RP-HPLC Method Development and Validation for the Simultaneous Estimation of Satranidazole and Ofloxacin in Pharmaceutical Dosage Form

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Abstract

A simple, rapid, and accurate reversed phase high-performance liquid chromatographic (RP-HPLC) method has been developed and subsequently validated for the simultaneous determination of ofloxacin (OFL) and satranidazole (SAT) in combination. The separation is carried out using a mobile phase consisting of 10mM phosphate buffer and methanol in the ratio of 50:50. The pH of the mobile phase is adjusted to 3.0 with 10% *o*-phosphoric acid. The column used is Kromasil-100 C_{18} (250 × 4.6 mm, 5 µm). with flow rate of 1.0 mL/min using UV detection at 294nm. The total run time is 5 min and the retention time of OFL and SAT is 2.59 min and 4.0 min respectively. The described method is linear for the assay of OFL and SAT over a concentration range of 10–24 µg/mL and 15–36 µg/mL respectively. Results of the analysis have been validated statistically and by recovery studies. The limit of quantitation for SAT and OFL has been found to be 0.042 µg/mL and 0.085 µg/mL respectively. The results of the studies showed that the proposed RP-HPLC method is simple, rapid, precise, and accurate, which is useful for the routine determination of SAT and OFL in bulk drug and its pharmaceutical dosage form.

Introduction

Ofloxacin (OFL) is a fluoroquinolone derivative. Chemically, it is (±)-9-fluoro-2,3-dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-7H-pyrido-[1,2,3-de]-1,4-benzoxazine-6-carboxylic acid. It is mainly used as an antibacterial, for the treatment of urinary tract infection and sexually transmitted diseases. It has been shown to effectively treat leprosy (1). Satranidazole (SAT) is a novel nitroimidazole derivative. Chemically, it is 1-methylsulfonyl-3-(1-methyl-5-nitro-2-imidazolyl)-2-imidazolidinone. It is used as antiprotozoal and antibacterial agent in the treatment of amoebiasis, trichomoniasis, and anaerobic infections (2). Both the drugs are marketed as combined dose tablet formulation in the ratio OFL–SAT (200:300 mg).

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Literature survey shows that various methods have been reported for estimation of OFL and SAT individually and in combination with other drugs (3–7). Available techniques are high-performance thin-layer chromatography HPTLC, HP liquid chromatography (LC), and colorimetric methods. OFL and SAT in combination with each other have been estimated by spectroscopy alone. The aim of this study was to develop a HPLC method for the combined dosage form OFL and SAT. Present work describes method development and validation of both drugs (i.e., OFL and SAT in combination according to ICH guidelines) (8).

Most of the HPLC methods developed for these two drugs so far have shown the use of acetonitrile as organic component of mobile phase for separation. Acetonitrile is a toxic reagent which causes environmental pollution and adverse health effects to humans and animals. An acute shortage of acetonitrile has led to increase in demand and thus higher prices. In an effort to practice "green chemistry," this study was planned so as to substitute acetonitrile with a safer and less toxic organic chemical. However ion pairing reagents like sodium lauryl sulfate and hepta sulfonic acid have been added to the methanol. The addition of these, however, leads to build up of back pressure in the column and damage. In this method, methanol alone with change in pH of buffer was used to increase retention time of the analytes, making the method easy and safe.

Experimental

Reagents and chemicals

HPLC grade methanol and water of HPLC grade were procured from E. Merck, Mumbai (India). Both orthophosphoric acid and potassium dihydrogen orthophosphate were of analytical grade delivered by S.D. Fine Chemicals, India. Satrogyl-O tablets manufactured by Alkem Laboratories, Ltd., were procured from local market (Mumbai, India). Satranidazole working standard was obtained as a gift sample from Alkem Laboratories, Ltd., (Mumbai, India) and ofloxacin working standard from Zhejianh Kangyo Pharmaceuticals Co., Ltd., Mumbai, India.

Preparation of standard stock solution and linearity solutions

The standard stock solutions of OFL and SAT (1 mg/mL) were prepared separately by dissolving 50 mg of each drug in 50 mL of methanol.

Several aliquots of these standard stock solutions were taken in different 10 mL volumetric flask and diluted up to the mark with mobile phase such that the final linearity concentrations of OFL and SAT were 10–24 µg/mL and 15–36 µg/mL, respectively.

Preparation of sample solution

Twenty tablets each containing 200 mg of OFL and 300 mg of SAT were weighed and powdered equivalent to dose, transferred to a 100 mL volumetric flask, and extracted with mixture of methanol and water (80:20). The mixture was sonicated for 20 min in an ultrasonic bath. The volume was adjusted to 100 mL with the same solvent mixture and then filtered. From this solution, 1.0 mL was pipetted and the volume was made up to 100 mL with mobile phase to get the concentration 20 µg/mL of OFL and 30 µg/mL of SAT.

Instruments and method

A surveyor HPLC system (Agilent 1100 series) consisting of G1379A degasser, a G1311A quaternary pump, a G1330B autosampler, a G1330B autosampler thermostat-ALS Therm, a G1316A column oven (COL COM) and a G1314A UV–vis detector were used for the experiment.

The HPLC column used was Kromasil – 100 C_{18} (250 × 4.6 mm) 5 µm. The mobile phase consisted of methanol–10mM potassium dihydrogen orthophosphate (50:50), pH adjusted to 3.0 with 10% *o*-phosphoric acid at a flow rate of 1.0 mL/min through the analytical column.

Results and Discussion

Method development and optimization

Some important parameters like pH of the mobile phase, concentration of the acid or buffer solution, percentage and type of the organic modifier, etc., were tested for a good chromatographic separation (9,10). Trials showed that an acidic mobile phase with reverse phase Kromasil-100 C_{18} column gives symmetric and sharp peaks. For this reason, 10 mM potassium dihydrogen orthophosphate solution was preferred as an acidic buffer. Methanol was chosen as the organic modifier because it dissolves drugs very well. Mobile phase composition of 50:50 (v/v) at a flow rate of 1.0 mL/min showed good resolution. When *o*-phosphoric acid was used as modifier, resolution between OFL and SAT was much better at pH 3.0, with a decrease in peak tailing. Retention time of the drugs obtained under these conditions were 2.59 and 4.06 min for OFL and SAT, respectively. For the quantitative analytical purposes the wavelength was set at 294 nm. The typical chromatogram of the sample is shown in Figure 1.

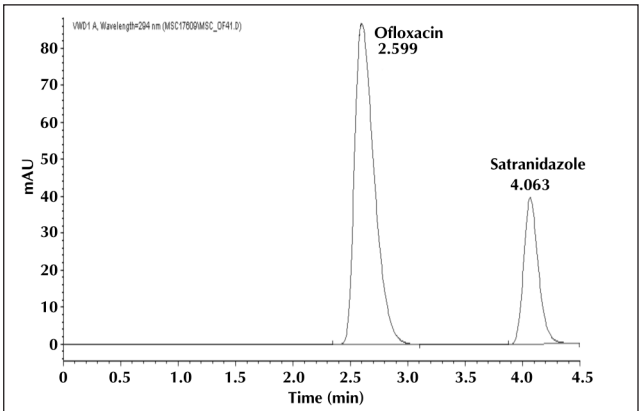

Figure 1. Typical chromatogram of authentic mixture containing Ofloxacin (20 µg/mL RT = 2.6 min) and Satranidazole (30 µg/mL RT = 4.06 min).

Method validation

System suitability studies

The column efficiency, resolution, and peak asymmetry were calculated for the standard solutions (6). The values obtained (Table I) demonstrated the suitability of the system for the analysis of this drug combination.

Solution stability

In order to demonstrate the stability of both the standard and sample solutions during analysis, both solutions were analyzed over a period of 24 h at room temperature. The results indicated that for both the solutions, the retention time and peak area of OFL and SAT did not show much variation (% RSD less than 2.0). There was no significant degradation within the indicated period. Hence, it was concluded that both the solutions were stable for 24 h at room temperature.

Limit of detection and limit of quantitation

The linearity for OFL was performed from 0.1–0.24 µg/mL and that for SAT from 0.15–0.36 µg/mL. Linearity graph was plotted and the correlation coefficient (*r*) determined. The limit of detection (LOD) was calculated from the linearity curve using the formula:

$$\text{LOD} = 3.3 \times ST_{yex} \left(\frac{Residual\ Standard\ Deviation}{Slope}\right)$$

The LOD for OFL was confirmed to be 0.024 µg/mL and for SAT it was confirmed to be 0.0418 µg/mL.

The limit of quantitation (LOQ) was calculated from the linearity curve using the formula:

$$\text{LOQ} = 10 \times \frac{St_{yex}}{Slope}$$

Table I. System Suitability Parameters

Test	Results		Acceptance criteria
	Ofloxacin	Satranidazole	
Retention time	2.6	4.06	–
RSD of replicate injections	0.135	0.324	Not more than 2%
Asymmetric factor	1.2857	1.4	Not more than 1.5
Theoretical plates	5187.0	4169.47	Not less than 3000
Resolution factor	–	2.56	More than 2

The LOQ for OFL was confirmed to be 0.0357 µg/mL and for SAT it was confirmed to be 0.126 µg/mL. The obtained values are reported in Table I.

Linearity study

The peak areas of OFL and SAT were linear with respect to the concentrations over the range of 15–36 µg/mL and 10–24 µg/mL. The slope and intercept value for calibration curve $Y = 54.7232X - 35.3577$ ($r = 0.999$) for OFL and $Y = 13.574X - 14.5753$ ($r = 0.999$) for SAT.

Table II. Linearity Study

Drug	Range*	Slope	Intercept	*r*	LOD*	LOQ*
Satranidazole	15–36	13.574	–14.5753	0.999	0.0418	0.126
Ofloxacin	10–24	54.7232	–35.3577	0.999	0.024	0.0357

* µg/mL

Table III. Recovery Studies for Spiked Concentration of Ofloxacin and Satranidazole

Drug	Level of % Recovery	Amount of Standard		% Recovery
		Added* (µg)	Recovered* (µg)	
OFL	50	10	10.0132	100.132
	100	20	21.857	109.282
	120	24	23.194	96.644
SAT	50	15	14.413	96.086
	100	30	30.9470	103.156
	120	36	35.190	97.751

* $n = 6$

The results showed that an excellent correlation exists between peak area and concentration of the drugs within the concentration range indicated previously. The data was analyzed by "linear regression least squares fit," and the parameters are listed in Table II.

Accuracy and precision

The accuracy of the recommended procedure was tested by the recovery test experiments. Tablet solutions were prepared according to the three standard concentrations ($n = 6$). The recovery rates were calculated and found to range between 97.64–106.28% for OFL and 98.75–103.15% for SAT, respectively. RSD ($n = 6$) ranged from 0.25–2.1% for OFL and 0.32–1.92% for SAT, respectively. The low values of RSD indicated that the method is accurate and percent assay shows that there is no interference from excipients. The % recovery values are tabulated in Table III.

Ruggedness and robustness

Ruggedness of the method was determined by carrying out the experiment on different instruments like JASCO – 1500 series by different operators using multiple Kromasil – 100 C_{18} (250 × 4.6 mm) 5 µm columns.

Robustness of the method was determined by subjecting the method to slight changes in the chromatographic conditions. No significant changes in the chromatograms were observed, proving that the developed method is rugged and robust. The obtained robustness results are presented in Table IV.

Tablet studies

The proposed method was successfully applied to the analysis of marketed products (Satrogyl-O) and the results obtained are given in Table V.

Table IV. Robustness Study

Change in flow rate			Change in pH			Change in Column temp.			Change in MeOH composition in mobile phase		
0.95*	1.0*	1.05*	2.8†	3.0†	3.2†	25°C	30°C	35°C	55:45‡	50:50‡	45:55‡
% Assay of Satranidazole											
96.15	99.42	104.48	100.46	99.76	100.20	100.3	100.1	99.76	100.3	99.76	99.73
% Assay of Ofloxacin											
98.19	98.48	103.46	100.0	99.09	99.80	100.0	99.53	99.09	100.0	99.097	99.17

* mL/min † pH ‡ B:M

Table V. Analysis of Formulation

Drug	Labeled Amount (mg)	Amount of mg/ tablet found*	% Label claim	% RSD
Satranidazole	300	294.5	98.17	0.433
Ofloxacin	200	198.3	99.15	0.684

* (n = 6)

Conclusion

The proposed method gave good resolution between OFL and SAT within short analysis time (< 5.0 min). Acetonitrile was successfully substituted with less toxic methanol in the mobile phase. Solution stability studies showed that the active pharmaceutical ingredients remained stable for 24 h at room temperature. The changes in flow rate, pH of mobile phase, composition of mobile phase, and temperature of column did not affect the percentage assay of drug, confirming the robustness of the method. Ruggedness of the method was confirmed as no significant changes were observed on analysis using different instrument. High percentage recovery of drug shows the method is free from interference of excipients present in the formulation.

Thus the proposed method is simple, rapid, sensitive, specific, accurate, and precise, and does not involve complicated sample preparation procedures.

Acknowledgments

We thank the California Separation Science Society (CASSS) for giving us the opportunity to present this work at the International Ion Chromatography Symposium (IICS) in September, 2009 at Dublin, Ireland. We are also grateful to Dr. A.M. Bhagwat, In-Charge, School of Science and the staff members of C.B. Patel Research Centre for their encouragement in carrying out this work.

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Manuscript received November 19, 2009.