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SPECTROPHOTOMETRIC DETERMINATION OF AMOXICILLIN IN PHARMACEUTICAL FORMULATIONS

Kemal ÜNAL, İ.Murat PALABIYIK, Elif KARACAN, *Feyyaz ONUR

Ankara University, Faculty of Pharmacy, Department of Analytical Chemistry,
06100 Tandoğan-Ankara, TURKEY

Abstract

In present study, three new spectrophotometric methods, original UV spectrophotometry, first and second order derivative UV spectrophotometry, were developed for the determination of amoxicillin in pharmaceutical preparations. In original UV spectrophotometry, absorbances were measured at 247.0 nm in the zero order UV spectra of the solution of amoxicillin in 0.1N NaOH in the range of 220 - 350 nm. In first derivative UV spectrophotometry, $dA/d\lambda$ values were measured at 255.8 nm in the first derivative UV spectra of the solution of amoxicillin in 0.1N NaOH in the range of 220 - 320 nm ($\Delta\lambda$= 2 nm). In second derivative UV spectrophotometry $d^2A/d\lambda^2$ values were measured at 249.2 nm in the second derivative UV spectra of the solution of amoxicillin in 0.1N NaOH in the range of 220 - 320 nm ($\Delta\lambda$= 4 nm). Linearity range was found as 3.2 – 48.0 μg/mL in all three methods. Mean recoveries and the relative standard deviations of the methods were found as 99.67 % and 1.20 % in original UV spectrophotometry at 247.0 nm, 99.04 % and 1.76 % in first derivative UV spectrophotometry at 255.8 nm and, 99.43 % and 2.34 % in second derivative UV spectrophotometry at 249.2 nm respectively. Three spectrophotometric methods developed were successfully applied to 8 tablets, 2 oral suspensions and 1 lyophilized powder formulation commercially available in Turkish drug market. All the results were compared statistically with those obtained by using the methods indicated in USP XXIII.

Keywords: *Amoxicillin, Spectrophotometry, Determination, Pharmaceutical Preparation*

Amoksisilin'in farmasötik preparatlarda spektrofotometrik miktar tayini

Bu çalışmada, amoksisilin'in farmasötik preparatlarda miktar tayini için üç yeni spektrofotometrik yöntem, orijinal UV spektrofotometri, birinci ve ikinci türev spektrofotometri, geliştirilmiştir. Orijinal UV spektrofotometride absorbans değerleri, amoksisilin in 0.1N NaOH içerisindeki çözeltilerinin 220-350 nm aralığındaki UV spektrumlarında 247.0 nm de ölçülmüştür. Birinci türevUV spektrofotometride, $dA/d\lambda$ değerleri, amoksisilin in 0.1N NaOH içerisindeki çözeltilerinin 220-320 nm aralığındaki birinci türev UV spektrumlarında ($\Delta\lambda$= 2 nm) 255.8 nm de ölçülmüştür. İkinci türev UV spektrofotometride $d^2A/d\lambda^2$ değerleri amoksisilin in 0.1N NaOH içerisindeki çözeltilerinin 220-320 nm aralığındaki ikinci türev UV spektrumlarında ($\Delta\lambda$= 4 nm) 255.8 nm de ölçülmüştür. Çalışmada doğrusal çalışma aralığı her üç yöntem için de 3.2 – 48.0 μg/mL olarak bulunmuştur. Yöntemlerdeki ortalama geri kazanım ve bağıl standart sapma değerleri sırasıyla orijinal UV spektrofotometride 247.0 nm de % 99.67 ve % 1.20, birinci türev UV spektrofotometride,255.8 nm de % 99.04 ve % 1.74 ve, ikinci türev UV spektrofotometride249.2 nm de % 99.43 ve % 2.35 olarak bulunmuştur. Geliştirilen üç yöntem Türkiye ilaç piyasasında bulunan 8 adet tablet, 2 adet oral suspansiyon ve bir adet liyofilize toz formulasyonuna başarıyla uygulanmıştır. Elde edilen tüm sonuçlar USP XXII de belirtilen yöntemlerle elde edilenlerle istatistiksel olarak karşılaştırılmıştır.

Anahtar kelimeler: *Amoksisilin, Spektrofotometri, Miktar Tayini, Farmasötik Preparat*

***Correspondence:** E-mail: onur@pharmacy.ankara.edu.tr

INTRODUCTION

Amoxicillin (Figure 1) is a moderate-spectrum β-lactam antibiotic used to treat bacterial infections caused by susceptible microorganisms. Amoxicillin is an antibiotic active against a wide range of Gram-positive, and a limited range of Gram-negative organisms. Amoxicillin acts by inhibiting the synthesis of bacterial cell walls. It inhibits cross-linkage between the linear peptidoglycan polymer chains that make up a major component of the cell wall of Gram-positive bacteria. It is usually the drug of choice within the class because it is better absorbed, following oral administration, than other beta-lactam antibiotics. Amoxicillin is susceptible to degradation by β-lactamase-producing bacteria, and so may be given with clavulanic acid to decrease its susceptibility.

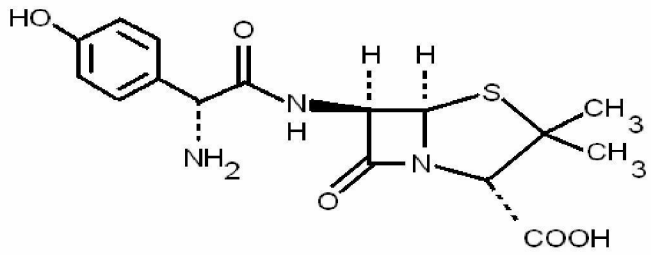

Figure 1. Amoxicillin

In previous studies; the determination of amoxicillin in pharmaceutical preparations containing only amoxicillin was made by using several methods including spectrophotometry (1-8), HPLC (9-11), spectrofluorimetry (12), flow-injection analysis (13-18), voltammetry and polarography (19,20) and titrimetry (21). Determination of amoxicillin in the presence of sulbactam sodium, clavulanic acid, fluxacilline and metronidazole was realized by using HPLC (22-28), TLC (29), CE (30) and chemometry (31). However, no information concerning with the determination of amoxicillin in pharmaceutical preparations by using classical UV spectrophotometry and derivative UV spectrophotometric methods could be seen in the literatures.

EXPERIMENTAL

Apparatus

Shimadzu 1601 PC double beam spectrophotometer with a fixed slit width (2 nm) connected to a computer loaded with Shimadzu UVPC was used for all the spectrophotometric measurements.

Materials

Amoxicillin trihydrate was kindly donated by BİLİM Pharm.Ind., Turkey and used without further purification.

All the materials used in the spectrophotometric analysis were of analytical reagent grade.

Commercial pharmaceutical preparations assayed

Commercial name	*Content*	*Batch no.*	*Firm*
a) Tablets:			
Alfoxil	500 mg Amoxicillin trihydrate	6083550	ABFAR
Amoksina	500 mg Amoxicillin trihydrate	7C05E	MUSTAFA NEVZAT
Atoksilin	500 mg Amoxicillin trihydrate	798	ATABAY
Demoksil	500 mg Amoxicillin trihydrate	213461	DEVA
Largopen	500 mg Amoxicillin trihydrate	7176001A	BİLİM
Moksilin	500 mg Amoxicillin trihydrate	9055589	İLSAN-İLTAŞ
Remoxil	500 mg Amoxicillin trihydrate	606002	İ.E.ULAGAY
Topramoxin	500 mg Amoxicillin trihydrate	1216	TOPRAK
b) Oral suspensions:			
Alfoxil	125 mg/ 5 mL Amoxicillin trihydrate	7020587	ABFAR
Largopen	125 mg/ 5 mL Amoxicillin trihydrate	007	BİLİM
c) Flakon			
Alfoxil	1 g / 4 mL Amoxicillin trihydrate	7010364	ABFAR

Standard solutions

Standard solutions of amoxicillin trihydrate (500 mg / 250 mL) were prepared in 0.1 M NaOH for spectrophotometric methods.

Sample preparation

Tablets: The content of 20 tablets were accurately weighed and powdered in a mortar. An amount of mass equivalent to one tablet was weighed in 100 mL volumetric flask and diluted to volume with 0.1N NaOH. After 45 min of mechanically shaking and 15 min of standing in the dark the solution was filtered through 4.5 μm milipore filter. Portion of the initial 5 mL was discarded and 1 ml of filtered solution was put into a 250 ml volumetric flask and the volume was completed to 250 mL with the same solvent. Final solution was used for the determinations.

Oral suspensions: 5 mL of suspension was put into 50 mL volumetric flask and diluted to volume with 0.1N NaOH. This solution was filtered through 4.5 μm milipore filter and 2 mL of the filtrat was put into 250 ml volumetric flask and the volume was completed to 250 mL with 0.1N NaOH. Final solution was used for the determinations.

Lyophilized powders: 4 mL of distilled water was injected into the flacon. After well stirring, 1 mL of the clear solution was put into 50 mL volumetric flask and diluted to volume with 0.1N NaOH. 1 mL of this solution was put into 250 ml volumetric flask and the volume was completed to the mark with 0.1N NaOH. Final solution was used for the determinations.

RESULTS

Original UV spectrophotometry

There are two maxima (247.0 and 289.8 nm) in zero-order UV spectra of the solution of amoxicillin trihydrate (**AMO**) in 0.1N NaOH in the range of 220-320 nm (Figure 2). The determination of **AMO** can be realized by measuring the absorbances at these wavelengths and using the calibration curve prepared by plotting the absorbances versus ten different concentrations of standard substance. Linearity range according to the Beer's law was found as 3.4 – 48.0 μg/mL in the method. LOQ was 3.4 μg/mL and LOD was calculated as 1.0 μg/mL by using the following equation; 3.3 SD/m (SD=Standard deviation, m=slope). Regression parameters were shown in Table 1. Recoveries and relative standard deviations were calculated by using standard solutions and the results were illustrated in Table 2.

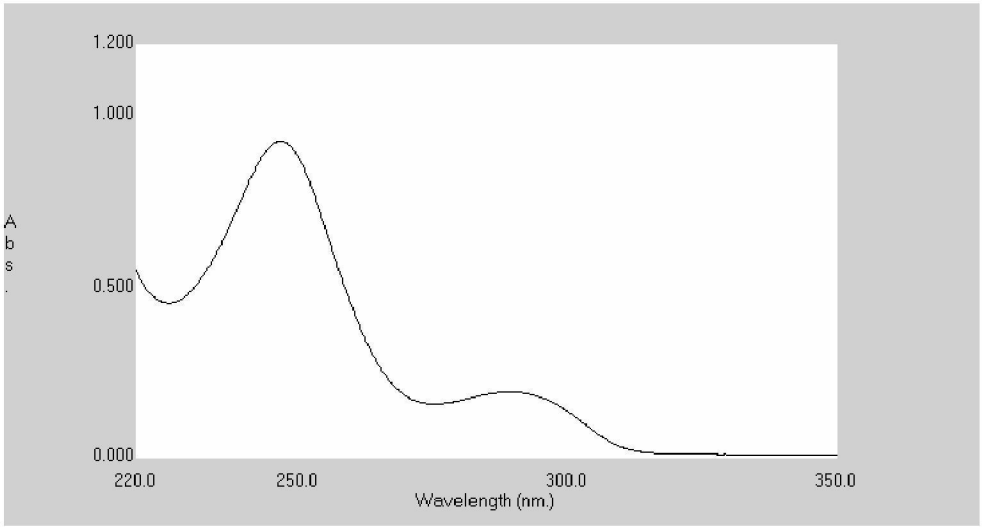

Figure 2. UV spectrum of the 36 μg/mL solution of **AMO** in 0.1 N NaOH .

First derivative UV spectrophotometry

There are two maxima (238.4 and 282.0 nm) and one minimum (255.8 nm) in the first derivative spectra of the solution of **AMO** in 0.1N NaOH in the range of 220-320 nm (Figure 3). Different Δλ values were tested and Δλ= 2 nm was found optimal in the method. The determination of amoxicillin can be realized by measuring the dA/dλ values at 238.4, 282.0 , and, 255.8 nm and using the calibration curve prepared by plotting the dA/dλ values versus ten doses of standard substance. Linearity range according to the Beer's law was found as 3.4 – 48.0 μg/mL in the method. LOQ was 3.4 μg/mL and LOD was calculated as 1.0 μg/mL by using the following equation; 3.3 SD/m (Regression parameters were shown in Table 1). Recoveries and relative standard deviations were calculated by using standard solutions and the results were illustrated in Table 3.

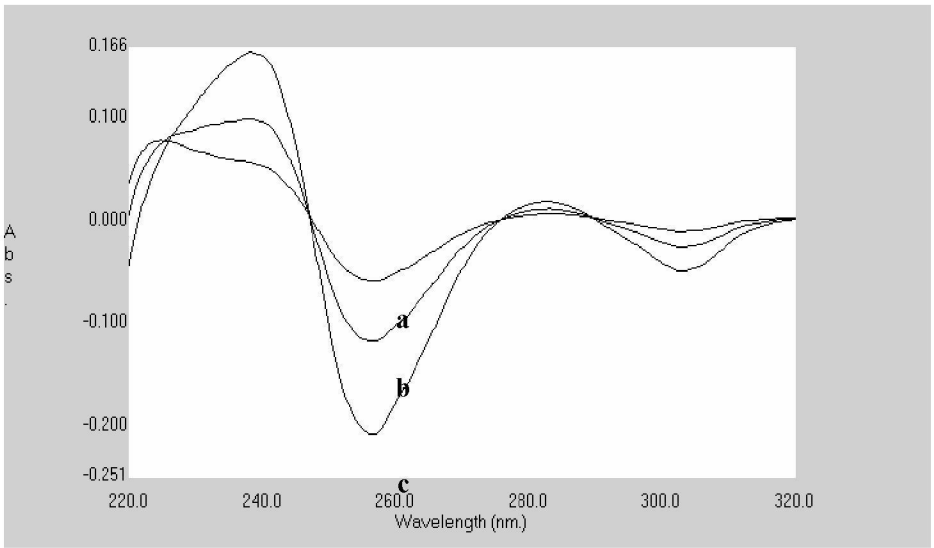

Figure 3. First derivative spectra of the solution of a) 8 μg /mL, b) 16 μg /mL , c) 28 μg/mL **AMO** in 0.1 N NaOH (Δλ = 2 nm) (Scaling factor = 5).

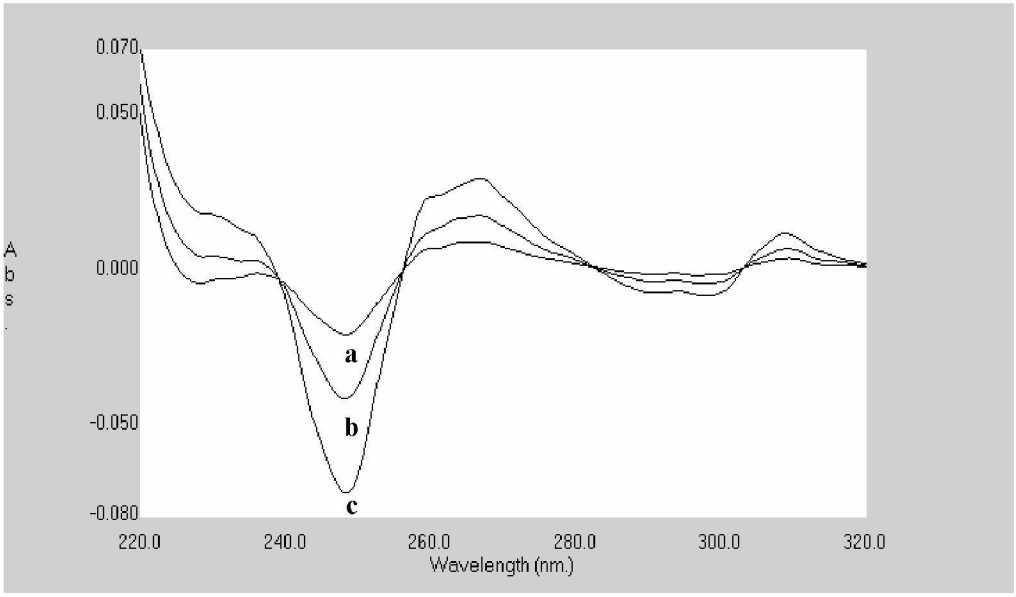

Figure 4. Second derivative spectra of the solution of a) 8 μg /mL, b) 16 μg /mL , c) 28 μg/mL **AMO** in 0.1 N NaOH ($\Delta\lambda$ = 4 nm) (Scaling factor = 10).

Second derivative UV spectrophotometry

There are two maxima (249.2 and 298.4 nm) and one minima (264.0 nm) in the second derivative spectra of the solution of **AMO** in 0.1N NaOH in the range of 220-320 nm (Figure 4). Different $\Delta\lambda$ values were tested and $\Delta\lambda$= 4 nm was found optimal in the method. The determination of amoxicillin
can be realized by measuring the $d^2A/d\lambda^2$ values at the wavelengths mentioned above and using the calibration curve prepared by plotting the $d^2A/d\lambda^2$ values versus ten doses of standard substance. Linearity range according to the Beer's law was found as 3.4 – 48.0 μg/mL in the method. LOQ was 3.4 μg/mL and LOD was calculated as 1.0 μg/mL by using the following equation; 3.3 SD/m Regression parameters were shown in Table 1. Recoveries and relative standard deviations were calculated by using standard solutions and the results were illustrated in Table 4.

Selectivity

According to official validation guidelines, in cases where it is impossible to obtain samples of all drug product components, it may be acceptable to add known quantities of the analyte to the drug product for determining recovery. For this reason, in order to know whether the excipients in the pharmaceutical preparation show any interference with the analysis, the recovery test was done by the standard addition method by adding known amounts of **AMO** at three different concentrations corresponding to 10, 25 and 50 % of the label claims. Each solution was prepared in triplicate and the methods were applied. According to the recoveries obtained for the amount of the added **AMO** (99.5 – 100.7 % for all the formulations selected) when applied three methods at selected wavelengths

Table 1. Regression parameters of the analytical methods

Yöntemler	λ (nm)	m	n	r	Working range (μg/mL)
original UV spectrophotometry	247.0	0.0275	-0.01	0.9998	3.2 - 48
	289.8	0.0057	-0.0184	0.9997	3.2 - 48
(^{1}D) First derivative spectrophotometry	238.4	0.0054	0.0119	0.9998	3.2 - 48
	255.8	-0.0073	-0.0025	0.9998	3.2 - 48
	282.0	0.0006	0.0002	0.9960	3.2 - 48
(^{2}D) Second derivative spectrophotometry	249.2	-0.0025	-0.0027	0.9989	3.2 - 48
	264.0	0.001	0.0008	0.9970	3.2 - 48
	298.4	-0.0004	0.00004	0.9979	3.2 - 48

m = slope, n = intercept, y = mx + n, r = correlation coefficient.

Table 2. Validation parameters in classical UV spectrophotometry using standard solutions of amoxicillin trihydrate in 0.1 N NaOH

	247.0 nm			289.8 nm	
No	**Added μg/mL**	**Found μg/mL**	**Recovery %**	**Found μg/mL**	**Recovery %**
1	6.4	6.40	100.00	6.81	106.40
2	6.4	6.25	97.66	6.39	99.84
3	6.4	6.28	98.13	6.23	97.34
4	24.0	24.00	100.00	23.82	99.29
5	24.0	24.11	100.45	24.05	100.20
6	24.0	23.85	99.37	23.76	99.00
7	40.0	40.57	101.43	41.20	103.00
8	40.0	40.44	101.10	40.62	101.56
9	40.0	39.67	99.18	39.54	98.85
10	40.0	39.76	99.40	40.12	100.29
n = 10		$\bar{X}$	99.67		100.57
		SD	1.19		2.56
		RSD	% 1.20		% 2.55

$\bar{X}$= mean, SD= standard deviation, RSD= relative standard deviation

Table 3. Validation parameters in first derivative UV spectrophotometric method using standard solutions of amoxicillin trihydrate in 0.1 N NaOH

		238.4 nm		255.8 nm		282.0 nm	
	Added µg/mL	**Found µg/mL**	**Recovery %**	**Found µg/mL**	**Recovery %**	**Found µg/mL**	**Recovery %**
1	6.4	5.96	93.13	6.16	96.25	5.87	91.72
2	6.4	6.31	98.59	6.23	97.34	6.33	98.90
3	6.4	6.39	99.84	6.19	96.71	6.20	96.87
4	24.0	23.99	99.95	24.27	101.13	21.25	88.54
5	24.0	23.64	98.50	23.94	99.74	2.00	91.67
6	24.0	23.72	98.84	24.01	100.04	22.99	95.79
7	40.0	39.71	99.27	40.02	100.05	37.62	94.05
8	40.0	40.35	100.87	40.41	101.03	38.52	96.30
9	40.0	39.46	98.66	39.80	99.49	38.00	95.00
10	40.0	39.37	98.42	39.44	98.60	37.22	93.05
n = 10		$\bar{X}$	98.61		99.04		94.19
		SD	2.08		1.74		3.03
		RSD	% 2.11		% 1.76		% 3.22

Table 4. Validation parameters in second derivative UV spectrophotometric method using standard solutions of amoxicillin trihydrate in 0.1 N NaOH

		249.2 nm		264.0 nm		298.4 nm	
	Added µg/mL	**Found µg/mL**	**Recovery %**	**Found µg/mL**	**Recovery %**	**Found µg/mL**	**Recovery %**
1	6.4	6.29	98.28	6.40	100.00	6.16	96.25
2	6.4	6.12	95.62	6.82	106.56	6.19	96.69
3	6.4	6.50	101.56	6.76	105.62	6.08	95.00
4	24.0	23.69	98.70	22.87	95.29	23.63	98.45
5	24.0	24.18	100.75	22.80	95.00	22.65	94.38
6	24.0	23.72	98.83	22.20	92.50	22.50	93.75
7	40.0	40.74	101.86	36.00	90.00	36.26	90.15
8	40.0	40.13	100.33	39.11	97.78	36.22	90.55
9	40.0	40.92	102.30	40.20	100.50	37.60	94.00
10	40.0	38.43	96.08	40.17	100.42	39.50	98.75
n = 10		$\bar{X}$	99.43		98.37		94.55
		SD	2.34		5.34		2.83
		RSD	% 2.35		% 5.43		% 2.98

(at 247.0 nm in original UV spectrophotometry, at 255.8 nm in first derivative UV spectrophotometry and at 249.2 nm in second derivative spectrophotometry) it was concluded that there was no interference from the ingredients placed in the formulations.

Accuracy and Precision

Accuracy in the methods was determined by the recovery studies using standard solutions of **AMO.** In original UV spectrophotometry**:** the mean recoveries were found as 99.67 and 100.57 % at 247.0 and 289.8 nm respectively. Relative standard deviations at these wavelengths were found as 1.20 and 2.55 % respectively (Table 2). In first derivative UV spectrophotometric method; the mean recoveries were found as 98.61, 99.04 and 94.19 % at 238.4, 255.8 and 282.0 nm respectively. Relative standard deviations at these wavelengths were found as 2.08, 1.76 and 3.22 % respectively (Table 3). In second derivative UV spectrophotometric method; the mean recoveries were found as 99.43, 98.37 and 94.55 % at 249.2, 264.0 and 298.4 nm respectively. Relative standard deviations at these wavelengths were found as 2.34, 5.34 and 2.98 % (Table 4).

Robustness

Robustness was tested by changing the concentration of NaOH. No significant difference was observed for 0.05 – 0.15 N NaOH range. We selected 0.1N NaOH for the methods proposed.

Solution Stability

Solution of **AMO** in 0.1N NaOH is stable for 8 hours at room temperature.

Analysis of Pharmaceutical Preparations

Developed three methods were applied to the determination of **AMO** in pharmaceutical preparations selected, 8 tablets, 2 oral suspensions and 1 flakon. Each pharmaceutical preparation was analyzed by performing ten independent determinations. In application, 247.0 nm in original UV spectrophotometry, 255.8 nm in first derivative spectrophotometry and 249.2 nm in second derivative spectrophotometry were selected by their lowest RSD values in the validation studies, Table 2-4. Satisfactory results were obtained for **AMO** and were found to be in agreement with the label claims (Table 5). The results obtained by the developed methods were compared with the official methods (HPLC, USP XXIII) statistically by using Student's *t* test and no significant difference was observed between them by the fact that *t* values calculated were lower than that of tabulated (theoretical) values for P = 0.05 level (Table 6). In USP XXIII, two HPLC methods were proposed for tablets and oral suspensions but there was no method indicated for the flakon formulations. So, we used HPLC method in USP XXIII indicated for oral suspensions for the determination of **AMO** in flakon formulation.

Table 5. Assay results of commercial formulations for **AMO.**

Methods / Pharmaceutical preparations	^{1}D Mean (mg) ± SD (% RSD)	^{2}D Mean (mg) ± SD (% RSD)	Original UV Spectr. Mean (mg) ± SD (% RSD)	****HPLC Mean (mg) ± SD (% RSD)
Alfoxil ® 500 mg Tablet	498.03 ± 14.58 (% 2.93)	497.14 ± 19.81 (% 3.98)	499.28 ± 16.75 (% 3.36)	499.84 ± 1.84 (%0.37)
Amoksina® 500 mg Tablet	485.19 ± 12.28 (% 2.53)	486.52 ± 16.98 (%3.49)	470.74 ± 5.86 (% 1.24)	470.30 ± 1.63 (% 0.35)
Atoksilin® 500 mg Tablet	490.90 ± 13.95 (%2.84)	503.49 ± 22.60 (% 4.49)	485.3 ± 2.57 (% 0.53)	489.23 ± 1.61 (%0.33)
Demoksil® 500 mg Tablet	500.34 ± 16.74 (%3.34)	497.61 ± 20.28 (% 4.08)	500.20 ± 15.17 (% 3.03)	499.84 ± 2.57 (%0.51)
Largopen® 500 mg Tablet	491.25 ± 8.42 (% 1.71)	496.18 ± 20.19 (% 4.07)	487.13 ± 13.74 (% 2.82)	487.17 ± 1.86 (% 0.38)
Moksilin® 500 mg Tablet	484.48 ± 15.93 (% 3.29)	479.33 ± 25.87 (% 5.40)	484.9 ± 4.06 (% 0.84)	488.66 ± 2.89 (% 0.59)
Remoksil 500 mg Tablet	487.30 ± 3.94 (% 0.81)	491.64 ± 19.65 (% 3.99)	486.30 ± 9.67 (% 1.99)	486.45 ± 1.95 (%0.40)
Topramoxin® 500 mg Tablet	493.59 ± 15.38 (%3.12)	478.37 ± 18.98 (% 3.97)	487.13 ± 22.87 (% 4.70)	487.73 ± 0.83 (%0.17)
Alfoxil® 1000 mg lyophilized powder	979.77 ± 35.48 (% 3.62)	994.60 ± 13.08 (% 1.31)	974.58 ± 4.83 (% 0.50)	978.06 ± 1.52 (% 0.16)
Alfoxil® 125 mg / 5ml Suspension	120.85 ± 4.30 (% 3.56)	120.51 ± 4.22 (% 3.50)	120.25 ± 1.44 (% 1.20)	121.89 ± 1.69 (% 1.39)
LARGOPEN® 125 mg / 5ml Suspension	122.22 ± 3.31 (% 2.70)	122.93 ± 3.62 (% 2.95)	120.48 ± 0.40 (% 0.33)	120.65 ± 0.37 (% 0.31)

* Mean of ten replicates
** SD = Standard deviation,
*** RSD = Relative Standard deviation
****Official method (USP XXIII)

Table 6. Calculated *t* values when compared the results with those obtained by official methods (USP XXIII)

Pharmaceutical preparations	0riginal UV – *HPLC	^{1}D – *HPLC	^{2}D – *HPLC
Alfoxil ® 500 Mg Tablet	0.08	0.30	0.95
Amoksina® 500 Mg Tablet	0.18	1.65	0.96
Atoksilin® 500 Mg Tablet	1.72	0.34	0.29
Demoksil® 500 Mg Tablet	0.63	0.14	1.51
Largopen® 500 Mg Tablet	0.01	1.16	0.96
Moksilin® 500 Mg Tablet	1.01	0.63	0.64
Remoksil 500 Mg Tablet	0.04	0.51	0.41
Topramoxin® 500 Mg Tablet	0.06	0.92	0.74
Alfoxil® 1000 Mg Flakon	1.88	0.60	0.13
Alfoxil® 125mg/5ml Suspension	2.02	0.62	0.22
Largopen® 125mg/5ml Suspension	1.72	1.30	2.10

*Tabulated value of *t* is 2.26 for P = 0.05

**Official method (USP XXIII)

CONCLUSION

Three methods, original UV spectrophotometry and, first and second derivative UV spectrophotometry, were developed and they were succesfully applied to the determination of **AMO** in 11 different formulations after their optimization and validation. Proposed methods are original and very simple methods for the determination of **AMO** in pharmaceutical preparations. These methods were found accurate and precise and, applicable for the routine analysis of the formulations. Good agreement was achieved in the assay results of pharmaceutical preparations, tablets, oral suspensions and flakons, widely used in Turkey, for three spectrophotometric methods proposed in the text. So, these methods can be apply accurately and precisely for the analysis of **AMO** in the pharmaceutical preparations mentioned above without prior separation procedure in place of HPLC methods (official methods) which are tedious, time consuming and expensive methods.

REFERENCES

1. **Mouayed, Q., Al-Abachi, H. H., Anas M. A.**, "Spectrophotometric determination of amoxicillin by reaction with N.N-dimethyl-p-phenylenediamine and potassium hexacyanoferrate(III)" *Anal. Chim. Acta*, 554(1-2), 184-189, **2005**.

2. **Duan, Y.L., Du, L.M., Chen, C.P.**, "Study on extractive spectrophotometry for determination of amoxicillin with ionic associate" *Guang Pu Xue Yu Guang Pu Fen Xi.* 25(11), 1865-1867, **2005**. PMID,16499066[PubMed-in process].

3. **Mohamed, G.G.,** "Spectrophotometric determination of ampicillin, dicluxacillin, flucloxacillin and amoxicillin antibiotic drugs, ion-pair formation with molybdenum and thiocyanate" *J.Pharm. Biomed. Anal.,* 24(4), 561-567, **2001.**.

4. **Belal, F., El-Kerdawy, M.M., El-Ashry, S.M., El-Wasseef, D.R.,** "Kinetic spectrophotometric determination of ampicillin and amoxicillin in dosage forms" *Farmaco,* 55(11-12), 680-686, **2000.**

5. **Fernandez-Gonzalez, A., Badia, R., Diaz-Garcia, M.E.,** "Comparative study of the micellar enhanced spectrophotometric determination of β-lactamic antibiotics by batch and flow injection analysis using a multisimplex design" *J. Pharm. Biomed. Anal.,* 29(4), 669-679, **2002**.

6. **Salem, H., Saleh, G.A.,** "Selective spectrophotometric determination of phenolic beta lactam antibiotics" *J. Pharm. Biomed. Anal*., 28(6), 1205-1213, **2002**.

7. **Nagaralli, B.S., Seetharamappa, J., Melwanki, M.B.,** "Sensitive spectrophotometric methods for the determination of amoxycillin, ciprofloxacin and piroxicam in pure and pharmaceutical formulations" *J. Pharm. Biomed. Anal.,* 29(5), 859-864, **2002**.

8. **Morgil, F.İ, Kestek, A.,** "Analytical investigation on penicillins-part VI determination of some selected semi-synthetic penicillins with ellman's reagent" *Gazi Univ. Eczacı. Fak. Derg..,* 7(1), 33-42, **1990.**.

9. **Samanidou, V.F., Evaggelopoulou, E.N., Papadoyannis, I.N.,** "Development of a validated HPLC method for the determination of four penicillin antibiotics in pharmaceuticals and human biological fluids" *J. Sep Sci.,* 29(11), 1550-1560, **2006**.

10. **Hong, C.C., Lin, C.L., Tsai, C.E., Kondo, F.**, "Simultaneous identification and determination of residual penicillins by use of high-performance liquid chromatography with spectrophotometric or fluorometric detectors" *Am. J. Vet. Res.,* 56(3), 297-303, **1995**.

11. **Lebelle, M.J., Wilson, W.L., Lauriault. G.,** "High-performance liquid chromatographic determination of amoxycillin in pharmaceutical dosage forms" *J. Chromatogr.,* 202(1), 144-147, **1980.**

12. **El Walily, A.F., Gazy, A.A., Belal, S.F., Khamis, E.F.,** "Selective spectrofluorimetric determination of phenolic beta-lactam antibiotics through the formation of their coumarin derivatives" *J. Pharm. Biomed. Anal.,* 20(4), 643-653, **1999**.

13. **Cao, W., Yang, J.H., Sun, C.X., Chen, Y.J., Gao, Q.F.,** "Flow injection chemiluminescence determination of amoxycillin using potassium permanganate and formaldehyde system" *Luminescence,* 20(1), 20-24, **2005**.

14. **Li, Y., Tang, Y., Yao, H., Fu, J.,** "Determination of ampicillin and amoxycillin by flow injection
chemiluminescence method based on their enhancing effects on the luminol-periodate reaction" *Luminescence,* 18(6), 313-317, **2003.**

15. **Li, Y., Lu, J.,** "Chemiluminescence flow-injection analysis of beta-lactam antibiotics using the luminol-permanganate reaction" *Luminescence*, 21(4), 251-255, **2006**.

16. **Fernandez-Gonzalez, A., Badia, R., Diaz-Garcia, M.E.,** "Comparative study of the micellar enhanced spectrophotometric determination of β-lactamic antibiotics by batch and flow injection analysis using a multisimplex design" *J. Pharm. Biomed. Anal.,* 29(4), 669-679, **2002.**

17. **Sun, Y., Tang, Y., Yao, H., Li, Y.,** "Flow injection chemiluminescence analysis of some penicillins by their sensitizing effect on the potassium permanganate-glyoxal reaction" *Anal. Sci.,* 21(4), 457-460, **2005.**

18. **Garcia, M.S., Sanchez-Pedreno, C., Albero, M.I., Rodenas. V.,** "Determination of ampicillin or amoxycillin in pharmaceutical samples by flow injection analysis" *J. Pharm. Biomed. Anal.,* 12(12), 1585-1589, **1994.**

19. **Kapetanovic, V., Veselinovic, D.,** "Determination of the Ni(II)- Amoxycillin couple by derivative polarography" *Archiv der Pharmazie*, 321, 559-560, **1988.**

20. **Uslu, B., Biryol, İ.,** "Voltammetric determination of amoxicillin using a poly (N-vinyl imidazole) modified carbon paste electrode" *J. Pharm. Biomed. Anal.,* 20(3), 591-598, **1999.**

21. **Kılıç, E., Köseoğlu, F., Akay, M.A.,** "The non-aqueous titrimetric assay of selected antibiotics using tetra-N-butylammonium hydroxide as titrant" *J. Pharm. Biomed. Anal.*, 12(3), 347-352, **1994.**

22. **Liu, H., Wang, H., Sunderland, V.B.,** "An isocratic ion exchange HPLC method for the simultaneous determination of flucloxacillin and amoxicillin in a pharmaceutical formulation for injection" *J. Pharm. Biomed. Anal.*, 37(2), 382-395, **2005**.

23. **Wang, P., Qi, M., Sun, Y., Yang, J.,** "A liquid chromatographic method for simultaneous determination of amoxicillin sodium and sulbactam sodium in a combination formulation" *J. Pharm. Biomed. Anal.*, 36(3), 565-569, **2004.**

24. **Aghazadeh, A., Kazemifard, G.,** "Simultaneous determination of amoxycillin and clavulanic acid in pharmaceutical dosage forms by LC with amperometric detection" *J. Pharm. Biomed. Anal.*, 25(2), 325-329, **2001.**

25. **Abounassif, M.A.., Abdel-Moety, E.M., Mohamed, M.E., Gad-Kariem, R.A.,** "Liquid chromatographic determination of amoxycillin and clavulanic acid in pharmaceutical preparations" *J. Pharm. Biomed. Anal.*, 9(9), 731-735, **1991**.

26. **Menelaou, A., Somogyi, A.A., Barclay, M.L., Bochner, F.,** "Simultaneous quantification of amoxycillin and metronidazole in plasma using high-performance liquid chromatography with photodiode array detection" *J. Chromatogr. B Biomed. Sci. Appl.*, 731(2), 261-266, **1999**.

27. **Tsou, T. L., Wu, J.R., Young, C.D., Wang, T.M.,** "Simultaneous determination of amoxycillin and clavulanic acid in pharmaceutical products by HPLC with beta cyclodextrin stationary phase" *J. Pharm. Biomed. Anal.*, 15(8), 1197-1205, **1997**.

28. **Wang, P., Qi, M., Sun, Y., Yang, J.,** "A liquid chromatographic method for simultaneous determination of amoxicillin sodium and sulbactam sodium in a combination formulation" *J. Pharm. Biomed. Anal.*, 36(3), 565-569, **2004**.

29. **Indrayanto, G., Sa, T.K., Widjaja, S.,** "Simultaneous determination of amoxicillin trihydrate and clavulanate potassium in pharmaceutical preparations by thin-layer chromatography/densitometry" *J. AOAC Int.*, 83(6), 1493-1496, **2000**.

30. **Pajchel, G., Pawlowski, K., Tyski, S.,** "CE versus LC for simultaneous determination of amoxicillin/clavulanic acid and ampicillin/sulbactam in pharmaceutical formulations for injections" *J. Pharm. Biomed. Anal.*, 29(1-2), 75-81, **2002.**.

31. **Pasamontes, A., Callao, M.P.,** "Sequential injection analysis for the simultaneous determination of clavulanic acid and amoxicillin in pharmaceuticals using second-order calibration" *Anal. Sci.*, 22(1) , 131-135, **2006**.

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