RESEARCH ARTICLE

Simultaneous Spectrophotometric Determination of phenylephrine hydrochloride and Amoxicillin via Derivative Spectrophotometry

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Received: 14th July, 19; Revised: 21th August, 19, Accepted: 12th September, 19; Available Online: 20th September, 2019

ABSTRACT

Simultaneous determination of phenylephrine hydrochloride and Amoxicillin via using derivative spectrophotometry is proposed in this work. The recommended derivative spectrophotometric method is suitable for the simultaneous analysis of univariate method due to its simplicity, low coast and short analysis time, nevertheless the effects of several instrument parameters on the derivative spectra causes a limitation in its application. The suggested method is simple, fast, inexpensive, and non-destructive and shows good linearity and sensitivity. The recommended method enables the estimation of the cited drugs either in the laboratory prepared as a single or in pharmaceutical formulations without prior separation.

Keywords: Amoxicillin, Derivative spectrophotometry method, Determination, Phenylephrine hydrochloride, Spectrophotometry.

International Journal of Drug Delivery Technology (2019); DOI: 10.25258/ijddt.9.3.32

How to cite this article: Aljeboree, A.M. and Alshirifi, A.N. (2019). Simultaneous Spectrophotometric Determination of phenylephrine hydrochloride and Amoxicillin via Derivative Spectrophotometry. International Journal of Drug Delivery Technology, 39(3): 502-508.

Source of support: Nil.

Conflict of interest: None

INTRODUCTION

Phenylephrine hydrochloride (HCl) is a pharmacological agent usually used in the practice of ophthalmology to reinforce the mydriatic effect of other cycloplegic agents such as tropicamide. It can also be used alone to keep the pupil dilated while keeping active accommodation, which is useful in the clinical evaluation of the accommodation mechanism.[1-3]

AMX is a semi-synthetic β-lactam antibiotic belonging to the group of penicillins. The chemical structure of amoxicillin consists of d-4-hydroxyphenylglycine side chain attached to 6-aminopenicillanic acid (6-APA) moiety. Because of its broad spectrum of bactericidal activity and, therefore, widespread use in medicines, various preparations of this drug alone including capsules, tablets, powder for oral suspension, and injections.[4,5]

Derivative spectroscopy is an analytical technique based on differentiation of the original, zero-order spectrum. The result of derivatization is called the derivative spectrum, which represents the values of absorbance differentials as a function of wavelength (λ), and it can be expressed as:

$dnA/d\lambda n = nDx,\lambda = f(\lambda)$

Where n denotes the derivative order, nDx, λ represents the value of the n-order derivative (i.e., the derivative amplitude) of the absorption spectrum of the analyte (x) at the given wavelength (λ), A-absorbance.[6] Derivative spectra often yield a characteristic profile where refined changes of curvature and gradient in the zero-order spectrums are observed as distinctive bipolar functions. The first derivative represents the gradient at all points of the spectrum and can be used to detect hidden peaks since dA/d λ is equal to zero at peak maxima. This bipolar function is typical of all odd-order derivative spectra. The distinctive feature of the second derivative spectrum (as well as all even-order derivatives) is a negative peak with a minimum at the λmax of the normal spectrum.[7-9]

Therefore, the purpose of the present work is to study the utility of the derivative spectrophotometric method in the estimation of the two drugs in bulk and pharmaceutical dosage.

EXPERIMENTAL

Instrumentation

All absorption spectra were recorded by U.V.-visible double beam 1800 Shimadzu (Kyoto-Japan) spectrophotometer with 1 cm quartz cells on a range of 200-300 nm with a scan speed of 5 $nm.sec^{-1}$, averaging of 1.0 nm, bandwidth of 1.8 nm, and data interval of 0.5 nm. The resulted absorption data were digitalized, plotted, and manipulated by Shimadzu 1800 software (UVProb 2.34) to obtain the first and second-order derivatives.

Materials

The PHE and AMXas the powder used in this study received in pure form 99.99% from the State Company for Drug Industries

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and Medical Appliances Samara-Iraq (SDI). Pharmaceutical formulations evaluated in this work were obtained from local pharmacies; The phenylephrine hydrochloride: Syrup (Soolan) (2.5mg /5mL) Alkaleej company of Pharmaceuticals Industries, Syrup (Norex) (5mg/5mL) SDI-Iraq , Capsules 10mg Tehran-Iran, Amoxycillin Capsules 250mg SDI-Iraq , Amoxmark Capsules 500mg Aurobindo Pharma–India, Neomox Syrup 250mg/5mL Neopharma -Abu Dhabi, UAE.

Preparation of standard solutions and calibration

Stock standard solutions containing 1g phenylephrine hydrochloride or Amoxicillin, were dissolved in 1000mL distilled water. Standard solutions of both drugs were prepared individually by dilution of the stock solutions with distilled water for spectrophotometric methods to obtain concentration range of 2–150 mg/L for phenylephrine hydrochloride and 2–240 mg/L for amoxicillin respectively.

The absorption spectra of phenylephrine HCl and amoxicillin and their mixture were recorded against distilled water as a blank. The absorption spectrum of phenylephrine HCl has a maximum wavelength of absorption at 272 nm, and the absorption spectrum of Amoxicillin which appears

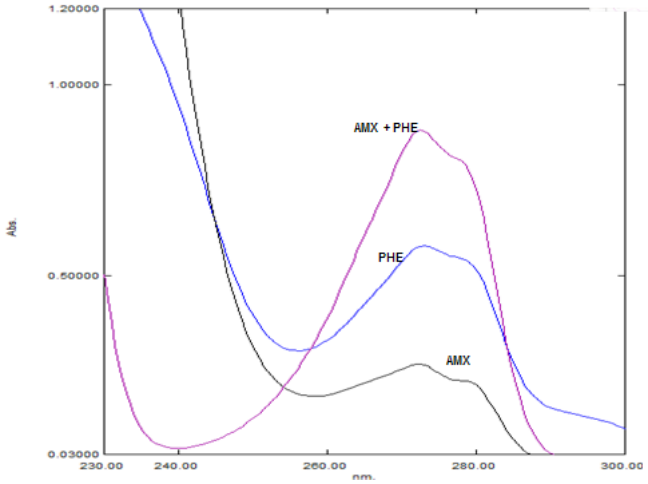

Figure 1: Absorption spectra of (–) 100 mg. L^{-1} phenylephrine HCl , (–) 100 mg. L^{-1} amoxicillin and (–) a mixture of 100 mg.L^{-1} phenylephrine HCl, 100 mg. L^{-1} amoxicillin

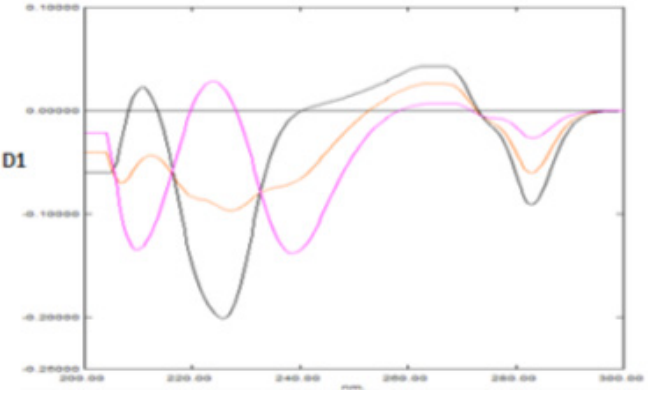

Figure 2: First derivative spectra of (–) 100 mg. L^{-1} phenylephrine HCl , (–) 100 mg. L^{-1} amoxicillin and (–) a mixture of 100 mg. L^{-1} phenylephrine HCl,100 mg.L^{-1} amoxicillin.

absorption maxima at 278nm in addition to the absorption spectrum of a mixture of two drugs which show a maximum wavelength of absorption at 272 nm which is related to the absorption maxima of the two compounds. Figure 1 displays the absorption spectrum of phenylephrine HCl, and Amoxicillin and drugs mixture.

RESULT AND DISCUSSION

First and Second Derivative Modes

The large overlap of the spectra of phenylephrine HCl and amoxicillin are obvious. Therefore, the direct determination of using zero-order absorption measurements, when they are present in the same solution, is very difficult using conventional methods.

The derivative spectrophotometric technique, as mentioned before, is of a particular utility in the determination of the concentration of a single component in such mixtures, with significant spectral overlapping. For this reason, derivative spectrophotometric methods have been applied. Both first and second-order modes were tested. The results obtained show that these techniques could successfully applied when the measurements are carried out under optimum selected conditions. To select derivative order, the first and second derivative spectra of phenylephrine HCl and amoxicillin were recorded.[10] The investigation reveals that first and second-order spectra were simple and gave results of the highest accuracy and detection limits. The first and second-order derivative spectra of phenylephrine HCl, and amoxicillin and their mixture are shown in Figures 2 and 3 respectively.

Calibration Curves for phenylephrine HCl , amoxicillin

To determine the values of derivative spectra, graphical techniques used zero-crossing have been used via UV-spectrophotometric method for qualitative analyses of phenylephrine HCl and amoxicillin individually. In zero-crossing technique, measurement of the absolute value of the total derivative spectrum at an abscissa value corresponding to the zero-crossing wavelength of the derivative spectra of individual components, which should be only a function of the concentration of other components.[11,12] The calibration curves were constructed by plotting the graphically measured

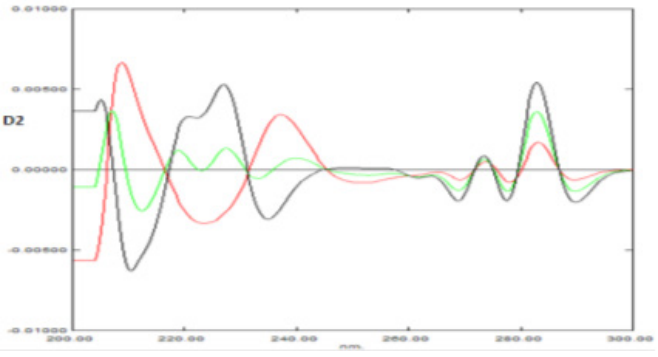

Figure 3: Second derivative spectra of (–) 100 mg. L^{-1} phenylephrine HCl, (–) 100 mg. L^{-1} amoxicillin and (–) a mixture of 100 mg. L^{-1} phenylephrine HCl, 100 mg. L^{-1} amoxicillin

(nm) amplitudes of the zero first and second-order derivatives spectra vs. the corresponding concentrations of the examined drugs. Figures 4–5 show first-order spectra for sets of phenylephrine HCl, amoxicillin, solutions containing various amounts of (2-150 mg. L^{-1}), (2-250) at the same order. In the first derivative techniques, the concentration of phenylephrine HCl varied, at zero crosses (228 and 258 nm) and amoxicillin (241nm).[13] The calibration curves for the phenylephrine HCl and amoxicillin were constructed at the mentioned wavelengths by plotting the values of zero-cross against the phenylephrine HCl, amoxicillin concentration as shown in Figures 6 and 7 at the same order.

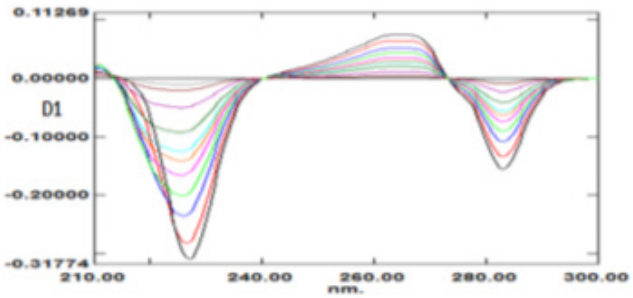

Figure 4: First derivative spectra of (2-150 mg. L^{-1}) PHE in distilled water

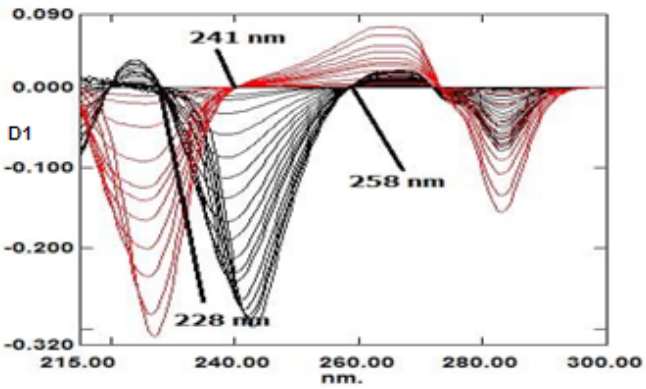

Figure 6: First derivative spectra of (2-150 mg. L^{-1}) PHE and (2-240 mg. L^{-1}) AMX in distilled water.

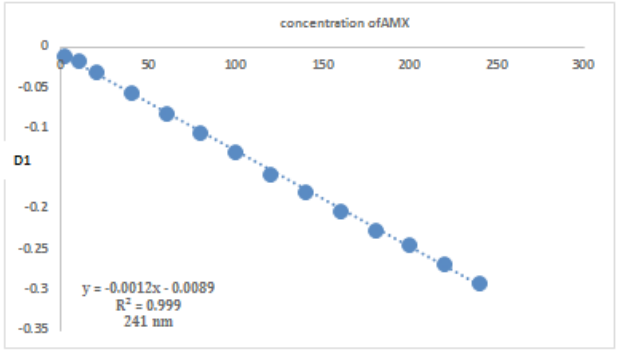

Figure 8: Calibration curve obtained via first mode derivative of AMX for height measurements at zero cross at 241nm.

Moreover, the second derivative spectra of same sets of solution (i.e., containing (2-100 mg. L^{-1}) phenylephrine HCl and (2-160) amoxicillin were also recorded and attempts were made to utilize them for finding the concentrations of the drug. Figures 8 and 9 show the D2 spectra for different concentrations of phenylephrine HCl and amoxicillin.[14] The delicate inspection of the second derivative spectra obtained for the mentioned of phenylephrine HCl and amoxicillin shows that t zero-cross (223, 238, 275 and 277 nm) and (226 nm) at the same order Calibration plots were constructed when the measured values were plotted against the concentration of t phenylephrine HCl and amoxicillin (Figures 10 and 11). Excellent calibration relations were obtained.

Therefore, calibration plot was constructed for the assay of PHE at 228,258 nm and AMX 241 nm by plotting the measured values of derivative D1 (as signals) versus the concentration of PHE, AMX drug A linear relation was obtained Figure 7-8,

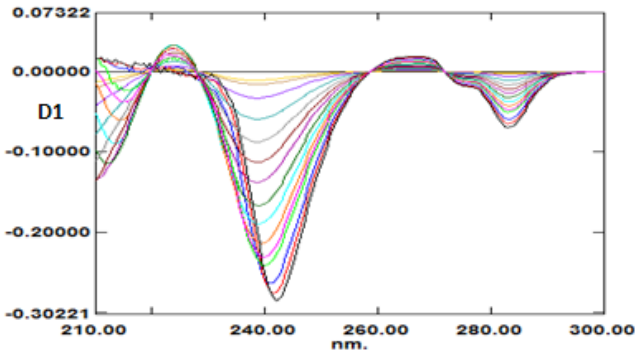

Figure 5: First derivative spectra of (2-240 mg. L^{-1}) AMX in distilled water.

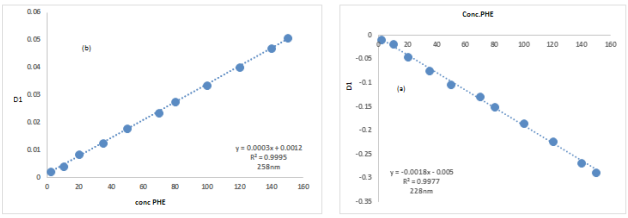

Figure 7: Calibration curve obtained via first mode derivative of PHE for height measurements at zero cross at (a)228, (b)258 nm.

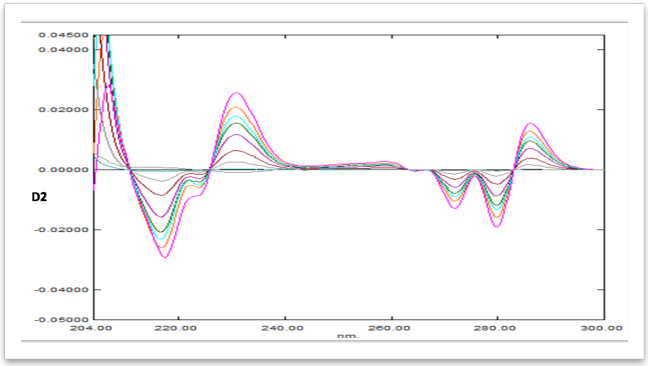

Figure 9: Second derivative spectra of (2-100 mg. L^{-1}) PHE in distilled water

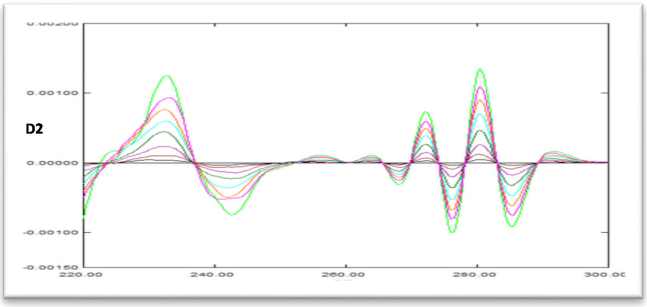

Figure 10: Second derivative spectra of (2-160 mg. L^{-1}) AMX in distilled water.

from which the regression equation and coefficient of determination (R^2) were calculated.

For the second derivative technique, the spectra were recorded for the same previous solutions of PHE and AMX . It is obvious from Figure 9–11

Some analytical characteristics, namely Beer's law limit, detection limit, slope, intercept, and correlation coefficient for the determination of PHE, AMX, in each derivative mode, were calculated, and the results are reported in Table 1.

Selectivity

Selectivity of the methods was achieved by the analysis of different laboratory prepared of PHE and AMX within

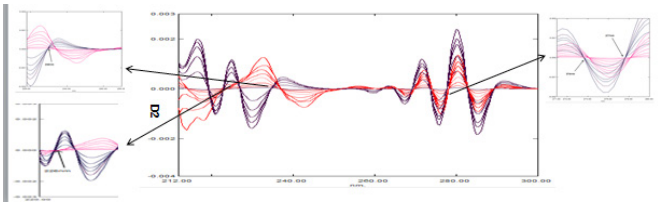

Figure 11: Second derivative spectra of (2-100 mg. L^{-1}) PHE and (2-160 mg. L^{-1}) AMX in distilled water

the linearity range, including the ratio present in the pharmaceutical dosage form. Satisfactory results were obtained as shown in Table1.

Stability

PHE and AMX working standard solutions in distilled water showed no spectrophotometric changes up to 2 weeks when stored at room temperature.

Accuracy and precision

The accuracy and precision of the determination of PHE and AMX via the proposed methods which were established by calculating the values of percentage of the relative error (E %) and relative standard deviation percent (RSD %), for different levels of analytes concentrations in the range of 20, 80, 100 mg. L^{-1} at the same day. The calculated analytical results show good accuracy with reasonable precision of the proposed methods, as reported in Table 2.

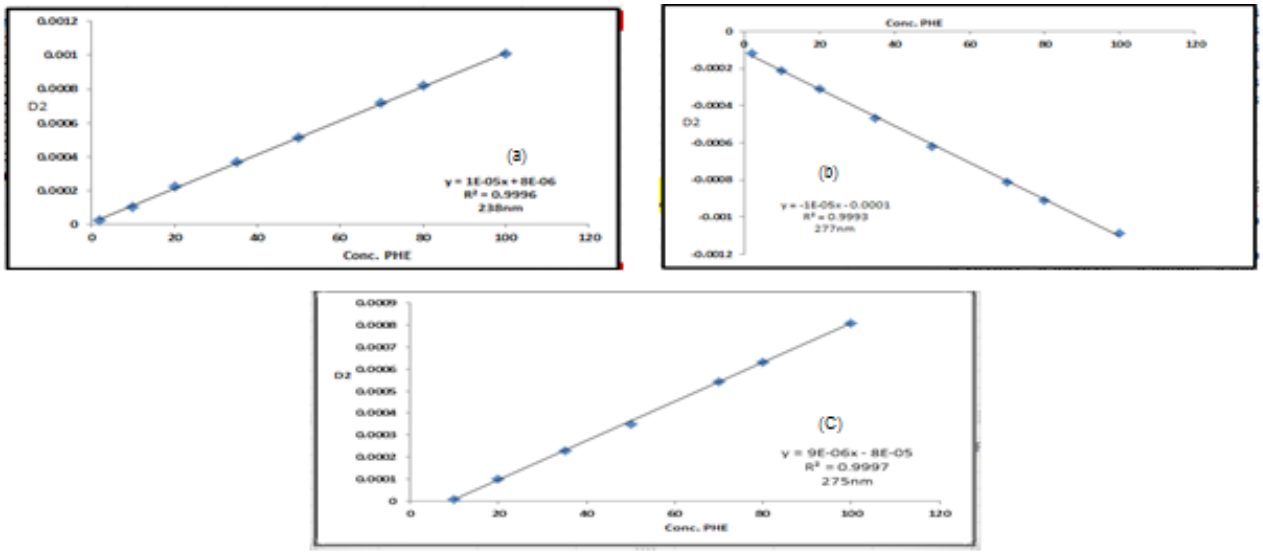

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Figure 12: Calibration curve obtained via second mode derivative of PHE for height measurements at zero cross at(a) 238,(b) 277,(c) 275 nm.

Table 1: Analytical parameters for the determination of phenylephrine HCl and amoxicillin using first, second derivative spectrophotometric techniques.

Drug	*Taken range (mg. L^{-1})*	*Derivative Mode*	*λ(nm)*	*Regression Equation*	R^2	*LOD (mgL-1)*	*LQD (mg. L^{-1})*
phenylephrine	2-150	D1 (*zero cross*)	228	y = 0.0018x - 0.05	0.9977	0.003185	0.010615
		D1 (*zero cross*)	258	y = -0.0003x- 0.0012	0.9995	0.01334	0.04444
	2-100	D2 (*zero cross*)	238	$y = 0.00001x - 8*10^{-6}$	0.9996	0.02636	0.08788
		D2 (*zero cross*)	275	$y = 9*10^{-6}x - 8*10^{-5}$	0.9997	0.12802	0.42673
		D2 (*zero cross*)	277	y = -0.00001 x - 0.0001	0.9985	0.10172	0.3390
Amoxicillin	2-240	D1 (zero cross)	241	y = - 0.0012x - 0.0089	0.999	0.004826	0.01608
	2-160	D2 (zero cross)	226	y = 0.00006x - 0.0003	0.9998	0.08387	0.2795

Application of the method in the assay of (Sharp, Capsules)

The proposed derivative spectrophotometric techniques calibration methods were applied for the determination of PHE and AMX drugs in their combined pharmaceutical formulation (Sharp, Capsules), as shown in Table 3. It shows that the developed methods are accurate and specific for the determination of the cited drugs in the presence of dosage. Table 3 shows the values of recovery percentage obtained for the analyzed samples by the application of the mentioned derivative techniques on the pharmaceutical formulations.

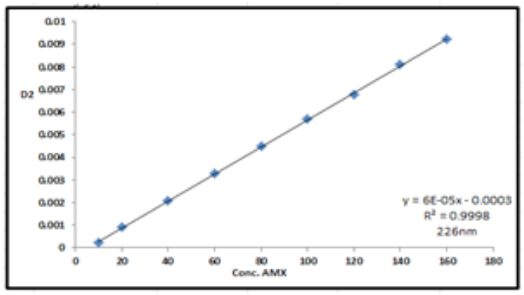

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Figure 13: Calibration curve obtained via second mode derivative of AMX for height measurements at zero cross at 226nm .

Table 2: Evaluation of accuracy and precision for the determination of AMX and PHE via derivative spectrophotometry.

			conc.(mg. L^{-1})				
Drugs	*Order of derivative*	*λ(nm)*	*Taken*	*Found*	*E%*	*Recovery*	*R.S.D%*
phenylephrine HCl	First	228	20	20.0611	0.3055	100.305	1.7546
		258		19.74	-1.3	98.7	0.7603
	second	238		20.3	1.5	101.5	1.1376
		275		20.111	0.555	100.55	0.0956
		277		20.1	0.9166	100.916	0.5243
	First	228	80	80.8888	1.1111	101.1111	0.6343
		258		79.3333	-0.833	99.1666	0.48504
	second	238		79.3	-0.87	99.12	0.2912
		275		80.11	0.1388	100.138	0.4804
		277		79.6666	-0.416	99.5833	0.24155
	First	228	100	99.3333	-0.666	99.333	0.3873
		258		99.6666	0.3333	99.666	0.193
	second	238		100.3	0.3	100.3	0.1151
		275		100.2	0.2	100.2	0.1152
		277		100.16	0.1666	100.16	0.0960
Amoxicillin	First	241	20	19.9166	-0.416	99.5833	0.2415
	second	226		20. 166	0. 833	100.83	5.248
	First	241	80	81.25	1.5625	101.562	0.8881
	second	226		81.666	2.083	102.08	0.2356
	First	241	100	100.583	0.5833	100.583	0.3347
	second	226		101.66	1.6666	101.66	0.9494

Table 3: Statistical validation data for quantitative assessment of commercial (Sharp, Capsules) formulation for PHE drug.

	Order of derivative	*Samples*	*λ(nm)*	*conc.(mg. L^{-1})*		*E%*	*Recovery%*	*R.S.D%*
				Taken	**Found*			
		Amoxycillin Capsules 250mg			19.8333	-0.8333	99.1667	0.4851`
	First	Amoxmark Capsules 500mg	241		19.9166	-0.4166	99.5833	0.24155
		NeomoxSyrup 250mg /5ml		20	20.0833	0.4166	100.416	0.2395
		Amoxycillin Capsules 250mg			20.3666	1.8333	101.833	1.0393
	second	Amoxmark Capsules 500mg	226		20.0166	0.08331	100.0833	0.04796
		NeomoxSyrup 250mg /5ml			20.0185	0.0925	100.092	0.0529
		Amoxycillin Capsules 250mg			79.25	-0.9375	99.0625	0.5463
Amoxicillin	First	Amoxmark Capsules 500mg	241		80.0833	0.1041	100.104	0.06
		NeomoxSyrup 250mg /5ml		80	80.833	1.0411	101.041	0.5951
		Amoxycillin Capsules 250mg			80.1667	0.2083	100.208	0.1198
	Second	Amoxmark Capsules 500mg	226		80.666	0.8333	100.833	0.4771
		NeomoxSyrup 250mg /5ml			81.666	2.0833	102.0833	1.1775
		Amoxycillin Capsules 250mg			100.0833	0.0833	100.0833	0.04806
	First	Amoxmark Capsules 500mg	241		100.916	0.9166	100.916	0.5243
		NeomoxSyrup 250mg /5ml		100	101.008	1.0083	1010083	0.5761
		Amoxycillin Capsules 250mg			99.1667	-0.8333	99.1667	0.4851
	second	Amoxmark Capsules 500mg	226		99.833	-0.166	99.833	0.0963
		NeomoxSyrup 250mg /5ml			100.1833	0.1833	100.1833	0.1056

Table 4: Statistical validation data for quantitative assessment of commercial (Sharp, Capsules) formulation for AMX drugs .

				conc.(mg. L^{-1})				
Drugs	*Order of derivative*	*Samples*	*λ(nm)*	*Taken*	**Found*	*E%*	*Recovery%*	*R.S.D%*
phenylephrine	***First***	PHE Syrup (Soolan) (2.5mg /5mL)	228	20	19.4444	-2.7777	97.2222	1.6495
			258		19.3333	-3.3333	96.6666	1.99081
		PHE Syrup (Norex) (5mg/5mL)	228		19.5055	-2.4722	79.5277	1.4634
			258		20.4333	2.1666	102.166	0.12282
		PHE Capsules 10mg	228		19.3889	-3.0555	96.9444	1.8197
			258		19.3	-3.5	96.5	2.09401
	second	PHE Syrup (Soolan) (2.5mg /5mL)	238		19.6	-2	98	1.1780
			275		20.0333	0.1666	100.166	0.0958
			277		20.1833	0.9166	100.916	0.5241
		PHE Syrup (Norex) (5mg/5mL)	238		20.4	2	102	1.1318
			275		19.666	-1.666	98.333	0.9783
			277		20.0333	0.1666	100.166	0.0959
		PHE Capsules 10mg	238		20.2	1.00	101	0.5693
			275		20.0033	0.01666	100.016	0.00961
			277		20.0332	0.01667	100.1	0.09599
	First	PHE Syrup (Soolan) (2.5mg /5mL)	228	80	80.5555	0.6944	100.6944	0.3981
			258		80.0666	0.0833	100.0833	0.0480
		PHE Syrup (Norex) (5mg/5mL)	228		81.1764	1.4705	101.4706	0.8367
			258		80.4333	0.5416	100.5417	0.311
		PHE Capsules 10mg	228		80.4705	0.5882	100.588	0.3376
			258		79.7333	-1.2	98.8	0.7012
	second	PHE Syrup (Soolan) (2.5mg /5mL)	238		79.6	-0.5	99.5	0.2900
			275		79.3333	-0.8333	99.1666	0.48504
			277		79.6666	-0.41667	99.5833	0.2415
		PHE Syrup (Norex) (5mg/5mL)	238		80.22	0.275	100.275	0.15831
			275		79.666	-0.4166	99.583	0.24155
			277		79.8333	-0.2083	99.791	0.1205
		PHE Capsules 10mg	238		80.18	0.225	100.22	0.1290
			275		80.033	0.04166	100.041	0.0246
			277		80.0166	0.0208	100.02	0.01202
	First	PHE Syrup (Soolan) (2.5mg /5mL)	228	100	99.4444	-0.5555	99.4444	0.3225
			258		99.6666	-0.3333	99.6666	0.193098
		PHE Syrup (Norex) (5mg/5mL)	228		100.588	0.5882	100.588	0.3376
			258		99.3666	-0.6333	99.3666	0.3679
		PHE Capsules 10mg	228		98.9529	-1.047	98.9529	0.6109
			258		99	-1	99	0.5831
			238		100.2	0.2	100.2	0.11517
			275		99.6666	-0.3333	99.666	0.19304
			277		100.1667	0.1666	100.166	0.096
	Second	PHE Syrup (Norex) (5mg/5mL)	238		100.2	0.2	100.2	0.1151
			275		100.033	0.0333	100.033	0.0192
			277		101.666	1.666	101.666	0.9464
		PHE Capsules 10mg	238		100.2	0.2	100.2	0.1152
			275		103	3	103	0.1346
			277		101.683	1.6833	101.683	0.9557

The results of the analysis were satisfactory, i.e., precise and accurate, as indicated by the excellent recovery percent.15 In addition, the results are in respectable agreement with the label claims, and this indicates the applicability of the proposed methods for the simultaneous estimation of the cited drugs in real samples (Tables 3 and 4).

CONCLUSION

Quick and accurate resolution of PHE, and AMX by using derivative spectrophotometric method was performed. PHE and AMX were determined by means of first (D1), second (D2) derivative spectrophotometric methods, The absorption spectrum of phenylephrine HCl has maximum wavelength of absorption at 272 nm, and the absorption spectrum of Amoxicillin which appears absorption maxima at 278 nm in addition to the absorption spectrum of mixture of two drugs which show a maximum wavelength of absorption at 272nm which is related to the absorption maxima of the two

compounds. The recommended methods were verified using a secondary laboratory prepared and then successfully applied for the pharmaceutical formulations analysis of the cited drugs.

REFERENCE

1. Aseel M Aljeboree , Abbas Noor Alshirifi, (2018). *Spectrophotometric Determination of phenylephrine hydrochloride drug in the existence of 4- Aminoantipyrine: Statistical Study. International* Journal of Pharmaceutical Research, 10(4).
2. Aseel Musthaq Aljeboree, A.N.A., (2018). *Colorimetric Determination of phenylephrine hydrochloride drug Using 4-Aminoantipyrine: Stability and higher sensitivity* J. Pharm. Sci. & Res., 10(7): p. 1774-1779
3. Aseel M. Aljeboree, A.N.A., (2018). *Adsorption of Pharmaceuticals as emerging contaminants from aqueous solutions on to friendly surfaces such as activated carbon: A review* J. Pharm. Sci. & Res., 10(9): p. 2252-2257
4. Fazelirad, H., et al., (2015). *Preparation of magnetic multi-walled carbon nanotubes for an efficient adsorption and spectrophotometric determination of amoxicillin.* Journal of Industrial and Engineering Chemistry, 21: p. 889-892.
5. Rojanarata, T., et al., *A simple, sensitive and green bienzymatic UV-spectrophotometric assay of amoxicillin formulations.* Enzyme and Microbial Technology. 46(3): p. 292-296.
6. M. Gallignani, R.A.R., J. F. Ovalles, M. R.Brunettoa, (2014). *Transmission FTIR Derivative Spectroscopy Estimation of Furosemide in Raw Material and Tablet Dosage Form.* Acta Pharmaceutica Sinica B, 4(5): p. 376-383.
7. O. Zaporozhets, I.T.a.M.I., (2012). *Determination of 8 Diuretics and Probenecid in Human Urine by Gas Chromatography-Mass Spectrometry: Confirmation Procedure.* American Journal of Analytical Chemistry, 3: p. 320-327.
8. Al-Shaalan, N.H., (2010). *Determination of phenylephrine hydrochloride and chlorpheniramine maleate in binary mixture using chemometric-assisted spectrophotometric and high-performance liquid chromatographic-UV methods.* Journal of Saudi Chemical Society, 14: p. 15-21.
9. J. Karpinska, (2012). *Basic Principles and Analytical Application of Derivative Spectrophotometry.* InTech, book edited by Jamal Uddin, p. 253-256.
10. Mali, A.D., (2015). *Simultaneous Determination of Carvedilol and Hydrochlorothiazide in Pharmaceutical Dosage Form by First Order Derivative UV Spectrophotometry.* Int J Pharm Phrm Sci, 7(9): p. 371-374.
11. A. Ansary, M.M.A.-M., F. M. Abdel-Gawad, E. A. Mohamed and M. M. Khater, (2012). *Simultaneous Determination of Carvedilol and Hydrochlorothiazide in Tablets and Spiked Human Plasma using Derivative Spectrophotometry.* Pharmaceut Anal Acta, 3(9): p. 6.
12. W. Zhang, X.Z., L. Cai, R. Chen, Q. Zhang, X. Wang, (2015). *Determination of Levan from Bacillus licheniformis by Ultraviolet Spectrophotometry.* Tropical Journal of Pharmaceutical Research, 14(4): p. 679-685.
13. H. E. Lakiss, M.I., D. Luiza Baconi, D. Bălălău, (2012). *Derivative UV Spectrophotometry Used for the Assay of Diazepam from Human Blood Plasma.* FARMACIA, 60(4): p. 565-570.
14. Ibrahim N, R.M., Ibrahim A, Tawakkol S, Ali S., (2014). *Simultaneous determination of amlodipine besylate and atorvastatin calcium by using spectrophotometric method with multivariate calibration and hplc method implementing "design of experiment".* Int J Pharmacy Pharm Sci., 6(1): p. 4.
15. C. Bosch Ojeda, F.S.R., (2013). *Recent Applications in Derivative Ultraviolet/Visible Absorption Spectrophotometry: 2009-2011.* Microchemical Journal, 106: p. 1-16.