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Simultaneous determination of Amoxicillin and Clavulanate in combined tablets by non-derivative and derivative UV spectrophotometric techniques

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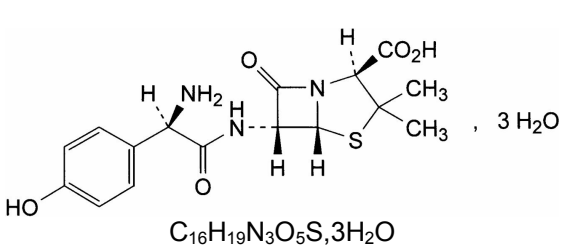
Abstract: Non-derivative and derivative UV spectrophotometric techniques using water as solvent have been developed for the simultaneous determination of amoxicillin and clavulanate in combined tablets. For UV spectrophotometric techniques, the concentrations of amoxicillin and clavulanate in conformity with the Lambert-Beer law are 100.0 – 160.0 µg/ml and 10.0 – 35.0 µg/ml, respectively. The derivative techniques include first-order derivative and ratio spectra first-order derivative; whereas the non-derivative techniques are absorbance ratio and compensation. UV spectrophotometric techniques have been extensively validated and compared to a HPLC technique showing their applicability in the routine analysis.
Keywords*: Amoxicillin; Clavulanate; UV derivative spectrophotometry; absorbance ratio; compensation; tablets.*

Introduction

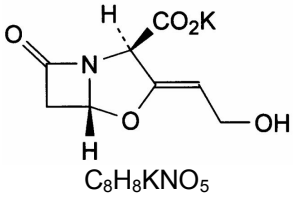
Disease-causing microbes that have become resistant to antibiotic therapy are recognized as an increasing public health problem. These bacterial disease burdens include paediatric infections and multiple drug resistance in both Gram-positive and Gram-negative microorganisms. The World Health Organization (WHO) in 2009 ranked acute respiratory (ARIs) as the leading cause of acute illnesses worldwide and the most important cause of infant and young children mortality [1]. This sets the scene for the requirement of antibacterials for community-acquired infections, especially for the treatment of ARIs antibiotic-resistant bacteria.

Amoxicillin (Figure 1a) is a betalactam antibiotic that has been extensively used to treat infections since 1970s [2]. Although it possesses good oral absorption and broad spectrum antimicrobial activity, amoxicillin is inactivated by betalactamases and therefore when administered by itself is not effective against bacteria (e.g. *Klebsiella* and *Proteus*) that produce these enzymes. The activity spectrum of amoxicillin may be extended by use with clavulanic acid (Figure 1 b), a potent betalactamase inhibitor with low antibacterial activity. This inhibitor not only reverses resistance to amoxicillin in betalactamase-producing strains of species otherwise sensitive but also enhances the activity of amoxicillin against several species not generally considered sensitive [3].



$C_{16}H_{19}N_3O_5S,3H_2O$

(a)



$C_8H_8KNO_5$

(b)

Figures 1: Structural formulae of (a) Amoxicillin trihydrate and (b) Potassium clavulanate.

Amoxicillin co-administered with clavulanic acid in tablet formulation in a ratio of amoxicillin (as the trihydrate) 2, 4, or 7 parts to 1 part of clavulanic acid (as the potassium salt) was launched as Augmentin in the UK in 1981[4].Almost 3 decades later, the combination of amoxicillin/clavulanate continues to play an important role in the treatment of a range of community-acquired infections, particularly those of the respiratory tract, in both adults and children worldwide [5].

In literature, these compounds (amoxicillin and clavulanic acid) in complex preparations could be co-assayed chromatographically[6–8] or spectrophotometrically [9–12]. It is noticeable that UV derivative spectrophotometry was also applied for the determination of clavulanic acid [13–15]and amoxicillin[16– 18] in the antibiotic pharmaceutical mixtures.

The aim of our study was to develop both non-derivative and derivative UV spectrophotometric techniques using water only as solvent, which differ from previous works, for the simultaneous determination of amoxicillin and clavulanate in their combined tablet. Furthermore, the spectrophotometric techniques were also compared with a modified United States Pharmacopoeia's HPLC technique[8] as a referee method to validate their applicability.

Experimental

Apparatuses and conditions

A UNICAM UV 300 double beam spectrophotometer (Thermo Spectronic, USA) with a fixed slit width (1.5 nm) connected to an IBM computer loaded with Thermo Spectronic VISION32 software and 1-cm quartz cell were used for the registration and treatment of absorption spectra. For all solutions, zero-order spectra were recorded over the range from 200.0 to 300.0 nm against a blank (water) at Intelliscan mode to enhance the signal-to-noise ratio of absorbance peaks without extended scan duration with a $\Delta\lambda$ = 0.1 nm (i.e. 30-120 nm/min). To get the best signal to noise ratio and resolution, spectra and their corresponding derivative ones were further smoothed by using Savitzky – Golay filter (order 3, number of coefficients 125).

High performance liquid chromatogram (HPLC) analysis was performed on an Agilent 1100 Series Diode-Array-Detector chromatograph (Agilent Technologies, USA) at ambient temperature. An Apollo silica (150×4.6 nm, 5μm) was used. The mobile phase with a flow rate of 2.0 $mLmin^{-1}$ was a mixture of phosphate buffer (0.78% w/v sodium dihydrogen orthophosphate monohydrate, adjusted to pH 4.4 with orthophosphoric acid) – methanol (95:5, v/v). Injection volume was 20 μL and detection wavelength was 220 nm.

Reagents and standard solutions

Amoxicillin trihydrate, AMO, (98.4%), potassium clavulanate, CLA, (84.0%) and all tablet excipients were kindly provided by Pharbaco Central Pharmaceutical Joint-Stock Company (Vietnam). De-ionized doubly distilled water was used throughout.

Stock solutions of AMO and CLA (1000 μg/ml) were prepared in water. Standard series of solutions were prepared in 25-mL calibrated flasks by using the same stock solutions and all dilutions freshly made.

Sample solutions

VIGENTIN, film-coated tablet commercially available in the domestic market (produced by Pharbaco Central Pharmaceutical Joint-Stock Company, Vietnam containing 500 mg amoxicillin and 125 mg clavulanic acid per tablet) was studied.

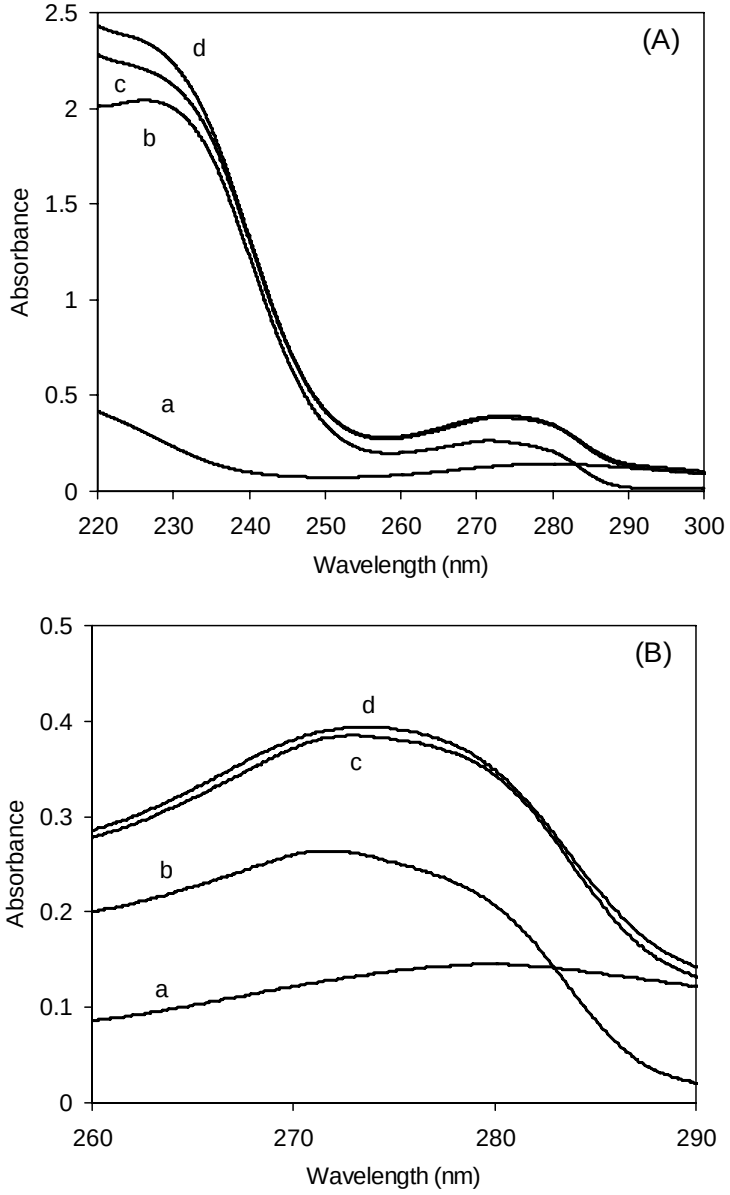
20 tablets were accurately weighed and powdered in a mortar. A mass corresponding to one tenth of a tablet was transferred to a 100-mL calibrated flask containing about 30 mL water, well shaken and ultrasonicated for 15 min. After the dissolution process, the solution was filtrated in a 100-mL calibrated flask through Whatman grade No. 42 filter paper. The residue was washed three times with 10 mL water and the volume completed to 100 mL with water. The resulting solution was further diluted to 1:5 in 25-mL calibrated flask with the same solvent for UV spectrophotometric measurement.

Results and discussion

Method development

Non-derivative UV spectrophotometric techniques

Figure 2 A shows the zero-order UV absorption spectra in water indicating that the two spectra of AMO and CLA overlapped greatly in the wavelength region studied. AMO exhibited a maximum at 270.4 nm, whilst CLA at 280.1 nm. It can be noted that the addition spectrum and mixture spectrum of corresponding concentrations were not completely identical over the range containing AMO and CLA maximum wavelengths (Figure 2 B). This eliminated the use of simultaneous equation technique [19] selecting extrema of the two compounds due to incorrect results potentially obtained.



Figures 2: (A) Zero-order spectra of 25 μg/ml CLA (a), 100 μg/ml AMO (b); addition spectrum (c) of (a) and (b), and spectrum of a mixture of 25 μg/ml CLA and 100 μg/ml AMO (d) in water and (B) corresponding zero-order spectra zoomed in.

Nevertheless, AMO and CLA were simultaneously determined in our study by other non-derivative techniques such as compensation and absorbance ratio. Unlike previously cited technique using derivative spectra [20, 21], the compensation technique in this study involves a comparison of several spectra (mixture – standard) using different concentrations of a standard solution as subtrahends. For any wavelength λ, the absorbance (A) of a mixture of two species X and Y (which do not interact with each other) is governed by the law of absorbance additivities: $A_m=A_X+A_Y$. If the absorption of Y is subtracted from A_m, the absorption characteristics of the mixture gradually approach that of X as C_Y increases. Finally, the absorption curve of mixture coincides with the absorption curve of X at the end-point, for which C_Y used as subtrahend is exactly the concentration of Y in the mixture. For a pure substance, the absorbance ratio at two wavelengths is constant over a certain range of concentration (i.e. independent of concentration and whether another absorbing component is present). Thus, the identification of Y is based on this ratio i.e. the absorbance ratio of the mixture is equal to that of pure Y meaning the concentration of Y in the sample solution is equal to that of pure Y. To determine this ratio, a series of solutions containing different concentrations of pure drugs, above and below that presented in the binary mixture solution were analyzed (Table 1).

Table 1: Experimental parameters calculated for the simultaneous determination of AMO and CLA in binary mixture by compensation technique.

	Concentration range (μg/ml)	Ratio	Mean (n=10)	RSD (%)
AMO	90-110	$A_{245.7}/A_{254.0}$	2,38	0.98
CLA	20-30	$A_{251.4}/A_{255.0}$	0.96	0.51

On the other hand, binary mixtures containing AMO and CLA could be analyzed by absorbance ratio technique [22]. The principle of this technique is based on the linear relationship between the absorbancy ratio value of a binary mixture and the relative concentration of such a mixture. The quantification analysis of AMO and CLA in a binary mixture are performed using the following equations:

$$C_1 = \frac{Q_1 - b_1}{a_1}\frac{A_{iso}}{a_{iso}} \times 10^3$$

$$C_2 = \frac{Q_2 - b_2}{a_2}\frac{A_{iso}}{a_{iso}} \times 10^3$$

where: $Q_1=A_1/A_{iso}$ for AMO, $Q_2=A_2/A_{iso}$ for CLA

C_1 and C_2: concentrations of AMO and CLA, respectively

A_{iso}: absorbance at isosbestic point (λ_{iso} = 251.9 nm)

a_{iso}: absorptivity at isosbestic point ($= \frac{A_{iso}}{C_1+C_2}$)

a_1: slope of regression equation (Q_1 versus $\frac{C_1}{C_1+C_2}$)

a_2: slope of regression equation (Q_2 versus $\frac{C_2}{C_1+C_2}$)

b_1 and b_2: intercept values of corresponding regression equations

A_1 and A_2: absorbances of mixture solution measured at AMO and CLA maximum wavelengths, 270.4 nm and 280.1 nm respectively

10^3: conversion factor of concentration unit from mg/ml to μg/ml

Derivative UV spectrophotometric techniques

Figure 3 displays the first derivative spectra of these pure drugs revealing that there existed two zero-crossing points at 244.9 nm and 272.0 nm for CLA and AMO respectively, and derivative values for each other at these wavelengths. The shape of the first derivative spectra was adequate for determining AMO in the presence of CLA and vice versa. This means AMO could be determined by the measurement of its derivative amplitude at 244.9 nm and in an analogous manner CLA at 272.0 nm.

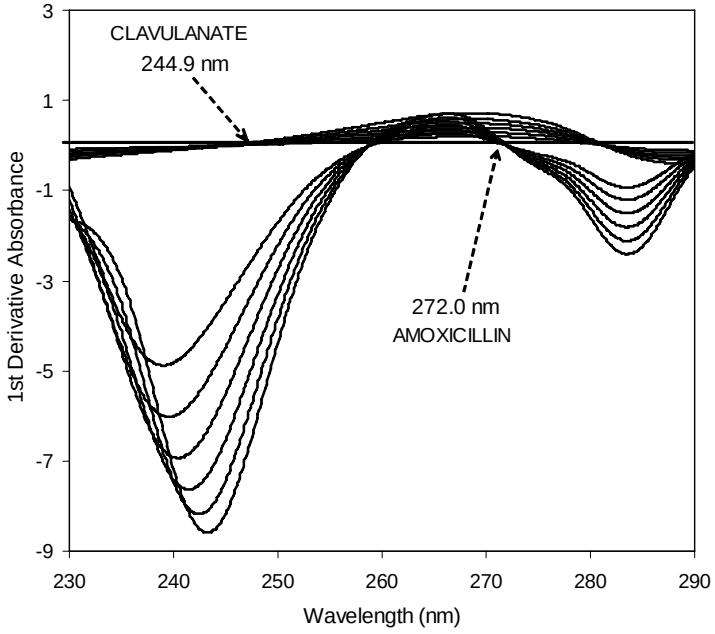
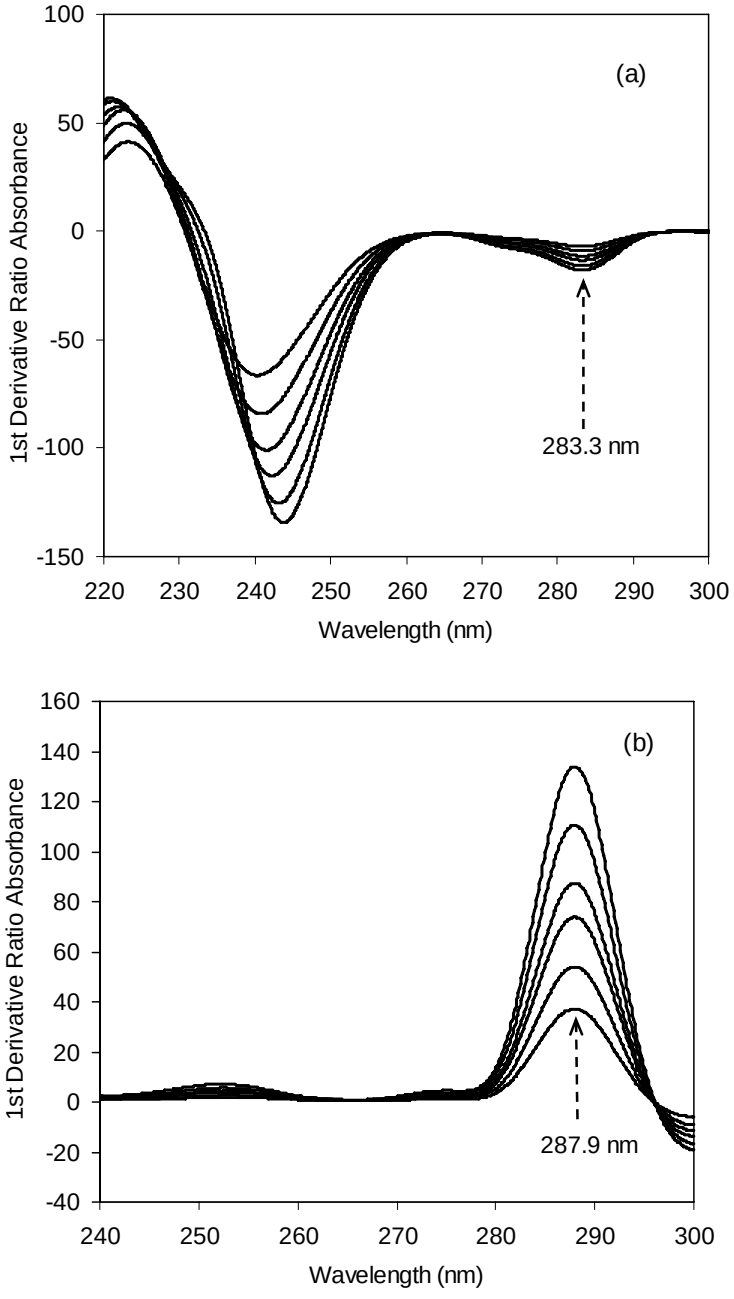


Figure 3: First-order derivative spectra of CLA (10.0 – 35.0 μg/ml) and AMO (60.0 – 160.0 μg/ml) in water. The dashed arrows indicate working wavelengths.

To optimize the simultaneous determination of AMO and CLA by using ratio spectra first-order derivative technique, the influence of divisor standard concentration was investigated with the concentration ranges for Lambert-Beer's law compliance of both drugs i.e. 100.0 – 160.0 μg/ml for AMO and 10.0 – 35.0 μg/ml for CLA. A standard spectrum of 10.0 μg/ml CLA was considered as suitable for the determination of AMO and a standard spectrum of 60.0 μg/ml AMO was suitable for CLA determination. The determination of each component was based on the proportionality of its concentrations to first-order derivative amplitudes at an extreme wavelength. For determining AMO and CLA, 283.3 nm and 287.9 nm were chosen as working wavelengths respectively (Figures 4 a, b).



Figures 4: Ratio spectra first-order derivatives of (a) mixtures of 60.0 – 160.0 μg/ml AMO and 25.0 μg/ml CLA using a 10.0 μg/ml CLA as divisor and (b) mixtures of 10.0 – 35.0 μg/ml CLA and 100.0 μg/ml AMO using a 60.0 μg/ml AMO as divisor in water.
The dashed arrows indicate working wavelengths.

Method validation and application

The validity and suitability of the proposed UV spectrophotometric techniques were assessed by accuracy (reported as percentage recovered), precision (reported as coefficient of variation, CV) and linearity (evaluated by regression equation). The proposed techniques were applied to simultaneously determine AMO and CLA in a synthetic mixture containing both analytes and excipients using the manufacturer's formula. The average percent recoveries obtained were 99.5 – 101.1 % for both AMO and CLA indicating the techniques' good accuracy.

The within-run precision (repeatability) of these techniques was evaluated by analyzing six replicates of the synthetic mixture. The within-laboratory precision (intermediate precision) of these techniques was also evaluated with this synthetic mixture being analyzed during six consecutive days. The low CV values (< 1.6 %) of intra-day and inter-day variations indicate the proposed techniques' good precision. A critical evaluation of the calibration graphs for UV derivative spectrophotometric determination was performed by the statistical analysis of the experimental data as shown in Table 2.

In our study, the applicability of the spectrophotometric techniques was also validated by comparison with the HPLC technique. Figure 5 displays a typical chromatogram of a commercial sample clearly showing that the two compounds, AMO and CLA, separated well under our chromatographic conditions (resolving power > 6.3, peak asymmetry factors < 1.1). This HPLC technique had good accuracy (100.7 – 100.9 % recovered) and precision (CV < 0.4 %) with the linearity ranges for AMO (100.0 – 400.0 μg/ml) and CLA (25.0 – 100.0 μg/ml).

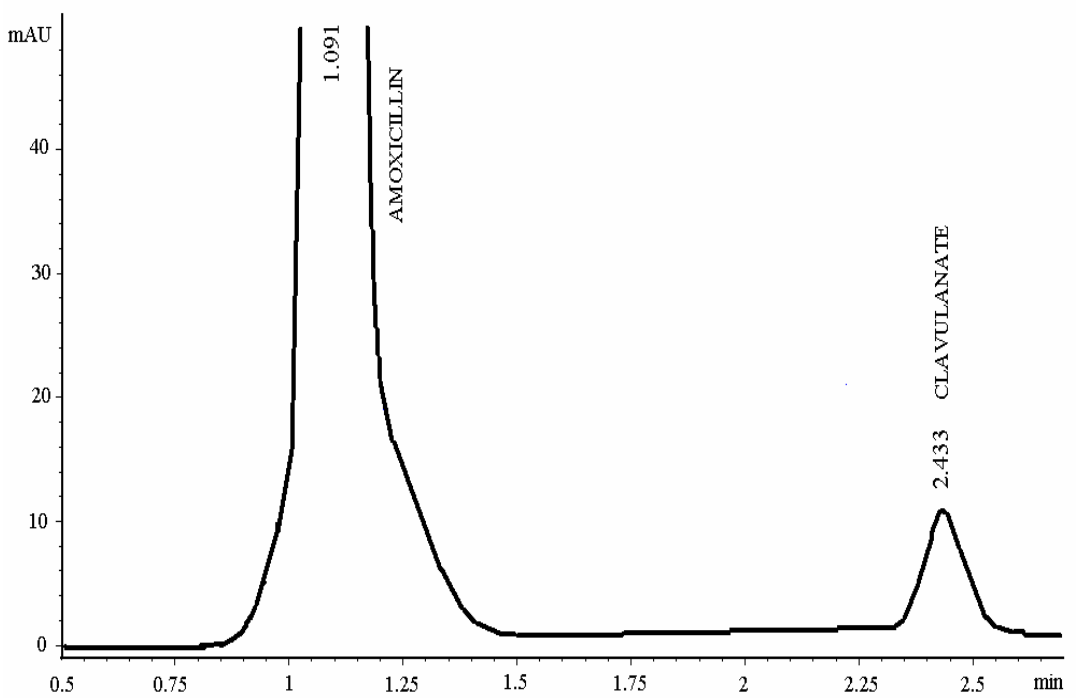


Figure 5: A typical chromatogram of a commercial sample (100.0 μg/ml AMO and 25.0 μg/ml CLA)

The proposed techniques were successfully applied to the simultaneous determination of AMO and CLA in their combined tablets. The spectrophotometric results were statistically compared with those obtained by the HPLC technique (Table 3). It is seen that at 95 % confidence level, there was no significant difference between the accuracy (evaluated by the Student t-value) and precision (evaluated by the variance ratio F-value) between the spectrophotometric techniques and HPLC one.

Table 2: Statistical analysis for the calibration graphs of AMO and CLA by use of UV derivative spectrophotometry.

	Analyte	Wavelength (nm)	Linearity range (μg/ml)	Linear aggression equation (n = 6)			
				Equation	r[a]	SE[b]	SE[c]	Sy.x[d]
Lambert-Beer law compliance	AMO	274.0	60.0 – 160.0	$Y = 0.0024C_{AMO} + 0.0185$	0.9999	0.0001	0.0015	0.0011
	CLA	280.1	10.0 – 35.0	$Y = 0.0130C_{CLA} – 0.0045$	0.9985	0.0004	0.0095	0.0080
First-order derivative	AMO	244.9	60.0 – 160.0	$Y = – 0.0504C_{AMO} – 0.4801$	– 0.9997	0.0007	0.0755	0.0460
	CLA	272.0	10.0 – 35.0	$Y = 0.0180C_{CLA} + 0.0036$	0.9998	0.0002	0.0045	0.0034
Ratio spectra first-order derivative	AMO	283.3	60.0 – 160.0	$Y = – 0.1098C_{AMO} – 0.1229$	– 0.9998	0.0011	0.1186	0.0721
	CLA	287.9	10.0 – 35.0	$Y = 3.4325C_{CLA} + 0.9938$	0.9982	0.1183	2.5090	1.8700

*a: correlation coefficient; b: standard error of slope; c: standard error of Y intercept; d: standard deviation of residuals from line.

Table 3: Assay results for the determination of AMO and CLA in VIGENTIN tablet, and their comparison with HPLC technique.

Analyte	Label claim (mg per tablet)	Recovery ± SD % (n = 6) and comparison with HPLC technique (p = 0.05, t = 2.228, F = 5.050)				
		HPLC	First-order derivative	Ratio spectra first-order derivative	Absorbance ratio	Compensation
AMO	500	99.68±0.73	99.21±1.32 t = 0.763 F = 3.270	100.14±1.46 t = 0.693 F = 4.000	100.42±1.17 t = 1.314 F = 2.569	99.45±0.85 t = 0.530 F = 1.356
CLA	125	99.28±0.89	99.72±1.64 t = 0.578 F = 3.398	99.93±1.39 t = 0.965 F = 2.439	98.75±1.68 t = 0.683 F = 3.565	99.05±0.54 t = 0.541 F = 2.716

Conclusion

Based on the data obtained in our study, the proposed UV spectrophotometric techniques are simple, rapid and precise. These UV spectrophotometric techniques are quite economical as proven by the use of water only as solvent. Both derivative and non-derivative techniques do not suffer from interference by excipients in the tablet formulation as confirmed by their recovery study and statistical comparison of assay results with the HPLC technique. It is worth mentioning the advantage of non-derivative techniques over derivative ones i.e. an ordinary spectrophotometer without a derivative mode is sufficient for the simultaneous determination of AMO and CLA. In conclusion, they can be exploited for the routine quality control of amoxicillin - clavulanic acid combined tablet, especially in the developing countries when HPLC machines often overcharged.

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References

1. http://www.who.int/vaccine_research/diseases/ari/en/index.html
2. Sutherland R., Croydon E.A.P and Rolinson G.N., Amoxycillin: a new semisynthetic penicillin, Br. Med. J., 1972, 3, 13 – 16.
3. Todd P.A. and Benfield P., Amoxicillin/clavulanic acid: an update of its antibacterial activity, pharmacokinetic properties and therapeutic use, Drugs, 1990, 39, 264–307.
4. White A.R., Kaye C., Poupard J., Pypstra R., Woodnutt G. and Wynne B., Augmentin (amoxicillin/clavulanate) in the treatment of community acquired respiratory tract infection: a review of the continuing development of an innovative antimicrobial agent, J. Antimicrob. Chemother., 2004, 53S, i3 – i20.
5. Alasdair M. G., Keith P.K. and George N.R., Introduction: historical perspective and development of amoxicillin/clavulanate, Int. J. Antimicrob. Agents, 2007, 30, S109 –S112.
6. Mohammad A.A, Ezzat M.A-M., Mohamed E.M. and El-Rasheed A.G-K., Liquid chromatographic determination of amoxycillin and clavulanic acid in pharmaceutical preparations, J. Pharmaceut. Biomed. Anal., 1991, 9, 731 – 735.
7. Mark F and Christopher R., Assay of amoxicillin and clavulanic acid, the components of Augmentin, in biological fluids with high-performance liquid chromatography, Antimicrob. Agents Chemother., 1982, 22, 753 – 762.
8. USP30-NF25, "Amoxicillin and clavulanate potassium tablets" and "Amoxicillin and clavulanate potassium for oral suspension" monographs, 2007, pp 1406 – 1407.
9. Ezzat M.A-M., Mohammad A.A., Mohamed E.M. and Nashaat A.K., Spectrophotometric determination of amoxycillin and clavulanic acid in pharmaceutical preparation, Talanta, 1989, 36, 683 – 685.
10. Ewa B-G, Determination of amoxycillin and clavulanic acid in some pharmaceutical preparations by derivative spectrophotometry, Mikrochim. Acta., 2001, 136, 31 – 34.
11. Muñoz de la Peña A., Espinosa-Mansilla A., Acedo Valenzuela M.I., Goicoechea H.C. and Olivieri A.C., Comparative study of net analyte signal-based methods and partial least squares for the simultaneous determination of amoxycillin and clavulanic acid by stopped-flow kinetic analysis, Anal. Chim. Acta, 2002, 463, 75 – 88.
12. Abdil Ö. and Ayşenur K., Comparative study of electrospray mass spectrometry and first derivative method and validation by HPLC method, J. Food and Drug Anal., 2007, 15, 118 – 125.
13. Murillo J.A., Lemus J.M. and Garcia L.F., Application of the ratio spectra derivative spectrophotometry to the analysis of cephradine and clavulanic acid in binary mixtures, Fresenius J. Anal. Chem., 1993, 347, 114 – 118.
14. Murillo J.A., Lemus J.M. and Garcia L.F., Resolution of binary mixtures of cephalothin and clavulanic acid by using first derivative spectrophotometry, Fresenius J. Anal. Chem., 1994, 349, 761 – 767.
15. Murillo J.A., Lemus J.M. and Garcia L.F., Simultaneous determination of the binary mixtures of cefsulodin and clavulanic acid by using first-derivative spectrophotometry, J. Pharmaceut. Biomed. Anal., 1995, 13, 769 – 776.
16. Murillo J.A., Rodríguez J., Lemus J.M. and Alañón A., Determination of amoxicillin and cephalexin in mixtures by second-derivative spectrophotometry, Analyst, 1990, 115, 1117 – 1119.
17. Ezzat M.A-M., Short communication: Spectrophotometric determination of amoxycillin and dicloxacillin in binary mixtures and in capsules, J. Pharmaceut. Biomed. Anal., 1991, 9, 187 – 190.
18. Akgül Y. and Mevlüt E, Second derivative spectrophotometric determination of amoxycillin in oral suspensions, Anal. Lett., 1991, 24, 2033 – 2041.
19. Glenn, A.L., The importance of extinction ratios in the spectrophotometric analysis of mixtures of

two known absorbing substances, J. Pharm. Pharmacol., 1960, 12, 595 – 608.
20. Wahbi A-A.M., El-Yazbi F.A., Barary, M.H. and Sabri S.M., Derivative spectrophotometric analysis of two-component mixtures using a compensation technique**,** Analyst, 1992, 117**,** 785 – 789.
21. Altun M.L. and Erk N., The rapid quantitative analysis of phenprobamate and acetaminophen by RP-LC and compensation technique, J. Pharmaceut. Biomed. Anal., 2001, 25, 85 – 92.
22. Enk N., Comparative study of the ratio spectra derivative spectrophotometry, derivative spectrophotometry and Vierordt's method applied to the analysis of lisinopril and hydrochlorothiazide in tablets, Spectrosc. Lett., 1998, 31, 633 – 645.

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