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Liquid chromatographic determination of amoxycillin and clavulanic acid in pharmaceutical preparations

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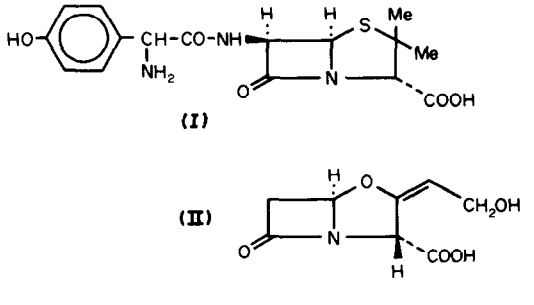
Abstract: A liquid chromatographic method for the simultaneous determination of amoxycillin and potassium clavulanate in tablet and suspension preparations is presented. The method specifies reversed phase column and a buffered mobile phase (CH_3OH + KH_2PO_4-buffer pH 6 + H_2O, 15:1:84) isocratically at a rate of 1.0 ml min^{-1}, with detection at 235 nm. The suitability of the chromatographic system developed is tested using replicate injections of the sample and standard preparations. The observed relative standard deviations (RSDs) were within 2%. Recovery experiments conducted utilizing the proposed method gives results of 101.51% ± 1.72 (n = 6) and 101.22% ± 1.93 (n = 6) for amoxycillin in tablets and powder for oral administration, respectively. Similarly, recovery experiments for clavulanic acid gave results of 100.33 ± 1.90 (n = 6) and 99.61 ± 1.32 (n = 6) in the tablets and suspension powder, respectively. Comparison of the proposed method with the USP method proved it to be satisfactory. The statistical F- and t-tests observed, indicated that there were no significant differences between the two methods regarding precision and accuracy.

Keywords: *Amoxycillin; potassium clavulanate; binary mixtures; LC determination; pharmaceutical analysis.*

Introduction

Amoxycillin (I), an aminopenicillin, is commonly prescribed with clavulanic acid (II) (as potassium salt), the naturally occurring β-lactamase inhibitor produced by fermentation of *Streptomyces clavuligerus*, for the treatment of illness induced by β-lactamase-producing bacteria that are resistant to amoxycillin alone.

A number of methods have been reported for the determination of amoxycillin including iodometric titration [1], spectrofluorometry [2, 3] and polarography [4]. Several HPLC assays also have been reported for the determination of the drug in biological samples [5–10] and the separation and quantitation of amoxycillin alone or in the presence of its decomposition products [11–16].



Scheme

Relatively few HPLC methods [17] have been cited for the quantitation of clavulanic acid in human serum and urine employing a pre-column reaction with 1,2,4-triazole and clavulanic acid as such using a hollow-fibre post-column reactor [18]. A spectrophotometric determination of the stability of clavulanic acid and its ether and amine derivatives in serum and urine has been reported [19].

The British Pharmacopoeia [20] adopts a spectrophotometric method of assay for amoxycillin in pharmaceutical formulation involving complexation with an imidazole–mercury reagent and measurement of absorbance values at 325 nm. However for amoxycillin trihydrate in bulk the British Pharmacopeial method is based on a potentiometric titration procedure.

Since the previously reported methods were for the separate determination of amoxycillin and clavulanic acid, it is deemed useful to develop a quantitative procedure for the simultaneous assay of mixtures of the two compounds.

This paper presents an LC method for the simultaneous determination of amoxycillin and

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clavulanic acid in binary mixtures and in some dosage formulations containing clavulanate-potentiated amoxycillin. The proposed method gives reproducible results with RSDs within 2% for replicate measurements. When compared with the USP method [21], the accuracy and precision are found to be satisfactory.

Experimental

Apparatus, materials and reagents

Liquid chromatograph. A Varian Model 5000, equipped with variable-wavelength UV 50 detector, Rheodyne Model 7125 injector fitted with a 20-μl loop, and a Varian Model 9179 recorder was used. Chromatographic parameters were controlled by a Varian data system (DCS 111L). Parameters were optimized as follows: mobile phase, methanol–phosphate buffer pH 6 (prepared by mixing 50 ml of 0.2 M KH_2PO_4 and 5.7 ml of 0.2 M NaOH and diluting with water to 200 ml)–water, (15:1:84); columns, μ-Bondapak C18, 10 μm (30 cm × 4 mm), (Waters Associates) flow rate, 1.0 ml min^{-1}; analysis time, 2.3 min for potassium clavulanate and 5.3 min for amoxycillin trihydrate; detection at 235 nm.

Solvents and standards. Chromatographic grade methanol (E. Merck, Darmstadt, Germany), amoxycillin trihydrate (Beecham Research Laboratories, Brentford, UK) BRL 2333 Std. No. 16; potassium clavulanate (Beecham Research Laboratories, Brentford, UK) BRL 14151 Std. No. 26, were used as reference substances without further treatment. Water was twice distilled and all other reagents were of analytical grade. Augmentin® tablets (labelled to contain 250 mg amoxycillin and 125 mg clavulanic acid per tablet), Augmentin® powder for suspension (labelled to contain 250 mg amoxycillin and 62.5 mg clavulanic acid per 5 ml of reconstituted powder) were kindly provided by Beecham Research Laboratories (Brentford, UK).

Standard solutions. Two working solutions containing amoxycillin trihydrate and potassium clavulanate in water with concentration ratios 200:50 μg ml^{-1} and 100: 50 μg ml^{-1} for amoxycillin:potassium clavulanate were prepared. Six serial dilutions for each working solution to cover the concentration range 4–20 μg ml^{-1} were made using the mobile phase. Duplicate readings of 20 μl injections for each concentration of the two components were recorded. From the average recorded values the calibration curves (peak area vs concentration) for amoxycillin trihydrate and potassium clavulanate were constructed.

Procedure for samples

For tablets. Twenty tablets were weighed and powdered. Six samples from the powder were accurately weighed such that each contained about 25 mg amoxycillin and 12.5 mg clavulanic acid. Each sample was transferred quantitatively into a 100 ml volumetric flask, 80 ml of water were added and the mixture was well shaken for 30 min. The volume for each was adjusted to 100 ml with water, and the mixture was shaken and filtered using Whatman filter paper No. 17 rejecting the first few millilitres of each filtrate. From the filtrate a 2 ml volume sample was pipetted into 50 ml volumetric flask and the volume adjusted using the mobile phase. Duplicate chromatograms for 20 μl injections were recorded for each sample and average peak area responses computed. The unknown concentration of the two ingredients were calculated by direct comparison with the standard solutions. Alternatively linear regression equations may be used.

For suspensions. Six samples each containing about 25 and 6.25 mg of amoxycillin and clavulanic acid, respectively, were weighed accurately and transferred quantitatively into six 100 ml volumetric flasks. A volume of 80 ml water was added to each sample which was then shaken for 30 min. The volume was made up to 100 ml and the mixture shaken and finally filtered. The first few millilitres of the filtrate were rejected. Duplicate chromatograms for 20 μl injections were recorded for each sample and the analyte concentrations were calculated as indicated above.

Results and Discussion

Amoxycillin and clavulanic acid mixture is not amenable to simultaneous determination by ordinary spectrophotometric methods due to severe spectral overlapping insofar as amoxycillin and clavulanic acid have absorption maxima at 274 and 270 nm, respectively (Fig. 1). A chromatographic method for the assay of the binary mixture therefore was deemed necessary to solve the problem.

Preliminary investigations revealed that with the μ-Bondapak C18 and 10 μm (30 cm ×

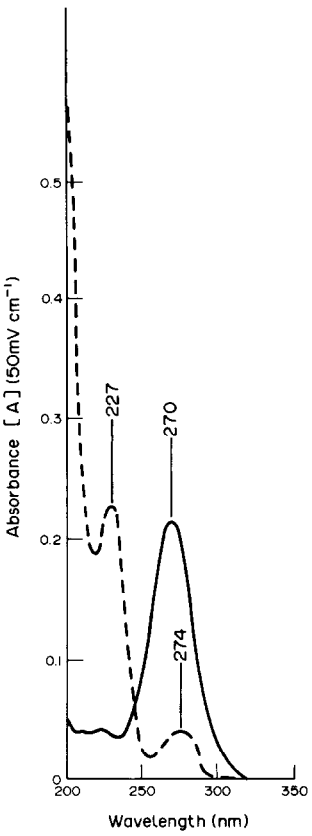

Figure 1
UV-absorption spectra for ethanolic solutions of amoxycillin triydrate [(– –), 10 μg ml^{-1}] and potassium clavulanate [(——), 5 μg ml^{-1}].

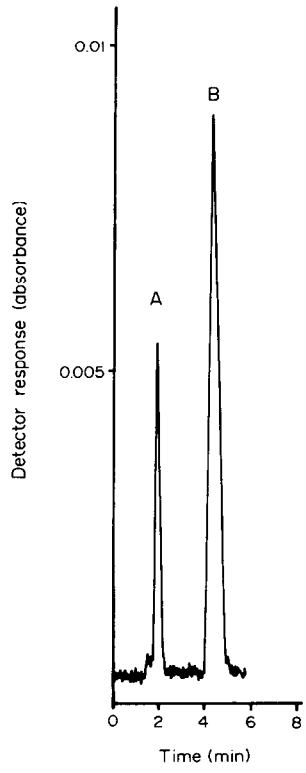

Figure 2
LC analysis of potassium clavulanate (A) concentration 6.73 μg ml^{-1} and amoxycillin trihydrate (B) concentration 18.68 μg l^{-1} AUFS = 0.01.

4 mm) column, the optimum composition of the mobile phase was: methanol–phosphate buffer (pH 6)–water, (15:1:84, v/v). By varying the detection wavelengths and the testing of different concentrations of amoxycillin and clavulanic acid, the peak responses at 235 nm were found adequate. Adoption of these experimental parameters gave rise to sharp symmetric peaks as shown in Fig. 2 indicating satisfactory column efficiency and good resolution. The observed retention times for amoxycillin trihydrate and clavulanic acid were 5.3 and 2.3 min, respectively. These conditions were regarded optimal since the resolution, retention times and column efficiency were good.

The reproducibility of the developed chromatographic method was tested by conducting replicate injections using standard and sample solutions in the dosage formulations ratios 2:1 and 4:1 (amoxycillin:calvulanic acid). Table 1 summarizes recovery experiments for added standard amoxycillin (6.7 μg ml^{-1}) and clavulanic acid solutions (6 μg ml^{-1}) to the sample solutions containing 10.2 and 5.0 μg ml^{-1} of amoxycillin and clavulanic acid, respectively. The added recovery experiments were conducted such that the total concentration re-

Table 1
Recovery of amoxycillin and potassium clavulanate added to pharmaceutical preparations

Preparation	Added (μg ml^{-1})*		Mean recovery %† ± SD	
	Amoxycillin	Clavulanic acid	Amoxycillin	Potassium clavulanate
Augmentin® tablets	6.7	6	101.51 ± 1.72	100.33 ± 1.90
Augmentin® powder for suspension	6.7	6	101.22 ± 1.93	99.61 ± 1.32

*Based on six replicate measurements.
†Samples contain 10.7 μg ml^{-1} (amoxycillin) and 5.0 μg ml^{-1} (clavulanic acid).

Table 2
LC determination of amoxycillin and potassium clavulanate in tablets and powder for oral suspension

Dosage formulation	Component	Proposed method Per cent found ± SD*	USP method Per cent found ± SD*	*F*- and *t*-tests
Augmentin tablets®	Amoxycillin trihydrate	100.60 ± 0.94 (n = 6)†	100.39 ± 1.56 (n = 6)	$F_{0.05}$ 2.75 (3.37)‡
				$t_{0.05}$ 0.79 (2.14)‡
	Potassium clavulanate	99.98 ± 1.65 (n = 6)	99.77 ± 2.00 (n = 4)	$F_{0.05}$ 1.47 (4.53)‡
				$t_{0.05}$ 0.35 (2.31)‡
Augmentin® powder for oral suspension	Amoxycillin trihydrate	99.48 ± 1.75 (n = 6)	100.25 ± 1.45 (n = 6)	$F_{0.05}$ 1.46 (4.28)‡
				$t_{0.05}$ 1.86 (2.23)‡
	Potassium clavulanate	100.68 ± 1.21 (n = 6)	101.54 ± 1.87 (n = 5)	$F_{0.05}$ 2.49 (4.38)‡
				$t_{0.05}$ 1.91 (2.26)‡

*Mean (X) ± standard deviation for n experiments.
‡Significant levels.
†Means of six different weighings.

mained within the linear range. The RSDs given were all within 2% indicating the good reproducibility of the proposed method.

The calibration curves (peak area vs concentration) were constructed for a series of concentrations (4–20 μg ml^{-1}) of amoxycillin and potassium clavulanate standard solutions in the molar ratios 2:1 and 4:1. Regression analysis indicated linear relationship between peak area (Y) and concentration (c, μg ml^{-1}) for each component; the equations being as follows:

$$Y \text{ (amoxycillin trihydrate)} = 2381 + 93038c, \quad (n = 6) \qquad \text{(A)}$$

and

$$Y \text{ (potassium clavulanate)} = 2588 + 49487c, \quad (n = 6). \qquad \text{(B)}$$

The correlation coefficients, r, were 0.9998 and 0.9999 for equations (A) and (B), respectively.

When the proposed chromatographic method was applied to the determination of the commercial tablets (Augmentin®) and the powder for oral suspension, the results obtained for amoxycillin were 100.60 ± 0.94 (n = 6), and 99.48 ± 1.75 (n = 6). Similarly results for potassium clavulanate were 99.98% ± 1.65 (n = 6) and 100.68% ± 1.21, (n = 6) for the tablets and the suspension, respectively. The USP method [21] was tested by utilizing the mobile phase prepared from a mixture of NaH_2PO_4 (pH 4.4) buffer and methanol (95:5). The mean percentages for amoxycillin were 100.39 ± 1.56 (n = 6) and 100.25 ± 1.45 (n = 6) for the tablets and the suspension, respectively. Similarly the recoveries for potassium clavulanate were 99.7 ± 2.00 (n = 4) and 101.54 ± 1.87, (n = 5).

The results found by applying the proposed and the USP methods to the analysis of Augmentin® tablets and the suspension were statistically compared (Table 2) with regard to precision and accuracy. Based on the F- and t-tests at 95% confidence level, there are no differences with regard to precision and accuracy between the two methods. In the light of these statistical inferences and the experimental data collected in Table 2, it may be concluded that the proposed method is reliable. Furthermore, the good agreement between the proposed method and the USP method indicates that the former can be used adequately for the simultaneous assay of amoxycillin and potassium clavulanate in their mixtures.

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