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Research Article

Spectrum Subtraction Method for Simultaneous Determination of Paracetamol and Diclofenac sodium in their Combined Pharmaceutical Dosage Forms

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Abstract

A simple, specific, accurate and precise spectrophotometric method was settled for simultaneous determination of paracetamol and Diclofenac Sodium in their pure form and in their pharmaceutical formulation. Spectrum subtraction technique has been used in simultaneous determination of both drugs without prior separation. Spectrum subtraction method parameters were validated according to ICH guidelines in which accuracy, precision, repeatability and robustness were found in accepted limits. Advantages and disadvantages of spectrum subtraction technique were discussed and statistical comparison between the proposed method and the reference one was also performed.

Keywords: Spectrophotometric; Paracetamol; Diclofenac Sodium; Spectrum subtraction; ICH guidelines; Statistical comparison.

Introduction

Paracetamol (PAR); N-(4-Hydroxyphenyl) acetamide (Figure 1) is related to NSAID (non-steroidal anti-inflammatory drugs) which can act both centrally and peripherally for the treatment of non-inflammatory conditions in patients having gastric symptoms [1]. Diclofenac sodium (DCL); 2-(2,6-dichloroamino)phenylacetic acid (Figure 1) [2], is an analgesic and anti-inflammatory agent which can act by the inhibition of the synthesis and the release of leukotrienes and prostaglandins [3]. DCL can be combined with PAR as the latter can provide basic relief before DCL and enhances its pain-relieving and antipyretic effect. PAR & DCL combination can be used in treatment of several diseases not only inflammation [4].The literature demonstrated that several methods were accomplished for the analysis of PAR and DCL in their mixture form or in combination with other drugs. PAR & DCL have been determined by spectrophotometric methods [5-9], HPLC methods [10-13], TLC methods [14,15], capillary zone electrophoresis method [16], voltammetric method [17], a method based on poly (diallyldimethylammonium chloride) functionalized graphene [18] and a method based on Au nanoparticles - functionalized graphene/poly (L-Arginine) glassy carbon electrode [19]. To the best of our knowledge, no method for the estimation of this drug mixture by using spectrum subtraction technique was yet reported. As such, the aim of the current work is to develop a new spectrophotometric method which is accurate, fast and non-complicated for determination of PAR & DCL combination without the interference of their additives or their excipients in pharmaceutical formulations.

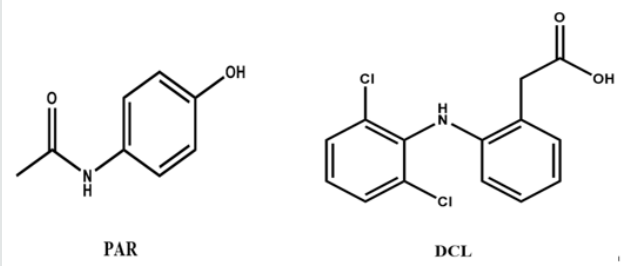

Figure 1: Chemical structures of Paracetamol (PAR) and Diclofenac sodium (DCL).

Experimental Apparatus

JASCO dual beam (Japan) UV-visible spectrophotometer model V-630, connected to an ACER compatible computer with spectra manager II software was used. The spectral slit width is 2 nm at speed up can be increased up to 8000 nm/min. All the measurements have been carried out in 1 cm quartz cell. The wavelength ranges were 200 - 400 nm at room temperature. Also, PASW statistics 18→ software program was used for statistical analysis.

Materials and Reagents

Pure standards

PAR and DCL were kindly provided by EIPICO(Egypt). Their purity was claimed to be as 99.50% and 99.80 % for PAR & DCL, respectively.

Pharmaceutical formulations

Diclocin® tablets were purchased from the market (label claim: PAR 250 mg + DCL 50 mg) produced by Cipcopharmaceuticals, India.

Solvents

HPLC grade Methanol was purchased from LiChrosolv, Merck KGaA (Germany). All of measurements have been accomplished by using 90% Methanol.

Standard solutions

Standard stock solutions (1 mg/mL) of PAR and DCL were prepared in 90% methanol. Working standard solution of PAR (40 µg/mL) and DCL (50 µg/mL) were prepared by further dilution with 90% methanol.

Laboratory prepared mixtures

Different ratios of PAR & DCL were performed by transferring aliquots from their standard solutions to volumetric flasks (10 mL) and then dilution was carried out with 90% methanol.

Procedure

Construction of calibration curves

For PAR: Working solutions equivalent to 4-22 µg/mL were prepared by addition of aliquots (1, 1.50, 2, 2.50, 3, 3.50, 4, 4.50, 5, 5.50 mL) of PAR working standard solution (40 µg/mL) to 10 mL volumetric flasks followed by dilution with 90% methanol. For DCL: Working solutions equivalent to 5-45 µg/mL were prepared by adding aliquots (1, 1.50, 2, 2.50, 3, 3.50, 4, 4.50, 5, 6, 7 mL) of DCL working standard solution (50 µg/mL) to 10 mL volumetric flasks followed by dilution with 90% methanol. Measurements of the absorption spectra were carried out at room temperature over the wavelengths (200-400 nm).

For Spectrum Subtraction method

The method relies on subtracting the spectrum of Y from the spectrum of the mixture (X + Y), therefore we can obtain the zero-absorption spectrum of X again. This can be summarized as the following:

$$(X + Y) - Y = X$$

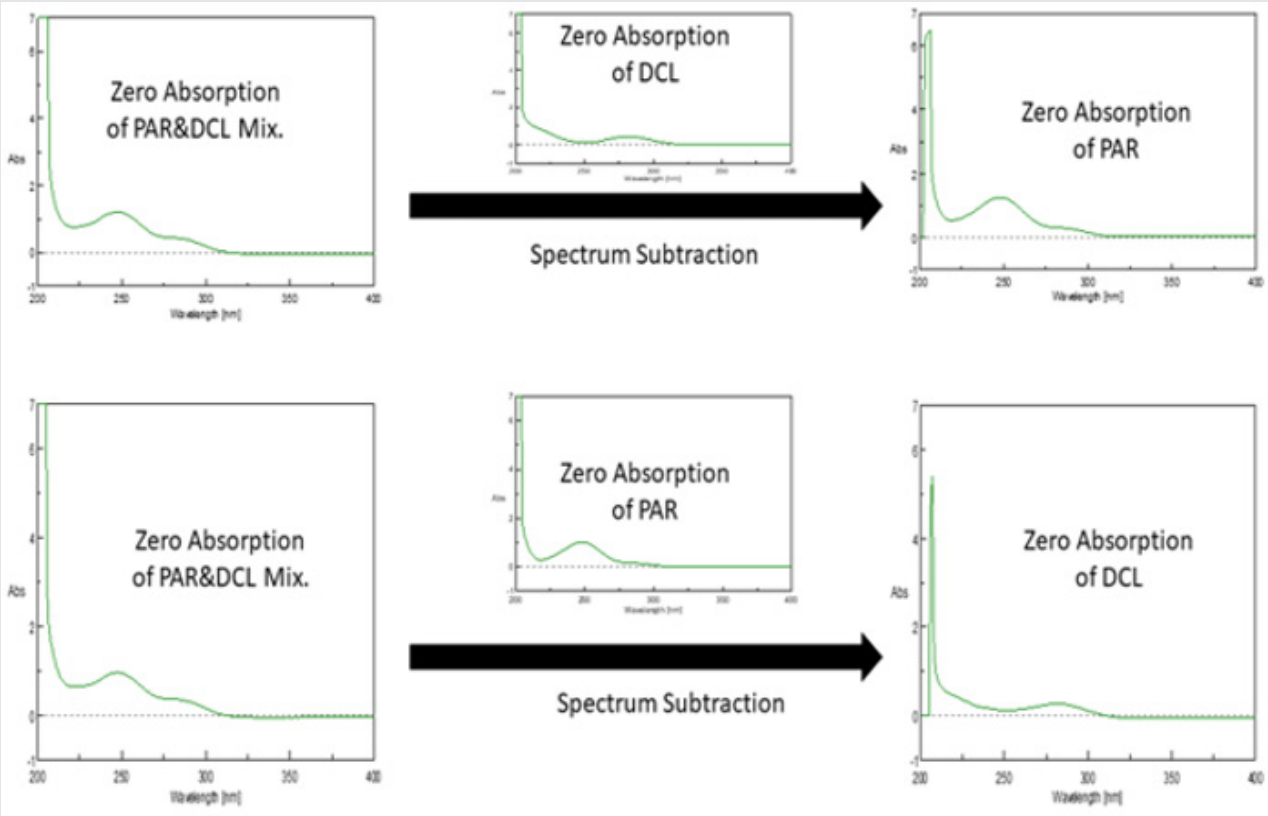

Figure 2: Spectrum subtraction of DCL & PAR from their mixture resulting in absorption spectra of PAR and DCL, respectively.

The concentration of X is calculated from the corresponding regression equation obtained by plotting the absorbance values of the zero order absorption spectra of X at its λmax against the corresponding concentrations. Zero absorption spectra of PAR & CAF can be recovered from their mixture through spectrum subtraction of CAF and PAR, respectively (Figure 2). Zero absorption spectra of CAF and PAR are shown in (Figure 3).

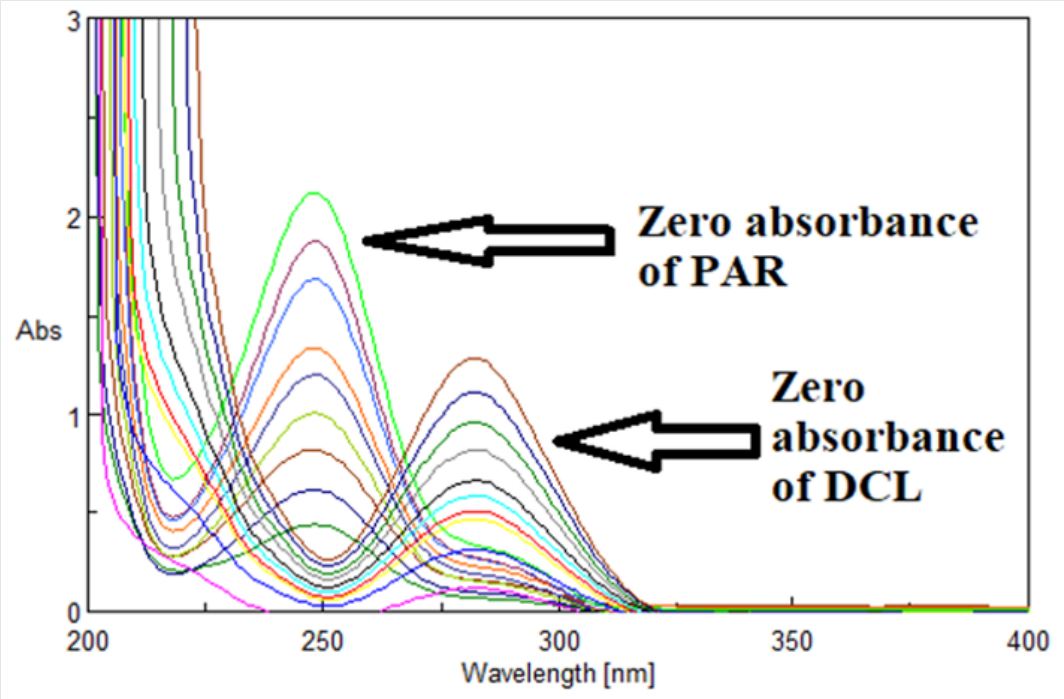

Figure 3: Zero absorption spectra of PAR overlaid with zero absorption spectra of DCL.

Analysis of laboratory prepared mixtures

The spectra of the mixtures were measured after preparation of different ratios of the laboratory prepared mixtures then handled in the same conditions as described under each method.

Application to pharmaceutical formulation

10 tablets of Diclocin® were weighed and crushed then an amount equivalent to 50 mg PAR and 10 mg DCL in each tablet was transferred into a volumetric flask (50 mL) and diluted with 90% methanol as follow: First, 30 mL of 90% methanol were added and sonicated then dilution was carried out to the mark and filtered. Second, 10 mL of the dilution was transferred into a 100 mL volumetric flask to give a concentration equivalent to 100 µg/mL PAR and 20 µg/mL DCL. Third, any further dilutions were carried out in volumetric flasks (10 mL) and treated in the same way as described under each method.

Results and discussion

Method Optimization

Two major problems were found during the analysis of PAR & DCL binary mixture; first, the overlapped spectra between the absorptivity of both drugs, and second, PAR, the main (major) constituent, had unfortunately very high absorbance, while DCL, the minor component, had low absorbance value. Intrinsically, sample enrichment technique [20] was used in which the concentration of DCL (the minor component) in their dual mixtures was increased to facilitate its determination. This was carried out by adding a fixed amount of standard DCL to each experiment when combined with PAR, then subtraction of its concentration before the calculation of the required concentration of DCL. Sample enrichment technique has been used for solving the same problem in the analysis of other drug mixtures of different drug ratios [21,22].

Spectrum Subtraction method

248 and 283 nm absorbances were used for determination of PAR & DCL in presence of each other, respectively. The calibration curves revealed accepted linear relationships between concentrations and absorbance in a range of 4-22 µg/mL for PAR and 7.5-45 µg/mL for DCL with correlation coefficients of ≥ 0.9990 for both drugs. The accuracy of the method illustrated accepted values with 99.94% ± 0.98 for PAR and 99.85% ± 0.55 for DCL. The specificity of the method demonstrated accepted values with 99.97% ± 1.51 for PAR and 100.86% ± 1.36 for DCL. The results are detailed in Table 1.

Spectrum subtraction is very easy and simple as it depends on zero absorption spectra without the need of extra processing. It is having few steps to get the zero order spectra of the desired drug, but it suffers from noise interference while acquiring the desired drug concentration by subtraction.

Method validation

The method was validated according to ICH guidelines [17] . The linear regression data for the calibration curve showed good linear

relationship. (Table 1). The accuracy was calculated by analyzing the standard addition where satisfactory results were obtained as shown in Table 1.The specificity of the method was calculated by assaying the laboratory prepared mixtures of PAR & DCL within the linearity range and good results were obtained (Table 1).The intra- and inter-day precisions were calculated by the analysis of 3 different concentrations of the drugs 3 times on the same day and on 3 successive days (Table 1).

Table 1: Assay parameters and validation results obtained by applying Spectrum subtraction method.

Mixture	PAR & DCL	
Method Parameters	**DCL**	**PAR**
Wavelength (nm)	283	248
Linearity range (µg/mL) (n=3)	7.5-45	22-Apr
Intercept	-0.0905	0.08
Slope	0.0303	0.0911
Correlation coefficient (r)	0.9991	0.999
Accuracy (Mean ± SD)	99.85 ± 0.55	99.94 ± 0.98
Precision (±%RSD)		
Repeatability	98.72 ± 0.18	99.11 ± 0.12
Intermediate precision	98.73 ± 0.14	99.81 ± 0.48
Specificity (Mean ± SD)	100.86 ± 1.36	99.97 ± 1.51

Application to Pharmaceutical Formulation

The proposed method was successfully applied for determination of PAR and DCL in their pharmaceutical formulation (Diclofenac Sodium plus→ tablets). The results were acceptable and with sufficient agreement with the labeled amounts. The standard addition technique was applied and showed that no interference of the excipients was observed (Table 2).

Table 2: Analysis of the pharmaceutical preparation (Diclocin® tablets) by applying proposed method.

	Spectrum subtraction							
	DCL				PAR			
			Recovery%				Recovery%	
	Tablet Taken (µg/mL)	Standard Added (µg/mL)	Tablet	Added	Tablet Taken (µg/mL)	Standard Added (µg/mL)	Tablet	Added
	2	8	101.91	100.04	10	9	101.29	101
		9.5	101.77	100.28		10	101.58	99.06
		10	99.61	99.24		11	101.46	99.76
Mean			101.1	99.85			101.45	99.94
SD			1.29	0.55			0.15	0.98

Statistical Analysis

Statistical comparison of the proposed method was performed through One-way ANOVA method by using PASW statistics 18→ software program in which there was no significant difference between the proposed method and the reference one [4] as shown in Table 3.

Table 3: Statistical comparison of the results obtained by the proposed method and the reference method using One-way.

Tablets	Drugs		Sum of Squares	df	Mean Square	F	Sig.
Diclocin® tablets	PAR	Between Groups	3.45	1	3.45	2.916	0.163
		Within Groups	4.734	4	1.183		
		Total	8.184	5			
	DCL	Between Groups	1.55	1	1.55	0.786	0.425
		Within Groups	7.886	4	1.971		
		Total	9.436	5			

Conclusion

Spectrum subtraction method was successfully applied for the determination of paracetamol and Diclofenac Sodium in their binary mixtures and in their dosage form. The proposed method is simple, sensitive and accurate and could be used for routine analysis by using simple technology or instruments. By comparison with the previous reported methods, it was concluded that spectrum subtraction method is very simple and doesn't require extra processing. Statistical comparison revealed that there was no observed significant difference between the proposed method and the reference one.

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