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Spectrophotometric determination of ampicillin, dicluxacillin, flucloxacillin and amoxicillin antibiotic drugs: ion-pair formation with molybdenum and thiocyanate

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Abstract

A sensitive spectrophotometric method is developed for the determination of some antibiotic drugs such as ampicillin (amp), dicluxacillin (dicl), flucloxacillin (fluc) and amoxicillin (amox). The method involves the formation of ion-pairs between these drugs under investigation and inorganic complex of Mo (V)–thiocyanate followed by its extraction with methylene chloride. The optimum conditions for the ion-pairs formation are established. The method permits the determination of amp, dicl, fluc and amox over a concentration range of 1.5–77.5, 3–75, 1.5–79 and 7.5–75 μg ml^{-1} respectively. The sensitivity (S) is found to be 0.017, 0.061, 0.014 and 0.073 μg cm^{-2} for amp, dicl, fluc and amox, respectively. The method is simple, rapid, reproducible and accurate within $\pm 1\%$. The method is applicable for the assay of the four drugs under investigation in different dosage forms and the results are in good agreement with those obtained by the official method.

Keywords: Pharmaceutical preparations; Extraction spectrophotometry; Ampicillin; Dicluxacillin; Flucloxacillin; Amoxicillin determination; Mo (V)-thiocyanate

1. Introduction

Different spectrophotometric methods were used for the determination of β-lactam antibiotics using complex formation with Cu (II) [1–3] and nickel (II) [4] ions or titration against Cu (II) ions using ion-selective electrode [5]. A modified spectrophotometric method was developed for the determination of some important antibiotics including amoxicillin and ampicillin through charge transfer complexation reaction with chloranil [6,7]. Amoxicillin and ampicillin were determined spectrophotometrically in pharmaceutical preparations using potassium iodate [8], picric and picramic acids [9], certain pi-acceptors [10] or with ammonium molybdate [11]. Amoxicillin was determined spectrophotometrically in a binary mixture with dicloxacillin [12] or in the presence of dicluxacillin or flucloxacillin [13]. Two spectrophotometric methods [14] (using *N*-bromosuccinimide and *N*-chlorosuccinimide) or two

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colourimetric methods [15] (using sodium hypochlorite and 1-chlorobenzotriazole) were described for the determination of amoxicillin. Ampicillin was determined spectrophotometrically in tablets, capsules, powder and granules with the use of Na 3,4-naphthaquinone-1-sulphonate [16] or with 1-fluoro-2,4-dinitrobenzene [17] reagents. An HPLC method [18] was used for the determination of amoxicillin in different pharmaceutical preparations.

This work was undertaken in order to study the analytical aspects of the reaction between the drugs under investigation (amp, dicl, fluc and amox) with Mo (V)–thiocyanate binary complex. This study also aims to test the sensitivity, accuracy and selectivity of the ion-pair formation method and to use this method for the spectrophotometric determination of these β-lactam drugs in pure form and in some pharmaceutical preparations.

2. Experimental

2.1. Materials and solutions

All reagents were of analytical-reagent grade and used without further purification. Water was always deionized.

Stock Mo (VI) solution (2% w/v) was prepared from AR grade ammonium molybdate in deionized water containing few drops of ammonia and standardized gravimetrically using 8-hydroxyquinoline [19]. The working solution was prepared by suitable dilution of the standardized stock solution.

Ammonium thiocyanate (10% w/v) and ascorbic acid (5% w/v) solutions were prepared in deionized water. 8 M HCl stock solution was prepared by accurate dilution from concentrated HCl solution (11.5 M). Dilute solution (4 M) was prepared by accurate dilutions.

2.1.1. Reference drug solution

200 mg of the drugs under investigation was weighed into 100 ml calibrated flask, dissolved in the least amount of methanol and diluted to the volume with deionized water.

2.1.2. Sample preparation solution

An accurately weighed amount of the capsules equivalent to 250 mg of the drugs was dissolved in methanol in a 100 ml calibrated flask. The solution was shaked for 15 min, diluted to volume and filtered through a whatman no. 41 filter paper.

2.2. Apparatus

A Perkin–Elmer spectrophotometer Model 601 with matched quartz cells of 1 cm optical path length was used for spectrophotometric measurements in the wavelength 467 nm.

2.3. Determination of amp, dicl, fluc and amox

A 4 ml of 80 μg ml^{-1} of ammonium molybdate, 2 ml of HCl (4 M), 5 ml of 10% each of ammonium thiocyanate and ascorbic acid were placed in a 50 ml capacity separating funnel. The mixture left for 15 min at room temperature (20 ± 5°C). Different volumes of 200 μg ml^{-1} of amp, dicl, fluc and amox solutions (0.1–7 ml) were added and diluted with deionized water up to 25 ml. After another 15 min, 10 ml of methylene chloride was added (twice with 5 ml portions), the mixture was shaked well for 1 min and allowed to stand to separate into two phases. The methylene chloride extract was dried over anhydrous sodium sulphate and the absorbance of the filtered extract was measured at 467 nm, against a reagent as a blank, which prepared similarly without the drugs.

2.4. Procedure for the capsules

The content of ten capsules of drugs under investigation was weighed and ground into a fine powder. A mass of powder containing about 250 mg of the drugs was weighed accurately, dissolved in the least amount of methanol, filtered through a Whatman no. 41 filter paper and washed with the methanol solvent. Then, the filtrate plus washings were diluted to 100 ml with deionized water in a calibrated flask. An aliquot was used for the determination of each drug according to the procedure mentioned above.

3. Results and discussion

The main objective of this study depends on the fact that, ion-pairs are formed between the tertiary amine group of amp, dicl, fluc and amox drugs and Mo (V)–thiocyanate binary complex via the protonated nitrogen atom of these drugs [20]. Mo (V) formed by the reduction of Mo (VI) with ascorbic acid combines with ammonium thiocyanate to form red Mo (V)–thiocyanate binary complex in HCl solution [21]. On adding amp, dicl, fluc or amox solutions, an orange red ion-pairs are formed in the same acid concentration. The ion-pairs are soluble in methylene chloride while Mo (V)–thiocyanate binary complex is insoluble. A double extraction is necessary to extract these ion-pairs quantitatively into the organic phase. The absorption spectra of the ion-pairs extracted in methylene chloride show a maximum at 467 for amp, dicl, fluc and amox against a reagent blank.

It was found that, the reduction probability of Mo (VI) to Mo (V) may occurs by ascorbic acid or SCN^- in acidic media [20]. But the rapidity, sensitivity and stability of Mo (V)–thiocyanate ion-pair binary complex is enhanced by using ascorbic acid. Ascorbic acid gives reproducible values and masks many interfering ions [22]. From the data shown in Fig. (1a), it is found that, 3–5 ml of 5% ascorbic acid is sufficient for the reduction of 80 μg Mo (VI) to Mo (V). The addition of excess amount of ascorbic acid more than the required volume has no effect on the absorbance of the ion-pairs.

It was found that, the Mo (V) ion-pairs were formed only in hydrochloric, sulphuric, nitric or phosphoric acid medium, but the absorbance readings of the methylene chloride extract from 1.5–4 M HCl is the maximum one [21]. Hence, HCl medium has been selected as the suitable medium for Mo (V) ion-pairs extraction. It was found that, 5 ml of 4 M HCl is suitable for the formation of Mo (V)–thiocyanate–β lactam ion-pairs (Fig. 1b).

The effect of ammonium molybdate on the ion-pairs formation and their extraction in methylene chloride is shown in Fig. (1c). The data show that, 3–4 ml of 2% (w/v) of ammonium molybdate is required for maximum absorbance in a final volume of 20 ml aqueous solution and in presence of 40 μg of amp, dicl, fluc and amox drugs. After this, the absorbance was nearly constant. Also, it was found that 3–5 ml of 10% ammonium thiocyanate in a final solution of 20 ml gave the maximum pronounced effect on the absorbance in the determination of amp, dicl, fluc and amox drugs (Fig. 1d).

From the above results, an equation representing the reaction of Mo (VI) with ammonium thiocyanate in 4 M HCl and in the presence of ascorbic acid is given as:

$$Mo(VI) \xrightarrow[4MHCl]{\text{Ascorbic acid}} Mo(V) \xrightarrow{6SCN^-} Mo(SCN)_6^-$$

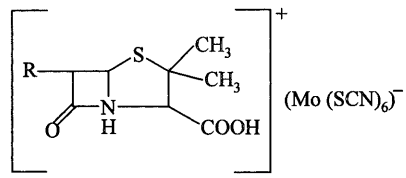
In this method, the complete formation of the ion-pairs needs 15 min before extraction with methylene chloride at 25°C. The absorbance of Mo (V)–thiocyanate binary complex is stable after 15 min while Mo (V)–thiocyanate drugs ion-pairs needs another 15 min for their complete formation (Fig. 1e).

Solvents like benzene, cyclohexane, acetone; methyl, *n*-pentyl, iso-butyl and ethyl alcohols; dioxane, dimethylformamide, carbon tetrachloride, diethyl ether and petroleum ether can not be used for the extraction of the ion-pairs while methylene chloride and dichloroethane extract the ion-pairs quantitatively. The molar absorptivity values for the ion-pairs in methylene chloride and dichloroethane are 6140 and 6130; 7025 and 7020; 5780 and 5755; and 6450 and 6643 $l.mol^{-1}cm^{-1}$ for amp, dicl, fluc and amox drugs, respectively, at $\lambda = 467$ nm which make them selected as the medium of extraction. Reproducible absorbance readings were obtained after either a single (extraction efficiency = 99.93, 99.85, 100.2 and 99.9% for amp, dicl, fluc and amox, respectively,) or double extraction (extraction efficiency = 100.02, 99.98, 100.1 and 99.96% for amp, dicl, fluc and amox, respectively,) with 10.0 ml of methylene chloride and 1 min shaking time. The studied ion-pairs are stable for more than 1 weak at 25°C in the organic solvents.

In order to prove the validity and the applicability of the proposed method and the reproducibility of the results obtained, four replicate experiments at different concentrations of amp,

dicl, fluc and amox drugs were carried out. Table 1 shows the values of the between–day relative standard deviations (S.D) for different concentrations of the drugs, obtained from experiments carried out over a period of 4 days. It was found that, the within-day relative S.D. were less than 1% which indicates that the proposed method is highly reproducible and Mo (V)–thiocyanate binary complex is successfully applied to determine amp, dicl, fluc and amox drugs.

The stoichiometry of the Mo (V) to each drug in the presence of excess amounts of ammonium thiocyanate was determined by the continuous variation method. The results indicate that, a 1:1 (metal:drug) ion-pairs are formed through the electrostatic attraction between positive protonated $ampH^+$, $diclH^+$, $flucH^+$, $amoxH^+$ and $(Mo(SCN)_6)^-$ complex as shown by the proposed structure [23]



Under the optimum conditions described above, the calibration graphs were constructed for the four drugs. The molar absorptivity, Sandell sensitivity (*S*) (the number of μg of the determi-

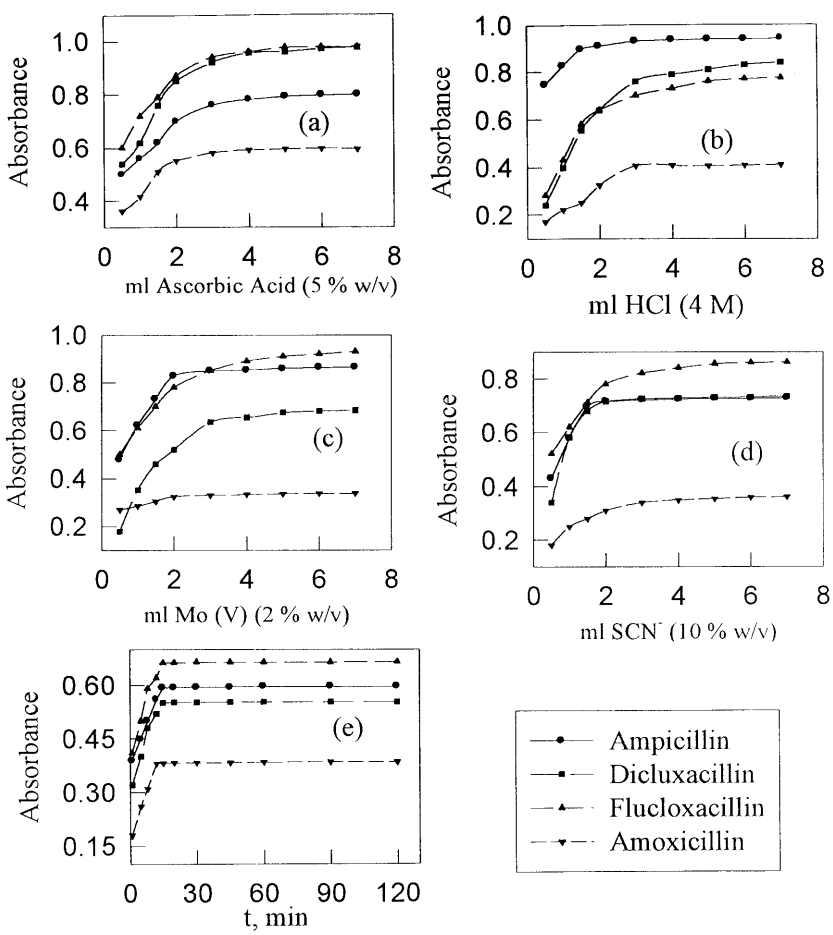


Fig. 1. Reaction condition of the colour formation of Mo (V) ion-pairs. (a) Effect of ascorbic acid (by volume); (b) effect of HCl (by volume); (c) effect of Mo (V) (by volume), (d) Effect of SCN^- (by volume) and (e) effect of time.

Table 1
Between–day precision of the determination of ampicillin, dicloxacillin, flucloxacillin and amoxicillin by the proposed method

Compound	Theoretical concentration (μg ml^{-1})	Found (μg ml^{-1})	Percentage recovery (%)	S.D	RSD[a]
Ampicillin	13.50	13.22	97.93	0.04	0.77
	24.0	23.98	99.92	0.06	0.58
	36.0	36.14	100.4	0.10	0.43
	48.0	47.93	99.85	0.08	0.52
Dicloxacillin	7.50	7.706	102.7	0.055	0.36
	18.0	17.98	99.89	0.046	1.05
	30.0	29.85	99.50	0.024	0.65
	60.0	59.89	99.82	0.035	0.92
Flucloxacillin	4.50	4.502	100.04	0.02	0.18
	15.0	15.11	100.7	0.061	0.44
	40.5	40.51	100.02	0.07	0.72
	52.5	52.44	99.89	0.043	0.68
Amoxicillin	10.0	10.03	100.3	0.03	1.02
	25.0	24.99	99.96	0.05	0.77
	40.0	40.06	100.15	0.06	0.25
	55.0	54.96	99.93	0.075	0.52

[a] Means and relative standard deviations (R.S.D.) for four experiments carried out on four different days.

Table 2
Analytical parameters for the determination of ampicillan, dicloxacillin, flucloxacillin and amoxicillin by the proposed method

Drug	λ_{max} (nm)	Conc. range (μg ml^{-1})	ε (l. mol.$^{-1}$ cm^{-1})	Sandell sensitivity (S) (μg cm^{-2})	$A = mc + z$		S.D.	R.S.D. (%)
					m	z		
Ampicillin	467	1.5–77.5	6.2×10^3	0.017	0.82	0.42	0.017–0.092	0.5–1.03
Dicluxacillin	467	3.0–75.0	4.98×10^3	0.061	1.12	−5.76	0.02–0.07	0.34–0.78
Flucloxacillin	467	1.5–79.0	7.25×10^3	0.014	0.76	2.6	0.032–0.067	0.22–0.83
Amoxicillin	467	7.5–85.0	5.28×10^3	0.073	1.06	−0.32	0.03–0.095	0.32–0.91

nand per ml of a solution having an absorbance of 0.001 for a path length of 1 cm, and given as $S = 10^{-3}$ per a, where a is the specific absorptivity) and regression equation for each drug was tabulated in Table 2. The correlation coefficients of the data obtained are 0.996, 0.999, 0.989 and 0.997 for amp, dicl, fluc and amox drugs, respectively. The S.D. are found to be 0.017–0.092, 0.02–0.074, 0.032–0.067 and 0.063–0.095 and the relative S.D. are 0.15–1.03, 0.24–0.75, 0.22–0.76 and 0.32–0.85 for amp, dicl, fluc and amox drugs, respectively, for five replicates determination. The low values of the relative standard deviations indicate the accuracy of the method. Beer's law was obeyed over the concentration range of 1.5–77.5, 3–75, 1.5–79.5 and 7.5–85 μg ml^{-1} for amp, dicl, fluc and amox, respectively. Moreover, Ringbom optimum concentration ranges can be calculated which give more accurate results at 5–72 and 5–70, 4–75 and 10–80 μg ml^{-1} for amp, dicl, fluc and amox, respectively. The apparent molar absorptivities of the resulting colour products were 6.0×10^3, 2.98×10^3, 6.25×10^3 and 3.28×10^3 l.mol^{-1}cm^{-1} for amp, dicl, fluc and amox, respectively.

The proposed method is successfully applied to the determination of amp, dicl, fluc and amox drugs in different pharmaceutical preparations and the results obtained are given in Table 3. From the calculated t- and F-values, it is clear

Table 3
Determination of ampicillin, dicloxacillin, flucloxacillin and amoxicillin in pharmaceutical preparations

Drug	Name of preparation	Drug (μg ml^{-1})	Recovery ± S.D. (%) ($n = 5$)		t-test	F-test
			Proposed method	official method		
Ampicillin	[a]Ampicillin	24.0	23.97	24.05	3.54	5.0
	[b]Ampicillin	36.0	35.92	35.97	5.10	4.10
Amoxicillin	[c]E-Mox	20.0	19.97	20.09	3.64	4.07
	[d]Ibiamox	38.0	37.89	38.0	4.10	3.0
	[e]Amoxil	40.0	39.95	39.99	5.10	5.2
Dicluxacillin	[f]Penestaph	20.0	19.97	20.05	4.45	4.25
Flucloxacillin	[g]Flucillin	30.0	29.98	30.03	4.20	5.10

[a] Ampicillin trihydrate (250 mg), Misr Co. for Pharm. Ind., SAA-Mataria, Cairo, Egypt.
[b] Ampicillin trihydrate (250 mg), ADWIC, El-Nasr Pharm. Chem. Co., Egypt.
[c] E-Mox (500 mg), Egyptian Int. Pharm. Ind. Co.(EIPICO), Egypt.
[d] Ibiamox (250 mg), Amoun Pharm. Ind. Co. (APIC), El-Salam City, Cairo, Egypt.
[e] Amoxil (500 mg), Medical Union Pharm. Co. (MUP), SAE Abou Sultan, Egypt.
[f] Penestaph (250 mg), Kahira Co. for Pharm. Ind., Cairo, Egypt.
[g] Flucillin (250 mg), Cid Co. for Pharm. Ind., Cairo, Egypt.

that, the results obtained by the proposed method are in good agreement with those obtained by the official method [24]. The proposed method was accurate, with high recoveries amounting to 99.67–100.95 ± 0.25–1.07 compared with 99.64–100.81 ± 0.3–0.95% using the official method.

3.1. Method validation

The aim of this method validation study [25] is to document successful implementation of the method for determining the drugs under study in their pharmaceutical preparations using spectrophotometric method. The calibration curves are found to be linear and the lines pass through the origin within 95% confidence limit. The limit of detection is found to be 0.5, 0.75, 0.9 and 0.75 for amp, dicl, fluc and amox, respectively. The S.D. within batches (S_w) and the S.D. between batches (S_b) values are in the required range from IQC (± 5%) and the results are, therefore, acceptable. The recovery (accuracy) is in the range 98–101.5, 97–105, 98–105 and 98–104% for amp, dicl, fluc and amox, respectively. This is satisfactory compared to the requirement of ± 20%. While, the average recovery (trueness) is from 99.28–100.64, 98.33–102.53, 98.56–102.48 and 98.46–101.48% for amp, dicl, fluc and amox, respectively. This is satisfactory compared with the requirement of ± 10%. The method has satisfactory precision in the whole working range. Repeatability (CV_w) is 0.58, 1.94, 0.155 and 0.95% for amp, dicl, fluc and amox, respectively, in the low end of the working range and 0.125, 0.117, 0.29 and 0.053% for amp, dicl, fluc and amox, respectively, for the higher concentrations. Between-day variability (CV_b) is 0.189, 1.41, 2.47 and 2.18% for amp, dicl, fluc and amox, respectively, at low concentration range and 0.282, 0.35, 0.3 and 0.27% for amp, dicl, fluc and amox, respectively, at higher concentrations. Therefore, according to the above data, the method could be applied satisfactory in the determination of amp, dicl, fluc and amox in pure and dosage forms with a high accuracy and precision.

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