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Ultrasonic/Soxhlet/supercritical fluid extraction kinetics of pyrethrins from flowers and allethrin from paper strips

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Abstract Three different extraction methods (ultrasonic extraction (USE), Soxhlet extraction (SOX) and supercritical fluid extraction (SFE)) were compared for the extraction of pyrethrins from chrysanthemic flowers and commercial insecticide powder. Allethrin was extracted from paper strips. All extracts and the kinetics were analyzed by supercritical fluid chromatography and flame ionization detector.

1 Introduction

Many types of plants produce insecticide active compounds. Only in a few cases synthesis and structure analysis of such natural insecticides are accomplished [1, 2]. Beside nicotine, rotenon or ryanodin, pyrethrin is one of the most explored insecticides, which is produced by certain species of chrysanthemum flowers [3]. The blossoms of the plant are harvested shortly after blooming. For commercial utilization the blossoms are either dried, powdered or extracted. Commercial extraction methods are: cold solvent extraction with solvents like methanol, acetone or petrolether or supercritical fluid extraction (SFE) with carbon dioxide [4].Using solvent extraction the undissolved plant matter from the ground pyrethrum flowers is removed by filtration and the solvent is flushed off to produce a crude oleoresin. It typically contains approx. 30% pyrethrins. Crude oleoresin requires further processing. During these refining steps pigments and extraneous plant matter, including vegetable waxes and resins, are removed [5]. Bunzenberger et al. described a SF-extraction of chrysanthemic flowers with carbon dioxide in a range of 6–10 MPa, where a simple refining step follows, to produce a pyrethrum extract of high quality [6]. For analytical determination of pyrethrins or pyrethroids in soil several extraction methods with different solvents were applied [7–11]. Synthetic derivatives of pyrethrins are called pyrethroids. Casamatta et al. described an extraction method using a 1 L laboratory batch extractor with hexane. In their investigations they found 1.25% pyrethrins in dried blossoms. They also measured the extraction kinetics [12]. SFE extraction kinetics of different pyrethroids were reported by Schipke [13].

Pyrethroids are more effective than natural pyrethrins while the latter are less toxic to mammals [14]. One common pyrethroid is allethrin. It is used in small paper strips for indoor release. In this study extraction kinetics of pyrethrins and allethrin were carried out by Soxhlet extraction (SOX) and ultrasonic extraction (USE) with isopropanol and supercritical fluid extraction (SFE) with carbon dioxide.

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2 Experimental

2.1 Chemicals. Allethrin was extracted from spiked paper strips (46.5 g/kg) (Jeyes Deutschland GmbH, Germany). One complete strip (35 mm × 20 mm, ~ 1 g) was prepared for extraction by cutting it in small pieces.

In all extraction methods 1 g of powdered chrysanthemic flowers ("Insektenblüten", Caesar&Loretz, Hilden, Germany obtained in 1992 and a second obtained in 1996 stored in a drawer) was applied. Another sample was a commercial insecticide powder (Spruzit® Staub, W. Neudorf GmbH, Emmerthal, Germany) against earth fleas, plant-louse, Colorado beetle and its larva. It contains 12 g/kg natural pyrethrum or 3 g/kg pyrethrins, respectively.

2.2 Extraction. For examination of the extraction kinetics chrysanthemic flowers (1992) and allethrin paper strips were applied.

For USE (Bransonic 2200, Branson Ultrasonic Corporation, Danbury, CT, USA) the sample was extracted 6 times with 10 mL isopropanol (p.A. Riedel de Haën, Seelze, Germany) each for about 20 min. The SOX extraction was performed with 50 mL (isopropanol) in a micro-Soxhlet apparatus. For determining the extraction kinetics, aliquots were taken at the beginning, after 10, 30, 40, 60 and 120 min. The samples were micro filtered (0.45 μm) and than inserted into the injector.

The extraction conditions for SFE (Model 206 D, ISCO, Axel Semrau, Sprockhövel, Germany) were a pressure of 30 MPa, a temperature of 100 °C and flowrates between 1.0 and 1.5 mL/min carbon dioxide (AGA, SF Grade, Düsseldorf, Germany). A heated (50 °C) regulated restrictor (Vari flow, ISCO Axel Semrau, Sprockhövel, Germany) was used to control expansion of carbon dioxide. The expanded CO_2 bubbled

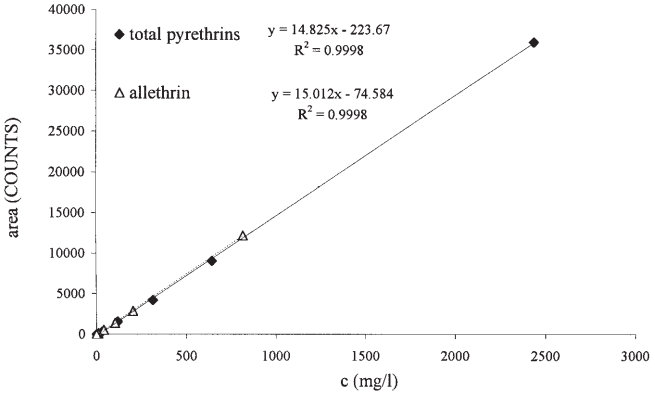

Fig. 1 Calibration plot of allethrin and total pyrethrins

Table 1 LOD and LOQ of allethrin and total pyrethrins

Substance	Method	LOD mg/L	LOQ mg/L
Allethrin	DFG	32.1	34.6
	DIN	11.5	38.4
Total pyrethrins	DFG	70.8	106.2
	DIN	35.4	117.8

through 50 mL isopropanol, contained in a 2 neck flask with reflux condenser, to trap the exhausting analytes [15]. After flushing the restrictor with 1 mL isopropanol, a 1 mL sample was taken out of the flask. The samples were taken at the start of the extraction, after 5, 15, 20, 45, 60, and 120 min. All measurements were performed 3 times.

2.3 Chromatography. For separation and determination of the analytes, supercritical fluid chromatography (SFC-FID) was used [16].The following substances were investigated: bioallethrin (93.9%) and World Pyrethrum Standard (19.2% total pyrethrins) (BAYER AG, Mohnheim, Germany). An isopropanolic solution of the standards was used for quantitative analysis. The SFC-equipment was a MPS/225 (SUPREX, Pittsburgh, PA, USA) with a four-port pneumatically driven, timed-split injection valve (0.1 µL internal loop) and a FID. The injection time was 0.5 s and the oven temperature was 110 °C. Carbon dioxide (AGA, Düsseldorf, Germany) with SFE grade quality was used as mobile phase. The separation was performed on a (DB5) fused silica capillary column (ID : 50 µm; L : 9 m; film thickness: 0.1 µm) from J&W Scientific (Folsom, CA, USA) using a linear pressure gradient from 11.1 MPa to 24.3 MPa with a rate of 0.2 MPa/min. A self-made Guthrie-restrictor was placed at the end of the column to expand the compressed carbon dioxide to ambient pressure.

3 Results

The calibration plot of allethrin and the sum of all pyrethrins is given in Fig. 1. The limit of detection (LOD) and limit of quantification (LOQ) based upon the method of Frehse and Thier are given in Table 1 [17].

Recoveries of allethrin were > 95% for SFE and 98 –100% for SOX and USE. After 60 min the extraction is complete for USE and SOX. SFE requires only 40 min. The kinetics are shown in Fig. 2.

Powdered chrysanthemum flowers were used to record the extraction kinetics of pyrethrins. Figure 3 shows a SF chromatogram of a pyrethrum flower SF extract after 20 min extraction time. The total amount of pyrethrins is 0.9% (w/w) using Soxhlet and ultrasonic extraction and 0.8% (w/w) using SFE as illustrated in Fig. 4. The SF extraction is very fast and finished after 20 min. The extraction time for SOX and USE is 60 min. The difference in yield between SFE and SOX/USE can be explained by the chosen SF conditions of 30 MPa, 100 °C. Higher pressure could increase the SFE yield but was not studied here. The difference in time is due to the different physical properties of supercritical fluids, mainly the binary diffusion coefficient (D_{12}) and viscosity.

In this study the amount is 0.9% (w/w) in USE and SOX and 0.8% (w/w) in SFE.

Comparing the total amount of pyrethrins in the solids, it could be shown that the yields using different extraction methods are very similar (Table 2). The pyrethrin content in flowers from 1992 is 0.8–0.9% (w/w), whereas the flowers from 1996 contain 0.9–1.1% (w/w). The pyrethrin content of 0.3% (w/w) in insecticide powder is confirmed.

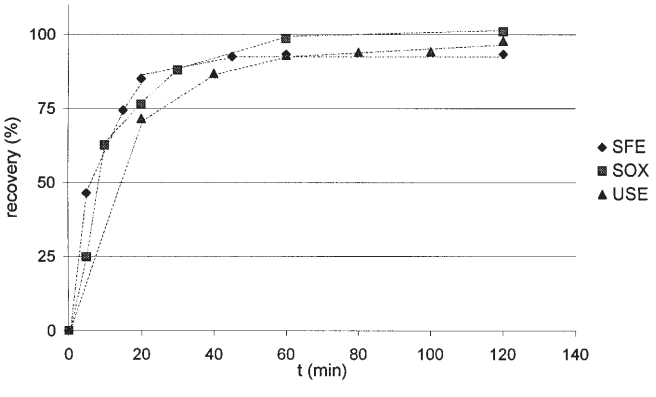

Fig. 2 Extraction kinetics and recoveries of allethrin for USE, SOX and SFE

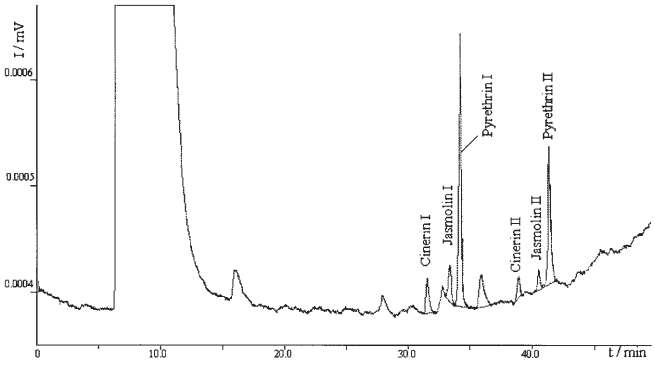

Fig. 3 Chromatogram of pyrethrum-SF extract using SFC with a pressure program: 11.1 MPa at 0.2 MPa/min to 24.3 MPa, temperature 110 °C; flame ionization detection

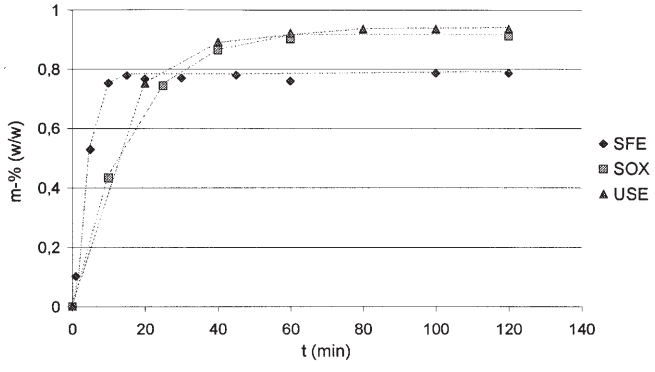

Fig. 4 Extraction kinetics and total amount of pyrethrins for USE, SOX and SFE

Table 2 Yield of pyrethrins in different samples using USE, SOX and SFE

Sample	Extraction method	m-% (w/w)	RSD (n = 5)
"Insektenblüten" (1996)	SFE	0.94	4.76
	SOX	1.04	3.69
	USE	1.13	2.20
"Insektenblüten" (1992)	SFE	0.78	1.31
	SOX	0.91	1.15
	USE	0.94	1.39
Spruzit (1998)	SFE	0.26	10.60
	SOX	0.31	9.83
	USE	0.30	1.30
	theoretical	0.30	

Considering the natural origin of the material, the age of the powdered flowers, where heat and oxygen could destroy a part of the pyrethrins, this compares favorably with literature and demonstrates that pyrethrins are stable even under very simple storage conditions. The total pyrethrin content in flowers is reported as 1–2% [18].

4 Conclusion

Recoveries of allethrin and total amount of pyrethrins in all three extraction methods are very similar. The extraction time in USE and SOX is slightly higher than in SFE.

SFC-FID can be used for the quantification of pyrethrins.

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