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H-Point Standard Additions method for the simultaneous determination of Paracetamol and Chlorzoxazone in Tablets using addition of both analytes and absorbance increment (ΔA)

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Abstract

The suitability of H-point standard additions method (HPSAM) to resolve of overlapping spectra corresponding to the paracetamol and chlorzoxazone was verified. The results showed that the H-point standard additions method with simultaneous addition of both analytes utilizing absorbance increment is suitable for the simultaneous determination of paracetamol and chlorzoxazone.

The results of applying the H-point standard additions method showed that the two drugs could be determined simultaneously with the concentration ratios of paracetamol to chlorzoxazone varying from 1:2 to 2:1 in the mixed samples. In addition the means of the calculated RSD (%) were 1.40 and 1.92 in synthetic mixtures with 1.29 and 1.68 in dosage form for paracetamol and chlorzoxazone, respectively.

Keywords: Spectrophotometric, Paracetamol, Chlorzoxazone, H-point standard additions method, absorbance increment.

1. Introduction

Paracetamol (PAR) chemically is N-(4-hydroxyphenyl) acetamide (Figure 1), also known as acetaminophen; it is a famous analgesic and antipyretic drug excessively used for management of pain and fever [1]. Chlorzoxazone (CHL) chemically is 5-chloro-3H benzooxazol-2-one (Figure 1). It is a skeletal muscle relaxant; it acts by inhibiting multi synaptic reflexes involved in producing and maintaining skeletal muscle spasm of varied etiology [2].

(a) (b)

Figure 1: Chemical Structures of (a) Paracetamol and (b) Chlorzoxazone

Chlorzoxazone and paracetamol combination is indicated as an adjunct to other measures, such as rest and physical therapy, for relief of pain and muscle spasm associated with acute, painful musculoskeletal conditions [3].

The combination of PAR and CHL is not official in any pharmacopoeia; hence no official method is available for the estimation of the two analytes in their combined synthetic mixture or dosage forms; however literature search revealed that different methods have been developed for the simultaneous determination of PAR and CHL as mixture; absorbance ratio technique and difference spectrophotometric method [4], derivative spectrophotometer [5], orthogonal functions-ratio spectrophotometry [6], thin layer chromatography densitometric method [7], gas liquid chromatography [8], and high performance liquid chromatography [9]. These methods required mathematical software programs or expensive instruments. The H-point standard additions method [10], was developed as an alternative to existing methods to overcome these difficulties.

In this work, a sensitive, selective, accurate and inexpensive spectrophotometric method was developed for the simultaneous determination of PAR and CHL by using H-point standard additions method with simultaneous addition of both analytes and utilizing the absorbance increment (ΔA) at pre-selected wavelengths pair for each analyte to determine its concentration. This method is a modification of regular H-point standard additions methods [10,11]. The method based on using absorbance increment (ΔA) value obtained after each standard addition as analytical signal for the determination of each analyte [12].

1.1 Theoretical considerations

The H-point standard additions method, HPSAM [10], allows the analyte concentration to be calculated free from all constant systematic and proportional errors even in the presence of a direct interferent in the sample to be assayed. It relies on the use of the analytical signals obtained at two wavelengths where the absorbance is the same for the interferent and different for the analyte; the application of the principal of standard addition method, under these conditions thus leads to two straight lines at each wavelength which intercept at the so-called H-point, defined by the coordinates $(-C_H, A_H)$, where C_H is the analyte concentration and A_H, is the analytical signal yielded by the interferent, which can thus be quantified as well.

A variant of the HPSAM that permits the simultaneous standard addition of the two analytes in order to obtain their concentration in the sample from a unique calibration set was developed [11]. The required data to apply the method are the absorbance of the sample and the absorbance of the sample spiked with known amounts of analytes at previously selected pairs of wavelengths. At these selected wavelengths the analyte signals must be linear with the concentrations and the interferent signal must remain equal, in the case where the analyte concentrations are changed, the analytical signal obtained from the mixture containing the analyte and the interfering should be equal to the sum of the individual signals of the two components. In addition, the difference in the slopes of the two straight lines measured at two selected wavelengths must be as large as possible while the difference in the slopes of the two straight lines measured at the other pair of wavelengths must be as small as possible (preferred to be equal zero) in order to get good accuracy and sensitivity [13]. The intersection point of the two lines resulting from the standard additions gives the concentration of the analyte.

Since under the HPSAM conditions the absorbance values of the interferent at the two wavelengths are the same, then the absorbance increments of the analyte depends exclusively on the analyte concentration, so the plot of the absorbance increment (ΔA) versus its added concentrations will be a straight line. The analyte concentration can thus be calculated from (ΔA) values by applying the HPSAM to the intercept of the straight line at the H-point.

The (ΔA) value obtained after each addition will be exclusively related to the analyte concentration as the interferent absorbance will be the same at the two wavelengths, so its contribution to (ΔA) will be zero even if the intensity of its analytical signal changes on successive analyte additions because of interaction with the analyte. Therefore, the analytical signals will be free from constant systematic errors [12].

When only the analyte concentration must be calculated, a single calibration plot of ΔA against the added analyte concentration allows one to calculate the unknown concentrations free from any bias error from the intercept of the line in the same way as with the method of standard addition MOSA [11,12].

2. Experimental

2.1 Instruments

UV-vis absorption spectra were measured on Shimadzu UV-Vis spectrophotometer, (UV 1800), with the use of 1.0cm quartz cells, ultrasonic bath (Life-care equipment-India) and centrifuge (Sartorius – Germany). Data analysis was performed using Microsoft Excel Spreadsheet 2003.

2.2 Materials and reagents

2.2.1 Materials

Paracetamol and Chlorzoxazone working standards were kindly provided by Blue Nile Pharmaceutical Company-Sudan. Nilogesic tablets labeled to contain 250 mg chlorzoxazone and 300 mg paracetamol per tablet were purchased from local market. Analytical grade sodium hydroxide pellets and methanol from Scharlau- Spain. Laboratory produced distilled water was used throughout this work.

2.2.2 Diluting solvent

Sodium hydroxide 0.1M was prepared by dissolving 4.0 gm of sodium hydroxide pellets in 1000 ml volumetric flask using distilled water.

2.3 Standards and solutions

2.3.1 Standards stock solutions

Standard stock solutions of paracetamol (300 µg/ml) and Chlorzoxazone (300 µg/ml) were prepared separately by dissolving 30 mg each into 100 ml volumetric flask using methanol.

2.3.2 Working standard mixture

Working standard solution containing both analytes was prepared by transferring 5 ml from each stock solution into a 100 ml volumetric flask and making the volume to the mark with 0.1 N NaOH.

2.3.3 Laboratory synthetic mixtures

Six laboratory synthetic mixtures containing different amounts of PAR and CHL were prepared by proper dilution of different aliquot volumes from their standards stock solutions in 100 ml volumetric flasks using the diluting solvent.

2.3.4 Samples preparation

An amount of the powdered tablets containing 300 mg paracetamol and 250 mg Chlorzoxazone was was accurately weighed and transferred into a 100 ml volumetric flask, about 70 ml methanol were added, the content of the flask was mechanically shaken for 20 minutes and then sonicated for another 10 minutes. The contents of the flasks were allowed to cool and volume was completed with methanol. A suitable portion of this mixture was centrifuged at 1500 rpm for 10 minutes, 2 ml of the clear supernatant solution were transferred into 100 ml volumetric flask and the volume was completed to the mark with the diluting solvent.

2.4 Methods

2.4.1 Optimum wavelengths selection

Two separate solutions containing PAR and CHL; 10 µg/ml were prepared by dilution from their respective standard stock solutions using the diluting solvent. The UV spectra of the two solutions were recorded over the range 220-320 nm. The pair of wavelengths for each analyte satisfying the requirement of applying the proposed method was selected from the spectra.

2.4.2 Linearity at the selected wavelength pairs

Aliquot volumes (1-5 ml) from each analyte standard stock solution were transferred into two separate series of five 100 ml volumetric flasks; the volume was then completed using the diluting solvent. The absorbance values of each concentration were measured at the selected wavelength pair for each analyte. The absorbance values obtained were plotted against the corresponding concentrations of PAR and CHL.

2.4.3 General procedure

Two ml volumes from the sample solution were transferred into six separate 25 ml volumetric flasks; each of the six flasks was spiked with a different volume of working standard mixture (2, 3, 4, 5, 6 ml) except one. The volumes of the flasks were made to mark with the diluting solvent. The absorbance of each solution was measured at the selected wavelength pair. Concentrations of PAR and CHL in the samples were obtained from straight line equation obtained from the added concentration versus the ΔA values.

3. Results and discussion

3.1 Optimum wavelengths selection

To select the appropriate wavelength pairs for using HPSAM the following principles should be applied. At these selected wavelengths, the analyte signals must be linear with concentrations and the interfering signals must remain equal and unchanged by changing the analyte concentration. The analytical signal obtained from a mixture containing the analyte and the interferent should be equal to the sum of the individual signals of the two components. In addition, the difference in the slopes of the two straight lines measured at two selected wavelengths must be as large as possible in order to get good sensitivity [13].

For the determination of PAR and CHL, we selected two pairs of wavelengths on their spectra. As it is observed from Fig. 2, the best wavelength pairs were 255 - 277.4 nm and 244 - 270.6 nm for determination of PAR and CHL respectively.

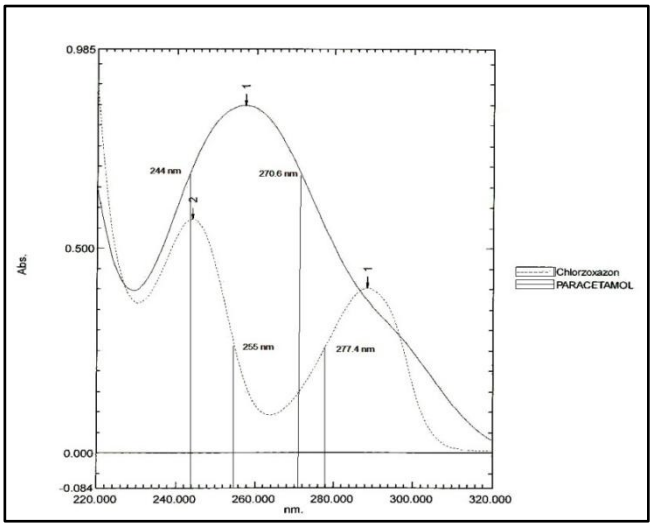

Figure 1: Absorption spectra of Chlorzoxazone 10 µg/ml and Paracetamol 10 µg/ml.

The suitability of the selected wavelength pairs was further verified from the data of the calibration lines obtained at the wavelength pair suitable for the determination of the other; the data is presented in Table 1.

Table 1: Linearity data of PAR and CHL at the selected wavelength

Parameter	Paracetamol		Chlorzoxazone	
Wavelength (nm)	244 nm	270.6 nm	255 nm	277.4 nm
Concentration range (µg/ml)	2 - 12 µg/ml		4 - 14 µg/ml	
Slope (b)	57.986	58.6286	25.429	24.6
Slope ratio	0.99		1.03	
Intercept (a)	0.0036	0.0016	0.0001	0.0013
Coefficient of determination (R^2)	0.9998	0.9999	0.9997	0.9994

Fig. 3 shows the H-point standard addition calibration lines constructed at the two selected pairs (255 - 277.4 nm) for determination of PAR and (244 - 270.6 nm) for CHL in the presence of each other.

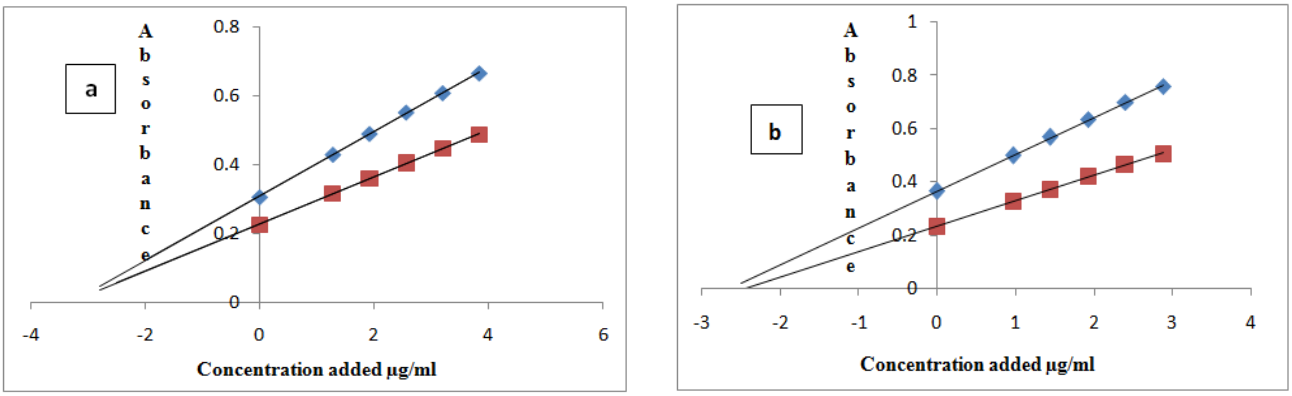

Figure 3: H-point standard additions plots for simultaneous determination of (a) paracetamol (3.2 µg/ml) and (b) chlozoxazone (3.2 µg/ml)

3.2. Analysis of laboratory synthetic mixtures:

Some mixtures of two compounds with known concentrations were initially tested to validate the applicability of the chosen wavelengths. The results of HPSAM for determination of PAR and CHL in some synthetic mixtures were tabulated in Table 1. As could be seen the accuracy of the results is satisfactory in all cases, when the concentration ratio of PAR and CHL vary from 1:2 to 2:1.

Table 1: Determination of PAR at (255-277.4 nm) and CHL at (244-270.6 nm) in some synthetic mixtures using ΔA method

Mix. No	Analytes	ΔA - c equation	R^2	Concentration (µg/ml)		% content
				Theoretical	Found	
1	Paracetamol	ΔA = 24.694c + 0.06	0.9990	2.40	2.42	101.26
	Chlorzoxazone	ΔA= 40.986 c + 0.199	0.9993	4.80	4.85	101.15
2	Paracetamol	ΔA = 24.556 c + 0.116	0.9996	4.80	4.72	98.33
	Chlorzoxazone	ΔA = 40.361 c + 0.099	0.9991	2.40	2.45	102.08
3	Paracetamol	ΔA = 25.469 c + 0.08	0.9994	3.20	3.14	98.13
	Chlorzoxazone	ΔA = 41.806 c + 0.133	0.9995	3.20	3.18	99.38
4	Paracetamol	ΔA = 24.167 c + 0.085	0.9993	3.60	3.51	97.50
	Chlorzoxazone	ΔA = 32.378 c + 0.168	0.9936	5.00	5.18	103.60
5	Paracetamol	ΔA = 23.741 c + 0.142	0.9937	6.00	5.98	99.67
	Chlorzoxazone	ΔA = 31.933 c + 0.098	0.9934	3.00	3.07	102.33
6	Paracetamol	ΔA = 25.444 c + 0.127	0.9985	5.00	4.99	99.80
	Chlorzoxazone	ΔA = 42.5 c + 0.209	0.999	5.00	4.92	98.40

3.3 Precision

The precision of the developed method was evaluated by the results obtained from between-days (intermediate precision) and within-day data (repeatability) for the six replicate samples analyzed. The calculated RSD values for both drugs were found to be within the accepted limit (less than 2%, Table 2).

Table 2: Precision data of the proposed method

Parameter	% of label claim			
	Chlorzoxazone		Paracetamol	
	Day 1	Day 2	Day 1	Day 2
Average (n =6)	104.29	103.00	102.29	102.94
STDEV	1.76	1.72	1.34	1.40
RSD%	1.68	1.67	1.29	1.38

4. Conclusion

The present study demonstrated that the H-point standard additions method with simultaneous addition of both analytes can be useful in resolving overlapped spectra of real samples. The proposed method offers a practical potential for the simultaneous determination of PAR and CHL, especially with its advantages of acceptable sensitivity, high selectivity, simplicity, and speed that were not present together in the previous literature.

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