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Determination of Etodolac in Commercial Formulations by HPLC-UV Method

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ABSTRACT

The aim of this study was to develop and verify a simple, rapid and sensitive high performance liquid chromatography method coupled with UV detector (HPLC-UV) method for the quantitative determination of etodolac in bulk and pharmaceutical dosage forms. Chromatographic separation was performed at ambient conditions on a reverse phase ACE C8 analytical column (250 mm x 4.6 mm ID, 5 umm) using the mobile phase containing acetonitrile-water (80:20, v/v) at a flow rate of 1.0 mL min^{-1}. A wavelength of 272 nm was used for etodolok and paracetamol (IS). A retention time of 4.21 min and 2.02 min were obtained for etodolac and IS, respectively. The method showed linearity in the range of 0.08-10 µg mL^{-1} for etodolac ($R \geq 0.9999$). The linear regression equations obtained by least square regression method were the ratio of peak area of etodolac and IS =1.559 concentration (etodolac µg/mL) - 0.139. The intra-day and inter-day RE% and RSD% values of the method were ≤10.0% and ≤2.65%, respectively. Limit of detection (LOD) and limit of quantification (LOQ) were found to be 0.04 and 0.06 µg mL^{-1} for etodolac, respectively. A new, simple and sensitive high performance liquid chromatography method was developed and validated for etodolac. The method can be applied for the quantification of etodolac without derivatization in bulk solutions and commercial formulations using the internal standard.

KEYWORDS: *HPLC, etodolac, validation, formulation*

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INTRODUCTION

Class of pharmaceuticals nominated as Non-steroidal anti-inflammatory drugs (NSAIDs) have several chemical structures and altered therapeutic potentials They have three well-known features which are exhibiting characteristical pharmacological effect, analogous adverse effects and metabolic behavior[1]. Etodolac whose chemical formula is 1,8-Diethyl-1,3,4,9-tetrahydropyrano (3,4-b)-indole-1-acetic acid (Figure 1), is a member of NSAIDs. It is mostly preffered in symptomatic therapy of patients who suufer from strong pain and severe inflammation due to surgery, rheumatoid arthritis and osteoarthritis[2]. Metabolisation of drug were achieved in liver[3].

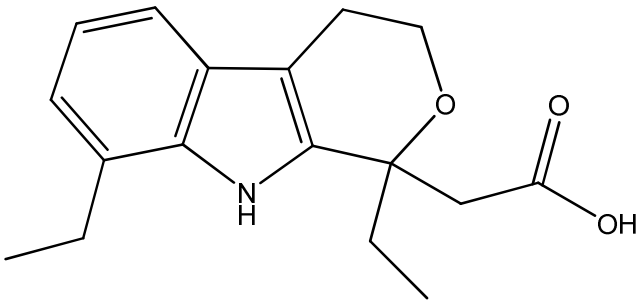

Figure1: Chemical structure of Etodolac

Several analytical methods as sequential injection analysis [2], voltammetric [4], capillary electrophoretic [5-7], spectrophotometric and spectrofluorimetric [8-14] and high-performance thin-layer chromatography [15, 16] have been reported for quantification of etodolac in bulk or pharmaceuticals in the literature. The official methods described are a nonaqueous acid base titration procedure and LC method for determination of etodolac in pure and dosage forms [10, 17-19]. Besides, several HPLC methods for analysis of etodolac in pharmaceutical preparations, and etodolac enantiomers, and the mechanisms of etodolac degradation have been reported [8, 20-25].

The aim of this work was to perform etodolac analysis in pure samples and commercial formulations by easy sample preparation and a precision HPLC method using the internal standard. So the method was developed by considering all optimization parameters as different from literature. The validation was carried out using the parameters of linearity, accuracy, analytical recovery, precision, specificity, precision and stability in accordance with the International Conference on Harmonization guidelines (ICH) [26].

MATERIALS AND METHOD

Chemicals:

Etodolac and Paracetamol were purchased from Sigma (St. Louis, Mo, USA). Acetonitrile, which is HPLC grade, was purchased from Merck (USA). For the mobile phase, high quality pure water prepared by the purification system (Millipore, Molsheim, France) was used. Etol Fort Tablet

(Nobel Ilac A.Ş.) and Tadolak Tablet (Saba Ilac A.Ş.), which were obtained from local pharmacies (Erzurum, Turkey), contained 400 mg etodolac.

Equipment and Chromatographic Conditions:
Chromatographic analysis was implemented on An Agilent 1200 series HPLC system, consisting of a quaternary pump, UV detector, a degasser and autosampler. Chromatographic separation was performed at ambient conditions on a reverse phase ACE C8 analytical column (250 mm x 4.6 mm ID, 5 umm). The best separation was obtained using the mobile phase containing acetonitrile-water (80:20, v/v) at a flow rate of 1.0 mL min^{-1}. The enjection volume was 10 µL. The mobile phase was used after filtration through a 0.22 µm membrane filter (Millipore).

For etodolok and IS, a wavelength of 272 nm was used and the etodolac and IS peak areas of each concentration were determined by the Agilent ChemStation software program. Etodolac and IS peak area ratios of the solutions at each concentration were calculated.

Preparation Of Calibration Standards And Quality Control Solutions:
5 mg of ethodolac was dissolved with 50 ml of acetonitrile. Solution with a final concentration of 100 µg ml^{-1} was used as the standard stock solution of etodolac. From this stock solution, working standard (WS) solutions (0.08, 0.5, 1.0, 2.5, 5.0 and 10.0 µg mL^{-1}) and quality control solutions (0.1, 4.5 and 9.5 µg mL^{-1}) were prepared by appropriate dilution in a 10 mL volumetric flask. QC samples were analyzed for assessing the precision and accuracy parameters. The paracetamol solution (100 µg mL^{-1}) used as the internal standard (IS) was diluted with acetonitrile to 1.0 µg mL^{-1}.

Tablets:
Separately 20 Etol fort tablets and Tadolak tablets were carefully weighed and pulverized in mortar. Correct weights equivalent to 400 mg of ethodolac were dissolved in 100 mL of acetonitrile. The solution was allowed to stand for about 10-15 minutes, then filtered through 12 mm filter paper. The filtrate was diluted with acetonitrile to obtain an etodolac concentration of 0.5, 4.0 and 8.0 µg mL^{-1} for both tablet samples.

RESULTS
System Suitability:
Sample of etodolac at a concentration of 5.0 µg mL^{-1} was analyzed by six replicates for assessing the system suitability. The acceptance criterion for system suitability was the percentage of standard deviation (% RSD) ± 2% for retention time and peak area of etodolac (Table 1). Column yield expressed by the number of theoretical plates (N) was 6712 ± 2% and the tail factor (T) was 1.11 ± 0.04 (mean ± RSD) for six replicates of injections.

	Retention time (min) [a]	Peak area[a]
	4.21	27.58
SD	0.12	0.55
RSD %	1.64	1.99

[a]Based on six analysis

Table 1 System suitability study of method (5.0 µg mL-1)

Linearity / Range:
The method developed for etodolac was found to have a linearity in the concentration range of 0.08 to 10 µg mL^{-1} in six replicates at six concentrations (0.08, 0.5, 1.0, 2.5, 5.0 and 10 µg mL^{-1}). Linearity is shown by linear regression analysis. At least square regression method was preferred for the necessary calculations. The linear regression equation [with standard error of intercept (S_a: 0.022) and slope (S_b: 0.0064)] and the correlation coefficient, R, was found to be y=1.559 x-0.139 (y: the ratio of peak areas of etodolac and IS; x: the concentration of etodolac) and 0.9999, respectively (Figure 2).

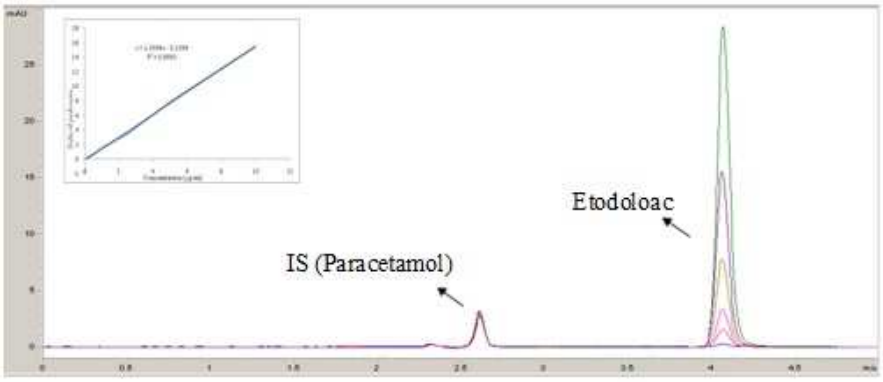

Figure 2: HPLC chromatograms of standard solutions of Etodolac (0.08, 0.5, 1.0, 2.5, 5.0 and 10.0 µg mL^{-1}) and IS (1.0 µg mL^{-1})

Limit of Detection and Limit of Quantification:
Under optimized conditions, the limit of detection (LOD) and limit of quantification (LOQ) parameters were used to determine the sensitivity of the method. In the method, LOD defined as signal / noise = 3 was determined as 0.04 µg mL^{-1} and LOQ defined as signal / noise = 10 was determined as 0.06 µg mL^{-1}. These values were considered appropriate for both accuracy and precision parameters (% RSD <20%).

Precision and Accuracy:
The precision and accuracy were determined by intra-day and inter-day analysis of QC samples at 0.1, 4.5 and 9.5 µg mL^{-1} concentrations. Intra-day accuracy and precision studies were evaluated by six replicates analysis of QCs on the same day. In the inter-day studies, the same QC samples were analyzed on different days (10 days) and compared. The precision was

calculated by RSD%, and the accuracy was determined by percent relative error (% RE). % RE expressed as a percentage of the average deviation from the known concentration. The RSD values for the precision were ≤2.65% (n=6). For the accuracy of the method, % RE values were found to be ≤10% (Table 2).

Added (µg mL^{-1})	Intra-day			Inter-day		
	Found±SD (µg mL^{-1})	RSD %	RE %	Found±SD (µg mL^{-1})	RSD%	RE%
0.1	0.093±0.001	1.08	-7.00	0.11±0.001	0.91	10.00
4.5	4.53±0.12	2.65	0.67	4.49±0.11	2.45	-0.22
9.5	9.52±0.11	1.16	0.21	9.49±0.09	0.95	-0.11

SD: Standard deviation, RSD: Relative Standard Deviation, RE: Relative error, average of six replicate determinations

Table 2: Precision and accuracy of the proposed method (n=6)

Analytical Recovery:

The standard addition method was used to calculate analytical recovery. A standard sample solution at a concentration of 1.0 µg mL^{-1} was added to tablet solutions of three different concentrations (0.5, 4.0 and 8.0 µg mL^{-1}) and tested by HPLC-UV method. The analytical recovery was calculated using the following equation:

$$\text{Analytical Recovery \%} = [(Ct-Cu) / Ca] \times 100$$

Cu indicates the concentration of the analyte in the tablet solutions and Ca indicates the standard analyte concentration put into the formulation. Ct is the total concentration of analyte in the prepared samples. The mean recovery percentage obtained was 98.0% and 101.0% for both tablets. The results showed that the method had good analytical recovery. Any interference was detected with the excipients in the preparation. RSD% values of recovery was between 1.32% to 3.23% (Table 3).

Commercial Formulations	Added (µg mL^{-1})	Taken (µg mL^{-1})	Found±SD (µg mL^{-1})	Recovery % (RSD%)
Etol Fort Tablet	1.0	0.5	1.51±0.02	101.0 (1.32)
		4.0	4.99±0.08	99.0 (1.60)
		8.0	8.99±0.29	99.0 (3.23)
Tadolak Tablet	1.0	0.5	1.48±0.04	98.0 (2.70)
		4.5	5.49±0.08	99.0 (1.46)
		8.5	9.48±0.24	98.0 (2.53)

SD: Standard deviation of six replicate determinations, RSD: Relative standard deviation

Table 3: Analytical recovery values of Etodolac in tablet (n=6)

Application Of The Method For Analysis Of Commercial Formulations:

The developed method was applied to two commercial preparations containing 400 mg of etodolac. According to the regression equation there is no statistically important difference between each calibration curves of the standard solution and commercial formulation. Six replicate analyzes confirmed that the tablets contained 400 mg of etodolac. HPLC chromatograms from commercial formulations are shown in Figure 3.

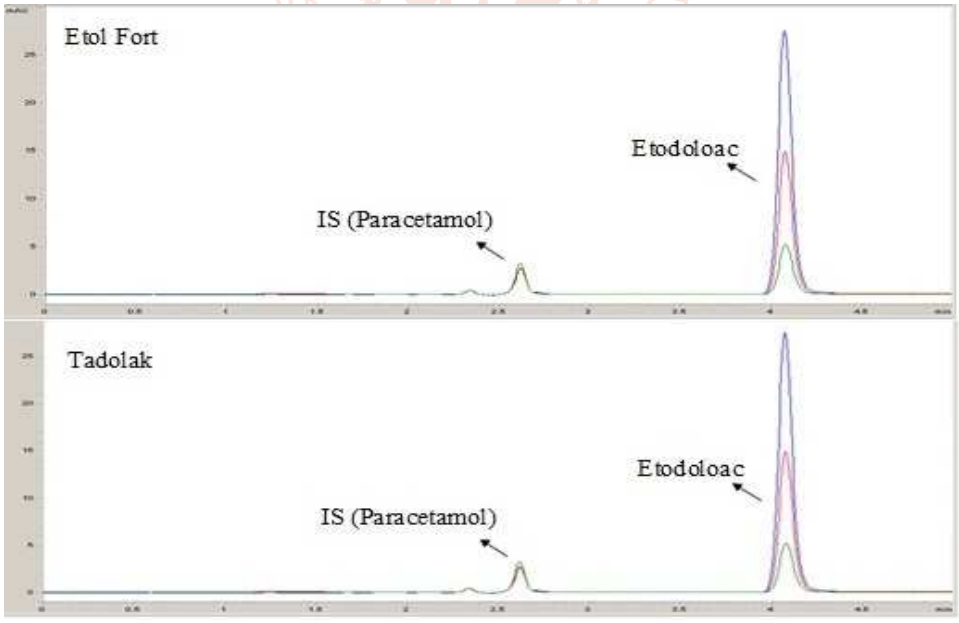

Figure 3: HPLC Chromatograms of solutions of commercial formulations

The results exhibited that the parameters of accuracy, precision and recovery from the pharmaceutical preparations were very good (Table 4). It has also been shown that there is no interference from the excipients in the pharmaceutical preparation to the results of the analysis. According to the analyzes repeated six times, the final recovery for each commercial preparation was about 99.95% and the RSD% was 4.25%. (n=6)

Commercial Formulations	Label claim	Mean±SD	Recovery (%)	RSD (%)
Etol Fort Tablet	400 mg	399.9±16.2	99.9	4.05
Tadolak Tablet	400 mg	400.1±17.8	100.0	4.45

Table 4: Determination of Etodolac in commercial formulations (n=6)

SD: Standard deviation of six replicate determinations, RSD: Relative standard deviation

DISCUSSION

Etodolac is a member of NSAIDs. In the proposed work, HPLC combined with UV detection was optimized and validated for quantitative analysis of etodolac without derivatization in pharmaceuticals using internal standard. HPLC method is mostly applied in pharmacopoeia analysis. For instance, the chromatographic seperation was preferred for the purity analysis and the quantitative determination of pharmaceuticals. On the other hand, this method can often be used to perform quality control analyzes.

Separation of enantiomers of etodolac using two different derivitization agents and three chiral stationary phases has been studied by HPLC method which was converted to its anilide derivative with either 1,3-dicyclohexyl-carbodiimide or 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride [25]. A isocratic chromatographic method for the separation, identification, and measurement of etodolac enantiomers using chiral stationary phase columns has been reported [24]. In another study, a HPLC method has been developed to measure etodolac in presence of three main degradants, 7-ethyl-2-(1-methylene-propyl)-1-H-indole-3-ethanol, the decarboxylated product of etodolac, and 7-ethyltryptophol [23]. A reversed-phase liquid chromatographic (RP-HPLC) method has also been proposed for the simultaneous determination of two substances in an oral formulation containing tolperisone hydrochloride (TOLP) and etodolac (ETD) [22]. However, Ficarra et al. also proposed the HPLC method for etodolac measurement in tablet formulation [20]. Chromatographic separation was actualized at 30 ^{o}C using a mobile phase of pH 6.0 phosphate buffer / methanol (60/40 v/v) and a C18 column (3.3 cm x 0.46 cm i.d.) at a flow rate of 1 mL min^{-1}. Etodolac detection was performed at 230 nm using a UV detector. Diazepam was used as an internal standard. They have been extracted with methanol the etodolac from tablets. A retention time of 1.46 min was obtained for etodolac under these conditions, and the method was found to be linear, precise and accurate over the concentration range of 0.01 to 0.1 mg mL^{-1}. In this proposed study, C8 column at ambient temperature and also asetonitril/water (80/20, v/v) as mobile phase and paracetamol as internal standard was used. Etodolac was detected at 272 nm wavelength on the basis of UV detection. A retention time of etodolac and IS were 4.21 min and 2.02 min under these conditions, respectively. Current study was accepted in terms of linearity, precision and accuracy parameters regarding to ICH criteria. It is also revealed that analytical recovery of the proposed method is in acceptable range (%98-101).

CONCLUSIONS

A rapid, specific, simple and accurate method has been successfully developed and validated in accordance with the ICH guidance. Proposed method was also applied onto commercial pharmaceuticals as Tadolak and Etol Fort. Thus, it is claimed that validated current procedure could be reliably used in routine analysis of both bulk samples and pharmaceutical preparations in quality control laboratories.

CONFLICTS OF INTERESTS

The authors declare no conflict of interest there are no conflicts, financial or otherwise.

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