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Research Article

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Spectrophotometric study of the interaction of ampicillin sodium with iodine in acetonitrile and formation of charge transfer complexes

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Abstract

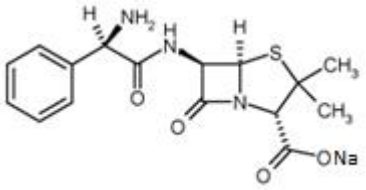
A spectrophotometric method for the determination of ampicillin sodium (Amp) with iodine has been studied. The method is based on charge transfer complexation reaction of the drug with iodine in acetonitrile medium. The absorbances were measured at λ_{max} 290 and 360 nm against the reagent blank. The first complex $AmpI^{+}.I^{-}$ is formed, after that the second complex $AmpI^{+}.I_3^{-}$ is formed. Under optimized experimental conditions, Beer's law is obeyed in the concentration ranges 0.743-14.856 µg.mL^{-1} for ampicillin sodium. The method was validated for specificity, precision, accuracy, linearity of the calibration curves, percent recoveries, limit of detection and quantitation. The method was successfully applied for determination of Amp in pure and pharmaceutical formulations samples with low RSD value. This is simple, specific, accurate and sensitive spectrophotometric method gives good results for the determination of Amp in pure and different dosage forms.

Keywords: Ampicillin sodium, Spectrophotometric method, Charge transfer complexation with iodine.

Introduction

Ampicillin sodium salt,6-{[amino(phenyl)acetyl]amino}-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate sodium, is a bactericidal antibiotic drug.

The chemical formula of ampicillin sodium (Amp) is $C_{16}H_{18}N_3NaO_4S$, its molecular weight is 371.39 g/mol. The chemical structure of Amp [1], see scheme 1.



Scheme 1: Chemical structure of ampicillin sodium

Various analytical techniques have been employed for the determination of ampicillin sodium in pure and pharmaceutical dosage, such as HPLC method [1, 2], spectrophotometry [3-7], spectrofluorimetry [8], capillary electrophoresis [9].Select ampicillin spectrally with some drugs as the sulbactam [10], amoxicillin [11-16], cloxacillin [14-18] and dicloxacillin [11, 17, 19].

In the present work, A spectrophotometric method for the determination of ampicillin sodium with iodine has been studied. The method is based on charge transfer complexation reaction of the drug with iodine in acetonitrile medium. The absorbances were measured at 290 and 360 nm against the reagent blank. The first complex $AmpI^{+}.I^{-}$ is formed, after that the second complex $AmpI^{+}.I_3^{-}$ is formed.

Experimental

Reagents

Ampicillin sodium (99.89%) was purchased from Lu Kang Drugs and Reagents Company (Shandong, China). Ampicillin sodium was assayed, according to English Pharmacopoeia by HPLC [1]. All reagents (iodine, acetonitrile and methanol) were of analytical grade from Merck.

Instruments and apparatus

Spectrophotometric measurements were made in T90+ UV-VIS spectrophotometer with 1.0 cm quartz cells. The diluter pipette model DIP-1 (Shimadzu), having 100 µL sample syringe and five continuously adjustable pipettes covering a volume range from 20 to 5000 µL (model Piptman P, GILSON).SARTORIUS TE64 (0.01 mg) electronic balance was used for weighing the samples.

A stock standard solution of iodine ($1x10^{-2}$ mol.L^{-1})

Dissolving 63.58 mg of iodine with acetonitrile into volumetric flask (25 mL) and diluting to mark by acetonitrile.

A stock standard solution of ampicillin sodium ($1x10^{-3}$ mol.L^{-1})

An accurately weighed 3.718 mg standard sample of ampicillin sodium was dissolved in 0.5 mL methanol, transferred into 10 mL standard flask and diluted to the mark with acetonitrile and mixed well.

Working solutions

The stock solutions were further diluted to obtain working solutions daily just before use in the ranges of Amp: 2.00, 3.00, 5.00, 10.00, 20.00, 30.00, and 40.00 µmol.L^{-1} (0.743, 1.114, 1.857, 3.714, 7.428, 11.142, and 14.856 µg.mL^{-1}) by dilution of the volumes: 0.020, 0.030, 0.050, 0.100, 0.200, 0.300, and 0.400 mL from stock standard solutions of ampicillin sodium $1x10^{-3}$ mol.L^{-1} were transferred into 10 mL volumetric flask, then added 0.100 mL from stock standard solution of iodine ($1x10^{-2}$ mol.L^{-1}) and diluted to 10 mL with acetonitrile.

Samples

Commercial formulations (as vial) were used for the determination of Amp. The pharmaceutical formulations were subjected to the analytical procedures:

(1) ***AMPI*** vial, **ELSAAD PHARMACEUTICAL**, Aleppo - SYRIA, each vial contains: 1000 mg of ampicillin sodium.
(2) ***AMPI*** vial, **ELSAAD PHARMACEUTICAL**, Aleppo - SYRIA, each vial contains: 500 mg of ampicillin sodium.
(3) ***AMPICILLIN*** vial, **ASIA PHARMACEUTICAL INDUSTRIES**, Aleppo - SYRIA, each vial contains: 1000 mg of ampicillin sodium.
(4) ***AMPICILLIN 1000*** vial, **SHIFA PHARMACEUTICAL INDUSTRIES**, Aleppo - SYRIA, each vial contains: 1000 mg of ampicillin sodium.
(5) ***AMPICILLIN PLUS 1500*** vial, **SHIFA PHARMACEUTICAL INDUSTRIES**, Aleppo - SYRIA, each vial contains: 1000 mg of ampicillin sodium and 500 mg of sulbactam sodium.
(6) ***MAXIM*** vial, **ELSAAD PHARMACEUTICAL**, Aleppo - SYRIA, each vial contains: 500 mg of ampicillin sodium and 500 mg of cloxacillin sodium.

Stock solutions of pharmaceutical formulations

Contents of 5 vials of each studied pharmaceutical formulations were weighted accurately and mixed well. An amount of the powder equivalent to the weight 3.718 mg of samples (1) to (4), 5.577 mg from sample (5) and 7.436 mg from sample (6) were solved in 0.5 mL methanol ,transferred into 10 mL standard flask and diluted to the mark with acetonitrile and mixed well; this solution contents $1x10^{-3}$ mol.L^{-1} of Amp for all studied pharmaceutical formulations.

Working solutions of pharmaceuticals

These solutions were prepared daily by diluting 100µL (0.100 mL) from stock solutions of pharmaceutical formulations into 10 mL volumetric flask, then added 0.100 mL from stock standard solution of iodine ($1x10^{-2}$ mol.L^{-1}) and diluted to 10 mL with acetonitrile.

Results and Discussion

Analytical procedure

A spectrophotometric method for the determination of ampicillin sodium with iodine has been studied. The method is based on charge transfer complexation reaction of the drug with iodine in acetonitrile medium. The absorbances were measured at 290 and 360 nm against the reagent blank. The first complex $AmpI^+.I^-$ is formed, after that the second complex $AmpI^+.I_3^-$ is formed.

Spectrophotometric results

UV-Vis spectra of Iodine, Amp, $AmpI^+.I^-$ and $AmpI^+.I_3^-$ solutions in acetonitrile were studied. The iodine solutions absorb at λ_{max} 465 nm (ε=830 L. mol^{-1}. cm^{-1}). The Amp solutions do not absorb in range 280-700 nm. The $AmpI^+.I^-$ complex absorb at λ_{max} 246 nm (ε=25000 L. mol^{-1}. cm^{-1}). The $AmpI^+.I_3^-$ complex absorb at $\lambda_{max,1}$ 290 nm and $\lambda_{max,2}$ 360 nm ($\varepsilon_{max,1}$=46000 and $\varepsilon_{max,2}$=23600 L. mol^{-1}. cm^{-1}). See figure 1.

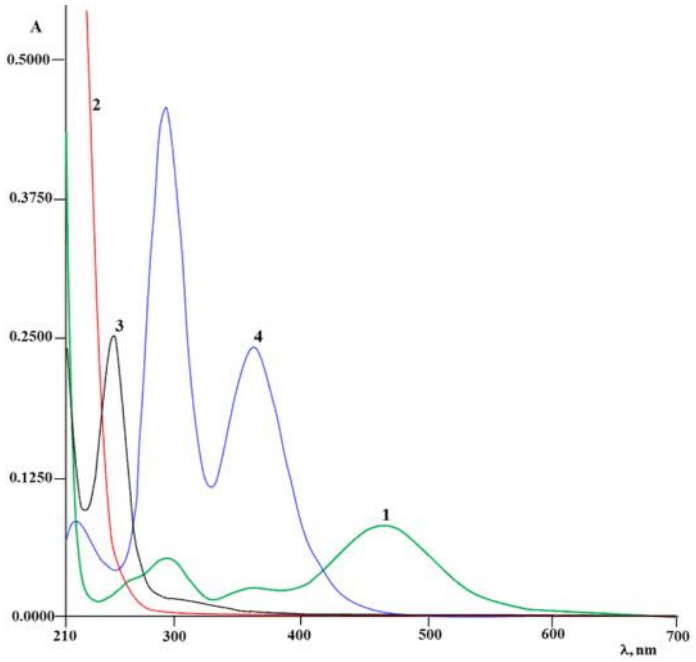
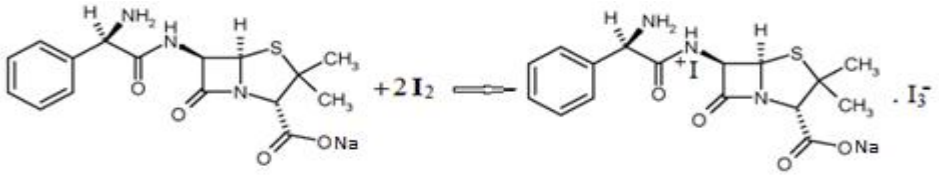


Fig. 1: UV-Vis spectra in acetonitrile of: 1- iodine $1.0x10^{-4}$ $mol.L^{-1}$; 2- ampicillin sodium (Amp) $1.0x10^{-4}$ $mol.L^{-1}$; 3- Amp $1.0x10^{-4}$ $mol.L^{-1}$ with I_2 $1.0x10^{-5}$ $mol.L^{-1}$; 4- Amp $1.0x10^{-5}$ $mol.L^{-1}$with I_2 $1.0x10^{-4}$ $mol.L^{-1}$ { blank for (1-2), (3) and (4) is acetonitrile, Amp $1.0x10^{-4}$ $mol.L^{-1}$ and I_2 $1.0x10^{-4}$ $mol.L^{-1}$, respectively, ℓ =1.0 cm}.

Studying of reaction ampicillin sodium with iodine:

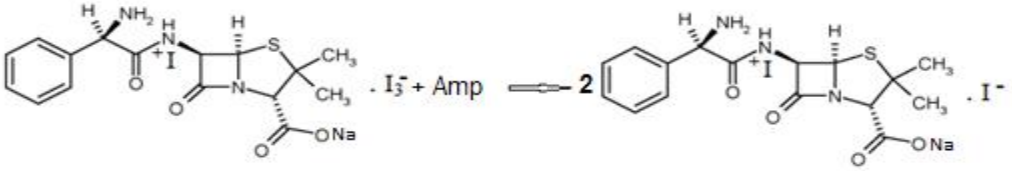
The effect of time on the interaction of ampicillin sodium (Amp) with iodine in the medium of acetonitrile with an excess of Amp was studied. It was found that in the beginning, the second complex is formed (because a formation constant of this complex is very large, pK 6.6 [20]) according to interaction (1), and because of an excess of Amp, it reacts with the complex $AmpI^+.I_3^-$ and is formed the first complex $AmpI^+.I^-$ according to reaction (2), see figure 2.

Figure 2 shows that at first the second complex $AmpI^+.I_3^-$ is formed and that its concentration is approximately half the concentration of iodine while the concentration of the first complex $AmpI^+.I^-$ is almost zero. After a sufficient time, the second complex changes its interaction with the excess drug (Amp) to the first complex and its concentration becomes equal to iodine concentration. The resulting interaction becomes as in equation (3). We also noted the emergence of an isoabsorptive point at a wave length equal to 264 nm.



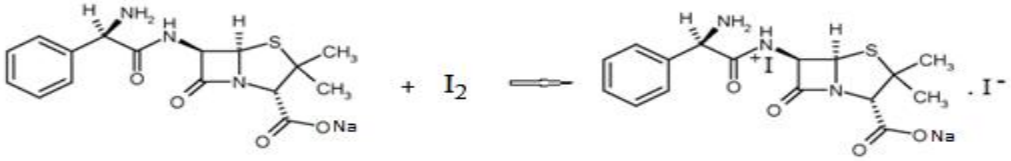
(1)

Ampicillin sodium (Amp) $AmpI^{+}.I_3^{-}$



(2)

$AmpI^{+}.I_3^{-}$ $AmpI^{+}.I^{-}$



(3)

Amp $AmpI^{+}.I^{-}$

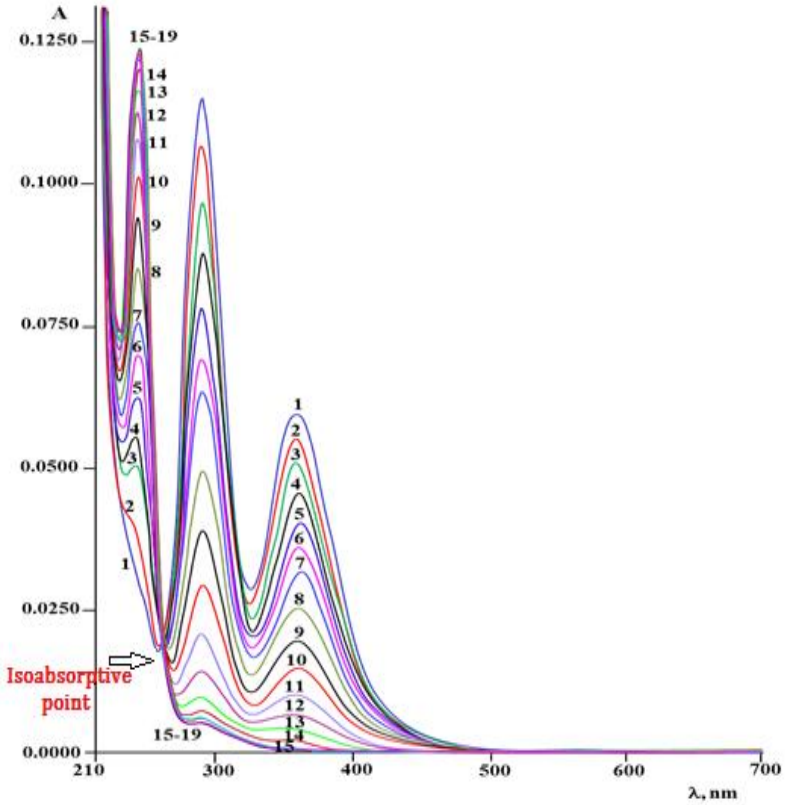


Fig. 2: The effect of time on UV-Vis spectra in acetonitrile of iodine $5.0x10^{-6}$ $mol.L^{-1}$ with $1.0x10^{-5}$ $mol.L^{-1}$ ampicillin sodium (Amp):1–7 the time 0, 5, 10, 15, 20, 25 and 30 min, respectively, and from 8 to 19 every 10 minutes {blank is acetonitrile, ℓ =1.0 cm}.

The effect of time on the interaction between ampicillin sodium and iodine in acetonitrile with an excess of iodine was also studied. The second complex ($AmpI^+.I_3^-$) only formed according to equation (1). It was found that the complex remained almost constant with increasing the time.

Calibration curve

The calibration curve of ampicillin sodium in pure form through complexation with iodine showed excellent linearity over concentration range of 0.743-14.856 $\mu g.mL^{-1}$ in presence of 1.0×10^{-4} $mol.L^{-1}$ of I_2 with good correlation coefficient (R^2= 0.9998) in acetonitrile. Regression equations at $\lambda_{max,1}$ 290 nm and $\lambda_{max,2}$ 360 nm were as the follows:

y=0.1247x+0.0026 and y=0.0638x+0.0006, respectively in acetonitrile. Figures 3 and 4 showed the spectra of complex $AmpI^+.I_3^-$ and calibration curve for determination of Amp according to optimal conditions at $\lambda_{max,1}$ and $\lambda_{max,2}$ (in present of 1.0×10^{-4} $mol.L^{-1}$ of I_2) where Amp: 0.743-14.856 $\mu g.mL^{-1}$ {blank is I_2 solution in acetonitrile 1.0×10^{-4} $mol.L^{-1}$; ℓ = 1.0 cm}.

The spectra characteristics of the method such as the molar absorptivity (ε), Beer's law, regression equation at λ_{max} (y=a.x+b); where y=absorbance, a=slope, x=concentration of ampicillin sodium by $\mu g.mL^{-1}$, b=intercept, the correlation coefficient, limit of detection (LOD) and limit of quantification (LOQ) and the optimum conditions for spectrophotometric determination of Amp through $AmpI^+.I_3^-$ complex is summarized in Table 1.

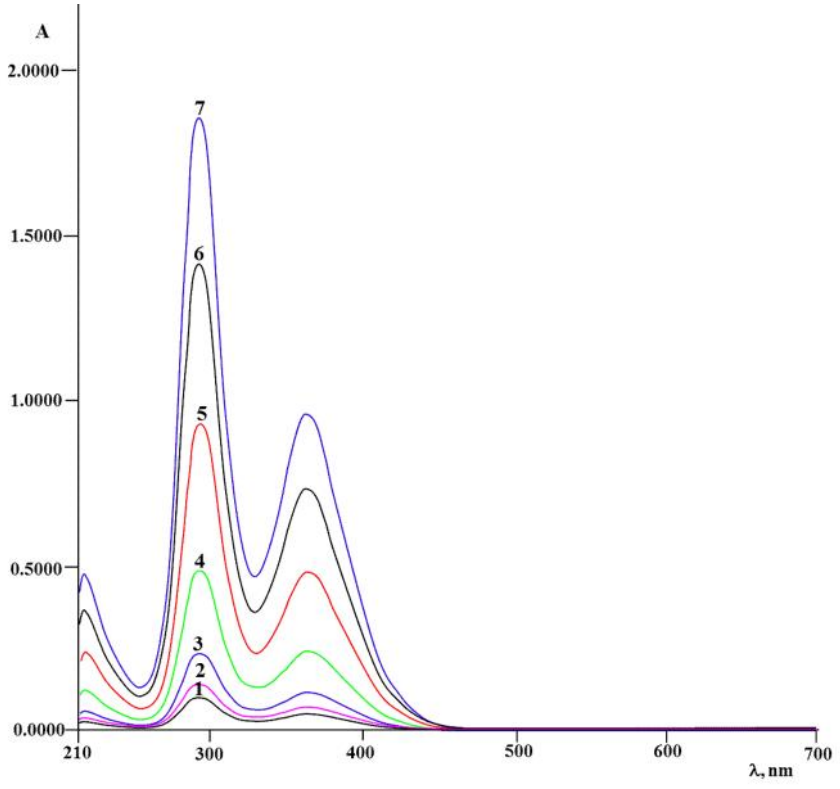


Fig.3. Spectra of $AmpI^+.I_3^-$ complex in presence of 1.0×10^{-4} $mol.L^{-1}$ of I_2; where C_{Amp} as the follows: 1 - 0.743, 2 - 1.114, 3 - 1.857, 4 - 3.714, 5 - 7.428, 6 - 11.142 and 7 - 14.856 $\mu g.mL^{-1}$. (ℓ = 1.0 cm, blank is 1.0×10^{-4} $mol.L^{-1}$ of I_2 in acetonitrile).

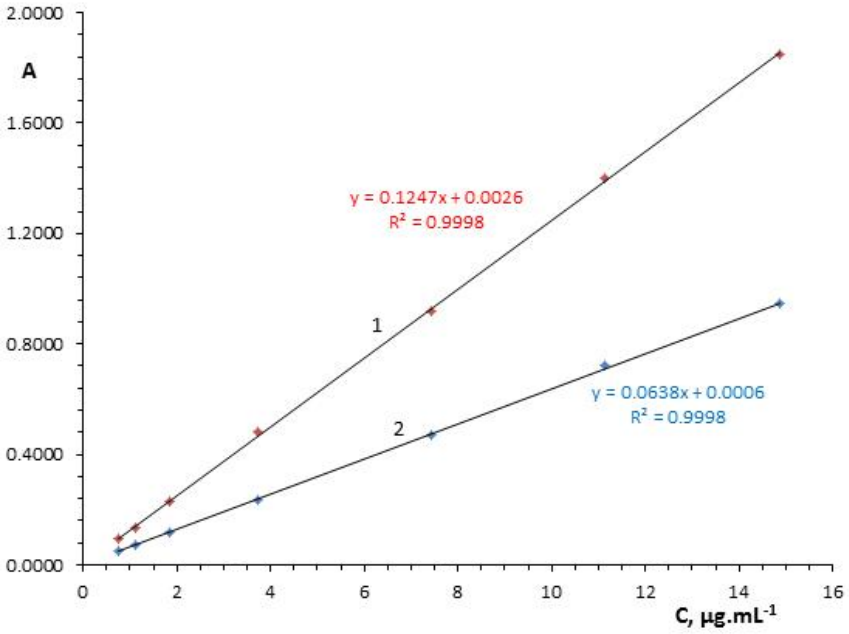


Fig. 4: Calibration curve of ampicillin sodium in pure form through complexation with iodine in presence of 1.0×10^{-4} mol.L^{-1} of I_2 in acetonitrile: 1 – at $\lambda_{max,1}$ 290 nm; 2 - at $\lambda_{max,2}$ 360 nm. (ℓ = 1.0 cm, blank is 1.0×10^{-4} mol.L^{-1} of I_2 in acetonitrile).

Table 1. The parameters established for spectrophotometric determination of ampicillin sodium by complex formation with I_2 in acetonitrile.

Parameters	Operating values
$\lambda_{max,1}$ of $AmpI^+.I_3^-$ complex, nm	290
$\lambda_{max.\ 2}$ of $AmpI^+.I_3^-$ complex, nm	360
Beer's Law Limit, for Amp by µM	2.00-40.00
Beer's Law Limit, for Amp by µg.mL^{-1}	0.743-14.856
Molar absorptivity of $AmpI^+.I_3^-$ complex (ε_1), L.mol^{-1}.cm^{-1}	46000
Molar absorptivity of $AmpI^+.I_3^-$ complex (ε_2), L.mol^{-1}.cm^{-1}	23600
Regression equation for $AmpI^+.I_3^-$ at $\lambda_{max,1}$ =290 nm:	
Slope	0.1247
Intercept	0.0026
Correlation coefficient (R^2)	0.9998
Regression equation for $AmpI^+.I_3^-$ at $\lambda_{max,2}$ =360 nm:	
Slope	0.0638
Intercept	0.0006
Correlation coefficient (R^2)	0.9998
C_{Amp}: C_{I_2}, mol.L^{-1}	≤1:2
Reaction time	5 min
Stability	24 h
Temperature of solution	25±5°C

n=5, t=2.776.

Analytical results

Spectrophotometric determination of ampicillin sodium through complexation with I_2 in acetonitrile within optimal conditions using calibration curve was applied. The results, summarized in Table 2, showed that the determined concentration of ampicillin sodium was rectilinear over the range of 0.743-14.856 $\mu g.mL^{-1}$, with relative standard deviation (RSD) not more than 2.8%. The results obtained from the developed method have been compared with the official HPLC method [1] and good agreement was observed between them.

Table 2: Spectrophotometric determination of ampicillin sodium through complex formation with I_2 within optimal conditions using calibration curve in acetonitrile.

X_i,(Taken)		[a], nm	* $\bar{x}$ ±SD, $\mu g.mL^{-1}$(Found)	$\bar{x} \pm \frac{t.SD}{\sqrt{n}}$ $\mu g.mL^{-1}$	RSD%	*X , $\mu g.mL^{-1}$ HPLC [1]
$\mu mol.L^{-1}$	$\mu g.mL^{-1}$					
2.000	0.743	290	0.757±0.021	0.757± 0.026	2.8	0.745
		360	0.759±0.020	0.759±0.025	2.7	
3.000	1.114	290	1.062±0.029	1.062±0.036	2.7	1.110
		360	1.135±0.029	1.135±0.036	2.6	
5.000	1.857	290	1.815±0.045	1.815±0.056	2.5	1.856
		360	1.871±0.045	1.871±0.056	2.4	
10.000	3.714	290	3.828±0.088	3.828±0.109	2.3	3.718
		360	3.658±0.084	3.658±0.104	2.3	
20.000	7.428	290	7.357±0.154	7.357±0.191	2.1	7.427
		360	7.357±0.154	7.357±0.191	2.1	
30.000	11.142	290	11.206±0.224	11.206±0.278	2.0	11.134
		360	11.276±0.214	11.276±0.266	1.9	
40.000	14.856	290	14.815±0.281	14.815±0.321	1.9	14.850
		360	14.802±0.266	14.802±0.330	1.8	

***** n=5, t= 2.776.

Applications

The developed spectrophotometric method was applied to determine ampicillin sodium in some Syrian pharmaceutical preparations through complex formation by I_2 in acetonitrile according to the optimal conditions. The results of quantitative analysis for ampicillin sodium in pharmaceutical preparations were summarized in Table 3.The proposed method was simple, direct, specific and successfully applied to the determination of ampicillin sodium in pharmaceuticals without any interference from excipients. Average recovery ranged between 98.7 to 100.4%. The results obtained by this method agree well with the contents stated on the vials and were validated by HPLC method [1].

Table 3: Determination of ampicillin sodium in some Syrian pharmaceutical preparations using spectrophotometric method through complex formation with I_2 within optimal conditions using calibration curve in acetonitrile, $\lambda_{max,2}$ 360 nm.

Dosage form	Label Claim of Amp, mg/vial	*Mean ±SD Amp, mg/vial	RSD %	Assay %	*Mean ±SD Amp, mg/vial by HPLC [1]	* Assay %, by HPLC [1]
AMPI vial, **ELSAAD PHARMACEUTICAL**	1000	987±23	2.3	98.7	1002±19	100.2
	500	498±12	2.4	99.6	497±10	99.4
AMPICILLIN vial, **ASIA PHARMACEUTICAL INDUSTRIES**	1000	1004±23	2.3	100.4	1010±20	101.0
AMPICILLIN 1000 vial, **SHIFA PHARMACEUTICAL INDUSTRIES**	1000	993±22	2.2	99.3	996±18	99.6

***** n=5.

Method validation

The developed method for simultaneous estimation of ampicillin sodium has been validated in accordance with the International Conference on Harmonization guidelines (ICH) [21].

Linearity

Several aliquots of standard stock solution of ampicillin sodium were taken in different 10 mL volumetric flask and diluted up to the mark with acetonitrile such that their final concentrations were 0.743-14.856 µg.mL^{-1} for ampicillin sodium. Absorbance was plotted against the corresponding concentrations to obtain the calibration graph, see Figure 4 and Table 2. Linearity equations obtained at $\lambda_{max,1}$ =290 nm and at $\lambda_{max,2}$=360 nm were y=0.1247x + 0.0026 and y=0.0638x+0.0006, respectively for the range 0.743-14.856 µg.mL^{-1} (R^2=0.9998).

Precision and Accuracy

The precision and accuracy of proposed method was checked by recovery study by addition of standard drug solution to pre-analyzed sample solution at three different concentration levels (80%, 100% and 120%) within the range of linearity for ampicillin sodium. The basic concentration level of sample solution selected for spiking of the ampicillin sodium standard solution was 7.428 µg.mL^{-1}. The proposed method was validated statistically and through recovery studies, and was successfully applied for the determination of ampicillin sodium in pure and dosage forms with percent recoveries ranged from 99.7% to 101.2%, see Table 4.

Table 4: Results of recovery studies (n=5).

Level	Recovery%	
	At $^{a}\lambda_{max}$ 290 nm	At $^{a}\lambda_{max}$ 360 nm
80%	99.7	99.9
100%	99.9	100.1
120%	101.2	100.9

Repeatability

The repeatability was evaluated by performing 10 repeat measurements for 7.428 µg.mL^{-1} of ampicillin sodium using the studied spectrophotometric method under the optimum conditions. The found amount of ampicillin sodium ($\bar{x}$ ± SD) 7.418 ± 0.147 µg.mL^{-1} at $\lambda_{max,1}$ and 7.422 ± 0.146 µg.mL^{-1} at $\lambda_{max,2}$. The percentage recovery was found to be 99.8% ± 2.2% with RSD of 0.022 at $\lambda_{max,1}$ and 99.9% ± 2.1% with RSD of 0.021 at $\lambda_{max,2}$. These values indicate that the proposed method has high repeatability for ampicillin sodium analysis.

Sensitivity (LOD and LOQ)

The sensitivity of the method was evaluated by determining the LOD and LOQ. The values of LOD and LOQ for ampicillin sodium at $\lambda_{max,1}$ are 0.082 and 0.248 µg.mL^{-1}, and at $\lambda_{max,2}$ are 0.068 and 0.206 µg.mL^{-1} respectively.

Robustness

The robustness of the method adopted is demonstrated by the constancy of the absorbance with the deliberated minor change in the experimental parameters such as the change in the concentration of excipients, C_{I_2}: C_{Amp} (2.5±0.3), temperature (25±5°C), stability (24±5% h) and reaction time (5±1 min), see Table 5, which indicates the robustness of the proposed method. The absorbance was measured and assay was calculated for five times.

Table 5: Robustness of the proposed spectrophotometric method at $\lambda_{max,2}$.

Experimental parameter variation	Average recovery (%)*	
	C_{Amp}	
	1.857 **µg.mL^{-1}**	7.428 **µg.mL^{-1}**
Temperature		
20°C	99.2	99.6
30°C	100.1	101.2
C_{I_2}:C_{Amp}		
2.2	99.4	99.8
2.8	99.8	100.9
Stability		
22.8 h	100.5	100.4
25.2 h	100.6	100.4
Reaction time		
4.0 min	99.8	99.7
6.0 min	100.2	100.1

* n=5.

Specificity

The specificity of the method was ascertained by analyzing standard ampicillin sodium in presence of excipients. There was no interference from most of the common excipients.

Interferences

Sulbactam sodium and cloxacillin sodium (which are found with ampicillin sodium in pharmaceuticals) are interfere.

Conclusion

A spectrophotometric method is based on charge transfer complexation reaction of the Amp with I_2 in acetonitrile. The absorbances were measured at 290 and 360 nm. The first complex $AmpI^+.I^-$ is formed, after that the second complex $AmpI^+.I_3^-$ is formed. Under optimized experimental conditions, Beer's law is obeyed in the concentration ranges 0.743-14.856 µg.mL^{-1} for Amp. The method was validated for specificity, precision, accuracy, linearity of the calibration curves, percent recoveries, limit of detection and quantitation. The method was successfully applied for determination of Amp in pure and pharmaceutical formulations samples with low RSD value. This is simple, specific, accurate and sensitive spectrophotometric method gives good results for the determination of Amp in pure and different dosage forms as vial.

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