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New and Sensitive Spectrofluorimetric Method for the Determination of Non-Steroidal Anti inflammatory Drugs, Etodolac and Diclofenac Sodium in Pharmaceutical Preparations through Derivatization with 7-fluoro-4-nitrobenzo-2-oxa-1,3-diazole

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ABSTRACT

A rapid, accurate and sensitive method was developed and validated for the determination of non-steroidal anti-inflammatory drugs, etodolac and diclofenac sodium in pharmaceutical preparations. The proposed method was based on the reaction of these drugs with 7-fluoro-4-nitrobenzo-2-oxa-1,3-diazole (NBD-F) in buffer of pH 8.5 to yield a yellow, fluorescent product. The method was validated for specificity, linearity, limit of detection, limit of quantification, precision, accuracy, recovery and robustness. Beer's law is obeyed over the concentration range of 40 - 600 ng/mL for etodolac, 25 - 500 ng/mL for diclofenac sodium, respectively. The detection limits were found to be 0.071 ng/mL for etodolac, 0.055 ng/mL for diclofenac sodium, respectively. Statistical analysis of the results has been carried out revealing high accuracy and good precision. The proposed methods were successfully applied for the determination of the drugs in their pharmaceutical preparations with good recoveries. The developed methods were sensitive, accurate and simple. The results obtained by the proposed method were comparable with those obtained by the official method.

Key words: spectrofluorimetry, derivatization, validation, anti-inflammatory drug

INTRODUCTION

Etodolac (ED), 1,8-diethyl-1,3,4,9-tetrahydropyrano-[3,4-b]indole-1-acetic acid, and diclofenac sodium (DC), 2-(2,6-dichloranilino) phenylacetic acid (Figure 1) are nonsteroidal anti-inflammatory drugs (NSAIDs)[1].

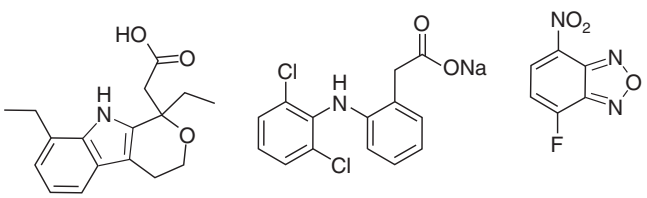

Figure 1. Chemical structures of ED, DC and NBD-F.

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NSAIDs are used in humans and domestic animals due to their anti-inflammatory, analgesic and anti-pyretic effects[2]. NSAIDs are non-selective inhibitors of prostaglandin biosynthesis in humans and indicated for the acute or long-term treatment of the signs and symptoms of rheumatoid arthritis and osteoarthritis[3].

Several methods have been described for the determination of ED in pharmaceutical preparations and plasma and urine, including high performance liquid chromatography (HPLC)[4-7], gas chromatography (GC)[8-10], spectrofluorimetry[11] and spectrophotometry[12,13].

Regarding DC, many reports have been described for its determination, in pharmaceutical preparations and plasma and urine. The reports include UV-Visible spectroscopy[14-19], chemometry[20] spectrofluorometry[21-23], liquid chromatography[24-32], titrimetry[33] and potentiometry[34-36].

In this study, a sensitive spectrofluorimetric method with high reproducibility has been developed for the

assay of ED and DC in pharmaceutical preparations by means of the derivative formed with NBD-F, which is a specific reagent in the analysis of primary and secondary aliphatic amines. In literature research, ED and DC, for the first time have been derivatized by a reagent and have been determined by spectrofluorimetry.

MATERIALS AND METHODS

I. *Materials*

Standard compounds of ED and DC were supplied from Sigma (St. Louis, MO, USA). Etol (400 mg) and Voltaren (25 mg) were obtained from local pharmacy. NBD-F was purchased from Fluka (Buchs, Switzerland). Other chemicals were purchased from Merck (Darmstadt, Germany). All solvents were of analytical grade. An aquaMAX^{TM} water system (Young Instrument, Korea) produced ultra pure analytical grade water.

II. *Apparatus*

Fluorescence measurements were performed with a Shimadzu spectrofluorimeter Model RF-1501 (Kyoto, Japan) equipped with a Xenon lamp.

III. *Preparation of Stock and Standard Solutions*

The stock solutions (ED and DC) were dissolved in methanol (1.0 mg/mL). The working standard solutions were prepared from stock solutions by dilution (50 μg/mL).

A borate buffer (0.1 M) was prepared by dissolving 0.620 g of boric acid and 0.750 g of potassium chloride in 100 mL of water. The pH was adjusted to 8.5 with 0.1 M sodium hydroxide solution and the volume was made up to 200 mL with water.

The NBD-F solution was freshly prepared at 0.2 mg/mL in methanol. Solutions were stored at 4°C.

IV. *General Procedure*

An appropriate volume of 50 μg/mL working solution (8.0 - 120 μL of ED; 5.0 - 100 μL of DC) was transferred into 12-mL stoppered tubes. To each flask 100 μL of borate buffer (pH 8.5) followed by 100 μL of NBD-F solution were added and mixed well. The solutions were heated in a thermo stated water bath at 70°C for 15 min (30 min for DC), then left to cool and acidified with 100 μL of 0.1 N HCI. The derivatives were extracted three times with 3.0 mL of chloroform. The combined organic phases were adjusted to 10 mL with the chloroform. The fluorescence intensity was measured spectrofluorimetrically at λ_{ex} 461 nm and λ_{em} 521 nm against blank prepared similarly. The DC-NBD derivative was determined at λ_{ex} 464 nm and λ_{em} 521 nm.

V. *Procedure for the Assay of the Tablets*

(I) *Etol (400 mg) Tablets*

Twenty tablets were weighed, finely powdered, and then a quantity of the powder equivalent to 100 mg of ED was transferred into a 100-mL volumetric flask, dissolved in 50 mL methanol, sonicated for 30 min, completed to volume with the methanol, shaken well for 5 min, and filtered into a 100-mL calibrated flask and then diluted to volume with methanol. Five milliliters of the filtrates were then adjusted to 100-mL with methanol in calibrated flask, and procedure followed as mentioned under general procedure.

(II) *Voltaren (25 mg) Tablets*

Twenty tablets were weighed and powdered, and then a quantity of the powder equivalent to 100 mg of DC was transferred to a 100-mL volumetric flask containing 50 mL of methanol. The flask was shaken for 30 min, diluted to 100 mL with the same solvent, and then filtered. Five milliliters volumes of the filtrates were then adjusted to 100 mL with methanol in calibrated flask, and procedure followed as mentioned under general procedure.

VI. *Method Validation*

The methods were validated according to International Conference on Harmonization guidelines for validation of analytical procedures[37].

(I) *Specificity*

The specificity of the method was investigated by observing any interference encountered from these tablet excipients, which did not interfere with the proposed methods.

(II) *Linearity*

The linearity was evaluated by linear regression analysis, which was calculated by the least square regression method. The calibration curves were constructed by plotting concentration *versus* intensities, using linear regression analysis. The calibration curves ($F = aC + b$) were constructed by the plots of the fluorescence intensitiy (F) of the drugs *versus* the concentrations (C) of the calibration standards.

(III) *Limit of Detection (LOD) and Quantification (LOQ)*

The limit of detection (LOD) and limit of quantification (LOQ) of drugs by the proposed methods were determined using calibration standards. LOD and LOQ were calculated as 3.3 σ/S and 10 σ/S, respectively, where S is

the slope of the calibration curve and σ is the standard deviation of intercept of regression equation.

(IV) *Precision and Accuracy*

The precision and accuracy of the assay were determined by repeatability (intra-day) and intermediate precision (inter-day). Repeatability was evaluated by assaying samples, at same concentration and during the same day. The intermediate precision was studied by comparing the assays on different days. Five sample solutions were prepared and assayed.

(V) *Recovery*

The % recovery of the added pure drug was calculated as, % recovery = $[(C_t - C_s)/C_a] \times 100$, where C_t is the total drug concentration measured after standard addition; C_s, drug concentration in the formulation sample; C_a, drug concentration added to the formulation.

(VI) *Robustness*

The robustness of the method was evaluated during the development by deliberate changes to the method parameters. The factors chosen for this study were the pH, temperature and volume of NBD-F.

RESULTS AND DISCUSSION

I. *Fluorescence Spectra*

In the present study, NBD-F forms highly fluorescent derivatives with secondary amines, therefore, it was chosen as a derivatizing reagent for ED and DC. Owing to the presence of labile floride in the chemical structure of NBD-F, a daily fresh solution was prepared and tested in the present study. It was found that ED and DC react with NBD-F and forms yellow-colored fluorescent derivatives.

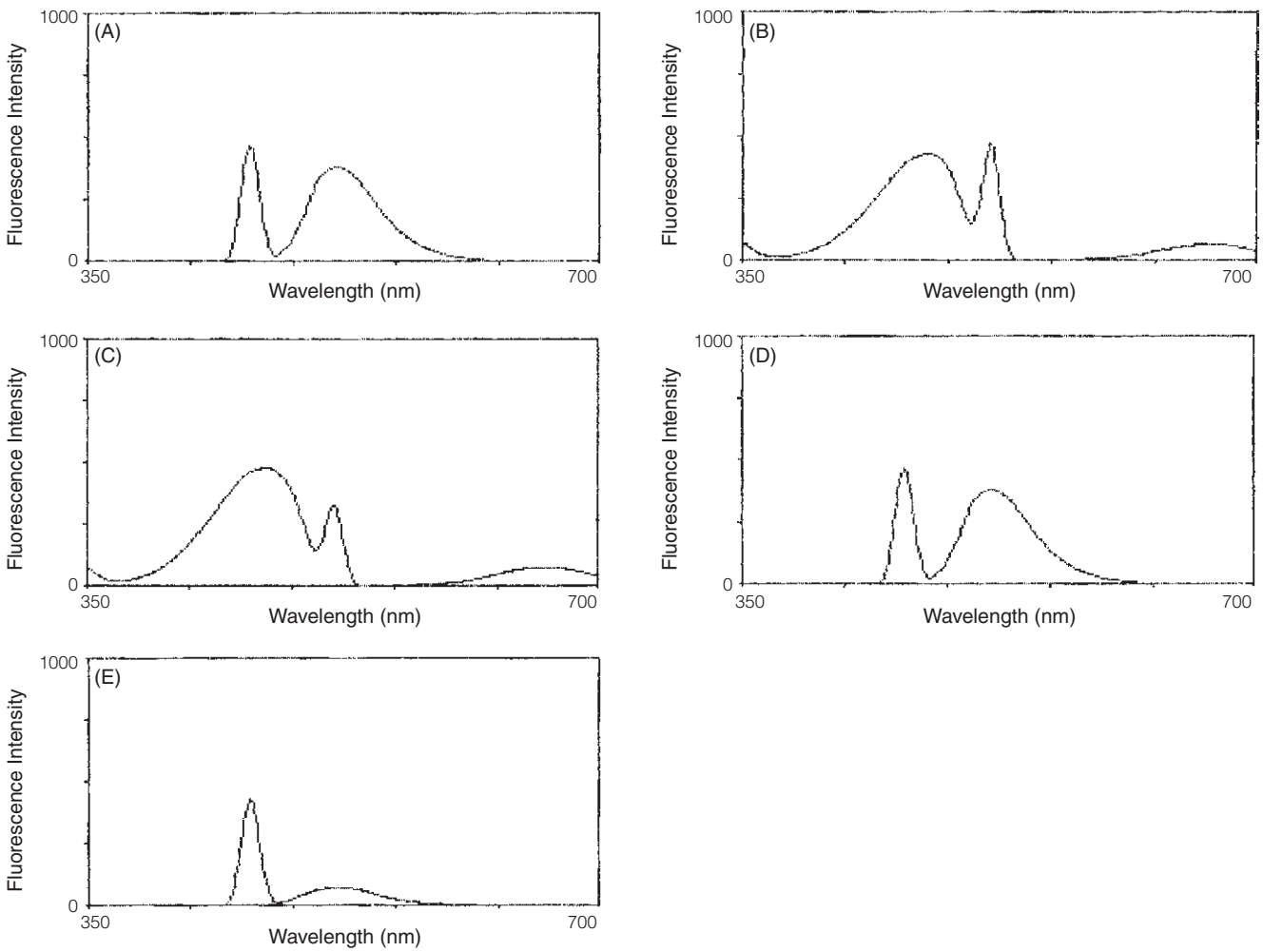

Figure 2. (A) Emission spectrum of the ED (400 ng/mL). (B) Exciation spectrum of the ED (400 ng/mL). (C) Exciation spectrum of the DC (400 ng/mL). (D) Emission spectrum of the DC (400 ng/mL). (E) Emission spectrum of the blank.

This derivative exhibited maximum fluorescence intensity (λ_{em}) at 521 nm after its excitation at wavelength (λ_{ex}) of 461 nm (464 nm for DC). The excitation and emission spectra for the reaction product of the drug with NBD-F are given in Figure 2A - E.

II. *Optimization of the Reaction Conditions*

The different experimental parameters affecting the development of the reaction product and its stability were carefully studied and optimized. Such factors were changed individually while others were kept constant. These factors include: pH, type of buffer, temperature, time and concentration of reagents.

(I) *Effect of pH*

The influence of pH on the fluorescence intensity of the reaction product was investigated using 0.1 M borate buffer over the pH range of 8.0 - 10.0 (Figure 3). Maximum fluorescence intensity was obtained at pH 8.5, after which the fluorescence intensity of the reaction product gradually decreased. Therefore, pH 8.5 was chosen as the optimum pH for such study. Other buffers having the same pH value such as phosphate buffer was tried and compared with 0.1 M borate buffer. The borate buffer was found to be superior to the phosphate buffer at the same pH value since it gave the highest fluorescence intensity value.

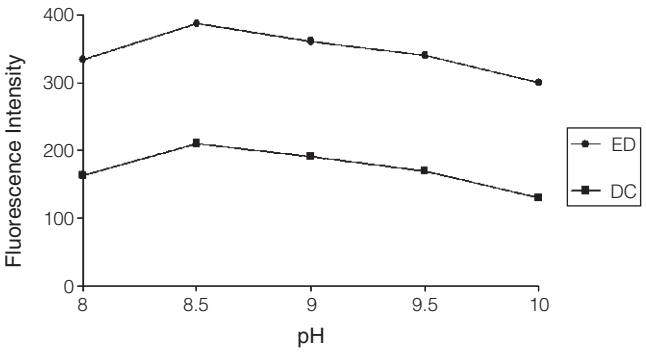

Figure 3. Effect of pH on fluorescence intensity.

(II) *Effect of Temperature and Time*

The effect of temperature on the color intensity was studied in the range from 50 to 80°C for different periods of time. The color intensity increased on increasing the applied temperature up to 70°C. The effect of the reaction time on the reaction course was studied by measuring the corresponding fluorescence intensity at constant temperature for different periods of time. It was found that the optimum reaction time is 15 min (30 min for DC).

(III) *Effect of Concentration of NBD-F*

Studying the effect of different NBD-F reagent concentrations on the produced fluorescence intensity revealed that highest fluorescence intensity was over the concentration of NBD-F reagent in the final solution between 0.01 and 0.04% (w/v). Therefore, the concentration of 0.02% (w/v) was selected for further experiments.

(IV) *Effect of Diluting Solvent*

Different solvents (methanol, acetonitrile, dichloromethane, chloroform and ethyl acetate) were tried to dilute the reaction mixture throughout the study. It was observed that chloroform gave the highest fluorescence intensity. The increase of fluorescence intensity due to the use of chloroform may be attributed to lowering the fluorescence of the blank reagent.

(V) *Effect of the Hydrochloric Acid*

The fluorescence of the hydrolysis product of NBD-F, namely, 4-hydroxy-7-nitrobenzo-2-oxa-1,3-diazole (NBD-OH), is quenched by decreasing the pH of the reaction medium to less than one. It is known that the maximum association between NBD-F and aimed compound is realized at the basic medium. But, NBD-F is also hydrolyzed in alkaline solution. Thus, the system stabilized by acidifying the reaction mixture to pH 2 (by adding 100 µL 1N HCl) before measurement[38] .

(VI) *Stability of the Fluorophore*

The NBD derivative was stable in chloroform for at stable at room temperature in the dark for 6 h, in daylight for 4 h and 8 days at 4°C in the dark.

III. *Method Validation*

(I) *Specificity*

The specificity of the method was investigated by observing any interference encountered from common excipients of the pharmaceutical formulation such as lactose**,** microcrystalline cellulose**,** povidone, magnesium stearate, starch-maize, iron oxide yellow and titanium dioxide. It was found that these compounds did not interfere with the results of the proposed methods.

(II) *Linearity*

By using the above procedure, a linear regression equation was obtained. The regression plots showed that there was a linear dependence of the fluorescence signal on the concentration of the drugs over the range cited in Table 1. The linear regression analysis of the data gave the following equation:

F = 0.5318C + 163.74 (r = 0.9996) for ED

F = 0.6461C + 177.72 (r = 0.9998) for DC
where F is the fluorescence signal, C is the concentration of the drug in ng/mL and r is the correlation coefficient. As can be seen from the data, the method is much more sensitive than most of the reported methods [11-23,32,34-36].

(III) *LOD and LOQ*

LOD were 0.071 ng/mL for ED, 0.055 ng/mL for DC. LOQ of ED and DC were 0.213 and 0.165 ng/mL, respectively. These results are given in Table 1, which is as good as that reported in other papers [11-23, 32,34-36].

(IV) *Precision and Accuracy*

Repeatability was determined by using different drug concentrations prepared from independent stock solution and analyzed (n = 5). Inter-day and intra-day variations were taken to determine precision of the proposed methods. Different levels of drug concentrations in triplicates were prepared thrice in a day and studied for intra-day variation. The same method was followed for three different days to study inter-day variation. The calculated relative standard deviation values were found to be very small below 2% indicating good repeatability and reliability of the proposed methods. The results and their statistical analysis were summarized in Table 2.

Table 1. Performance data of the proposed procedure (n = 5)

Parameter	ED-NBD	DC-NBD
λ_{ex}	461	464
λ_{em}	521	521
Linear range (ng/mL)	40 - 600	25 - 500
Correlation coefficient (r)	0.9996	0.9998
Slope	0.5318	0.6461
Intercept	163.74	177.72
Standard deviation of the intercept	1.15×10^{-2}	1.08×10^{-2}
LOD (ng/mL)	0.071	0.055
LOQ (ng/mL)	0.213	0.165

(V) *Recovery*

The recovery study was performed by adding known amounts of the compounds studied to a known concentration of the commercial pharmaceutical tablets (standard addition method, Table 3). These values are better than those obtained by other methods[16,25].

(VI) *Robustness*

The robustness of the method adopted is demonstrated by the constancy of the fluorescence intensity with the deliberated minor change in the experimental parameters such as the change in the volume of NBD-F (0.02%) ± 5.0 µL and the change in the heating temperature (70 ± 1°C) and pH 8.5 ± 0.1. These minor changes that may take place during the experimental operation did not affect the fluorescence intensity of the reaction product (Table 4).

(VII) *Stoichiometry of the Reaction*

The Job's method[39] of continuous variation was employed. Master equimolar (1.74×10^{-4} M for ED and

Table 2. Intra-day and inter-day precision and accuracy of ED and DC-NBD derivatives (n = 5)

Intra-day				Inter-day			
Added (ng/mL)	Mean ± SD Found (ng/mL)	RSD (%)	RME (%)	Added (ng/mL)	Mean ± SD Found (ng/mL)	RSD (%)	RME (%)
ED-NBD							
40	39.3 ± 0.6	1.5	-1.8	40	39.5 ± 0.5	1.2	-1.3
100	101.2 ± 0.5	0.5	1.2	100	101.7 ± 0.4	0.4	1.7
600	599.2 ± 0.6	0.1	- 0.1	600	601.3 ± 1.1	0.2	0.2
DC-NBD							
25	25.1 ± 0.1	0.4	0.4	25	24.8 ± 0.2	0.8	-0.7
250	251.2 ± 1.2	0.5	0.5	250	249.2 ± 0.8	0.3	-0.3
500	498.8 ± 1.0	0.2	-0.2	500	501.5 ± 0.9	0.2	-0.3

Table 3. Recovery studies of ED and DC (n = 5)

Concentration of drug in formulations (ng/mL)	Concentration of pure drug added (ng/mL)	Recovery ± RSD (%)
ED		
50	550	101.3 ± 0.2
50	250	101.1 ± 0.3
50	150	99.1 ± 0.5
DC		
50	450	99.5 ± 0.2
50	250	99.2 ± 0.3
50	150	98.7 ± 0.7

Table 4. Robustness of the proposed method

Conditions	Mean ± SD	
	ED-NBD (100 ng/mL)	DC-NBD (250 ng/mL)
Reagent volume		
95 µL	101.3 ± 0.6	250.2 ± 0.8
105 µL	101.6 ± 0.8	250.2 ± 1.2
pH		
8.4	101.2 ± 0.6	250.1 ± 0.6
8.6	101.2 ± 0.6	250.1 ± 0.7
Reaction temp.		
69°C	101.2 ± 0.6	250.1 ± 0.6
71°C	101.2 ± 0.6	250.1 ± 0.6

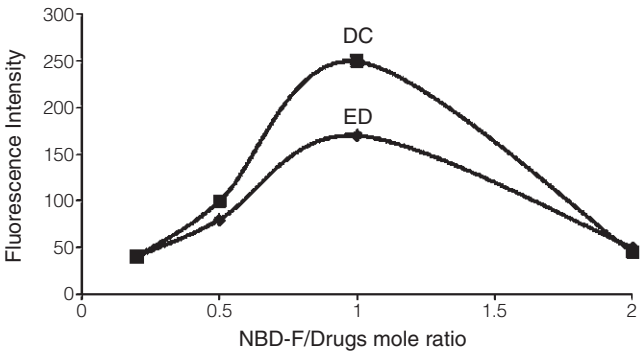

Figure 4. Mole ratio of DC and ED to NBD-F.

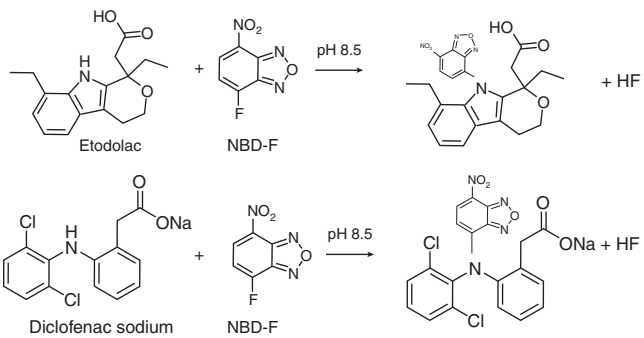

Figure 5. The reaction between drugs and NBD-F.

Table 5. Determination of ED and DC in its pharmaceutical formulations (tablets) by the proposed spectrofluormetric and the official methods

	% Mean ± SD			
	Proposed method	Official method [40,41]	*t*	*F*
Etol (400 mg)	101.2 ± 0.6	101.5 ± 1.0	0.9	2.7
Voltaren (25 mg)	99.4 ± 0.4	99.8 ± 0.6	1.9	2.2

*n = 6, p = 0.05, t = 2.23, F = 5.05

1.27 × 10^{-4} M for DC) solutions of drugs and NBD-F were prepared. Series of 10 mL portions of the master solutions of drugs and NBD-F were made up comprising different complementary proportions in 12-mL screw-capped test tubes containing 100 µL of buffer solution (pH 8.5). The solution was further manipulated as described under the general procedures. It is clear that only one amino group of ED and DC molecules are involved in the reaction with NBD-F and hence the molar ratio is 1:1 (Figures 4 and 5).

(IV) *Application*

The proposed method has been successfully applied to the determination of the studied drugs in commercial tablets. The results obtained are shown in Table 5. According to the *t*- and *F*-tests, no significant difference were found between the calculated and theoretical values of both the proposed and the official methods[40,41] at 95% confidence level. This indicates good level of precision and accuracy.

CONCLUSIONS

The proposed method is accurate, time saving, simple, sensitive and reproducible. The proposed method is sensitive enough to determine small amounts of these drugs, therefore can be used for quality control and routine determination of drugs in pharmaceutical tablets

where precision, time and cost effectiveness of analytical methods are important.

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REFERENCES

1. Merck & Co. Inc. 2001. The Merck Index. 13th ed. Etodolac and Diclofenac. pp.542-685. Whitehouse Station, NJ, U.S.A.
2. Radi, Z. A. and Khan, N. K. 2006. Effects of cyclooxygenase inhibition on the gastrointestinal tract. Exp. Toxicol. Pathol. 58: 163-173.
3. Radwan, M. A. and Aboul-Enein, H. Y. 2004. *In vitro* release and stereoselective disposition of flurbiprofen loaded to poly(D,L-lactide-co-glycolide) nanoparticles in rats. Chirality 16: 119-125.
4. Becker, S. U. and Blaschke G. 1993. Evaluation of the stereoselective metabolism of the chiral analgesic drug etodolac by high performance liquid chromatography. J. Chromatogr. 621: 199-207.
5. Wright, M. R. and Jamali, F. 1993. Limited extent of stereochemical conversion of chiral non-steroidal anti-inflammatory drugs induced by derivatization methods employing ethyl chloroformate. J. Chromatogr. 616: 59-65.
6. Jamali, F., Mehavan, R., Lemko, C. and Eradiri, O. 1998. Application of a stereospecific high-performance liquid chromatography assay to a pharmacokinetic study of etodolac enantiomers in humans. J. Pharm. Sci. 77: 963-966.
7. Ficarra, R., Ficarra, P., Calauro, M. L. and Costantino, D. 1991. Quantitative high performance liquid chromatographic determination of etodolac in pharmaceutical formulations. Farmaco. 46: 403-407.
8. Singh, N. N., Jamali, F., Pasutto, F. M., Coutts, R. T. and Russell, A. S. 1986. Stereoselective gas chromatographic analysis of etodolac enantiomers in human plasma and urine. J. Chromatogr. 382: 338-342.
9. Giachetti, C., Assandri, A., Zanolo, G. and Brembilla, E. 1994. Gas chromatography-mass spectrometry determination of etodolac in human plasma following single epicutaneous administration. Biomed. Chromatogr. 8: 180-183.
10. Srinivas, N. R., Shyu, R. W. C. and Barbhaiya, H. 1995. Gas chromatographic determination of enantiomers as diastereomers following pre-column derivatization and applications to pharmacokinetic studies. Biomed. Chromatogr. 9: 1-9.
11. El Kousy, N. M. 1990. Spectrophotometric and spectrofluorimetric determination of etodolac and aceclofenac. J. Pharm. Biomed. Anal. 20: 185-194.
12. Duymus, H., Arslan, M., Kucukislamoglu, M. and Zengin, M. 2006. Charge transfer complex studies between some non-steroidal anti-inflammatory drugs and π-electron acceptors. Spectrochimica. Acta. Part. A. 65: 1120-1124.
13. Amer, S. M., El-Saharty, Y. S., Metwally. F. H. and Younes, K. M. 2005. Spectrophotometric study of etodolac complexes with copper (II) and iron (III). J. AOAC. Int. 88: 1637-1643.
14. Fabre, H., Sun, S. W. Mandrou, B. and Maillols, H. 1993. Assay validation for an active ingredient in a pharmaceutical formulation: practical approach using ultraviolet spectrophotometry. Analyst 118: 1061-1064.
15. Botello, J. C. and Pérez-Caballero, G. 1995. Spectrophotometric determination of diclofenac sodium with methylene blue. Talanta 42: 105-108.
16. Garcia, M. S., Albero, M. I., Sánches-Pedre˜no C. and Molina, J. 1998. Flow-injection spectrophotometric determination of diclofenac sodium in pharmaceuticals and urine samples. J. Pharm. Biomed. Anal. 17: 267-273.
17. Micalizzi, Y. C., Pappano, N. B. and Debattista, N. B. 1998. First and second order derivative spectrophotometric determination of benzyl alcohol and diclofenac in pharmaceutical forms. Talanta 47: 525-530.
18. Córdova, M. L. F., Barrales, P. O. and Díaz, A. M. 1998. Sensitive and selective determination of diclofenac sodium in pharmaceutical preparations by solid phase ultraviolet absorptiometry. Anal. Chim. Acta. 369: 263-268.
19. Sena, M. M., Chaudhry, Z. F., Collins, C. H. and Poppi, R. J. 2004. Direct determination of diclofenac in pharmaceutical formulations containing B vitamins by using UV spectrophotometry and partial least squares regression. J. Pharm. Biomed. Anal. 36: 743-749.
20. Castellano, P. M., Vignaduzzo, S. E., Maggio, R. M. and Kaufman, T. S. 2005. Application of a chemometric method for simultaneous determination of acetaminophen and diclofenac in content-uniformity and drug-dissolution studies. Anal. Bioanal. Chem. 382: 1711-1714.
21. Carreira, L. A., Rizk, M., El-Shabrawy, Y., Zakhari, N. A. and Toubar, S. S. 1995. Europium(III) ion probe spectrofluorometric determination of diclofenac sodium. J. Pharm. Biomed. Anal. 13: 1331-1337.
22. Damiani, P. C., Bearzotti, M., Cabezón, M. A. and Olivieri, A. C. 1999. Spectrofluorometric determination of diclofenac in tablets and ointments. J. Pharm. Biomed. Anal. 20: 587-590.
23. Arancibia, J. A., Boldrini, M. A. and Escandar, G. M. 2000. Spectrofluorimetric determination of diclofenac in the presence of α-cyclodextrin. Talanta 52: 261-268.
24. Patil, S. T., Sundaresan, M., Bhoir, I. C. and Bhagwat, A.M. 1998. Packed column supercritical fluid chromatographic separation and estimation of acetaminophen, diclofenac sodium and methocarbamol in pharmaceutical dosage forms. Talanta 47: 3-10.

25. González, L., Yuln, G. and Volonté, M. G. 1999. Determination of cyanocobalamin, betamethasone, and diclofenac sodium in pharmaceutical formulations, by high performance liquid chromatography. J. Pharm. Biomed. Anal. 20: 487-492.
26. Klimes, J., Sochor, J., Dolezal, P., Körner, J. 2001. HPLC evaluation of diclofenac in transdermal therapeutic preparations. Int. J. Pharm. 217: 153-160.
27. Abdel-Hamid, M. E., Novotny, L. and Hazma, H. 2001. Determination of diclofenac sodium, flufenamic acid, indomethacin and ketoprofen by LC-APCI-MS. J. Pharm. Biomed. Anal. 24: 587-594.
28. Hájková, R., Solich, P., Pospisilová, M. and Sicha, J. 2002. Simultaneous determination of methylparaben, propylparaben, sodium diclofenac and its degradation product in a topical emulgel by reversed-phase liquid chromatography. Anal. Chim. Acta 467: 91-96.
29. Mayer, B. X., Namiranian, K., Dehghanyar, P., Stroh, R., Mascher, H. and M. Müler, 2003. Comparison of UV and tandem mass spectrometric detection for the high-performance liquid chromatographic determination of diclofenac in microdialysis samples. J. Pharm. Biomed. Anal. 33: 745-754.
30. Chmielewska, A., Konieczna, L., Plenis, A. and Lamparczyk, H. 2006. Sensitive quantification of chosen drugs by reversed-phase chromatography with electrochemical detection at a glass carbon electrode. J. Chromatogr. B 839: 102-111.
31. Nebot, C., Gibb, S. W. and Boyd, K. G. 2007. Quantification of human pharmaceuticals in water samples by high performance liquid chromatography-tandem mass spectrometry. Anal. Chim. Acta 598: 87-94.
32. Panusa, A., Multari, G., Incarnato, G. and Gagliardi, L. 2007. High-performance liquid chromatography analysis of anti-inflammatory pharmaceuticals with ultraviolet and electrospray-mass spectrometry detection in suspected counterfeit homeopathic medicinal products. J. Pharm. Biomed. Anal. 43: 1221-1227.
33. Cakirer, O., Kilic, E., Atakol, O. and Kenar, A. 1999. The non-aqueous titrimetric assay of the selected anti-inflammatory agents using tetra-n-butylammonium hydroxide as titrant. J. Pharm. Biomed. Anal. 20: 19-26.
34. Pimenta, A. M., Araújo, A. N. and Montenegro, S. M. 2002. Simultaneous potentiometric and fluormetric determination of diclofenac in a sequential injection analysis system. Anal. Chim. Acta 470: 185-194.
35. Shamsipur, M., Jalali, F., Ershad, S. 2005. Preparation of a diclofenac potentiometric sensor and its application to pharmaceutical analysis and to drug recovery from biological fluids. J. Pharm. Biomed. Anal. 37: 943-947.
36. Hassan, S. S. M., Mahmoud, W. H., Elmosallamy, M. A. F. and Almarzooqui, M. H. 2005. Iron(II)-phthalocyanine as a novel recognition sensor for selective potentiometric determination of diclofenac and warfarin drugs. J. Pharm.Biomed. Anal. 39: 315-321.
37. Validation of Analytical Procedures, Methodology ICH Harmonised Tripartite Guideline, Having Reached Step 4 of the ICH Process at the ICH Steering Committee meeting on, November 6, 1996.
38. Imai, K. and Watanabe, Y. 1981. Fluormetric determination of secondary amino acids by 7-fluoro-4-nitrobenzo-2-oxa-1,3-diazole. Anal. Chim. Acta 130: 377-383.
39. Huang, C. Y., 1982. Determination of Binding Stoichiometry by the Continuous Variation Method, The Job Plot. Methods in Enzymology 87: 509-525.
40. British Pharmacopoeia Commission. 2001. British Pharmacopoeia. p.2018. Stationery Office. London.
41. British Pharmacopoeia Commission. 2001. British Pharmacopoeia. p. 2070. Stationery Office. London.