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Quantitative analysis of Amoxicillin and Dicloxacillin in Combined Dosage Form by First Derivative and Simultaneous Equation Method in Application to the determination of Content Uniformity

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Abstract

Two simple, accurate and precise spectrophotometric methods have been developed for the simultaneous estimation of Amoxicillin and Dicloxacillin. Method A was quantitative determination of Amoxicillin and Dicloxacillin by First Order Derivative zero crossing method. The first order derivative absorption at 231.02 nm (zero cross point of Dicloxacillin) was used for Amoxicillin and 246.26nm (zero cross point of Amoxicillin) for Dicloxacillin. Both the drugs obeyed the limit 15-35µg/ml (correlation coefficient r^2<1). No interference was found between the both determined constituents and those of matrix. Method B was developed for estimation of content uniformity of Amoxicillin and Dicloxaciliin in its combined tablet dosage form. The method involves solving the simultaneous equation using 245nm and 227nm as two wavelengths for Amoxicillin and Dicloxacillin respectively. From the results, it was concluded that all brands are within the limits of content uniformity (85-115%). 0.1 N sodium hydroxide was used as a solvent for both methods. Developed method was employed to determine the Amoxicillin and Dicloxacillin content in ten individual capsule units of four marketed formulations. Both the methods were validated statistically and recovery studies were carried out to confirm the accuracy of the methods.

Keywords: Amoxicillin, Dicloxacillin, First Derivative Spectroscopy, Content Uniformity, Simultaneous equation method.

1. Introduction

Amoxicillin (AMC) is oral semi synthetic amino penicillin similar to ampicillin. It is more stable to gastric acids and more bioavailable than other penicillin's and is a moderate-spectrum, bacterolytic, β-lactam antibiotic used to treat bacterial infections, skin infections, urinary tract infections caused by susceptible micro organisms. It is chemically 6-[D-(-)-α-amino-p-hydroxy phenylacetamido) pencillanic acid]. Literature survey reveals that several analytical methods have been reported for the quantitative determination of AMC individually in pharmaceutical formulations or in combination or in biological fluids and they are HPLC[1,2], UV[3], Bioanalytical method[4,5], Spectrophotometry[6,7], Simultaneous Spectroscopy[8,9,10], Second order multivariate analysis[11], LC-MS/MS[12], LC-ESI-MS[13].

Dicloxacillin (DIC) is a narrow – spectrum β lactam antibiotic of the penicillin class. It is chemically (2S, 5R, 6R)-6-[(3-(2, 6-dichlorophenyl)-5-methyl-oxazole-4-carbonyl) amino]-3, 3-dimethyl-7-oxo-4-thia-1-azabicyclo (3.2.0) heptanes-2-carboxylic acid. It acts by inhibiting the synthesis of bacterial cell walls and active against β-lactamase producing organisms such as Staphylococcus aureas. It is used to treat infections caused by susceptible gram-positive bacteria. Literature survey reveals that it is official in United States Pharmacopoeia. Several analytical methods have been reported for the quantitative determination of DIC individually in pharmaceutical formulations or in combination and they are HPLC[14,15] and Spectroscopy[16]. The developed methods were more precise with 0.1N NaOH as solvent. The first derivative and content uniformity was determined at less linearity range.

2. Materials and methods

2.1. Instrumentation

Analysis was performed on double beam UV/Visible spectrophotometer (LABINDIA UV 3092) having two matched quartz cells with 1 cm path length. Weighing electronic balance (Shimadzu Model No EPA-151 WT -12) was used.

2.2. Reagents

Sodium hydroxide (0.1N NaOH): 4 gm of sodium hydroxide was dissolved in distilled water and made up to 1000 ml.

Four brands of Amoxicillin and Dicloxacillin combination capsules (250-250mg) are NOVACLOX (Mfg by Cipla), STARCLOX (Mfg by Invision Medi sciences Pvt Ltd.), BAMICILLIN (Mfg by Cruisa Pharmacia Pvt Ltd.), and AMPOXIN PLUS (Mfg by Unichem)

2.3. Standard stock solution (Amoxicillin & Dicloxacillin)

Standard stock solutions of AMC and DIC (1000µg/ml) were prepared by dissolving and diluting with 0.1 N NaOH and were further diluted with 0.1N NaOH to obtain final concentration of 100 µg/ml (working standard solution).

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3. Results and Discussion

3.1. Method A: First Derivative Spectroscopy

Suitable aliquot of standard stock solutions were diluted with 0.1N NaOH to obtain a concentration of 25 µg/ml and scanned in the range of 200-400 nm. The absorption spectra thus obtained were derivatized to first order. From the over line spectra of both the drugs (fig.1) wavelength was selected for quantization which is 231.02 nm (zero cross point of DIC) for AMC and 246.26 nm (zero cross point of AMC) for DIC.

Linearity for both the drugs was obtained in the concentration range of 15-35 µg/ml. For all the solutions the derivative spectra were obtained over 200-400 nm range. At 231.02 nm there were well developed first order derivative absorption spectra for varying concentrations of AMC for its determination(fig.2a) and no interference was observed with DIC as D1=0 (fig 2b). So any change in DIC concentration has no effect on quantitative determination of AMC.

To determine DIC the first order derivative spectra were used by making measurements at 246.26 nm (fig.3a) at which D1=0 for AMC. No interference was observed with AMC at different concentrations (fig.3b) for quantitative determination of DIC. Over line spectra of both the drugs were given in (fig.4)

The calibration curves were constructed by plotting drug concentration versus absorbance values of first derivative spectrum (D1) 231.02nm for AMC and 246.26 nm for DIC. Statistical data for calibration curves is depicted in (Table 1). The concentrations of individual drugs present in the mixture were determined from the calibration curves in quantization mode.

Fig 1: First order derivative overlain spectra of Amoxicillin (AMC, 25 µg/ml) and Dicloxacillin (DIC, 25 µg/ml)

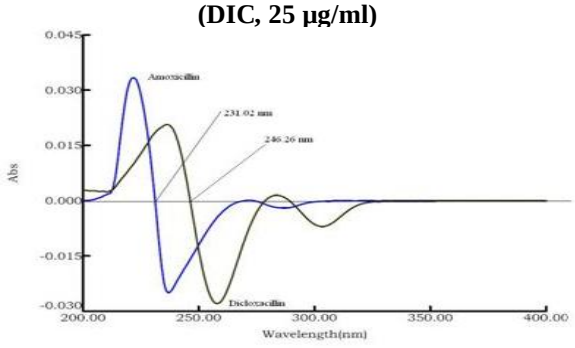


Fig 2a: First order derivative spectra for AMC (20, 25, 30, 35 µg/ml) and at 246.26 nm

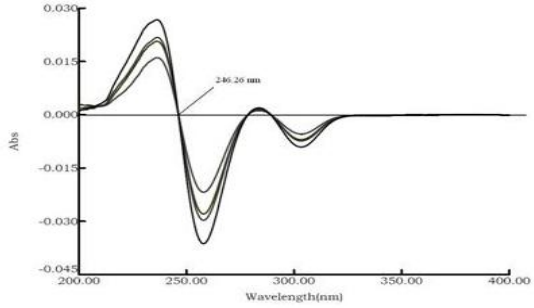


Fig 2b: First order derivative spectra for AMC (25 µg/ml) and DIC (20, 25, 30, 35µg/ml)

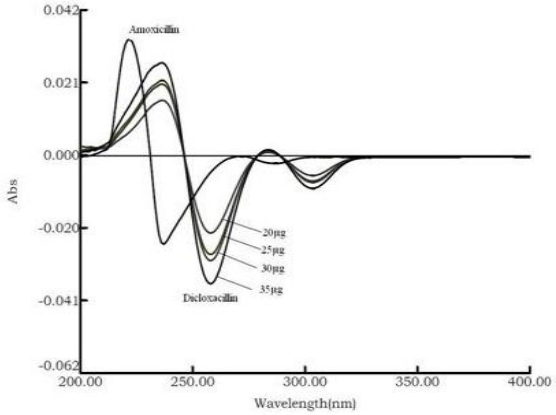


Fig 3a: First order derivative spectra for DIC (20, 25, 30, 35 µg/ml) and at 231.02nm

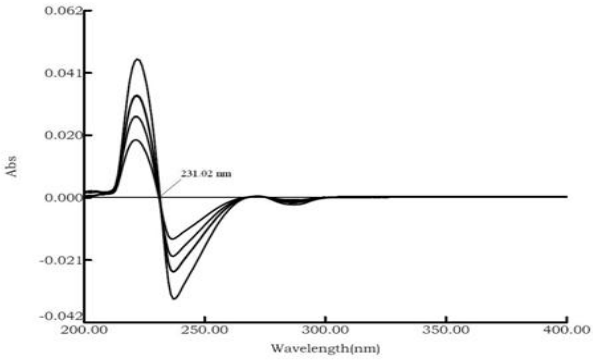


Fig 3b: First order derivative spectra for DIC (25 µg/ml) and AMC (20, 25, 30, 35µg/ml)

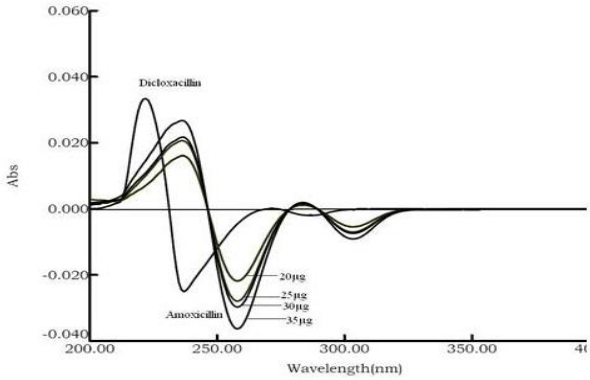
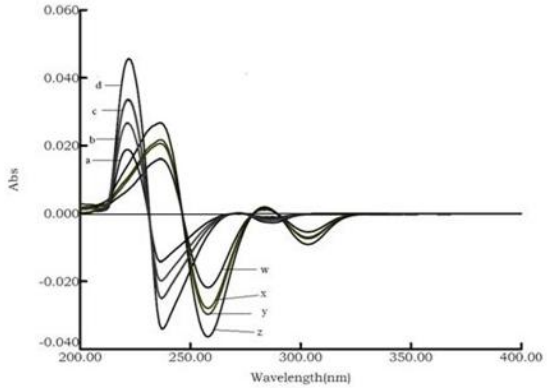


Fig 4: First order derivative spectra of (a) 20, (b) 25, (c) 30, and (d)35 µg/ml solution of AMC and (w) 20, (x) 25, (y) 30, and (z)35 µg/ml solution of DIC



3.1.1. Sample preparation

10 tablets were weighed accurately; average weight was determined and finely powdered. The powder equivalent to 10 mg of AMC and 10 mg of DIC were weighed accurately and transferred to 100 ml volumetric flask and diluted with 0.1N sodium hydroxide solution. The solution was filtered through whatman filter paper (no.41) and the volume was adjusted up to the mark with 0.1N NaOH. 25µg/ml of AMC and DIC were prepared with 0.1N NaOH .The concentration of both AMC and DIC were determined by measuring the absorbance at 231.02nm and 246.26 nm in first order spectrum mode and the results of tablets analysis were calculated from the calibration curve in quantization mode.

3.1.2. Validation

The method was validated statistically as per ICH/ USP 16 guidelines for all the parameters like accuracy, linearity, precision, ruggedness and specificity. Accuracy of the method was ascertained on the basis of recovery studies, carried out by standard addition method, in which pre-analyzed samples were taken and standard drug was added at three different levels (80%,100%,120% of the test concentration).The % recovery±SD lies in the range of 99.28±0.302 to 99.72±0.52 for AMC and 99.39±0.62 to 100.05±0.532 for DIC (Table2).The linearity of the method was established from the first derivative spectra by the measurement of absorbance of standard solutions containing varying concentrations of each compound in the presence of constant concentrations of other one. Linearity was constructed in the range of (15-35 µg/ml) (r^2<1). Precision was studied by analyzing six replicates of sample solutions and concentrations were calculated.Ruggednesss was established by carrying out the experiment at different conditions like intra-day, inter-day and by different analyst. Specificity of the method was ascertained by analyzing standard drug sample. There was no interference of the excipients present in the formulation. By observing validation parameters (Table 3), the method described was found to be specific, accurate, precise, and economical, can be successfully applied to analyze commercially available tablets contains AMC and DIC. The results obtained were in good agreement with the labeled content (Table 4).

Table 1: Statistical Data of Calibration Curve.

S.No	Parameters	AMC	DIC
1	Wave length(nm)	231.02	246.26
2	Beers law limit	15-35	15-35
3	Regression equation*	Y=0.001X	Y=0.001X
4	Correlation coefficient	0.998	0.997
5	LOD(µg/ml)	10	15
6	LOQ(µg/ml)	15	20

Y=mx; where x is the concentration of drug in µg/ml; y is the amplitude at specific wavelength, m is the slope.

Table 2: Recovery Study Data

S.No	Level of standard addition (%)	% Recovery ±SD*	
		AMC	DIC
1	80	99.28±0.302	99.39±0.62
2	100	99.87±0.38	100.10±0.55
3	120	99.72±0.52	100.05±0.532

Table 3: Result of Validation Studies of Proposed Method

S.No	Parameters	AMC	DIC
1	Linearity	± 15% of test conc.	± 15% of test conc.
2	Precision (%label claim ± SD,n=5)	99.9 ± 0.32	100.05 ± 0.532
	Ruggedness (%label claim,n=3)		
3	Intra day	100.10	99.28
4	Inter day	99.85	100.05
5	Different analyst	99.50	99.75
6	Specificity	Specific & passes	Specific & passes

SD is standard deviation

Table 4: Results of Analysis of Commercial Formulations

S. No	Drug	% Label Claim*(mg)	± SD*
1	AMC	99.50	0.325
2	DIC	99.90	0.510

*mean of three determinations. SD is standard deviation.

3.2. Method B: Simultaneous equation in application to content uniformity

3.2.1. Sample preparation (Amoxicillin and Dicloxacillin)

10 tablets were individually dissolved in 30 ml of 0.1 N NaOH, further diluted to 250 ml with 0.1 N NaOH solutions and was filtered using sintered glass filter. From the filtrate, 25µg/ml was prepared with 0.1 N NaOH.

3.2.2. Procedure:

Analytical wave length for simultaneous equation method, was selected by preparing standard solutions of AMC (25 µg/ml) and DIC (25 µg/ml) scanned in UV range (fig.5) represents the overlain spectrum of both the drugs. The wavelengths of AMC and DIC for simultaneous equation were 245 nm and 227 nm respectively.

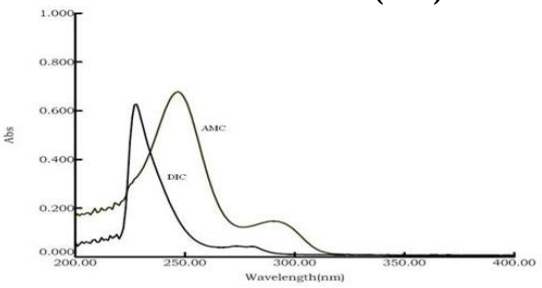
Working standard solutions (100µg/ml) of both the drugs were diluted to prepare solutions having concentrations 15, 20, 25, 30, 35µg/ml of AMC and DIC. All the solutions were measured at both the wavelengths and four calibration curves were constructed.

Absorptivities at each wavelength for AMC and DIC were determined and used to form the equation. The absorbance and absorptivity values at particular wavelengths were submitted in the following equations to obtain concentration.

Cx = (A2ay1-A1ay2)/ (ax2ay1-ax1ay2)
CY = (A1ax2-A2ax1)/ (ax2ay1-ax1ay2)
CX = concentration of Amoxicillin.
Cy = concentration of Dicloxacillin.
A1=absorbance of samples at 245nm.
A2= absorbance of samples at 227 nm.
ax1 is the absorbivity of Amoxicillin at 245nm.
ax2 is the absorbivity of Amoxicillin at 227 nm.
ay1 is the absorbivity of Dicloxacilin at 245nm.
ay2 is the absorbivity of Dicloxacilin at 227 nm.

Absorptivity of DIC at 245 nm (ay1) and 227 nm (ay2) was found to be 184.4 and 204 respectively. Absorptivity of AMC at 245 nm (ax1) and 227 nm (ax2) was found to be 271.2 and 68 respectively.

Fig.5: Overlain spectra of Amoxicillin (AMC) and Dicloxaciliin (DIC)



3.2.3. Validation parameter

Linearity was obtained in the range of (15-35 µg/ml) for AMC and DIC .The developed method was validated by determing accuracy, precision and repeatability. For the determination of intraday precision, solutions contains AMC and DIC in the range of 15-25 µg/ml were analyzed 6 times on the same day and % CV was calculated. For inter day precision, same range was analyzed on 3 different days and % CV was calculated. Repeatability was determined by analyzing 3 concentrations of the calibration curve, prepared in triplicate and % CV was calculated and given in (Table 5). Accuracy of analysis was determined by calculating percentage recovery of combination of AMC and DIC by standard addition method.

To a fixed amount of sample solution, increasing aliquots of mixture of standard stock solution (1.5, 2.0 & 2.5 ml) was spiked and diluted to 10 ml with water. The solutions were measured at 227 & 245 nm and % recovery of DIC and AMC was calculated.

Four different brands of AMC and DIC were analyzed for their content uniformity. Content of individual tablet unit (10 replicates) for all formulations were calculated using simultaneous equation method. None of the results fall outside the prescribed range i.e. 85-115%. The results for all four brands complied with pharmacopoeia requirements. The corresponding data along with % content of both the combinations are given in (Table 6).The method can be used for routine quality control of dosage form in industry.

Table 5.Validation parameters for AMC and DIC

S.NO	Parameter	Results of DIC		Results of AMC	
1	Correlation coefficient(r2)	227 nm 0.998	245nm 0.999	227nm 0.997	245nm 0.998
2	Linearity range (µg/ml)	15-35		15-35	
3	Precision				
a	Repeatability(n=3)(%CV)	0.35-0.75		0.55-0.88	
b	Intraday(n=5) (%CV)	0.92-1.20		0.77-0.97	
c	Interday(n=5) (%CV)	1.30-1.70		1.01-1.65	
4	Mean % Recovery	100.21-100.05		99.99-100.25	

Table 6: Percentage content of AMC and DIC in different brand formulations

Tablet Number	A		B		C		D	
		Diclox	Amox	Diclox	Amox	Diclox	Amox	Diclox
1	90.90	95.60	91.05	96.50	97.50	95.50	98.90	91.50
2	91.20	94.90	92.50	95.30	96.20	96.25	99.10	92.05
3	90.30	94.10	91.90	96.20	96.90	95.90	98.20	91.40
4	89.90	96.20	92.30	97.10	95.90	96.50	99.50	92.50
5	90.80	94.90	90.90	96.90	95.50	94.20	99.90	91.10
6	92.30	93.90	91.20	96.50	96.20	94.00	100.05	92.10
7	91.45	94.90	93.50	95.90	96.40	94.50	98.10	91.60
8	92.45	96.20	91.90	96.10	96.70	93.90	98.20	91.90
9	90.30	97.10	92.10	96.30	95.20	96.90	99.50	92.50
10	91.00	97.05	91.50	95.10	95.30	94.30	100.10	92.60
Average	91.06	95.48	91.886	96.19	96.18	95.19	99.13	91.92
SD	0.832	1.128	0.780	0.633	1.510	1.139	0.728	0.513
%CV	0.914	1.181	0.849	0.658	1.570	1.197	0.734	0.550

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