.P Kardile, N.V. Kalyane T.H. Thakkar, M.R. Patel R.K Moradiya Simultaneous estimation of amlodipine besylate and olmesartan medoxomil drug formulations by HPLC and UV-spectrophotometric methods. Journal of pharmaceutical science and research 2010; 599-614.

Y Asmit. Kamble , V Mahadeo Mahadik , D Laxman Khatal & R Sunil Dhaneshwar validated HPLC and HPTLC Method for Simultaneous Quantitation of Amlodipine Besylate and Olmesartan Medoxomil in Bulk Drug and Formulation 2009;251-258.

V Chintan Patela, P Amit Khandharb, D Anandi Captaina, T Kalpesh Patelc Validated Absorption Factor Spectrophotometric and Reversed-Phase High-Performance Liquid Chromatographic Methods for the Determination of Ramipril and Olmesartan Medoxomil in Pharmaceutical Formulations. Eurasian journal of analytical chemistry 2007;159-17.