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ORIGINAL ARTICLE

Spectrophotometric methods for the determination of ampicillin by potassium permanganate and 1-chloro-2,4-dinitrobenzene in pharmaceutical preparations

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Abstract Two simple and sensitive kinetic methods for the determination of ampicillin (AMP) are described. The first method is based on kinetic investigation of the oxidation reaction of the drug with alkaline potassium permanganate at room temperature for a fixed time of 25 min. The absorbance of the colored manganate ions is measured at 610 nm. The second method is based on the reaction of AMP with 1-chloro-2,4-dinitrobenzene (CDNB) in the presence of 0.1 mol L^{-1} sodium bicarbonate. Spectrophotometric measurement was achieved by recording the absorbance at 490 nm for a fixed time of 60 min. All variables affecting the development of the color were investigated and the conditions were optimized. Plots of absorbance against concentration in both procedures were rectilinear over the ranges 5–30 and 50–260 μg mL^{-1}, with mean recoveries 99.80 and 99.91, respectively. The proposed methods were successfully applied for the determination of AMP in bulk powder and in capsule dosage form. The determination of AMP by the fixed concentration

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method is feasible with the calibration equations obtained, but the fixed time method proves to be more applicable.

1. Introduction

Ampicillin AMP, (6*R*)-6-(a-phenyl-D-glycylamino) penicillanic acid (Fig 1) is a semisynthetic penicillin (Goodman, 1991). It is prepared from the benzylpenicillin or penicillin-G (Wilson, 1982) which is prepared by a biosynthetic process using various strains of *Penicillium notatum* and *Penicillium chrysogenum* (Bentley, 1969). Penicillin-G was the first antibiotic to be used in the chemotherapy. It is a bacterio static drug of choice for the treatment of the infections caused by most of the Gram-positive and Gram-negative bacteria (Delgado and Remers, 1995) and a broad spectrum antibiotic (Rang et al., 1996). AMP is acidic in nature, and it acts by inhibiting the protein synthesis (Satoskar and Bhandarkar, 1990) of the bacterial cell wall. The basic nature of the ampicillin is 6-aminopenicillanic acid, which consists of a thiazolidine ring linked to β-lactam ring. The side chain determines the antibacterial and pharmacological characteristics of this compound (Rang et al., 1998). The several procedures reported for the determination of ampicillin in pure form or in pharmaceutical formulations as well as in biological fluids included by spectrophotometric (Askal et al., 1991; Al-Khamees et al., 1995; Sun et al., 1996; Sastry et al., 1998), polarographic (Belal et al., 1998; El-Sayed et al., 1994), flow injection analysis (Garcia et al., 1994), HPLC methods (Verdon and Couedor, 1999; Ishida et al.,1999) and the complexes of ampicillin with different metal ions have also been studied (Mukherjee and Ghosh, 1995; Alekseev and Samuilova, 2008; Lyle and Yassin, 1993). Kinetic-based methods of pharmaceutical analysis are not widely applied although they do not suffer any interference from additives which probably affects other methods. Furthermore, some specific advantages in the application of the kinetic methods can be expected (Mansilla et al., 1998):

1. Selectivity due to the measurement of the evolution of the absorbance with the time of reaction instead of measuring a concrete absorbance.
2. Possibility of no interference from the colored/turbid background of the samples.

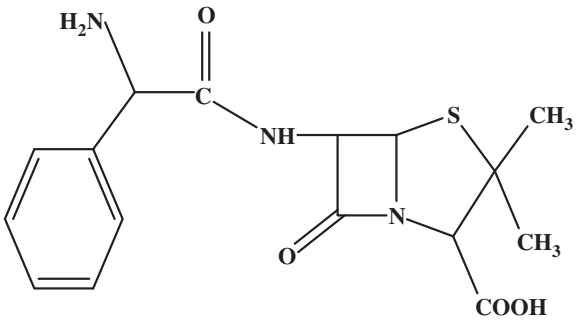


Figure 1 Chemical structure of AMP.

In the present work, kinetically based methods are proposed for the determination of AMP by measuring the absorbance at 610 nm after oxidation with alkaline $KMnO_4$ or at 490 nm after addition of CDNB in the prescience of borate buffer. All variables affecting the development of the color were investigated and the conditions were optimized. The proposed methods were successfully applied for the determination of AMP in bulk powder and in capsule dosage form. The determination of AMP by the fixed concentration method is feasible with the calibration equations obtained, but the fixed time method proves to be more applicable.

2. Experimental

2.1. Apparatus

A Shimadzu UV–visible 1601 spectrophotometer was used for all spectral measurements, pH-metric measurements were done with Elico-Li 120 pH meter and a water bath shaker NSW 133, India was used to control the temperature.

2.2. Materials

AMP was purchased from Sigma (New Delhi, India). The $KMnO_4$, NaOH and 1-chloro-2,4-dinitrobenzene (CDNB) were purchased from Merck (Mumbai, India). Pharmaceutical preparations containing the studied compounds were purchased from commercial sources in the local market. Double distilled, de-ionized water was used throughout. The chemicals used were of analytical grade. Stock solutions of the compounds were wrapped with carbon paper to protect them from photodecomposition.

2.3. Method A

Transfer aliquots equivalent to 5–30 μg mL^{-1} AMP (solution A) into a series of 50 mL volumetric flasks. Add to each flask 1 mL of 0.5 mol L^{-1} NaOH and 2 mL of 5×10–3 mol L^{-1} $KMnO_4$, mix well, dilute to volume with water, and leave to stand for 25 min. Measure the absorbance of the resulting solution at 610 nm at 5-min intervals at ambient temperature (25 °C) against a blank solution prepared simultaneously. To obtain the standard calibration curve, plot the values of absorbance against the drug concentration in μg mL^{-1} after 25 min.

2.4. Method B

Transfer aliquots equivalent to 50–260 μg mL^{-1} AMP (solution B) into a series of 10-mL volumetric flasks. Add to each flask 2 mL of 3.5×10^{-3} mol L^{-1} CDNB with 0.2 mol L^{-1} borate buffer of shake well, dilute to volume with distilled water, and leave to stand for 60 min. Measure the absorbance of the reaction mixture at 490 nm at 10-min intervals at

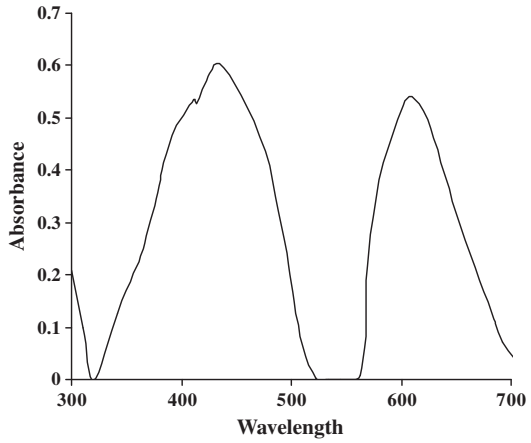


Figure 2 Absorption spectrum of AMP ($25\ \mu g\ mL^{-1}$) after reaction with $KMnO_4$: (a) oxidation product, (b) manganate ions (Method A).

ambient temperature (25 °C) against a blank solution prepared simultaneously. To obtain the standard calibration curve, plot the values of absorbance against the concentration of AMP after 60 min.

2.5. Procedures for formulations

The entire content of 20 capsules containing AMP were weighed and mixed well. Amount of the powder equivalent to 500 mg of AMP was weighed into a 100 mL volumetric flask containing about 75 mL of distilled water. It was shaken thoroughly for about 15–20 min, filtered through a Whatman filter paper No. 40 to remove the insoluble matter and diluted to the mark with distilled water. A volume of 25 mL of the filtrate was diluted to 100 mL and a suitable aliquot was analyzed using the general procedures followed in the concentration ranges mentioned above.

3. Results and discussion

3.1. Optimization of the reactions conditions

3.1.2. Method A (oxidation with $KMnO_4$)

The reaction between AMP and $KMnO_4$ in alkaline solution yields a green color as a result of the manganate species, which absorbs at 610 nm (Fig. 2). The intensity of the color produced increases gradually reaching its maximum after 25 min, when it remains stable for at least 1 h. As the intensity of color increases with time, it was deemed useful to elaborate a kinetically based method for the determination of AMP in bulk and in pharmaceutical forms. The reaction was investigated under various conditions of reagent concentration and alkalinity. Water was used to dissolve the drug since $KMnO_4$ oxidizes with the production of green manganate ions. At room temperature the reaction increased substantially with time, as revealed by the intensification of the developed color and subsequent increase in the slope of the calibration graph (Table 1) indicating high analytical sensitivity.

3.2. The influence of $KMnO_4$

The reaction rate and absorbance increases with increasing $KMnO_4$ concentration. The absorbance was studied in the range 1×10^{-4} to 1×10^{-3} mol L^{-1} keeping all other parameter constant. It was found that 7.5×10^{-4} mol L^{-1} $KMnO_4$ is the optimum concentration for the absorbance of AMP as shown in (Fig 3). The effect of the color development was investigated by adding different volumes (0.1–2.0 mL) of 7.5×10^{-4} mol L^{-1} potassium permanganate to a drug. The maximum absorbance of the green color was attained with 1.8 mL of the reagent, and remained constant even when higher volumes were added (Fig 4). Therefore, 2 mL of the reagent was used throughout the experimental investigations.

3.3. The influence of the NaOH

The reaction rate and absorbance increases with increasing KMnO4 concentration on the formation of MnO_4^{2-} was also examined at constant concentration of drug, permanganate ion and varying volume (0.2–2.0 mL) of 0.5 mol L^{-1} NaOH at 25 °C. The optimum absorbance was obtaine with 0.9 mL of 0.5 mol L^{-1} NaOH, after which increase in volume of NaOH caused no changed in absorbance. Hence 1 mL of 0.5 mol L^{-1} NaOH was used throughout the experimental investigations (Fig. 5).

3.4. Method B (complexation with CDNB)

The reaction between the investigated drugs and CDNB in slightly alkaline borate buffer produces an orange-yellow color with maximum absorbance at 477 nm (Fig. 6). This bathochromic shift from 370 nm CDNB to 490 nm may be attributed to the formation of a charge transfer complex through n–π^* interaction, where the electron donor is the amine moiety of the drug and the π-acceptor is the CDNB moiety (Amin et al., 2002).The possibility of the reaction of AMP with CDNB was investigated under various conditions. It was found that the reaction proceeds in alkaline medium and at room temperature. The absorbance of the colored adducts remains stable for at least one and half hour. The extent of formation of this species depends on the concentration of reactants, alkalinity, temperature, pH and therefore the effects of these variables were carefully studied.

3.5. The influence of the CDNB

The effect of CDNB concentration on the absorbance of yellow colored Meisenheimer complex was studied in the range of 1×10^{-4} to 4×10^{-3} mol L^{-1}. It was found that 3.5×10^{-3} mol L^{-1} CDNB is the optimum concentration for the absorbance of AMP as shown in Fig. 7. Therefore, the optimum concentration of 3.5×10^{-3} mol L^{-1} CDNB was chosen for further work.

3.6. The influence of pH

The influence of pH on the absorbance value of the reaction product was evaluated. Maximum absorbance value was obtained at pH 7.8 after which the absorbance of the reaction

Table 1 Calibration equations at different fixed times for AMP in the ranges 5–30 and 50–260 μg mL^{-1} applying methods A and B, respectively.

Time (min)	Regression equation	Correlation coefficient (*r*)
Method A (oxidation with $KMnO_4$)		
5	$A = -0.05554 + .04126\ C$	0.9912
10	$A = -0.04602 + .04406\ C$	0.9945
15	$A = -0.02212 + 0.05130\ C$	0.9975
20	$A = -0.00122 + 0.0286\ C$	0.9932
25	$A = -0.00015 + 0.0311\ C$	0.9982
Method B (reaction with CDNB)		
10	$A = -0.04041 + 0.03805\ C$	0.9968
20	$A = -0.02110 + 0.04102\ C$	0.9959
30	$A = -0.0076 + 0.0032\ C$	0.9982
40	$A = -0.00078 + 0.0032\ C$	0.9991
50	$A = -03.05 \times 10^{-3} + 0.03826\ C$	0.9989
60	$A = 2.12 \times 10^{-3} + 0.03932\ C$	0.9994

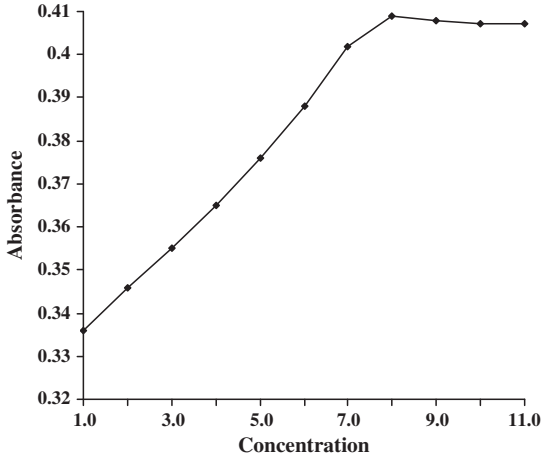


Figure 3 Effect of the concentration ranges 1×10^{-4} to 1×10^{-3} mol L^{-1} of $KMnO_4$ on the intensity of the color produced during the reaction (AMP 25 μg mL^{-1}; 1 mL of 0.5 mol L^{-1} NaOH).

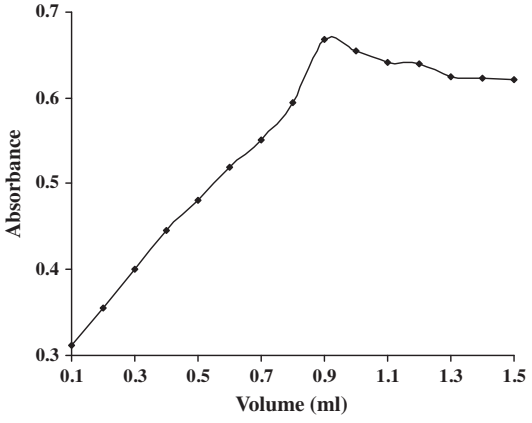


Figure 5 Effect of the volume of 0.5 mol L^{-1} NaOH on the intensity of the color produced during the reaction (AMP 25 μg mL^{-1}; 2 mL of 7.5 mol L^{-1} $KMnO_4$).

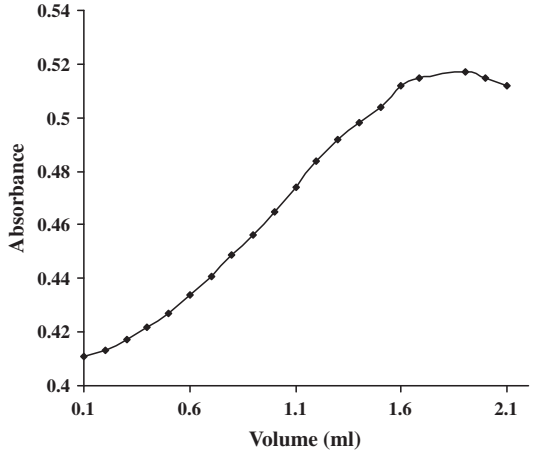


Figure 4 Effect of the volume of 2 mol L^{-1} $KMnO_4$ on the intensity of the color produced during the reaction (AMP 25 μg mL^{-1}; 1 mL of 0.5 mol L^{-1} NaOH).

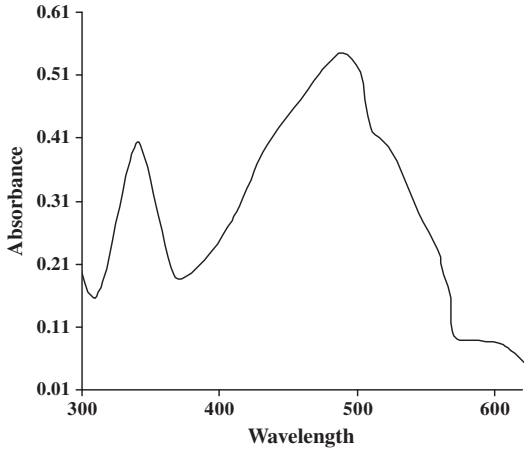


Figure 6 Absorption spectrum of AMP (250 μg mL^{-1}) after reaction with CDNB at pH 7.8 (Method B).

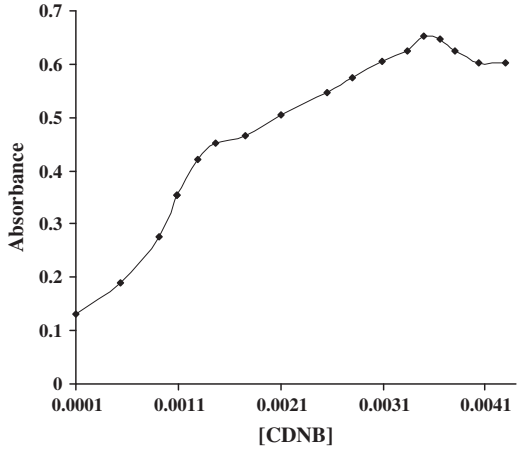


Figure 7 Effect of the concentration ranges 1×10^{-4} to 4×10^{-3} mol $L^{-1}$of CDNB on the absorbance value of the reaction product of AMP (250 μg mL^{-1}) with 0.2 mol L^{-1} borate buffer.

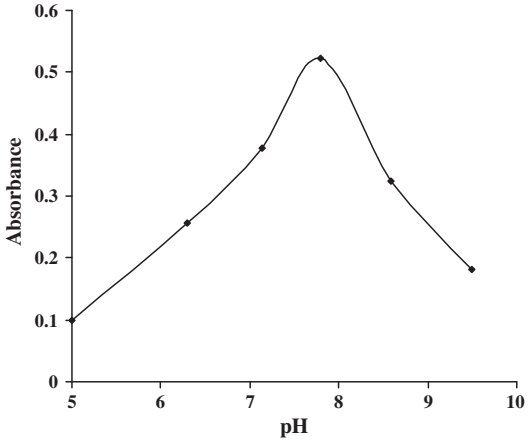


Figure 8 Effect of the concentration of CDNB (3.5×10^{-3} mol L^{-1}) on the absorbance of the colored product keeping AMP (250 μg mL^{-1}).

product began to decrease gradually until pH 9.5. Therefore, pH of 7.8 was chosen as the optimum pH (Fig. 8). Other buffers having the same pH value such as phosphate buffer and hexamine buffer were tried and compared with 0.2 mol L^{-1} borate buffer. The borate buffer was found to be superior to the phosphate and hexamine buffers having the same pH value since it gave the highest absorbance value.

3.7. Calibration graphs

After optimizing the reaction conditions, the fixed time was applied to the determination of AMP in pure form over the concentration ranges 5–30 and 50–260 μg/mL for both methods, respectively.

Analysis of the data gave the following regression equations:

$A = 0.1606 + 0.01054\ C$ ($r = 0.9906$) Method A
$A = 0.3068 + 0.001126\ C$ ($r = 0.9919$) Method B

The calibration graphs were shown in (Figs. 9 and 10).

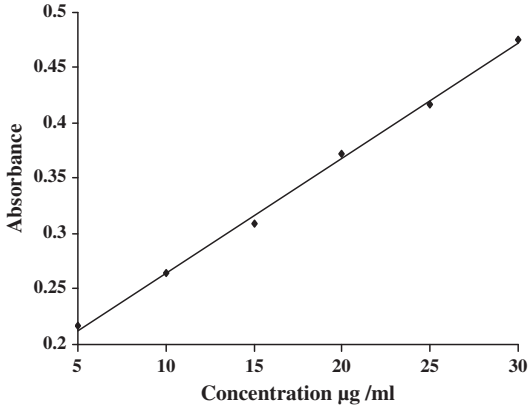


Figure 9 Spectrophotometric calibration curves for Method A.

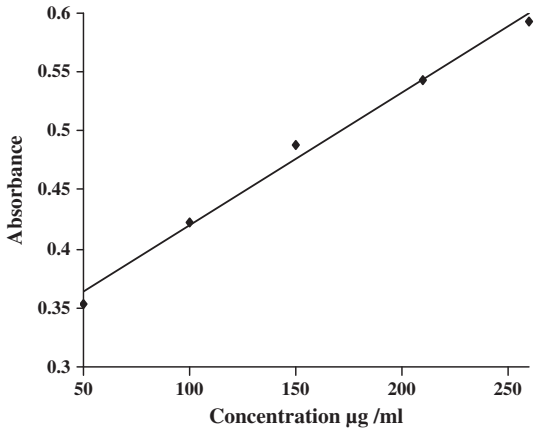


Figure 10 Spectrophotometric calibration curves for Method B.

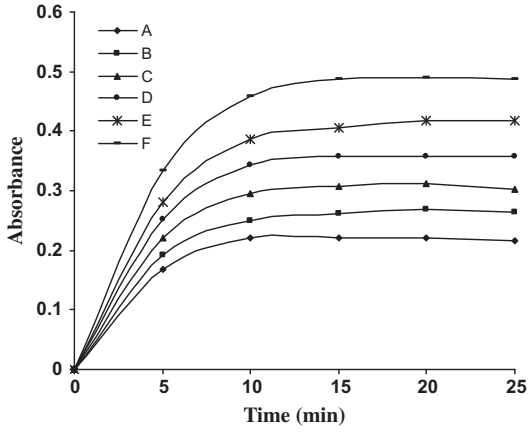


Figure 11 Plots of absorbance vs. time for the oxidation of AMP with alkaline $KMnO_4$ (Method A) concentration of AMP: (1) 1.4×10^{-5}, (2) 2.8×10^{-5}, (3) 4.2×10^{-5}, (4) 5.6×10^{-5}, (5) 7.0×10^{-5}, (6) 8.4×10^{-5} mol L^{-1}.

3.8. Kinetic study of the reactions

The rate of the reactions was also found to be dependent on the concentration of AMP. The rates were followed at room temperature with various concentrations of AMP:

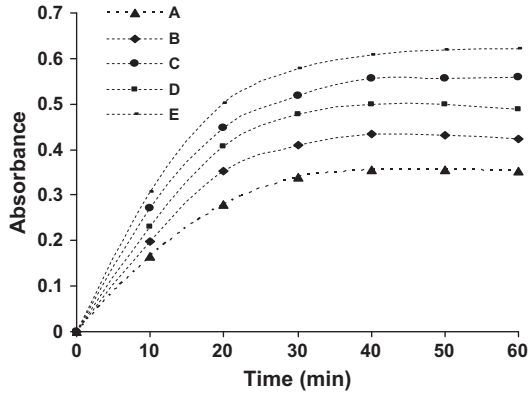


Figure 12 Plots of absorbance vs. time for the reaction of AMP with CDNB (Method B) concentration of AMP: (1) 1.5×10^{-4}, (2) 3×10^{-4}, (3) 4.5×10^{-4}, (4) 6×10^{-4}, (5) 7.5×10^{-4} mol L^{-1}.

1. In the range 5–30 μg mL^{-1}, keeping $KMnO_4$ and NaOH constant at high concentration as described in the general procedures, applying method A; and
2. In the range 50–260 μg mL^{-1}, keeping the other reactants, and CDNB constant at high concentration as described in the general procedures, applying method B.

From the graphs shown in Figs. 11 and 12, obtained by applying methods A and B, respectively, it is clear that the rate increases as the AMP concentration increases, indicating that the reactions rates obey the equation:

$$\text{Rate} = K'[\text{AMP}]^n \quad (1)$$

where K' is the pseudo first-order rate constant of the reaction and n is the order of the reaction. The rate of the reaction may be estimated by the variable-time method measured as $\Delta A/\Delta t$, where A is the absorbance and t is the time in seconds (Weisberger et al., 1953).

Taking logarithms of rates and concentration, as shown in Table 2, Eq. (1) is transformed into:

$$\log(\text{rate}) = \log \Delta A/\Delta t = \log k' + n\log[\text{AMP}] \quad (2)$$

Regression of log (rate) versus log (AMP) gave the regression equations:

log (rate) = −0.9172 + 0.9923 log C
r = 0.9936 Method A
log (rate) = −0.6790 + 0.8808 log C
r = 0.9914 Method B

Hence K' = 0.121 S^{-1} or 0.209 S^{-1}, applying methods A or B, respectively, and the reactions can be approximated to first order ($n \approx 1$) with respect to AMP concentration.

3.9. Evaluation of the kinetic methods

The quantitation of drug under the optimized experimental conditions outlined above would result in a pseudo-first order with respect to their concentrations where $KMnO_4$ concentration was at least 50 times of the initial concentration of AMP and NaOH concentration was at least 100 times the initial concentration of AMP applying method A; and CDNB concentration was at least four times of the concentration of AMP applying method B.

Table 2 Logarithms of the rates for different concentrations of AMP applying methods A and B.

Log $\Delta A/\Delta t$	Log [AMP], (mol L^{-1})
Method A (oxidation with $KMnO_4$)	
−3.942	−4.854
−3.690	−4.553
−3.491	−4.376
−3.360	−4.244
−3.281	−4.155
−3.150	−4.075
Method B (reaction with CDNB)	
−4.066	−3.824
−3.750	−3.523
−3.552	−3.251
−3.500	−3.222
−3.452	−3.125

Table 3 Values of K' calculated from slopes of log A versus t graphs multiplied by −2.303, for different concentrations of AMP, by applying methods A and B.

K' (s^{-1})	Log [AMP], (mol L^{-1})
Method A (oxidation with $KMnO_4$)	
-9.4773×10^{-4}	1.4×10^{-5}
-7.9414×10^{-4}	2.8×10^{-5}
-5.6421×10^{-4}	4.2×10^{-5}
-4.9483×10^{-4}	5.6×10^{-5}
-5.3669×10^{-4}	7.0×10^{-5}
-4.8899×10^{-4}	8.4×10^{-5}
Method B (reaction with CDNB)	
-4.9344×10^{-4}	1.5×10^{-4}
-4.6647×10^{-4}	3.0×10^{-4}
-3.7702×10^{-4}	4.5×10^{-4}
-1.5545×10^{-4}	6.0×10^{-4}
-1.0255×10^{-4}	7.5×10^{-4}

However, the rate will be directly proportional to drug concentration in a pseudo-first rate equation as follows:

$$\text{Rate} = K'[\text{drug}] \quad (3)$$

where K' is the pseudo-order rate constant. Several experiments were then carried out to obtain drug concentration from the rate data according to Eq. (3). The rate constant, fixed-concentration and fixed time methods (Yatsimirskii, 1966; Laitinen and Harris, 1975) were tried and the most suitable analytical method was selected taking into account the applicability, the sensitivity, the correlation coefficient (r) and the intercept.

4. Rate-constant method

Graphs of log (absorbance) versus time over the concentration ranges 1.4×10^{-5} to 8.4×10^{-5} mol L^{-1}, and 1.5×10^{-4} to 7.5×10^{-4} mol L^{-1} AMP, were plotted by applying methods A or B, respectively. The pseudo-first order rate constants corresponding to different AMP concentrations were then calculated from the slopes multiplied by −2.303; they are presented in Table 3.

Regression of (C) versus K' gave the equations:

$K' = -4.5 \times 10^{-3} + 76.96\ C\ (r = 0.9831)$ Method A
$K' = -4.1 \times 10^{-3} + 0.5119\ C\ (r = 0.8739)$ Method B

The value of r is indicative of poor linearity, probably because of inconsistency of K'.

4.1. Fixed-concentration method

Reaction rates were recorded for different AMP concentrations in the range 5.6×10^{-5} to 8.4×10^{-5} mol L^{-1}, and 4.5×10^{-4} to 7.5×10^{-4} mol L^{-1}, applying methods A or B, respectively. A pre-selected value of the absorbance was fixed and the time was measured in seconds. The reciprocal of time (i.e. $1/t$) versus the initial concentration of AMP (Table 4) was plotted. The following equations for calibration graphs were obtained by linear regression:

$1/t = -4.5 \times 10^{-3} + 76.97\ C\ (r = 0.9831)$ for Method A
$1/t = -4.1 \times 10^{-3} + 1.862\ C\ (r = 0.9962)$ for Method B

4.2. Fixed-time method

Reaction rates were determined for different concentrations of AMP. At a preslected fixed time, which was accurately determined, the absorbance was measured. Calibration graphs of absorbance versus initial concentration of AMP were established at fixed time of 5, 10, 15, 20 and 25 min applying method A and a fixed times of 10, 20, 30, 40, 50 and 60 applying method B with the regression equation shown in Table 1.

It is clear that the slope increases with time and the most acceptable values of the correlation coefficient (r) and the intercept were obtained for a fixed times interval for measurements when applying methods A and B, respectively (Table 5).

4.3. Mechanism of the reaction

The stoichiometry of the reaction was studied adopting the limiting logarithmic method (Rose, 1964). The ratio of the reaction between (log Abs versus log [AMP], log [$KMnO_4$] and log [CDNB] were calculated by dividing the slope of $KMnO_4$ and CDNB over the slope of the drug curve. It was found that, the ratio was 1:2 (AMP to $KMnO_4$) while for (AMP to CDNB) the ratio was 1:1. The proposal pathway of the reaction is presented in scheme 1.

5. Validation of the proposed method

5.1. Accuracy and precision of the proposed methods

Accuracy and precision was checked according to USP validation guidelines (TUSP, 2002) at three concentration levels within the specified range, six replicate measurements were recorded at each concentration levels. The results are summarized in Table 6.

5.2. Limit of detection (LOD)

LOD was calculated based on standard deviation of response and the slope of calibration curve. The limit of detection was expressed as:

Table 4 Values of reciprocal of time taken at fixed absorbance (0.4 and 0.3) for different rates of various concentrations of AMP applying methods A and B.

$1/t$ (s^{-1})	Log [AMP], (mol L^{-1})
Method A (oxidation with $KMnO_4$)	
1.11×10^{-3}	5.6×10^{-5}
8.40×10^{-4}	7.0×10^{-5}
6.70×10^{-4}	8.4×10^{-5}
Method B (reaction with CDNB)	
3.34×10^{-4}	4.5×10^{-4}
3.03×10^{-4}	6.0×10^{-4}
2.78×10^{-4}	7.5×10^{-4}

Table 5 Analytical parameters for fixed time method of the kinetic spectrophotometric determination of investigated AMP in the pure form by applying methods A and B.

Parameters	Method A	Method A
Optical characteristics		
λ_{max} (nm)	610	490
Linearity range (μg mL^{-1})	5–30	50–260
Regression equation		
Intercept (a)	0.0863	0.0994
Standard deviation of intercept (Sa)	0.6814	0.5814
Slope (b)	0.0138	0.0022
Standard deviation of slope (Sb)	0.002	0.0004
Correlation coefficient (r)	0.9977	0.9919
LOD (μg mL^{-1})	0.162	0.866
LOQ (μg mL^{-1})	0.493	0.2625

$$LOD = 3\sigma/S$$

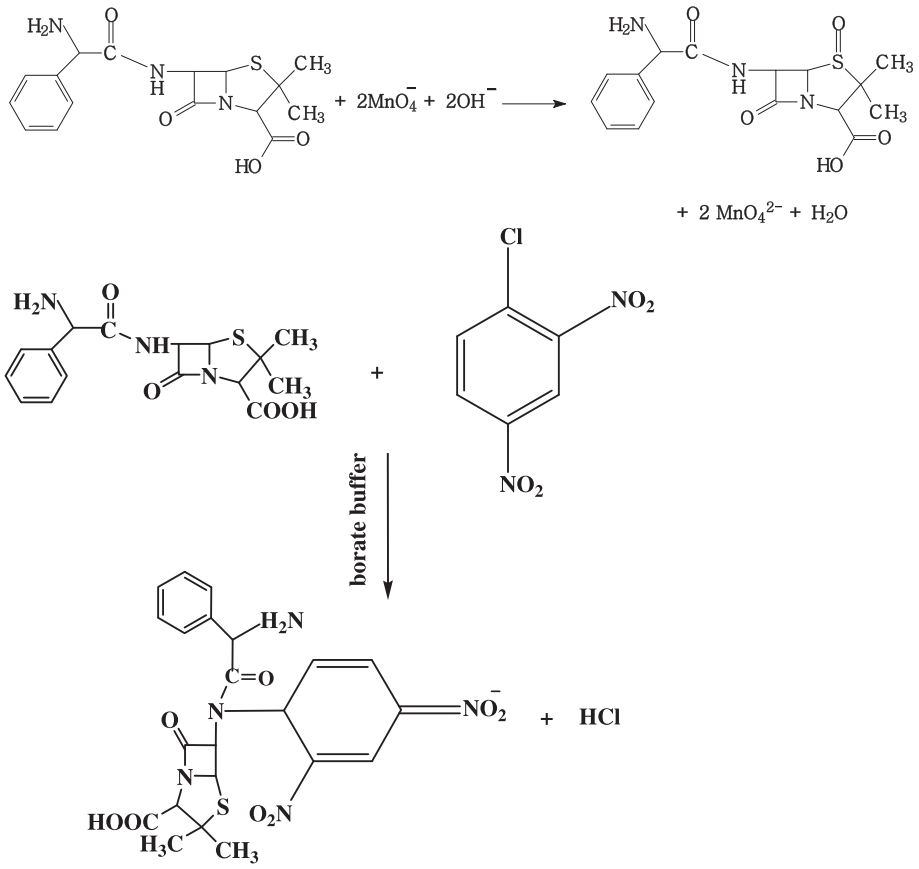
where σ is the standard deviation of intercept, S is the slope of calibration curve. The results were summarized in Table 5 indicating good sensitivity of the proposed method. According to USP validation guidelines (TUSP, 2002), the calculated LOD values should be further validated by laboratory experiments. In our work, good results were obtained where the calculated drug concentration by LOD equations were actually detected in these experiments.

5.3. Limit of quantitation (LOQ)

LOQ was calculated based on standard deviation of intercept and slope of calibration curve. In this method, the limit o quantitation is expressed as:

$$LOQ = 10\sigma/S$$

The results were summarized in Table 5 indicating good sensitivity of the proposed method. According to USP validation guidelines (TUSP, 2002), the calculated LOQ values should be further validated by laboratory experiments. In our work, good results were obtained where the calculated drug concentration by LOQ equations were actually quantitated in these experiments.



Scheme 1 A detailed mechanistic scheme of the oxidation and reaction of AMP.

Table 6 Evaluation of precision of the proposed kinetic spectrophotometric method for determination of investigated AMP by applying methods A and B.

Amount taken (μg mL^{-1})	Amount found (μg mL^{-1})	% Recovery ± S.D.	±RSD[a] (%)	SAE[b]
Method A				
10	9.96	99.86	0.520	0.02
20	19.2	99.34	0.478	0.04
30	30.05	100.06	0.368	0.05
Method B				
55	54.95	99.97	0.432	0.02
150	150.02	100.04	0.428	0.03
260	259.05	99.72	0.424	0.06

[a] Mean for five independent analyses.
[b] Standard analytical error.

6. Application to pharmaceutical dosage forms

The rate constant and fixed time methods of the proposed kinetic spectrophotometric method for determination of investigated AMP have been tested on commercial pharmaceutical dosage forms. The concentration of investigated AMP was computed from its responding regression equations. The results of proposed methods were statistically compared with those of reported methods (Saleh et al., 2003; Ayad et al., 1999; Taha, 2003), in respect to accuracy and precision. The obtained mean recovery values of the obtained amount were 99.80 and 99.91 respectively, which ensures that there is no interference of other active compounds present in the capsule. The calculated and theoretical value of both the proposed and the reported methods at 95% confidence level. This indicates good precision and accuracy in the analysis of investigated of AMP in pharmaceutical dosage forms.

7. Conclusion

Different methods were established to determine AMP concentration kinetically, the reaction rate method, rate constant and fixed time methods were applied. Applying the fixed time method, it is clear that the slope increased with time and the most acceptable values of correlation coefficients (r) and intercepts were obtained for a fixed time, which was therefore chosen as the most suitable time interval for measurements. The proposed method is sensitive enough to enable determination of lower amounts of drug, these advantage encourage the application of proposed method in routine quality control of investigated AMP in industrial laboratories. Finally our method provide advantages of improving selectivity, avoiding interference of colored and turbidity background of samples as our methods measure the increase in absorbencies with time against blank treated similarly and possibility avoiding interference of other active compound present in commercial product.

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