Simultaneous determination and stability indicating HPLC assay method for cefatrizine pentahydrate and potassium clavulanate

Singh Loveraj*, Gupta Pallavi, Kona.S.Srinivas, Dwivedi Dhananjay, Kishor Kamal

Ranbaxy Research Laboratories, ARD-NDDR, PlotNo.20, Sector18, Udyog Vihar Industrial Complex, Gurgaon-122001, Haryana, (INDIA)

E-mail : loveraj.singh@ranbaxy.com

ABSTRACT

A rapid and specific high-performance liquid chromatographic gradient method was developed and validated for the simultaneous determination of cefatrizine and potassium clavulanate in bulk drug. The present paper describes the simultaneously determination and stability-indicating assay method by HPLC. Forced degradation studies were performed for cefatrizine pentahydrate and potassium clavulanate bulk drug using acid (0.1N hydrochloric acid), base (0.01N sodium hydroxide), and oxidation (0.1% hydrogen peroxide). Chromatography conditions were phosphate buffer pH 5.5 (20mM potassium dihydrogen orthophosphate), acetonitrile, orthophosphoric acid and triethylamine.The mobile phase initial composition was (buffer: acetonitrile- 100:0). A (150×4.6 mm) Kromasil column with particle size 5μm was used. The flow rate was 1ml/min, the column temperature was 30°C, and the detection performed out a wavelength of 210nm. The samples were kept at 5°C in an autosampler cooler. The developed HPLC method was statistically validated and method was linear, précised, rugged and robust. © 2009 Trade Science Inc. - INDIA

INTRODUCTION

Potassium clavulanate (Figure 1), produced by *Streptomyces clavuligerus*, is a potent inhibitor of β-lactamase enzymes which are responsible for the protection of micro-organisms against β-lactam antibiotics[1]. Microorganisms which are resistant to certain antibiotics are increasingly posing serious problems in the treatment of infectious diseases. β-Lactam antibiotics are one of the most frequently used antimicrobial agents[2]. However, with the increased prevalence of β-lactamase-producing bacteria, penicillins and cephalosporins have become less effective. One modern strategy to cope with these problems is the discovery and development of potent and selective enzyme inhibitors lacking intrinsic antimicrobial activity, such that the antibiotics are protected from the hydrolytic activity of diverse β-lactamases. Cefatrizine is an orally active semisynthetic cephalosporin antibiotic with broad-spectrum antibacterial activity and is similar or superior to other oral cephalosporins in terms of activity against a wide range of Gram-positive and Gram-negative bacteria. Like other β-lactam antibiotics, cefatrizine is degraded and becomes inactive by various types of β-lactamases[3].

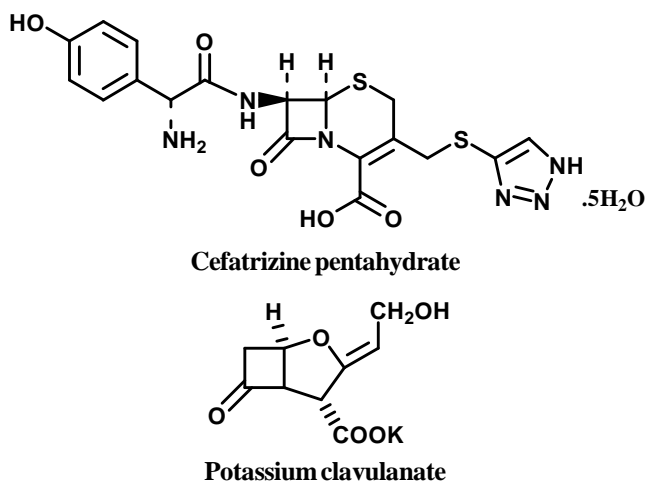Cefatrizine and potassium clavulanate in combina-

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Figure 1 : Chemical structures of cefatrizine pentahydrate and potassium clavulanate.

tion showed a good activity against laboratory strains of Gram-positive and Gram-negative bacteria and exhibited an excellent antibacterial activity not only against β-lactamase-producing strains but extended-spectrum β-lactamase-producing strains. Based on these results, new formulations containing cefatrizine and potassium clavulanate were developed to exploit this strongly synergistic effect. However, no methods have been reported for the simultaneous determination of cefatrizine and potassium clavulanate.

The present paper describes a gradient reversed-phase HPLC method to simultaneously quantify cefatrizine and potassium clavulanate in a mixture.

An HPLC assay method for the simultaneous determination and stability indicating analysis of cefatrizine and potassium clavulanate was develop under a variety of conditions, to provide the information on the drugs inherent stabilities and helps in the validation of analytical methods to be used in stability studies. Therefore, the objective of the present study was to develop a stability-indicating HPLC assay method for cefatrizine and potassium clavulanate.

EXPERIMENTAL

Equipment

The HPLC system consisted of a Waters® 2965 controller solvent delivery module (Waters Chromatography Division, Milford, MA, USA), a Waters auto sampler, a solvent degasser, a Waters 2996 PDA detector (Waters Chromatography Division, Milford, MA, USA).

A Empower® (Waters Chromatography Division, Milford, MA, USA) Chromatographic software used to record and evaluate the data collected during chromatographic analysis.

Reagents and materials

Clavulanic acid was used as a potassium salt and cefatrizine was used as a pentahydrate. The compounds were kindly provided by the Ranbaxy Research laboratories, Department of Analytical Research and Development, New Drug Discovery Research, Plot No 20, Sector 18, Udyog Vihar Industrial Area, Gurgaon, Haryana, India.

Potassium dihydrogen phosphate (Analytical grade, Merck Ltd, Shiv Sagar Estate A, Dr.Annie Besant Road Worli,Mumbai-400018 india).

Sodium carbonate anhydrous (Analytical grade, Qualilgens Fine Chemicals, A division of GlaxoSmith Kline Pharmaceuticals Limited, Dr.Annie Besant Road, Worli, Mumbai-400025).

Acetonitrile (HPLC grade, J.T.Baker, MallinckrodtBaker, inc. 222 Red School Lane, Phillipsburg NJ 08865.).

Ortho-phosphoric acid (85% w/w, Analytical grade Qualilgens Fine Chemicals A division of GlaxoSmith Kline Pharmaceuticals Limited, Dr.Annie Besant Road. Worli, Mumbai 00025).

Water (HPLC grade, MILLIPORE (INDIA) PVT. LTD. 304-306, Oriental House, Community Center, Gulmohar Enaclave, New Delhi 110049) were also used.

Chromatographic conditions

The mobile phase consist of a phosphate buffer (20 mM potassium dihydrogen ortho-phosphate and 2.0ml triethylamine in one litre HPLC grade water) maintained at pH 5.5 with the help of orthophosphoric acid and

TABLE -1 : Gradient profile

Time	Phosphate buffer pH 5.5 (%)	Acetonitrile (%)
0	100	0
3	100	0
12	85	15
16	85	15
18	100	0
20	100	0

acetonitrile which was filtered through a 0.45-µm filter (Millipore), and deaerated ultrasonically prior to use. The elution rate and injection volume were 1ml/min and 20µl respectively, the wavelength was set at 210nm for cefatrizine pentahydrate and potassium clavulanate. The column oven temperature was maintained at 30°C.

Preparation of standard solutions

Standard stock solution of Potassium clavulanate (standard stock solution A)

Weighed equivalent to 10.0mg of clavulanate acid working standard in a 50 ml volumetric flask, added 30 ml water, sonicated in ice cold water for 10 min, waited till the solution attained the room temperature, made up to volume with water.

Standard stock solution of Cefatrizine pentahydrate (standard stock solution B)

Weighed cefatrizine pentahydrate equivalent to 50.0 mg of cefatrizine working standard in a 50ml volumetric flask added about 6.2 mg sodium carbonate anhydrous and 30ml water, sonicated in ice cold water for 10 min, waited till the solution attained the room temperature, made up to volume with water.

Standard solution

Accurately transferred 5ml of standard stock solution A and 10 ml of standard stock solution B to a 50ml volumetric flask and made up the volume to 50 ml with diluent and filtered through 0.45µm nylon filter.

Test item

Potassium clavulanate and cefatrizine pentahydrate were mixed in the ratio of 1:10 w/w.

Method validation

The method was validated with respect to parameters including linearity, precision,

Solution stability, robustness, ruggedness and Force degradation[4,5].

RESULTS

Precision

System precision

Precision of the assay method was evaluated by carrying out six independent injection of the working standard. The relative standard deviation (RSD) should not be more than 2.0%. In system precision RSD was found for cefatrizine pentahydrate 0.66 and 0.41 for Potassium clavulanate.

Method precision

In method precision, the assay method was evaluated by carrying out six independent assays of the test item. The relative standard deviation (RSD) should not be more than 2.0%. The percentage RSD of six determinations was found for cefatrizine pentahydrate 0.94 and 0.98 for Potassium clavulanate.

Linearity

Linearity of the assay method was obtained from stock solutions at five concentration levels from 80% to 120%. The peak area versus concentration data was analyzed by least-squares linear regression. The correlation coefficient should be more than 0.99. In this method, the correlation coefficient was greater than 0.99 i.e. 0.999 for both the drugs.

Ruggedness

The method was found to be rugged when the cefatrizine pentahydrate and potassium clavulanate were assayed on different days, using different lot number columns, by different analysts, and by using different systems. The overall relative standard deviation was not more than 2.0%.

Robustness

To determine the robustness of the method the experimental conditions were deliberately altered and the cefatrizine pentahydrate and potassium clavulanate assayed. Variation in the chromatographic conditions (flow rate $\pm$10%, organic ratio $\pm$2% absolute, column temperature $\pm$5°C, pH of buffer $\pm$0.2) overall relative standard deviation produced an of less than 2.0%, and it was concluded that the assay of the samples was in close agreement with the initial result thus illustrating the robustness of the method.

Forced degradation

Degradation studies were carried out in different solvents and using different conditions (e.g. HCl, NaOH, H_2O_2)[6]. Force degradation studies were performed

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TABLE 2 : Summary of force degradation result

Sr. No.	Stress condition	Time	Degradation CLA (%)	Degradation CFZ (%)
1	Acid hydrolysis (0.1N HCl)	Initial	13	12
2	Alkaline hydrolysis (0.01N NaOH)	Initial	12	15
3	Oxidation (0.1% H_2O_2)	Initial	26	94

to develop a stability indicating HPLC method for the quantitative and purity evaluation of cefatrizine pentahydrate and potassium clavulanate bulk drugs.

It was found that the degraded products were well resolved from the analyte peaks. The developed HPLC method was confirmed to be specific by photo diode array for cefatrizine pentahydrate, potassium clavulanate, and the degradation products.

Acid degradation studies

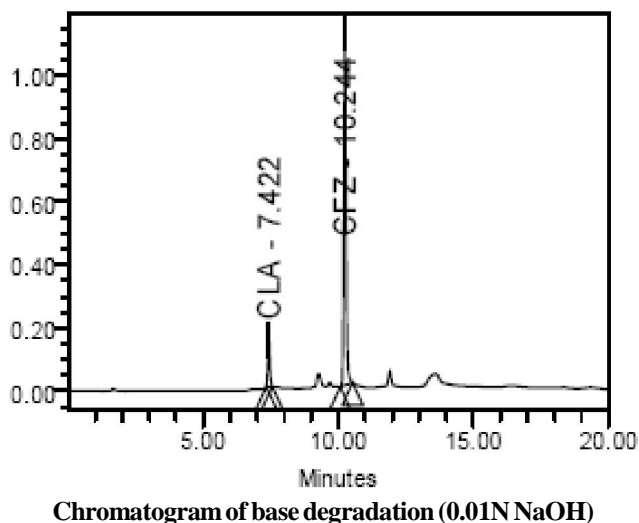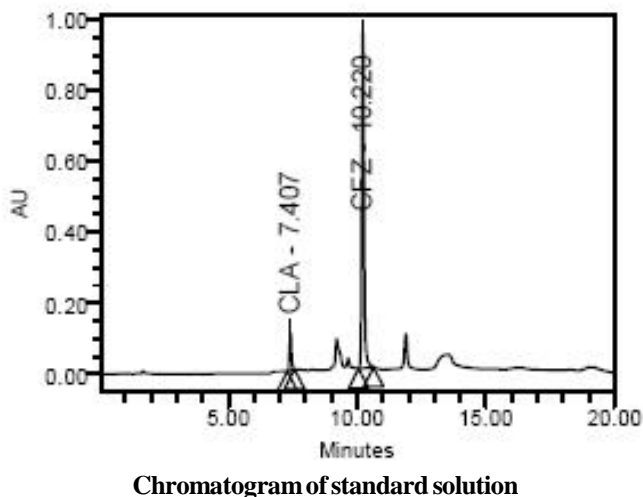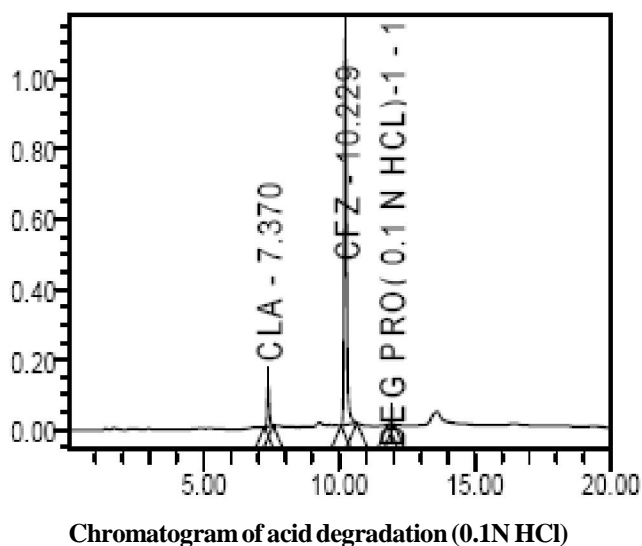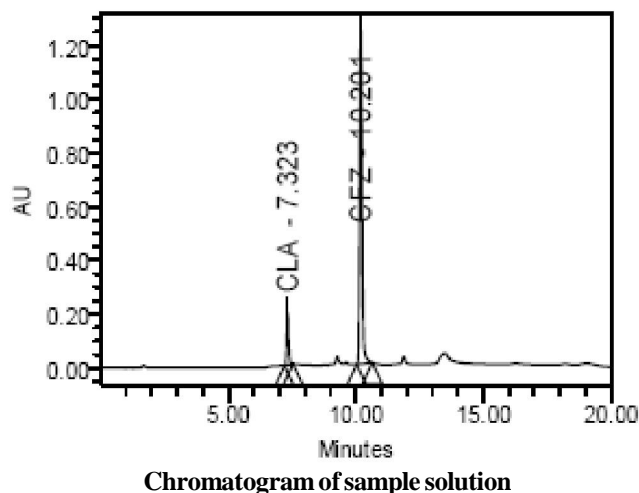Solutions for acid degradation studies were prepared in diluent and added 1mL of 0.1N Hydrochloric acid and injected immediately. Degradation of about 13% in Clavulanic acid and 12% in Cefatrizine pentahydrate were observed.

Alkali degradation studies

Solutions for alkali degradation studies were prepared in diluent and added 1 mL of 0.01N, sodium hydroxide and injected immediately. Degradation of about 12% in Clavulanic acid and 15% in Cefatrizine pentahydrate were observed.

Oxidative degradation studies

Solutions for oxidative degradation studies were prepared in diluent and added 2 mL of 0.1% hydrogen peroxide and injected immediately. Degradation

Chromatogram of sample solution

Chromatogram of acid degradation (0.1N HCl)

Chromatogram of standard solution

Chromatogram of base degradation (0.01N NaOH)

of about 26% in Clavulanic acid and 94% in Cefatrizine pentahydrate were observed. In stress oxidative conditions, cefatrizine pentahydrate was almost completely degraded.

Solution stability

The solution stability in the assay method was carried out by maintaining the sample solutions in tightly capped volumetric flasks at 2-8°C and 25°C. The sample solution was assayed at regular intervals. The Cumulative RSD of the assay of Cefatrizine pentahydrate and potassium clavulanate was less than 2.0%. The solution stability experiment shows that the sample solution at 2-8°C stable for Cefatrizine pentahydrate upto 14 hours and for Clavulanic acid up to 10 hours, and sample solution at 25°C stable for Cefatrizine pentahydrate up to 2 hours and for Clavulanic acid up to 2 hours.

CONCLUSIONS

The developed and validated HPLC assay method for the quantitative determination of cefatrizine pentahydrate and potassium clavulanate is rapid, precise, rugged and robust.

The analytical validation of the assay method shows satisfactory data for the parameters tested. The method produces good separation of the drugs and their degradation products.

The developed method is stability indicating and can be used for assessing the stability of cefatrizine pentahydrate and potassium clavulanate in bulk drug samples and formulations.

ACKNOWLEDGEMENTS

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