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Short communication

An isocratic ion exchange HPLC method for the simultaneous determination of flucloxacillin and amoxicillin in a pharmaceutical formulation for injection

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Abstract

An isocratic ion exchange high performance liquid chromatography method was developed for the simultaneous determination of flucloxacillin and amoxicillin in pharmaceutical formulations for injections. The separation was made by a ZORBAX 300-SCX column using 0.025 M ammonium dihydrogen phosphate (adjusted to pH 2.6 with phosphoric acid)–acetonitrile (95:5) as mobile phase. The validation of the method was performed, and specificity, reproducibility, precision and accuracy were confirmed. The limits of quantification were approximately 0.2 μg/ml for each drug. Due to its simplicity and accuracy the method is particularly suitable for routine pharmaceutical quality control.

Keywords: Amoxicillin; Flucloxacillin; Ion exchange; HPLC simultaneous analysis

1. Introduction

Penicillins with an extended spectrum of activity such as amoxicillin have been used in antibiotic therapy for many years. However, the more frequent occurrence of β-lactamase producing clinically important bacterial strains has limited the usage of these antibiotics. Co-administration of the labile β-lactam together with another antibacterial capable of inhibiting the β-lactamase was developed to improve the activity and overcome bacterial resistance. Flucloxacillin is bactericidal with a mode of action similar to that of benzylpenicillin, but is resistant to staphylococcal penicillinase. Flucloxacillin may be administered in combination with other antibiotics, including amoxicillin (in proportion 1:1, known as flumocin or flomoxin), to produce a wider spectrum of activity. The standard prophylactic regimen for patients undergoing cardiac surgery is flucloxacillin and amoxicillin [1]. It was also suggested that if *Staphylococcus aureus* is the likely pathogen, a macrolide or combination of flucloxacillin with amoxicillin is appropriate [2].

Production of combination drugs always creates a challenge for the pharmaceutical analyst. The modern analytical investigation of antibiotic drugs, content and purity estimations of active compounds, very often involve high performance liquid chromatography (HPLC). An isocratic reverse-phase ion-pair HPLC method [3] and a gradient reverse-phase ion-pair HPLC method [4] were reported for the simultaneous determination of flucloxacillin and amoxicillin in pharmaceutical formulations for injections. However, amoxicillin was weakly retained in these two HPLC systems, which resulted in its being co-eluted with some related substances of these two antibiotics. Moreover, the gradient method suffers from slow column equilibration due to the more hydrophobic ion-pair reagent and detection at relatively long wavelengths

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due to baseline drift. Hence, ion-pair HPLC in a gradient mode is usually not recommended [5]. In this investigation, a simple and sensitive isocratic ion exchange HPLC method was developed and validated for the simultaneous determination of flucloxacillin and amoxicillin in pharmaceutical formulations for injections.

2. Experimental

2.1. Standards and reagents

Flucloxacillin sodium [6] was provided by Bright Future Pharm. Fty., amoxicillin sodium [7] was from Shanghai Asia Pioneer Pharmaceuticals Co. Ltd. Flucloxacillin sodium from British Pharmacopoeia Chemical Reference Standard of activity 90.2% and amoxicillin trihydrate from Chinese National Chemical Reference Standard of activity 86.2% were used. The commercially available drug for injection from Biflocin® (Esseti) was used for quantitative determination.

Ammonium dihydrogen phosphate, phosphoric acid (85%, w/w) were of analytical grade and purchased from SCR (Shanghai, PR China). Acetontrile was HPLC grade and obtained from Merck (Darmstadt, Germany).

All solutions were prepared with distilled water for HPLC.

2.2. Instrumentation

The HPLC system was a model 1100 (Agilent, Waldbronn, Germany) composing quaternary pump, autosampler, mobile phase degaser, heated column thermostat, and variable-wavelength UV detector. The mobile phase contained 0.025 M ammonium dihydrogen phosphate (adjusted to pH 2.6 with phosphoric acid)–acetonitrile (95:5) and the flow rate was maintained at 1.5 ml/min and monitored at 225 nm. Chromatographic separations were performed at 25 °C on a 250 mm × 4.6 mm i.d. column packed with 5 μm ZORBAX 300-SCX (Agilent) and the injection volume was 10 μl.

2.3. Standard solution and sample solution

A combined standard solution containing flucloxacillin and amoxicillin was prepared in water with the concentration of each active compound at 0.1 mg/ml.

A sample solution was prepared in water with the concentration of flucloxicillin and amoxicillin both at 0.1 mg/ml.

3. Results and discussion

The goal of this study was to develop a single isocratic HPLC assay for the simultaneous determination of flucloxacillin and amoxicillin. Due to the relatively large difference in hydrophobicity between flucloxacillin and amoxicillin, amoxicillin was eluted near the dead volume, which co-eluted with some of its related substances in a typical reversed phase HPLC system suitable for analysis of flucloxacillin [6]. For this reason, an ion-pair reagent was added to the mobile phase to increase the retention of amoxicillin [3,4]. However, the effect on retention increase was not significant enough to improve separation and a gradient system was necessary to elute the strongly retained flucloxacillin [4].

Therefore, the main analytical challenge during development of a new method was obtaining adequate retention of the polar amoxicillin, while maintaining a reasonable elution time for the less-polar flucloxacillin in order to reduce analysis time and improve chromatographic performance. Since amoxicillin possess net positive charge in acid solution an ion exchange column may exhibit reverse-phase and ion exchange behavior, i.e., mixed-mode separation [5]. The use of a cation-exchange column with an acid buffered aqueous-organic mobile phase was investigated. Although ion exchange HPLC is used infrequently today, when compared with reverse-phase or ion-pair HPLC, ion exchange HPLC may have certain advantages: unique selectivity, easier control over selectivity and resolution, and more stable and reproducible columns [5]. Thus, ZORBAX 300-SCX, a polar bonded-phase column packing used for cation-exchange HPLC, was investigated. This packing consists of an aromatic sulfonic acid moiety covalently bonded to ZORBAX PSM 300 through Si–O–Si bonds.

The results indicated that addition of acetonitrile to the mobile phase resulted in decreased retention of the two drugs. Within pH 2.3–3.6, and maintaining the concentration of ammonium at 0.025 M, the retention times of the two drugs increased as the pH of the buffer decreased. Maintaining the pH of the buffer at 2.6, as the concentration of ammonium was increased from 0.025 to 0.040 M, the retention time of amoxicillin reduced significantly while that of flucloxacillin remained nearly constant, and the elution order of the two drugs even changed at about 0.035 M. Clearly, amoxicillin with a net positive charge opposite to that of the column (negative charge) was retained predominantly by an ion exchange mechanism. Considering the retention of amoxicillin was more sensitive to the concentration of its displacer (ammonium), while flucloxacillin was retained by reverse-phase behavior only. In addition, the experimental results also showed that the major related products of flucloxacillin were eluted before flucloxacillin, and most related products of amoxicillin were eluted after amoxicillin and strongly retained on the column. A gradient elution must be selected if elution of all the products was necessary. For this reason, a buffer that contained 0.025 M ammonium was chosen in order to cause amoxicillin to be eluted after flucloxacillin.

The final mobile phase consisted of pH 2.6 buffer–acetonitrile (95:5) and a cation-exchange column, provided a chromatogram (Fig. 1) with the specificity required for the simultaneous determination of flucloxacillin and amoxicillin in pharmaceutical formulations for injections.

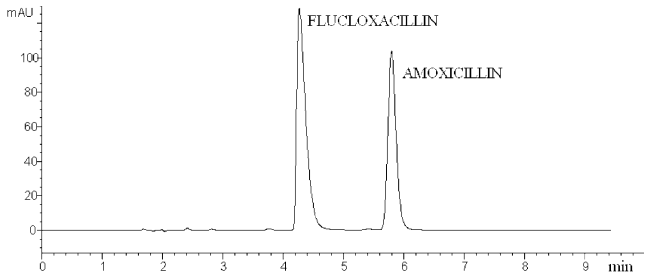

Fig. 1. Typical chromatogram of sample solution (0.1 mg/ml flucloxacillin and 0.1 mg/ml amoxicillin).

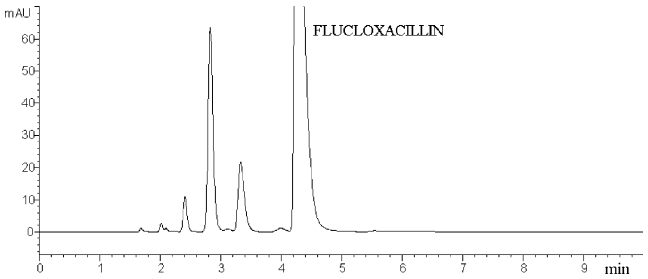

Fig. 2. Typical chromatogram of flucloxacillin sodium solution (0.2 mg/ml flucloxacillin) stored at room temperature for 7 days.

For confirmation of the method specificity, chromatograms of flucloxacillin sodium solution (0.2 mg/ml flucloxacillin) and amoxicillin sodium solution (0.2 mg/ml amoxicillin) after storage at room temperature for 7 days were performed (Figs. 2 and 3). The related substances of the two drugs were well separated from each active compound.

3.1. Linearity

For the construction of a calibration curve, six calibration standard solutions were prepared over the concentration range 50–150% of the nominal concentrations of active compounds (50–150 μg/ml). The areas exhibited linear responses with $r^2 = 1.0000$ for both flucloxacillin and amoxicillin.

3.2. Precision

Within-day precision of the assay was determined by analysis of replicate ($n = 5$) samples of three different concentration (0.08, 0.10, and 0.12 mg/ml) on the same day. Within-day relative standard deviation (R.S.D.) values for flucloxacillin and amoxicillin assay ranged from 0.9 to 1.2% and 0.6 to 1.0%, respectively.

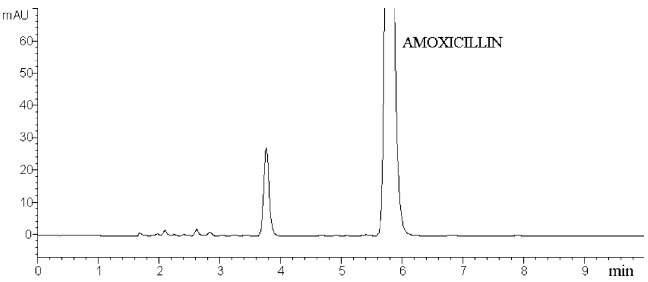

Fig. 3. Typical chromatogram of amoxicillin sodium solution (0.2 mg/ml amoxicillin) stored at room temperature for 7 days.

Table 1
Recoveries obtained in the analysis of synthetic mixtures

	Mean recovery, $n = 3$%		
Drugs (%)	80	100	120
Flucloxacillin	100.2	100.1	100.1
Amoxicillin	100.1	100.0	100.1

3.3. Accuracy

Accuracy was determined by applying the described method to synthetic mixtures containing known amounts of each drug corresponding to 80, 100 and 120% of label claim. The accuracy was then calculated as the percentage of analyte recovery by the assay. Mean recoveries for flucloxacillin and amoxicillin from the formulations are shown in Table 1.

3.4. Limits of quantification

The limits of quantification (LOQ) of flucloxacillin and amoxicillin were both estimated at 0.2 μg/ml (signal-to-noise ratio of 10).

3.5. Repeatability

Six individual sample weighings of the same batch were taken and analyzed. The R.S.D.'s of the results were 0.5% (flucloxacillin) and 0.4% (amoxicillin), respectively.

3.6. Stability of standard solution and sample solution

Although using the mobile phase as solvent was more suitable for chromatography, water was used instead owing to the more rapid degradation of flucloxacillin and amoxicillin in acid aqueous solution. Two standard solutions and three sample solutions (stored at room temperature: around 25 °C) were separately injected at 0, 0.2, 0.5, 1, 2 and 4 h. The results remained almost unchanged and no significant degradation was observed within the given period, indicating that the standard solution and sample solutions were stable for at least 4 h.

4. Conclusion

The proposed HPLC method in this study has the advantage of simplicity, precision, accuracy, and convenience for the simultaneous determination of flucloxacillin and amoxicillin in pharmaceutical formulations for injections. In conclusion, the developed method can be used for the assay of the two drugs in this combination formulation.

References

[1] M.R. Adrianzen Vargas, M.H. Danton, S.M. Javaid, J. Gray, C. Tobin, W.J. Brawn, D.J. Barron, Eur. J. Cardio-thoracic Surf. 25 (2004) 256–260.
[2] British Thoracic Society of Standards of Care Committee, BTS guidelines for the management of community acquired Pneumonia in childhood, Thorax 57 (2002) i1–i24.
[3] L.N. Li, J.F. Song, L.Q. Mo, J. Instrum. Anal. 14 (1995) 87–89.
[4] Y. Chen, Chin. J. Antibiot. 29 (2004) 15–18.
[5] L.R. Snyder, J.J. Kirkland, J.L. Glajch, Practical HPLC Method Development, 2nd ed., Wiley, New York, 1997, pp. 338–346.
[6] British Pharmacopoeia Commission, British Pharmacopoeia, vol. I, The Stationery Office, London, 2003, pp. 801–802.
[7] British Pharmacopoeia Commission, British Pharmacopoeia, vol. 1, The Stationery Office, London, 2003, pp. 132–134.