Direct Analysis of Fenvalerate Isomers by Liquid Chromatography. Application to Formulation and Residue Analysis of Fenvalerate

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Summary
The separation of the optical isomers of fenvalerate [cyano(3-phenoxyphenyl)methyl 2-(4-chlorophenyl)-3-methylbutyrate] has been carried out by high-performance liquid chromatography (HPLC) on a chiral column with (R)-N-3,4-dinitrobenzoyl-phenyl-glycine (DNBPG) covalently bonded on aminopropyl silica and eluted with mixtures of methanol, 2-propanol, and hexane. The system was applied to the analysis of Pydrin®, an emulsifiable concentrate formulation of fenvalerate, and to the residue analysis of fenvalerate in milk samples. For the analysis of Pydrin® the only requirement was the proper dilution of Pydrin® with hexane. For the analysis of residues in milk, fenvalerate was extracted with hexane after precipitating the milk proteins with acetonitrile and removing the precipitate by filtration; the hexane extract was concentrated to small volume and filtered before being analyzed by HPLC.

Introduction
Fenvalerate (Sumicidin, Ectrin, S-5602) is a broad spectrum pyrethroid insecticide. It has two asymmetric carbon atoms and therefore is a mixture of four isomers. Due to the very strict stereospecificity of the pyrethroid molecule for biological activity only one of the four isomers is particularly insecticidally active [1]. Therefore, it is important and necessary to be able to estimate the optical purity.

The separation of the optical isomers of unmodified fenvalerate has not yet been reported. The optical isomers of 2-(4-chlorophenyl)isovaleric acid (CPIA) which is the acid moiety of fenvalerate have been resolved either after being derivatized to diastereomeric l-methyl esters and analyzed by GC or after being derivatized to enantiomeric isopropylamides and analyzed by GC on a capillary column coated with a chiral liquid phase [2, 3]. Also the technique of preferential crystallization with achiral amines was utilized for the resolution of CPIA enantiomers [4]. An HPLC system capable of resolving directly all four enantiomers of fenvalerate is herewith reported.

Experimental
The liquid chromatograph consisted of a Waters Associates Model 6000 A pump and a Model 440 UV detector connected to an Omniscribe chart recorder. Samples were injected by means of a valve-type Model 7120 Rheodyne injector equipped with 20μl loop.

Two commercially available chiral HPLC columns have been tested and evaluated. These columns are the BAKER-BOND (J.T. Baker Co.) column which contains (R)-N-3,4-dinitrobenzoyl-phenyl-glycine covalently bonded to modified 5μm silica (column A) and the PIRKLE (Regis) column which contains phenylglycine instead (column B). Both columns were 250 x 4.6mm. For the analysis of fenvalerate residues in milk extracts the chiral column was protected with a 30 x 4.6mm silica gel cartridge (Brownlee Labs MPLC Guard Column).

The solvents used for the chromatography were HPLC grade (J.T. Baker Co.) while all the solvents utilized for sample preparation were technical grade solvents redistilled in the laboratory. The HPLC solvents were degassed by filtration under vacuum through a 0.4μm filter (Millipore) just before use.

Fenvalerate [cyano(3-phenoxyphenyl)methyl 2-(4-chlorophenyl)-3-methylbutyrate] (99.9% purity) analytical standard, as well a sample of Pydrin® (SD 43775, 2.4lbAlgal-1) formulated material were in-house stocks donated by Shell Chemical Company. Standards of the pyrethroids fluvalinate [cyano(3-phenoxyphenyl)methyl 2-[2-chloro-4-(trifluoromethyl)anilino]-3-methylbutanoate], cypermethrin [cyano(3-phenoxyphenyl)methyl 3-[2,2-dichloroethenyl]-2,2-dimethylcyclopropanecarboxylate], permethrin [3-(3-phenoxyphenyl)methyl 2,2-dimethylcyclopropanecarboxylate], and resmethrin [5-(phenylmethyl)-3-furyl 2,2-dimethyl-3(2-methyl-1-propanyl)cyclopropane-
carboxylate] were also in-house stocks. Milk samples were either provided by the University Farm (Aristotelian University, Greece) or obtained from the local market.

Sample preparation and analysis. A 0.33% (v/v) solution of Pydrin® in hexane was prepared, filtered, and analyzed directly by HPLC with a mobile phase consisting of 0.1% 2-propanol in hexane (v/v) and at a flow rate of 1 ml min⁻¹. For the analysis of fenvalerate residues in milk 40 ml acetonitrile were added to 20 g of a milk sample and the mixture was shaken vigorously for 15 min in a mechanical shaker. The precipitated proteins were removed by filtration under suction through No. 2 Whatman filter paper. An aliquot of the filtrate equivalent to 10 g milk was transferred into a separating funnel containing 20 ml hexane and the mixture was shaken smoothly for 30 sec. Eighty ml 4% NaCl solution were added and the funnel was shaken again for 30 sec. The two phases were allowed to separate and the aqueous phase was discarded. The hexane phase was washed twice with 10 ml distilled water and drained through anhydrous Na₂SO₄ into a round bottom flask. The separating funnel was rinsed with 10 ml hexane and the hexane rinse was also drained through the anhydrous Na₂SO₄. The hexane extract was evaporated to small volume by use of a rotary evaporator under vacuum and was transferred to a centrifuge tube where its volume was further reduced to 1 ml by a stream of nitrogen. The final concentrated extract was filtered and 20 µl from it were injected onto the HPLC column. For the analysis of fenvalerate residues in milk samples the mobile phase consisted of 0.1% methanol, 0.3% 2-propanol, and 99.6% hexane (v/v/v) and the flow rate 1 ml min⁻¹.

Results and Discussion

The pyrethroid insecticides have 1 to 3 asymmetric atoms and due to the lack of stereospecific synthetic methods most of the commercially available compounds are mixtures of 2 to 8 isomers. The group of pyrethroids containing a cyclopropane ring have cis/trans isomerism. Fenvalerate is commercially available as a mixture of four isomers, but only the isomer with the SS configuration is mainly insecticidally active [4]. With the chiral column A and utilizing a mobile phase consisting of 0.1% 2-propanol in hexane (v/v) all four isomers of fenvalerate can be resolved completely. A sample chromatogram from the analysis of Pydrin® is shown in Fig. 1. The first peak to elute is the solvent peak whereas the other peaks that elute before the optical isomers of fenvalerate are apparently positional isomers of fenvalerate present as impurities in technical materials. The resolution achieved (Rₛ) between adjacent enantiomeric peaks is 1.78, 2.1 and 1.2, respectively. As resolution (Rₛ) between two peaks with retention times tᵣ(1) and tᵣ(2), respectively, the ratio between 2[tᵣ(2) - tᵣ(1)] and the sum of the base width of the respective peaks was taken. As much as 1 mg fenvalerate can be injected in each run and the resolved isomeric peaks can be recovered. This column has been also applied to the analysis of fenvalerate residues in milk extracts. A sample chromatogram is shown in Fig. 2. In this case by using a mobile phase consisting of 0.1% methanol, 0.3% 2-propanol, and 99.6% hexane (v/v) a baseline separation of all isomers has been obtained.

Column A has also been found appropriate for the separation of the optical isomers of fluvinate. A sample chromatogram from the analysis of an emulsifiable concentrate formulation of fluvinate is shown in Fig. 3. The mobile phase consisted of 0.07% 2-propanol in hexane (v/v) and the flow rate was set to 1 ml min⁻¹. The resolution between the first pair of eluting isomers was 2.25 and the second pair was 0.92, however the resolution of the two middle peaks was 0.57. This same column with mobile phases consisted either of binary or tertiary mixtures of dichloromethane, acetonitrile, hexane or methanol, 2-propanol, isobutyl alcohol, and hexane was also tested for the analysis of cyclopropane ring-containing pyrethroids (permethrin, resmethrin, and cypermethrin). Only the cis/trans and diastereomeric pairs were resolved.