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Pyrocatechol violet in pharmaceutical analysis. Part I. A spectrophotometric method for the determination of some β-lactam antibiotics in pure and in pharmaceutical dosage forms

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Abstract

A fairly sensitive, simple and rapid spectrophotometric method for the determination of some β-lactam antibiotics, namely ampicillin (Amp), amoxycillin (Amox), 6-aminopenicillanic acid (6APA), cloxacillin (Clox), dicloxacillin (Diclox) and flucloxacillin sodium (Fluclox) in bulk samples and in pharmaceutical dosage forms is described. The proposed method involves the use of pyrocatechol violet as a chromogenic reagent. These drugs produce a reddish brown coloured ion pair with absorption maximum at 604, 641, 645, 604, 649 and 641 nm for Amp, Amox, 6APA, Clox, Diclox and Flucolx, respectively. The colours produced obey Beer's law and are suitable for the quantitative determination of the named compounds. The optimization of different experimental conditions is described. The molar ratio of the ion pairs was established and a proposal for the reaction pathway is given. The procedure described was applied successfully to determine the examined drugs in dosage forms and the results obtained were comparable to those obtained with the official methods.

Keywords: β-Lactam antibiotics determination; Spectrophotometry; Pyrocatechol violet; Dosage forms

1. Introduction

The importance of penicillins as broad-spectrum antibiotics is well known. They demonstrate clinical efficacy for a large number of infectious and revolutionized the treatment of infection diseases. They are known to inhibit protein synthesis in bacteria by causing misreading of the genetic code [1]. They are active against gram-positive and gram-negative bacteria. They inhibit cell wall synthesis in *Escherichia coli* [2]. Ampicillin trihydrate (Amp), amoxycillin trihydrate (Amox), 6-amino-penicillanic acid (6APA), cloxacillin sodium (Clox), dicloxacillin sodium (Diclox) and flucloxacillin sodium (Fluclox) are some of the penicillin group of antibiotic drugs. Several spectrophotometric procedures for their determination based on charge transfer and ion pair complex formation [3–10] and UV derivative [11,12] have been reported. Also, various potentiometric [13,14], polarographic [15–17], voltammetric [18], iodometric [19], titrimetric [20], microbiological [21] and high-performance liquid chromatographic (HPLC) [22–25] procedures have been reported for the determination of such drugs. Yet, no work has been performed to determine β-lactam antibiotics using pyrocatechol violet (PCV) as a reagent for spectrophotometric determination.

In this paper, the development of an analytical procedure based on the formation of ion pair products with PCV is reported for the analysis of the drugs cited above. This highly sensitive procedure is simple, rapid, reproducible and readily adaptable to unit dose analysis.

2. Experimental

2.1. Apparatus

A Perkin–Elmer Lambda 3B spectrophotometer was used for all spectral measurements. The pH of solutions was checked using an Orion Research Model 601 A/ Digital Ionalyzer. The IR spectrum was recorded on

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Table 1
UV–Vis spectral characteristics and precision data

Parameter	Amp	Amox	6APA	Clox	Diclox	Fluclox
Beer's law limits (μg/ml)	0.2–28	0.2–25	0.2–35	0.2–22	0.2–38	0.2–44
Molar absorptivity (l/mol/cm)	8.65×10^3	9.23×10^3	2.35×10^3	6.6×10^3	6.18×10^3	7.52×10^3
Sandell sensitivity (μg/cm^2)	0.047	0.045	0.085	0.072	0.083	0.066
Detection limit (μg/ml)	0.050	0.020	0.025	0.060	0.015	0.020
Correlation coefficient	0.9988	0.9996	0.9985	0.9998	0.9990	0.9999
Regression equation (A^* [a])						
Slope (b)	0.021	0.022	0.012	0.014	0.013	0.015
Intercept (a)	−0.017	−0.026	0.033	−0.021	0.018	0.029
Relative standard deviation (%)	1.56	1.42	1.36	1.08	1.19	1.24
Range of error (%) (95% confidence limit)	±1.33	±1.07	±1.48	±1.25	±1.03	±1.13
Stability of drug–PCV complex (h)	4.0	4.0	6.0	6.0	6.0	8.0
Logarithmic stability constants	8.8	9.0	8.3	8.1	9.2	8.6
t-test [b] (2.57) [c]	1.74	2.11	1.36	1.23	1.67	1.92
F-test [b] (5.05) [c]	1.49	1.69	1.82	1.57	1.31	1.77

[a] $A^* = a + bC$, where C is the concentration in μg/ml.
[b] Comparison with the official methods [27,28].
[c] Values in parenthesis are the theoretical t-and F-values for five degrees of freedom at $P = 0.05$.

Pye Unicam SP 9700 infrared spectrophotometer, by KBr disc technique. ^{1}H NMR spectra were run on a Varian EM 390-90 spectrometer in DMSO-d_6; chemical shifts are reported relative to TMS as internal standard.

2.2. Reagents

All chemicals and reagents were of analytical-reagent grade. PCV reagent (5×10^{-3} M) was prepared by dissolving 0.1932 g of the product (Aldrich) in 10 ml bidistilled water and diluting to the mark with water in a 100 ml measuring flask. Borate buffer solutions of different pH values (5–11) were prepared as recommended [26].

2.3. Materials

Amp, Amox, Fluclox and Clox were obtained from Egyptian International Pharmaceutical Industries Company (EIPICO). 6APA was obtained from Aldrich and Diclox was obtained from Medical Union Pharmaceutical Company, Egypt. Stock solutions (1×10^{-3} M) of the β-lactam agents were prepared by dissolving the appropriate weight of each drug separately in the minimum volume of 0.01 M sodium hydroxide solution and diluting to 100 ml with water. Working standard solutions were prepared as required by suitable dilution of the stock solution with water.

2.4. General procedures

An aliquot containing the drug was transferred over a working concentration range (Table 1) into a 25-ml measuring flask and 3 ml of 5×10^{-3} M PCV solution and 15 ml of pH 8.5 buffer solution were added. The solution was diluted to volume with water and allowed to stand for 3 min for Amp, Amox and Fluclox, whereas for 6APA, Colx and Diclox, the flasks were left to stand in a water bath at 60 ± 2°C for 10 min until complete colour development and then allowed to stand at room temperature for 5 min. The absorbance was measured at the optimum λ_{max} after the specified time against a reagent blank prepared in the same way for each drug. The absorbance was plotted against the final drug concentration in order to obtain a calibration graph.

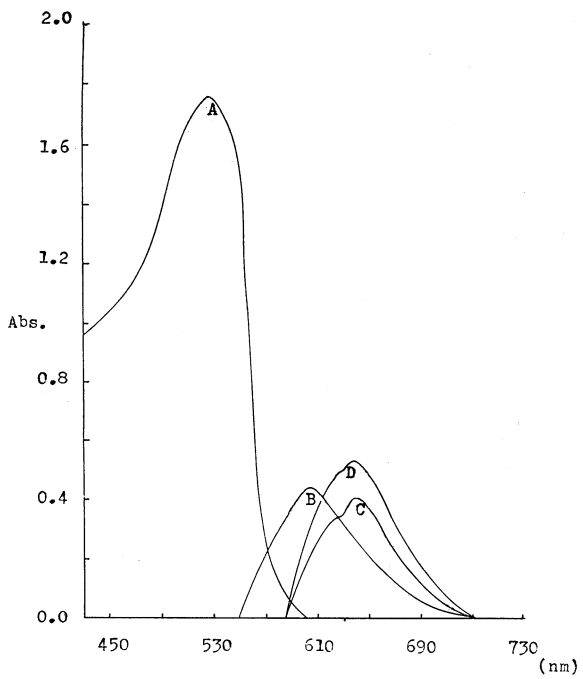


Fig. 1. Absorption spectra of (A) 6×10^{-4} M PCV; (B) A + 20 μg/ml Amp vs. A; (C) A + 18 μg/ml Amox vs. A; and (D) A + 32 μg/ml fluclox vs. A at room temperature 25°C.

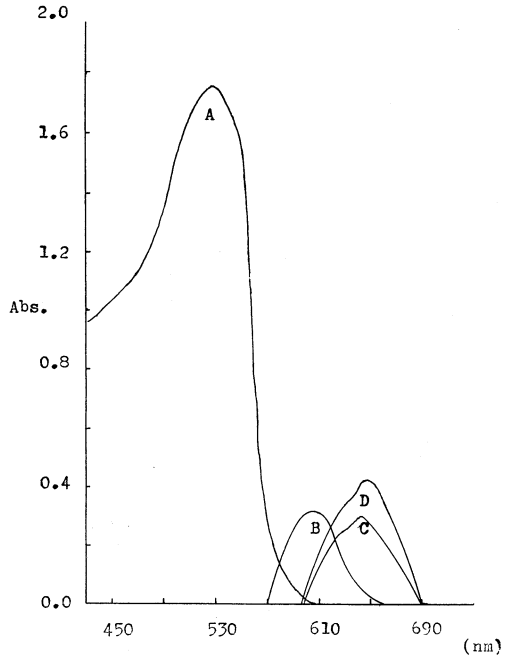


Fig. 2. Absorption spectra of (A) 6×10^{-4} M PCV; (B) A + 25 μg/ml Clox vs. A; (C) A + 25 μg/ml 6APA vs. A; and (D) A + 35 μg/ml Diclox vs. A; after 10 min at 60°C.

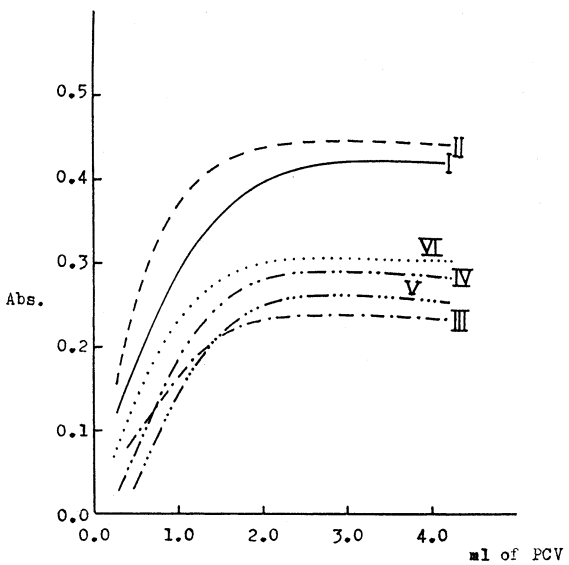


Fig. 3. Effect of reagent PCV (5×10^{-3} M) on the complex formation for: I: Amp; II: Amox; III: 6APA; IV: Clox; V: Diclox; and VI: Fluclox.

2.5. Assay procedures for dosage forms

2.5.1. Capsules

The average content weight of a capsule was determined and 100 mg was dissolved in the minimum volume of 0.01 M sodium hydroxide and the volume was made up to 500 ml with water. An aliquot containing the drug in the working range (Table 1) was transferred. The concentration of each drug was determined using the general procedure described above.

2.5.2. Syrup and vials

A volume of syrup or vials contents containing 100 mg of drug was transferred, dissolved in the minimum amount of 0.01 M NaOH, filtered if necessary and the volume was made up to 500 ml with water. A volume of this solution, which contained the drug, was transferred in the working range (Table 1). Using the above general procedure, the concentration of each drug was determined.

2.5.3. Procedure for mixture of Amp and Clox in dosage forms

Since Amp reacts with PCV at room temperature and Clox at 60 ± 2°C after 10 min, we can determine both drugs in their pharmaceutical dosage forms. A volume of solution prepared from capsules, syrup or vials containing 50 mg of each drug was taken and the volume made up to 500 ml with water. A volume of this solution containing the drugs in the working range (Table 1) was transferred into 25-ml measuring flask and 3 ml of 5×10^{-3} M PCV solution and 15 ml of pH 8.5 buffer solution were added. The solution was diluted to volume with water and the absorbance was measured after 3 min of mixing at room temperature against reagent blank prepared in the same manner. The concentration of Amp was determined using its calibration curve. The flasks were placed in a water bath for 10 min, at 60 ± 2°C, then allowed to stand for 5 min at room temperature. The absorbance was measured at 604 nm against a reagent blank. The increase in absorbance was due to Clox. Alternatively, Amp in the mixture was determined by measuring the absorbance of the solution prepared in cold and the total Amp and Clox in another identical volume after heating, as above.

2.5.4. Procedure for mixture of Amox and Fluclox in dosage forms

Since Amox and Fluclox react rapidly with PCV at room temperature and the band due to Fluclox disappears on raising the temperature (60 ± 2°C), but not the band of Amox ion pair which remains constant for 4.0 h, we can determine both drugs in their pharmaceutical dosage forms simultaneously without separation. A volume of prepared solution from capsules, syrup or vials containing 50 mg of each drug was taken and the volume was made up to 500 ml with water. A volume of this solution containing the drugs in the working range (Table 1) was transferred into a 25-ml measuring flask containing 15 ml of pH 8.5 buffer solution; 3 ml of 5×10^{-3} M PCV solution was then added, diluting to the mark with water. The absorbance was measured after 3 min of mixing at room temperature against blank prepared in the same manner. The absorbance value was due to both the ion pairs formed. The flasks were placed in a water bath for 10 min at 60 ± 2°C,

then allowed to stand for 5 min in cold. The absorbance was then measured at 641 nm against a reagent blank treated similarly. The absorbance in this case was due to Amox ion pair complex, whereas, the decrease in the absorbance value was due to Fluclox. The concentration of each drug was determined using a calibration curve prepared earlier.

2.6. Preparation of the solid ion pairs

Ion pairs were prepared by mixing ethanolic 0.01 mol of PCV with an appropriate weight of each drug equivalent to 0.01 mol dissolved in ethanol. The mixtures were refluxed in a water bath of 70 ± 2°C for 1.5 h, and then cooled at room temperature. The precipitates obtained were filtered, thoroughly washed with bidistilled water and dried. They were subjected to elemental microanalysis (C, H and N) (microanalytical centre of Cairo University), infrared and ^{1}H NMR spectral analysis.

3. Results and discussion

Figs. 1 and 2 show the absorption spectra of PCV–β-lactam ion pair complexes, formed in aqueous buffer medium, and reagent blank. These spectra have absorption maximum at 604, 641, 645, 604, 640 and 641 nm for Amp, Amox, 6APA, Clox, Diclox and Fluclox, respectively. Hence, these wavelengths were used for all subsequent measurements.

Investigations were carried out to establish the most favourable conditions for the complexation reaction of PCV with some β-lactams to achieve maximum colour development in their determination. The influence of some variables on the reaction has been tested as follows.

3.1. Effect of time and temperature

Sample solutions containing antibiotics and the blank were treated identically with the reagent and

Table 2
Evaluation of accuracy and precision of the official (O) proposed (P) method using PCV as reagent

Drug	Taken (μg/ml)	Found [a](μg/ml)				Standard error	Confidence limits
		O	P	s	S_r (%)		
Amp	9.0	8.90	8.96	0.05	0.69	0.020	8.96 ± 0.060
	18.0	18.20	17.90	0.08	1.04	0.033	17.90 ± 0.095
	27.0	27.35	27.10	0.06	0.75	0.024	27.10 ± 0.070
Mean					0.83	0.026	
Amox	8.0	7.90	8.95	0.03	0.45	0.012	8.05 ± 0.040
	16.0	16.20	16.10	0.07	0.91	0.029	16.10 ± 0.080
	24.0	24.33	23.90	0.04	0.52	0.016	23.90 ± 0.050
Mean					0.63	0.019	
6APA	11.0	10.85	11.08	0.06	0.74	0.024	11.08 ± 0.070
	22.0	22.20	21.90	0.09	1.08	0.037	21.90 ± 0.110
	33.0	33.40	32.85	0.05	0.66	0.020	32.85 ± 0.060
Mean					0.82	0.027	
Clox	7.0	7.11	6.96	0.04	0.51	0.016	6.96 ± 0.050
	14.0	13.80	14.09	0.10	1.16	0.041	14.09 ± 0.12
	21.0	21.30	20.86	0.08	0.99	0.033	20.86 ± 0.095
Mean					0.88	0.030	
Diclox	12.0	12.15	11.95	0.05	0.68	0.020	11.95 ± 0.060
	24.0	23.75	24.15	0.09	1.05	0.037	24.15 ± 0.110
	36.0	36.45	36.20	0.07	0.94	0.029	36.20 ± 0.080
Mean					0.89	0.029	
Fluclox	14.5	14.35	14.55	0.04	0.53	0.016	14.55 ± 0.050
	29.0	28.70	29.15	0.07	0.86	0.029	29.15 ± 0.080
	43.5	43.80	43.40	0.08	0.99	0.033	43.40 ± 0.095
Mean					0.79	0.026	

[a] Average of five determinations.

Table 3
Assay of Amp, Amox and Fluclox in pharmaceutical dosage forms using PCV in aqueous buffer media by the proposed (P) and official (O) methods [a]

Dosage forms	Company	Content (mg)	Found [b]					
			Amp		Amox		Fluclox	
			P	O	P	O	P	O
Capsules								
Epicocillin	EIPICO	250	251	245				
Epicocillin	EIPICO	500	497	506				
E-mox	EIPICO	250			248	255		
E-mox	EIPICO	500			504	510		
Flucillin	CIDCO	250					251	247
Flucillin	CIDCO	500					498	508
Syrup								
Pentraxyl	CIDCO	125	126	122				
Pentraxyl	CIDCO	250	247	255				
Amoxicid	CIDCO	125			125	127		
Amoxicid	CIDCO	250			254	244		
Vials								
Ampicillin	Misr Co.	500	506	512				
Ampicillin	Misr Co.	1000	995	980				
Ibiamox	APIC	250			249	255		
Ibiamox	APIC	500			505	491		
Flucillin	CIDCO	500					503	495
Flucillin	CIDCO	1000					994	988

[a] EIPICO: Egyptian International Pharmaceutical Industries Company, Egypt. CIDICO: Chemical Industrial Development Company, Egypt. Misr Co: Misr Company for Pharmaceutical Industries, Egypt. APIC: Amount Pharmaceutical Industries Company, Egypt.
[b] Average of six determinations.

buffer within different time and temperature. The results obtained indicated that Amp, Amox and Fluclox–PCV ion pair complexes were formed after 3 min of starting the reaction at room temperature (25 ± 2°C), whereas for 6APA, Clox and Diclox, the full colour development was achieved after 60 min. To overcome this problem, heating on a water bath of different temperature for variable time was useful. Maximum absorbance values for all complexes formed were attained after 10 min at 60 ± 2°C. For Amp and Amox complex, the absorption spectra were not altered by varying temperature and remained stable for 4 h, whereas for the Fluclox complex it decayed completely at this temperature (60 ± 2°C). For 6APA, Clox and Diclox complexes, the absorbance remained stable at room temperature for 6 h, after which it began to fade slowly.

3.2. Effect of pH

In order to establish the optimum pH range, each antibiotic under consideration was allowed to react with PCV in aqueous buffered solutions of pH 5–11. The reddish brown ion pair complex formed was measured spectrophotometrically. Constant absorbances were obtained over the pH range 8.0–9.0 in borate buffer solution. Hence a pH 8.5 was used in all subsequent work.

3.3. Effect of reagent concentration

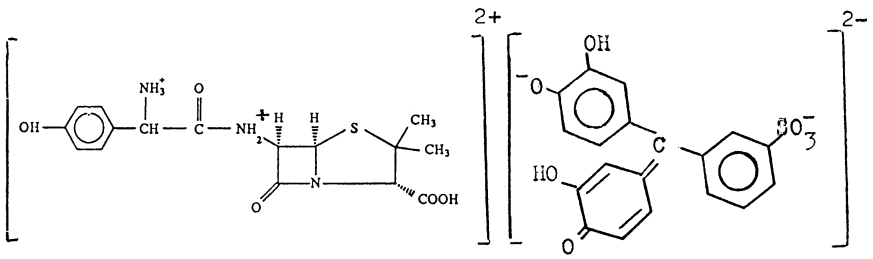
To establish the optimum concentration of the reagent, different volumes of a 5×10^{-3} M reagent solution were used. Aliquots of 5×10^{-3} M PCV solution (3 ml) was used for the production of maximum and reproducible colour intensity. Higher concentrations of the reagent did not affect the colour intensity (Fig. 3).

3.4. Composition of the complex

The stoichiometry of the complexes formed between different antibiotics and PCV was investigated at pH 8.5 applying the molar ratio and continuous variation methods. The results indicated the formation of 1:1 ion pair complexes. The logarithmic stability constants was found to be 8.8, 9.0, 8.3, 8.1, 9.2 and 8.6 for Amp, Amox, 6APA, Clox, Diclox and Fluclox, respectively. The presence of the ion pair complexes may be supported by the bathochromic shift observed from 526 nm for PCV to 604, 641, 645, 604, 649 and 641 nm, respectively, for the above-mentioned antibiotics. To

deduce the structure, the solid ion pair complexes were prepared and analysed using elemental analysis and IR spectra. The fundamental IR spectra of the complexes are the sum of PCV and drugs spectra with PCV predominating in the 4000–400 cm^{-1} region. Significant differences in the region 3200–1700 cm^{-1}, which are attributed to amine, amide and sulphonic groups were observed. On the other hand, no change occurred in the bands at 1580 cm^{-1} (COO^- asymmetric stretching), 1675 cm^{-1} (C=O amide stretching) and at 3550–3400 (phenolic OH), respectively. The presence of the β-lactam carbonyl at $\cong 1620$ in all complexes indicates the stability of the examined drug in the ion pair complex formation. ^1H NMR spectra of PCV and its complexes with β-lactam antibiotics were recorded in DMSO-d_6 using TMS as internal reference. The signal at $\delta = 9.7$ ppm assigned to the sulphonic proton disappeared in all complexes, whereas the integral of the signal at $\delta = 11.8$ ppm due to three hydrogen of the hydroxyl groups in PCV was reduced indicating the presence of two hydrogen only in PCV moiety of the complexes. So two protons from PCV moiety were transferred to the drug moiety to form 1:1 ion pair complex.

The coloured reaction product can be represented taking Amox–PCV ion pair complex as an example by the following structure:



3.5. Quantification

Regression plots showed that there was a linear dependence of absorbance on concentration over the

Table 4
Assay for simultaneous determination of the studied drugs in their dosage forms by the proposed (P) and official (O) methods [a]

Dosage forms	Company	Labelled content (mg)	Found [b]							
			Amp		Clox		Amox		Fluclox	
			P	O	P	O	P	O	P	O
Capsules										
Ampiclox (250 mg)	1	125	124	123						
		125			126	123				
Flumox (250 mg)	2	125					126	127		
		125							126	122
Ampiclox (500mg)	1	250	253	146						
		250			249	245				
Flumox (500 mg)	2	250					252	245		
		250							248	255
Syrup										
Ampiclox (250mg)	1	125	125	122						
		125			124	127				
Hi-Flucil (250 mg)	3	125					126	123		
		125							124	127
Vials										
Ampiclox (1000mg)	1	500	504	510						
		500			497	490				
Flumox (500mg)	2	250					252	247		
		250							249	245
Hi-Flucil (1000 mg)	3	500					498	494		
		500							505	510

[a] 1. Medical Union Pharmaceutical Company, Egypt. 2. Egyptian International Pharmaceutical Industries Company, Egypt. 3. Chemical Industrial Development Company, Giza, Egypt.
[b] Average of six determinations.

Beer's law ranges. The optimum conditions were those used in the procedure. The molar absorptivity, Sandell sensitivity, slope, intercept and correlation coefficient were obtained by a linear least-squares treatment of the results for ion pairs in solution. The detection limits for each drug were also calculated as three times the standard deviation of the blank. For a more accurate analysis, Ringbom optimum concentration ranges were calculated (Table 1).

The reproducibility of the proposed method was determined by analysing six replicate samples of each β-lactam antibiotic (15 μg/ml Amp, 12 μg/ml Amox, 18 μg/ml 6APA, 10 μg/ml Clox, 20 μg/ml Diclox and 24 μg/ml Fluclox; at these concentrations, the relative standard deviations and ranges of error obtained are given in Table 1.

The performance of the proposed method was assessed by calculation of the *t*-(for accuracy) and *F*- (for precision) values compared with the official methods [based on potentiometric titration using 0.02 M mercury nitrate for Amp, Amox, Clox and Diclox [27], whereas HPLC was used for 6APA [28]. For Fluclox, a spectrophotometric determination was introduced using imidazol–mercury reagent [27]. Mean values were obtained in a Student's *t*-and *F*-test and 95% confidence limits for five degrees of freedom [29] and the results showed that the calculated *t*-and *F*-values did not exceed the theoretical values (Table 1).

In order to determine the accuracy and precision of the proposed method, solutions containing six different concentrations of the examined drug were prepared and analysed in quintuplicate. The measured standard deviation, relative standard deviation (s_r), the standard analytical error and confidence limit values (Table 2) can be considered satisfactory, at least for the levels of concentrations examined.

3.6. Interference

Interference studies were carried out in order to investigate the effect of additives, excipients and degradation products that might be present in β-lactam antibiotics dosage forms. Addition of PCV aqueous solution to the different degradation products results in the formation of colourless solutions. Thus, no interference was observed from benzylpenicilloic acid, benzylpenicillenic acid, penicillic acid, penicillamine and ultimately penilloaldyhyde which are the main degradation products of antibiotics under consideration resulting from thermal and hydrolytic cleavage of the studied drugs. Also sodium acetate, magnesium stearate, talc powder, starch, glucose, lactose and fructose do not interfere in the determination indicating that complexation does not occur with these ingredients under the same reaction conditions used, confirming high selectivity in their determination with the proposed method.

Since Amp reacts with PCV at room temperature without interference of Clox at this temperature and the absorbance of the ion pair complex formed is not affected by raising the temperature up to 70°C, the determination of Amp and Clox in their mixture can be performed without any separation by the proposed method. Also, while Amox and Fluclox react rapidly with PCV at room temperature, the complex due to Fluclox is destroyed rapidly at higher temperature (60 ± 2°C) whereas that of Amox remain stable for 4.0 h. This phenomenon can be used for simultaneous determination of both drugs without any separation. Satisfactory results were obtained for the recovery of both the compounds, indicating that the proposed method is effective in the simultaneous determination of (Amp and Clox) and (Amox and Fluclox) in their mixtures.

3.7. Analytical applications

The proposed and official methods [27,28] were applied to the determination of the studied drugs in their pharmaceutical dosage forms (Table 3). The concentration of the drug in their preparations is calculated from the corresponding calibration graph or regression equation. Statistical analysis of the results obtained, compared with those of the official methods, showed no significant difference in the accuracy and precision of the two methods. The method is reproducible, accurate and precise for the simultaneous determination of a mixture of (Amp with Clox) and (Amox with Fluclox) in their dosage forms (Table 4).

4. Conclusions

The proposed method is fairly simple, less time-consuming and more sensitive than the official methods. Although the colour development of Clox, Diclox and Fluclox complexes at room temperature requires 60 min for completion, this can be shortened to 10 min by raising the temperature to 60 ± 2°C. Simultaneous determination of Amp and Clox mixture in their dosage forms can be successfully applied without any separation, since Amp react with PCV at room temperature and Clox at 60 ± 2°C. Also, a simultaneous determination of Amox and Fluclox mixture can be done, since they rapidly react with PCV at room temperature, whereas that of Amox appeared only on raising the temperature for 10 min at 60 ± 2°C. The proposed method is suitable for the determination of the studied β-lactam drugs in their dosage forms without interference from excipients and additives such as starch, glucose, magnesium stearate or from common degradation products, suggesting applications in bulk drug analysis.

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