ELSEVIER

Journal of Pharmaceutical and Biomedical Analysis
20 (1999) 185–194

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

Spectrophotometric and spectrofluorimetric determination of etodolac and aceclofenac

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Received 3 April 1998; received in revised form 27 May 1998; accepted 15 August 1998

Abstract

Two simple, sensitive and reproducible spectrophotometric and spectrofluorimetric methods were adopted for the analysis of the anti-inflammatory drugs, etodolac and aceclofenac. The first method is based on the formation of coloured complexes between the drugs and *p*-dimethylaminobenzaldhyde reagent (PDAB) 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. Regression analysis of Beer's plots showed good correlation in the concentration ranges 10–80 and 8–55 μg ml^{-1}, respectively. The second was the spectrofluorimetric method in which samples of etodolac in ethanol showed native fluorescence at a $\lambda = 345$ nm when excitation was at 235 nm and samples of aceclofenac in the phosphate buffer pH 8 showed native fluorescence at $\lambda = 355$ nm when excitation was at 250 nm. The calibration graph was rectilinear from 96 to 640 ng ml^{-1} for etodolac and from 2 to 8 μg ml^{-1} for aceclofenac. The proposed methods are applied successfully for the determination of the two drugs in bulk powder with a mean accuracy of 100.48 ± 0.85 and 100.03 ± 0.38 in the PDAB method and of 100.61 ± 0.79 and 99.88 ± 0.45 in the spectrofluorimetric method. Applicability of the proposed methods was examined by analysing dosage forms of the investigated drugs. Recoveries were 98.77–101.46 and 98.65–102.10% for the two methods, respectively and RSD values were 0.6–0.7 and 0.35–1.06%, respectively.

Keywords: Spectrophotometry; Spectrofluorimetry; Etodolac; Aceclofenac

1. Introduction

Etodolac and aceclofenac are nonsteroidal anti-inflammatory antirheumatic drugs. Few methods have appeared in the literature for the determination of etodolac in biological fluids, pharmaceutical formulations and in presence of its enantiomer. The techniques used in this connection include only chromatographic methods, HPLC [1–4] and GC [5–7].

Aceclofenac is determined by a single technique, namely stripping voltammetry [8].

Although spectrometric methods are the instrumental methods of choice commonly used in industrial laboratories, no colorimetric or fluorimetric method has been reported so far for the determination of the investigated drugs. Therefore, the need for fast, low cost selective methods is obvious, especially for the routine quality control analysis of pharmaceutical formu-

0731-7085/99/$ - see front matter
PII: S0731-7085(99)00019-9

lations containing etodolac and aceclofenac. This paper describes spectrophotometric and spectrofluorimetric methods for the determination of the cited drugs.

2. Experimental

2.1. Apparatus

2.1.1. Shimadzu-160 A, UV-visible spectrophotometer.

2.1.2. Shimadzu spectrofluorimeter, data recorder, DR 3.

2.1.3. Hanna pH meter.

2.2. Materials

2.2.1. Pure samples

2.2.1.1. Etodolac working standard, supplied by Pharco, Egypt. Purity was found to be $100.35 \pm 0.64\%$ according to the Pharco method [9] in which the absorbance of 0.002% w/v etodolac solution in 0.1 N sodium hydroxide was measured at 276 nm.

2.2.1.2. Aceclofenac working standard was supplied by Squibb, Egypt. Purity is found to be $99.78 \pm 0.49\%$ according to Squibb method [10]. The absorbance of 0.002% w/v methanolic solution of aceclofenac was measured at 274 nm.

2.2.2. Market samples

2.2.2.1. Etodine capsules 300 mg/capsule, supplied by Pharco.

2.2.2.2. Bristaflam tablets 100 mg/tablet, supplied by Squibb, Egypt.

2.2.3. Reagents

2.2.3.1. 1. 0.1 and 0.2% w/v PDAB solution in 50% v/v sulfuric acid.

2.2.3.2. 2. 2.5% w/v aqueous solution of ferric chloride.

2.2.3.3. 3-Phosphate buffer pH 8. Add 48.6 ml of 0.2 M sodium hydroxide solution to 50 ml of 0.2 M potassium dihydrogen orthophosphate solution. Complete to volume in a 200 ml volumetric flask using distilled water and check the pH using a pH meter.

2.3. Preparation of standard solutions

2.3.1. For the spectrophotometric method

Prepare solution of etodolac or acedofenac in ethanol in a concentration 0.5 mg ml^{-1}.

2.3.2. For the spectrofluorimetric method

2.3.2.1. Prepare solution of concentration 1 mg ml^{-1} of etodolac, transfer 10 ml to a 100 ml volumetric flask and make up to volume with ethanol. Carry out further dilution to obtain solution with a concentration of 1 μg ml^{-1}.

2.3.2.2. Prepare solution of concentration 1 mg ml^{-1} of aceclofenac, transfer 2 ml to a 100 ml volumetric flask and make up to volume using phosphate buffer pH 8 (20 μg ml^{-1}).

2.4. Preparation of test solution

2.4.1. For the spectrophotometric method

In a 100 ml volumetric flask, shake an amount of the powdered tablets or capsule contents equivalent to 50 mg etodolac or aceclofenac with 70 ml of ethanol for 20 min, make up to volume mix and filter.

2.4.2. For the spectrofluorimetric method

2.4.2.1. Into a 100 ml volumetric flask, transfer 20 ml of the test solution of etodine capsules (prepared for the spectrophotometric method) and make up to volume using ethanol. Carry out further dilution with ethanol to obtain a solution of concentration 1 μg ml^{-1} of etodolac.

2.4.2.2. Into a 100 ml volumetric flask, transfer 4 ml of the test solution of bristaflam tablets (prepared for the spectrophotometric method) and make up to volume using phosphate buffer pH 8 to obtain a solution of concentration 20 μg ml^{-1} of aceclofenac.

2.5. Procedures

2.5.1. Construction of calibration curves

2.5.1.1. For the spectrophotometric method. Into two series of 10 ml volumetric flasks, separately transfer aliquot amounts of standard solution equivalent to 80–900 μg of etodolac or 50–600 μg of aceclofenac. To the first series, add 6 ml of 0.1% w/v PDAB solution and to the second series, add 8 ml of 0.2% w/v solution of the same reagent. To each flask, transfer 0.2 ml of 2.5% w/v ferric chloride solution and heat on a water bath at 65 ± 5°C for 20 min. Cool, complete to volume using 50% v/v sulfuric acid and measure the absorbance at 591.5 and 545.5 nm in the case of etodolac and aceclofenac, respectively. Construct the calibration curves and calculate the regression equations.

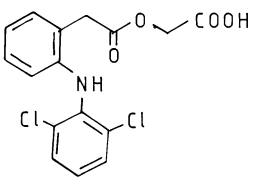
2.5.1.2. For spectrofluorimetric method. Into two series of 10 ml volumetric flasks, separately transfer different aliquots of standard solution equivalent to 1–7 μg of etodolac and 10–100 μg of aceclofenac. Complete to the mark using ethanol for the first series and phosphate buffer pH 8 for the second. The fluorescence was recorded at 345 nm with an excitation at 235 nm for etodolac and at 355 nm with an excitation at 250 nm for aceclofenac. Construct the calibration curves and calculate the regression equations.

2.5.2. Determination of dosage forms

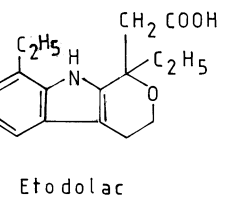
2.5.2.1. For the spectrophotometric method. The same procedure mentioned under the construction of calibration curves for the spectrophotometric method is repeated using different aliquots of the test solution equivalent to 200–400 μg etodolac and 150–250 μg aceclofenac. The standard addition technique is applied by adding aliquots of the standard solution equivalent to 100–400 μg etodolac and 100–300 μg aceclofenac.

2.5.2.2. For the spectrofluorimetric method. Repeat the same procedure mentioned in Section 2.5.1.2 using different aliquots of the test solution equivalent to 1–3 μg etodolac and 20–60 μg aceclofenac. The standard addition technique is applied by adding aliquots of the standard solution equivalent to 1–2 μg etodolac and 20–40 μg aceclofenac.

3. Results and discussion



Aceclofenac



Etodolac

Different pharmaceutical substances were determined using PDAB reagents such as primary amines, secondary amines [11] and indole derivatives [12–14]. PDAB dissolved in sulfuric acid and in the presence of ferric chloride is used for the identification and determination of furosamide [15,16].

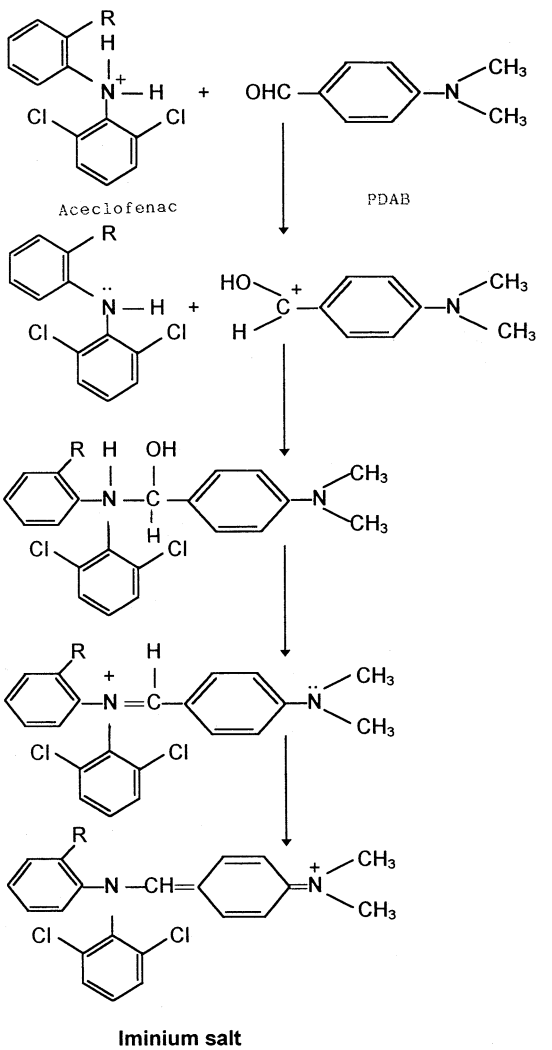
The structure of the investigated drugs was promising for the successful determination by using PDAB as etodolac is an indole derivative and aceclofenac is a secondary amine in nature.

Certain amines and alicyclic amines such as pyrrole and indole derivatives condense with various aldehydes such as PDAB in strongly acid media to give products including Schiff bases that are oxidised by ferric ions to give coloured species [17,18]. Scheme 1 represents a suggestion for the reaction of aceclofenac with PDAB in acid medium via the protonated amino group [19]. Some pyrrole and indole derivatives react with PDAB by condensation with a $-CH_2-$ group [20], consequently the reaction of the indole derivative, etodolac with this reagent is suggested to proceed as shown in Scheme 2. The suggested mechanisms agree with the molar ratio of the investigated drugs to PDAB (1:1) obtained by applying the continuous variation technique [21].

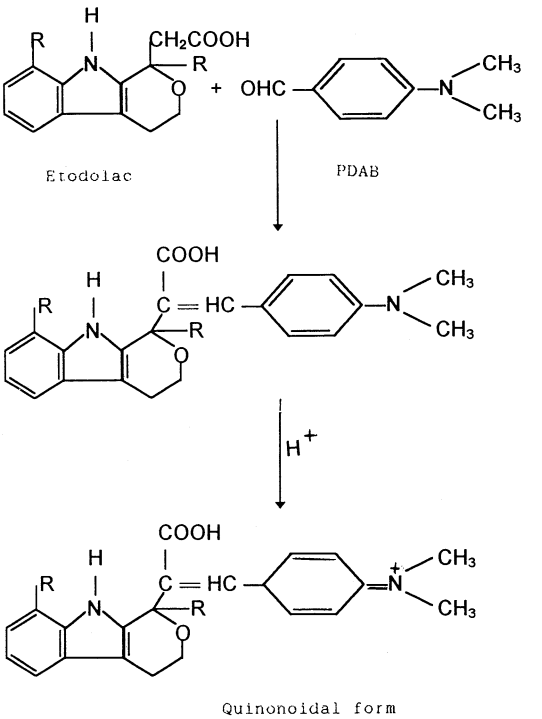
The optimum conditions for the development of the proposed methods were established by varying the parameters one at a time and observ-

ing the effect produced. The absorption spectra of the coloured complexes were constructed and maximum absorption was attained at 591.5 nm for etodolac and at 545.5 nm for aceclofenac (Fig. 1).

The effect of volume and concentration of the PDAB reagent was studied using different amounts ranging from 1 to 10 ml of 0.1 and 0.2% w/v solutions. It was observed that 6 ml of 0.1% w/v solution and 8 ml of 0.2% w/v solution are the optimum for etodolac and aceclofenac, respectively. The sensitivity of the method is highly



Scheme 1.



Scheme 2.

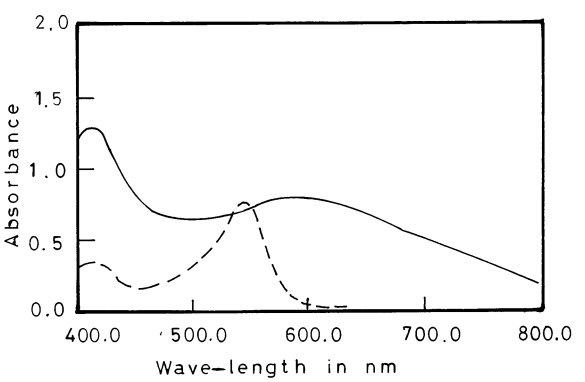


Fig. 1. Absorption spectra of etodolac (60 μg ml^{-1}) and aceclofenac (40 μg ml − 1) complexes with PDAB: —, etodolac; – – – –, aceclofenac.

improved by using 0.2 ml of 2.5% w/v solution of ferric chloride. Study of the effect of time of standing showed that 1 h is essential for the complete reaction between the investigated drugs and PDAB but heating at 65°C enhances the reaction where 20 min was required. Higher re-

sults were observed on making up the volume of the final solution by using 50% v/v sulfuric acid than by using ethanol. Interference in the PDAB method is expected from primary amines, secondary amines, hydrazines, pyrrole and indole, derivatives [11–14].

By applying the proposed colorimetric method, a linear correlation was obtained between absorbances and concentration over the ranges 10–80 and 8–55 μg ml^{-1} for pure etodolac and aceclofenac, respectively. The regression equations were calculated to be as follows:

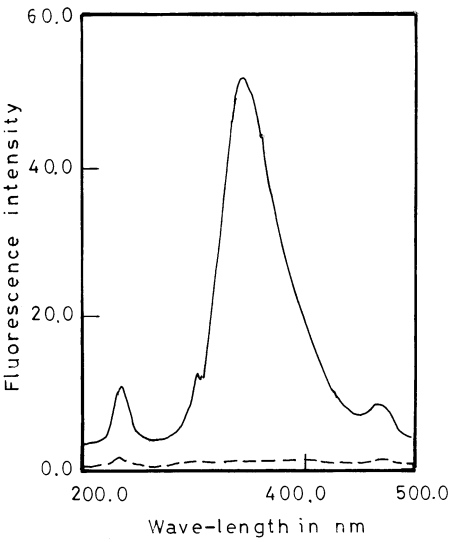


Fig. 2. Excitation and emission spectrum of etodolac (400 ng ml^{-1}) in ethyl alcohol: —, etodolac; – – – –, blank.

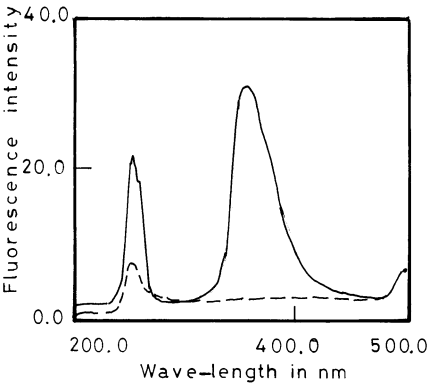


Fig. 3. Excitation and emission spectrum of aceclofenac (8 μg ml^{-1}) in phosphate buffer pH 8.

1. $A = 0.013C + 0.00$, $r = 1$ (Etodolac)
2. $A = 0.019C + 0.00$, $r = 1$ (Aceclofenac)

where A is the absorbance at 591.5 and 545.5 nm, respectively, C is the concentration in μg ml^{-1} and r is the correlation coefficient. The intercepts were found to be zero in both cases as the calibration curves pass through the origin.

The cited drugs showed native fluorescence in ethanol in the case of etodolac and in phosphate buffer pH 8 in the case of aceclofenac. Solutions of the cited drugs exhibited the strongest fluorescence at 345 nm when excited at 235 nm (Fig. 2) and at 355 nm when excited at 250 nm (Fig. 3), respectively.

A linear correlation was obtained between the fluorescence intensity and concentration in the

Table 1
Statistical comparison of results obtained by the suggested methods and the reference methods on the analysis of etodolac and aceclofenac in a pure drug[b]

Data	Spectrophotometric method		Reference method[a]		Spectrofluorimetric method	
	Etodolac	Aceclofenac	Etodolac	Aceclofenac	Etodolac	Aceclofenac
Mean %	100.48	100.03	100.35	99.78	100.61	99.88
SD	0.85	0.38	0.64	0.49	0.79	0.45
n	5	5	5	5	5	5
$t(P = 0.05)$ [2.306]	0.174	0.90			0.58	0.2
$F(P = 0.05)$ [6.4]	1.742	1.664			1.49	1.19

[a] Pharco and Squibb UV methods [9,10].
[b] Figures in parentheses represent corresponding tabulated values for t and F at $P = 0.05$.

Table 2
Determination of etodolac in dosage form by the spectrophotometric and spectrofluorimetric methods

Spectrophotometric method						Spectrofluorimetric method					
Claimed ($\mu g\ ml^{-1}$)	Found ($\mu g\ ml^{-1}$)	%[a]	Added ($\mu g\ ml^{-1}$)	Found ($\mu g\ ml^{-1}$)	%[b]	Claimed ($ng\ ml^{-1}$)	Found ($ng\ ml^{-1}$)	%[a]	Added ($ng\ ml^{-1}$)	Found ($ng\ ml^{-1}$)	%[b]
40	40.46	101.15	20	20.08	100.4	100	101.86	101.86	200	201.99	100.99
40	40.62	101.55	40	40.15	100.38	150	152.63	101.75	150	151.54	101.02
20	20.38	101.90	10	10.05	100.5	200	204.17	102.08	100	100.00	100.00
20	20.00	100.00	20	20.15	100.75	250	255.38	102.15	150	152.31	101.54
20	20.54	102.70	40	40.23	100.58	300	308.01	102.67	100	100.00	100.00
Mean	—	101.46	—	—	100.52	—	—	102.10	—	—	100.71
SD	—	0.72	—	—	0.15	—	—	0.36	—	—	0.68

[a] Recovery of etodolac contents in the capsules.
[b] Recovery of the added etodolac standard.

Table 3
Determination of aceclofenac in dosage form by the spectrophotometric and spectrofluorimetric methods

Spectrophotometric method						Spectrofluorimetric method					
Claimed ($\mu g\ ml^{-1}$)	Found ($\mu g\ ml^{-1}$)	%[a]	Added ($\mu g\ ml^{-1}$)	Found ($\mu g\ ml^{-1}$)	%[b]	Claimed ($\mu g\ ml^{-1}$)	Found ($\mu g\ ml^{-1}$)	%[a]	Added ($\mu g\ ml^{-1}$)	Found ($\mu g\ ml^{-1}$)	%[b]
20	19.63	98.15	10	10.00	100.00	2	1.99	99.5	4	4.02	100.5
20	19.63	98.15	20	20.10	100.50	3	2.92	97.33	3	3.04	101.33
15	14.84	98.93	30	29.90	99.67	4	3.99	99.75	2	2.02	101.00
15	14.95	99.67	15	14.95	99.67	5	4.94	98.83	3	3.04	101.33
25	24.74	98.96	25	25.00	100.00	6	5.87	97.83	2	1.99	99.64
Mean		98.77			99.97			98.65			100.76
SD		0.64			0.34			1.05			0.71

[a] Recovery of aceclofenac contents in the tablets.
[b] Recovery of the added aceclofenac standard.

Table 4
Statistical comparison of results obtained by the suggested methods and the reference methods on analysis of etodolac and aceclofenac contents in etodine capsules and bristaflam tablets[b]

Data	Spectrophotometric method		Reference method[a]		Spectrofluorimetric method	
	Etodolac cap.	Bristaflam tab.	Etodine cap.	Bristaflam tab.	Etodine cap.	Bristaflam tab.
Mean %	101.46	98.77	102.16	98.65	102.52	98.65
SD	0.72	0.64	0.73	0.88	0.15	1.05
n	5	5	5	5	5	5
$t(P=0.05)$ [2.306]	1.532	0.247			0.17	0
$F(P=0.05)$ [6.4]	1.035	1.866			4.17	1.42

[a] Pharco and Squibb UV methods [9,10].
[b] Figures in parentheses represent the corresponding tabulated values for t and F at $P=0.05$.

range 96–640 ng ml^{-1} and 2–8 μg ml^{-1} for etodolac and aceclofenac, respectively.

The regression equations were calculated and found to be as follows:

1. $F=0.13C-0.32$, $r=1$ (etodolac)
2. $F=3.56C-0.11$, $r=0.9928$ (aceclofenac).

where F is the intensity of the fluorescence at 345 and 355 nm, respectively and C is the concentration in ng ml^{-1} and μg ml^{-1} for etodolac and aceclofenac, respectively and r is the correlation coefficient.

On comparing the results of the analysis of the developed and published methods, it was observed that the PDAB method has more or less the same sensitivity as that of the HPLC [11] and UV [10] methods for the determination of etodolac and aceclofenac, respectively. It was also noticed that the proposed fluorimetric methods are ~80- and 3-fold more sensitive than the UV reference methods [9,10] for etodolac and aceclofenac, respectively. These methods are valid with respect to linearity, sensitivity, accuracy, reproducibility and precision. Linearity between concentration and absorbance or fluorescence intensity lies in the concentration range 10–80 or 8–55 μg ml^{-1} and 96–640 ng ml^{-1} or 2–8 μg ml^{-1} of etodolac or aceclofenac in the spectrophotometric and fluorimetric methods, respectively. The proposed methods are highly sensitive and detect up to 8–10 or 0.10–2 μg ml^{-1} of the cited drugs in the two methods. The mean percentage recoveries and the standard deviation of pure samples of etodolac and aceclofenac are found to be 100.48 ± 0.85 or 100.03 ± 0.38 in the spectrophotometric method and 100.61 ± 0.79 or 99.88 ± 0.45 in the fluorimetric method for etodolac or aceclofenac, respectively, indicating accuracy and reproducibility. The results of the determination of the drug in pure form demonstrated good precision as shown in Table 1. Values for t and F also showed that there was no significant difference between the results obtained by the suggested procedures and the reference methods [9,10]. Etodine capsules and Bristaflam tablets were analysed for their contents of etodolac or aceclofenac by the proposed methods, using aliquots of test solutions equivalent to 200–400 or 150–250 μg of etodolac or aceclofenac for the PDAB method and 1–3 or 20–60 μg of the cited drugs for the fluorimetric method. The validity of the procedures was assessed by applying the standard addition technique. The results obtained are represented in Tables 2 and 3, i.e. the mean percentage recovery of added standard ranges from 99.97–100.52 and 100.71–100.76 in the spectrophotometric and fluorimetric methods, respectively. Further comparative determination of the cited drugs in its pharmaceutical preparation by the suggested procedures and the reference methods [9,10] was performed. The results obtained are illustrated in Table 4, where t and F values showed that there was no significant difference between the results obtained by the suggested and reference methods.

The main degradation products of etodolac are identified as 7-ethyl-2(1-methylene propyl)-1H-indole-3-ethanol, 1,8 diethyl-1-methyl-1,3,4,9-tetrahydropyrano-[3,4-b]indole and 7-ethyl tryp-

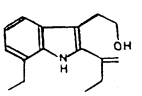
tophol [22] and those of aceclofenac are diclofenac and 1-(2,6-dichlorophenyl)oxindole [23]. Schemes 3 and 4 represent the structure formulae of the degradation products of etodolac and aceclofenac from which it is clear that they are mainly indole derivatives except diclofenac which is a secondary amine. Consequently, interference of the degradation products in the determination of the cited drugs by PDAB method is expected. The proposed fluorimetric method may be suitable for stability indicating assay as the difference between the structure formulae of the degradation products and the parent drugs with respect to the number and conjugation of double bonds may lead to differences in the excitation wavelength, thus excluding interference.

4. Conclusions

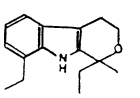
PDAB is a sensitive chromogenic reagent for the determination of the cited drugs and up to 8–10 μg ml^{-1} of each can be determined in pure form or in dosage form using this reagent. The fluorimetric method is more sensitive as up to 96 ng ml^{-1} of etodolac and 2 μg ml^{-1} of aceclofenac can be determined using this technique.

The suggested methods are simple, rapid, sensitive and suitable for routine analysis in control laboratories. Student's *t*-test and variance ratio *F*-tests showed no significant differences between the performance of the proposed and reference methods [9,10] with respect to accuracy and precision.

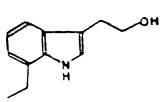
Degradation products of etodolac



7- ethyl – 2 (1-methylenepropyl) – 1H – indole – 3 – ethanol



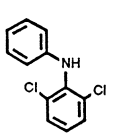
1,8 – diethyl –1- methyl – 1,3,4,9 – tetra–hydropyrano- [3,4-b] indole



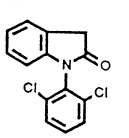
7 – ethyltryptophol

Scheme 3.

Degradation products of aceclofenac



Diclofenac



1 – (2,6- dichlorophenyl) oxindole

Scheme 4.

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