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A UV Spectrophotometric method for simultaneous determination of Ibuprofen and Paracetamol concentration in suspensions

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ABSTRACT

INTRODUCTION: Paracetamol is a standard antipyretic and analgesic which is widely used since the 19th century. Currently, paracetamol is the first-line treatment for pain management of different types. Ibuprofen, on the other hand, is an antipyretic and an analgesic. It is safe and has been used in the treatment of a number of conditions including mild to moderate pain, dysmenorrhea, inflammations and fever to name few.

METHODS: This study aimed at developing and validating a simultaneous UV spectrophotometric method for analysis of ibuprofen and paracetamol in fixed-dose combination suspensions. The proposed method is based on the simultaneous equation principle, which involves measurement of absorbance at wavelengths of maximum absorbance for ibuprofen and paracetamol. The method was validated for linearity, accuracy, repeatability, intermediate precision and robustness as per USP and ICH guidelines.

RESULTS: The two molecules showed wavelength of maximum absorbance at 222 nm and 243 nm for ibuprofen and paracetamol respectively, using phosphate buffer as a diluent. The method was also linear ($R^2 \geq 0.995$), precise (RSD ≤ 2), and robust with accuracy ranging between 98.1%-105% and 109.8 %- 134.9% for paracetamol and ibuprofen, in the range of 0.0032 – 0.0048 mg/ml for ibuprofen and 0.004-0.006 mg/ml for paracetamol, respectively.

CONCLUSION: We have developed an accurate and robust method which can be used to analyze quantitatively paracetamol in suspensions which contain both paracetamol and ibuprofen. The limits of quantification of this method cover the concentration range recommended by the USP (80%- 120%), which justifies the application of the method in routine analysis.

Keywords: Ultraviolet rays, Ibuprofen, Paracetamol, Spectrometry, Spectrophotometry

INTRODUCTION

The number of medicines brought to the market increases annually. Some of these medicines are new products, while some are structural variations of existing drugs [1]. Typically, there is a period of time between the introduction of the drug on the market and its incorporation into the pharmacopeia [1]. Analysis of two or more absorbing molecules by UV-Vis spectrophotometry

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is based on the additivity principle, which states that the absorbance at any wavelength of the mixture is equal to the sum of the absorbance of each component in the mixture at that wavelength. One of the methods used in mixture analysis is the simple simultaneous equation method [2]. In this method, maximum absorbance for each component is selected and the molar absorptivity for each component at a selected wavelength is determined using the absorbance of pure standard solutions of known concentrations [3]. The presence of π bonds (chromophores) in these molecules gives them the property of absorbing UV-Vis light between 185 and 1000 nm [3].

The ultraviolet spectroscopy and HPLC technique play an important role in qualitative and quantitative analysis of pharmaceutical products. Ultraviolet spectroscopy is preferable in pharmaceutical analysis, since it is easy to manipulate, economic, rapid, robust, and offers high precision [4]. While several UV methods for simultaneous determination of ibuprofen and paracetamol in tablet form have previously been reported [5–12], there is no available UV method that can be used for simultaneous determination of ibuprofen and paracetamol in suspension. Hence there is a need to develop a spectrophotometric method to test ibuprofen and paracetamol simultaneously in suspension, which is the subject of this study [3].

METHODS

Equipment: Agilent Cary 60 and Agilent Cary 100 double beam UV-Vis spectrophotometers with a wavelength range of 190 nm to 1100 nm equipped with 10 mm quartz cuvette were used. A calibrated Kern ABT analytical balance model 120-5DM was also used.

Chemicals, samples, and reference standards: Both ibuprofen (purity of 99.8%, BN R024X0) and paracetamol (purity of 99.8%, BN K2M244) USP primary reference standards were used. Sekalgic sample with BN: GO19041 was used in method development and validation. In addition to these, Milli-Q water was used during the preparation of analytical solutions.

Preparation of the phosphate buffer: The phosphate buffer (pH =7.2) was prepared by dissolving 6.80 g of potassium dihydrogen phosphate R and 1.40 g of sodium hydroxide R in sufficient water to produce 1000 mL [13].

Wavelength selection: Separate solutions of 0.1 mg/mL of ibuprofen and paracetamol reference standards were prepared in phosphate buffer and scanned in a range of 200 nm to 400 nm. The wavelength of maximum absorbance was recorded and used for further analysis [3].

Study of Beer's law for ibuprofen and paracetamol: The Lambert-Beer's law was investigated by using ten different concentrations of ibuprofen and paracetamol prepared separately and ten standard solutions containing a mixture of ibuprofen and paracetamol in the range of 0.001 - 0.01 mg/ml. The absorbances of these solutions were recorded at wavelengths of maximum absorbances of ibuprofen and paracetamol. Additionally, the plot of absorbance against concentration and coefficient of determination was evaluated.

Determination of molar absorptivity: Determination of molar absorptivity for ibuprofen $(E_1)_{\lambda 1}$ and $(E_1)_{\lambda 2}$ at a wavelength of maximum absorbance λ_1 and λ_2, respectively and molar absorptivity for paracetamol $(E_2)_{\lambda 1}$ and $(E2)_{\lambda 2}$ at a wavelength of maximum absorbance λ_1 and λ_2, respectively, was done using ten different standard concentrations of ibuprofen and paracetamol, used in the study of Beer's law for ibuprofen and paracetamol. The absorbance of each solution was recorded and used to calculate the molar absorptivity using the equation below [2].

$$A=E*L*C \quad \text{(Equation 1)}$$

Development of a simultaneous equation: After determining $(E_1)_{\lambda 1}$ $(E_1)_{\lambda 2}$, $(E_1)_{\lambda 3}$ and $(E_1)_{\lambda 4}$, each value was substituted in the system of equations 1 and the concentrations of ibuprofen and paracetamol, C1 and C2 respectively, were deduced.

$$A_{\lambda 1} = C_1(E_1)_{\lambda 1} + C_2(E_2)_{\lambda 1}$$

$$A_{\lambda 2} = C_1(E_1)_{\lambda 2} + C_2(E_2)_{\lambda 2} \quad \text{(System of equations 1)}$$

Determination of linearity: Five different concentrations of standard solutions of ibuprofen, paracetamol and mixtures of ibuprofen and paracetamol were prepared in the range of 0.0032 – 0.0048 mg/ml and 0.004 -0.006 mg/ml for ibuprofen and paracetamol, respectively. The absorbances were recorded at 222 nm and 243 nm and the response curves were plotted as absorbance versus concentration [14]. In addition to this, the coefficient of determination (R2) was

also evaluated.

Determination of accuracy: The accuracy of the developed method was determined by spiking the pre-analyzed standard solutions of SEKALGIC containing 0.0023 and 0.00352 mg/ml ibuprofen and paracetamol respectively at three levels namely 80%, 100% and 120%. These solutions were analyzed by the proposed method in triplicates. The recovery was calculated to evaluate the accuracy of the method [14].

Determination of precision: The precision of the method was evaluated through repeatability and intermediate precisions.

Determination of repeatability: Repeatability of the analytical method was demonstrated by preparing six replicates of sample solution containing 0.004 and 0.005 mg/ml of ibuprofen and paracetamol respectively, using phosphate buffer as diluent. All solutions for repeatability study were prepared by one analyst in one period and the absorbances were recorded using Cary 100 UV-Vis spectrophotometer [14].

Determination of intermediate precision: Intermediate precision was demonstrated by preparing six replicates of SEKALIC sample solutions containing 0.004 and 0.005 mg/ml of ibuprofen and paracetamol respectively by a second analyst on different days. Absorbances from different sample solutions were recorded at 222 nm and 243 nm using Cary 60 UV-Vis spectrophotometer [14].

Determination of robustness: As the absorbance of the solution depends on the wavelength of analysis, the solvent, the pH, and the temperature [15], the robustness of this method was studied by analyzing the sample solution by changing the pH of the phosphate buffer by ± 0.1, changing the wavelength of analysis at ± 2 nm and by varying the temperature of the sample solution by 30 ± 5 0C.

RESULTS AND DISCUSSION

Wavelength selection: The wavelength of maximum absorbance for ibuprofen and paracetamol were determined from the spectra of both molecules. Ibuprofen and paracetamol showed the absorbance of maximum absorbance at 222 nm and 243 nm, as shown in Figure 1.

These wavelength values of maximum absorbance are like those used by the United States Pharmacopoeia in dissolution analysis of ibuprofen and paracetamol tablets, which are 221 and 243 nm respectively [14]. These wavelengths (222 nm for ibuprofen and 222 nm and 243 for paracetamol) were selected for further analysis of the drug.

Study of Beer's law for ibuprofen and paracetamol: The two molecules obeyed Beer's law in the range of 0.001–0.01 mg/ml for ibuprofen and paracetamol, as shown in Figure 2 and Figure 3. The higher values of determination coefficient

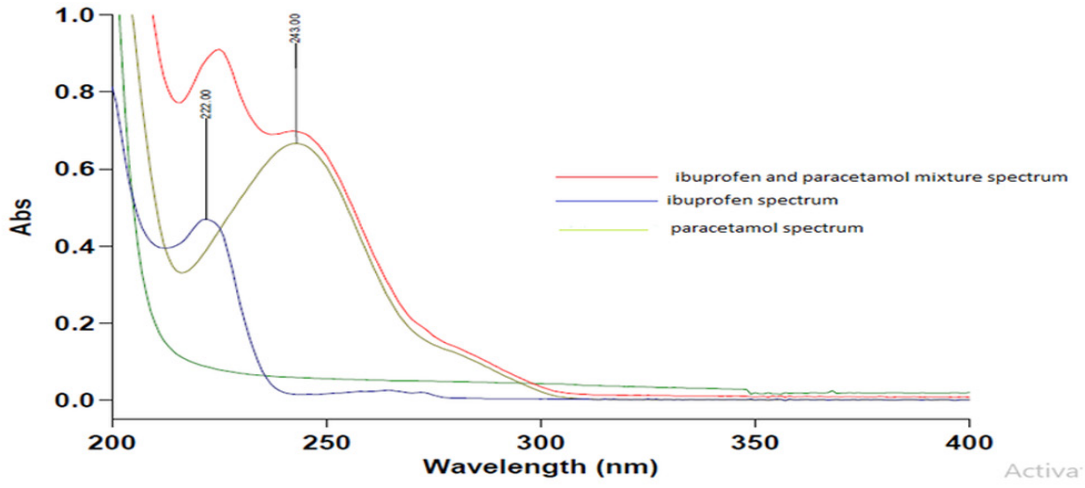


Figure 1: UV Spectra of ibuprofen, paracetamol, the mixture of ibuprofen and paracetamol and the blank (unlabeled)

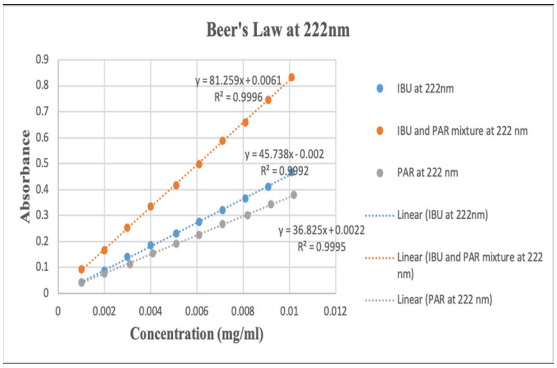


Figure 2: Calibration curve for ibuprofen, paracetamol and the mixture of ibuprofen and paracetamol at 222nm

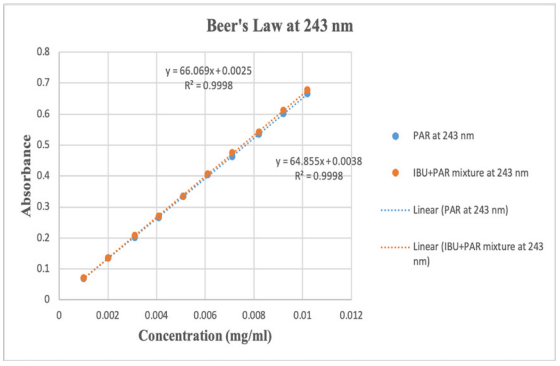
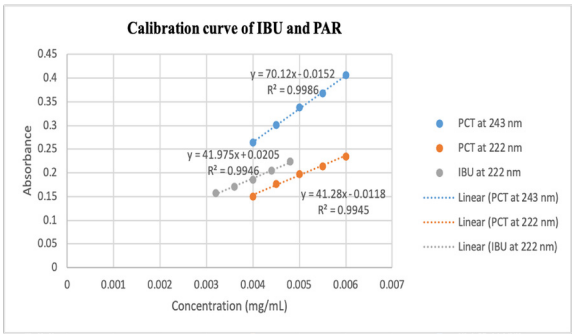


Figure 3: Calibration curve for ibuprofen, paracetamol and the mixture of ibuprofen and paracetamol at 243 nm

(R2≥ 0.995) indicate good linearity of calibration curves for both drugs as in each situation [5].

Molar absorptivity: The molar absorptivities of ibuprofen and paracetamol at 222 nm and 243 nm were calculated using the above-mentioned equation 1 and were found to be approximately the same for all the concentrations (Table 1). The calculated molar absorptivities for the first solution at 222 nm and 243 nm were considered as outliers, as per Grubbs' test for outlier since the G > Gcritical (2.5, 2.8 and 2.4 > 2.1) at 95%



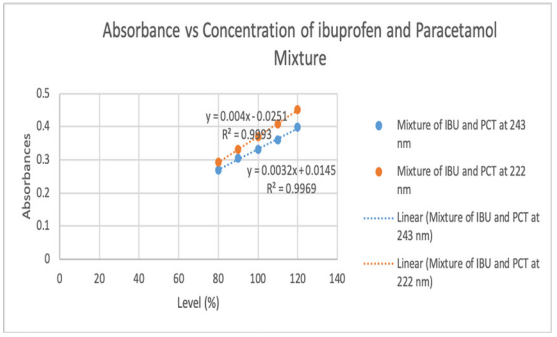


Figure 4: Linearity study for a mixture of ibuprofen (IBU) (Right) and paracetamol (PCT) (Left) at wavelengths of maximum absorbances.

Table 1: Molar absorptivities of ibuprofen (IBU) and paracetamol (PCT) at 222 nm and 243 nm

SN	$(E1)_{222nm\ for\ IBU}$	$(E1)_{222nm\ for\ PCT}$	$(E_2)_{243nm\ for\ PCT}$
1	40.6	40.9	70.2
2	43.7	38.3	68.1
3	46.7	36.2	65.1
4	46.5	37.7	64.9
5	45.3	37.5	65.4
6	45.3	36.9	66.2
7	45.1	37.5	65.3
8	45.2	36.5	65.2
9	44.9	37.2	65.3
10	46.1	37.2	65.3
Average (n=9)	45.4 ± 0.9	37.2 ± 0.6	65.6 ± 1

Table 2: Linearity data for ibuprofen and paracetamol

Concentration (mg/mL)		Absorbance		
Ibuprofen	**Paracetamol**	**PCT at 243 nm**	**PCT at 222 nm**	**IBU at 222 nm**
0.0032	0.004	0.2644	0.1505	0.1568
0.0036	0.0045	0.3011	0.1764	0.1708
0.004	0.005	0.3377	0.1973	0.186
0.0044	0.0055	0.3673	0.214	0.2043
0.0048	0.006	0.4066	0.2349	0.224

PCT: paracetamol; IBU: ibuprofen

confidence level with the degree of freedom df= 9 [16]. Ibuprofen did not show any absorbance at 243 nm and this means $(E_1)_{243nm}$= 0. Development of a simultaneous equation: After determining $(E_1)_{222nm}$ $(E_1)_{243nm}$, $(E_2)_{222nm}$ and $(E_2)_{\lambda 243nm}$, each value was substituted in the system of equations 1 for the determination of ibuprofen and paracetamol concentrations. The equations were found to be the following:

$A_{222nm}=45.4C_1+37.2C_2$
$A_{243nm}=65.6C_2$

By solving the system of equations 1 given above, the concentrations of ibuprofen C_1 and paracetamol C_2 can be estimated.

Method validation: The method was developed following the United States Pharmacopoeia

Table 3: Accuracy data for paracetamol and ibuprofen using UV method

Accuracy data for paracetamol				
Level	**Added conc (mg/ml)**	**Absorbance at 243nm**	**Practical conc (mg/ml)**	**% Recovery**
80%	0.00052	0.2644	0.00403	98.1
	0.00052	0.2645	0.00403	98.4
	0.00052	0.2643	0.00403	97.8
100%	0.00156	0.3378	0.00515	104.6
	0.00156	0.3383	0.00516	105.1
	0.00156	0.3384	0.00516	105.2
120%	0.0026	0.4061	0.00619	102.9
	0.0026	0.407	0.0062	103.4
	0.0026	0.4068	0.0062	103.3
Accuracy data for ibuprofen				
Level	ABS at 222 nm	Added conc (mg/ml)	Practical conc (mg/ml)	% Recovery
80%	0.2575	0.0004	0.0028	133.8
	0.2574	0.0004	0.0028	133.2
	0.2577	0.0004	0.0028	134.9
100%	0.293	0.0012	0.0036	109.8
	0.2929	0.0012	0.0036	109.6
	0.293	0.0012	0.0036	109.8
120%	0.3379	0.002	0.0046	115.3
	0.338	0.002	0.0046	115.4
	0.3378	0.002	0.0046	115.2

Table 4: Precision and robustness data

Precision data					
SN	**Absorbance at 222 nm**			**Absorbance at 243 nm**	
Repeatability (Day 1 Analyst 1)					
1	0.3400			0.328	
2	0.3401			0.3282	
3	0.3428			0.3275	
4	0.3427			0.3285	
5	0.3432			0.328	
6	0.3442			0.3305	
Average (1-6)	0.3422			0.3285	
% RSD (1-6)	0.5			0.3	
Intermediate precision (Day 2 Analyst 2)					
7	0.3312			0.3273	
8	0.3284			0.3254	
9	0.3321			0.328	
10	0.3305			0.327	
11	0.3322			0.3286	
12	0.3349			0.3299	
Average (7-12)	0.3315			0.3277	
% RSD (7-12)	0.6			0.5	
% RSD (1-12)	1.7			0.4	
Robustness data					
Parameter		**Average for standard absorbance (n=3)**	**RSD (%) for standard absorbance**	**Average for sample absorbance (n=3)**	**RSD (%) for sample absorbance**
Wavelength (222 ±1 nm)	221	0.3824	1.4 0.416 0.4213	0.4093	1.4
	222	0.3863			
	223	0.3933			
Wavelength (243±1 nm)	242	0.3612	1.4 0.3886 0.3896	0.3985	1.4
	243	0.352			
	244	0.3541			
pH at 222 nm	7.1	0.402	2 0.416 0.4286	0.427	1.6
	7.2	0.389			
	7.3	0.3874			
pH at 243 nm	7.1	0.3619	0.4 0.3999 0.4014	0.4044	0.6
	7.2	0.3601			
	7.3	0.3592			
Temperature (°C) at 222 nm	25	0.411	1.8 0.4389 0.4389	0.4387	0
	30	0.4114			
	35	0.3987			
Temperature (°C) at 243 nm	25	0.3507	1.5 0.3935 0.3936	0.3946	0.2
	30	0.3479			
	35	0.3581			

(USP) 2018 and The International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) 2005 guidelines. The following performance characteristics were evaluated: precision, linearity, accuracy and robustness.

Linearity: The least square regression was used to obtain calibration curves. A good linearity was proven in the range of 0.0032 - 0.0048 mg/ml for ibuprofen and 0.004 - 0.006 mg/ml for paracetamol (Table 2), with related coefficients of determination being greater than or equal to 0.995 as required by the USP 2018 [14].

Accuracy: In this study, the accuracy results revealed the percentage recovery of paracetamol at three levels of concentration (80%, 100%, and 120%) in the range of 98.1%–105.0% as shown in Table 3, while the percentage recovery for ibuprofen at three concentration levels (80%, 100%, and 120%) range between 109.6% – 134.9% as shown in Table 3. These results indicate that this method is only accurate in the determination of paracetamol absorbance, since the percent recovery lies between 95%- 105% [14].

Precision: The precision of this method was evaluated through repeatability and intermediate precisions. Table 4 summarizes the results obtained under repeatability and intermediate conditions. The relative standard deviation below 2% for each analyst and relative standard deviation of less than 3% between two analysts on different days indicates that the proposed method is precise in determination of ibuprofen and paracetamol in suspension [14].

Robustness: The impact of slight variation of wavelength, pH and temperature on absorbance was assessed during this study and compared with previous literature [15]. Evaluation of robustness data demonstrated that a minor modification of the method conditions including the wavelength, the pH and the temperature was found to be robust within the desired range (RSD less than 2.0%). Different absorbances obtained under different conditions are demonstrated in Table 4.

CONCLUSION

The results of this study within the statistical parameters demonstrated that the proposed UV spectrophotometric method was simple, rapid, specific, accurate and precise. Therefore, this method may be adopted to determine paracetamol quantitatively for routine analysis in prepared formulations of suspensions, without the interference of commonly used excipients and related substances. A UV spectrophotometric method for determination of ibuprofen and paracetamol in suspension without prior separation was developed and validated using a simultaneous equations approach. The obtained results and the statistical parameters showed that this UV spectrophotometric method for determination of ibuprofen and paracetamol in suspension was simple, rapid, linear, precise, robust and accurate in determination of paracetamol only. Therefore, the proposed method can be successfully used to analyze quantitatively paracetamol in suspensions.

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