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Short communication

Resolution of (±)-ibuprofen using L-arginine-impregnated thin-layer chromatography

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Abstract

The resolution of (±)-ibuprofen into its enantiomers was achieved by TLC on silica gel plates impregnated with optically pure L-arginine (0.5%), as the chiral selector, using acetonitrile–methanol–water (5:1:1, v/v/v) as the solvent system. The detection limit was 0.1 μg.

1. Introduction

Enantiomers of a compound exhibit different biological, physiological and chemical behaviour, and most common commercially available pharmaceuticals and drugs are racemic mixtures. It is very important to develop methods for their resolution and for establishing enantiomeric purity. Ibuprofen is one of the most effective non-steroidal analgesic and anti-inflammatory agents with fewer side effects, and is used extensively worldwide. It is marketed as a racemic mixture.

The liquid chromatographic resolution of enantiomeric mixtures of a variety of compounds [1–4] has been reported, and there has been particular emphasis on the resolution of enantiomers by TLC [5–9]. Wainer [10] discussed specifically the resolution of ibuprofen. (±)-Ibuprofen has been resolved by HPLC via derivatization with (*S*)-α-methylbenzylamine [11], ethyl chloroformate [12], the fluorescence derivatization reagent (−)-2-[4-(1-aminoethyl)-phenyl-6-methoxybenzoxazole [13] and α_1-acid glycoprotein [14,15] and (*R*)-(−)-(1-naphthyl)ethylurea [12] as a chiral stationary phase; there is no report on the TLC resolution of (±)-ibuprofen on arginine-impregnated plates. This paper reports a direct approach for the resolution and determination of the enantiomeric purity of (±)-ibuprofen by TLC on silica gel plates impregnated with L-arginine.

2. Experimental

(±)-Ibuprofen was obtained from Ferman Chemical (Solan, India) and silica gel G with 13% calcium sulphate as binder, having chloride, iron and lead impurities up to 0.02% and with pH 7.0 in a 10% aqueous suspension, from Merck (Bombay, India). The other reagents and chemicals used were of analytical-reagent grade and were obtained from SISCO Research Laboratory, BDH and Merck.

Impregnated thin-layer plates (20 cm × 20

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cm × 0.5 mm) were prepared by spreading a slurry of silica gel G (50 g) in distilled water (100 ml containing 0.5 g of L-arginine) with a Stahl-type applicator. L-Arginine has a p*I* of 10.8 and therefore a few drops of acetic acid were added to maintain the pH below the isoelectric point and to keep the amino acid in the cationic form. The plates were dried overnight at 60°C. The solutions of racemic ibuprofen and its (+)-isomer (10^{-3} *M*) were prepared in 70% ethanol and were applied to the plates at the 10-μl level. Separate plates were run for one- and two-dimensional modes. In the one-dimensional mode the spots of (±)-ibuprofen and the (+)-isomer were applied side-by-side on the same plate; in the two-dimensional mode first the spot of (±)-ibuprofen was applied to the L-arginine-impregnated plate and then the spot of the (+)-isomer was applied after the first run at the side of the former spot. Spots of (±)- and (+)-ibuprofen were also applied separately on two different plates and both were developed under identical conditions in the two-dimensional mode side-by-side. Chromatograms were developed at 32 ± 2°C for 30 min in acetonitrile–methanol–water (5:1:1, v/v/v) in a paper-lined rectangular glass chamber, pre-equilibrated with the solvent system for 10–15 min. The developed plates were dried at 60°C for 15 min and the spots were located in an iodine chamber. Iodine was allowed to evaporate from the plate; it was then sprayed with dilute HCl, heated for 15 min, cooled and sprayed with ninhydrin (0.2% in acetone).

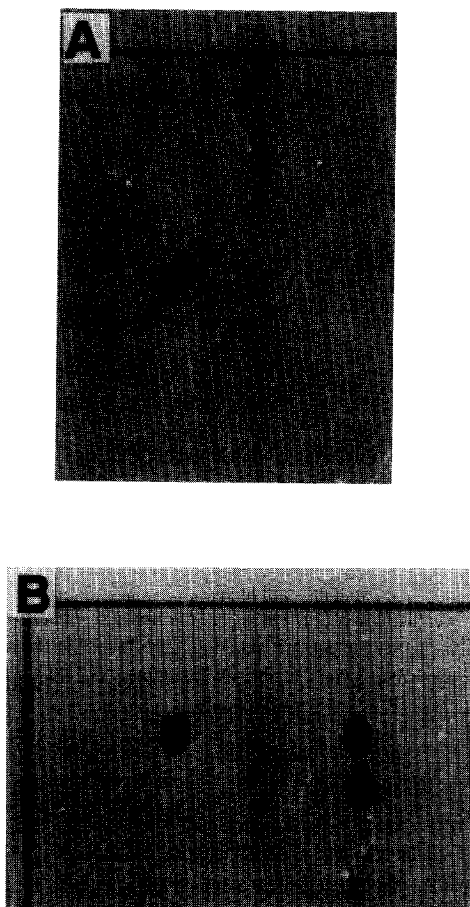

Fig. 1. Photographs of thin-layer chromatograms. (A) (±)-Ibuprofen, one-dimensional mode. (B) 1 = Pure (+)-ibuprofen; 2 = (+)- and (−)-ibuprofen resolved in two-dimensional mode.

3. Results and discussion

The hR_F values for the resolved (+)- and (−)-isomers of ibuprofen are 80 and 77, respectively (development: 15 min in the first dimension and 20 min in the second dimension); the hR_F value of the pure (+)-isomer is 80 under the same conditions. The results are averages of at least five identical runs. Fig. 1 shows the chromatograms.

Arginine has a p*I* of 10.8 and is cationic below this point. It interacted with (±)-ibuprofen to give two diastereomeric salts, (+)(−) and (−)(−), leading to enantiomeric separation; the enantiomers were located by exposure to iodine vapour. The treatment with ninhydrin gave a characteristic colour indicating the presence of arginine in both the spots. It showed that arginine formed diastereomeric salts with the components of the racemic mixture. The experiment with ninhydrin clearly detected the presence of (−)-arginine in both spots, thereby confirming the formation of diastereomers and the resolution of enantiomers of (±)-ibuprofen via the in situ formation of diastereomers with (−)-arginine. The reaction taking place on the chro-

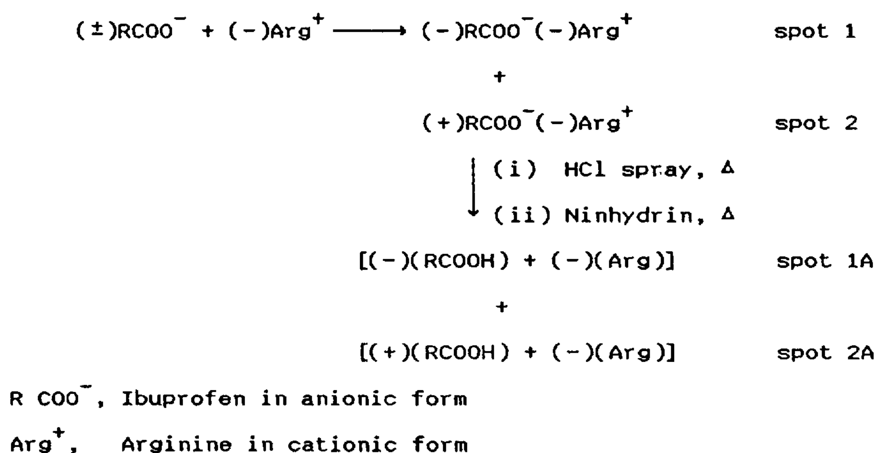

Fig. 2. Resolution of (±)-ibuprofen on L-arginine-impregnated silica gel. Spots 1 and 2 located in iodine chamber; spots 1A and 2A due to ninhydrin showing the presence of (−)-arginine in both spots and confirming the formation of diastereomers.

matogram is represented in Fig. 2. Although the whole plate is derivatized, the resolved spots are visible with a greater characteristic colour intensity and sharpness of the spots on a light pink background. Nevertheless, the detection is satisfactory with iodine vapour.

The method was successful in resolving as little as 0.1 μg of the enantiomeric mixture (i.e., 0.05 μg of each enantiomer), which is much lower than the reported [13] minimum quantifiable concentration of 0.2 μg/ml by RP-HPLC using fluorescene detection.

Ibuprofen has a weak chromophore and UV detection of nanogram amounts is difficult, and to detect low levels of ibuprofen in biological samples a moiety with a high UV molar absorptivity or a fluorescent label is added by derivatization. The resolution system reported here provides the direct separation of (±)-ibuprofen even in very small amounts, and therefore has an advantage over general indirect methods involving chiral derivatization prior to chromatography.

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