SIMULTANEOUS DETERMINATION OF CEFTRIAXONE SODIUM AND STATIN DRUGS IN PHARMACEUTICAL FORMULATIONS AND HUMAN SERUM BY RP-HPLC

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ABSTRACT

An accurate, sensitive and least time consuming reverse phase high performance liquid chromatographic (RP-HPLC) method for the estimation of ceftriaxone in the presence of statin drugs in formulation and human serum has been developed and validated. Chromatographic separation was conducted on prepacked Purospher Star, C18 (5 mm, 250 x 4.6 mm) column at room temperature using methanol:water:acetonitrile (70:15:20 v/v/v) as a mobile phase, pH adjusted at 2.8 with ortho-phosphoric acid and at a flow rate of 1.0 mL/minute, while UV detection was performed at 240 nm. The results obtained showed a good agreement with the declared content. The method shows good linearity in the range of 2.5–25 µg/mL with a correlation coefficient 0.9966 – 0.9998 (inter- and intra-day RSD < 2 %). The limit of detection and quantification for ceftriaxone and statins in pharmaceutical formulation and serum were in the range 0.124 -1.006 µg/mL. Analytical recovery was >98.72 %. The proposed method may be used for the quantitative analysis of commonly administered statin's i.e. simvastatin, rosuvastatin atorvastatin and pravastatin alone or in combination with ceftriaxone from raw materials, dosage formulations and in serum. The established HPLC method is rapid, accurate and selective, because of its sensitivity and reproducibility.

Keywords: ceftriaxone, statin's, simvastatin, rosuvastatin, atorvastatin, pravastatin and RP-HPLC.

INTRODUCTION

Ceftriaxone (fig.1) is a (6R,7R)-7-{2-(2-amino-4-thiazolyl)-(Z)-2-[methoxyimino-*o*-acetamido]-3-[(2,5-dihydro-6-hydroxy-2-methyl-5-oxo-1,2,4-triazin-3-yl)thio]methyl}-8-oxo-5-thia-1-azobicyclo [4,2,0] oct-2-ene-2-carboxylic acid, in the hydrated disodium salt and contains approximately 83 mg of sodium per gram of ceftriaxone. It is a third generation parenteral cephalosporin with a relatively long half life which is stable to β -lactamases particularly those produced by Gram-negative organisms [1- 6]. It has excellent anti Gram-negative activity. It contains a highly acidic heterocyclic system on the 3-thiomethyl group, this unusual ring system is believed to confer the unique pharmacokinetic properties to this agent. It kills bacteria by interfering in the synthesis of the cell wall. Ceftriaxone has been effective in treating infections due to other 'difficult' organisms such as multi drug-resistant *Enterobacteriaceae*, [7- 12].

Fig. 1: Ceftriaxone

Statin's are a group of drugs used primarily in lowering cholesterol. They are generally capable of lowering cholesterol levels by 20 to 60 percent. The discovery of HMG-CoA (3-hydroxy-3-methylglutaryl-CoA) reductase inhibitors, called statins, was a breakthrough in the prevention of hypercholesterolemia and related diseases.[13-16]

Cholesterol plays an important role in the everyday functioning of the body. Unfortunately, it can also have a negative effect, contributing to the development of atherosclerosis. These plaques can block the arteries, cutting off blood flow, or rupturing and causing a clot that increases blockage. The results of such blockages are very serious and can include angina, claudication, heart attack, and stroke [17-19].

Available in tablet or capsule form, statin's are usually taken with dinner or at bedtime. Results are typically evident after a period of four to six weeks of use. Medications in this group are usually easy to tolerate and cause few, if any, side effects [20].

Fig. 2

The statins differ with respect to their ring structure and substituents. These differences in structure affect the pharmacological properties of the statins. Statins have sometimes been grouped into two groups of statins according to their structure. Statins that belong to type 1 are pravastatin and simvastatin. Statins that are fully synthetic and have larger groups linked to the HMG-like moiety are often referred to as type 2 statins. Statins that belong to this group are atorvastatin and rosuvastatin [21-23].

The scope of study was to investigate the possible effect of these statin's on emergency treatment with ceftriaxone. The present method was actually developed in our laboratories for drug-drug interaction studies between ceftriaxone and statin's in order to study the effect of these on the availability of ceftriaxone. Such a method was needed for co-administration of both drugs is possible in multiple drug therapy and there is no such method reported in the literature for the simultaneous determination of ceftriaxone and statin's. Furthermore, we report a specific and sensitive HPLC method for the simultaneous determination of ceftriaxone and statin's in pharmaceutical preparations and human serum.

There are a number of liquid chromatographic methods reported in the literature for the individual assays of ceftriaxone in aqueous and biological sample [24-29]. Owens achieved chromatographic separation by using cetyltrimethylammonium bromide (0.01 M) as ion-pairing agent. The mobile phase consisted of methanol-acetonitrile-phosphate buffer, pH 7.4 (20:20:60, v/v/v) but it was a difficult mobile phase [24]. Another HPLC method for the determination of ceftriaxone has been developed by Eric [25]. Chromatogram evaluation in the reflectance/absorbance mode. Traument [26] developed a

method for determination of ceftriaxone in plasma, urine and bile by means of ion-pair reversed phase chromatography using phosphate buffer of pH 8 at 254 nm. One method for the analysis of ceftriaxone in clinical microbiology in the biological fluids has been developed by the Knoeller et al.[27]. Nahata [28], Jehl and Birkel [29] also developed a method using phosphate buffer of pH 7 as a mobile phase. All of these methods consisted buffers combination as mobile phase. Several methods also reported for determination of Statin's [30-34].

However, no method for simultaneous determination of Statin's and ceftriaxone in active and in dosage formulations has been studied so far. On this basis, it became apparent to develop and validate for the first time a simultaneous method for the estimation of these drugs in bulk material, dosage formulations and in human serum using RP-HPLC. Several problems were resolved in the simultaneous determination of compounds investigated. The first was the selection of separation conditions to ensure efficient extraction of the drugs and from human serum with minimum interference from serum endogenous compounds. The second was the choice of proper chromatographic conditions to obtain separation of all components from the endogenous compounds. Thirdly, the method had to be sufficiently sensitive to measure concentrations of the investigated drugs in serum within their therapeutic range. The present work describes a simple RP-LC method for the simultaneous determination of ceftriaxone, Simvastatin, Rosuvastatin, Atorvastatin, and Pravastatin bulk raw materials, dosage formulations and in human serum.

EXPERIMENTAL

Instrumentation and analytical conditions

Two identical LC systems were used for the separation in two different labs: Shimadzu HPLC system equipped with LC-10 AT VP pump, DGU-14 AM on-line degasser, Rheodyne manual injector fitted with a 20 μ L loop, column and SPD-10 A VP UV-VIS detector and a Purospher star C18 (5 μ m, 25 X 0.46 cm) column separation was utilized. Chromatographic system was integrated via Shimadzu model CBM-102 Communication Bus Module to P-IV computer. Shimadzu CLASS-GC software (Version 5.03) was used for data acquisition and mathematical calculations.

Materials and reagents

HPLC grade acetonitrile, methanol and phosphoric acid were obtained from Merck Germany. Ceftriaxone (bestrix Injection) was a gift from Pharmevo Pvt Ltd. The statins used were simvastatin (limitrol® 10 mg), rosuvastatin (x-plended® 5 mg), atorvastatin (derot® 10 mg) of Pharmevo (Pvt) Ltd and pravastatin (pravachol® 20 mg) of Bristol Myer Squibb which were purchased from the local pharmacy. All these drugs had an expiry of not less than one year at the time of study.

Stock and working solution preparation

Stock standard solutions of 100 μ g/mL of ceftriaxone, simvastatin, atorvastatin, rosuvastatin and pravastatin were prepared individually by dissolving 10 mg of each in 100 mL volumetric flask using mobile phase as a diluent. Working solutions were also prepared separately by diluting from the standard solution to obtain concentration between 2.5-25 μ g/mL. These solutions were stored at 20°C. They were prepared once and analyzed daily for inter-day and inter-operator variations of the method and analyzed each time before drug analysis in biological samples. 20 μ L of these solutions were injected into LC system and chromatographed.

Sample preparation

For testing the suitability of the proposed method for the estimation of the drugs in dosage form, 20 tablets of each drug were powdered and equivalent to 10 mg of statin's (simvastatin, atorvastatin, rosuvastatin and pravastatin) and 10 mg of ceftriaxone were transferred to 100 mL volumetric flask dissolved and diluted with mobile phase. The resulting solutions were filtered through watman filter paper no. 41 and diluted to the desired concentration and analyzed for the drug content.

Drug-plasma solution

These solutions were prepared once and stored at 20°C for the preparation of drug serum solution. To 1 mL of plasma 10 mL of acetonitrile was added and vortexed for 1 minute and subsequently centrifuged for 10 minutes at 10,000 rpm. Supernatant was filtered through a 0.45-micron pore size membrane filter. Serum obtained was added to the above solutions to produce the desired concentrations and stored at 20°C. In order to evaluate linearity in serum five concentration levels ranging from 2.5-25 μ g/mL were prepared and linearity and % R.S.D values were evaluated.

RESULTS AND DISCUSSION

This work was designed to develop an isocratic method based on RP-LC separation for statin's and ceftriaxone assay in pharmaceutical dosage formulations, bulk material as well as in human serum. The goal of this study was to develop a rapid, more accurate, precise, reliable, least time consuming HPLC method for the five drugs individually as well as simultaneously. This analytical method was developed taking in account the therapeutic and overdose concentration range, has been validated and holds well for the determination of drugs in raw materials, dosage formulations and especially in human serum.

Method optimization and chromatographic conditions

To optimize the operating conditions for isocratic RP-LC detection of all analytes, a number of parameters such as the mobile phase composition, pH and the flow rate were varied. Various ratios (80:20, 70:30, 60:40 v/v) of methanol: water was tested as starting solvent for system suitability study. The variation in the mobile phase leads to considerable changes in the chromatographic parameters, like peak symmetry, capacity factor and retention time. The pH effect showed that optimized conditions are reached when the pH value is 2.8, producing well resolved and sharp peaks for all drugs assayed. Henceforth, in the present method pH adjusted to 2.8 using wavelength 240 nm (isosbestic point). However, the peak shape and resolution were found to be good when the mobile phase comprising of the methanol: water: acetonitrile pH adjusted to 2.8 with phosphoric acid was used in the ratio of (70:15:20 v/v/v) at a flow rate of 1 mL/min (filtered through a 0.45 micron filter). For simultaneous determination of ceftriaxone with statin's drugs, simvastatin, rosuvastatin, atorvastatin and pravastatin in individual drug solutions were injected into the column at the concentration of 100 μ g/mL⁻¹ and both elution pattern and resolution parameters were studied. System suitability data given in table 2. Type 1 statin's and type 2 statin's run separately with ceftriaxone due to overlapping of pravastatin and rosuvastatin. (Figure 3 and 6).

Figure 3. A representative chromatogram of ceftriaxone, Pravastatin and Simvastatin in raw material.

Figure 4. A representative chromatogram of ceftriaxone, pravastatin and simvastatin in serum

Figure 5. A representative chromatogram of ceftriaxone, pravastatin and simvastatin in formulation

Figure 6. A representative chromatogram of ceftriaxone, rosuvastatin and atorvastatin in raw material.

Wavelength selection

In addition, the UV spectra of individual drugs were recorded in the wavelength range from 200 to 400 nm and compared. The choice to use a common wavelength set at 240 nm was considered satisfactory, permitting the detection of all drugs with adequate sensitivity.

Method validation

For validation of analytical methods, the guidelines of the International Conference on the Harmonization of Technical Requirements for the Registration of Pharmaceuticals for Human Use (ICH) [35] and USP [4] have recommended the accomplishment of accuracy tests, precision, specificity, linearity and robustness of the method.

System suitability

The system suitability studies were carried out as specified by ICH [35] by analyzing the symmetry of the ceftriaxone and statin's peaks (symmetry factor <2), theoretical plates of the column >3,000

Accuracy and precision

Inter and intra-day precision and accuracy of the method were evaluated at different independent concentrations by adding known quantities of the analytes to the drug product (spiking method). The results of accuracy (table 3 - 5) revealed that the method was accurate for all above purposes. The method passed the test for repeatability (system suitability test table 2) as determined by %RSD of the area of the peaks of six replicate injections at 100% test concentration.

Figure 7. A representative chromatogram of ceftriaxone, rosuvastatin and atorvastatin in serum.

Figure 8. A representative chromatogram of ceftriaxone, rosuvastatin and atorvastatin in formulation.

Specificity

Representative chromatograms Figure. 4 and 8 were generated to show other components that could be present in the sample matrix are resolved from the parent analytes. No change was observed in the chromatogram of ceftriaxone and Statin's in the presence of common excipients. The specificity was also determined by injecting human plasma samples. Figure 5 and 7 represent human serum containing all drugs. Therefore, the proposed method is selective and specific for the drugs.

Detection and quantitation limit

The LOD and LOQ values for ceftriaxone and Statin's were determined and are presented in table 1. The calculated LOD and LOQ values confirmed that method were sufficiently sensitive.

Range and linearity

Solutions, as described above, were injected at the interval of 4 hour on day one. This process was repeated for 4 days. Standard curves were constructed at concentrations 2.5, 5, 10, 15, 20 and 25 µg/mL of all statin's and ceftriaxone. The standard calibration curves were shown to be linear in the above mentioned range in human serum. Curves were obtained by plotting the peak area against concentrations of these drugs. Linear calibration curves were generated by linear regression analysis and obtained over the respective standard concentrations ranges. The suitability of the calibration models were confirmed by back-calculating the concentrations of the calibration standards. The standard curve, slope, intercept and the correlation coefficient were determined. The regression statistics are shown in table 1.

Ruggedness & robustness

Ruggedness of this method was evaluated in two different labs with two different instruments. Lab 1 was in the Research Institute of Pharmaceutical

Sciences, Faculty of Pharmacy University of Karachi, while Lab 2 was in the Department of Chemistry, Faculty of Science University of Karachi. The method did not show any notable deviations in results from acceptable limits. Robustness was evaluated by slight changes in pH levels of mobile phase and it was found that the %R.S.D. values did not exceed more than 2 %. The developed method has been applied for the determination of different drug contents in formulations. The assay results shown in, demonstrates the suitability of method. Similarly the % recoveries of drugs in presence of serum reveal the applicability of method for therapeutic purposes.

CONCLUSION

In short, our method is specific, sensitive, rapid and easy to perform for simultaneous determination of ceftriaxone and statin's (simvastatin, rosuvastatin, atorvastatin and pravastatin). The limit of quantification, small sample volume and short chromatographic time of this method makes it advantageous for adaptation to routine assay requirements and enables simultaneous determination of statin's and ceftriaxone because of good separation and resolution of the chromatographic peaks. The obtained results are in good agreement with the declared contents of dosage formulations. Results are accurate and precise and are confirmed by the statistical parameters. Reliability, rapidness, simplicity, sensitivity, economical nature, good recovery and precision of this method give it advantage over the other reported methods.

Table 1: Regression statistics

Drugs	Linearity range ($\mu\text{g/mL}$)	%-RSD	%-Recovery	r^2	LOD	LOQ
Ceftriaxone	2.5 - 25	0.55	100.75	0.9966	0.284	0.860
Rosuvastatin	2.5 - 25	0.44	100.52	0.9997	0.172	0.520
Atorvastatin	2.5 - 25	0.62	100.70	0.9977	0.124	0.376
Pravastatin	2.5 - 25	1.26	101.39	0.9998	0.332	1.006
Simvastatin	2.5 - 25	1.17	101.25	0.9995	0.290	0.879

Table 2: System suitability parameters.

Drugs	Ret. Time (minutes)	k	Tailing	Resolution	Separation factor
Ceftriaxone	2.4	0	1.45		
Pravastatin	3.2	0.34	1.34	3.59	0
Rosuvastatin	3.3	0.43	1.41	3.28	0
Atorvastatin	4.0	0.71	1.46	1.84	1.68
Simvastatin	8.0	2.29	1.41	13.51	6.79

Table 3: Interday accuracy and precision of ceftriaxone and statin's

Drugs	Conc.*	Conc.*	Conc. found	%R.S.D.	% Recovery
		$\mu\text{g/mL}$	$\mu\text{g/mL}$	Precision	Accuracy
Ceftriaxone	80%	8	8.00	0.01	99.99
	100%	10	10.12	0.83	101.19
	120%	12	12.20	1.19	101.69
Rosuvastatin	80%	8	8.00	0.04	99.94
	100%	10	10.13	0.88	101.26
	120%	12	12.02	0.13	100.19
Atorvastatin	80%	8	7.99	0.13	99.82
	100%	10	10.25	1.75	102.50
	120%	12	12.01	0.08	100.12
Pravastatin	80%	8	8.00	0.04	99.95
	100%	10	10.11	0.76	101.08
	120%	12	12.10	0.58	100.83
Simvastatin	80%	8	7.99	0.05	99.93
	100%	10	10.10	0.70	100.99
	120%	12	12.07	0.43	100.61

*Concentration

Table 4: Precision and recovery of ceftriaxone and statin's in formulation.

Drugs	Conc. injected	Conc. found($\mu\text{g/mL}$)			%RSD
	($\mu\text{g/mL}$)	24hours	48hours	72hours	Mean
Ceftriaxone	8	8.01	7.90	8.04	0.35
	10	10.13	10.20	10.20	1.13
	12	11.68	11.91	11.95	0.98
Rosuvastatin	8	8.08	8.02	7.87	0.52
	10	9.83	10.23	10.23	1.34
	12	11.93	11.95	12.00	0.21
Atorvastatin	8	7.91	8.04	8.02	0.38
	10	10.00	10.07	10.07	0.69
	12	11.97	11.81	11.93	0.44
Pravastatin	8	8.20	8.05	8.99	0.46
	10	9.76	9.76	10.18	0.56
	12	12.09	11.47	12.12	0.26
Simvastatin	8	8.22	8.13	8.05	1.10
	10	10.09	9.78	10.05	0.43
	12	12.08	10.95	11.95	0.29

Table 5: Precision and accuracy of ceftriaxone and statin's in human serum

Drug	with in day			between day		
	Conc. ($\mu\text{g/mL}$)	Amount recovered	% RSD	Amount recovered	Mean %recovery	Mean % RSD
Ceftriaxone	8	8.00	0.01	7.98	99.81	0.47
	10	10.12	0.83	10.18	101.75	1.23
	12	12.20	1.19	11.85	98.72	0.91
Rosuvastatin	8	8.00	0.04	7.99	99.88	0.68
	10	10.13	0.88	10.10	100.96	1.49
	12	12.02	0.13	11.96	99.69	0.24
Atorvastatin	8	7.99	0.13	7.99	99.86	0.46
	10	10.25	1.75	10.05	100.48	0.34
	12	12.01	0.08	11.90	99.21	0.57
Pravastatin	8	8.00	0.04	8.05	100.67	0.99
	10	10.11	0.76	9.93	99.25	0.53
	12	12.10	0.58	12.07	100.55	0.58
Simvastatin	8	7.99	0.05	8.11	101.40	0.98
	10	10.10	0.70	10.07	100.69	0.48
	12	12.07	0.43	12.03	100.23	0.57

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