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Research Article

H-Point Assay 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 were settled for simultaneous determination of paracetamol and diclofenac sodium in pure form and in their pharmaceutical formulation. H-Point assay has been used in simultaneous determination of both drugs without prior separation. H-Point assay method parameters were validated according to ICH guidelines in which accuracy, precision, repeatability and robustness were found in accepted limits. Advantages and disadvantages of H-point assay were discussed and statistical comparison between the proposed method and the reference method was performed.

Keywords: Spectrophotometric; Paracetamol; Diclofenac sodium; H-Point assay; ICH guidelines

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].

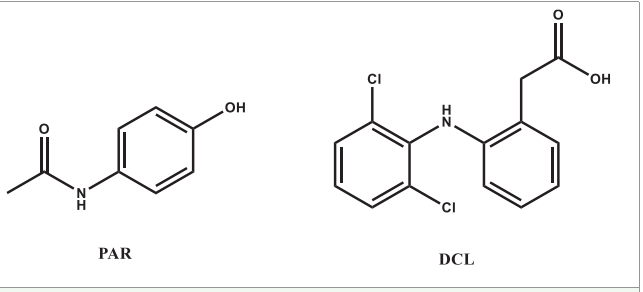

Figure 1: Chemical structures of paracetamol (PAR) and diclofenac (DCL).

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, there is no reported method for the determination of this drug mixture using H-point assay technique. As such, the aim of work is to develop a 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.

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.

PROCEDURES

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).

H-Point assay method

In the proposed method, two wavelengths were selected, 225 nm and 267 nm at which PAR exhibited the same absorptivity, in contrast to DCL that demonstrated sufficient difference in absorptivity at each of the selected wavelengths as shown in figure 2. Two calibration curves were constructed for PAR at 225 nm and 267 nm and its zero absorbance spectrum is showed in figure 3.

On the other hand, DCL was the component, from which

standard solutions of increased concentration could be added to a mixture of both components to determine the concentration of both by H-point standard addition method.

Aliquots containing 7.5, 12.5, 17.5, 20, 22.5, 25, 30, 35 µg/mL DCL were accurately added to the laboratory prepared mixture or pharmaceutical dosage form prepared solutions to be determined by this method. Curves of standard addition method were constructed where absorbances of solutions after DCL standard addition were represented on Y-axis while concentrations of the added DCL standard were represented on X-axis at the two selected wavelengths (225 nm and 267 nm). By plotting the absorbance versus added DCL concentration, two straight lines of different slopes and intercepts were obtained. As the absorbance value of PAR is constant at the two selected wavelengths (225 nm and 267 nm), all straight lines obtained at the different wavelengths by applying the standard additions method will have a common point. This point is known as the H point (Figure 4), the abscissa refers to DCL concentration (C_{DCL}) alone and the Y coordinate is the absorbance of PAR (A_{PAR}) alone in the corresponding mixture. The concentration of PAR (C_{PAR}) in mixture was then determined by substitution in any of regression line equations at 225 nm and 267 nm.

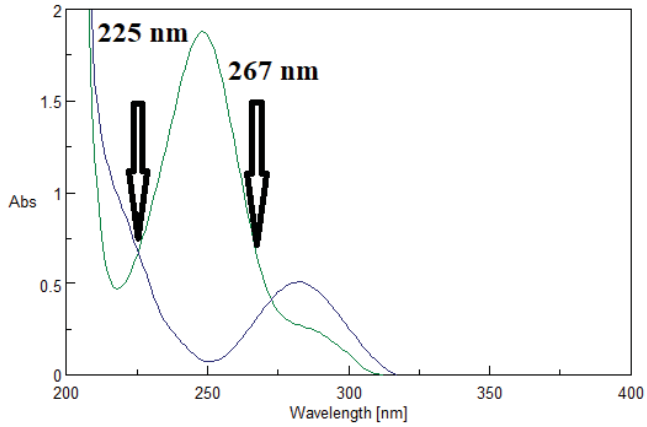

Figure 2: Zero absorption spectrum of 20 µg/mL DCL (blue line) overlaid with 20 µg/mL PAR (green line) revealed that 225 and 267 nm has the same absorbance for PAR spectrum.

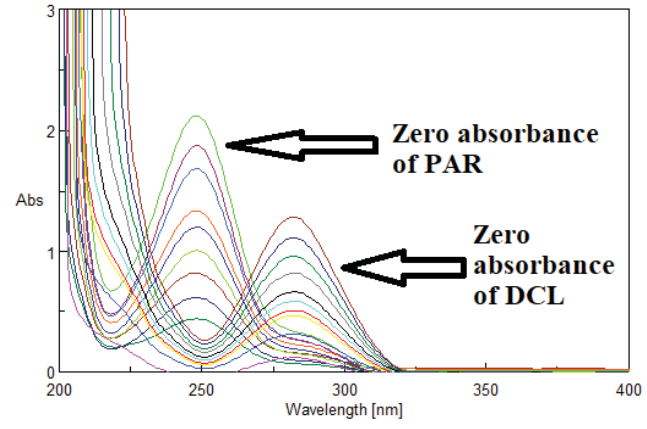

Figure 3: Zero absorption spectra of PAR and DCL.

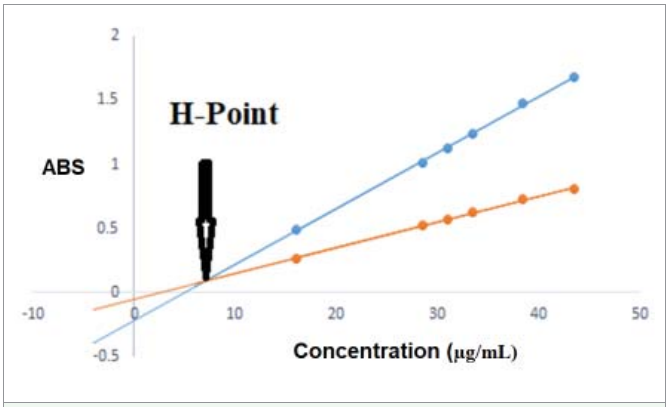

Figure 4: H-Point in the intercept of the two straight lines at 225nm & 267 nm after standard addition of DCL.

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].

H-Point assay method

The determination of concentration of drug X using H-point assay method requires the selection of two wavelengths λ_1 and λ_2, at which the interferent species, Y, has the same absorbance. Then, known amounts of X are successively added to the mixture and the resulting absorbances are measured at the two wavelengths and expressed by the following equations:

$$A_{(\lambda 1)} = b_0 + b + M_{\lambda 1} C_i \quad (1)$$

$$A_{(\lambda 2)} = A_0 + A + M_{\lambda 2} C_i \quad (2)$$

Where, $A_{(\lambda 1)}$ and $A_{(\lambda 2)}$ are the analytical signals measured at λ_1 and λ_2, respectively. b_0 and A_0 (b_0 and A_0 are not equal) are the original analytical signal of X at $A_{(\lambda 1)}$ and $A_{(\lambda 2)}$, respectively.

b and A' are the analytical signals of Y at $A_{(\lambda 1)}$ and $A_{(\lambda 2)}$, respectively. $M_{\lambda 1}$ and $M_{\lambda 2}$ are the slopes of the standard addition calibration lines at λ_1 and λ_2, respectively and C_i is the added X concentration. The two straight lines obtained intersect at the so-called H-point ($-C_H$, A_H).

At H-point, since $A_{(\lambda 1)} = A_{(\lambda 2)}$, $C_i = C_H$, from Eqs. (1) and (2) it follows that:

$$b_0 + b + M_{\lambda 1}(-C_H) = A_0 + A' + M_{\lambda 2}(-C_H) \quad (3)$$

$$-C_H = [(A_0 - b_0) + (A' - b)]/(M_{\lambda 1} - M_{\lambda 2}) \quad (4)$$

225 and 267 nm absorbances were used for determination of PAR & DCL in presence of each other at the same wavelengths. The calibration curves revealed accepted linear relationships between concentrations and absorbance in a range of 4-22 µg/mL for PAR and 7.50-45 µg/mL for DCL with correlation coefficients of ≥ 0.9990 for both drugs. The accuracy of the method illustrated accepted values with 100.25% ± 1.29 for PAR and 100.32% ± 0.51 for DCL. The specificity of the methods demonstrated accepted values with 100.32% ± 0.51 for PAR and 100.25% ± 1.29 for DCL. The results are detailed in table 1.

Table 1: Assay parameters and validation results obtained by applying H-Point assay spectrophotometric method.

Mixture	PAR & DCL			
Method Parameters	DCL		PAR	
Wave length (nm)	225	267	225	267
Linearity range (µg/mL) (n = 3)	7.50-45	7.50-45	4-22	4-22
Intercept	-0.1622	-0.083	0.0322	0.019452
Slope	0.0436	0.0193	0.0349	0.02717
Correlation coefficient (r)	0.9994	0.9991	0.999	0.9996
Accuracy (Mean ± SD)	100.25 ± 1.29	100.25 ± 1.29	100.32 ± 0.51	100.32 ± 0.51
Precision (±%RSD)				
Repeatability	100.19 ± 0.98		98.94 ± 0.48	
Intermediate precision	100.45 ± 1.01		98.83 ± 0.89	
Specificity (Mean ± SD)	100.39 ± 0.90		100.76 ± 1.12	

H-Point assay is very easy and simple as it depends on zero absorption spectra without the need of extra processing. On the other hand, it has two limitations; which are the need for some specific calculations to determine the values of H-Point in addition to requiring more time for performing the standard addition on each mixture.

Method validation

All methods were legalized as demonstrated by ICH guidelines [23]. The linear regression data for the calibration curves showed good linear relationships (Table 1). The accuracy was assessed by analyzing the standard addition method where satisfactory results were achieved as shown in (Tables 1,2). The specificity of this technique was assessed 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 computed by the analysis of 3 different concentrations of the drugs 3 times on the same day in addition to 3 successive days (Table 1).

Table 2: Analysis of the pharmaceutical preparation (Diclocin® tablets) by applying H-Point assay method.

	H-Point assay							
	DCL				PAR			
			Recovery%				Recovery%	
	Tablet	Standard Added	Tablet	Added	Tablet	Standard Added	Tablet	Added
	Taken (µg/mL)	(µg/mL)			Taken (µg/mL)	(µg/mL)		
	2	8	99.47	101.54	10	9	101.97	100.24
		9.5	100.42	100.24		10	100.57	100.87
		10	101.26	98.97		11	99.75	99.85
Mean			100.39	100.25			100.76	100.32
SD			0.9	1.29			1.12	0.51

Application to pharmaceutical formulation

H-point assay method was successfully applied for determination of PAR & DCL in its pharmaceutical formulation (Diclocin® tablets) the results were acceptable in agreement with the labelled quantities. The standard addition method was used and revealed that no interference of the excipients was observed (Table 2).

Statistical analysis

Statistical comparison between the proposed technique and the reference method was done by One-way ANOVA method through utilizing PASW statistics 18® software program. The calculated F values were less than the theoretical ones indicating that there was no significant difference between them (Table 3).

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

Tablets	Drugs		Sum of Squares	df	Mean Square	F	Sig.
Diclocin® tablets	PAR	Between Groups	1.591	1	1.591	1.179	0.339
		Within Groups	5.397	4	1.349		
		Total	6.988	5			
	DCL	Between Groups	6.181	1	6.181	7.001	0.057
		Within Groups	3.532	4	0.883		
		Total	9.713	5			

CONCLUSION

H-Point assay 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 H-point assay method doesn't require extra processing but it needs more time and calculations. Statistical comparison revealed that there is no observed significant difference between the proposed method and the reference one.

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