The HPLC Analysis of Process Materials of Semi-synthetic Penicillins

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Key Words

Column liquid chromatography
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Process effluents

Summary

An HPLC method for the determination of D(-)-p-hydroxyphenylglycine, D(-)-α-phenylglycine and 6-aminopenicillanic acid has been developed using a 5 μm Hypersil Spherisorb ODS 1 column (250 × 4.6 mm). These analytes were separated in a single run under isocratic conditions. Various chromatographic parameters including linearity, precision and accuracy have been evaluated. The method was found to be suitable for the analysis of process materials and effluents associated with the manufacture of amoxycillin, ampicillin and cloxacillin sodium. The run time is less than five minutes.

Introduction

D(-)-p-Hydroxyphenylglycine and D(-)-α-phenylglycine are used in the manufacture of amoxycillin [1,2] and ampicillin [3–8], respectively, whereas 6-aminopenicillanic acid is used in the manufacture of all these semi-synthetic penicillins [1–10]. These materials may end up in the finished product as impurities or in effluents as pollutants. It is desirable to have a good control on the presence of such impurities with a view to: i) improve the quality of the product, ii) prevent the loss of materials by an effective control of the process, and iii) prevent contamination of the effluent water.

It is also desirable to know the level of D(-)-p-hydroxyphenylglycine and 6-aminopenicillanic acid in the reaction mixture at the penultimate stage of amoxycillin manufacture. Similarly the levels of D(-)-α-phenylglycine and 6-aminopenicillanic acid in the manufacture of ampicillin and 6-aminopenicillanic acid in case of cloxacillin sodium should be known precisely to control the processes.

To our knowledge there is no single method available in the literature, which allows simultaneous determination of these chemicals in a mixture and it seemed likely that an HPLC method would be most appropriate for this purpose.

Experimental

Materials

The following chemicals were used without further purification. Acetonitrile (Hiper Solv. BDH, UK), potassium dihydrogenphosphate (Merck, Germany), D(-)-p-hydroxyphenylglycine (Recordati Farmaceutica, Italy), D(-)-α-phenylglycine (DSM Dertil, Spain), 6-aminopenicillanic acid (Beecham, UK)

Preparation of Solutions

Buffer Solution

0.05 M potassium dihydrogenphosphate in distilled water, pH adjusted to 5.0 with 0.5 M potassium hydroxide solution.

Mobile Phase

Filtered degassed mixture of the buffer solution and acetonitrile mixed in the ratio 97:3, *v/v*.

Standard Solutions

Accurately weighed quantities, about 20 mg each of D(-)-p-hydroxyphenylglycine, D(-)-α-phenylglycine and 6-aminopenicillanic acid were dissolved separately in the mobile phase and volumes made up to 100 mL. A standard solution was prepared by mixing 4 mL of the D(-)-p-hydroxyphenylglycine, 30 mL of the D(-)-α-phenylglycine and 25 mL of the 6-aminopenicillanic acid solutions.

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Table I. Chromatographic Parameters

Substance	Precision (RSD %) With in Day Area	Rt.	Between Days Area	Rt.	Resolution	Theoretical plates m^{-1}	LOD ($ng\ mL^{-1}$)	LOQ
D(-)-p-hydroxyphenylglycine (PHPG)	0.03	0.12	0.21	0.31	Between PHPG and APG = 4.43	10 600	0.14	0.28
D(-)-α-phenylglycine (APG)	0.02	0.39	0.17	0.62	Between APG and 6-APA = 5.13	10 300	32.73	65.46
6-aminopenicillanic acid (6-APA)	0.03	0.17	0.18	0.52	Between PHPG and 6-APA = 8.97	13 100	20.00	40.00

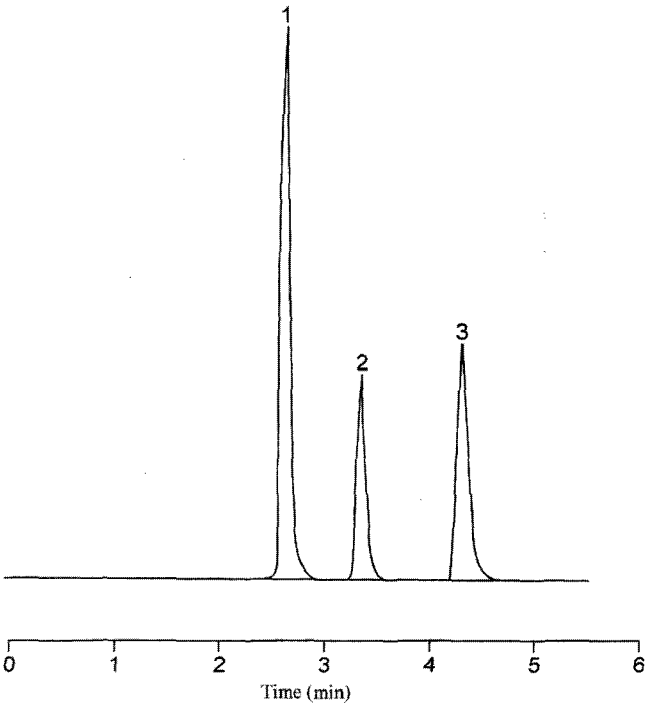

Figure 1
Chromatogram of a mixture of **1** = D(-)-p-hydroxyphenylglycine (8.08 $\mu g\ mL^{-1}$), **2** = D(-)-α-phenylglycine (60.66 $\mu g\ mL^{-1}$), and **3** = 6-aminopenicillanic acid (50 $\mu g\ mL^{-1}$).

In-process Mixture

An accurately weighed quantity, about 10 g, of in-process mixture obtained during production of amoxycillin, ampicillin and cloxacillin sodium was dissolved in the mobile phase and diluted to 100 mL with the mobile phase.

Effluent Solution

10 mL of the effluent was mixed with the mobile phase, filtered and the volume made up to 100 mL with the mobile phase.

Chromatographic Conditions

The column was stainless steel, 250 × 4.6 mm, packed with 5 μm Spherisorb ODS 1, Hypersil. The detector wavelength was 230 nm; injection volume 20 μL; column temperature 25±2 °C, and flow rate 1.0 mL min^{-1}.

Procedure

Precision

Ten replicate measurements were made by using the following solutions in the mobile phase: i) 0.84 mg of D(-)-p-hydroxyphenylglycine per 100 mL, ii) 6.0 mg of D(-)-α-phenylglycine per 100 mL, and iii) 2.06 mg of 6-aminopenicillanic acid per 100 mL.

Linearity

Ten different concentrations of D(-)-p-hydroxyphenylglycine, D(-)-α-phenylglycine and 6-aminopenicillanic acid were prepared in the mobile phase. 20 μL of each concentration was injected.

Limit of Detection

The solutions of D(-)-p-hydroxyphenylglycine, D(-)-α-phenylglycine and 6-aminopenicillanic acid in the mobile phase were diluted to known concentrations to a final response equal to twice the signal-to-noise ratio.

Results and Discussion

All the substances under investigation were separated under the chromatographic conditions used. The resolution was good, ranging from 4.43 to 8.97 (Table I). A representative chromatogram is shown in Figure 1.

Precision

The within-day and between days (10 days) precision of the method was determined for both peak area and retention time of D(-)-p-hydroxyphenylglycine (8.4 $\mu g\ mL^{-1}$), D(-)-α-phenylglycine (60.0 $\mu g\ mL^{-1}$) and

Table II. Linearity Parameters

Substance	Correlation coefficient	Slope	Intercept at Zero concentration	Concentration range ($\mu g\ mL^{-1}$)
D(-)-p-hydroxyphenylglycine (PHPG)	0.9999	0.994	0.0500	8.72 to 0.87
D(-)-α-phenylglycine (APG)	1	0.996	0.0033	65.47 to 6.55
6-aminopenicillanic acid (6-APA)	0.9999	0.986	–0.0606	20.00 to 2.00

6-aminopenicillanic acid (20.6 $\mu g\ mL^{-1}$) by measuring the response of the replicate injections. The results are given in Table I. The very low values of relative standard deviations indicate an excellent reproducibility.

Linearity

Detector response was measured at 230 nm from ten solutions containing the analyte and a graph of peak area versus concentration was plotted. A straight line was obtained. The statistical parameters including correlation coefficient, slope and intercept are given in Table II. The response was found to be excellent as slope and correlation coefficient were very near to one and intercept was much lower than the limit of ± 2 % of the detector at 100 % of the analyte level [11].

Limits of Detection and Quantitation

The limits of detection (LOD) and quantitation (LOQ) are given in Table I. The very low levels indicate that the method is very sensitive for the determination of the substances under investigation and suitable for determination of these analytes in effluents.

Accuracy

The accuracy of the proposed method for the determination of D(-)-p-hydroxyphenylglycine, D(-)-α-phenylglycine and 6-aminopenicillanic acid in the in-process mixtures and the effluent was established in the presence of each other by measuring the response of solutions of the analytes of known concentrations in triplicate. The concentrations of the analytes were calculated and a linear regression was performed of the mean of the concentrations. The resulting regression equations had slopes of 1 and intercepts of zero (within 95 % confidence limits) with $r^2 \geq 0.994$.

Conclusion

A method for simultaneous determination of D(-)-p-hydroxyphenylglycine, D(-)-α-phenylglycine and 6-aminopenicillanic acid in in-process materials and effluents produced in the manufacturing of amoxycillin, ampicillin and cloxacillin sodium has been developed and validated. The method is simple, rapid and does not require special sample pre-treatment. The mobile phase does not include any special agent such as ion-pairing and the analysis can be performed at room temperature. The linearity of the response and precision are good. The method can be applied for the routine monitoring of the analytes in process streams and effluents with good reliability. The method is being successfully used for such determinations for about two years at Pharmagen Beximco Ltd. Lahore for the manufacture of amoxycillin, ampicillin and cloxacillin sodium.

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