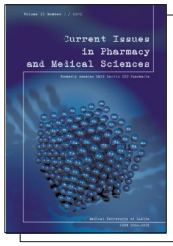

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Development of spectrophotometric method for simultaneous estimation of diclofenac sodium and papaverine hydrochloride in tablets based on simultaneous equation method

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ABSTRACT

A simple, rapid, accurate and precise spectrophotometric method for simultaneous estimation of diclofenac sodium and papaverine hydrochloride in combined tablet and the dissolution media used in the release studies has been developed. It employs formation and solving of simultaneous equation using two analytical wavelengths corresponding to diclofenac sodium and papaverine hydrochloride in the dissolution medium. This method obeys Beer's Law in the employed concentration ranges of 2.5-25 μg/mL for two active substances. The method was validated.

Keywords: Diclofenac sodium, papaverine hydrochloride, spectrophotometric method, simultaneous equation

INTRODUCTION

Diclofenac sodium, sodium 2-[(2,6-dichlorophenyl)amino] phenyl]-acetate, is a potent non-steroidal anti-inflammatory drug, therapeutically used in the treatment of acute and chronic pain, rheumatoid arthritis and related conditions [8,14]. Papaverine hydrochloride, 1-(3,4-dimetoxybenzyl) -6,7-dimetoxyisoquinoline hydrochloride, as a spasmolytic agent is used [8]. In order to increase the therapeutic effect, and decrease the number of adverse reactions, the tablets combining diclofenac sodium and papaverine hydrochloride were prepared and patented [7]. However, simple and rapid simultaneous determination of these two active substances was not easy using a spectrophotometric method.

Diclofenac sodium alone is reported to be estimated by spectrophotometric method in the pharmaceutical preparations [1, 12] and in the dissolution media at different pH which was used in the release studies from the sustained release tablets [2] or matrix tablets [17].

Papaverine hydrochloride can be determined by spectrophotometric method in pharmaceutical dosage forms and in the dissolution media [10, 11].

The simultaneous estimation of diclofenac sodium and papaverine hydrochloride in dosage powders using spectrophotometric method has been described previously. This method was developed for determination only in alkaline solution and a step of the extraction of papaverine hydrochloride with chloroform was required [6].

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A simple and rapid spectrophotometric method for simultaneous estimation of two active substances was described [4, 5, 13]. The method involving formation and solving of simultaneous equation is very simple for routine analysis of two drugs in combined dosage forms. Once the equations are formed, then only measurement of the absorbance of sample solution at two wavelengths and simple calculations are required [4].

The aim of this study was to develop and validate the spectrophotometric method based on simultaneous equation for simultaneous determination of diclofenac sodium and papaverine hydrochloride in tablets and in eight dissolution media used in the release studies.

MATERIAL AND METHODS

Substances and reagents. Diclofenac sodium (DIC) produced by Caesar and Loretz, GmbH, Hilden, Germany, papaverine hydrochloride (PAP) obtained from Galfarm PPH, Cefarm Lublin, Poland, polyvinylpyrrolidone (PVP), mannitol (M), potato starch (PS), trisodium citrate dehydrate, citric acid monohydrate, potassium dihydrogen phosphate, methanol and water were the products of Merck, Germany. Sodium hydroxide solution (0.1 mol/L) and hydrochloric acid solution (0.1 mol/L) were freshly prepared. All other reagents used were of analytical grade (pure for analysis). The composition and preparation of the tablets has been presented in the Polish Patent [7]. One tablet (300 mg) consisted of 50 mg DIC, 20 mg PAP, 70 mg PVP, 70 mg M and 90 mg PS.

Apparatus. UV-visible spectrophotometer Spectromom 195, Hungary, was used.

S o l u t i o n s. An accurately weighed 10 mg of DIC and 10 mg of PAP were transferred into a 100 ml volumetric flask using 30 mL of methanol. After dissolving these substances, the flask was completed to volume with methanol and obtained the stock solution at concentration 100 µg/mL of DIC and 100 µg/mL of PAP.

Accurate volumes of this stock solution were diluted with methanol until obtaining six final concentrations of solutions in the range from 5.0 µg/mL to 50.0 µg/mL.

These methanolic solutions were mixed with the dissolution medium in ratio (1:1, v/v), so those final concentrations of DIC and PAP in the range from 2.5 µg/mL to 25.0 µg/mL (standard solutions) were achieved.

D i s s o l u t i o n m e d i a. Water, HCL 0.1 mol/L, phosphate buffer at pH 4.5; 6.5; 6.8 and citric buffer at pH 4.5; 6.5; 6.8 were used as a dissolution media.

S o l u t i o n s f r o m t a b l e t s. Ten tablets were weighted and average mass of these tablets was calculated, next tablets were powdered. An accurately weighed of the powder mixture (equivalent to about 40 mg DIC and 16 mg PAP, 50 mg DIC and 20 mg PAP, 60 mg DIC and 24 mg PAP) was transferred to a 100 mL volumetric flask using 50 mL of methanol and shaken about 5-7 min. The flask was completed to volume with methanol. The obtained solution was filtered by the Whatman filter paper. An accurate volume of the effluent was diluted with methanol (to obtaining concentrations of active substances in the range from 2.5 µg/mL to 25.0 µg/mL). The obtained solution was mixed with the dissolution medium in the ratio 1:1 (v/v).

Similarly, the solution from tablets without active substances (obtained from powder mixture about 244 mg, 230 mg and 216 mg) was prepared and determined.

D e t e r m i n a t i o n o f U V s p e c t r u m. The absorbance of the methanolic solution of DIC and PAP at concentration 10 µg/mL mixed with each of the dissolution medium in ratio 1:1 (v/v) were measured in the range of UV spectrum from 230 nm to 330 nm. At the analytical wavelengths for DIC and PAP the maximum absorbance was achieved. The analytical wavelengths of active substances (λ_{max}) in each of the dissolution medium are presented in Table 1.

Table 1. Values of the Sandell's sensitivity (a) calculated for analytical wavelengths (λ_{max}) of DIC and PAP in eight dissolution media

Dissolution media	λ_{max}, nm	a_D, µg/cm^2	a_p, µg/cm^2
Water	λ_1 = 276 λ_2 = 239	a_{D1}= 0.02929 a_{D2}= 0.01886	a_{p1}= 0.01298 a_{p2}= 0.12374
HCL 0.1 mol/L	λ_1 = 274 λ_2 = 251	a_{D1}= 0.01054 a_{D2}= 0.00657	a_{p1}= 0.01534 a_{p2}= 0.16758
Phosphate buffer pH 4.5	λ_1 = 274 λ_2 = 240	a_{D1}= 0.02908 a_{D2}= 0.02281	a_{p1} = 0.01645 a_{p2}= 0.09289
Phosphate buffer pH 6.5	λ_1 = 278 λ_2 = 238	a_{D1}= 0.03078 a_{D2}= 0.02497	a_{p1}= 0.01650 a_{p2}= 0.10998
Phosphate buffer pH 6.8	λ_1 = 278 λ_2 = 238	a_{D1}= 0.03276 a_{D2}= 0.02433	a_{p1}= 0.01692 a_{p2}= 0.12728
Citric buffer pH 4.5	λ_1 = 276 λ_2 = 238	a_{D1}= 0.03190 a_{D2}= 0.02346	a_{p1}= 0.01526 a_{p2}= 0.10037
Citric buffer pH 6.5	λ_1 = 278 λ_2 = 238	a_{D1}= 0.03402 a_{D2}= 0.02693	a_{p1}= 0.02063 a_{p2}= 0.14696
Citric buffer pH 6.8	λ_1 = 278 λ_2 = 238	a_{D1}= 0.03939 a_{D2}= 0.02838	a_{p1}= 0.01861 a_{p2}= 0.10773

C a l c u l a t i o n o f t h e S a n d e l l ' s s e n s i t i v i t y (a). The Sandell's sensitivity is the concentration of the analyte (c, µg/mL) which will give an absorbance (A) of 0.001 in a cell of path length (l) 1 cm and is expressed as µg/cm^2. It was calculated from the following equation:

$$a = \frac{A}{l \cdot c}$$

F o r m a t i o n o f s i m u l t a n e o u s e q u a t i o n. Based on Beer's Law for calculation of the concentrations of DIC and PAP two equations were created [9, 13]. When = 1 cm equations can be expressed:

$$A_1 = a_{D1} \cdot C_D + a_{p1} \cdot C_p$$

$$A_2 = a_{D2} \cdot C_D + a_{p2} \cdot C_p$$

hence

$$C_D = \frac{A_1 \cdot a_{p2} - A_2 \cdot a_{p1}}{a_{D1} \cdot a_{p2} - a_{p1} \cdot a_{D2}}$$

$$C_P = \frac{A_2 \cdot a_{D1} - A_1 \cdot a_{D2}}{a_{D1} \cdot a_{p2} - a_{p1} \cdot a_{D2}}$$

where
A_1 – is the absorbance at analytical wavelength λ_1
A_2 – is the absorbance at analytical wavelength λ_2
λ_1 is λ_{max} – for DIC, nm
λ_2 is λ_{max} – for PAP, nm
a_{D1}, a_{D2} – are Sandell's sensitivity of DIC at analytical wavelengths λ_1 and λ_2, respectively, µg/cm^2
a_{p1}, a_{p2} – are Sandell's sensitivity of PAP at analytical wavelengths λ_1 and λ_2, respectively, µg/cm^2
C_D, C_P – are concentrations of DIC and PAP, respectively, µg/mL.

The values of Sandell's sensitivity of DIC and PAP at analytical wavelengths in eight dissolution media are shown in Table 1.

P r o c e d u r e f o r t h e c a l i b r a t i o n c u r v e. The absorbances of the standard solutions of DIC and PAP at concentrations 2.5-25.0 µg/mL were measured spectrophotometrically at two analytical wavelengths for DIC and PAP. The calibration curves are presented in Fig.1.

V a l i d a t i o n o f t h e m e t h o d

Selectivity. To check selectivity, the absorbance of the solutions from tablets without active substances were measured in the range of UV spectrum from 230 nm to 330 nm.

Linearity. To evaluate linearity, the standard solutions were assayed and the regression equations and regression coefficients for the calibration curves for DIC and PAP were presented. The linearity data are shown in Fig. 1.

Accuracy. To check the accuracy of the proposed method, recovery studies were carried out at 80, 100 and 120% of the concentration of active substances in powdered tablets. The recovery study was performed three times at each level. The results are reported in Table 2.

Precision. To check the degree of repeatability of the methods, suitable statistical evaluation was carried out. Repeatability was performed for six times with tablets formu-

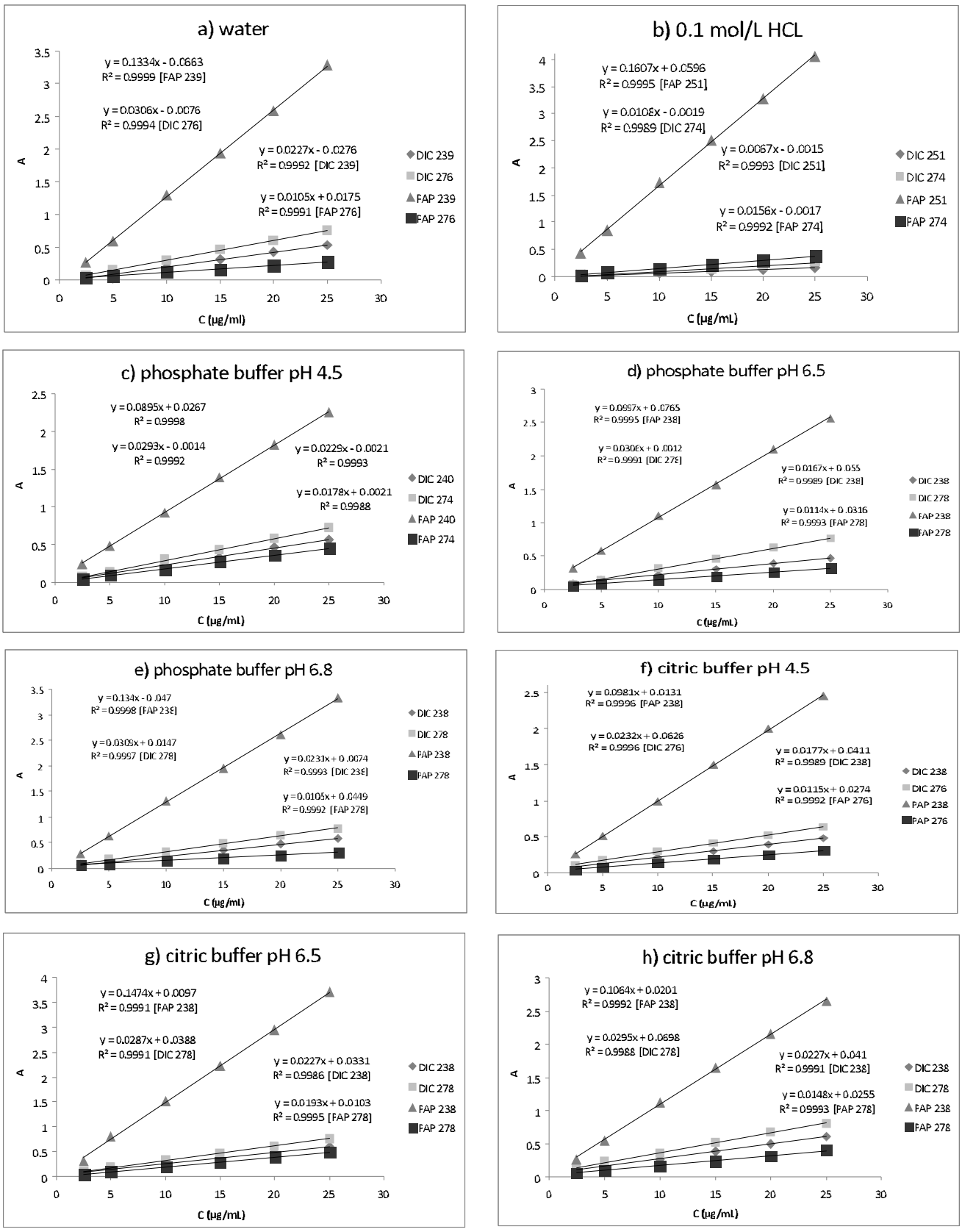

Fig. 1. Calibration curves of DIC and PAP at corresponding analytical wavelengths in the dissolution medium: a) water, b) HCL 0.1 mol/L, c) phosphoric buffer pH 4.5, d) phosphoric buffer pH 6.5, e) phosphoric buffer pH 6.8, f) citric buffer pH 4.5, g) citric buffer pH 6.5, h) citric buffer pH 6.8

lation. The standard deviation, coefficient of variation and standard error were calculated. The results of statistical evaluation are given in Table 2.

RESULTS AND DISCUSSION

For determination of DIC and PAP in different dissolution media used in the release study, a spectrophotometric

method for simultaneous assay of two active substances must be developed. The chosen spectrophotometric method was based on simultaneous equation. This method was used for simultaneous estimation of ibuprofen and paracetamol in gelatin capsules [4], in tablets [5] and for determination of acetylsalicylic acid and paracetamol in tablets [13]. This method is based on assays of absorbance of the solution composed of two active substances in their analytical wavelengths.

Table 2. Estimation of content of DIC and PAP in the powdered tablets

Dissolution medium	Amount taken (mg)		Amount found (%)		% Recovery ± SD CV (%)	
	DIC	PAP	DIC	PAP	DIC	PAP
Water	40	16	98.88 ± 1.13	99.25 ± 0.78	99.44 ± 1.07 1.076	99.75 ± 0.90 0.902
	50	20	100.17 ± 0.85	99.87 ± 1.24		
	60	24	99.26 ± 1.22	100.13 ± 0.67		
HCL 0.1 mol/L	40	16	99.56 ± 0.80	98.55 ± 1.31	98.94 ± 0.86 0.869	98.95 ± 1.07 1.081
	50	20	100.23 ± 1.03	99.43 ± 0.85		
	60	24	99.02 ± 0.76	98.87 ± 1.04		
Phosphate buffer pH 4.5	40	16	98.45 ± 1.25	99.21 ± 0.59	99.21 ± 0.93 0.937	99.8 ± 0.72 0.721
	50	20	99.69 ± 0.89	100.04 ± 0.61		
	60	24	99.50 ± 0.64	100.15 ± 0.97		
Phosphate buffer pH 6.5	40	16	101.13 ± 1.31	98.68 ± 1.20	100.26 ± 1.0 0.997	99.23± 0.83 0.836
	50	20	100.21 ± 0.76	100.05 ± 0.55		
	60	24	99.43 ± 0.94	99.76 ± 0.74		
Phosphate buffer pH 6.8	40	16	99.56 ± 1.05	99.20 ± 0.95	99.50 ± 1.12 1.126	99.28 ± 0.97 0.977
	50	20	100.11 ±0.95	100.07 ± 0.82		
	60	24	98.84 ±1.35	98.57 ± 1.14		
Citric buffer pH 4.5	40	16	98.93 ± 0.64	100.13 ± 0.68	99.3 ± 0.77 0.775	100.27 ± 1.03 1.027
	50	20	99.76 ± 1.14	101.03 ± 1.36		
	60	24	100.21 ± 0.54	99.65 ± 1.05		
Citric buffer pH 6.5	40	16	99.58 ± 0.73	99.78 ± 0.54	99.97 ± 0.75 0.750	99.95 ± 0.64 0.640
	50	20	100.09 ± 0.55	100.00 ± 0.72		
	60	24	100.25 ± 0.98	100.08 ± 0.65		
Citric buffer pH 6.8	40	16	100.53 ± 0.65	98.77 ± 0.91	98.36 ± 0.98 0.996	99.50 ± 0.95 0.955
	50	20	99.36 ± 1.28	99.58 ± 0.69		
	60	24	100.18 ± 1.01	100.16 ± 1.24		

As shown in Table 1, the analytical wavelengths in which observed maxima absorbance for DIC and PAP are different in water, HCL 0.1 moL/L, citric and phosphoric buffers at pH 4.5, in turn in citric and phosphoric buffers at pH 6.5 and 6.8 are the same and equal for DIC 278 nm and for PAP 238 nm.

The solutions from tablets without active substances had no absorbance. This fact indicated that the auxiliary substances did not interfere with determination of DIC and PAP and the method can be used for determination of two active substances.

Saleh et al. [16] determined maximum absorbance of DIC at wavelengths depending on pH solution: at 273 nm at pH 1.2; 2.5; 4.5 or at 275 nm at pH 5.3; 6.5; 7.2 using the UV spectrum.

Bucci et al. [3] estimated the UV spectrum for aqueous DIC solution (0.1 %, w/v) at pH 7.7. The data showed that there are two maximum absorbances for DIC at wavelengths 200 nm and 276 nm.

For calculation of concentrations of active substances from a simultaneous equation the Sandell's sensitivity () values for DIC and PAP in their analytical wavelengths were calculated and shown in Table 1.

Having analytical wavelengths for DIC and PAP in each of dissolution medium and the Sandell's sensitivity values, the standard solutions were measured and the calibrated curves were done. As shown in Fig. 1, the linearity range for DIC and PAP were 2.5-25.0 μg/mL. The correlation coefficients of calibration curves ($R^2 > 0.99$) and its confirmed good linearity of the method are satisfactory. The values of regression equations show that the method is not charged with a systematic error.

The results of the recovery studies are found to be satisfactory as shown in Table 2. The results obtained from accuracy study indicated that the percentage recovery was in the range 98.36-100.26 % for DIC and 98.95-100.27 % for PAP. Repeatability studies were found to be satisfactory withRSD 0.750-1.126 and 0.640-1.081 for DIC and PAP, respectively. Average values of recovery and average related coefficients were different at no more than 2 % , which suggests that the method is accurate and precise [15].

The limit of quantification (LOQ) was determined to be 1.5 μg/mL for DIC and 1.8 μg/mL for PAP. The limit of detection (LOD) was calculated at 0.5 μg/mL and 0.6 μg/mL for DIC and PAP, respectively.

CONCLUSIONS

The proposed spectrophotometric method for simultaneous estimation of diclofenac sodium and papaverine hydrochloride in different dissolution media is simple, rapid, accurate, and precise. The validation method confirms that the analytical procedure employed for the analysis is suitable and reliable for its intended use. In the presented study, all validation parameters for quantitative analysis of diclofenac sodium and papaverine hydrochloride in tablets were tested and the data were elevated according to their acceptance criteria.

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