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Original Article

New Method Development and Validation of Simultaneous Estimation of Ezetimibe and Simvastatin by Using RP-HPLC in Tablet Dosage Form

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ABSTRACT

Pharmaceutical analysis plays a vital role in the pharmaceutical product development. Pharmaceutical analysis is a specialized branch of analytical chemistry. Analytical chemistry involves separating, identifying, and determining the relative amounts of components in a sample matrix. Pharmaceutical analysis derives its principles from various branches of sciences like physics, microbiology, nuclear science, and electronics etc. Qualitative analysis reveals the chemical identity of the sample. Quantitative analysis establishes the relative amount of one or more of these species or analytes in numerical terms. According to literature survey, it is found that few analytical methods such as HPLC, LCMS are available for the analysis (assay) of Simvastatin & Ezetimibe. The objective of this work is to develop validatable, transferable, robust, reliable, accurate and precise methodology for the determination of Simvastatin & Ezetimibe in pharmaceutical dosage forms by using RP-HPLC. A suitable chromatographic method was developed through optimization by changing various parameters such as the mobile phase, injection volume, flow rate etc.

Keywords: Simvastatin & Ezetimibe, RP-HPLC, chromatographic method. Injection volume.

1. INTRODUCTION

Qualitative analysis is required before a quantitative analysis can be undertaken. A separation step is usually a necessary part of both a qualitative and quantitative analysis. The results of typical quantitative analysis can computed from two measurements[1]. One is the mass or volume of sample to be analyzed and second is the measurement of some quantity that is proportional to the amount of analyte in that sample and normally completes the analysis. Instruments play a key role in the quantitative analysis of pharmaceutical

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chemistry[2].Reversed phase liquid chromatography (RPLC) is considered as the method of choice for the analysis of pharmaceutical compounds for several reasons, such as its compatibility with aqueous and organic solutions as well as with different detection systems and its high consistency and repeatability. Sensitive and accurate RPLC analysis, whether in the pharmaceutical or bioanalytical field, necessitates the use of stationary phases which give symmetrical and efficient peaks. Therefore, manufacturers of stationary phases are continuously improving and introducing new RPLC products, and the selection of various types of reversed phase stationary phases is high[3]. The needs for consistency as well as the globalization of the pharmaceutical companies require that the methods will be transferred from site to site, using either the same column brands or their equivalents. Therefore, an extensive categorization or characterization of the rich selection of stationary phases has been done in recent years[4].

2. MATERIALS AND METHOD

Drug profile[5]

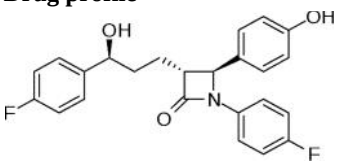

Fig 1: Structure of Ezetimibe

Name : EZETIMIBE[6]

Category : Anticholesteremic, Cholesterol Absorption Inhibitor

Molecular formula :$C_{24}H_{21}F_2NO_3$

Molecular weight : 409.4 g·mol^{-}

Melting point : 164–166 °C (327–331 °F)

Solubiity : Ezetimibe is highly soluble in alcohols (methanol, ethanol, 1-propanol)

pKa : 14.4

Chemical name : (3*R*,4*S*)-1-(4-fluorophenyl)- [(3*S*)-3-(4-fluorophenyl)-3-ydroxypropyl]-4- (4-hydroxyphenyl)azetidin-2-one

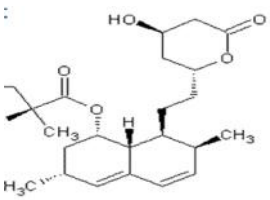

Fig 2: Structure of Simvastatin

- Name : Simvastatin
- Category : Anticholesteremic Agents, Antilipemic Hydroxy methyl glutaryl-CoA Reductase Inhibitors
- Molecular formula:$C_{25}H_{38}O_5$
- Molecular weight: : 418.566 g/mol
- Melting point: : 135-138oC
- Solubiity : soluble in water.
- pKa: : 13.4

Methodology

The objective of this experiment was to optimize the assay method for simultaneous estimation of simvastatin and Ezetimibe based on the literature survey made and the methods given in official pharmacopoeias. Trials done for optimization are as follows[7] (Table 1):

Table 1: Trial and Error Report

Trail	Column	Flow rate (ml/min)	Temp	Mobile phase	Wave length	PH	Remark
1	INERTSIL C_{18}(250mm,4.6 mm i.d., 5 microns)column	1.2	AMBIENT	Meoh :WATER 60:40	225	No	It was not satisfactory
2	INERTSIL C_{18}(250mm,4.6 mm i.d., 5 microns)column	1.2		ACN: WATER 80:20	225	No	It was not satisfactory
3	SYMMETRY C_{18}(250mm,4.6 mm i.d., 5 microns)column	1.5	AMBIENT	ACN: WATER 70:30	225	No	It was not satisfactory
4	SYMMETRY C_{18}(250mm,4.6 mm i.d., 5 microns)column	1.8	45˚C	0.01M POTASSIUM PHOSPHATE BUFFER : ACN 30: 70	225	9.5	It was not satisfactory
5	Sunfire C_{18}(250mm,4.6 mm i.d., 5 microns) column	1.8	45˚C	0.01M POTASSIUM PHOSPHATE BUFFER : ACN 20: 80	225	8.5	It was not satisfactory
6	Sunfire C_{18}(250mm,4.6 mm i.d., 5 microns) column	1.8	50˚C	0.05M Phosphate buffer : ACN 40: 60	225	p^H= 7.2	System suitability factors are satisfied

Table 2: Optimised chromatographic conditions for simultaneous estimation of Ezetimibe and Simvastatin by RP HPLC

Optimised Chromatographic Conditions	
Mode of separation	Isocratic elution
Mobile phase	ACN:Phosphate buffer(62%: 38%)
Column	Sunfire C_{18} (250mm,4.6mm(id), 5µ)
Flow rate	1.5 ml/min
Detector wavelength	225 nm
Injection volume	12µl
Oven temperature	Ambient
Run time	10min

Preparation of Required Solutions For Simultaneous Estimation Of Ezetimibe And Simvastatin By RP HPLC

Preparation of 0.05M of Potassium dihydrogen Phosphate Buffer Solution: (pH 7.2)

6.8 g of Potassium dihydrogen Phosphate was dissolved in 1000 ml of ml water. The solution was adjusted to a PH of 7.2 with Triethylamine. Then it was degassed in ultrasonicator for 10 minutes and then filtered through 0.45 µ pore size membrane filter[8].

Preparation of mobile phase:

Mix a mixture of above buffer 400 ml and 600 ml of Acetonitrile HPLC grade and degas in ultrasonic water bath for 10 minutes. Filter through 0.45 µ filter under vacuum filtration[9].

Preparation of standard solution of Ezetimibe and simvastatin:

10mg of simvastatin and 10mg of ezetimibe working standard were taken in 100ml volumetric flask. It was dissolved in 50ml methanol and made up to the mark with the methanol to get a concentration of 100µg/ml and 100µg/ml. It was degassed in ultrasonicator and then filtered through membrane filter of 0.45µ pore size[10].

Preparation of sample solution of Ezetimibe and simvastatin

10 tablets were crushed and powder equivalent to 10mg was taken into 100ml volumetric flask .It was made to dissolve with methanol and made upto the mark with methanol to get the concentration of 100µg/ml solution . The solution was degassed and filtered through membrane filter of pore size 0.45µ.

3. RESULTS AND DISCUSSION

Table 1: Solution State Stability

	Compound	RT	Area	% Assay
0 hour	**Ezetimibe**	2.39	2088264	100.24
	Simvastatin	6.46	2650097	99.48
	Ezetimibe	2.30	2047165	100.06
12th hour	**Simvastatin**	6.18	2697237	100.1
	Ezetimibe	2.19	2029552	98.25
24th hour	**Simvastatin**	6.9	2668187	97.76

Table 2: Heat-Degradation studies

S.No	Sample Weight(mg)	Ezetimibe Area	Simvastatin Area	% Assay of Ezetimibe	% Assay of Simvastatin
Acid-Degradation	173.00	2000235	2121378	96.92	96.56
Base-Degradation	173.00	2001354	2212143	95.54	95..52
Peroxide-Degradation	173.00	2001187	2122465	94.54	92.26
Water-Degradation	173.00	2001298	2131764	96.89	96.36
Heat-Degradation	173.00	2001265	2123476	96.24	96.78

Table 3: Linearity-150% Chromatogram of Ezetimibe and Simvastatin

Ezetimibe		**Simvastatin**	
Con. («g/ml)	**Area**	**Con. («g/ml)**	**Area**
50	1035618	50	1342439
75	1552937	75	2010764
100	2061491	100	2688600
125	2586635	125	3350466
150	3098704	150	4045287
Correlation Coefficient	**1**	**Correlation Coefficient**	**1**

An effort has been made to identify a Simple, Precise, Specific and Accurate method fo estimation of ezetimibe and simvastatin in formulation by using RP-HPLC method. During the selection of mobile phase several solvents were tried at various levels and finally selected mobile phase system was Acetonitrile: 0.01M Potassium dihydrogen phosphate of pH 7.2 at ratio 60:40 at ambient temperature[11]. The Standard concentration (100µg/ml) of Ezetimibe & Sivastatin was prepared by using mobile phase. After considering all the system suitability parameters, Acetonitrile: Phosphate Buffer (60:40) adjusted with Orthophosphoric Acid to pH 7.2 was selected for analysis at optimized flow rate of 1.8 ml/min. The Retention time of ezetimibe & simvastatin was found to be 2.35 min, 7.23 min respectively.

The Linearity of Ezetimibe & Simvastatin was carried out at different concentrations ranging from 50-150µg/ml and correlation coefficient was found to be using1, 1 which indicates that the concentration had given good linearity as shown in Table 24 & Fig 21, 22, 23, 24, 25, 26, and 27. Accuracy was confirmed by Recovery Studies. The % recovery of ezetimibe & simvastatin was found to be 99 %,100 % which were in the acceptance limit of 98 to 102%. The Precision has done in two ways i.e., System Precision and Method Precision. The % RSD values of EZETIMIBE & SIMVASTATIN for System Precision and Method Precision was found to be 0.6 & 0.8 and 0.61 & 0.81 respectively. Which were in the acceptance limit of less than 2%. The Specificity for these two drugs was determined by using 0.1N HCl, 0.1N NaOH and 1%H2O2 and upon refluxing drug solution at 600c for 30min When drug was mixed with 0.1N HCL, 0.1N NaOH and 1% H_2O_2 Upon refluxing to 600c. It was found to be occurrence of irregular peak and peak elution was not good

4. CONCLUSION

A suitable chromatographic method was developed through optimization by changing various parameters such as the mobile phase, injection volume, flow rate etc., In the present method a Sunfire C_{18}(250×4.6mm I.D,5µ) column has been used for Ezetimibe & Simvastatin drugs respectively. Mobile phase used was Acetonitrile:Phosohate buffer (60:40%v/v) for drugs Ezetimibe & Simvastatin respectively, Retention of Ezetimibe & Simvastatin have more dependence on the

mobile phase. The separation of the two peaks was also dependent on the buffer and the percentage of mobile phases[12]. Ezetimibe & Simvastatin were eluted at acceptable retention times and got good resolution. Several assay methods has been developed for the determination of Ezetimibe & Simvastatin in formulations and biological fluids but this method is most economic and accurate so this method is very useful for the determination of Ezetimibe & Simvastatin in tablet formulations. This method was validated as per ICH-Q2 (R1) guidelines and met the regulatory requirements for selectivity, accuracy and stability. Considering the obtained data, it was possible to affirm that the proposed method was fast, simple and suitable for the accurate determination of drug Ezetimibe & Simvastatin in tablet formulation[13].

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