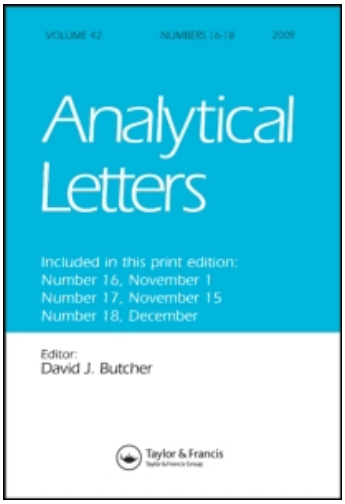

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Colorimetric Determination of Ampicillin and 6-Aminopenicillanic Acid Using Acenaphthenequinone as a Chromophoric Reagent

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COLORIMETRIC DETERMINATION OF AMPICILLIN AND 6-AMINOPENICILLANIC ACID USING ACENAPHTHENEQUINONE AS A CHROMOPHORIC REAGENT

Key words: Colorimetric determination, ampicillin, 6-aminopenicillanic acid, acenaphthenequinone, pharmaceutical formulations, serum and urine analysis

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ABSTRACT

A colorimetric method for the determination of ampicillin (Amp.) and 6-aminopenicillanic acid (6-APA) are described, based on the reaction of these drugs with acenaphthenequinone in basic media to give a highly intense red coloured product. The latter exhibits an absorption maximum at 610 nm with apparent molar absorptivities of 2.83 and 1.45 x 10^4 l. mol^{-1} cm^{-1} and Sandell sensitivities of 0.013 and 0.015 μg cm^{-2} for Amp. and 6-APA, respectively. The optimum concentration ranges are 0.4-10 and 0.4-14 μg ml^{-1} for Amp. and 6-APA, respectively. For more accurate results, Ringbom optimum concentration ranges are 1-8.5 and 1-12 μg ml^{-1} for Amp. and 6-APA, respectively. Statistical analysis indicated that there was no significant difference between the results obtained by the described method and those of the official methods. The mean recoveries percentage were found to be

99.5 ± 1.1% for pharmaceutical formulations and 99.1 ± 1.6% for serum and urine samples. The method is selective for the determination of Amp. or 6-APA in the presence of their degradation products, additives and excipiences that are normally encountered in dosage forms. The proposed method was applied successfully to the determination of Amp. in pharmaceutical formulations. Also, applicability of the proposed method to human serum and urine is presented and the validity assessed by applying the standard addition technique.

INTRODUCTION

Ampicillin [7177-48-2] is (6R)-6-(α-D-phenylglycylamino) penicillanic acid (Amp.), present as the trihydrated form. Several spectrophotometric methods for assay of ampicillin are cited in the literature[1-4]. The reported polarographic[5] and titrimetric[6,7] procedures for estimation of ampicillin are not satisfactory for microgram quantities. 6-Aminopenicillanic acid [551-16-6] (6-APA) is one of the penicillin group of antibiotic drugs. The importance of penicillins as broad spectrum antibiotics is well known. Since the penicillin nucleus itself has no absorption maximum in the ultraviolet and visible spectrum, all the spectrophotometric methods are mainly dependant on indirect measurements[8-11]. Potentiometric studies on complex formation of some transition metal ions with 6-APA have been reported[12]. The official method for the assay of ampicillin[13] is based on the potentiometric titration with mercuric nitrate using a platinum indicator electrode and mercury-mercury(I) sulphate reference electrode, while that for the assay of 6-aminopenicillanic acid is based on the iodometric assay[14]. The present work deals with the determination of Amp. and 6-APA using the condensation reaction of the amino group with acenaphthenequinone with the purpose of increasing sensitivity and selectivity. The proposed method has been successfully applied in various pharmaceutical preparations, serum and urine samples.

EXPERIMENTAL

Apparatus

All absorption spectral measurements were performed on a Perkin-Elmer λ3B spectrophotometer equipped with 10 mm quartz cells.

Reagents and samples

All reagents were of AnalaR grade chemicals. Acenaphthenequinone [82-86-0] was obtained from Aldrich; a 5 x 10^{-3} M solution in acetone was prepared and kept in a refrigerator and fresh solutions were prepared every week. A standard solution of ampicillin or 6-aminopenicillanic acid (100 mg l^{-1}) was prepared by dissolving 0.025 g of pure sample in 5 ml of 0.1 M sodium hydroxide and diluted in a 250 ml calibrated flask with bidistilled water. A 1.00 M sodium hydroxide solution was prepared by dissolving the appropriate weight in a 500 ml calibrated flask.

Pharmaceutical samples of ampicillin were obtained from the Egyptian International Pharmaceutical Industrial Company (EIPICO), Egypt; the Chemical Industrial Development Company (CID), Egypt; the Misr Company for Pharmaceutical Industry, Cairo, Egypt; the Advanced Biochemical Industrial Company, El-Salam, Cairo, Egypt and the Nile Company for Pharmaceutical and Chemical Industries, Cairo, Egypt.

Synthetic mixtures of 6-APA were prepared from pure drugs representing the actual situation in several formulations (due to the absence of such drug formulations in the local market in Egypt).

Biological samples, serum and urine specimens containing ampicillin were collected from the hospital of Benha University, Benha, Egypt. No sample pretreatment, extraction, or derivatization procedure was applied other than a standard deproteinizing technique using trichloroacetic acid. The samples were first

analysed by the proposed method, then the method of standard addition was applied to ensure that there was no interference from endogenous compounds.

General procedure

A portion of Amp. or 6-APA solution (0.1-3.5 ml) was pipetted into a 25-ml calibrated flask. After addition of 5 ml of 5 x 10^{-3} M reagent solution, 5 ml pure acetone was added to achieve 40 % (v/v) acetone-water media. 10 ml of 1.0 M NaOH was added and completed to the mark with bidistilled water. The flasks were placed in a water bath at 60 ± 1 °C for 15 or 10 min. on using Amp. or 6-APA, respectively, and allowed to stand at room temperature for 5 min. The absorbance was measured against a blank solution containing the same ingredients except the drug solution at 610 nm for Amp. or 6-APA. The drug concentration was obtained from a calibration graph prepared under identical conditions.

Determination of Amp. in pharmaceutical preparations

An amount of powdered tablet, capsule, injection or oral suspension equivalent to 100 mg of Amp. was extracted with water (four 15-ml portions). The combined extracts were collected in a 100-ml calibrated flask and the volume was made up to the mark with water. This solution was then analysed as described under general procedure. The results were compared with the official method and summarized in Table 1.

Determination of Amp. in serum and urine

To a sample of serum or urine (1.00 ml) appropriate amounts of Amp. or 6-APA were added. Deproteinization was achieved by adding 20 ml of trichloroacetic acid solution (240 g l^{-1}). After centrifugation, 1.0 ml of the supernatant solution was treated with 10 ml of 1.0 M NaOH and followed as mentioned under general procedure. The concentration of Amp. or 6-APA in serum or urine was calculated from a calibration graph prepared as mentioned above. Spiked serum and urine

Table 1. Analytical data for Amp. and 6-APA using the proposed and official procedures.

	proposed method		official method	
Parameters	Amp.	6-APA	Amp.[13]	6-APA[14]
Validity of Beer's law, μg ml^{-1}	0.4-10.0	0.4-14.0		
Ringbom optimum range, μg ml^{-1}	1.0-8.5	1.0-12.0		
Molar absorptivity, l.mol^{-1}cm^{-1}	2.83 x 10^4	1.45 x 10^4		
Sandell sensitivity, μg cm^{-2}	0.013	0.015		
Detection limit, μg ml^{-1}	0.100	0.140		
Regression equation[1]				
Intercept (a)	-0.036	+0.017	+0.095	-0.068
Slope (b)	0.076	0.067	0.015	0.009
Correlation coefficient	0.9985	0.9997	0.9975	0.9968
Standard deviation, %	0.59	0.43	1.25	1.08
Relative standard deviation[2], %	1.55	1.35	3.61	3.17
Range of error, %	±1.5	±1.8	±2.4	±2.7
Calculated t-test (2.57)[3]	1.27	1.56	1.27	1.56
Calculated F-test (5.05)[4]	3.12	3.51	3.12	3.51

1 A = a + bc where c is the concentration in μg ml^{-1}.
2 Relative standard deviation (six replicate determinations).
3 Theoretical t-value at the 95% confidence level.
4 Theoretical F-value at the 95% confidence level.

samples were analysed for their Amp. and 6-APA content applying the proposed and the official methods.

RESULTS AND DISCUSSION

Acenaphthenequinone reacts with Amp. or 6-APA in alkaline medium to produce the corresponding Schiff base. The absorption spectra of the reaction product shows a characteristic maximum at 610 nm. A detailed study of the optimum experimental conditions for complete colour development was performed.

Effect of alkalinity

The reaction of acenaphthenequinone with Amp. and 6-APA in alkaline medium is suggested to give highly sensitive colour development. The results

showed that NaOH is the best medium for complete colour development. The optimum concentration of alkali was in the range 0.5-1.0 M NaOH. Hence 1.0 M NaOH was selected for further study of Amp. and 6-APA, since the results are highly consistent at this concentration. The amount of 1.0 M NaOH added to 25 ml of solution was also investigated and 10 ml gives the highest colour intensity [Fig. 1].

Effect of solvent

Several organic solvents were examined to select the optimum one for complete colour development (e.g methanol, ethanol, propan-1-ol, acetone and dioxane). Acetone was found to be the best solvent, resulting in the highest absorbance and stability of colour. The percentage of acetone was also investigated and it was found that 40% (v/v) acetone-water gave the highest absorbance values [Fig. 2].

Effect of time and temperature

The maximum colour intensity was attained after allowing the original reaction mixture to stand at room temperature (24 ± 2°C) for 90 and 60 min for Amp. and 6-APA, respectively. Bathing in a thermostatic water bath at 60 ± 1°C reduced the reaction time to 15 and 10 min for Amp. and 6-APA, respectively. Further increase in the heating time does not cause any change in colour intensity and the absorbance starts to decay. For this reason; raising the temperature of the reaction to 60 ± 1°C for 15 and 10 min for Amp. and 6-APA, respectively was selected for further studies [Fig. 3]. The absorbance of Amp. complex remained stable for 24 hrs, then decreases gradually, while for
6-APA complex, the absorbance remains stable for 3 days.

Effect of reagent concentration

The obtained results showed that at least 4 ml of the prepared reagent solution should be present to achieve maximum colour intensity [Fig. 4]. However,

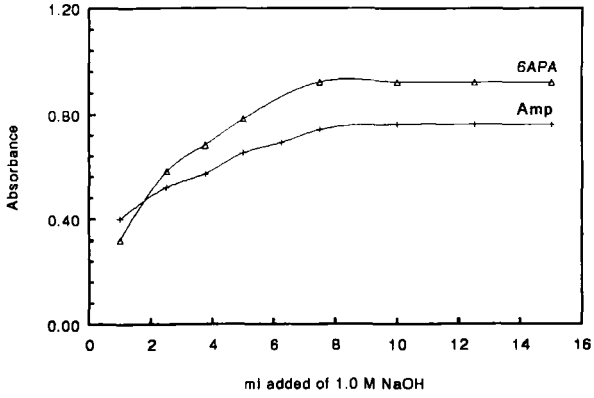

Fig. (1). Effect of 1.0 M NaOH on the absorbance of the formed complexes

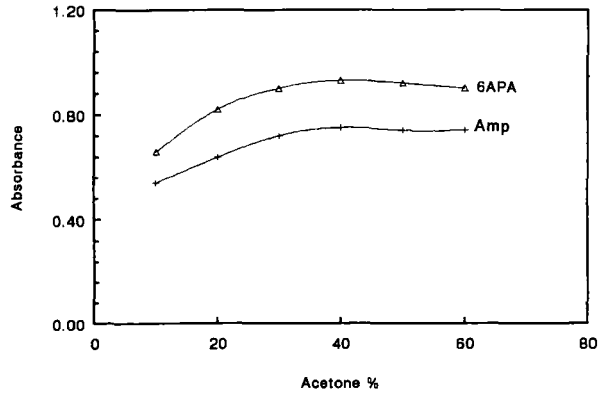

Fig. (2). Effect of acetone% on the absorbance of the formed complex.

5 ml of 5 x 10^{-3} M acenaphthenequinone was used in the present study to insure complete colour formation and quantitative reaction at the upper limit of the calibration curves.

Quantification

A linear correlation was found between absorbance and concentration in the range 0.4-10.0 and 0.4-14.0 μg ml^{-1} for Amp. and 6-APA, respectively. For more

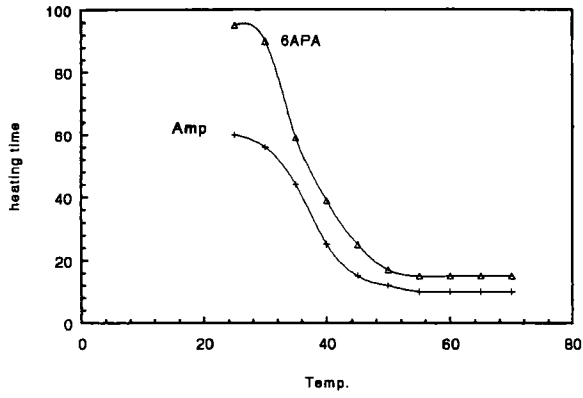

Fig. (3). Relation between time and temperature for attaining maximum absorbance value.

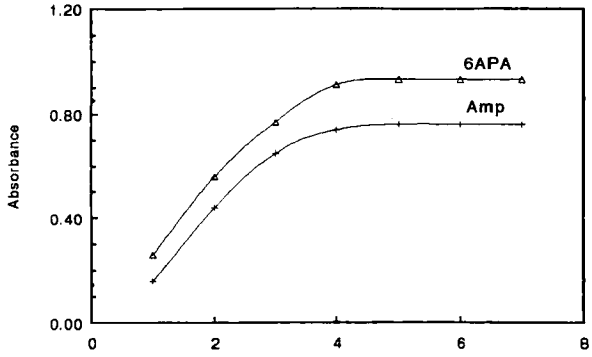

Fig. (4). Effect of 5×10^{-3} M reagent added on the complex formation

accurate analysis, the Ringbom optimum concentration range was 1.0-8.5 and 1.0-12.0 g ml^{-1} for Amp. and 6-APA, respectively. The apparent molar absorptivities of 2.83×10^4 and 1.45×10^4 l. mol^{-1} cm^{-1}, and Sandell sensitivities of 0.013 and 0.015 µg cm^{-2} for Amp. and 6-APA, respectively are obtained.

Regression analysis indicated a linear relationship between absorbance and concentration (Table 1). The correlation coefficients were found to range between

Table 2. Recovery of ampicillin in pharmaceutical formulations by official and proposed methods.

		Proposed method		Official method	
Sample	Label claim	Found*	Recovery %, ± SD	Found*	Recovery %, ± SD
Capsules	250 mg/cap[1]	251	100.4 ± 0.33	253	101.2 ± 0.93
	250 mg/cap[2]	246	98.4 ± 0.57	245	98.0 ± 1.13
	500 mg/cap[1]	495	99.0 ± 0.46	491	98.2 ± 1.06
	500 mg/cap[2]	503	100.6 ± 0.63	508	101.6 ± 0.98
Syrup	125 mg/5ml[3]	125.5	100.4 ± 0.37	126	100.8 ± 0.78
	125 mg/5ml[4]	124	99.2 ± 0.44	123	98.4 ± 0.71
	250 mg/5ml[3]	247	98.4 ± 0.59	254	101.6 ± 0.83
	250 mg/5ml[4]	246	98.4 ± 0.66	244	97.6 ± 1.84
Vials	250 mg/vial[5]	248	99.2 ± 0.49	246	98.4 ± 1.41
	250 mg/vial[6]	251	100.4 ± 0.40	255	102.4 ± 1.57
	500 mg/vial[5]	494	98.8 ± 0.48	490	98.0 ± 1.67
	500 mg/vial[6]	502	100.4± 0.36	507	101.4 ± 1.22
	1 g/vial[5]	1.005	100.5 ± 0.45	1.013	101.3 ± 0.87
	1 g/vial[6]	0.985	98.5 ± 0.62	0.980	98.0 ± 1.54

* Average of five determinations.
1 Epicocillin [EIPICO].
2 Ampicillin (Nile Co. for Pharm & Chem. Ind. Cairo, Egypt).
3 Ampicyn (Adv. Biochem. Ind., El-Salam city, Cairo, Egypt).
4 Pentrexyl (Chem. Ind. Development Co., Egypt).
5 Ampicillin (Misr Co. for Pharm. Ind. Egypt).
6 Amfipen (Misr Co. for Pharm. Ind. Egypt).

0.9985 and 0.9997. Six replicates determinations at different concen-tration levels were carried out to test the precision of the method. The relative standard deviations were found to be less than 1.6%, indicating excellent reproducibility of the method. The limits of detection, where the absorbance of the sample reading was triple the blank reading, were found to be 0.1 and 0.14 μg ml^{-1} for Amp. and 6-APA, respectively. The performance of the proposed method was assessed by comparing with the official methods[13,14]. Mean values obtained in a student's t- and F- tests[15] showed the absence of any systematic error in the method (Table 1).

Table 3. Precision and relative recovery in the determination of Amp. and 6-APA in spiked human urine and blood samples applying the standard additions method.

Amp.			6-APA		
Added	Recovery* %, ± SD		Added	Recovery* %, ± SD	
μg ml^{-1}	Proposed method	Official method[13]	μg ml^{-1}	Proposed method	Official method[14]
1.2[a]	97.50 ± 1.37	97.50 ± 1.53	1.5	98.00 ± 1.43	97.33 ± 1.87
2.4[b]	100.42 ± 0.51	101.66 ± 1.14	3.0	100.67 ± 0.71	98.33 ± 1.41
3.6[a]	98.61 ± 0.77	98.06 ± 1.27	4.5	97.78 ± 1.58	101.11 ± 1.14
4.8[b]	98.96 ± 0.63	101.67 ± 1.72	6.0	100.50 ± 0.51	101.67 ± 0.82
6.0[a]	100.50 ± 0.48	98.33 ± 1.17	7.5	100.67 ± 0.74	98.67 ± 1.33
7.2[b]	98.61 ± 0.81	101.39 ± 0.98	9.0	100.56 ± 0.66	101.11 ± 1.17
8.4[a]	99.41 ± 0.68	101.19 ± 0.89	10.5	99.05 ± 1.11	101.90 ± 1.61
9.6[b]	98.96 ± 0.61	102.08 ± 1.84	12.0	99.17 ± 1.23	98.33 ± 1.37

* Average of six determinations

a: Urine samples

b: Blood samples

Interferences

The possibility of interference from the co-existing additives and bases was studied. No interference was observed from these excepiences. or from the serum components which remain after deproteinization. Also, no interference from, penicilloic-, penilloic-, penillic-, and penaldic acids, penicillamine and ultimately penilloaldehyde, obtained from which are the thermal and hydrolytic degradation products of Amp. and 6-APA.

Applications

The proposed procedure was applied to the determination of Amp. in dosage forms (commercial products randomly collected from local pharmacies. and Amp. or 6-APA in spiked cerum and urine samples. Tables 2 and 3 list the results obtained by the proposed and official methods.[13] The results indicate good agreement between the two methods.

Conclusion

The proposed colorimetric method is simple, accurate, precise, highly sensitive and suitable for the determination of the investigated drugs in pure form, pharmaceutical formulations and in different biological fluids. In particular, the method is much simpler in technique than that for Amp. in pharmacopoeial method, based on the potentiometric titration with mercuric nitrate, or the official method for 6-APA (based on the iodometric assay). Furthermore, the method can be used for the determination of Amp. in the presence of its degradation products and excipiences, present in the pharmaceutical formulations, an advantage over the official method. The proposed method can be recommended for the routine analysis in drug control laboratories .

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