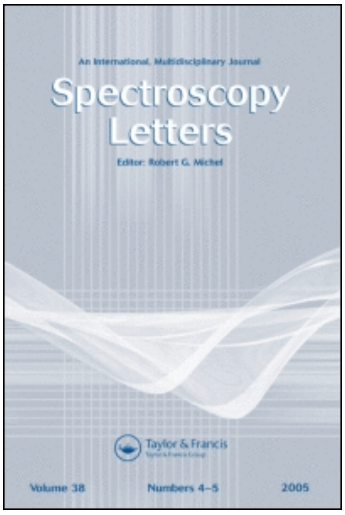

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Modified Spectrophotometric Method for the Determination of Some Important Antibiotics Through Charge - Transfere Complexation Reaction with Chloranil

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Modified Spectrophotometric Method for the Determination of Some Important Antibiotics Through Charge - Transfere Complexation Reaction with Chloranil

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A new modified spectrophotometric procedures are presented for the determination of three pharmaceutical antibiotic compounds through charge-transfere complexation reaction with chloranil. The complex shows an absorption mAxima at 550 nm, having a molar absorptivity coefficient of $5.55X10^3$,$7.81x10^3$ and $1.38x10^4$ l. $mol^{-1}cm^{-1}$ for ampicillin, amoxycillin and neomycin, respectively. Optimization of the reaction conditions has been investigated. Obediance to Beer's law (40μg/ml) permitted the assay of thes drugs in its dosage forms. A variety of pharmaceutical dosage forms containing ampicillin, amoxycillin and neomycin are successfully analysed by the proposed procedure.

INTRODUCTION

Several spectrophotometric methods for the assay of ampicillin trihydrate (1-4), [(6R)-6-(α-D-phenylgly cylamino)-penicillanic acid [7177-48-2], amoxycillin trihydrate (2,5-7) [(6R)-6-(α-D- hydroxy phenylgly-cylamino)-penicillanic acid [61336-70-7] and neomycin sulphate (8,9) [O-2,6-diamino-2,6-dideoxy-α-D-glucopyranosyl-(1→3) O-β-D-ribofuranosyl-(1→5)-O-[2,6-diamino-2,6-dideoxy-α-D-glucopyranosyl-(1→4)]-2-deoxy-D-streptamine sulphate][4146-30-9] were reported. Chloranil reagent has been found to be a valuable reagent for the determination of several pharmaceutical compounds through charge transfere complexation(10-13). Al-Ghabsha et al. (14,15) has reacted ampicillin, amoxycillin and neomycin

sulphate with chloranil in aqueous basic medium. The chromophore formed has λ_{max} at 347nm. The present work describes a modification of the chloranil reagent method for colorimetric estimation of ampicillin (Amp.), amoxycillin (Amox.) and neomycin sulphate (Neom.) in which they are reacted in non aqueous medium to produce a yellowish a red color complex which have an additional peak in the visible regoin at 550 nm. The procedure has been successfully applied to analyse a variety of pharmaceutical preparation of the studied drugs.

EXPERIMENTAL

Apparatus

A Perkin-Elmer λ 3B recording spectrophotometric equipped with 10 mm matched silica cells was used for all spectral measurements.

Reagents

All chemicals and reagent used were of analytical or pharmaceutical grade. Ampicillin and amoxycillin trihydrates were obtained from the Egyptian International Pharmaceutical Industries Company (EIPICO) whereas neomycin sulphate was obtained from Memphis Chemical Company (Egypt)

Stock standard solution

A 200 μg/ml solution of Amp, Amox. and Neom. was prepared by dissolving 100 mg of pure drug in least amount of methanol in a 500 ml measuring flask and complete to the mark with acetonitrile.

2×10^{-3} M of chloranil was prepered by dissolving the apropriate weigh in acetonitrile in 100 ml measuring flask and complete to the mark with acetonitrile.

General procedure

Aliqout (0.1-5.0 ml) of drug solution dissolved in acetonitrile was placed in a 25 ml measuring flask. 2.5 ml of 2×10^{-3} M chloranil solution was added and heated the flask content on a water bath at 60 °C for 10 min, cooled and diluted to volume with acetonitrile. The absorbance was measured at 550 nm against a reagent blank prepared in the same manner.

Determination of Amp., Amox. and neom. in dosage forms

An amount of powdered capsule, injection, tablets or oral suspension equivalent to 20mg of Amp., Amox. or Neom. was extracted with least amount of methanol. The combined extracts were collected in a 100 ml measuring flask and the volume was made up to the mark with acetonitrile. This solution was then analysed as above. The results were compared with the official method and are summarized in Table (1).

RESULTS AND DISCUSSION

Absorption spectra

The absorption spectrum of the reaction product from Amp., Amox., or Neom. with chloranil in non aqueous media shows characteristic maxima at 347 (UV) and 550 nm (visible region). All the measurements for analytical parposes were made at 550 nm.

The optimum conditions for the charge transfere complex formation were established by varying of one parameter at a time. For the complexation process, acetonitrile was found to be the optimum solvent, and the non-aqueous media was found optimal. This compares with the basic (pH 9.O) aqueous media used by AL-Ghabsha et al. (14,15) for the UV method. The reaction time was established by increasing it in increments of 2 min, and it was found that 10 min on using a water bath of 60±1°C is sufficient to yield maximum absorbance [Fig. (1)]. The final temperature after dilution was not critical. The use of 2.5 ml of $2x10^{-3}$ M of reagent solution was considered optimal [Fig. (2)].

Job's continuous variations graph for the complexation of Amp., Amox. or Neom. with chloranil shows that the interaction between these two compounds occurs on an equimolar basis. The reaction of Amp., Amox. or Neom. with chloranil occurs through the formation of a charge-transfer complex. The coloured reaction product can be represented, taking Amp. as an example, by the following structure.

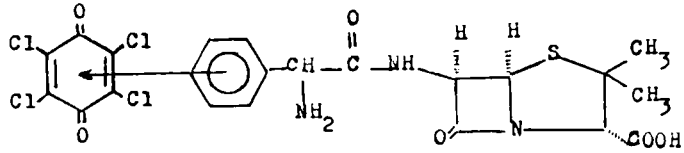

Table (1) : Quantitative parameters for the complexation of Amp., Amox. and Neom. using chloranil

Parameters	Drugs		
	Amp.	Amox.	Neom.
Beer's law limits μg ml^{-1}	0.8 - 40	0.8 - 36	0.8 - 28
Molar absorptivity l.mol, cm^{-1}	5.55×10^3	7.81×10^3	1.38×10^4
Sandell sensitivity ng cm^{-2}	7.72	5.37	4.45
Ringbom optimum conc. mg ml^{-1}	2.0 - 37	2.0 - 33	2.0 - 25
Range of error (%)	± 0.9	± 0.7	± 1.2
Standard deviation (%)	0.54	0.41	0.67
Regression equation*			
Slope (b)	0.014	0.019	0.023
Intercept (a)	0.018	- 0.025	- 0.033
Correlation coefficient (r)	0.9988	0.9996	0.9984
Calculated "t" value (2.310)**	0.936	0.783	1.265
Calculated "F" value (2.450)**	1.107	0.989	1.422

* $A = a + bc$ where c is the concentration in μg ml^{-1}

** Values in paranthesis are the theoretical t. and F. values for five degree of freedom and 95% confidence limit

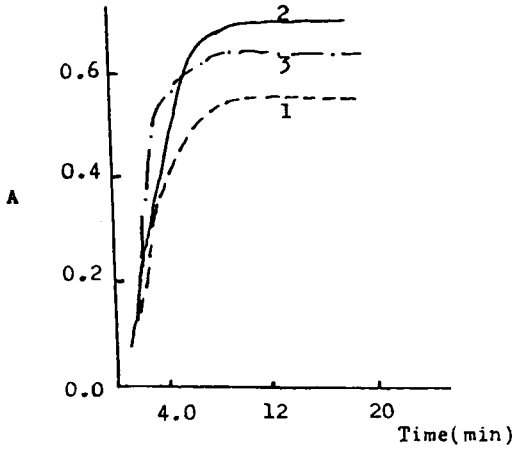

Fig. (1) Effect of time on the reaction.

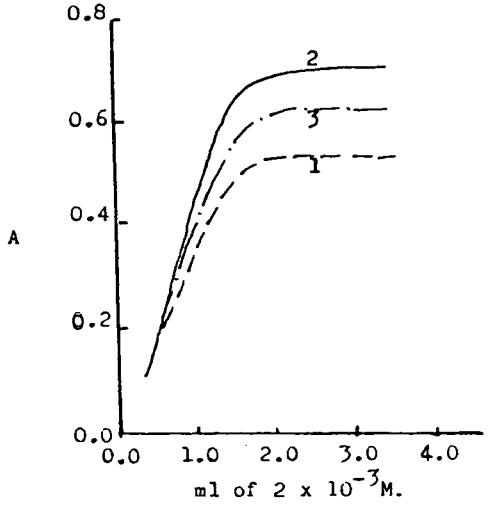

Fig. (2) Effect of reagent concentration.

Quantification

A linear correlation was found between absorbance and concentration in the ranges given in Table (1). The correlation coefficients, slopes and intercepts for the calibration data are calculated using the least-squares method.

The reproducibility of the procedure was determined by running seven replicate samples, each containing 15 μg of drug per ml in the final assay solution. At this concentration, the relative standard deviation was 0.75, 0.63 and 0.89% for Amp., Amox. and Neom. complexes, respectively.

The performance of the proposed method was assessed by calculation of the t-and F-values compared with the official method. Mean values were obtained in a student's t-and F-test and 95% confidence limits for five degrees of freedom (16), and the results showed that the calculated t-and F-values did not exceed the theoretical values.

Sensitivity, Accuracy and precision

The mean molar absorptivity (ε) and Sandell sensitivity (SS) as calculated from Beer's law are presented in Table (1). For more accurate analysis, Ringbom optimum concentration ranges were also obtained (Table 1).

In order to determine the accuracy and precision of the method, solutions containing five different concentrations of the drug were prepared and analysed in quintuplicate. The measured standard deviations (S), relative standard deviation (Sr), the standard analytical errors and confidence limits (Table 2) can be considered satisfactory, at least for the levels of concentrations examined.

Comparison of the results obtained by the proposed methods with those obtained by AL-Ghabsha et al.(14,15) using the same reagent in aqueous basic buffer medium showed a wider range of determination, higher accuracy, more sensitve and less time consumption with the non-aqueous method proposed here.

Comparison of the recovery obtained with the proposed method with the purity of the studied compounds as determined according to the British Pharmacopoeia (17) showed a high accuracy of the present method. The proposed method is simpler, more sensitive and less time consuming than the official method. Moreover the proposed method could be used for the routine analysis of pure drug form or in pharmaceutical formulations.

Table (2) : Evaluation of accuracy and precision of the proposed method

Drug	Taken µg ml^{-1}	Found[a] (µg ml^{-1}) Proposed method	Found[a] (µg ml^{-1}) Official method	S (µg ml^{-1})	Sr (%)	Standard error	Confidence limits
Ampicillin	8	7.98	7.90	0.03	0.49	0.012	7.98 ± 0.035
	16	16.10	16.20	0.04	0.56	0.016	16.10 ± 0.050
	24	24.20	23.60	0.06	0.69	0.024	24.20 ± 0.070
	32	31.80	32.50	0.08	1.03	0.033	31.80 ± 0.095
	40	39.70	40.70	0.10	1.13	0.041	39.70 ± 0.120
	Mean				0.78	0.025	
Amoxycillin	6	6.05	5.85	0.05	0.61	0.020	6.05 ± 0.060
	12	11.90	12.30	0.07	0.88	0.029	11.90 ± 0.080
	18	17.80	17.50	0.09	1.10	0.037	17.80 ± 0.110
	24	24.25	23.40	0.11	1.19	0.045	24.25 ± 0.130
	30	29.70	30.50	0.12	1.27	0.050	29.70 ± 0.145
	Mean				1.01	0.036	
Neomycin	5	4.97	5.10	0.06	0.77	0.024	4.97 ± 0.070
sulphate	10	10.08	9.80	0.04	0.57	0.016	10.08 ± 0.050
	15	15.10	14.75	0.08	0.98	0.033	15.10 ± 0.095
	20	19.80	20.50	0.09	1.06	0.037	19.80 ± 0.110
	25	24.50	25.60	0.12	1.23	0.050	24.50 ± 0.145
	Mean				0.92	0.032	

a : Average of six determinations.

Applications

The proposed method was applied to some pharmaceutical formulations containing Amp., Amox. and Neom. The results in Table (3) showed an excellent correlation with those of the British pharmacopoeia procedure (17). The proposed method is suitable for the determination of Amp., Amox., and Neom. in drug formulation without interferences for addatives and excipients such as glucose, lactose, fruactose sucrose, starch, magnesium stearate, acacia and dicalcium phosphate. Also the common degradation products of each drug do not interfere in the determination by the proposed method

Table (3) : Determination of ampicillin, amoxycillin and neomycin sulphate in different drugs using chloranil.

Sample	Company	label calaim	Found, mg*		Recovery %
			official	Proposed	± SD
Capsules					
Hiconcil (Amox)	Pharco-pharm-Alex	250 mg/cap	244	247.5	99.0 ± 0.41
Hiconcil	Pharco-pharm-Alex	500 mg/cap	509	503.0	100.6 ± 0.63
Ampicillin (Amp.)	Mataria-Cairo, Egypt	250 mg/cap	255	248.0	99.2 ± 0.37
	Mataria-Cairo, Egypt	500 mg/cap	492	495.5	99.1 ± 0.78
Injection					
Pentrexyl (Amp.)	Chem. Ind. Develop. - Giza	500 mg/amp	493	504.0	100.8 ± 0.81
Pentrexyl	Chem. Ind. Develop - Giza	1g/amp	1.02	1.010	101.0 ± 0.62
Ibiamox (Amox.)	Amoun pharm. Ind. Co. Egypt	250 mg/amp	245	252.0	100.8 ± 0.56
Ibiamox	Amoun pharm. Ind. Co. Egypt	500 mg/amp	510	503.0	100.6 ± 0.49
Tabletes					
Neomycin (Neom)	Memphis chem. Co. Egypt	500 mg/tab	485	495.0	99.0 ± 0.66
Syrup					
Ampicyn (Amp.)	Adv. Biochem. Ind. Co. Egypt	125 mg/5ml	122	126.0	100.8 ± 0.39
Ampicyn	Adv. Biochem. Ind. Co. Egypt	250 mg/5ml	243	247.0	98.8 ± 0.74
Amoxicid (Amox.)	Chem. Ind. Develop. - Giza	125 mg/5ml	127	123.0	98.4 ± 0.93
Amoxicid	Chem. Ind. Develop. - Giza	250 mg/5ml	256	254.0	101.6 ± 0.98

* Average of six determinations.

CONCLUSION

The proposed method is simpler, more sensitive and less time consuming than that of the official method (17) (based on the potentiometric titration of the drug dissolved in boric acid and acetic anhydride with 0.02 M mercury nitrate). Although the colour development of the charge transfer-complexes at room temperature requires from 75-90 min for completion, this can be shortened to 10 min by raising the temperature to 60 ± 1 °C. Moreover, the advantage

over an aqueous method (14,15) of a wider range of determination, higher sensitivity and less time consumption. The proposed method is suitable for the determination of Amp. Amox. and Neom. in pharmaceutical formulations without interferences of additives and excipients or from the dergradation products, suggesting applications in bulk drug analysis.

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