A validated RP-HPLC method for estimation of Oseltamivir in pharmaceutical formulation

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Abstract:

A simple, selective, rapid, precise and economical reverse phase high pressure liquid chromatographic method has been developed for the estimation of Oseltamivir from pharmaceutical formulation.The method was carried out on a Princeton SPHER C_{18} (25 cm x 4.6 mm i.d., 5 µ) column with a mobile phase consisting of acetonitrile: potassium Dihydrogen ortho phosphate (adjusted to pH 3.5 using orthophosphoric acid) (50:50 v/v) at a flow rate of 1.0 ml/min**.** Detection was carried out at 254 nm. Theophylline was used as an internal standard.The retention time of theophylline and oseltamivir was 5.56 and 9.61 min, respectively.The developed method was validated in terms of accuracy, precision, linearity, limit of detection, limit of quantitation and solution stability.The proposed method can be used for the estimation of oseltamivir in pharmaceutical dosage form.

Keywords: Oseltamivir, Pharmaceutical Formulation

Introduction

Oseltamivir phosphate (OP) is an antiviral drug that is used in the treatment and prophylaxis of both influenza A and influenza B. It is effective against all known influenza viruses than can infect humans, including pandemic influenza viruses and may be the most appropriate antiviral option against avian influenza caused by H5N1 virus. Tamiflu®, the registered trademark used under exclusive license by Roche laboratories with OP as active pharmaceutical ingredient, is considered the best treatment for the bird flu disease.Only one method was reported for the estimation of oseltamivir in dosage form by HPLC method [1-4]. The present work describes the development of a validated RP-HPLC method with internal standard. The present RP-HPLC method was validated following the ICH guidelines [5].

Materials and Methods

Reagents and chemicals

Acetonitrile HPLC grade was procured from E.Merck (India) Ltd, Mumbai. Potassium Dihydrogen ortho phosphate AR grade were procured from Qualigens fine chemicals, Mumbai. Water HPLC grade was obtained from a Milli-QRO water purification system. A reference standard of Oseltamivir was procured from Neopharma Abudhabi and theophylline was procured from Cadila Pharmaceuticals Ltd, Ahmedabad.

Apparatus and chromatographic conditions

Chromatographic separation was performed on a Shimadzu® liquid chromatographic system equipped with a LC-10AT-vp solvent delivery system (pump), SPD M-10AVP photo diode array detector, Rheodyne 7725i injector with 50 µl loop volume. Class-VP 6.01 data station was applied for data collecting and processing (Shimadzu, Japan). Princeton SPHER C_{18} (25 cm x 4.6 mm i.d., 5 µ) column with a mobile phase consisting of acetonitrile: potassium dihydrogen ortho phosphate (adjusted to pH 3.5 using orthophosphoric acid) (50:50 v/v) at a flow rate of 1.0 ml min**.** Detection was carried out at 254 nm. Theophylline was used as an internal standard. The mobile phase was filtered through a 0.2µ membrane filter and degassed. The injection volume was 50 µl and the analysis was performed at ambient temperature.

Preparation of standard solutions

Standard stock solutions of 1.0 mg mL Oseltamivir were prepared by using a mixture of water and acetonitrile (1:1 v/v). From the standard stock solution, mixed standard solution was prepared to contain 7.0 µg ml of Oseltamivir and 50.0 µg ml of theophylline as internal standard.

Preparation of sample solutions

Twenty tablets, each containing 70.0 mg of Oseltamivir were weighed and finely powdered; a quantity of powder equivalent to 7.0 mg of Oseltamivir was weighed and transferred to a sintered glass crucible. To this 5.0 ml of 1.0 mg ml solution of Theophylline was added and the drugs were extracted with three quantities, each of 20 ml of mixture of acetonitrile and water (1:1 v/v). The combined extracts were made up to 100 ml with mobile phase and further dilutions were made to get a concentration of 7.0 µg mL of Oseltamivir, 50.0 µg ml of Theophylline as internal standard and this solution was used for the estimation.

Assay method

With the optimized chromatographic conditions, a steady baseline was recorded, the mixed standard solution was injected and the chromatogram was recorded. The retention time of Theophylline and oseltamivir was found to be 5.56 and 9.61 min, respectively. This procedure was repeated for the sample solution obtained from the formulation. The response factor (peak area ratio of standard peak area and internal standard peak area) of the standard solution and sample solution were calculated.

Results and Discussion

Estimation of Oseltamivir in dosage forms

The HPLC procedure was optimized with a view to develop precise and stable assay method. Both the pure drugs Oseltamivir were run in different mobile phase compositions with different C_{18} columns (Kromacil 25 cm x 4.6 mm i.d., 5µ) Phenomenex C_{18} column (25 cm x 4.6mm i.d., 5µ).The flow rate was also varied from 0.5 ml to 1.2 ml min. Finally Princeton SPHER C_{18} (25cm x 4.6mm i.d., 5µ) with a mobile phase of a mixture of acetonitrile and 50mM potassium dihydrogen ortho phosphate (adjusted to pH 3.5 using orthophosphoric acid) at a flow rate of 1.0 ml min with a detection at 254nm gave sharp and symmetrical peaks with retention time 5.56 and 9.61 for theophylline and oseltamivir respectively. The resolution factor at the above said condition was 1.7. The typical chromatogram of sample solution is shown in Fig. 1. Detection was done at 254 nm. The peak area ratio of standard and sample solutions was calculated. The assay procedures were repeated for six times and mean peak area and mean weight of standard drugs was calculated. The percentage of individual drugs found in formulations, mean, standard deviation in formulations were calculated and presented in Table 1. The results of analysis shows that the amounts of drugs were in good agreement with the label claim of the formulation.

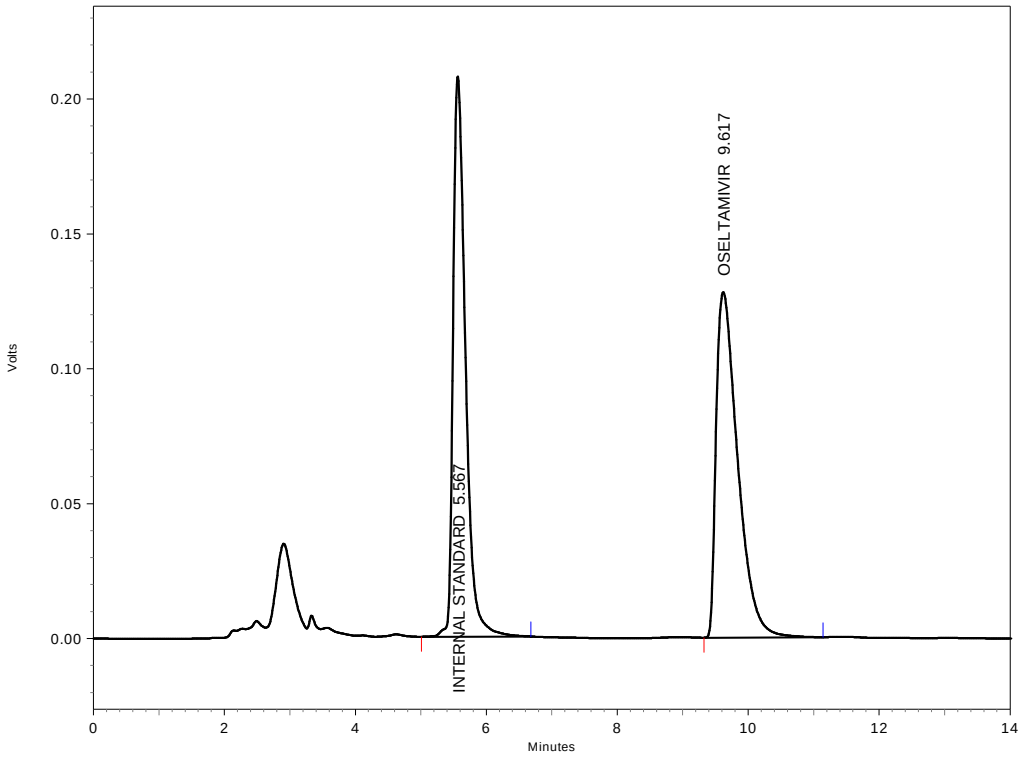

Fig. 1. Typical Chromatogram of Sample Solution

Table 1. Results of Analysis of Formulation and Recovery Studies

Drug	Amount mg/tablet		% Label claim*	% Recovery*
	Labelled	Found *		
Oseltamivir	70.00	68.51 ± 1.73	97.88 ± 1.69	98.05 ± 1.035

Validation

Accuracy and precision

The accuracy of the method was determined by recovery experiments. The recovery studies were carried out six times and the percentage recovery and standard deviation of the percentage recovery were calculated and presented in (Table 1). From the data obtained, added recoveries of standard drugs were found to be accurate.

The precision of the method was demonstrated by inter day and intra day variation studies. In the intra day studies, six repeated injections of standard and sample solutions were made and the response factor of drug peaks and percentage RSD were calculated. In the inter day variation studies, six repeated injections of standard and sample solutions were made for three consecutive days and response factor of drug peaks and percentage RSD were calculated and presented in Table 2. From the data obtained, the developed RP-HPLC method was found to be precise.

Linearity and Range

The linearity of the method was determined at five concentration levels ranging from 1.0µg ml to 11. 0µg mL for Oseltamivir. The calibration curve was constructed by plotting response factor against concentration of drugs. The slope and intercept value for calibration curve was y = 0.0588x-0.0167 (R^2=0.9913) for Oseltamivir. The results show that an excellent correlation exists between response factor and concentration of drugs within the concentration range indicated above (Table 3). The calibration curve are shown in Fig. 2.

Limit of Detection and Limit of Quantification

The Limit of Detection (LOD) and Limit of Quantification (LOQ) of the developed method were determined by injecting progressively low concentrations of the standard solutions using the developed RP-HPLC method. The LOD is the smallest concentration of the analyte that gives a measurable response (signal to noise ratio of 3). The LOD for theophylline and Oseltamivir were found to be 10 ng mL and 5.0 ng/ml respectively. The LOQ is the smallest concentration of the analyte, which gives response that can be accurately quantified (signal to noise ratio of 10). The LOQ was 30 ng/ml and 15 ng/ml for theophylline and Oseltamivir, respectively.

Ruggedness and Robustness

The ruggedness of the method was determined by carrying out the experiment on different instruments like Shimadzu HPLC (LC2010A_{HT}), Agilent HPLC and Water's Breeze HPLC by different operators using different columns of similar type like Hypersil C_{18}, Phenomenex Gemini C_{18} and Hichrom C_{18}. Robustness of the method was determined by making slight changes in the chromatographic conditions. It was observed that there were no marked changes in the chromatograms, which demonstrated that the RP-HPLC method developed, are rugged and robust.

Table 2. Intraday and Interday Precision Studies

Intraday studies		**Interday studies**		
RF* of Oseltamivir	**Mean ± RSD***	**Day**	**RF of Oseltamivir**	**Mean ± RSD***
			0.5545	
0.1532			0.6789	
0.2497		Day 1	0.5698	
0.3218	0.3947		0.5487	0.4259
0.4125	(0.66321)		0.4562	
(0.0015)				
0.5341			0.3564	
0.6973			0.5246	
			0.3691	
		Day 2	0.4521	
			0.5748	
			0.2698	
			0.4102	
			0.3987	
			0.4210	
		Day 3	0.5934	
			0.2748	
			0.3597	
			0.5975	

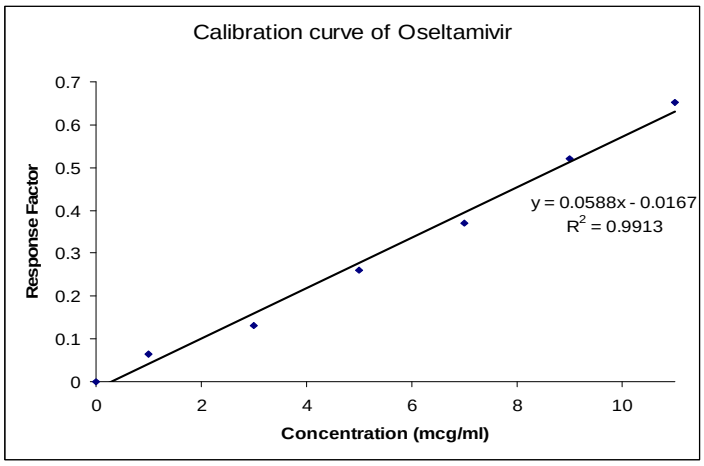

Fig . 2. Calibration curve of Oseltamivir

Table 3. Linearity and Range

Concentration (µg ml)	Response factor
1.0	0.0651
3.0	0.1303
5.0	0.2605
7.0	0.3708
9.0	0.5211
11.0	0.6513

Solution stability

In order to demonstrate the stability of both standard and sample solutions during analysis, both solutions were analyzed over a period of 5 h at room temperature. The results show that for both solutions, the retention time and peak area of Oseltamivir remained almost unchanged (% R.S.D. less than 2.0) and no significant degradation within the indicated period, thus indicated that both solutions were stable for at least 5 h, which was sufficient to complete the whole analytical process.

System suitability studies

The column efficiency, resolution and peak asymmetry were calculated for the standard solutions. The values obtained demonstrated the suitability of the system for the analysis of this drug and

the system suitability parameters fall within ± 3 % standard deviation range during routine performance of the method.

Conclusion

The proposed RP-HPLC method for the simultaneous estimation of Oseltamivir in pharmaceutical dosage form is accurate, precise, linear, rugged, robust, simple and rapid. Hence the present RP-HPLC method is suitable for the quality control of the raw materials, formulations and dissolution studies.

References

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