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Note

Liquid chromatographic separation of positional isomers of suprofen on a cyclodextrin-bonded phase

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Suprofen, [(±)-α-methyl-4-(2-thienylcarbonyl)benzene acetic acid], is a potent peripherally active analgesic with both anti-inflammatory and antipyretic properties. The acid is a white to off-white powder with a pK_a value of 3.91 and a melting point of 123.5°C.

Two positional isomers of suprofen may be formed during synthesis. Since these isomers are not completely removed by crystallization, a method was needed to identify and quantitate these compounds. These materials are potential process impurities. They need to be observed and quantified if present to ensure the quality of the drug substance. Although suprofen can be assayed by conventional reversed-phase high-performance liquid chromatography (HPLC) and/or gas chromatography, neither of these methods can adequately resolve the two isomers from each other or from suprofen. Additionally, the gas chromatographic assay requires a derivatization step involving the use of potentially hazardous diazomethane.

A number of methodological approaches were tried, *e.g.*, different stationary phases (phenyl, C_8, cyano), combinations of methanol, acetonitrile and aqueous buffers at various pH values on reversed-phase packings, and the addition of metal cations to the buffer in an attempt to form chelated species as suggested by Karger *et al.*[1]. All of these approaches proved to be ineffective. A β-cyclodextrin column was subsequently investigated.

β-Cyclodextrin is a helical arrangement of seven gluco-pyranose units. Because of its inherent chirality (each glucose unit is chiral), β-cyclodextrins have been employed as enantioselective stationary phases[2]. However, these columns can also be used to separate structural isomers[3]. Cyclodextrins are known for their ability to form selective inclusion complexes for a variety of organic molecules. Therefore, as a stationary phase in HPLC, these materials should offer separation possibilities different from conventional packings. The mechanism of separation may involve many factors including molecular size or bulk, dipole–dipole interaction, hydrogen bonding and the hydrophobicity of the solute. These inclusion complexes are formed in aqueous media and therefore, chromatography is usually performed using high concentrations of water. The degree and strength of complex formation, owing to the physical orientation of the solute in the toroid structure and the symmetrical arrangement of hydrogen bonding sites of the cyclodextrin, enhances retention of symmetrical structures over those found for traditional alkyl-bonded phases.

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A facile separation was developed using a β-cyclodextrin column and a mobile phase of acidified water and acetonitrile. No special sample preparation is required and all three isomers are baseline resolved.

EXPERIMENTAL

Materials, equipment and liquid chromatographic conditions

A Waters Associates HPLC system with a fixed-wavelength (254 nm) detector and Hewlett-Packard Model 3354 laboratory data system was used. The HPLC column was a Cyclobond I, β-cyclodextrin, 5-μm particle size, 25 cm × 4.6 mm I.D. (Advanced Separations Technologies) and the mobile phase was water–acetonitrile (70:30). The water was prepared by adding 1 ml triethylamine to 1 l of water and adjusting to pH 4.5 $\pm$ 0.1 with glacial acetic acid. The acetonitrile was HPLC grade as were the water and its additives. The column was maintained at 35°C and the flow-rate was maintained at 2.0 ml/min.

Solution, standard and sample preparation

Acidified water. HPLC-grade water was adjusted using 1.0 ml triethylamine and dropwise addition of glacial acetic acid until pH 4.5 $\pm$ 0.1 was reached as determined by a pH meter.

Sample solvent. A volume of 300 ml of acetonitrile was added to 700 ml of HPLC-grade water, thoroughly mixed and degassed.

Mobile phase. A volume of 300 ml of acetonitrile was added to 700 ml of acidified water, thoroughly mixed and degassed.

Sample or standard. About 40 mg of sample and standard were accurately weighed into separate 100-ml volumetric flasks, dissolved and diluted to volume with sample solvent.

Resolution mixture. About 50.0 mg of standard was accurately weighed into a 100-ml volumetric flask. About 25 mg of the isomer eluting closest to suprofen was accurately weighed into a separate 100-ml volumetric flask, and diluted to volume with sample solvent. A 5.0-ml aliquot of this solution was transferred to the volumetric flask containing the suprofen standard, and the mixture dissolved and diluted to volume with sample solvent.

Diluted resolution mixture. Accurately measured 10.0-ml aliquots of the resolution solution and sample solvent were transferred to a suitable container.

Assay procedure

The instrument was set as previously indicated and the column equilibrated for at least 20 min with the mobile phase flowing. The system suitability was determined prior to sample analysis. The resolution solution and the diluted resolution solution were injected and the chromatograms and integrated areas for the components obtained. The resolution between suprofen and its isomer should be at least 1.0 using the standard resolution equation. The integrated area of suprofen in the diluted resolution mixture should be between 48 and 52% of that of suprofen in the resolution mixture. The precision of the system was evaluated using the relative standard deviation of the response factors (area/μg) for the injections of the standard solution. Typically, the relative standard deviation was less than 2.0%.

Calculations

Integrated sample area counts were normalized for the amount of compound injected (area/μg sample) divided by normalized standard area counts and multiplied by the percent purity of the standard to determine the percent assay. The isomers were calculated in a similar fashion, with a sensitivity factor (*F*) added; this factor accounts for the difference in sensitivity between the major compound and a known isomer under analytical assay conditions. Unidentified peaks were assigned a sensitivity factor of 1.0.

RESULTS AND DISCUSSION

Suprofen and each of the positional isomers were used as racemic mixtures. The HPLC procedure described was used to assess the positional isomeric purity of suprofen. The suprofen was quantified using a standard of known purity, while sensitivity factors were employed in quantifying the amount of isomers. These sensitivity factors were obtained experimentally by chromatographing known concentrations of authentic samples of the isomers and dividing their response factor (area/μg injected) by the suprofen response factor.

The structures, retention times, sensitivity factors and detection limits of suprofen and its positional isomers are presented in Table I, demonstrating the procedure to be specific and sensitive. A typical chromatogram is shown in Fig. 1.

The linearity of response for each compound is shown in Fig. 2. The suprofen plot represents up to 110% of the normal amount chromatographed, while the isomer plots range from 0.05% to 2.2% of the nominal suprofen injection. The lower limit of detection was determined from these plots.

Precision was evaluated using twelve injections of a suprofen sample spiked with each of the two isomers. The concentration of each impurity isomer component was adjusted to represent approximately 0.5% of the nominally injected suprofen.

TABLE I

STRUCTURES, RETENTION TIMES, SENSITIVITY FACTORS AND DETECTION LIMITS OF SUPROFEN AND ITS ISOMERS

NA = Not applicable.

Compound	*Structure*	*Retention time (min)*	*F*	*Detection limit (%)*
McN-JR-26283	CH_3, CH—COOH, C, S, O	8.8	0.87	0.04
McN-JR-44379	CH_3, C, CH—COOH, O, S	10.9	1.70	0.04
Suprofen	CH_3, S, C, CH—COOH, O	12.2	1.0	NA

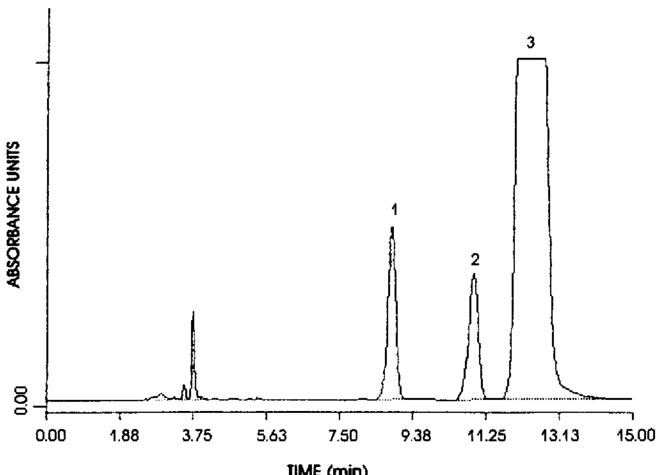


Fig. 1. Typical chromatogram of a spiked suprofen sample. Peaks: 1 = McN-JR-26283; 2 = McN-JR-44379; 3 = suprofen.

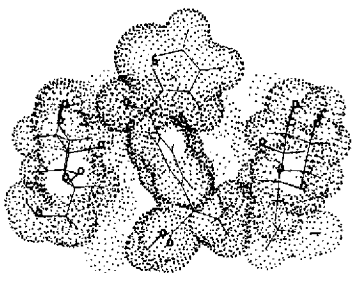
Fig. 2. Linearity plots for suprofen and each isomer.

The data indicated the integrated peak areas for each component were reproducible, with the following relative standard deviations: 0.24% for suprofen (McN-JR-25061), 1.6% for the 3-phenyl isomer (McN-JR-26283), and 1.5% for the 3-thienyl isomer (MCN-JR-44379). The quantitation of and resolution between each of the two positional isomers and suprofen was found to be rugged with respect to column temperature, flow-rate, pH of the mobile phase, percent acetonitrile in the mobile phase, and the amount of triethylamine in the aqueous portion of the mobile phase. The resolution between the 3-thienyl isomer and suprofen, and the quantitation of suprofen and its two isomers with respect to changes in the above-mentioned parameters are shown in Table II. The resolution between the 3-thienyl and 3-phenyl isomers was sufficiently large that chromatographic alterations resulted in inconsequential resolution changes under the condition investigated. The mobile phase composition could be varied from 25 to 35% acetonitrile although resolution begins to decrease at 35% acetonitrile. Increasing the amount of aqueous component increased the resolution but also increased retention time. The pH of the aqueous buffer did not appear to affect the resolution within the limits investigated. However, at pH values greater than 4.5, the retention times for all components began to decrease. The amount of triethylamine did not seem to affect the resolution to any significant degree, but without the amine, none of the components eluted. At higher temperatures (*i.e.*, 50°C) the resolution decreased sharply. The flow-rate over the range investigated

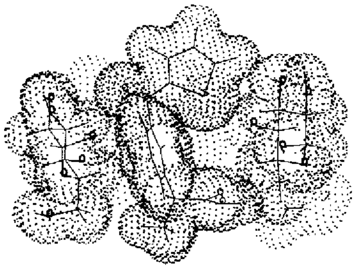
TABLE II

EFFECT OF CHANGES IN CHROMATOGRAPHIC PARAMETERS ON RESOLUTION AND QUANTITATION OF SUPROFEN AND ITS POSITIONAL ISOMERS

Parameter	*Value*	*Resolution*	*% (w/w)*		
			Suprofen	*McN-JR-26283*	*McN-JR-44379*
Flow-rate (ml/min)	1.0	1.21	98.93	0.83	0.71
	1.5	1.16	98.78	0.83	0.74
	2.0	1.06	98.76	0.83	0.73
Column temperature (°C)	35	1.16	98.78	0.83	0.74
	40	1.09	98.66	0.85	0.73
	45	1.03	98.58	0.83	0.73
	50	0.83	98.72	0.83	0.73
Acetonitrile content (%)	25	1.35	98.70	0.81	0.71
	30	1.16	98.78	0.83	0.74
	35	0.74	98.63	0.81	0.73
pH aqueous phase	4.0	1.06	98.73	0.83	0.73
	4.5	1.16	98.78	0.83	0.74
	5.0	1.20	98.48	0.85	0.73
Triethylamine content (ml/l)	0.0	No elution of any components			
	0.5	0.91	98.55	0.83	0.73
	1.0	1.16	98.78	0.83	0.74
	2.0	1.17	98.67	0.83	0.73



SUPROFEN In CYCLODEXTRIN



McN-JR-26283 In CYCLODEXTRIN

Fig. 3. Computer model of racemic suprofen isomers and β-cyclodextrin.

did not affect the resolution. Discussion of operational parameters, general selectivity and the ruggedness of cyclodextrins have recently been published[4–6].

Computer modeling of the β-cyclodextrin with the racemic suprofen isomers using the program SYBAL (Tripos Assoc.) revealed interesting docking arrangements. The computer program positioned the suprofen positional isomers to minimize steric and electronic hindrances. The 3-thienyl and 4-phenyl isomers have essentially the same conformation when inserted into the β-cyclodextrin, while the 3-phenyl isomer differs. Computer-generated figures of cross-sections of the 3-phenyl and 4-phenyl racemic isomer complexes are shown in Fig. 3. Although these represent low-energy arrangements, the ease with which the isomers can attain these docking positions is unknown and solvation effects are not incorporated. Thus, strict interpretation of these data to predict elution order is currently unclear. However, the observed elution order (first to last) *m*-, *o*-, *p*- is corroborated by ref. 3.

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