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Method development and validation of HPLC for simultaneous determination of Etodolac

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ABSTRACT

The resolution of etodolac by reversed phase HPLC applied by using methanol as the injection solvent and the system of gradient elution, which leads to peak broadening as well as poor separation of etodolac from the impurities such as 2-(7-ethylindol-3-yl) ethanol respectively.

Keywords: Etodolac, Liquid dispersion, Phase solubility study, TPGS, Dissolution studies.

INTRODUCTION

Etodolac is a non-steroidal anti-inflammatory agent (NSAIDs). It is a white crystalline compound insoluble in water but soluble in alcohols and chloroform. Chemically etodolac is 2-{1,8-diethyl-1H,3H,4H,9H-pyrano[3,4- b]indol-1-yl}acetic acid. It is used as anti-inflammatory agent, cyclooxygenase inhibitor, analgesic and antipyretic. Etodolac is official in the British Pharmacopoeia and United States Pharmacopoeia[1-4]. The etodolac is used for treatment of osteoarthritis and rheumatoid arthritis.

Several analytical methods for the determination of etodolac have been proposed in the last years, high performance liquid chromatographic (HPLC) determination with ultraviolet detection being the most common applied method, though often laborious and time consuming. A differential pulse and square wave voltametric method, and high performance thin-layer chromatographic determination were also suggested. Other techniques have been used for the separation of etodolac and its metabolites, but not for its quantification, such as capillary electro chromatography or electrophoresis coupled with electro spray ionization mass spectrometry, or gas chromatography–mass spectrometry after extractive methylation. spectrophotometry or spectrofluorimetry, though widely used in pharmaceutical analysis, were only used by a few authors for the determination of etodolac using basic methylene blue dye, *p*-dimethylaminocinnamaldehyde or cupper acetate, *p*-dimethilaminobenzaldehyde, or nitrous acid.

MATERIALS AND METHODS

Reagents and solutions

All chemicals were of analytical grade and were used without further purification. All solutions were prepared using high purity water (Milli-Q), with specific conductivity less than 0.1 μ cm^{-1}. Etodolac was kindly supplied by Sofex Pharmaceutical, (Queluz, Portugal). The stock solution of etodolac (5×10−4 mol L−1) was prepared by dissolving the powder in ethanol and then the appropriate volume of water was added to obtain a solution with 5% (v/v) of ethanol. This solution was stored at 20 ◦C for a maximum of 1 week, and working solutions(1–4.5×10−5 mol L−1) were prepared daily in a 5% (v/v)ethanol solution

Method development

Stock and working sample solutions

Stock solution was prepared by accurately weighing 10 mg pure ETD and quantitatively transferred into a 10-ml volumetric flask and complete the volume with mobile phase. For the preparation of the working solutions serial dilution was done to get three different concentrations (0.1, 0.5, 1 mg/ml) for gradient elution method. Also, another serial dilution was done to get three different concentrations (0.003, 0.01, 0.1 mg/ml) for isocratic elution method. Stock and working solutions of CON were prepared with the same procedure of ETD solutions preparation**.** For preparation of the drug-impurity mixture, the CON impurity solution was prepared as 1 % v/v in ETD sample solution. That done by transferring100 µl impurity working solution by micropipette to 10-ml volumetric flask and complete the volume with ETD working solution. (Working solution for ETD and CON should be at the same concentration)

Potassium dihydrogen phosphate buffer

Accurately weighed 13.6 g potassium dihydrogen phosphates were quantitatively transferred to one-liter beaker by small amount of distilled water. The obtained solution was completed to 900 ml bi-distilled water to get clear solution. The pH was adjusted to 7 by addition of drops of potassium hydroxide solution (300g/L) (5.6 M).

Mobile phase

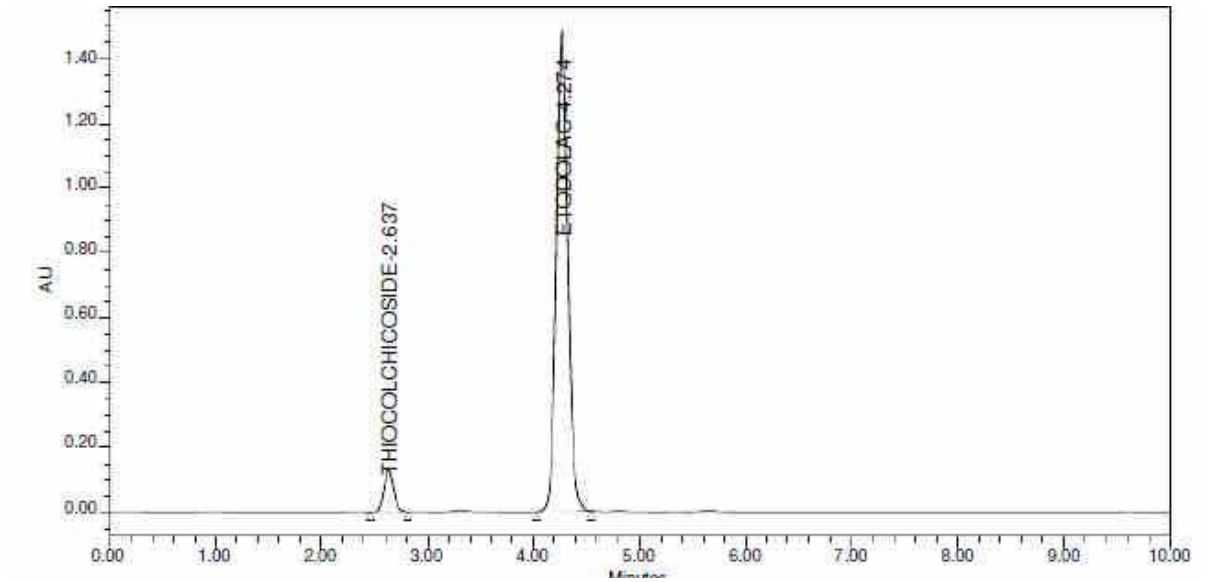
Accurately measured 688 and 312 ml of potassium dihydrogen phosphate buffer and methanol respectively were transferred to one-liter volumetric flask. distilled water was added to complete to the volume (A) was acetonitrile (B) HPLC-grade. The mobile phase was filtered through 0.45 µm Millipore membrane filters and degassed by sonication in an ultrasonic bath before use.

Chromatographic conditions of gradient elution system

Solvent A and B mixture was used as a mobile phase which pumped with flow rate (1.5 ml/min) in a time programming starting with 96:4 till 92:8 for A: B ratios along 20 min run time. The stationary phase was Lichro CART RP-18 column. Twenty micro liters was the sample volume and the detection wavelength was 225 nm. The column temperature was 40 ºC.

Chromatographic conditions of isocratic elution system

The isocratic mobile phase consists of solvent A and B, 70:30 v/v, which pumped at F= 1.2 ml/min. The stationary phases were Lichro CART RP-18, monolithic column, fused core, and luna columns. The Injection volume was 20 µl and 225nm was used as detection wavelength. All separations were performed at ambient temperature.



Typical chromatogram of etodolac

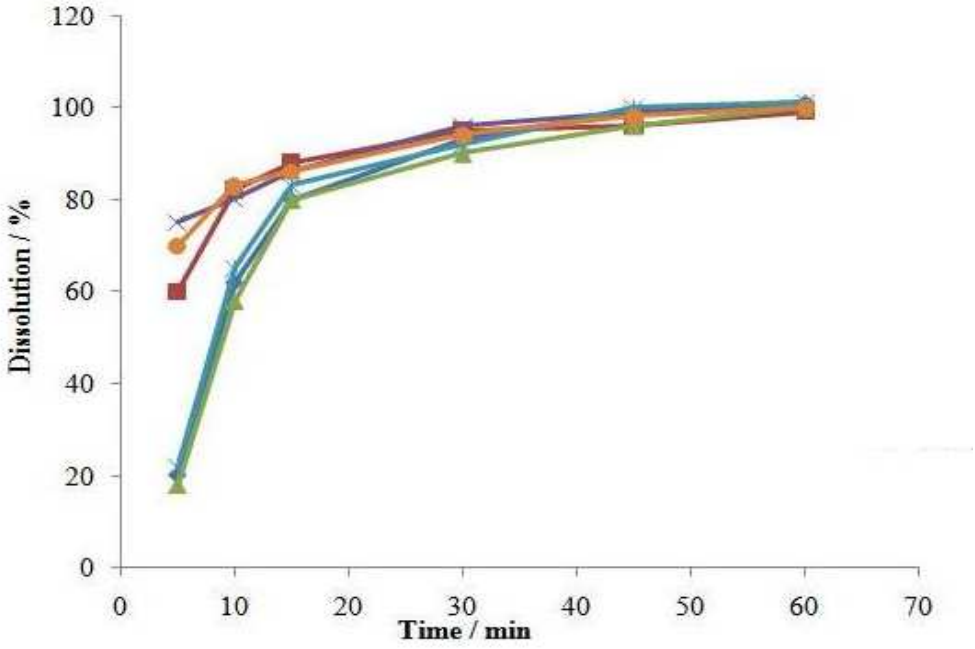
TLC analysis

Sample preparation

Ten milligrams pure ETD powder were accurately weighted and transferred quantitatively to 10-ml volumetric flask. The drug powder was dissolved in acetone and complete to volume with acetone giving 1 mg/ml solution. The sample of ETD- impurity CON was prepared with the same procedure and at the same concentration of ETD sample. The drug-impurity mixture, was prepared by transferring 500 µl impurity sample (1mg/ml) by micropipette to 10-ml volumetric flask and complete the volume with ETD sample (1mg/ml) to get 0.05mg of CON/ ml of ETD sample.

Chromatographic conditions

TLC and HPTLC plates were pre-washed with methanol, and then activated at 120 °C for 1 hr. The plates were left in desiccators for 15 min to lower its temperature to room temperature. Three and five micro liter sample volumes were spotted on the HPTLC and TLC plates respectively by the use of glass micro tubes. The mobile phase consists of toluene: acetone, 1: 1 (v/v).The plates were dried by using hot air (hairdryer). After drying stage the plates were placed in a horizontal chamber which contained 2 ml of the same used mobile phase for 3 and 1.5 min by using TLC and HPTLC plates respectively.

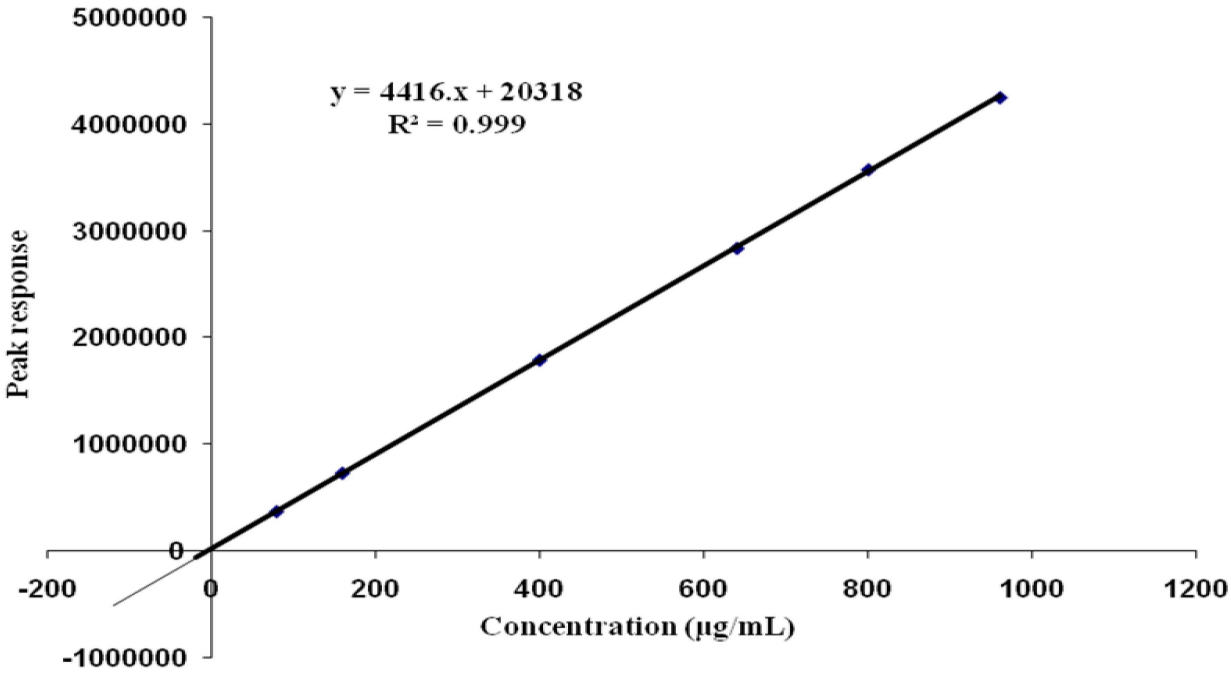


Dissolution profile data Etodolac

RESULTS AND DISCUSSION

HPLC analysis

The chemical stability of etodolac during the fusion process was determined by HPLC assay. The assay was tested for accuracy, linearity and sensitivity. The correlation coefficients of the calibration curves (greater than r = 0.9967) confirm good linearity in the range of 0.5 µg/ml–20 µg /ml). The area of the etodolac in the HPLC chromatograms of samples taken from all the final formulations accounted for greater than 99.4 of the total peak area. This proved to be in good agreement with the theoretical values. The absence of other peaks indicates that etodolac didn't undergo chemical decomposition during the fusion process or appear to have interacted with the carriers.



Calibration curve of Etodolac

Table:-1 Summary of analytical method for Etodolac determination

Detector	Internal standard	Column	Mobile phase	Extraction method	LOQ/ LOD
HPLC-UV 250 nm	C18 Nucleosil 250 x 4 mm, i.d. 5 µm	Methanol-0.2 M ammonium acetate (63:37, v/v)	LLE	5 µg/ml (LOD)	HP :102%
HPLC-UV 195nm	C18 Bio-sil ODS 250 mm i.d. 10 µm	Acetonitrile-Potassium dihydrogen phosphate pH3.8-4 (40:60, v/v)	LLE	1 ng/ml (LOD)	HP :78%
HPLC-UV 254 nm	C18 Absorbosphere 100 x 4.6 mm, i.d. 3 µm	Acetonitrile-0.4 ml concentrated sulfuric acid, 1.1 g tetramethyl ammonium chloride, 0.5 ml triethylamine per liter (45:55, v/v)	LLE	2 ng/ml (LOD)	HP :80%
	Bondapak CN 300 x 3.9 mm, i.d. 10 µm		LLE	0.5 ng/ml (LOD)	HP:92% RH:93%
HPLC-EC	C8 bonded RP 250 x 4.6 mm, i.d. 5 µm	Acetonitrile-potassium phosphate buffer pH 6.8 (45:55, v/v)	LLE	1µg/l (LOD)	HP:89%
HPLC-UV 254 nm	C18 250 x 4.6 mm, i.d. 10 µm	Acetonitrile-methanol-16.5mm disodium hidrogen phosphate pH5 (45:25:30, v/v/v) Acetonitrile-100 mm	LLE	2 ng/ml (LOD)	HP:86% RH:92%

The solubility of pure etodolac in water is poor, but the literature gives no exact data. In this study the solubility of etodolac in water was found to be about 75 µg/ mL. The solubility phase diagram representing the effect of increasing the concentrations of Gelucire 44/14 and TPGS on the apparent solubility of etodolac in water at 37 °C and 45 °C. Comparing the two polymers, aqueous solutions of TPGS increased the solubility of etodolac more than

that of Gelucire 44/14. Solubility experiment showed that the concentration of Etodolac in water at 37 °C, 45 °C increased as a function of Gelucire or TPGS concentration. The increase in solubility was linear with respect to the weight fraction of the carrier. The shape of all solubility diagrams followed an AL-type system where a linear increase of etodolac solubility was observed as function of Gelucire, TPGS concentrations, over the entire concentration range studied. At 15% concentration of Gelucire 44/14 and TPGS, the increase in etodolac solubility was approximately at 37 °C, respectively, and Gelucire at 45 °C. The stability constant values vary with the carrier (129 and 161 g−1 for Gelucire at 37 °C and 45 °C, respectively, and 171 g^{-1} for TPGS at 37 °C). The increase in solubility of etodolac by Glacier and TPGS may probably be explained by increased wet ability of etodolac and micellar solubilization. Indeed, Gelucire and TPGS being surfactants cause a decrease of the interfacial tension between the drug and the solving solution. The same effect of Gelucire was observed for the solubility of temperature at 37 °C.

CONCLUSION

Analytical method had been developed and validated for etodolac, a fixed dose combination tablet which determines the dissolution profile of components simultaneously using phosphate buffer pH 6.8 and USP Apparatus 1 (basket) at 100 rpm. This circumvents the problem previously described with the USP single entity dissolution media, and provides the optimum dissolution conditions for this product. Also, an HPLC method was developed to measure concomitantly, etodolac in the dissolution samples, thus eliminating the need for separate HPLC methods for the etodolac components.

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