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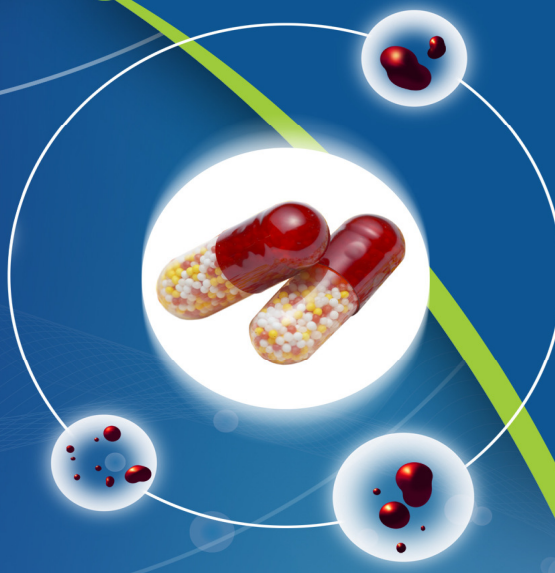

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Research Article

SIMULTANEOUS DETERMINATION OF CEFTRIAXONE SODIUM AND H_2 RECEPTOR ANTAGONISTS 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 H_2 receptor antagonists in formulation and human serum has been developed and validated. Ceftriaxone and H_2-receptor antagonists analysis was conducted on a Purospher STAR, C_{18} (5µm, 250 x 4.6 mm) column and mobile phase was water and methanol (60:40, v/v), pH adjusted at 2.8 with ortho-phosphoric acid. Flow rate was 1 $mLmin^{-1}$ and UV detector was set at 240 nm for cimetidine, ranitidine and Famotidine. The results obtained showed a good agreement with the declared content. The method shows good linearity in the range of 2.5–25 μgmL^{-1} with a correlation coefficient 0.9995 – 0.9999 (inter-and intra-day RSD < 2 %).The limit of detection and quantification for ceftriaxone and H_2 receptor antagonists in pharmaceutical formulation and serum were in the range 0.06-0.41 μgmL^{-1}. Analytical recovery was 98.6 - 101.29%. The proposed method may be used for the quantitative analysis of commonly administered H_2 receptor antagonists i.e. cimetidine, ranitidine and Famotidine 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, H_2 receptor antagonists, Cimetidine, Ranitidine, Famotidine and RP-HPLC.

INTRODUCTION

Ceftriaxone is a (6R,7R)-7-{2-(2-amino-4-thiazolyl)-(Z)-2-[methoxyiminuteo-acetamido]-3{[(2,5-dihydro-6-hydroxy-2-methyl-5-oxo-as-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.

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It is a third generation parenteral cephalosporin with a relatively long half-life which is stables to β-lactamases particularly those produced by Gram-negative bacteria [1-6]. It has excellent anti Gram-negative activity. 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 multidrug-resistant *Enterobacteriaceae* [7-12]. Histamine is a physiologically active

endogenous substance (autocoid), produced within the body by the decarboxylation of the amino acid, histidine [13]. H_2-recepter antagonists are reversible competitive blockers of histamine at H_2 receptors, they bind with H_2 receptors on the parietal cells of stomach and inhibit gastric acid secretion and also decrease hydrogen ion concentration of gastric juice, therefore, useful in the treatment of peptic and duodenal ulcer, Zollinger-Ellison syndrome, reflux oesophagitis, stress ulcer, short-bowel syndrome, hypersensitivity states and also given as a pre-anesthetic medication in emergency operation to reduce the danger of aspiration of acidic contents [14-15].

H_2-receptor antagonists are commonly prescribed to patients complaining of GI irritations, which may be a consequence of inflammations in the tract. In addition, all cephalosporins cause gastric upset even in normal subjects. H_2-receptor antagonists may be co administered in patients having cephalosporin therapy and complaining of about gastric irritations specially the peptic ulcer patients, who are the real victims of this simultaneous drug therapy. Sometime be aware of possible life threatening adverse reactions to commonly used H_2-receptor antagonists, such as famotidine with cephalosporin's [16-17].

The scope of study was to investigate the possible effect of these H_2 receptor antagonists on emergency treatment with ceftriaxone. The present method was actually developed in our laboratories for drug–drug interaction studies between ceftriaxone and H_2 receptor antagonist in order to study the effect of these on the availability of ceftriaxone. A method was developed for the simultaneous determination of ceftriaxone and H_2 receptor antagonist. Furthermore, we report a specific and sensitive HPLC method for the simultaneous determination of ceftriaxone and H_2 receptor antagonists 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. 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) [18]. Another HPLC method for the determination of ceftriaxone has been developed by Eric [19]. Traument [20] 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., [21] Nahata [22], and Jehl and Birckel [23] also developed a method using phosphate buffer of pH 7 as a mobile phase. All of these methods consisted of buffers combination as mobile phase. Several methods have been reported for the determination of ranitidine and/or cimetidine in pharmaceutical preparations using high performance liquid chromatography (HPLC) [24-27]. Many HPLC methods have been reported for the analysis of individual H_2 antagonists, for cimetidine [28-30], famotidine [31-33] and ranitidine [34] in biological samples comprising urine or urine and plasma.

However, no method for simultaneous determination of H_2 receptor antagonists 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 reverse phase high performance liquid chromatography (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 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 reverse phase high performance liquid chromatography (RP-HPLC) method for the simultaneous determination of ceftriaxone, cimetidine, ranitidine and famotidine 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 µL loop, column and SPD-10 A VP UV–VIS detector and Purospher star C18 (5 µm, 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 H_2 receptor antagonists used were cimetidine (Cimetamat ® 200 mg), ranitidine (Anzol ® 150 mg) from Indus pharma and famotidine (Acicon ® 20 mg) of Barrett Hadgson (Pvt) Ltd which were purchased from the local pharmacy. All these drugs had an expiry date of not less than one year at the time of study.

Stock and working solution preparation

Stock standard solutions of 100 μgmL^{-1} of ceftriaxone, cimetidine, ranitidine and famotidine were prepared individually by dissolving 10 mg of each in 100 mL volumetric flask using mobile phase. Working solutions were also prepared separately by diluting from the standard solution to obtain 2.5-25 μgmL^{-1}. These solutions were stored at 20 ºC and analyzed daily for inter-day and inter-operator variations of the method and analyzed each time before drug analysis in biological samples. Twenty micro liters 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 H_2 receptor antagonists (Cimetidine, Ranitidine and Famotidine) and 10 mg of ceftriaxone were transferred to 100 mL volumetric flask dissolved and diluted with mobile phase. The resulting solutions were filtered through 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 ºC for the preparation of drug serum solution. To 1 mL of plasma (human blood plasma) 10 mL of acetonitrile were 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 (human blood plasma) obtained was added to the above solutions to produce the desired concentrations and stored at 20 ºC. In order to evaluate linearity in serum five concentration levels ranging from 2.5–25 μgmL^{-1} were prepared and linearity and % R.S.D values were evaluated.

METHOD DEVELOPMENT

Experimental design and optimization of isocratic HPLC 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 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 (isobestic point). However, the peak shape and resolution were found to be good when the mobile phase comprising of the water: methanol pH adjusted to 2.8 with phosphoric acid was used in the ratio of (60:40 v/v) at a flow rate of l $mLmin^{-1}$ (filtered through a 0.45 micron filter). For simultaneous determination of ceftriaxone with H_2 receptor antagonists; cimetidine, ranitidine and famotidine in individual drug solutions were injected into the column at the concentration of 100 μgmL^{-1} and both elution pattern and resolution parameters were studied.

Table 1: Calibration curves and limits of detection and quantification of ceftriaxone and H_2 receptor antagonists

Analytes	**Regression equation**	**r^2**	**Slope**	**LOD**	**LOQ**
				(μgmL^{-1})	(μgmL^{-1})
Ceftriaxone	y = 58848x - 54419	0.9995	11157.27	0.10	0.30
Cimetidine	y = 25013x - 21770	0.9996	4882.507	0.09	0.28
Ranitidine	y = 26914x - 24989	0.9998	10842.7	0.06	0.20
Famotidine	y = 54324x - 57135	0.9999	5381.072	0.14	0.41

Table 2: System suitability parameters

Ret. time	**Drugs**	**K**	**Tailing**	**Resolution**	**Theoretical plates**
3.00	Cimetidine	0.3	1.18	2.92	2553
2.93	Ranitidine	0.41	1.09	4.22	3721
2.84	Famotidine	0.40	1.31	4.35	2247
3.97	Ceftriaxone	0.71	1.26	4.20	1934

Wavelength selection

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

All validation steps were carried out according to the ICH guidelines [35-38].

Method validation establishes that the method performance characteristics are suitable for the intended use. Validation entails evaluation of various parameters of the method such as system suitability, selectivity, specificity, linearity (concentration–detector response relationship), accuracy, precision, sensitivity, detection and quantitation limit recovery from the matrix**.**

System suitability

The system suitability was assessed by five replicate analyses of the drug at a concentration of 10 μgmL^{-1}. System suitability of the method was evaluated by analyzing the repeatability, peaks symmetry (symmetry factor), theoretical plates of the column, resolution between the peaks of ceftriaxone and H_2 antagonists, mass distribution ratio (capacity factor) and relative retention. Typical system suitability results are summarized in table 2, all the values for the system suitability parameters are within limits. Two clear peaks were observed for the individual drugs. The separation of ceftriaxone and H_{2-} antagonists obtained by this method is significantly better.

Selectivity and Specificity

Specificity is the ability of a method to discriminate between the analytes of interest and other components that are present in the sample. Representative chromatograms 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 H_2 receptor antagonists in the presence of common excipients (Figure 1-3). The specificity was also determined by injecting human plasma samples.

Linearity and Range

Standard curves were constructed at concentrations 2.5, 5, 10, 15, 20 and 25 μgmL^{-1} of all H_2 receptor antagonists and ceftriaxone. Calibration curves were linear within the quantification ranges for all the assayed drugs using a linear regression. Beer's law is obeyed over this concentration range (table 2). Accordingly, using an intercept, excellent linearity was obtained in all cases with correlation coefficients ≤ 0.9995.

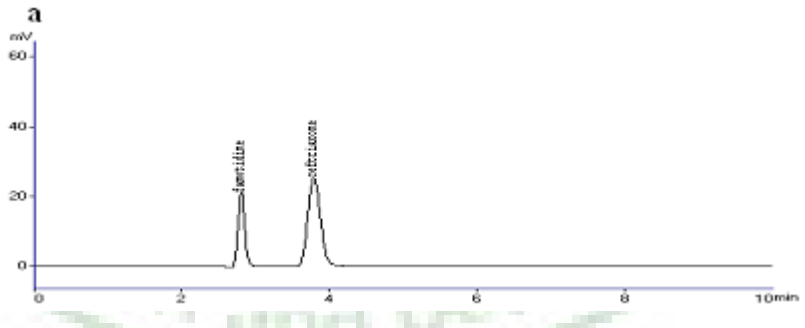

Figure 1: Chromatogram of famotidine [1] and ceftriaxone [2](a),

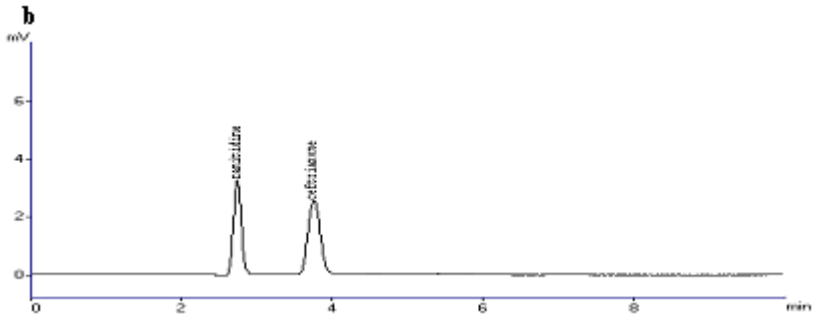

Figure 2: Chromatogram of ranitidine [1] and ceftriaxone [2] (b)

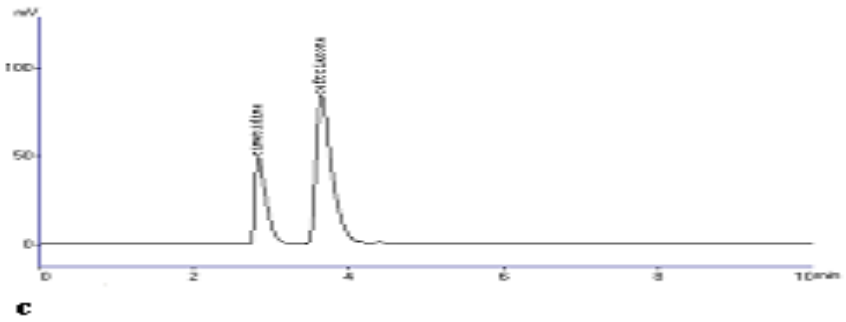

Figure 3: Chromatogram of cimetidine [1] and ceftriaxone [2] (c)

Table 3: Accuracy and precision of ceftriaxone and H_2 receptor antagonists in formulation

Analytes	Conc.* (μgmL^{-1})	Conc. found (μgmL^{-1})	%R.S.D.	% Rec.
Ceftriaxone	8	8.10	0.91	101.29
	10	10.12	0.86	101.23
	12	11.91	0.51	99.28
Cimetidine	8	8.09	0.80	101.13
	10	10.00	0.03	99.96
	12	12.06	0.34	100.49
Ranitidine	8	8.14	1.24	101.77
	10	10.13	0.89	101.26
	12	11.83	1.00	98.60
Famotidine	8	7.98	0.14	99.81
	10	10.02	0.12	100.17
	12	12.06	0.37	100.53

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. The results of accuracy revealed that the method was accurate for all above purposes. The results demonstrated that the values were within the acceptable range and the method was sufficiently accurate and precise. The method passed the test for repeatability as determined by % RSD of the area of the peaks of six replicate injections at 100% test concentration.

Table 4: Precision and recovery of ceftriaxone and H_2 receptor antagonists in formulation

		Intra-day			Inter-day		
Analytes	**Conc. injected**	**Conc. Found**	**%RSD**	**%Rec.**	**Conc. Found**	**%RSD**	**%Rec.**
	μgmL^{-1}	μgmL^{-1}			μgmL^{-1}		
Ceftriaxone	2.5	2.45	1.36	98.09	2.50	0.02	99.97
	5	5.10	1.35	101.93	4.92	1.15	98.38
	10	10.12	0.86	101.23	10.02	0.14	100.19
	15	15.14	0.65	100.93	15.03	0.13	100.18
	20	20.04	0.13	100.18	19.7	1.93	98.50
	25	24.92	0.22	99.70	25.40	1.13	101.61
Cimetidine	2.5	2.46	1.23	98.27	2.49	0.34	99.52
	5	4.98	0.25	99.64	4.98	0.25	99.65
	10	10.00	0.03	99.96	9.95	0.33	99.54
	15	15.00	0.02	100.03	14.98	0.09	99.88
	20	20.33	1.17	101.67	19.80	0.71	99.00
	25	24.57	1.24	98.27	25.12	0.34	100.48
Ranitidine	2.5	2.46	1.28	98.21	2.49	0.29	99.59
	5	5.09	1.24	101.77	5.05	0.65	100.93
	10	10.13	0.89	101.26	9.88	0.88	98.76
	15	15.18	0.85	101.21	15.11	0.52	100.74
	20	19.65	1.26	98.24	20.24	0.85	101.21
	25	25.07	0.21	100.29	25.09	0.26	100.37
Famotidine	2.5	2.51	0.29	100.41	2.49	0.41	99.42
	5	5.07	0.99	101.40	4.98	0.24	99.66
	10	10.02	0.12	100.17	10.09	0.63	100.89
	15	15.11	0.51	100.73	15.11	0.53	100.75
	20	20.40	1.42	102.02	20.10	0.37	100.52
	25	24.73	0.78	98.91	25.03	0.08	100.11

Detection and quantitation limit

The LOD and LOQ values for ceftriaxone and H_2 receptor antagonists were determined and are presented in table 1. The calculated LOD and LOQ values confirmed that methods were sufficiently sensitive.

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 H_2 receptor antagonists (Cimetidine, Ranitidine and Famotidine). 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 H_2 receptor antagonists 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.

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