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Simultaneous determination of amoxycillin and clavulanic acid in pharmaceutical dosage forms by LC with amperometric detection

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Abstract

A simple and sensitive liquid chromatographic method with electrochemical (EC) detection is described for the quantitative determination of amoxycillin and clavulanic acid in pharmaceutical dosage forms. Sample components were separated by a reversed phase C18 column, using a mixture of methanol-phosphate buffer (pH 3.2–3.4) (5:95, v/v) as the mobile phase. Detection of antibiotics was performed amperometricaly by applying a potential of +1.25 V. High linearity over a concentration range of 15.625–500 ng was demonstrated for amoxycillin ($r = 0.9999$) and clavulanic acid ($r = 0.9979$). Detection limits were 0.8 ng ml^{-1} for amoxycillin and 15 ng ml^{-1} for clavulanic acid. This method was found to be convenient and reproducible for analysis of these two components in oral suspensions and tablets and might be useful for other pharmaceutical dosage forms.

Keywords: Amoxycillin; Amperometric detection; Clavulanic acid; Reversed phase-HPLC-EC

1. Introduction

Amoxycillin is commonly prescribed with clavulanic acid as potassium salt, the naturally occurring β-lactamase inhibitor produced by fermentation of streptomyces clavuligerus, for the treatment of infection caused by β-lactamase producing bacteria that are resistant to amoxycillin alone.

The determination of antimicrobial drugs is mainly carried out using microbiological techniques [1]. The relatively long time required for analysis by such techniques is a disadvantage, when results are urgently needed. Also in such case, when more than one antibiotic is in the formulation, some interaction may occur.

A number of analytical methods in addition to microbiological assay have been reported for the determination of these two components in pharmaceutical preparation, which include enzymatic assay [2], iodometric titration [3], spectrofluorometry [4,5], UV spectrophotometry [6,7],

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polarography [8,9] and high performance liquid chromatography (HPLC) assay involving pretreatment of amoxycillin and clavulanic acid with imidazole [10]. Precolumn [11] and postcolumn derivatization [12–15] and ion-pair HPLC [16] methods have also been reported. Some methods for simultaneous assay of these two components in pharmaceutical products have also been reported, including reversed phase high performance liquid chromatography (RP-HPLC) with UV detection [17] and HPLC with β-cyclodextrin stationary phase [18].

The present study describes a new RP-HPLC with electrochemical (EC) detection method for simultaneous determination of amoxycillin and clavulanic acid in pharmaceutical preparations. This method was found to be highly sensitive and there is no need for special column and precolumn or postcolumn treatment of sample.

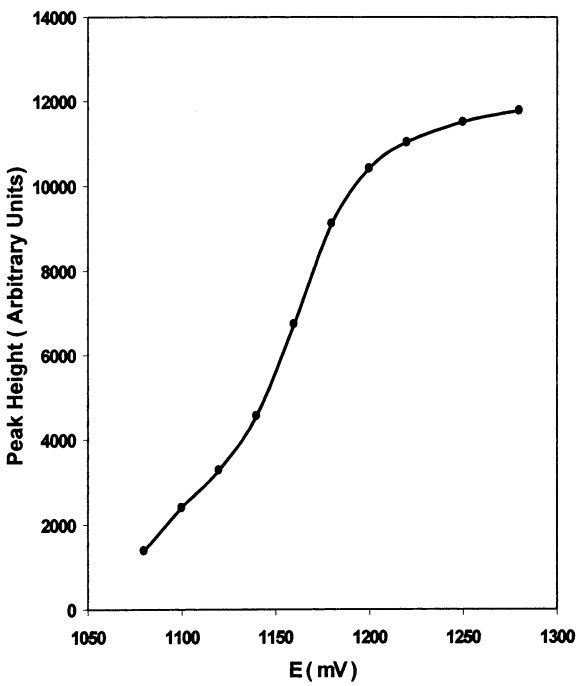

Fig. 1. Voltammogram of amoxycillin, which was obtained by successive determination of amoxycillin in standard solution of amoxycillin and clavulanic acid (10 μg ml^{-1} of each) at potential range 1080–1280 mV by increment of 20 mV.

2. Experimental

2.1. Materials

All the materials were of HPLC grade. Methanol and potassium dihydrogen phosphate were obtained from E. Merck (Darmstadt, Germany). Amoxycillin trihydrate and lithium clavulanate (U.S.P.C. INC., Rockville, MD, USA) were used as reference substance without further treatment. Augmentin® (Beecham, Singapore) and co-amoxyclave (Kosar Co. Iran) tablets (labeled to contain 500 mg amoxycillin and 125 mg clavulanic acid per tablet) and co-amoxyclave powder for suspension (labeled to contain 125 mg amoxycillin and 31.25 mg clavulanic acid per 5 ml of reconstituted powder) were purchased from local drug store.

2.2. HPLC apparatus and chromatographic conditions

The HPLC apparatus consisted of a solvent delivery system (Perkin–Elmer series 4), a fixed loop injector with a loop of about 50 μl (7125–075 Cotati, California, USA) and an EC detector with glassy carbon working electrode (TL-5A BAS, West Lafayette, USA) which was controlled by an amperometric controller (LC-4b BAS, West Lafayette, USA). It was operated in the direct current mode at +1.25 V versus Ag/AgCl and attenuation range of 100–500 nAV^{-1}. Chromatographic recordings were made on an integrator (Perkin–Elmer LCI-100). The chromatographic column was a RP-C18 150 mm × 4.6 mm i.d. (Perkin–Elmer/HS-5 C18).

The mobile phase was 0.05 M phosphate buffer in methanol (95:5, v/v) adjusted to pH 3.2–3.4 with 84–85% phosphoric acid and was filtered and degased prior to use and the flow rate was 1 ml min^{-1}. The optimal detector cell potential for the oxidation of amoxycillin and clavulanic acid was explored using reference compounds dissolved in mobile phase. The resulting hydrodynamic voltammograms are shown in Figs. 1 and 2. Based on these results, the potential of +1.25 V was chosen for simultaneous detection of amoxycillin and clavulanic acid.

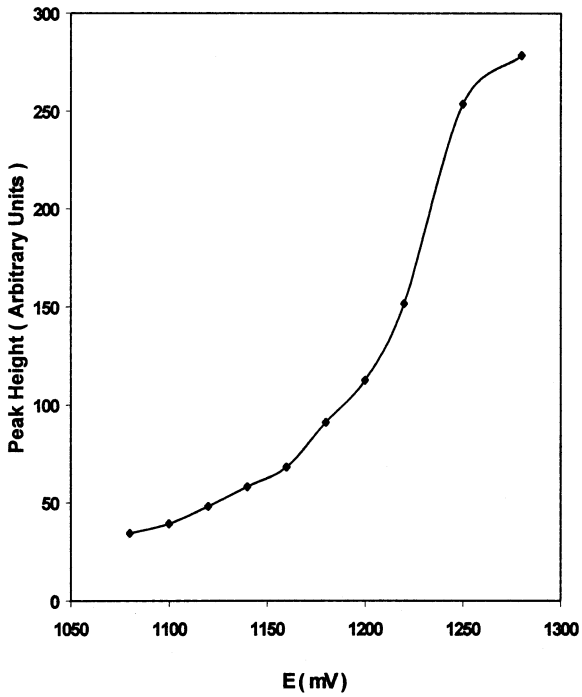

Fig. 2. Voltammogram of clavulanic acid, which was obtained by successive determination of clavulanic acid in standard solution of amoxycillin and clavulanic acid (10 μg ml^{-1} of each) at potential range 1080–1280 mV by increment of 20 mV.

2.3. Standard solutions

A stock solution containing 100 μg ml^{-1} amoxycillin and clavulanic acid were prepared by dissolving required amount of amoxycillin trihydrate and lithium clavulanate in the mobile phase. Although amoxycillin is stable moderately, due to instability of clavulanic acid the stock solutions were stored at 8°C for a week [19]. Working standard solutions containing 10, 5, 2.5, 1.25, 0.625 and 0.3125 μg ml^{-1} of amoxycillin and clavulanic acid were prepared by dilution of stock solution with mobile phase. To construct the standard curves six replicate (50 μl) of each standard solution were injected immediately after preparation to column and peak height of the chromatograms were measured. Then, the standard curves were constructed using mean peak values (Table 1).

2.4. Sample solutions

Ten tablets from each brand were weighed and powdered separately. Then, six samples from each powder, equivalent to 500 mg amoxycillin and 125 mg clavulanic acid, were accurately weighed and transferred quantitatively into separated volumetric flasks. A 800 ml mobile phase was then added to each flask and the mixture was well shaken for 10 min. Then, the volume of each mixture was adjusted to 1000 ml by mobile phase. After filtration of samples, the filtrates were diluted with the mobile phase to reach a suitable concentration. Finally, 50 μl of each diluted sample was injected into the column and data were recorded. Antibiotic concentrations in samples were then calculated using peak data and standard curves.

For suspensions, six samples, each equivalent to 125 mg amoxycillin and 31.25 mg clavulanic acid, were weighed and transferred quantitatively into six separate volumetric flasks and the volume adjusted to 1000 ml with mobile phase. The procedure was then continued as above.

3. Results and discussion

Typical chromatograms of amoxycillin and clavulanic acid standard solutions and formula-

Table 1
Calibration data for the standard curves of the peak height versus concentration of amoxycillin and clavulanic acid[a]

Compound	Range of concentration (μg ml^{-1})	Correlation coefficient (r)	Slope	Intercept
Amoxycillin	0.3125–10	0.9999	996.67	−140.68
Clavulanic acid	0.3125–10	0.9979	499.32	216.19

[a] Results are the mean of six replicate analyses.

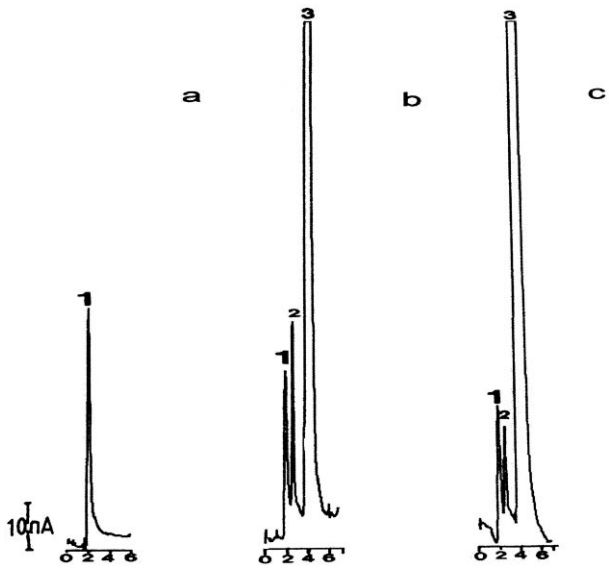

Fig. 3. Typical chromatograms of (a) mobile phase, (b) amoxycillin and clavulanic acid standard solution (5 μg ml^{-1} of each) in the mobile phase and (c) amoxycillin (10 μg ml^{-1}) and clavulanic acid (2.5 μg ml^{-1}) in the mobile phase prepared from co-amoxyclav 625 tablets. Peaks: 1, mobile phase; 2, clavulanic acid and 3, amoxycillin.

tion samples are shown in Fig. 3. Other component of pharmaceutical preparations, due to their lack of electroactivity, did not show any peak (see Fig. 3b and c) and the resolution factor for this study was 1.7, which is in acceptable range. Linearity of the standard graphs of amoxycillin and clavulanic acid over the concentration range 0.3125–10 μg ml^{-1} was verified. Straight lines for amoxycillin and for clavulanic acid were obtained, when the heights of the peaks were plotted versus concentration (Table 1). The assay method showed relative standard deviations (R.S.D.s) less than 5.149% (Table 2), which indicate a good reproducibility of the proposed method. Detection limits were determined as the concentration of compounds giving a signal to noise ratio greater than 3:1. The limits of detection for amoxycillin and clavulanic acid were found to be 0.8 and 15 ng ml^{-1}, respectively.

To determine the intra-day variation of assay method, the standard solutions of amoxycillin and clavulanic acid were injected in three separate occasions in the same day (Table 2). The inter-day variation study standard solutions of amoxycillin were assayed in three different times from day 1 to 20 after preparation (Table 3). The same assay for clavulanic acid was done using freshly prepared solutions (5.0 μg ml^{-1}) that gave R.S.D. equal to 8.3%.

The antibiotic content of the dosage forms determined in this study and those claimed by the manufacturer are shown in Table 4. The differences between the amount claimed and those measured were very low and the deviations were within the acceptable windows mentioned by the USP XXIV.

Table 2
Intra-day variability of amoxycillin and clavulanic acid assay method[a]

Compound	Actual concentration (μg ml^{-1})	Measured concentration (μg ml^{-1})		
		Mean	±S.D.	R.S.D (%)
Amoxycillin	0.625	0.64	0.02	3.13
	2.5	2.44	0.12	4.92
	5.0	5.03	0.19	3.78
	10.0	10.02	0.18	1.80
Clavulanic acid	0.625	0.505	0.026	5.149
	2.5	2.59	0.03	1.16
	5.0	5.41	0.04	0.74
	10.0	9.79	0.04	0.41

[a] Results are the mean of six replicate analyses.

Table 3
Inter-day variability of amoxycillin assay method[a]

Compound	Actual concentration (μg ml^{-1})	Measured concentration (μg ml^{-1})		
		Mean	±S.D.	R.S.D (%)
Amoxycillin	0.625	0.625	0.006	0.960
	2.5	2.43	0.11	4.53
	5.0	5.09	0.07	1.38
	10.0	10.04	0.04	0.40

[a] Results are the mean of six replicate analyses.

Table 4
Determination of amoxycillin and clavulanic acid in pharmaceutical dosage forms[a]

Dosage Form	Dosage strength (mg per unit)	Amount found (mg per unit)	Assay amount (%)	
			Mean	±S.D.
Augmentin (tablet)	Amoxycillin 500	508.12	101.62	2.08
	Clavulanic acid 125	124.35	99.48	0.76
Co-amoxyclav (tablet)	Amoxycillin 500	518.35	103.67	1.38
	Clavulanic acid 125	126.46	101.19	2.45
Co-amoxyclav (powder for susp.)[b]	Amoxycillin 125	125.46	100.36	1.22
	Clavulanic acid 31.25[b]	30.94	99.10	0.78

[a] Results are the mean of six replicate analyses. See text for more explanation.
[b] Each 5 ml of reconstituted suspension labeled to contain the amount shown.

4. Conclusions

In this study a HPLC-EC method was used for simultaneous determination of amoxycillin and clavulanic acid. The method was shown to be simple, rapid and reproducible. This method can be used as the method of choice for analysis of electroactive β-lactam antibiotics in dosage forms.

References

[1] A.P. Ball, P.G. Davey, A.M. Geddes, I.D. Farrei, G. Brookes, Lancet 1 (1980) 620–623.
[2] W. Cullmann, W. Dick, Immun. Infect. 14 (1986) 188–190.
[3] Code Federal Regulation, Title 21 436, 1976, 204.
[4] D.F. Davidson, Clin. Chem. Acta 69 (1976) 67–71.
[5] R.H. Barhaiya, P. Turner, E. Shaw, Clin. Chem. Acta 77 (1977) 373–377.
[6] P. Izquierdo, A. Gomez-Hens, D. Perez-Bendito, J. Pharm. Biomed. Anal. 11 (1993) 927–931.
[7] A.E. Bird, J.M. Bellis, B.C. Gasson, Analyst 107 (1982) 1241–1245.
[8] C.G. Perez, I.G. Martin, B.R.V. De Aldana, J. Pharm. Biomed. Anal. 9 (1991) 383–386.
[9] L.J. Nunez-Vergara, J.A. Sequella, M.M. Silva, Farmaco, Ed. Prat. 35 (1980) 409–415.
[10] M. Foulstone, C. Reading, Antimicrob. Agents Chemother. 22 (1982) 753–762.
[11] J. Martin, R. Mendez, J. Liq. Chromatogr. 11 (1988) 1697–1705.
[12] J. Haginaka, H. Yasuda, T. Uno, T. Nakagawa, Chem. Pharm. Bull. 31 (1983) 4436–4447.
[13] J. Haginaka, J. Wakai, H. Yasuda, T. Uno, T. Nakagawa, J. Liq. Chromatogr. 8 (1985) 2521–2534.
[14] J. Haginaka, J. Wakai, H. Yasuda, Chem. Pharm. Bull. 34 (1986) 1850–1852.
[15] J. Haginaka, J. Wakai, H. Yasuda, Anal. Chem. 59 (1987) 324–333.
[16] S. Chulavatnatol, B.G. Charles, J. Chromatogr. 615 (1993) 91–96.
[17] M.A. Abounassif, E.M. Abdel-Moety, M.E. Mohamed, E.A. Gad-Karim, J. Pharm. Biomed. Anal. 9 (1991) 731–735.
[18] T. Tsou, J. Wu, C. Young, T. Wang, J. Pharm. Biomed. Anal. 15 (1997) 1197–1205.
[19] A.C. Mehta, S. Hart-Davies, J. Payne, R.W. Lacey, J. Clin. Pharm. Ther. 19 (1994) 313–315.