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Improvement of the etodolac purity test by reversed phase high-performance liquid chromatography

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Received August 11, 2008, accepted September 4, 2008

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Pharmazie 63: 913–914 (2008)
doi: 10.1691/ph.2008.8233

The resolution of etodolac by reversed phase HPLC applied in the European Pharmacopeia (EP) was found to be greatly affected 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. Changing the type of injection solvent (methanol) by the mobile phase, which is a mixture of methanol, buffer (KH_2PO_4, PH = 7), and acetonitril as well as monitoring the gradient program (increasing the polarity) leads to enhance the selectivity and efficiency of the analysis of etodolac by eliminating the peak broadening and markedly improving the separation of etodolac from 2-(7-ethylindol-3-yl) ethanol. The method was validated by parameters such as selectivity, repeatability, and intermediate precision.

Etodolac, (±)-1,8-diethyl-1, 3, 4, 9-tetrahydropyrano-(3,4-b)indole-1-acetic acid (Jamali et al. 1988) possesses anti-inflammatory, analgesic and antipyretic activities (Turner 1987; Martel and Klicius1982) and is used in rheumatology (Casey et al. 1997, Brocks and Jamali 1994). Reversed phase high performance liquid chromatography (RP-HPLC) is applied to test the purity of drugs including etodolac. Broadening of the etodolac peak, as well as the poor efficiency in separating etodolac from its contaminants such as 2-(7-ethylindol-3-yl) ethanol are the main disadvantages of the procedure used in the European Phar-

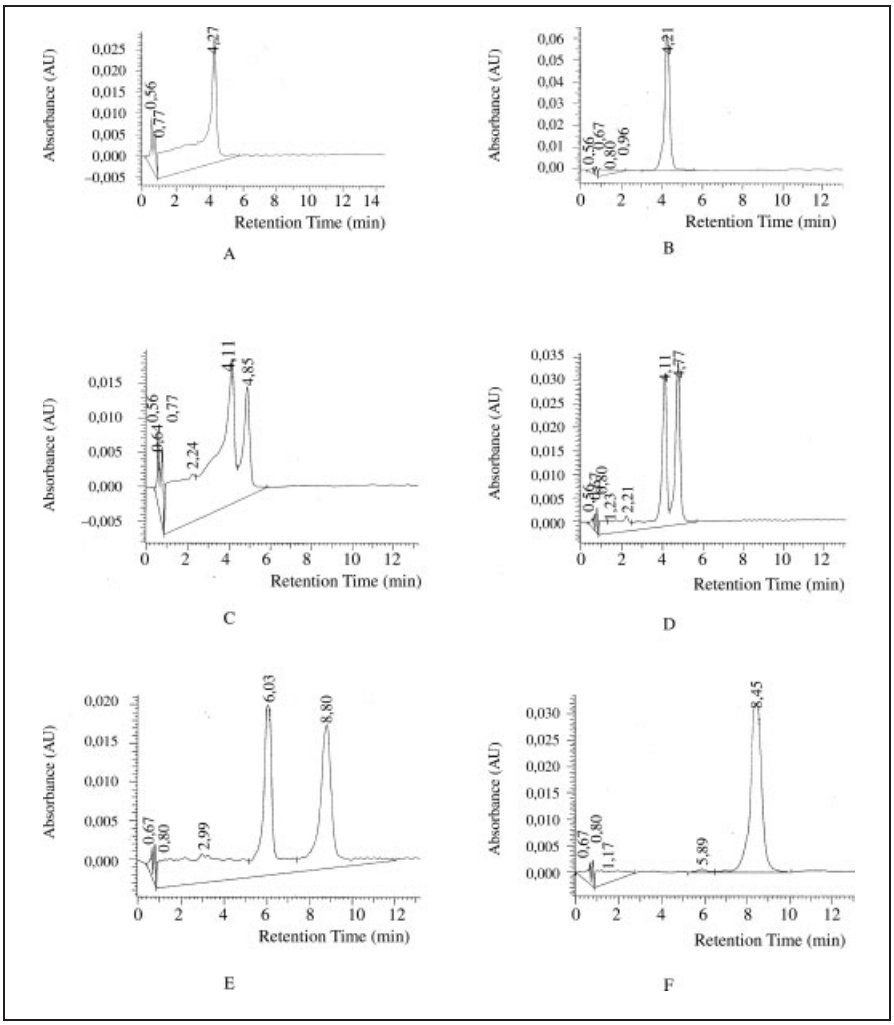

Fig.: Influence of several parameters on etodolac purity testing by RP-HPLC
A: Effect of methanol as injection solvent on the peak shape, B: Effect of the mobile phase as injection solvent on the peak shape, C: Effect of methanol as injection solvent on the separation of etodolac from its contaminant, D: Effect of the mobile phase as injection solvent on the separation of etodolac from its contaminant, E: Influence of the gradient elution system on the column resolution of etodolac and the contaminant, F: Efficiency of the developed method on the separation of small amount of contaminant

macopoeia (EP) to test the purity of etodolac. Therefore the present study was undertaken to optimise the method for purity testing of etodolac by RP-HPLC.

We found that using methanol as an injection solvent for the sample (etodolac) as mentioned in the European Pharmacopoeia (2005) leads to broadening of the peak (Fig. A). This result can be explained by the fact that the injected solvent containing the sample is not immediately diluted with the mobile phase which is a mixture of solvent A (methanol + buffer) and solvent B (acetonitril) in the proportion 90 : 10 and therefore some of the sample might migrate down the column with stronger solvent. Using the mobile phase (solvent A and solvent B) in the ratio 97 : 3 as an injection solvent for the sample instead of methanol resulted in a well-shaped and symmetrical peak (Fig. B). The applied injection solvent of the sample became in equilibrium with the mobile phase (solvent A: solvent B 90 : 10) immediately and consequently a distortion of the peak is avoided. A similar result was obtained by applying the mobile phase as injection solvent (MAC-MOD Analytical).

Applying methanol as an injection solvent and a gradient elution with a mixture of solvent A: solvent B (90 : 10 to 80 : 20 during 20 min runtime) to separate etodolac from its contaminant 2-(7-ethylindol-3-yl) ethanol according to EP leads to the same results mentioned above (a broadening of the peak) and to a less efficient separation as well. An efficient separation could be achieved using an injection solvent made of solvent A: solvent B 97 : 3 and the same gradient elution (Fig. C, D).

Increasing the resolution of the method and therefore improvement of the separation of etodolac from its contaminant could be attained by using the aforementioned injection solvent mixture and monitoring the gradient elution with solvent A: solvent B starting with the ratio 96 : 4 until 92 : 8 during a 20 min run (Fig. E). The column resolution Rs as well as the selectivity factor is increased ($Rs = 2.6$; $\alpha = 1{,}5$) compared to the method of EP ($Rs < 1$; $\alpha = 1.18$) respectively. The efficiency of the developed method for the analysis of etodolac was justified by the ability of the method to detect and separate a small amount of the contaminant (1% of the etodolac amount) from the sample (Fig. F).

Experimental

1. Chemicals and reagents

Glass, distilled-grade solvents, acetonitril, methanol, were obtained from Baker (Deventer-Holland). Potassium dihydrogen phosphate was acquired from Merck (Darmstadt, Germany). Potassium hydroxide was purchased from Ferak (Berlin, Germany). 2-(7-Ethylindol-3-yl) ethanol was a gift from Prof. Dr. Loewe. Etodolac was obtained from CILAG AG (Schaffhausen, Switzerland). The separation was carried out on column Lichro-CART 125–4 mm, RP 18 (5 μm, Merck, Darmstadt, Germany).

2. Method

The etodolac purity test was performed according to the method of the European Pharmacopoeia (2005). We applied some modifications to the method such as the changing of the injection solvent from methanol to a mixture of solvent A (methanol: KH_2PO_4, pH 7.0, 1 : 2.2) and solvent B (acetonitril) 97 : 3 and increasing the polarity of the gradient elution system, which consists of the components A (a mixture of buffer and methanol), and B (acetonitrile).

The method was validated by parameters such as selectivity, repeatability, and intermediate precision ($n = 10$; $SD = 0{,}27$; mean = 8.68; $RSD = 3.1\%$; $SE = 0.085$; $RSE = 0.98\ \%$; selectivity factor = 1.5). The confidence interval was used to test significance (mean value + $t \times SE$, $P < 0.05$).

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