Bivariate Analysis for the Determination of Paracetamol and Caffeine in Drug Formulations

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Abstract

The UV absorption spectrum of paracetamol/caffeine combination shows strongly overlapped features. Bivariate spectrophotometric analysis was applied to quantify a paracetamol/caffeine combination in commercial drug samples. The proposed method was tested according to ICH guidelines and compared to the validated HPLC-UV method. The results obtained by the bivariate method are in a good agreement with the labeled contents. Statistical evaluation using student's *t*-test and *F*-test showed no significant differences between the results obtained by the bivariate method and the HPLC method for commercial drug samples. The bivariate spectrophotometric method is rapid, precise and accurate; it could effectively be an alternative method to HPLC.

Keywords: *Bivariate analysis, Paracetamol, Caffeine, HPLC, Derivative spectroscopy, Binary Drug.*

Introduction

Combination drug products, also known as fixed-dose combinations (FDC), are combinations of two or more active drugs in a single pill.[1] The active drugs in such combinations are commonly acceptable to act by different mechanisms. In recent years, an increasing interest in the pharmaceutical industry to develop FDC drug products was noticed since FDCs are cheaper to manufacture and easier to distribute than single drug formulations.[2] Panadol Extra® and Panda® compound tablets, which are binary FDC drugs, are formulated to contain paracetamol (PAR) and caffeine (CAF) at various proportions. PAR, also known as acetaminophen or APAP, is N-(4-hydroxyphenyl)-acetamide; it is used as a pain reliever and a fever reducer.[3] CAF is 1,3,7-Trimethylpurine-2,6-dione and is the most widely consumed central nervous system stimulant.[3]

Several methods have been reported for the analysis of PAR and CAF such as high performance liquid chromatography (HPLC),[4-8] capillary electrophoresis (CE),[6] derivative spectrophotometry,[9] flow injection analysis with multiple pulse amperometric (FIA-MPA) detection,[10] liquid chromatography-mass spectrometric

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method LC-ESI-MS,[11] micellar electrokinetic capillary chromatography,[12] multiparameter-responding flow-through system with solid phase UV spectrophotometric detection,[13-15] micellar liquid chromatography[16] as well as thin layer chromatography.[17] Although most of these methods are accurate and sensitive, the HPLC methods are officially the standard ones for the quantitative analysis of PAR and CAF in pharmaceutical formulations (tablets [4-8] and syrup[16]) as well as in the human plasma.[17]

The HPLC methods suffer, however, from some drawbacks such as the use of low resolution power columns, long run time, limited choice of detectors and using different solvents for sample and mobile phase.[4,18-20] Moreover, the availability of HPLC instruments could be a major challenge for researchers working on the analysis of FDCs in small scale industries and universities. Spectroscopic based techniques have come to provide simple and fast alternatives for the analysis of FDC drug products. One of these techniques is the bivariate analysis method.[4] The feasibility of the bivariate method compared to the HPLC methods was demonstrated in the analysis of drug tablets such as hydrochlorothiazide/enalapril maleate, hydrochlorothiazide/bisoprolol fumarate, prifinium bromide/paracetamol and moxicillin/potassium clavulanate.[21, 22]

The main advantage of the bivariate spectrophotometric analysis is its ability to produce optimal wavelengths for strongly overlapped absorption spectra of binary mixtures without the need to use derivatization procedures.[23] Optimal wavelengths chosen for analysis are thereby not necessarily the wavelengths of maximum absorption,[21,22] reducing thus background and matrix interferences.[24-28] The simultaneous determination of individual components in binary mixtures is based on the additive absorbance of the two components at two wavelengths. The chosen two wavelengths are correlated with the maximum sensitivity value according to Kaiser`s sensitivity matrix (**K**).[23]

The objective of this work is to provide a simple and fast spectrophotometric method to simultaneously quantify PAR and CAF in pharmaceutical dosage forms, which could be applied in a routine drug quality control according to ICH guidelines.

Materials and Methods

Experimental setups for bivariate spectrophotometric method, instrument, sample preparation, procedures and liquid chromatography method were described in details in a previously published work by Wedian *et al*.[21,22]

Results and Discussion

The bivariate spectrophotometric method

Triple distilled water was used as a solvent and a blank in this. The absorption spectra of 1.0 mg/L of paracetamol (purity = 93%, batch No. 9510610, Hikma

Pharmaceuticals, Jordan) and 5.0 mg/L of CAF (purity = 99.9%, Aldrich chemicals) are shown in Figure 1. PAR shows a maximum absorbance at a wavelength of 246 nm, while CAF has three maximum absorbances at wavelengths of 207, 234, and 275 nm. PAR maximum absorbance is in complete overlapping with the absorption spectrum of CAF between 205–300 nm. The bivariate analysis was applied on this mixture, the absorption at six wavelengths of 216, 230, 244, 262, 277 and 282 were measured. Standard calibration curves were constructed at each selected wavelengths and the slopes of these curves were obtained. The slopes were then analyzed by Kaiser`s determinant (**K**) in order to produce an optimum pair of wavelengths; the absorption at 216 and 244 nm shows the highest absolute sensitivity (Table 1).

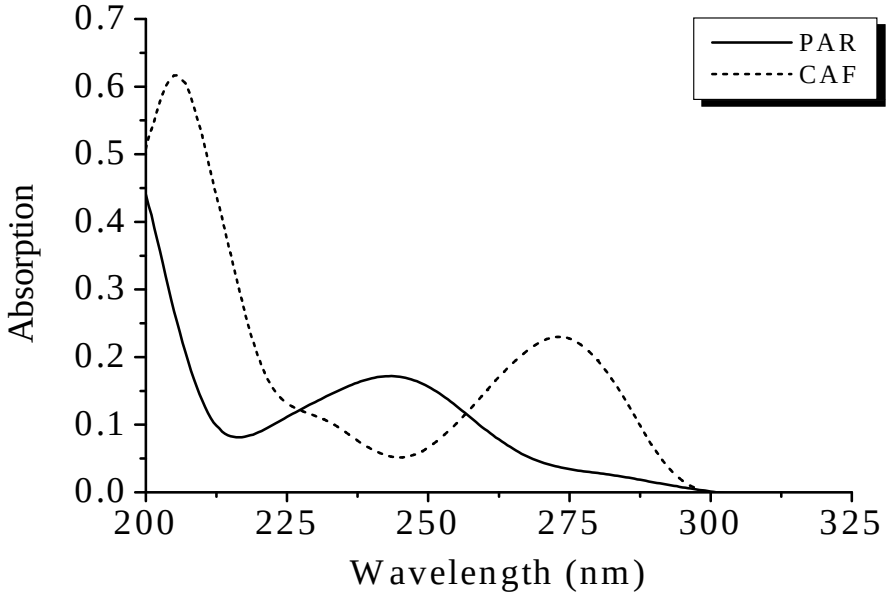

Figure1: Absorption of an aqueous solution of 1.0 mg/L of PAR (solid line) and 5.0 mg/L of CAF (dash line).

Table 1: Values of the absolute selectivity of Kaiser`s determinant ($K \times 10^5$).

λ_1/λ_2 (nm)	216	230	244	262	277	282
216	–	263.2	**405.8**	103.1	51.63	19.18
230	–	–	109.1	100.8	20.32	11.76
244	–	–	–	198.5	292.9	17.44
262	–	–	–	–	99.66	53.74
277	–	–	–	–	–	8.412
282	–	–	–	–	–	–

Linearity

The Linearity of the method was evaluated at the selected wavelengths for PAR and CAF in the concentration range of 1–50 mg/L in triple distilled water. Good determination coefficients higher than 0.997 were obtained. The calibration equations for PAR and CAF at the proposed wavelengths are presented in Table 2.

Table 2: The bivariate linear calibration equations.

Component	Calibration Equations	
	λ_1 = 216 nm	λ_2 = 244 nm
PAR	Y=0.028X– 0.013 r^2= 0. 997	Y=0.064X– 0.003 r^2= 0.997
CAF	Y=0.069X– 0.002 r^2= 0.999	Y=0.013X– 0.009 r^2= 0.998

Y is the absorbance at given wavelength; X is the concentration in ppm. r^2 is the coefficient of correlation.

Analytical recovery of spectrophotometric method

The analytical performance of the method proposed to a routine analysis was confirmed by analyzing laboratory prepared mixtures in which the concentration ratios of PAR to CAF were in the range 1:5 to 5:1. The results of the percentage recoveries (mean value ± S.D., *n*=4) are shown in Table 3. High recoveries between 96.2 to 100.6% were obtained. The recovery data appears to be quite independent on the concentration ratio of the two components in the mixtures. The bias of all mixtures was less the 8%. The relative standard deviation (R.S.D.) was less than 2% in all mixtures. Therefore, the results suggest that the method is accurate and precise, since these values are within the permissible limits suggested by the ICH guidelines.[29]

Table 3: Spectrophotometric training sets of different PAR/CAF mixtures.

Mixture	PAR			CAF		
PAR:CAF	Concentration (mg/L)	Found[b] (mg/L)	%Recovery ±S.D.	Concentration (mg/L)	Found[b] (mg/L)	% Recovery ±S.D.
1 (1:3)	10.0	9.9	99.0	30.0	29.3	97.7
2 (2:3)	20.0	19.5	97.7	30.0	28.2	94.0
3 (3:2)	30.0	29.2	97.3	20.0	20.5	100.3
4 (3:1)	30.0	29.1	97.1	10.0	9.89	98.9
5 (5:2)	50.0	49.2	98.4	20.0	19.1	97.6
6 (5:1)	50.0	50.3	100.6	10.0	9.63	96.2
Mean ±S.D.[a]			98.4± 1.3			97.5 ± 2.2

[a] Standard deviation; [b] average of 4 measurements (S.D < 0.35).

Analytical Frequency

The whole proposed analytical procedure (including sample treatment, preparation and measurement) takes about 10 minutes per sample; thus, the analytical frequency is about 6 samples per hour. The proposed method is, therefore, considerably less time consuming compared to the used chromatographic methods.

HPLC Studies

The PAR/CAF combination was analyzed using a reversed-phase C-18 column (250 mm × 4.6 mm i.d., 5µm) applying isocratic elution of a mobile phase consisting of 1.0 mM phosphate buffer (pH=3.0) – acetonirtile (TEDIA, OH, USA) – triethylamine (Sigma-Aldrich chemicals) (84.5:15:02,v/v/v) with 1.5 mL/min flow rate and detected at

216 nm.[30]The retention times of Paracetamol and Caffeine were found to be 3.21±0.20 min and 7.11±0.20 min, respectively (Fig. 2). Under these conditions, the PAR/CAF peaks were well defined and resolved with acceptable tailing.

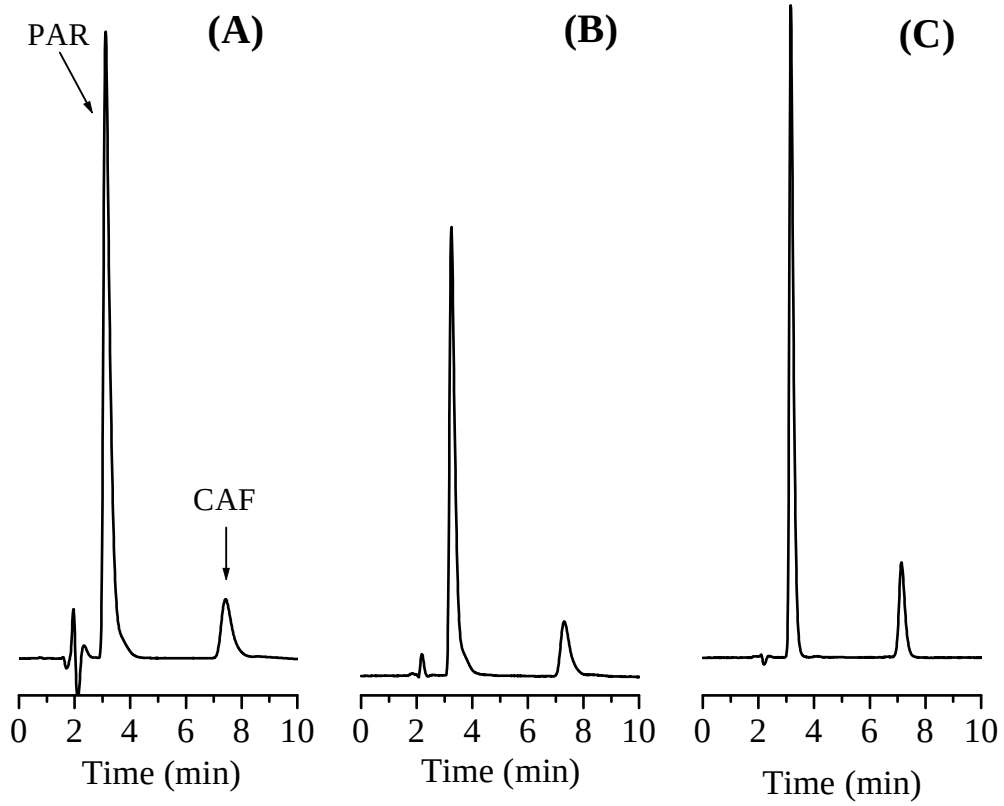

Figure 2: Representative chromatograms of a lab mixture, 50.0 mg/L PAR and 10.0 mg/L CAF in the mobile phase (panel A), Panda® Compound, 40.0 mg/L PAR and 5.2 mg/L CAF in the mobile phase (panel B), Panadol Extra® Compound, 40.0 mg/L PAR and 5.2 mg/L CAF in the mobile phase (panel C).

Linearity and Reproducibility of the HPLC

The calibration curves for PAR and CAF were linear over the concentration range of 1–50 mg/L with determination coefficients greater than 0.997. The calibration curves were established from an average of eight determinations for each point. For both compounds, a good linear relationship between peak area and standard concentration was found, as described by the linear regression equations: Y=43056×[PAR] – 16998 (r^2=0.9998) for PAR and Y=13429×[CAF] – 65851 (r^2=0.9998) for CAF. The method was accurate for both components (bias<10%) and reproducible (inter and intra-day R.S.D<2%).

HPLC Analytical Recovery

The HPLC-UV assay[30] was used to quantify PAR and CAF in laboratory prepared mixtures of various proportions (Table 4). The average recovery of the method is 99.5% with a percentage RSD for the method precision less than 3% which strongly confirms that the HPLC-UV method is precise, accurate and selective for the simultaneous determination of PAR and CAF in mixtures.

Table 4: Chromatographic training sets of different PAR/CAF mixtures.

Mixture	PAR			CAF		
(PAR:CAF)	Concentration (mg/L)	Found[b] (mg/L)	%Recovery ±S.D.	Concentration (mg/L)	Found[b] (mg/L)	% Recovery ±S.D.
1 (1:3)	10.0	9.99	99.9	30.0	31.0	103.3
2 (2:3)	20.0	21.1	105.6	30.0	30.9	97.5
3 (2:5)	20.0	20.8	96.3	50.0	49.2	98.4
4 (5:1)	50.0	52.0	96.0	10.0	9.91	99.1
5 (3:2)	30.0	29.7	99.0	20.0	20.0	100.0
Mean ±S.D.[a]			99.4±3.9			99.7± 2.2

[a] Standard deviation; [b] average of three determinations (S.D < 0.52).

Applicability on Commercial Products

The absorption spectra for Panadol Extra® and Panda® (commercial products) working solutions and a spiked mixture of the PAR and CAF (mixture number 6, Table 3) are shown in Figure 3. The similarity in absorption behavior and the relative intensities of the absorption peaks indicate that the filtration of the aqueous working solutions of Panadol Extra® and Panda® is sufficient to remove insoluble tablet additives such as starch pregelatinised, calcium carbonate, alginic acid, magnesium stearate, sodium ethyl (E 215) and sodium propyl (E 217) parahydroxybenzoates. This result indicates that the filtration procedure shows low interferences, thus, it can be applied in the analysis of commercial products.

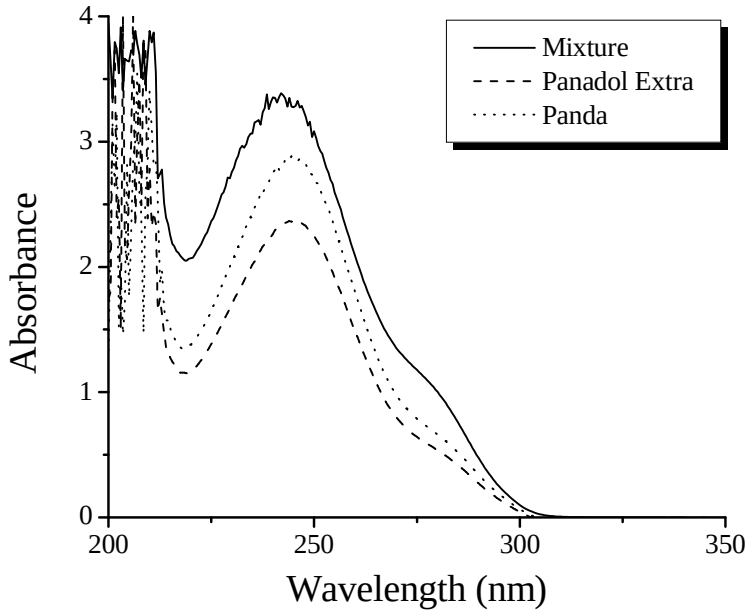

Figure 3: UV absorption of aqueous solutions of Panadol Extra® (40.0 mg/L PAR, 5.2 mg/L CAF mixture (dash line)), Panda® (40.0 mg/L PAR, 5.2 mg/L CAF mixture(dotted line)) in comparison to a spiked mixture of 50.0 mg/L PAR and 10.0 mg/L PAR (solid line).

The bivariate method was applied for the analysis of marketed drug, Panadol Extra® Compound and Panda® Compound, containing 500 mg PAR and 65 mg CAF per tablet. The proposed method yielded percentage recoveries (mean value ± S.D., *n*=5) of 87.53±1.12 for PAR and 85.11±0.32 for CAF in Panadol Extra® Compound and

93.84±0.95 for PAR and 96.44±0.32 for CAF in Panadol® Compound. In comparison, the HPLC method was applied to the same mixture yielding 86.32±0.57 for PAR and 84.90±0.41 for CAF in Panadol Extra® Compound and 95.17±0.78 for PAR and 97.32±0.61 for CAF in Panadol® Compound. Percentage recoveries of both compounds are presented in Table 5. The results in Table 5 indicate that percentage recoveries of both compounds are satisfactory according to the ICH guidelines.[29]

Statistical analysis of the results

The results obtained for PAR/CAF in the pharmaceutical tablets in Table 5 were statistically analyzed using Student's *t*-test and the variance ratio *F*-test; the results are given in Table 5. The *t* and *F* values at 95% confidence level for Panadol Extra® Compound were found to be less than the tabulated values which indicates that there are no significant differences between the accuracy and the precision of proposed method and that of the HPLC method. It was noticed that the accuracy for Panadol Extra® was less than 15% different from the claim labels which can be attributed to the matrix effect since it is accustomed to use insoluble additives (Fig. 2). The analysis of Panda® shows no significant difference in the precision of the two methods used. Moreover, the accuracy was less than 8% from the claimed label value.

Table 5: Statistical analysis for the results obtained by the bivariate method and the HPLC method for Panadol Extra® and Panda® Compounds.

Drug	Label Claim (mg/L)		Average percentage recoveries ± S.D.			
			Bivariate Method[a]		HPLC[b]	
	PAR	**CAF**	**PAR**	**CAF**	**PAR**	**CAF**
Panadol Extra®	500	65	87.53±1.12	85.11±0.32	86.32±0.57	84.90 ±0.41
Parameters						
N			5	5	4	4
Variance			1.25	0.102	0.32	0.17
t (2.37)*			2.07	0.864	–	–
F (5.19)*			3.91	1.67	–	–
Panda®	500	65	93.84±0.95	96.44±0.32	95.17±0.78	97.32 ±0.61
Parameters						
N			5	5	4	4
Variance			0.91	0.10	0.61	0.37
t (2.37)*			2.24	1.48	–	–
F (5.19)*			1.49	3.72	–	–

[a] average of five determinations; [b] average of four determinations; * values in parentheses correspond to the theoretical values of *t* and *F* at (p = 0.05).

Conclusions

The bivariate spectrophotometric analysis was applied successfully to quantify PAR/CAF combinations in pharmaceutical tablets of Panadol Extra® and Panda®. This simple assay demonstrates good reproducibility, short analysis duration and little sample manipulation. It limits also the consumption of organic solvents (a green method) when compared to the HPLC method. Based on the *F*- and *t*- tests at 95%

confidence level, there are no significant differences with regard to precision and accuracy between the bivariate method and the HPLC method. Finally, the proposed method may represent an alternative rapid assay for the analysis of these two components in pharmaceutical dosage forms.

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