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Simpler and Faster Spectrophotometric Determination of Diclofenac Sodium in Tablets, Serum and Urine Samples

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Abstract

This work describes a simple, sensitive, rapid and economical analytical procedure for direct spectrophotometric evaluation of diclofenac sodium (DS) using aqueous medium without using a chemical reagent. Parameters like time, temperature, acidic and basic conditions and interference by analgesic drugs were studied for a 5μg ml^{-1} solution of DS at 276 nm. Under optimized parameters, a linear working range of 0.1–30 μg ml^{-1} with regression coefficient of 0.9998 and lower detection limit of 0.01 μg ml^{-1} was obtained. The method was applied for DS contents in tablets, serum and urine samples.

Keywords: Diclofenac sodium; UV-Visible spectrophotometer; tablets; serum; urine

Introduction

DS or sodium 2-[(2, 6-dichlorophenyl) amino] phenyl acetate, is a broadly used non-steroidal anti-inflammatory drug [1] for the treatment of inflammatory conditions such as rheumatoid arthritis, osteoarthritis and ankylosing spondilytis [2]. Accurate assay of the drug in pharmaceutical preparations as well as biological fluids are of prime importance from clinical as well as physiological point of view. Different analytical methods have been employed for the quantification of DS, such as spectrophotometry [1, 3] fluorimetry [4, 5] FT-Raman spectroscopy [6, 7] potentiometry [5] chromatography [2, 8] voltammetry [9] and polarography [10]. Most of these methods face certain problems such as the use of additional reagents, complex formation, long time and ha.00zardous matrices. In contrast simplicity, economy, green chemistry and faster assay are the actual factors which make a method far more superior to those lacking one or more of these characteristics. We have already reported a simpler, sensitive, cheaper and environmental friendly spectrophotometric method for investigation of paracetamol (PC) in all types of liquid samples and pharmaceutical preparations which is very much useful for quick analyses [11]. In continuation to our previous work we report another simple, sensitive, rapid, economical, highly reproducible and accurate analytical procedure for direct spectrophotometric determination of diclofenac sodium (DS) in the aqueous medium employing no chemical reagent for determining DS in various types of drugs and body fluids. The method has extended range of calibration with quite lower detection limit as compared to several reported methods and successfully applied for determining DS in pharmaceutical tablets, urine and serum samples.

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Materials and Methods

Apparatus

All the spectrophotometric experiments were carried out by Lambda 2 UV/visible spectrometer of Perkin-Elmer. Julabo HC5 water bath Model, GMBH D-7633 Germany, fitted with test tube netted tray held in vertical position was used for controlling temperature.

Washing of glassware

All glassware was washed by soaking in 3 M HNO_3 overnight followed by washing with detergent water. It was then thoroughly washed with tap water and finally rinsed at least 3 times with doubly distilled water. The glassware was then dried in an oven at 110 ^0C.

Reagents and solutions

All the reagents used in this study were of analytical grade ultra pure quality from Merck, Fluka and BDH, etc. Diclofenac sodium and other pharmaceutical standards were provided by Birds Chemotec Karachi, Pakistan. The identity of pharmaceutical standards was checked by Fourier Transform Infrared spectra and comparing these with the relevant data found in literature. Stock standard solution (w/v) of diclofenac sodium (1 mg ml^{-1}) was prepared in 100 ml calibrated volumetric flask and diluted to the mark with doubly distilled water. Dilute working standards were prepared from time to time as per requirement. Solutions of other reagents were also prepared in doubly distilled water in the desired concentration.

Procedure for determining DS in tablets

Four brands of tablets containing DS from different manufacturers were purchased from local market and analyzed by using the current method. Ten tablets from each brand were finely powdered and mixed. An amount equal to the average weight of one tablet was collected randomly, transferred to a 100 ml volumetric flask, dissolved and made up with doubly distilled water. Dilute solutions were made from each sample and analyzed by UV-Vis Spectrometer. The concentration of DS per tablet was calculated with the help of equation for linear calibration curve of DS and multiplying with relevant dilution factor.

Procedure for DFS in serum and urine samples

The blood and urine samples were collected before the intake of DS (blank) at day 1 and after taking DS at day 2. The individuals were instructed not to use any analgesics including DS one week before examination and use the same the common diet at day 1 and day 2. The DS was administered just after the collection of blank urine samples. The blood samples were collected after 2 hours of dosage while urine samples were collected after 12 hours of administration of 50 mg of tablet. Serum was obtained as supernatant from blank and DS containing clotted blood samples by centrifugation method. In order to freed the urine and serum sample from water insoluble impurities, 1 ml of blank as well as DS containing sample were passed through a column DSC-18 (used in solid phase extraction), which was pre-washed with 2 ml of methanol. The recovered urine or serum sample was diluted to 10 ml with doubly distilled water and analyzed for DSF contents by UV-Visible spectrometer taking water as actual blank (reference) to record the spectra of blank and DS containing serum and urine samples. The difference of absorbance between blank and actual sample gave the concentration of DS after fitting the value in linear equation and multiplying the result with dilution factor. This treatment was carried out due to the difficulty in finding out the absorbance in case of taking actual serum or urine sample as blank against DS containing serum or blood sample. Standard solution of DS was spiked to each serum or urine sample in order to confirm the peak of DS.

Results and Discussion

The absorption spectra of DS in aqueous medium have been described by several workers [1, 12]. However up to the best of our knowledge, no attempt has been made so far to utilize the aqueous medium as one of the most reliable matrices for determining DS with excellent working linear range, sufficiently low detection limits and its application to many types of samples. So, various parameters were studied for their effect upon the UV-Visible spectrophotometric determination of DS.

Influence of time

The effect of time on the absorbance behavior of 5 µg ml^{-1} aqueous solution of DS at 276 nm was studied in the range of 1–90 minutes at room temperature of 25 ± 1^0C using double distilled water as a blank solution (Fig. 1). It is observed that the absorbance remains constant throughout the whole period and thus independent of time.

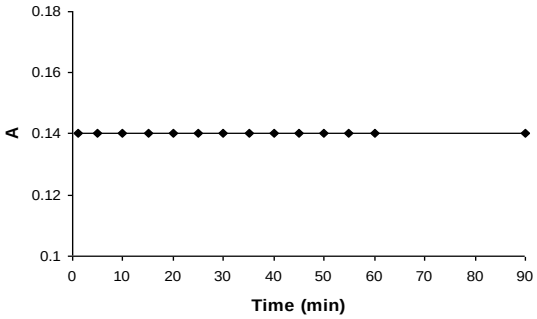

Figure 1. **Time effect on absorbance of diclofenac sodium.**

Time effect has been described by other workers [1, 11] as well. The constant absorbance with time confirms the stability of the analyte and faster analysis of DS in aqueous solution.

Effect of temperature

The effect of temperature on absorbance of 5µg ml^{-1} DS at 276 nm was observed in the range of 5–60 ^{0}C (Fig. 2). It can be seen that lower temperature range of 5–25 ^{0}C shows maximum absorbance, with a little bit decreased value for the range of 30–45^{0}C followed by a further decrease thereafter.

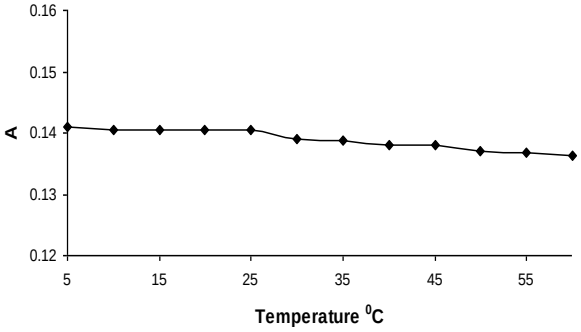

Figure 2. **Temperature effect on UV absorbance of 5 µg ml^{-1} DS solution.**

The possible reason of higher absorbance value at lower temperature may be due to the adsorption of DS and/ water molecules on the wall of quartz cell and thus absorbing a little bit higher amount of UV light [11]. The lower absorbance at higher temperature may be due to the instability of DS molecules. However the room temperature (25 ± 1^{0}C) was taken as optimum temperature in order to make the process simple by avoiding additional steps. Temperature studies have also been reported before [1] regarding the assay of DS.

Interference by various analgesic drugs

The interference of various analgesic drugs at the absorbance of 5µg ml^{-1} DS solution in various proportions was checked to investigate the possibility of its determination in the presence of other drugs (Table 1).

Table 1. **% interference by various analgesics in different ratios on 5 µg ml^{-1} DS.**

Ratio to DFS	(%) Interference				
	Caffeine	Paracetamol	Aspirin	Ibuprofen	Ascorbic acid
1:1	+5.47	+39.72	-18.49	+ 1.36	-7.53
5:1	+49.72	+13.69	-11.64	-6.16	-58.90
10:1	0.00	-12.32	+13.01	-10.27	-62.32

In a 1:1 ratio caffeine and Ibuprofen show little interference while paracetamol and aspirin are the major positive and negative interferers respectively. The positive interference by paracetamol may be due to its somewhat structural similarity or interaction with DS. The negative value shown by aspirin may be due to interaction of central N atom of DS with the –COO group of aspirin thus hindering its presence to some extent as actual diclofenac. Other higher values of interference for a 5:1 ratio showing +49.72 % interference in case of caffeine may be due to indistinguishable wavelength (275 nm) [12] to that of DS (276 nm) which adds up to increase the total absorbance of DS. No interference at 10:1 ratio of caffeine to DS may be due to rate of interaction of DS with UV light in a first order manner where the higher concentration of second analyte is usually constant. The highest negative interference value for 5:1 and 10:1 ratio of ascorbic acid:DS may be due to the interaction of –COO groups of DS with attached -OH groups of ascorbic acid through H-bonding which then hinders the actual absorbing activity of DS. As the lower ratio (1:1, 1:2 or 2:1) is usually taken in most of combined drugs, hence we can say that at usual combination level the mentioned drugs are showing interferences in acceptable values. However, the presence of paracetamol or ascorbic acid will interfere with the true analytical value of DS.

This has been observed that the amount of caffeine is quite high in the biological fluids such as urine, serum or whole blood because various natural foods contain sufficient amount of caffeine which is ultimately transferred in these fluids. So, no interference by the highest combination of caffeine and DS in 10:1 ratio is a good indicator for application of this method in the presence of high amount of caffeine especially in biological samples.

Effect of acidic and alkaline conditions

Spectrophotometric study of 5µg ml^{-1} DS solution was carried out in various concentrations of strong and weak acids and bases. UV spectra of a 5 µg ml^{-1} DS solution are given (Fig. 3) to describe the shift in wavelength and absorbance of DS at extreme conditions of acidity and alkalinity.

Moreover, a detailed sketch of such effects upon the addition of different strong and weak acids and bases was also observed (Table 2). According to the data, more negative interference is true at lower pH values of DS solution. However as the pH is increased, the negative interference is decreased and reaches to acceptable limit. The value of λ_{max} shifted from 273 nm to 276 nm by entering from acidic to basic conditions.

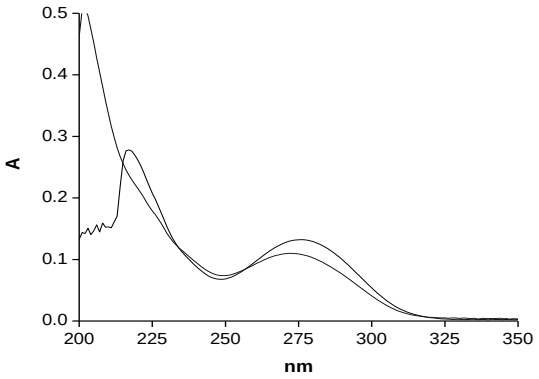

Figure 3. **UV spectra of DS at acidic pH, 3.56 (lower) and basic pH, 11.68 (higher).**

Table 2. **Effect of strong and weak acids and bases on DFS determination.**

Acid / base (1M)	Volume of acid / base (ml)	% effect	pH of DS solution	λ_{max} (mid point) (nm)
Hydrochloric acid	0.1	-10.67	2.44	273
	0.5	-14.44	2.07	273
	1.0	-18.31	1.73	273
Acetic acid	0.1	-5.97	3.78	273
	0.5	-8.32	3.56	273
	1.0	-12.06	3.24	273
Ammonia	0.1	0.00	9.85	276
	0.5	-2.23	10.30	276
	1.0	-2.98	10.65	276
NaOH	0.1	-1.0	10.93	276
	0.5	-1.86	11.68	276
	1.0	-2.23	12.34	276

Lower absorbance value of DS is linked with 2 factors. First is the solubility which depends upon pH and it is reported that solubility of DS is decreased in acidic solution. Secondly, DS is subjected to intramolecular cyclization at acidic pH and hence inactivated [13]. In basic conditions a reverse of cyclization restores the actual molecule along with maximum efficiency. However, a different situation is presented elsewhere [1] where acidic medium (HNO_3) results in the formation of yellowish nitrated derivative of DS which absorbs heavily. According to our opinion in a proton rich (highly acidic) medium all the Na^+ ions are not easy to freed the diclofenac ions available for maximum absorption because the available protons (H^+ ions) try to repel the formation of free Na^+ ions due to similar charges. So when the number of H^+ ions decreases, the number of free diclofenac ions is increased accordingly that results in increased absorption. In case of basic solution, the Na^+ ions are accepted by the negative OH^- ions and hence sufficient diclofenac ions are available for increased absorption. However the very little negative effect in basic solution is attributed to base hydrolysis of very limited number of diclofenac ions into smaller metabolites.

Calibration plot

UV spectra were recorded at 276 nm for standard solutions of DS with different concentrations in the range of 0.1–30 µg ml^{-1} (Fig. 4). Linear equation obtained from calibration plot is represented as; Y= 0.0269X+0.0041 with regression coefficient of 0.9998 and detection limit of 0.01 µg ml^{-1} at 276 nm. A relative standard deviation of 0.33 % was observed for a solution of 5 µg ml^{-1} DS (n=11) which describes an excellent reproducibility and repeatability of the method.

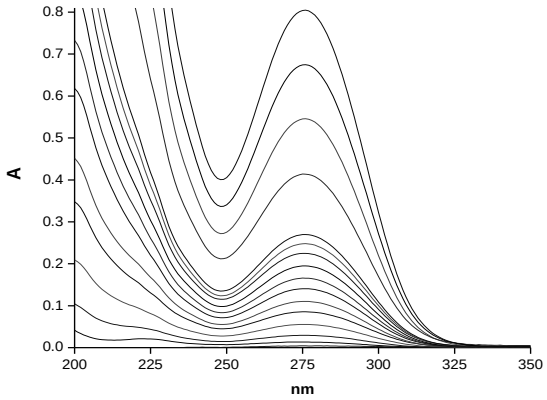

Figure 4. **Calibration plot of absorbance verses concentration for DS solutions from below to above as 0.1, 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 and 30 µg ml^{-1}.**

Comparison with other reported spectroscopic methods

A comparison of linear calibration range and detection limits of the current method with those of some other spectroscopic methods was also described (Table 3). It is quite clear that despite using various complexing agents, still the working ranges and detection limits of the reported methods [1, 14, 15, 16, 17] could not compete with that of the currently developed method. The addition of other reagents for complex formation makes the other methods time consuming, complicated and expensive. Due to the lack of the mentioned problems, better sensitivity, broader linear range and environmental friendly nature, the newly investigated method has a clear edge over described methods. Moreover, the method is also better than some other reported methods described in section 1, which possess nearly similar problems as true in case of reported spectroscopic methods.

Table 3. **Comparison of current method with other spectroscopic methods for determination of DS.**

Method	Reference	Linear range	LOD
Spectrofluorimetry	[14]	0.2 - 5.0 µg ml^{-1}	0.2 µg ml^{-1}
FI spectroscopy	[15]	0.2 - 8 µg ml^{-1}	0.023 µg ml^{-1}
UV-Vis. Spectrometry	[1]	1-30 µg ml^{-1}	0.46 µg ml^{-1}
UV-Vis. Spectrometry	[16]	0.8 -6.4 µg ml^{-1}	0.37 µg ml^{-1}
UV-Vis. Spectrometry	[17]	1.59-38.18µg ml^{-1}	1.29 µg ml^{-1}
UV-Vis. Spectrometry	Current method	0.1-30 µg ml^{-1}	0.01 µg ml^{-1}

Analysis of tablets

A representative UV spectrum of DS in a randomly selected sample of tablets (Voltral) diluted to 5 µg ml^{-1} DS according to the mentioned concentration is described (Fig. 5). The clarity of the signal proves no interference from the matrix. The average results of various locally manufactured tablets containing DS with mentioned and determined concentration for 5 replicate runs were recorded (Table 4). Each actual result was obtained after multiplying the determined concentration with dilution factor of 100.

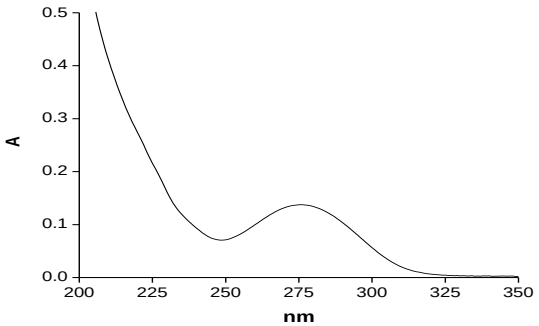

Figure 5. **Representative UV-spectrum of expected 5 µg ml^{-1} DS in (Voltral) tablet.**

Table 4. **Determination of DS in tablets of various companies by proposed method.**

Chemical Formulation	Mentioned Concentration (mg unit^{-1})	Actual Concentration [a] mg unit^{-1}	Recovery (%)
Fenac	50	51.153 ± 0.005	102.31± 0.011
Dichloran	50	50.646 ± 0.005	101.21 ± 0.011
Voltral	50	49.388 ± 0.008	98.68 ± 0.016
Ardifenac	50	50.923 ± 0.004	101.85 ± 0.008

a, average value; ±, standard deviation (n=5)

The closeness of mentioned and calculated concentrations of DS in the collected tablets samples proves the validity of the method and lack of interference from the excipients.

Analysis of urine and serum samples

10 fold of diluted urine or serum sample was processed according to the procedure mentioned in experimental section. Representative spectra of each of urine and serum sample are demonstrated respectively (Fig. 6 and Fig. 7). For each spectral observation, the diluted blank and SD containing urine or serum sample of the same individual were processed.

In case of blank urine sample a hump is seen which reflects the presence of sufficient amount of caffeine because it has nearly similar λ_{max} (275 nm) [12] as DS (276 nm). In all samples, the absorbance of blank was considered as zero for DS. A clear signal represented by blue line indicates the presence and absorption spectrum of DS in the urine sample of the individual. The value is however blue shifted due to the acidic pH and presence of some other ingredients in case of urine samples. In case of serum sample the lower spectrum shows the presence of caffeine at lower concentration indicating that very little of it is retained by the serum while most is removed in urine. The results also show that DS containing serum sample from the individual administered with 50 mg tablet has a higher absorbance value as compared to his blank serum sample showing the presence of DS because caffeine is constant for both samples. The spiking of each diluted urine and serum sample was performed with 40 µl of DS solution in order to confirm the peak signal and hence the presence of DS in the serum sample. The results of DS in 4 urine and 4 serum samples by the currently developed method were observed (Table 5). Each sample of urine and serum with specific number (e.g. urine 1 and serum 1) was collected from same individual at different times as described in experimental section.

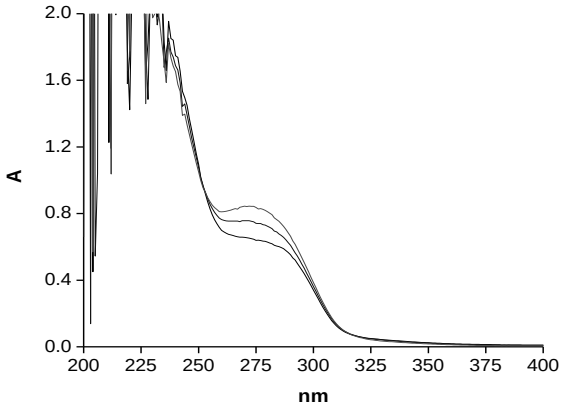

Figure 6. **UV spectra showing blank (black), DS containing (blue) and DS spiked (red) urine.**

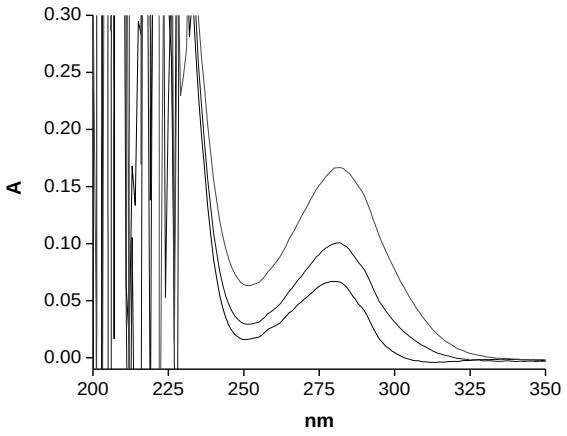

Figure 7. **UV spectra showing blank (black), DS containing (blue) and DS spiked (red) serum.**

Table 5. **Concentration of determined DS and its relation with ingested DS by oral administration.**

Sample type	DS found (µg ml^{-1}) [a]	Actual DS (µg ml^{-1}) [a]	DS % of ingested tablet
Urine 1	3.49 ± 0.010	34.9 ± 0.10	69.8
Urine 2	2.93 ± 0.0120	29.3 ± 0.12	58.6
Urine 3	2.34 ± 0.0160	23.4 ± 0.16	46.8
Urine 4	2.56 ± 0.020	25.6 ± 0.20	51.2
Serum 1	1.14 ± 0.004	11.4 ± 0.04	11.8
Serum 2	1.09 ± 0.004	10.9 ± 0.04	21.8
Serum 3	1.22 ± 0.005	12.2 ± 0.05	24.4
Serum 4	1.15 ± 0.006	11.5 ± 0.06	23.0

Symbols, as true for previous table

DS in each sample was determined with the help of linear equation and multiplied by dilution factor 10 in order to get the actual concentration of the ingested DS transferred to respective urine or serum sample. The data show that the urine and serum contents of DS have the range of 46.8–62.0 % and 10.09–12.2 % respectively for a 50 mg ingested tablet. The remaining DS may be present in plasma and/ or converted to inactive metabolites. As there is no satisfactory data available for DS contents in these fluids hence we rely upon the currently investigated data. Further studies in this regard could throw sufficient light upon actual kinetics of this drug and its fate in the body fluids.

Conclusion

The newly developed method for determining DS is more superior to reported spectrophotometric methods due to its better sensitivity, more simplicity, stability, economy, environmental safety, broader linear working and lower detection limits. Application of the developed method for quantification of DS in tablets with good recovery and lower standard deviation proves its suitability for analysis of DS in other pharmaceutical preparations. Its successful use in the determination of DS in urine and serum samples of patients makes this method as biomarker for identification and hence diagnosis of some diseases recognized by elevated or decreased level of DS.

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