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Multi-wavelength Spectrophotometric Determination of Chlorzoxazone and Paracetamol in Bulk and Capsules

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Abstract

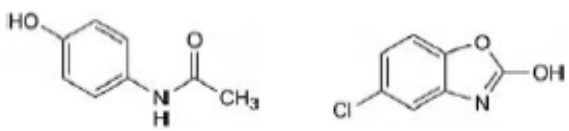
A simple, cost effective spectrophotometric method has been developed for the simultaneous determination of paracetamol (PAR) and chlorzoxazone (CHL) in bulk and dosage forms. The method permits data to be taken at multiple wavelengths to generate linear plots, from which the concentrations can be determined. The absorbance values of the two analytes were linear with the concentration at the wavelengths taken at 10 nm interval over the range of 230 -300 nm. The accuracy and the precision of the developed method were very good (RSD < 2%). The validity of the proposed method was confirmed through the statistical comparison of the obtained data with those obtained by a reference method utilizing H-point for the determination of the two actives.

Keywords: Spectrophotometry, Multi-wavelength, Paracetamol, Chlorzoxazone.

1. Introduction

Paracetamol chemically is N-(4-Hydroxyphenyl) acetamide (Figure 1), it has analgesic and antipyretic properties and weak anti-inflammatory activity. Paracetamol is often the analgesic or antipyretic of choice, especially in the elderly and in patients in whom salicylates or other nonsteriodal anti-inflammatory drugs (NSAIDs) are contra-indicated [1].

Chlorzoxazone chemically is 5-Chlorobenzoxazol-2(3H)-one (Figure 1), it is a centrally acting skeletal muscle relaxant with sedative properties. It is claimed to inhibit muscle spasm by exerting an effect primarily at the level of the spinal cord and subcortical areas of the brain [1].



(a) **(b)**

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

Paracetamol and chlorzoxazone 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 [2].

The combination of paracetamol (PAR) and chlorzoxazone (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 [3], derivative spectrophotometer [4], orthogonal functions-ratio spectrophotometry [5], thin layer chromatography densitometric method [6], gas liquid chromatography [7], high performance liquid chromatography [8], and H-point standard addition [9].

For the simultaneous spectrophotometric determination of two sample components, the choice of an analytical procedure is strictly related to the observed

resolution between the individual absorption peaks of these components. Such a determination is not problematic, if the absorption peaks are satisfactorily resolved, but if the individual component signals are partly or totally overlapped, then chemometric techniques are needed. These techniques require full-spectrum information and the spectral data to be processed using highly specialized software. Hence the aim of the present work was to develop a simple, cost effective and accurate spectrophotometric method based on multi-wavelength regression analysis for the simultaneous determination of paracetamol and chlorzoxazone in bulk and capsules.

1.1 Theoretical considerations

Multi-Wavelength Linear Regression Analysis (MLRA) [10]; enables determination of the composition of a binary mixture with overlapping spectra without determining molar absorptivities or complicated mathematics. The method is very simple it requires only three measurements, the absorbance of a standard solution for each component, and the unknown mixture itself.

Assuming additivity, the absorbance of a mixture is the sum of the absorbencies of its components. If we have a mixture consisting of two components, 1 and 2, with an unknown concentration of C_1 and C_2, then the absorbance of the unknown mixture,

$$A_{mixture} = A_1 + A_2$$

Applying Beer's law: $A_1 = \epsilon_1 b C_1$ and $A_2 = \epsilon_2 b C_2$

Substituting: $A_{mixture} = \epsilon_1 b C_1 + \epsilon_2 b C_2$.

However, the absorbancies of standard solutions of the same substances will follow the same Beer's law relationship and have the same molar absorbance, ϵ, and one centimeter path length, b, as the unknown solutions under the same conditions.

Therefore, we can write:

$$A_{standard\ 1} = \epsilon_1 b C_{standard\ 1} \text{ and } A_{standard\ 2} = \epsilon_2 b C_{standard\ 2}$$

Rearranging these relationships:

$$\epsilon_1 b = \frac{A_{standard\ 1}}{C_{standard\ 1}} \text{ and } \epsilon_2 b = \frac{A_{standard\ 2}}{C_{standard\ 2}}$$

Substituting,

$$A_{mixture} = \frac{A_{standard\ 1}}{C_{standard\ 1}} C_1 + \frac{A_{standard\ 2}}{C_{standard\ 2}} C_2$$

or

$$A_{mixture} = \frac{C1}{C_{standard\ 1}} A_{standard1} + \frac{C2}{C_{standard\ 2}} A_{standard2}$$

Dividing by $A\ standard1$ and simplifying we obtain:

$$\frac{A_{mixture}}{C_{standard\ 1}} = \frac{C1}{C_{standard\ 1}} + \frac{C2}{C_{standard\ 2}} \times \frac{A_{standard\ 2}}{A_{standard\ 1}}$$

Therefore, a plot of

$$\frac{A_{mixture}}{C_{standard\ 1}} \text{ versus } \frac{A_{standard\ 2}}{A_{standard\ 1}}$$

is straight line with the following slope and intercept

$$\text{slope} = \frac{C2}{C_{standard\ 2}} \text{ and intercept} = \frac{C1}{C_{standard\ 1}}$$

That is, the concentration of the unknown component 2 (C_2) in the mixture, equals the slope times the concentration of the standard solution for component 2. Likewise, the concentration of the unknown component 1 (C_1) in the mixture equals the product of the intercept times the concentration of the standard solution for component1.

or simply

C_1= intercept x $C_{standard\ 1}$ and C_2 = slope x $C_{standard\ 2}$

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 and ultrasonic bath (Life-care equipment-India). Data analysis was performed using Microsoft Excel Spreadsheet 2003.

2.2 Materials

Paracetamol and Chlorzoxazone working standards were kindly provided by Blue Nile Pharmaceutical Company-Sudan. Relaxone capsules (Jamjoom Pharma -KSA): labeled to contain 500 mg of paracetamol and 300 mg of chlorzxoazone were purchased from Kingdom of Saudi Arabian. Analytical grade sodium hydroxide pellets from Scharlau- Spain. Laboratory produced distilled water was used throughout this work.

2.3 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.

2.4 Standards and solutions

2.4.1 Standards stock solutions

Standard stock solutions of paracetamol (160 µg/ml) and Chlorzoxazone (160 µg/ml) were prepared separately by dissolving 16 mg each into 100 ml volumetric flask using 0.1M NaOH as a solvent.

2.4.2 Linearity standards

Separate linearity standards of the two analytes were prepared by proper dilution of suitable aliquots from their corresponding stock standard solutions with 0.1M NaOH to give concentrations in the range of (2 -16 µg/ml) of each analyte.

2.4.3 Working standards

Working standards were prepared by quantitative dilution with 0.1M NaOH of suitable volumes from the stock standard solutions and used in different parts of the analytical work.

2.4.4 Laboratory synthetic mixtures

Five laboratory synthetic mixtures containing different amounts of PAR and CHL were prepared by proper dilution of aliquots from their corresponding stock standard solutions.

2.4.5 Sample preparation

The content of twenty capsules was accurately weighed and mixed well. A quantity of the resulted powder equivalent to about 100 mg paracetamol was accurately weighed and transferred into a 100 ml volumetric flask, 50 ml 0.1 M NaOH were added and the mixture was sonicated for 5 minutes then the volume was made to the mark with 0.1 M NaOH , the solution was filtered using 0.45 µm nylon filter. Five ml of the clear filtrate were transferred into 50 ml volumetric flask and the volume was completed to the mark with 0.1 M sodium hydroxide, further 5 ml of this solution were transferred into 50 ml volumetric flask and the volume was completed to the mark with 0.1 N sodium hydroxide.

2.5 Methods

2.5.1 Spectral characteristics

The individual spectra of the analytes were obtained by scanning their corresponding working standard solutions over the wavelength range of 230-300 nm.

2.5.2 Linearity

The absorbances of the linearity standard solutions were read at10 nm intervals over the wavelength range of 230-300 nm.

2.5.3 General procedure

The absorbance values of the two working standards, synthetic mixtures and samples were read at10 nm intervals over the wavelength range of 230-300 nm.

The concentrations of CHL and PAR in the synthetic mixtures and the samples were calculated according to the MLRA principle using the slopes and intercepts of the straight lines obtained.

3.1 Spectral characteristics

The individual spectra of the analytes showed extensive overlapping over the wavelength range of 230-300 nm (Fig 2); hence resolutions of their photometric signals by classical spectrophotmetric methods are not possible.

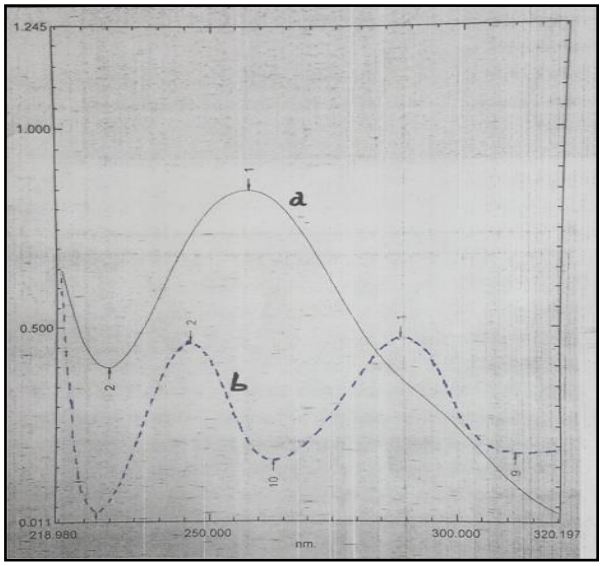


Figure 1: Absorption spectra of (a) Paracetamol 10 µg/ml and (b) Chlorzoxazone 10 µg/ml.

3.2 Linearity

Application of MLRA method requires obeying Beer's law at each wavelength over the selected wavelength range [18]. The two analytes showed linear relation between the concentration and absorbance over the entire wavelengths range investigated ($r^2 < 0.99$). Table 1 reveals the regression equations parameters.

3. Results and discussion

Table 1: Linearity data of Paracetamol and Chlorzoxazone

λ	230 nm	240 nm	250 nm	260 nm	270 nm	280 nm	290 nm	300 nm
Paracetamol (2 – 16 µg/ml)								
Slope	0.02748	0.05016	0.07061	0.07439	0.06248	0.04333	0.03037	0.02102
Intercept	-0.0004	0.0078	0.0099	0.008	0.0077	0.0058	0.0025	-0.0015
r^2	0.9989	0.9997	0.9996	0.9997	0.9996	0.9997	0.9996	0.9995
Chlorzoxazone (2 – 16 µg/ml)								
Slope	0.036	0.0576	0.04964	0.0084	0.01504	0.03384	0.04412	0.01816
Intercept	-0.034	-0.021	-0.014	-0.001	-0.018	-0.014	-0.011	-0.011
r^2	0.9972	0.9998	1.0000	0.9856	0.9991	0.9997	0.9995	0.9998

3.3 Determination of synthetic mixtures (accuracy)

The accuracy of the proposed method was tested by analyzing five laboratory prepared synthetic mixtures containing different concentration ratios of PAR and CHL. The analytes concentrations in the synthetic mixtures were in close agreement with the actual ones irrespective of their concentration ratios. The results obtained indicated good accuracy of the method supporting the suitability of its application for the analysis of the two drugs in capsules Table 2.

Table 2: The accuracy results of the synthetic mixtures

Sample	Paracetamol (µg/ml)		% content	Chlorzoxazone (µg/ml)		% content
	Theoretical	Found		Theoretical	Found	
m1	13.36	13.04	98.61	9.60	9.68	100.83
m2	13.36	12.92	98.67	12.80	12.83	100.27
m3	6.68	6.67	99.83	9.60	9.51	99.01
m4	13.36	13.22	98.98	6.40	6.56	102.50
m5	10.02	10.02	100.03	9.60	9.36	98.45

m= synthetic mixture

3.4 Method precision

The precision of the developed method was evaluated by the results obtained from between-days (intermediate precision) and within-day data (repeatability) for five sample replicates containing 100% of their corresponding expected concentrations in the pharmaceutical product. The calculated RSD values for both drugs were found to be within the accepted limit (less than 2%, Table 3).

Table 3: Precision of the proposed method (n=5)

	Percent relative standard deviation (%RSD)		
Analyte	**Day 1**	**Day 2**	**Over all**
Paracetamol	0.70	0.32	0.68
Chlorzoxazone	1.59	1.37	1.47

3.5 Analysis of commercial sample

The proposed method was applied to the analysis five samples taken from commercial capsules dosage. The results obtained were in good agreement with the labelled amounts 103.12% and 104.34% with relative standard deviations of 1.59% and 0.70% for CLX and PAR respectively. The capsules analysis data is presented in Tables 4.

Table 4: Samples assay results

Sample	Paracetamol µg/ml		% content	Chlorzoxazone µg/ml		% content
	Theoretical	Found		Theoretical	Found	
S1	10.05	10.43	103.72	8.38	8.71	103.96
S2	10.16	10.56	103.98	8.47	8.75	103.39
S3	10.03	10.57	105.33	8.36	8.49	101.63
S4	10.07	10.57	104.89	8.39	8.84	105.27
S5	10.00	10.37	103.76	8.33	8.44	101.35
	Average		104.34	**Average**		103.12
	Standard deviation		0.73	**Standard deviation**		1.64
	RSD%		0.70	**RSD%**		1.59

The validity of the method was further assessed by comparing the statistical results obtained with those of our earlier published method; based on H-point standard addition developed for the determination of the two analytes (9). As the calculated t- values were less than tabulated ones (n =5, P=0.05), the result of the developed method can be considered as accurate and precise as the official liquid chromatographic method (Table 5).

Table 5: Results of the proposed method compared to the reference method (9)

		% content ± sd	t - calculated (t - tabulated)
Proposed method	Paracetamol	104.34 (0.73)	1.76 (2.78)
	Chlorzoxazone	102.79 (1.59)	2.56 (2.78)
Reference method	Paracetamol	103.68 (0.50)	
	Chlorzoxazone	102.29 (1.68)	

4. Conclusions

Multi-wavelength Linear Regression Aanalysis is a straightforward procedure allowing the accurate resolution of binary mixtures of compounds with overlapped spectra without prior separation, the method is very simple requires only measurement of absorbance values of the two standards and the sample. The concentrations of the two analytes are calculated using easy to understand simple mathematics.

The cost effectiveness in term of time and money renders the method as suitable alternative to other expensive techniques e.g. chromatographic methods for the analysis of binary mixtures of compounds with overlapped spectra in laboratories and countries where such sophisticated equipments are not affordable. The accuracy and simplicity of the method suggest it suitability in cases where quick results are demanded e.g. as in-process analysis procedure during blend analysis in industrial setups.

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Conflict of interest: None Declared

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