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Research Article

Determination of Diclofenac in Pharmaceutical Preparations by UV- and First-Order Derivative Spectrophotometry Methods

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ABSTRACT

In this study, new, rapid UV spectrophotometry (UV) and first-order derivative spectrophotometry (^{1}D) methods were developed for the determination of diclofenac in pure and tablets. The solvent system and wavelength of detection were optimized in order to maximize the sensitivity of the proposed methods. Parameters such as linearity, precision, accuracy, specificity, stability, limit of detection and limit of quantification were studied according to the International Conference on Harmonization Guidelines. Calibration curve was linear between the concentration range of 2-14 μg ml^{-1}. Within- and between-day precision values for diclofenac were less than 4.27%, and accuracy (relative error) was better than 2.71%. The mean recovery value of diclofenac was 100.1% for pharmaceutical preparations. The developed method was successfully applied to tablet formulations and the results were compared statistically with each other.

INTRODUCTION

Diclofenac (Fig. 1) is a nonsteroidal anti-inflammatory drug (NSAID) that is widely prescribed for the treatment of rheumatoid arthritis, osteoarthritis, musculoskeletal injuries and post surgery analgesia in human and veterinary medicine.

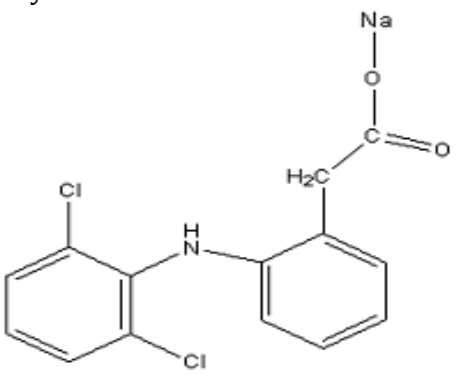

Figure 1: Chemical structure of diclofenac.

Patients are frequently given special formulations of diclofenac or a co-treatment agent as a therapeutic strategy to attenuate the gastrointestinal tract complications that limit the use of diclofenac and other NSAIDs [1-3].

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Many patients prescribed diclofenac for arthritis also take additional drugs for other chronic health problems such as hypertension [4, 5].

Several methods have been reported for determination of diclofenac including gas chromatography-mass spectrometry (GC-MS) [6-8], high-performance liquid chromatography (HPLC) [9-23] and LC-MS-MS [24] in human plasma and other biological fluids.

However, to our knowledge, there is no individual first-order derivative spectrophotometric method for the determination of diclofenac in pharmaceutical preparations in literature. Derivative spectrophotometry is an analytical technique of great utility for extracting both qualitative and quantitative information from spectra composed of unresolved bands, and for eliminating the effect of baseline shifts and baseline tilts. It consists of calculating and plotting one of the mathematical derivatives of a spectral curve [25]. Last year, this technique rapidly gained ground in application in the analysis of pharmaceutical preparations.

We wanted to develop new spectrophotometric methods for the determination of diclofenac in pharmaceutical preparations without the necessity of sample pre-treatment. After

developing spectrophotometric methods were also carried out and all optimization parameters were also considered. Also, the developed methods were applied to commercial preparations (Diclomec, Dicloflam and Voltaren) as tablet. The results obtained were statistically compared.

MATERIALS AND METHODS

Chemicals

Diclofenac was obtained from Sigma (St. Louis, MO, USA). Diclomec, Dicloflam and Voltaren tablets (100 mg diclofenac) were obtained the pharmacy (Erzurum, Turkey).

Equipments

A Thermospectronic double-beam UV-Visible spectrophotometer (HEλIOSβ) with a fixed slid width 2 nm and with a data processing system was used. UV and ^{1}D spectra (N=6. Δλ=4.0 nm) of standard and sample solutions were recorded in 1 cm quartz cells between wavelength ranges of 250-320 nm at scan speed of 600 nm min^{-1} and derivation interval (Δλ) 21.0 nm.

Preparation of Standard Curve for UV and ^{1}D Methods

Stock solution of diclofenac (100 µg ml^{-1}) was prepared by dissolving 10 mg diclofenac in 100 mL of methanol. Working solutions (WS) containing 2, 4, 6, 8, 10, 12 and 14 µg ml^{-1} of diclofenac were daily prepared by diluting the stock solution with a constant volume of methanol. The WS were prepared daily in analysis. Solutions were transferred to quartz cells for analysis. Stock solution was stored at -20 ºC in glass flask and brought to room temperature before use. Quality control (QC) samples were prepared by adding aliquots of standard solution of diclofenac to final concentrations of 3, 7 and 11 µg ml^{-1}.

Data Analysis

All statistical calculations were performed with the Statistical Product and Service Solutions (SPSS) for Windows, version 10.0. Correlations were considered statistically significant if calculated P values were 0.05 or less.

RESULTS AND DISCUSSION

Optimization of Spectrophotometric Conditions

To develop a sensitive UV and ^{1}D spectrophotometric method, the experimental conditions such as the solvent, the degree of derivation, the wavelength range and smoothing were optimized. Optimum results were obtained by measuring the wavelength range 250-320 nm through using high smoothing (Δλ = 21.0 nm) for UV and first-order derivative spectrophotometry. In this assay, various solvent systems such as water, methanol, ethanol and acetonitrile were tried either individually or in combinations of different proportions. The final decision of using methanol was based on sensitivity, interference, and easy preparation, suitability for drug, content estimation and cost, respectively. Methanol was used in this study because it has no toxicity. Figs. 2 and 3 present the overlay of UV and ^{1}D spectra of progesterone in the concentration of 2-14 µg ml^{-1} in methanol, respectively. Each spectrum can be used for the determination of this drug.

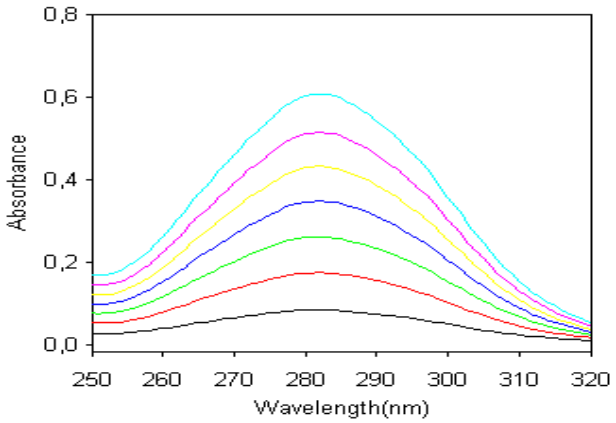

Figure 2: Spectra obtained from UV method (2, 4, 6, 8, 10, 12 and 14 µg ml^{-1}).

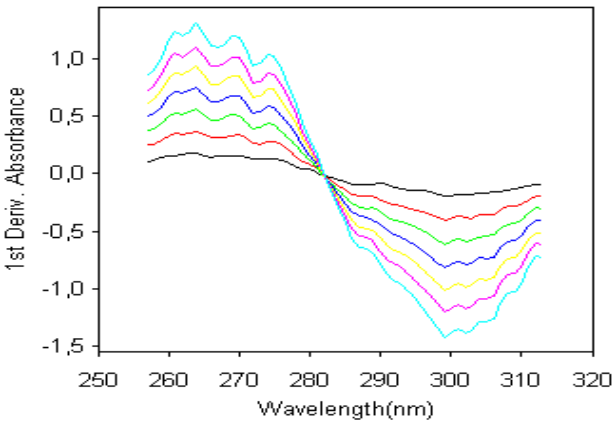

Figure 3: Spectra obtained from first-order derivative method (2, 4, 6, 8, 10, 12 and 14 µg ml^{-1}).

The maximum peak at 282 nm was observed in UV spectra of diclofenac. The maximum peak at 264 nm and a minimum peak at 298 nm were observed in the ^{1}D spectra of diclofenac (Fig. 3).

Method Validation

Linearity of Calibration Curves

In the UV and ^{1}D spectrophotometry method, the working solutions were scanned at 250-320 nm against a similarly prepared blank. The 282 nm wavelength for UV and the 264 and 298 nm wavelengths for ^{1}D method were used for calibration curves. Six-level calibration series with six analyses at each concentration level were measured. The standard calibration curves of progesterone in all wavelengths were constructed by plotting the A (Absorbance values for UV spectrophotometric method) and the $dA/d\lambda$ (first derivative values for first-order derivative spectrophotometric method) versus diclofenac concentrations. For all calibration curves, a good linearity within the concentration range of 2-14 μg ml^{-1} was shown for UV and ^{1}D methods. The regression equations were obtained by the least-square regression method. The calibration curves, regression equations and correlation coefficients found for UV and ^{1}D method were given in Table 1.

The correlation coefficient of standard calibration curves at 282 nm for UV spectrophotometric method and at 264 and 298 nm wavelength for ^{1}D method of diclofenac in methanol was higher.

Sensitivity

Spectrophotometrically, the limit of detection (LOD) and the limit of quantification (LOQ) were determined by an empirical method that analyzed a series of standard solutions which were containing decreasing amounts of diclofenac. LOQ was defined as the lowest concentration of measured value [(RSD< 10%) and accuracy (80-120 %)] of standard solutions in calibration curves. LOD determined (RSD< 10%) as the lowest concentration of analyte which was distinguished from the blank with reasonable confidence was also calculated. LOQ and LOD values for both methods of standard diclofenac solutions were found as 0.50 μg ml^{-1} and 0.20 μg ml^{-1}, respectively. All the RSD values were found lower than 10 %.

Repeatability

Repeatability is given as within-day and between-day precision and accuracy where it was evaluated via analysis of three different concentrations of diclofenac on six different days. Six replicate determinations at three different concentrations (3, 7 and 11 μg ml^{-1}) in 282 nm wavelength (for UV method) and, 264 and 298 nm wavelengths (for ^{1}D spectrophotometry method) were carried out to test the precision of these methods. The precision of the methods were given as the relative standard deviation (RSD=100 x Standard deviation/Mean) and the accuracy of these methods were given as the percent of mean deviation from known concentration [relative error; (concentration found-known concentration) x 100 / known concentration]. All samples were freshly prepared. For UV and ^{1}D spectrophotometry methods, the within-day precision showed that acceptable RSD% values which were <3.45% and <3.76% (n=6), respectively, and the between-day precision (intermediate precision) showed that acceptable RSD% values which were <2.27% and <4.27% (n=6), respectively (Table 2).

Accuracy of UV and ^{1}D spectrophotometry methods showed that acceptable relative error values which were <2.57% and <2.71% (n=6), respectively (Table 2).

Analytical Recovery

To check accuracy of the proposed spectrophotometry methods, the standard addition technique was applied. The three different concentrations (2.5, 7.5 and 12.5 μg ml^{-1}) of pure sample solution were added to 5.0 μg ml^{-1} concentration of tablet solution and assayed. The analytical recovery of the added standard to the assay samples was calculated from followed equation.

$$\text{Recovery \%} = [(C_t - C_u) / C_a] \times 100$$

Where C_t is total concentration of the analyte determined; C_u is the concentration of the anlyte present in the formulation; and C_a is the concentration of the pure analyte added to the formulation. The results of analysis of the commercial tablet and the recovery study were given in Table 3.

The average percent recoveries obtained were quantitatively as 99.8% for UV method and 100.3% for ^{1}D method, indicating good accuracy of the methods. No interference from the common excipients was observed.

Stability

Spectrophotometrically, to determine the stability of diclofenac standard solutions in the refrigerator and at room temperature, diclofenac solutions of 2.5, 7.5 and 12.5 μg ml^{-1} concentrations and stock solution were stored in the refrigerator and at room temperature for four days. Then, the stability measurements were carried out.

Table 1: Results of regression analysis of proposed methods

Methods	Range (μg ml^{-1})	λ (nm)	LR[a]	Sa	Sb	R	LOD (μg ml^{-1})	LOQ (μg ml^{-1})
UV	2-14	282	y=0.0433x+0.0005	0.0026	6.32^{E-5}	0.9998	0.200	0.500
^{1}D	2-14	264	y=0.1011x-0.0054	0.0061	8.37^{E-05}	0.9995	0.200	0.500
	2-14	298	y=0.0932x-0.0047	0.0056	0.0005	0.9997	0.200	0.500

λ: Wavelength [a] Based on six calibration curves LR: Linear regression Sa: Standard deviation of intercept of regression line Sb: Standard deviation of slope of regression line R: Coefficient of correlation x: diclofenac concentration (μg ml^{-1}), A: Absorbance, ^{1}D: First order-absorbance, LOD: Limit of detection, LOQ: limit of quantification

Table 2: Precision and accuracy of proposed methods

Method	λ (nm)	Added (μg ml^{-1})	Within-day			Between-day		
			Found±SD (μg ml^{-1})	Accuracy	Precision RSD%[a]	Found±SD (μg ml^{-1})	Accuracy	Precision RSD%[a]
UV Method	$A_{282\ nm}$	3.0	3.07±0.085	2.33	2.77	3.06±0.048	2.00	1.57
		7.0	7.18±0.248	2.57	3.45	7.17±0.163	2.43	2.27
		11	11.23±0.341	2.09	3.04	11.20±0.221	1.82	1.97
First-order Spectro photometry Method	$^1D_{264\ nm}$	3.0	3.06±0.007	2.00	0.23	3.08±0.098	2.67	3.18
		7.0	7.10±0.137	1.43	1.93	7.13±0.209	1.86	2.93
		11	11.24±0.423	2.18	3.76	11.28±0.482	2.55	4.27
	$^1D_{298\ nm}$	3.0	3.04±0.065	1.33	2.14	2.95±0.057	-1.67	1.93
		7.0	7.19±0.225	2.71	3.13	7.14±0.213	2.00	2.98
		11	10.84±0.228	-1.45	2.10	10.88±0.171	-1.09	1.57

SD: Standard deviation of six replicate determinations, RSD%: Relative standard deviation, Accuracy:(%relative error)(found-added/addedx100)

Table 3: Results of analytical recovery studies by standard addition method (n=6)

Method	Amount taken (μg ml^{-1})	Amount added (μg ml^{-1})	Total amount found (μg ml^{-1}) (Mean ±SD)	Recovery±RSD (%)
UV	5.0	2.5	7.49±0.11	99.6±1.46
		7.5	12.51±0.35	100.1±2.80
		12.5	17.48±0.13	99.8±0.743
$^1D_{264\ nm}$	5.0	2.5	7.55±0.10	103.0±1.32
		7.5	12.49±0.15	99.9±1.20
		12.5	17.25±0.28	98.0±1.62

Table 4: Determination of diclofenac in pharmaceutical preparations

Commercial Preparation	Method	λ (nm)	Found±SD (mg)	Recovery (%)	RSD (%)	Confidence Interval
Diclomec	UV	282	99.7±0.50	99.7	0.50	98.0-101.2
	^{1}D	264	101.1±1.02	101.1	1.01	99.0-102.5
Dicloflam	UV	282	98.7±2.50	98.7	2.53	97.3-101.2
	^{1}D	264	101.1±3.10	101.1	3.07	100.0-102.5
Voltaren	UV	282	99.4±2.28	99.4	2.29	98.2-101.2
	^{1}D	264	101.1±3.06	101.1	3.03	99.0-102.5

SD: Standard deviation of six replicate determinations, RSD: Relative standard derivation, [a]Average of six replicate determinations

Table 5: Statistical comparison (t-test) of the results obtained by proposed methods

Commercial preparation	Statistical Values	UV Method	^{1}D Method	t values
Diclomec	n	18	18	
	X	99.7	101.1	t_c=1.28
	SD	0.50	1.02	t_t=1.69
	CI	98.0-101.2	99.0-102.5	
Dicloflam	n	18	18	
	X	98.7	101.1	t_c=1.42
	SD	2.50	3.10	t_t=1.69
	CI	97.3-101.2	100.0-102.5	
Voltaren	n	18	18	
	X	99.4	101.1	t_c=1.34
	SD	2.28	3.06	t_t=1.69
	CI	98.2-101.2	99.0-102.5	

n: Number of determination. X: mean, SD: Standard deviation, CI: Confidence interval, t_c: Calculated F values, t_t: Tabulated t values, H_o: Hypothesis: no statitically significant difference exists between two methods, $t_t > t_c$: H_o hypothesis in accepted (α=0.05)

The results were evaluated by comparing these measurements with those of standards and expressed as percentage deviation. The stability of diclofenac solutions were determined by keeping them for three days in the refrigerator and for two days in room temperature. A significant change in concentration (recovery = 100 ± 1%) were not found under both conditions. In addition to this, stock solution was found to be stable for a week in refrigerator.

Specificity

The specificities of the two methods were investigated by observing interferences between diclofenac and the excipients. Standard diclofenac and drug formulation solutions were prepared and analyzed. No interference was found from tablet excipients at the selected assay conditions.

Assay Sample Preparation

The average tablet mass was calculated from the mass of tablets of Diclomec, Dicloflam and Voltaren (100 mg diclofenac tablet, which was composed of diclofenac and some excipients). They were then finely ground, homogenized and portion of the powder was weighed accurately, transferred into a 100 mL brown measuring flask and diluted to scale with methanol. The mixture was sonicated for at least 10 min to aid dissolution and then filtered through a Whatman 42 paper. An appropriate volume of filtrate was diluted further with methanol so that the concentration of diclofenac in the final solution was within the working range and then recorded against methanol.

The percent analytical recovery values were calculated by comparing concentration obtained from the spiked samples with actual added concentrations. The mean recoveries of UV and ^{1}D spectrophotometry methods were 100.9 and 101.2%, respectively (Table 4).

Comparison of Two Spectrophotometric Methods

The results show the high reliability and reproducibility of two methods. The best results obtained at 282 nm and 264 nm for zero- and first-order derivative spectrophotometric methods were statistically compared using the t-test. At 95 % confidence level, the calculated t-values do not exceed the theoretical values (Table 5). Therefore, there is no significant difference between zero- and first-order derivative spectrophometric methods. This is suggested that the two methods are equally applicable. The proposed methods are very effective for the assay of diclofenac in tablets. The validity of the proposed methods was presented by recovery studies using the standard addition method. For this purpose, a known amount of reference drug was spiked to formulated tablets and the nominal value of drug was estimated by the proposed methods. Each level was repeated six times. The results were reproducible with low SD and RSD. No interference from the common excipients was observed.

CONCLUSION

In the present report, simple, rapid, sensitive, reliable, specific, accurate and precise UV and ^{1}D spectrophotometry methods for the determination of diclofenac in pharmaceutical preparations were developed and validated. The proposed methods can be used effectively, without separation and interference, for routine analysis of diclofenac in pure form and its formulations and can also be used for dissolution or similar studies. On the other hand, UV and ^{1}D spectrophotometry methods are also suitable for analysis of sample during accelerated stability studies, routine analysis of formulations and raw materials.

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REFERENCES

[1] Davies NM, Saleh JY, Skjodt NM. Detection and prevention of NSAID-induced enteropathy. J Pharm Pharm Sci 2000; 3: 137-55.

[2] Fortun PJ, Hawkey CJ. Nonsteroidal antiinflammatory drugs and the small intestine. Curr Opin Gastroenterol 2005; 21: 169-75.

[3] Wallace JL and Del Soldado P. The therapeutic potential of NO-NSAIDs. Fundam Clin Pharmacol 2003; 17: 11-20.

[4] Einarson TR, Metge CJ, Iskedjian M, Mukherjee J. An examination of the effect of cytochrome P450 drug ınteractions of hydroxymethylglutaryl-coenzyme A reductase ınhibitors on health care utilization: A canadian population-based study. Clin Ther 2002; 24: 2126-36.

[5] Izhar M, Alausa T, Folker A, Hung E, Bakris GL. Effects of COX inhibition on blood pressure and kidney function in ACE inhibitor-treated blacks and hispanics. Hypertension 2004; 43: 573-77.

[6] Borenstein MR, Xue Y, Cooper S, Tzeng TB. Sensitive capillary gas chromatographic-mass spectrometricselected-ion monitoring method for the determination of diclofenac concentrations in human plasma. J Chromatogr B 1996; 685: 59-66.

[7] Kadowaki H, Shiino M, Uemura I, Kobayashi K. Sensitive method for the determination of diclofenac in human plasma by gas chromatography-mass spectrometry. J Chromatogr 1984; 308: 329-33.

[8] Puppo M.D, Cighetti G, Kienle MG, Paroni R, Borghi C. Determination of diclofenac in human plasma by selected ion monitoring. Biol Mass Spectrom 1991; 20: 426-30.

[9] Schweizer A, Willis JV, Jack DB, Kendall MJ. Determination of total monohydroxylated metabolites of diclofenac in urine by electron-capture gas-liquid chromatography. J Chromatogr 1980; 195: 421-24.

[10] El-Sayed YM, Abdel-Hameed ME, Suleiman MS, Najib NM. A rapid and sensitive high-performance liquid chromatographic method for the determination of diclofenac sodium in serum and its use in pharmacokinetic studies. J Pharm Pharmacol 1988; 40: 727-29.

[11] Torres-Lopez JE, Robles MB, Perez-Urizar J, Flores-Murrieta FJ, Granados-Soto V. Determination of diclofenac in micro-whole blood samples by high-performance liquid chromatography with electrochemical detection. Application in a pharmacokinetic study. Arzneimittelforschung 1997; 47: 1040-43.

[12] Silva LC, Simoes IG, Lerner FE, Belém GR, De Moracs MEA, De Nucci G. Comparative bioavailability of two different diclofenac formulations in healthy volunteers. Arzneimittelforschung 1999; 49: 920-24.

[13] Hirai T, Matsumoto S, Kishi I. Simultaneous analysis of several non-steroidal anti-inflammatory drugs in human urine by high-performance liquid chromatography with normal solid-phase extraction. J Chromatogr B 1997; 692: 375-88.

[14] Paroni R, Fioravanti A, Fattorini L, Giordano N, Borghi C, Galli Kienle M, Marcolongo R. Bioavailability of diclofenac sodium with vaginal administration in healthy women. Curr Ther Res Clin E 1991; 50: 200-04.

[15] Lansdorp D, Janssen TJ, Guelen PJM, Vree TB. High-performance liquid chromatographic method for the determination of diclofenac and its hydroxy metabolites in human plasma and urine. J Chromatogr B 1990; 528: 487-94.

[16] Zecca L, Ferrario P, Costi P. Determination of diclofenac and its metabolites in plasma and cerebrospinal fluid by high-performance liquid chromatography with electrochemical detection. J Chromatogr 1991; 567: 425-32.

[17] Hinz B, Auge D, Rau T, Rietbrock S, Brune K, Werner U. Simultaneous determination of

aceclofenac and three of its metabolites in human plasma by high performance liquid chromatography. Biomed Chromatogr 2003; 17: 268-75.

[18] Avgerinos A, Karidas TH, Malamataris S. Extractionless high-performance liquid chromatographic method for the determination of diclofenac in human plasma and urine. J Chromatogr B 1993; 619: 324-29.

[19] Miller RB. High-performance liquid chromatographic determination of diclofenac in human plasma using automated column switching. J Chromatogr B 1993; 616: 283-90.

[20] Roskar R, Kmetec V. Liquid chromatographic determination of diclofenac in human synovial fluid. J Chromatogr B 2003; 788: 57-64.

[21] Kaphalia L, Kaphalia BS, Kumar S, Kanz MF, Treinen-Moslen M. Efficient high performance liquid chromatography/ultraviolet method for determination of diclofenac and 4'-hydroxydiclofenac in rat serum. J Chromatogr B 2006; 830: 231-37.

[22] Arcelloni C, Lanzi R, Pedercini S, Molteni G, Fermo I, Pontiroli A, Paroni R. High-performance liquid chromatographic determination of diclofenac in human plasma after solid-phase extraction. J Chromatogr B 2001; 763: 195-200.

[23] Giagoudakis G, Markantonis SL. An alternative high-performance liquid-chromatographic method for the determination of diclofenac and flurbiprofen in plasma. J Pharm Biomed Anal 1998; 17: 897-901.

[24] Sparidans RW, Lagas JS, Schinkel AH, Schellens JHM, Beijnen JH. Liquid chromatography-tandem mass spectrometric assay for diclofenac and three primary metabolites in mouse plasma. J Chromatogr B 2008; 872: 77-82.

[25] Ojeda CB, Rojas FS. Recent developments in derivative ultraviolet/visible absorbption spectrophotometry. Anal Chim Acta 2004; 518: 1-24.