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SENSITIVE SPECTROPHOTOMETRIC DETERMINATION OF ACECLOFENAC FOLLOWING AZO DYE FORMATION WITH 4-CARBOXYL-2,6-DINITROBENZENE DIAZONIUM ION

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Abstract: The study is a description of a sensitive spectrophotometric determination of aceclofenac following azo dye formation with 4-carboxyl-2,6-dinitrobenzenediazonium ion (CDNBD). Spot test and thin layer chromatography revealed the formation of a new compound distinct from CDNBD and aceclofenac. Optimization studies established a reaction time of 5 min at 30°C after vortex mixing the drug/CDNBD for 10 s. An absorption maximum of 430 nm was selected as analytical wavelength. A linear response was observed over 1.2–4.8 μg/mL of aceclofenac with a correlation coefficient of 0.9983 and the drug combined with CDNBD at stoichiometric ratio of 2 : 1. The method has a limit of detection of 0.403 μg/mL, limit of quantitation of 1.22 μg/mL and is reproducible over a three day assessment. The method gave Sandell's sensitivity of 3.279 ng/cm^2. Intra- and inter-day accuracies (in terms of errors) were less than 6% while precisions were of the order of 0.03–1.89 % (RSD). The developed spectrophotometric method is of equivalent accuracy ($p > 0.05$) with British Pharmacopoeia, 2010 potentiometric method. It has the advantages of speed, simplicity, sensitivity and more affordable instrumentation and could found application as a rapid and sensitive analytical method of aceclofenac. It is the first described method by azo dye derivatization for the analysis of aceclofenac in bulk samples and dosage forms.

Keywords: aceclofenac tablets, CDNBD, azo dye derivatization, spectrophotometric analysis

Chemically, aceclofenac is 2-(2-{2-[(2,6-dichlorophenyl)amino]phenyl}acetyl)oxyacetic acid. It is an analogue of diclofenac belonging to the class of non-steroidal anti-inflammatory drugs. It is commonly available in tablet form. The British Pharmacopoeia stated its purity to be between 99.0 to 101% (1). Aceclofenac is a phenylacetic acid derivative. It occurs as a white or almost white, crystalline powder. It has melting point of 149–150°C (2). It is practically insoluble in water, freely soluble in acetone, soluble in ethanol (96 percent).

Aceclofenac is a potent inhibitor of cyclooxygenase enzyme which is involved in the production of prostaglandins. It also inhibits the synthesis of mediators of inflammatory response e.g., cytokines, interleukin, tumor necrosis factor and PGE_2 (3). Action of aceclofenac has been found to be due to stimulation of synthesis of interleukin receptor antagonist and a decrease of production of nitric oxide in human articular chondrocytes subjected to inflammatory stimuli (4).

Aceclofenac is well absorbed from the gastrointestinal tract. Peak plasma concentration is reached in 1–3 h after oral dose. It is more than 99% bound to plasma proteins. The plasma $t_{1/2}$ is about 4 h. About two-third of a dose is excreted in the urine as hydroxymetabolites (5). The drug is used for the relief of pain and inflammation associated with rheumatoid arthritis, osteoarthritis and ankylosing spondylitis.

Various methods have been reported in the literature for the assay of aceclofenac either alone or in combined form in drug formulations. The analytical techniques include: spectrophotometric colorimetric, spectrofluorimetric, densitometric, HPLC, RP-HPLC and titrimetric. El-Moghazy et al. (6) reported the assay of aceclofenac in pure form and in pharmaceutical formulation by spectrophotometric method using *p*-dimethylaminocinnamaldehyde in acidified methanol to give a stable colored complex after heating at 75°C for 20 min. Absorption measurements were carried out at 665.5 nm (6). Also, El-

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Kousy demonstrated that aceclofenac in combination with etodolac could be assayed by the formation of colored complexes between the drugs and *p*-dimethylaminobenzaldehyde reagent in the presence of sulfuric acid and ferric chloride. Measurement of the absorbances was carried out at 591.5 and 545.5 nm for etodolac and aceclofenac, respectively (7). Colorimetric estimation of aceclofenac and indapamide was shown by Shingvi and Goyal (8) using Folin-Ciocalteu (FC) as a chromogenic reagent. The method is based on the formation of blue and green colored products. Aceclofenac shows absorbance maxima at 642.6 nm and linearity in the concentration range of 80–160 µg/mL (8). Shah et al. (9) reported a validated spectrophotometric assay of aceclofenac in tablet formulation by measuring the UV absorbance in a phosphate buffer of pH 7.4. The absorption maximum of aceclofenac in the buffer was 273 nm at the different dilutions prepared (20 and 1000 µg/mL).

Various stability-indicating spectrophotometric methods for the determination of aceclofenac in the presence of its main degradation product, diclofenac have also been described. Three methods were described by Hasan et al. Method A utilizes third derivative spectrophotometry at 242 nm. Method B is the spectrophotometric method based on the simultaneous use of the first derivative of ratio spectra and measurement at 245 nm. Method C is a pH-induced difference spectrophotometry using UV measurement at 273 nm. Regression analysis of a Beer's plot showed good correlation in the concentration ranges 50, 100 and 150 µg/mL for methods A, B, and C, respectively (10). In a similar work (11) two methods were developed for the determination of aceclofenac. In the first method, third-derivative spectrophotometry (D_3) was used. The D_3 absorbance was measured at 283 nm where the hydrolytic degradation product – diclofenac, does not interfere. The suggested method shows a linear relationship in the range of 4–24 µg/mL with mean percentage accuracy of 100.050 ± 0.88. This method determines the intact drug in the presence of up to 70% of degradation product with mean percentage recovery of 100.42 ± 0.94. The second method depends on Ratio-Spectra first-Derivative (RSD_1) spectrophotometry at 252 nm for aceclofenac and at 248 nm for determination of degradation product over concentration ranges of 4–32 µg/mL for both aceclofenac and diclofenac with mean percentage accuracy of 99.81 ± 0.84 and 100.19 ± 0.72 for pure drugs and 100.170 ± 0. 94 and 99.73 ± 0.74 for laboratory-prepared mixtures, respectively (11).

A number of chromatographic and densitometric techniques had been employed in the determination of aceclofenac in the biological system, bulk and pharmaceutical formulations. A stability-indicating assay of aceclofenac by HPLC and densitometry in the presence of its degradation products over a concentration range of 20–70 µg/mL and 1–10 µg per spot and mean recoveries of 99.590 ± 0.90 and 99.45 ± 1.09 was described (6). Simultaneous determination of aceclofenac, paracetamol and chlorzoxazone by RP-HPLC was described by Mangaonkar et al. (12).

In a simultaneous determination of aceclofenac, paracetamol and tizanidine (13) chromatographic separation of the three drugs were performed on a Hypersil C_{18} column (250 mm × 4.6 mm, 5 µm) as stationary phase. RP-HPLC method developed by Hasan et al. depended on using ethanol : water (60:40, v/v) as mobile phase at a flow rate of 1 mL/min and UV detection at 275 nm. Regression analysis of a Beer's plot revealed good correlation in the concentration range 1–50 µg/mL (10). Other methods include densitometric method for analysis of aceclofenac and paracetamol reported by Gandhi et al. (14) and spectrofluorimetric determination of etodolac and aceclofenac (7).

British Pharmacopoeia 2010 described a potentiometric titration for the determination of aceclofenac. In this method, 0.3 g of the drug was dissolved in 40 mL of methanol. Then, the solution was titrated with 0.1 M NaOH with end point determined potentiometrically.

A majority of these methods suffers from the disadvantage of extensive extraction procedures, long analytical time, high calibration range and low sensitivity.

In continuation of previous efforts in the analysis of organic compounds of pharmaceutical importance through azo dye chemical derivatization with 4-carboxy-2,6-dinitrobenzene diazonium ion (15–23), we report in this paper a sensitive, cost effective and rapid method for the assay of aceclofenac in tablets.

EXPERIMENTAL

Chemicals and reagents

Aceclofenac reference substance, acetone, glacial acetic acid, ethyl acetate, methanol, Zerodol®100 mg/ tablet (Ipca Laboratories Ltd.), Oetco® 100 mg/tablet (Laborate Pharmaceutical Ltd.).

Equipment

Electrochemical melting points apparatus (Stuart, England), analytical balance (Mettler H80),

precoated TLC aluminium plates, UV lamp 254/364mm (PW Allen and Co., England), digital colorimeter (6051 Jenway, UK), UV–Vis spectrophotometer (Perkin Elmer Lambda 25, UK), Vortex mixer (Griffin and George Ltd., UK), thermostated water bath (Langford, UK).

Methods

Preparation of stock solutions

A 3.0 mg quantity of aceclofenac crystals were weighed and dissolved in 10 mL of glacial acetic acid in a volumetric flask to give a solution of 9.18×10^{-4} M. CDNBD was prepared in concentrated sulfuric acid with sodium nitrite as previously reported (24).

Evidence of diazo coupling reaction

A 0.1 mL aliquot of the stock solution of aceclofenac was coupled with 0.5 mL of CDNBD by vortex mixing the reaction mixture for 10 s. After 20 min, the reaction tube was transferred into an ice bath with 5 mL of ice-cold water. The azo adduct formed was extracted into 2 mL of ethyl acetate. The ethyl acetate was concentrated to 1 mL. A control containing only CDNBD was similarly treated.

The samples (adduct, CDNBD and pure aceclofenac) were spotted on a 10×5 cm TLC pre-coated aluminium plate. The plate was developed using ethyl acetate and methanol (9 : 1, v/v) as mobile phase. The developed plate was examined using UV lamp at 254 and 365 nm.

Optimization studies

This was done based on the method of steepest ascent. The effect of temperature was studied at four levels of 30, 50, 60 and 70°C with each maintained for 5 min and 20 min. The stock solution of aceclofenac (0.1 mL) was coupled with CDNBD (0.5 mL). At the end of each time, the azo adduct formed was extracted into 5 mL of ethyl acetate and the absorbance readings at λ_{max} of 430 nm were recorded. The procedure was repeated with replicate samples.
A 0.1 mL of aceclofenac stock solution was added to 0.5 mL of the reagent in test tubes. The effect of varied time allowed for coupling taking place at 30°C between aceclofenac and CDNBD was studied at 0, 2, 5, 10, 15, 20, 25 and 30 min. The azo adduct formed was processed as described above. The procedure was repeated with replicate samples.

Determination of stoichiometric ratio for the formation of azo dye

Aliquots of CDNBD reagent; 0, 0.2, 0.25, 0.33, 0.50, 0.67, 0.75, 0.8 and 1.0 mL were added into nine test tubes. Each tube was in turn made up to 1.0 mL with the drug stock solution. The mixture was vortex mixed for 10 s and processed. The azo adduct formed in each case was extracted into 5 mL of ethyl acetate. A series of blank determinations were carried out in which the volume of the drug stock solution was replaced with glacial acetic acid. The absorbance was measured at 430 nm against the blank and values obtained were plotted against the mole fraction of the reagent solution. Each determination was carried out in duplicate.

Validation studies

Calibration curve for the adduct was prepared using concentration range of 0–4.8 µg/mL from the stock of aceclofenac solution in glacial acetic acid. To seven test tubes, each containing 0.67 mL of CDNBD reagent solution, 0, 0.02, 0.03, 0.04, 0.05, 0.06 and 0.08 mL corresponding to 0, 1.2, 1.8, 2.4, 3.0, 3.6 and 4.8 µg/mL of aceclofenac stock solution were added, respectively. The mixture was vortex mixed for 10 s. After 5 min, the azo adduct was processed and the absorbance readings were recorded at 430 nm and replicate samples were prepared. The regression line equation and correlation coefficient were obtained from the calibration curve.

Accuracy and precision are the analytical parameters to be determined through recovery studies. These were determined using quadruplicate samples at three concentration levels of 1.2, 2.4 and 3.6 µg/mL on three different days. The volume of aceclofenac solution corresponding to these concentrations were measured i.e., 0.02, 0.04 and 0.06 mL. Each volume was added into test tubes containing 0.67 mL of CDNBD reagents solution one after the other. The reaction mixture was vortex mixed for 10 s. At the end of the coupling time, the adduct formed was extracted into 5 mL of ethyl acetate. This was done four times for each volume. The absorbance readings were recorded at 430 nm using ethyl acetate as blank solvent. A three-day recovery study was done and the accuracy and precision were determined from the regression equation.

Application to tablet analysis

Two brands of aceclofenac tablets (100 mg) were analyzed: Zerodol (Ipca Laboratories Ltd.) and Oetco (Laborate Pharmaceutical Ltd.). Uniformity of weight test was carried out on all the brands using 20 tablets.

The assay of active ingredient was determined based on the new and official methods. For the assay of the two brands of aceclofenac tablets, two different analyte stock solutions (A and B) of the same

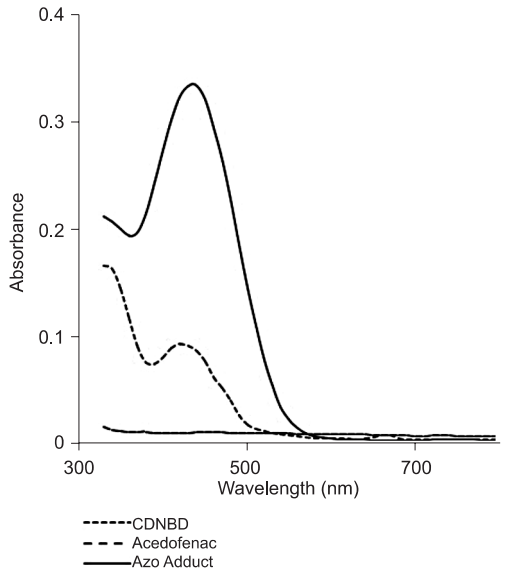


Figure 1: Absorption spectra of aceclofenac, CDNBD and aceclofenac-CDNBD adduct

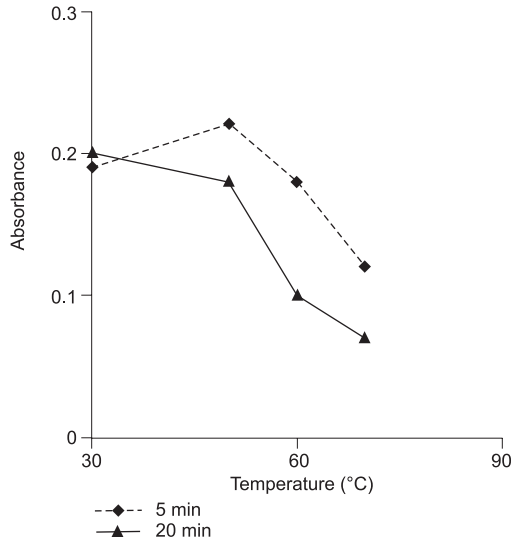


Figure 2: Optimization of temperature of coupling reaction

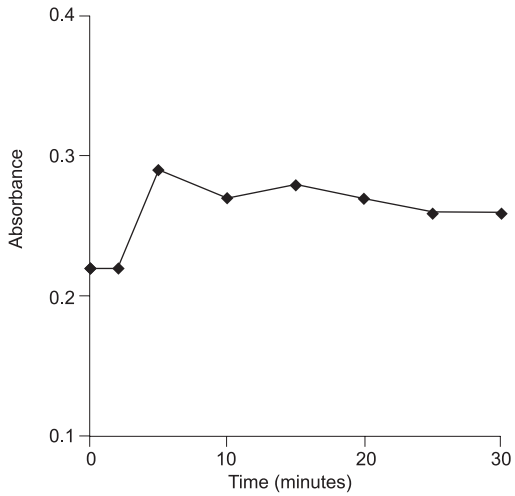


Figure 3: Optimization of coupling reaction time at 30°C

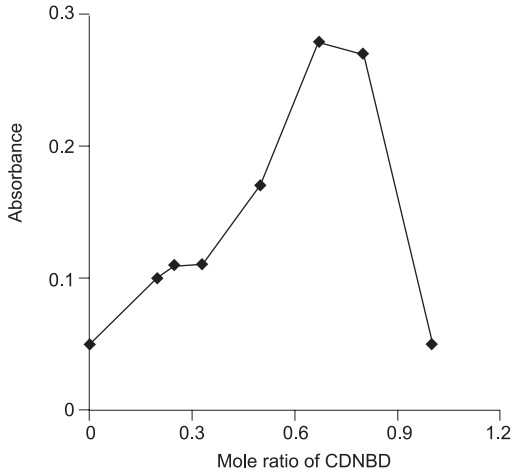


Figure 4: Determination of stoichiometric ratio for the formation of aceclofenac-CDNBD adduct

concentration were prepared. Based on weight uniformity test, 6 mg of Zerodol® and 20 mg Oetco® powdered tablets (each corresponding to 3 mg of aceclofenac) were weighed and dispersed in 10 mL of glacial acetic acid in a volumetric flask. The suspension was left overnight for complete dissolution, after which it was filtered. The filtrate was then kept in sample vial and used as drug stock solution.

From each analyte stock solution A and B, 0.05 mL was transferred into six tubes containing 0.67 mL CDNBD reagent solution. The mixture was vortex mixed for 10 s. At the end of 5 min, the tubes were transferred into an ice bath and 5 mL of ice-cold distilled water was added to each test tube. The adduct formed was extracted into 5 mL ethyl acetate and transferred into vials wrapped with aluminium foil. The absorbance readings were recorded at λ_{max} of 430 nm on colorimeter.

For the reference potentiometric titration, the titrant, sodium hydroxide was standardized. Zerodol® powder (0.563 g) and Oetco® powder (1.452 g) equivalent to 0.3 g of aceclofenac were weighed and dissolved in 40 mL of methanol. The solution was left for 10 min and was titrated with 0.1

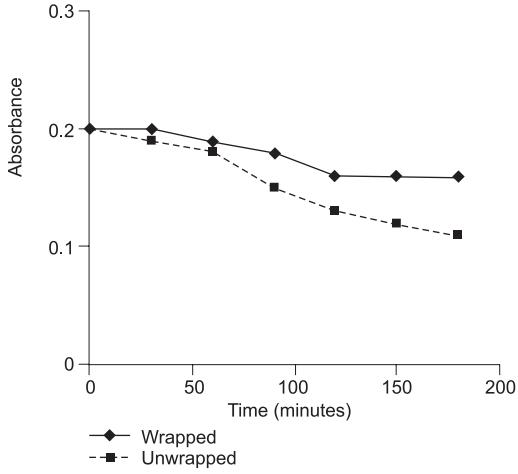


Figure 5: Analytical signal stability at 430 nm

M NaOH. For every 2 mL of sodium hydroxide added, pH was determined. After a sharp increase in pH was observed, volume of titrant was reduced to 1 mL. Titration was done four times for each brand. Values of pH obtained were plotted against volume. Then, a derivative curve of $\Delta pH/\Delta V$ against volume, V was plotted. The titre value was used in calculating weight of aceclofenac and its % content.

Analytical signal stability

A 0.05 mL quantity of aceclofenac stock solution was added into each of four test tubes containing 0.67 mL of CDNBD reagent solution. After processing the reaction mixture as before, the ethyl acetate extract was kept in vials labelled A, B, C and D. Samples A and B were wrapped with aluminium foil, while C and D

Table 1. Analytical and validation parameters for the assay of Aceclofenac by CDNBD method.

Parameter	Value
Maximum wavelength of absorption (λ_{max})	430 nm
Beer's law limits, (µg/mL)	1.2 – 4.8
Limit of detection, (µg/mL)	0.4032
Limit of quantitation, (µg/mL)	1.222
Molar absorptivity (Mol^{-1} cm^2)	$(1.19 \pm 0.09) \times 10^4$
Sandell's sensitivity, (ng/cm^2 per 0.001 absorbance unit)	3.279
Regression equation[a]	
Slope, b	0.0305
95% confidence interval of slope	0.0048
Intercept, a	0.1006
95% confidence interval of intercept	0.0143
Correlation coefficient	0.9983

[a] y = bx + a, where y is the absorbance for concentration x µg/mL

Table 2. Intra-day accuracy of the proposed CDNBD spectrophotometric method.

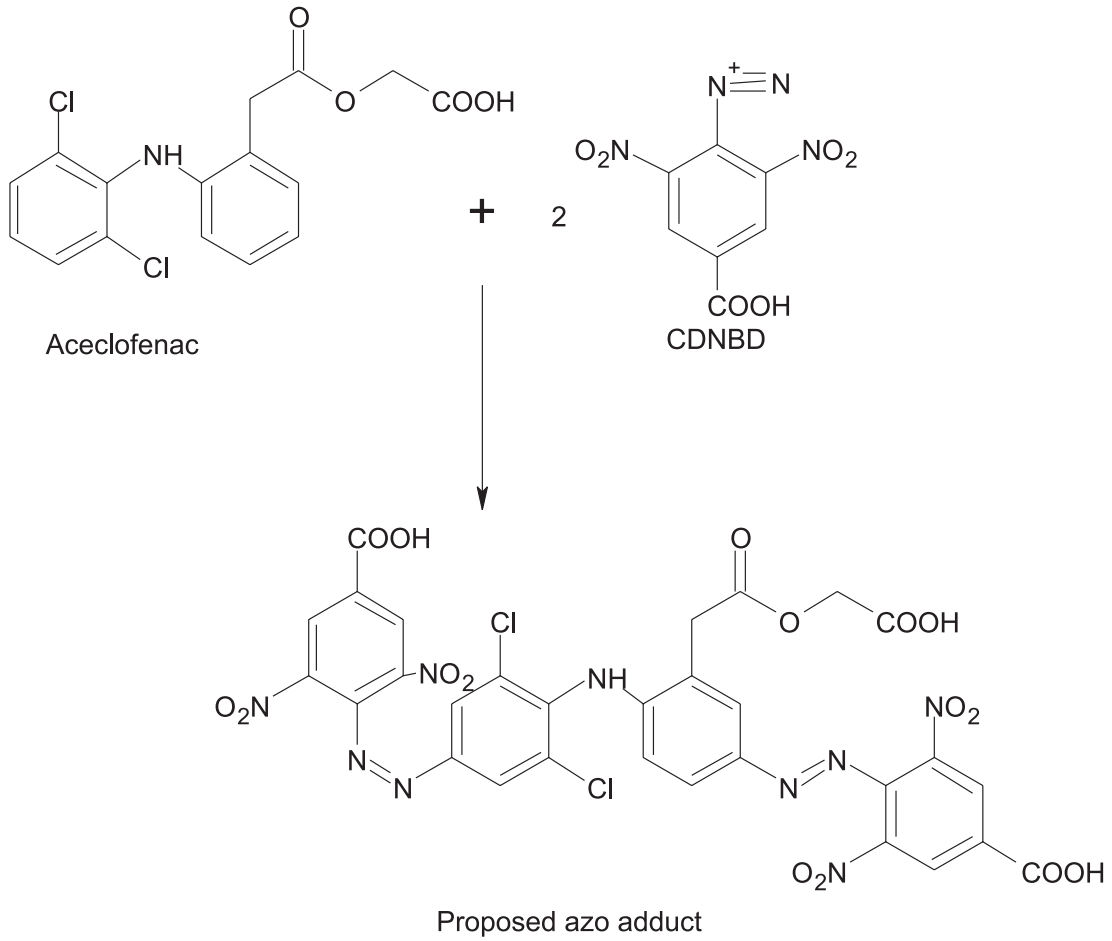
Drug	Amount taken (µg/mL)	Amount found (µg/mL)	Recovery (%)[a]	RSD (%)[b]	Relative error (%)
Aceclofenac	1.20	1.27	105.88	0.205	5.55
	2.40	2.44	102.05	0.698	2.01
	3.60	3.70	102.91	1.887	2.83

[a] Average of six determinations; [b] RSD, relative standard deviation

Table 3. Inter-day accuracy of the proposed spectrophotometric method.

Drug	Amount taken (µg/mL)	Amount found (µg/mL)	Recovery (%)[a]	RSD (%)[b]	Relative error (%)
Aceclofenac	1.20	1.27	105.63	0.54	5.33
	2.40	2.44	101.75	0.79	1.72
	3.60	3.73	103.71	1.28	3.58

[a] Average of twelve determinations; [b] RSD, relative standard deviation



Scheme 1. Proposed coupling pattern between Aceclofenac and CDNBD

were unwrapped. Both groups were kept on the laboratory bench. Absorbance readings were recorded at 30 min interval for a period of 3 h at 430 nm.

Interference studies

A 0.05 mL quantity of the stock solution corresponding to 3 µg/mL of aceclofenac was spiked into tablet excipients containing 5 mg each of lactose, starch, gelatin, magnesium stearate, talc and a mixture of the five excipients.

The procedure for coupling, extraction and sample determination were carried out as described earlier. Blank determinations with CDNBD in the absence of the drug were carried out.

Statistical analysis

The F-test and *t*-test were determined and used to compare equivalence of the new CDNBD method with the official potentiometric method. A p-value less or equal to 0.05 was taken as significant.

RESULTS AND DISCUSSION

Evidence of diazo coupling reaction and selection of analytical wavelength

The drug produced an instant light orange-red coloration which became intense with time when coupled with CDNBD reagent.

The TLC revealed an evidence of coupling reaction with the adduct having an R_f value (0.63), CDNBD (0.62) and pure aceclofenac (0.79). Further evidence on TLC for the formation of a new compound was the fluorescence exhibited by the azo adduct at 365 nm, which was absent in both CDNBD and aceclofenac.

The absorption spectrum of aceclofenac shows absorption maximum at 277 nm. The spectrum of the adduct shows three maxima at 274 nm 346 nm and 430 nm. The blank reagent exhibits absorption maxima at 258 nm, 333 nm and 423 nm. The overlaid absorption spectra of aceclofenac, the

azo adduct and CDNBD reagent are presented in Figure 1.

Aceclofenac has nearly no absorbance at 430 nm while CDNBD has very small absorbance of about 0.09. The spectrum of the azo adduct shows a bathochromic shift with respect to aceclofenac due to chromophoric elongation caused by azo linkage. A hyperchromic shift observed in the spectrum of the adduct when compared with CDNBD was due to higher number of chromophores. The peak at 430 nm was selected as analytical wavelength, because at this wavelength, the difference in absorptivity between the blank reagent and the adduct was maximal.

Optimization studies

The first set of optimization studies conducted was the determination of the optimum temperature and time required for the diazo coupling reaction between aceclofenac and CDNBD. The temperature profile is presented in Figure 2. There was an increase in absorbance from 30 to 50°C at 5 min incubation period. After 50°C, absorbance decreased. At 20 min of incubation period, absorbance decreased slightly from 30 to 50°C. After 50°C, there was a sharp decrease in absorbance. This could have been due to thermal decomposition of the adduct formed. It has been previously reported that the reagent, CDNBD is reasonably stable at elevated temperatures (24). However, the drastic reduction in the absorbance with an increase in temperature can be attributed to thermal decomposition of the azo adduct. Since a gain in time of analysis will be achieved on using the most convenient temperature, 30°C was selected as the temperature for the reaction of aceclofenac with CDNBD. Further investigation of the reaction time was carried out at 30°C. It was observed, as shown in Figure 3, that the absorbance obtained at 0 and 2 min was the same. Absorbance rose for 5 min and then decreased steadily until 15 min after which it was constant. This means that coupling reaction is very fast and completed after vortex mixing of the drug/reagent mixture for 10 s.

The optimal ratio for the formation of the azo adduct was investigated using Job's method of continuous variation. The result is presented in Figure 4. The highest possible absorbance reading was obtained at a mole fraction of 0.67 for the reagent solution and the absorbance was found to decrease at lower or higher mole fractions. This shows that CDNBD and aceclofenac react in a 2:1 stoichiometric ratio. Aceclofenac has two aromatic rings joined by an amino bridge. Both rings are favorably attacked by the strong electrophile, CDNBD. The amino group (-NH) of the aceclofenac direct the electrophile to the para position of the two aromatic rings as shown in Scheme 1. This justifies the 2:1 reagent to drug stoichiometric ratio obtained. TLC analyses of the coupling reaction mixture revealed the presence of a single adduct thereby ruling out formation of multiple products. The isolated nature of the aromatic rings might have promoted the simultaneous aromatic electrophilic substitution. The proposed reaction pattern between aceclofenac and CDNBD is presented in Scheme 1. However, further evidence suggesting the probability of the adduct proposed comes from the reaction of indomethacin with CDNBD, where ^{1}H NMR revealed disubstitution by CDNBD once again due to the isolated nature of the aromatic skeletons in indomethacin (18).

Validation studies

Calibration lines were prepared on three successive days and the average absorbance readings were used to describe linear regression line for the assay of aceclofenac by the CDNBD method. The assays of aceclofenac were linear over the range 1.2–4.8 µg/mL of aceclofenac with a linear regression of y = 0.0305x + 0.1006 with correlation coefficient of 0.9983. The limits of detection (LOD) and quantitation (LOQ) were estimated as stipulated by the ICH guidelines (25) using the expressions below:

$$LOD = \frac{3.3\sigma}{s} \quad \text{and} \quad LOQ = \frac{10\sigma}{s}$$

where σ is the standard deviation of the blank signals and *s* is the slope of the calibration graph. The LOD and LOQ values obtained are 0.4032 and 1.222 µg/mL, respectively. The Sandell's sensitivity obtained is 3.279 ng/cm^2. These low values point to the high sensitivity related to this new spectrophotometric method. This is the first report of such high sensitivity for the estimation of aceclofenac. The various analytical and validation parameters are presented in Table 1.

Accuracy and precision were estimated over three days by recovery studies. The intra- and inter-day data obtained are presented in Tables 2 and 3, respectively. The intermediate precision is presented in Table 4. This represented the precision of the analytical method over the three assessment periods. The accuracy of the new spectrophotometric method using CDNBD ranged from 0.8 to 5.8% (relative error). Lower relative errors were obtained with higher concentration ranges than the low concentration values. The overall recoveries of aceclofenac over three-day assessment was found to be 103.69 ± 1.85 (RSD 1.784%).

Table 4. Assessment of intermediate precision.

Drug	Recovery (% ± S.D.)[a]		
	Day 1	Day 2	Day 3
Aceclofenac	103.61 ± 1.64	103.25 ± 1.87	104.22 ± 1.37

[a]values are the mean of three determinations ± S.D.; overall recovery: 103.69 ± 1.85 (RSD = 1.78 %)

Table 5. Mean recoveries of aceclofenac in the presence of common tablet excipients.

Excipients	Starch	Magnesium stearate	Gelatin	Lactose	Talc	Mixture of excipients
Recovery (% ± S.D.)[a]	103.18 ± 0.57	103.80 ± 0.57	103.73 ± 0.62	103.84 ± 0.57	103.84 ± 0.57	103.84 ± 0.57

[a] n = 4

Table 6. Assay of aceclofenac in tablets using the CDNBD and official potentiometric methods.

Drug (Amount on label, mg)	Tablet brands	Found (mg)	Amount RSD (%) (New method)	Mean % ± S. D. [a]		Statistics	
				New method	Official method	F-test	*t*-test
Aceclofenac (100 mg)	Zerodol®	103.39	0.67	103.39 ± 0.69	102.59 ± 0.05	0.95	0.25
	Oetco®	74.95	2.51	74.95 ± 1.88	74.17 ± 0.87	0.06	0.49

[a] Average of six determinations

Interference studies

The influence of commonly utilized excipients on the recovery of aceclofenac from the reaction medium was studied using starch, magnesium stearate, gelatin, lactose, talc and a mixture of the five excipients. The results obtained are presented in Table 5. Good recoveries were obtained in the presence of all the excipients implying that the method is free from undue interference by the commonly utilized tablet excipients.

Analytical signal stability

The absorbance readings of the aceclofenac-CDNBD azo adduct was monitored over three hours and absorbance values were recorded at 30 min intervals for sets of samples wrapped and those exposed to diffuse light of the laboratory. The result is presented in Figure 5. There is no appreciable change in absorbance for wrapped sample extract from 0 to 60 min after which there is a marked decline up to 180 min. The unwrapped sample extract shows slight change in absorbance from 0 to 30 min, thereafter, there is a great decline up to 180 min. The greater loss in absorbance by the unwrapped samples might be due to the greater light intensity passing through the solution, compared with the wrapped samples, which might probably allow the passage of light *via* the plastic cover of the plastic vial. From the result, it can be inferred that the azo adduct is maximally stable for 1 h when wrapped. The significant loss of absorptivity after this time implies that this new method shares the common disadvantage of most color-forming derivatization procedures, where stability of the colored complex or solution to diffuse light is low. Thus, it is recommended that sample solutions of aceclofenac adduct are wrapped and determined within 1 h after preparation.

Tablet analysis

The new spectrophotometric method developed using aromatic ring derivatization with CDNBD was applied to the assay of aceclofenac in two tablet brands and the results were compared with potentiometric titration using NaOH (Table 6). No significant difference was found in the content of aceclofenac in the tablets by both methods thereby establishing their equivalence.

Some clearly defined advantages of this new spectrophotometric method are: low concentrations of the calibration ranges compared to the use of Folin-Ciocalteu and *p*-dimethylaminocinnamalde-

hyde as derivatizing agents. Singhvi and Goyal (8) and El-Moghazy et al. (6) reported ranges of 80–160 μg/mL and 20–100 μg/mL, respectively. These two Beer's limits are higher than that of CDNBD method (1.2–4.8 μg/mL) conferring a great advantage of sensitivity to the latter. Furthermore, CDNBD method is simple, fast and rapid with an instant formation of a stable orange-red adduct compared with the procedure reported (6), where a stable complex is formed after heating at 75°C for 20 min. In addition, dilution of stock solution with phosphate buffer pH 7.4 and distilled water to obtain a working solution as reported by Shah et al. (9) may compromise accuracy and precision. The CDNBD method is the first described method for the analysis of aceclofenac by azo dye derivatization methodology.

CONCLUSIONS

The new spectrophotometric method described in this report is simple, accurate, precise and highly sensitive compared to previously reported methods. It is of equivalent accuracy with the reference potentiometric titration. The method can find application as a simple and rapid in-process quality control procedure for the assay of aceclofenac.

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