December 2008 Volume 7 Issue 11

Trade Science Inc.

Analytical CHEMISTRY

An Indian Journal

Full Paper

ACAIJ, 7(11) 2008 [807-811]

Determination of residual formic acid in ceftazidime drug substances using ion chromatography by facile non-suppressed conductivity detection

Ramalingam Murugan[1]*, S.Sriman Narayanan[2]

[1]Department of Analytical Research and Development, Orchid Research Laboratories Ltd., Sholinganallur, Chennai-600 119 , (INDIA)

[2]Department of Analytical Chemistry, University of Madras, Guindy Campus, Guindy, Chennai-600 025, (INDIA)

Tel: 91-044-24501474

E-mail : murugan.r@orchidpharma.com

Received: 8th September, 2008 ; Accepted: 13th September, 2008

ABSTRACT

A simple and sensitive ion chromatographic method, for the determination of residual formic acid in sterile ceftazidime drug substance has been developed and validated. The formate ion was separated from the drug substance by an acid selective ion exchange column. The mobile phase was 10% acetone and 90% of aqueous sulfuric acid (5×10^{-4} M). This method was linear over the concentration in the range of 0.25µg/ml to 25.33µg/ml with $r^2 > 0.98$ and the signal-to-noise ratio 0.065. This method was validated in terms of Selectivity, Linearity, Precision, Accuracy, Robustness, Limit of detection (LOD), Limit of quantitation (LOQ). This method has been succefully applied for formulated and drug substance of ceftazidime.

KEYWORDS

Ceftazidime;
Cephalosporins;
Formic acid;
Ion chromatography.

1. INTRODUCTION

Ceftazidime, a β-lactam antibiotic is commonly used for the treatment of nosocomial gram-negative bacilli infections, particularly *Pseudomonas aeruginosa* infection (Richards and Brogden, 1985). Ceftazidime inhibits one of the enzymes involves in the synthesis of bacterial cell walls[1]. It is 1-[[(6R, 7R)-7-[2-(2-Amino-4-thiazolyl) glyoxylamido]-2-carboxy-8-oxo-5-thia-1-azabicyclo[4.2.0] oct-2-en-3-yl] methyl] pyridinium hydroxide, inner salt, 72-(Z)-[O(1-carboxy-1-methylethyl) oxime], pentahydrate. (Figure 1). The molecular formula is $C_{22}H_{22}N_6O_7S_2.5H_2O$ representing a molecular weight of 636.65[2-4]. Formic acid was used in the synthetic process of ceftazidime pentahydrate. Formic acid falls in class III solvent as per International Conference on Harmonization[ICH] guidelines and it should not be more than 0.5% in any active pharmaceutical ingredients. Consequently the analysis of class

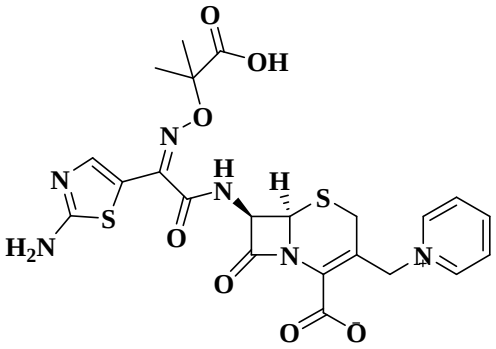

Figure 1: Chemical structure of ceftazidime

III type of solvent such as formic acid becomes vital to observe long-term effects such as genotoxicity, carcenogenicity[5]. The determination of formic acid by capillary electrophoresis in various products like milk butter, soil, urine and rubber has been reported[6,7]. Another method using ion exclusion chromatography has been used to determine the content of formic acid

Full Paper

in fruit juices by UV detection[8]. There is no reported method to analyze the residual formic acid content in the ceftazidime drug substances. This paper explicates the method development and validation to determine the formic acid in the ceftazidime drug substances and formulated products[9-11].

2. EXPERIMENTAL

2.1. Samples and reagents

The well-examined samples of ceftazidime pentahydrate bulk material in powdered form (B.No-CFTZ/165/2005) were obtained from Orchid Chemicals & Pharmaceuticals Ltd., Chennai, India.

Formic acid AR grade were obtained from Merck, Germany. Dimethyl Sulfoxide of AR grade obtained from s.d-fine-chem Ltd, India and Sulfuric acid AR grade from Ranbaxy Ltd, India. Commercial Ceftazidime formulated samples (Glaxo SmithKline & Biochem Pharmaceuticals) were purchased from the local market and used as such. High pure Milli-Q water was used with the help of Millipore Milli-Q plus purification system (MILLIPORE SA, 67120 MOL SHEM, France).

2.2. Apparatus

The Metrohm 732 Ion-exchange chromatograph (Metrohm, Switzerland) equipped with a serial dual pump with flow range 0.05 to 5.0 ml/min and a conductivity detector having the conductivity measuring range between 0.05μS/cm and 100μS/cm. A stainless steel column of 250mm length and 7.8 mm internal diameter packed with polystyrene divinylbenzene (Metrohm, Switzerland) functionalised with sulfonic acid groups. Metrohm IC Net 2.3 was used as a data handling system. Samples were weighed in Sartorius ME 235 S (Sartorious, Germany) microbalance.

2.3. Ion chromatography conditions

An in-house ion chromatography method was developed for the determination of formic acid in Ceftazidime, where a stainless steel column of 250mm length and 7.8 mm internal diameter packed with polystyrene divinylbenzene (PS-DVB) functionalised with sulfonic acid groups with a mobile phase consisting of a mixture of sulfuric acid (5×10^{-4}M) and acetone in the ratio of (90:10), flow rate of 0.5 ml/min. The overall analysis was performed at ambient temperature, the injection volume 10μL and the overall run time was 30 minutes.

2.4. Evaluation of system suitability

Equal quantity of formic acid working standards were accurately weighed in separate flasks and diluted to make required concentration using mobile phase (0.02 mg/ml). The % RSD of the area of formic acid from six replicated injections will not be more than 2.0.

3. RESULTS AND DISCUSSION

3.1. Optimization of IC conditions

In order to obtain a precise and rugged method, several attempts have been made to quantify the residual formic acid in ceftazidime drug substance. In preliminary experiments an anion exchange resin column having polymethacrylate with quaternary ammonium groups was used with an anion buffer eluent by Ion chromatographic technique. In order to improve the peak symmetry, a cation exchange resin column having Poly Styrene-Divinyl Benzene[PS-DVB] copolymer with sulphonic acid group was used to elute out target

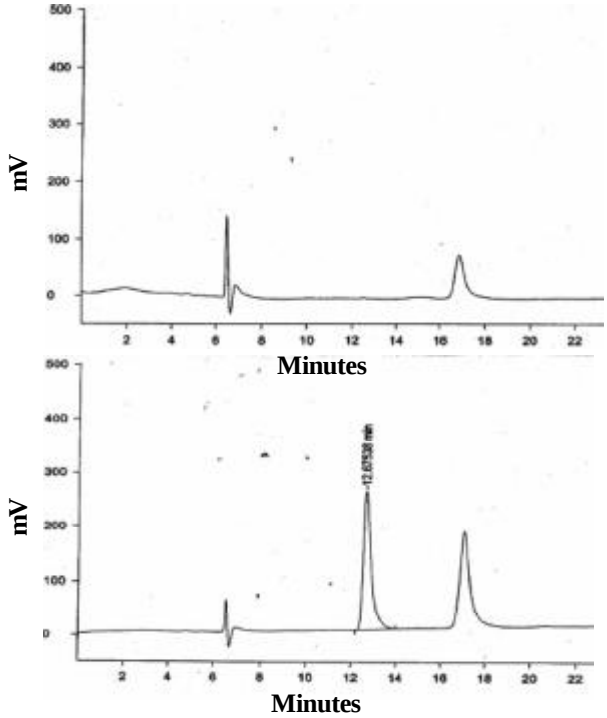

Figure 2 : (a) Chromatogram of diluent for formic acid, (b) chromatogram of formic acid standard

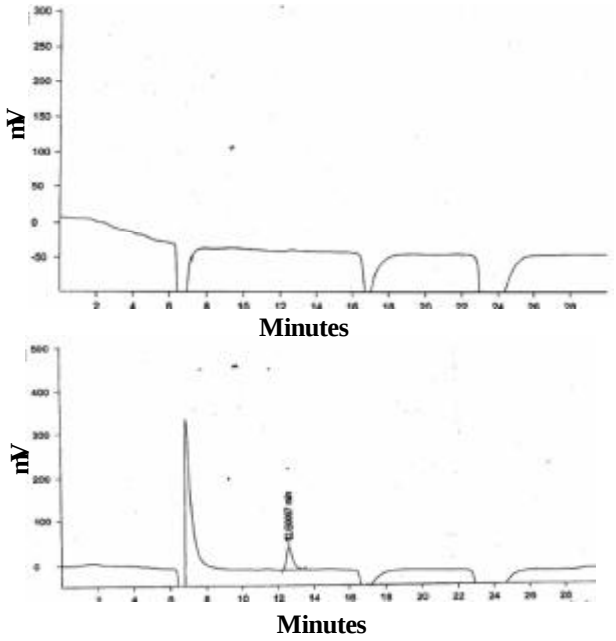

Figure 3 : (a) Chromatogram of diluent for Ceftazidime, (b) Chromatogram of Ceftazidime sample with formic acid

TABLE 1: Limit of quantification (LOQ)

Preparation	Area
1	277.929
2	329.830
3	278.391
4	307.666
5	277.468
6	303.197

% RSD = 7.27

TABLE 2: Limit of detection (LOD)

Preparation	Area
1	96.970
2	62.278
3	118.584
4	95.782
5	91.237
6	90.064

% RSD = 19.53

analyte. The use of organic solvent (acetone) resulted in a better separation of formic acid from the degraded drug. The content of organic solvent in the eluent was optimized to 10% after varying it in the range of 5% to 25%. A value of 10% was fixed in order to have reasonable analysis time without compromising resolution from interfering peaks from the sample. Formic acid eluted at retention time of 12.5minutes (Figures 2(a, b)).

3.2. Validation of determination of formic acid

After optimization of analytical conditions, the evaluation of parameters such as specificity, linearity, LOD, LOQ, precision, accuracy, ruggedness and robustness were completed for the validation of the method.

Specificity

In order to show this method is highly specific, formic acid was injected individually in the concentration about 0.02 mg/ml. Further to confirm the specificity, the formic acid was spiked with the sample in 1% level to the ceftazidime concentration 4.0 mg/ml. It was observed that the formic acid is well separated from each other and also from the ceftazidime peak (Figures 3(a, b)).

This method is not only specific in the normal analysis, but also in the analysis of ceftazidime samples, which endure in stressed conditions like thermal and photolytic degradation.

Linearity

The solution of ceftazidime and the formic acid was prepared at low concentrations from 0.25 µg/ml and at higher concentrations 25µg/ml, and the relationship between peak area (Y) and concentration (X) was observed. An excellent linearity[for formic acid Y = 13654 X + 563 (r = 0.98)] was obtained within the above concentration range. Microsoft Excel software used to plot the peak areas versus micrograms injected.

Limit of quantitation and detection

The limit of quantitation (LOQ) of known related substances of ceftazidime were determined by using the residual standard deviation[STEYX, that is the standard error of the predicted Y value for each X in the regression. The standard error is a measure of the amount of error in the prediction of Y for an individual X] and the slope values from the linearity data of respective related substances using the following formula[LOQ = (STEYX / slope) ×10]. The each related substance solutions were prepared at about the predicted LOQ concentration level and its precision was verified. (TABLE 1).

Similarly the limits of detection (LOD) of known related substances of ceftazidime were determined by using the following formula[LOD = (STEYX / slope) × 3.3]. The each related substance solutions were prepared at about the predicted LOD concentration level and its precision was verified. (TABLE 2).

Full Paper

TABLE 3: Ruggedness data (Precision and intermediate precision

Formic acid content (% w/w/, as is)							
S.no.	1	2	3	4	5	6	% RSD overall
Analyst-1	0.102	0.103	0.099	0.102	0.102	0.102	1.37
Analyst-2	0.107	0.107	0.108	0.107	0.109	0.106	0.93

% RSD = 3.05

TABLE 4: Recovery of formic acid

Level*	Added	Recovered	%
	µg/ml	µg/ml	Recovery
20%	3.895	3.742	96.08
50%	9.737	9.072	93.15
100%	19.475	18.980	97.46
150%	29.212	29.326	100.39

*** = each 3 determination**

TABLE 5: Solution stability at room temperature

Time (min)	Area	Cumulative % RSD
Intial	7910.806	-
62	7900.764	0.090
121	7886.185	0.160
180	7971.729	0.470
241	8206.397	1.670
300	7949.194	1.500
360	8005.194	1.380

Precision or reproducibility and ruggedness

The precision of the method was determined by preparing a sample solution of formic acid (in the concentration of 0.02 mg/ml) six times and analyzed as per the proposed method. The formic acid of ceftazidime was calculated against the standard formic acid peak. Two different analysts conducted the six replicate determination of ceftazidime drug substance in the same concentration on different days using different instruments in two different columns of same brand. The comparative results are summarized in TABLE 3. There is no significant deviation between the results of two different values, it has clearly indicates that this method is precise and rugged.

Accuracy

Method accuracy was demonstrated by spiking a known amount of formic acid in the sample preparation (1.0 mg ml^{-1}) in four different concentration levels from 3.5µg/ml to 30µg/ml in triplicate. There is no significant change in the values between the amount added and the amount recovered after the corrections of the known formic acid, which is already present. The percentage recoveries of all substances were in between 91 to 102. (The acceptance criteria is 80 % to 120 %) The %

TABLE 6: Robustness data

Preparation	-2% flow	+2% flow
	Area	Area
1	6777.233	5620.529
2	6895.602	5650.997
3	6922.993	5579.368
4	6842.301	5641.475
5	6864.100	5664.988
6	6855.732	5628.419
	% RSD = 0.76	% RSD = 0.53
Preparation	**-10% organic**	**+10% organic**
	Area	**Area**
	6803.282	5347.134
	6698.569	5406.545
	6661.619	5386.127
	6725.925	5384.522
	6808.470	5424.861
	6748.166	5463.156
		% RSD = 0.86

RSD of recovery of three levels were <3.0.(TABLE 4).

Stability of analytical solution

The solution (4.0 mg/ml) of ceftazidime with the known concentration of formic acid (spiked in 1% level) was studied at room temperature at different time intervals. The cumulative %RSD of each related substances were calculated and concluded that the ceftazidime and formic acid were stable for about 6 hrs at room temperature (≅25°C) (TABLE 5).

Robustness

The chromatographic conditions were deliberately changed to demonstrate the robustness. The flow rate (± 10 %), the composition of acetone (± 2 % absolute) was changed to check the difference in the resolution between the formic acid and other peaks of ceftazidime. There is no noteworthy variation in results were clearly indicates that this method is robust (TABLE 6).

System suitability

The system suitability testing, which is part of an integral part of chromatographic methods, and used to verify that the reproducibility of the system are adequate for the analysis to be performed.

4. CONCLUTIONS

According to complete validation studies, the formic acid peak of ceftazidime was free of interference from the related substances and its degradation prod-

ucts, point out that the proposed ion chromatography method is simple, precise, accurate, rugged and robust in all situation.

ACKNOWLEDGMENTS

The authors wish to thank Dr.Gopalan, CEO, Orchid Research Laboratories Ltd., and the management for permitting this work to be published. Cooperation extended by all contemporaries of analytical research department and the appreciatively acknowledged.

REFERENCES

[1] A.S.Benko, D.M.Cappelletty, J.A.Kruse, M.J. Rybak; Antimicrob Agents Chemother, **40**, 691-5 **(1996)**.
[2] E.R.Branhart; 'Physicians Desk Reference', 44th ed., New Jersey, Medical Economics, **1**, 1007-10 **(1990)**.
[3] W.A.Craig, S.C.Ebert; Antimicrob Agents Chemother, **36**, 2577-83 **(1992)**.
[4] B.Fantin, R.Farinotti, A.Thabaut, C.Carbon; J.AntimicrobChemother, **33**, 563-9 **(1994)**.
[5] ICH; Harmonized Tripartite Guideline - Impurities: Guideline for Residual Solvents, **17**, **(1997)**.
[6] J M Izco, M Tormo, R Jimenez-Flores; J.Agric.Food Chem., **50(7)**, 1765-73 **(2002)**.
[7] V.Galli, N.Olmo, C.Barbas; J.Chromatogr A, **894(1-2)**, 135-44 **(2000)**.
[8] D.H.Guo, L.Xia, Se Pu; Medline, **19(3)**, 276-8 **(2001)**.
[9] General chapter <621> Chromatography, United States Pharmacopoeia 27-National Formulary22, USP Pharmacopeial Convention Inc., Rockville, 2272-2284 **(2004)**.
[10] General Chapter <1225> Validation of Compendial Methods, United States, Pharmacopoeia 27-National Formulary 22, USP Pharmacopeial Convention INC., Rockville, 2622-2625 **(2004)**.
[11] ICH Guidelines, Q2B- Validation of analytical procedures: Methodology.