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# In situ transesterification of the natural pyrethrins to methyl esters by heterogeneous catalysis using a supercritical fluid extraction system and detection by gas chromatography–mass spectrometry

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**Abstract**

The natural pyrethrins were transesterificated in situ at 270°C and 40 MPa in the presence of acidic alumina and methanol in a static supercritical fluid extraction step. The extraction fluid and reaction medium was carbon dioxide. The following step was the dynamic extraction with pure carbon dioxide at 70°C and 40 MPa. Analytes were collected in toluene and analysed and identified by GC–MS detection. To achieve a quantitative conversion high temperature together with a catalyst were necessary. Supercritical carbon dioxide was necessary to dissolve the pyrethrins and to bring them in contact with alumina as the catalyst. Because this catalyst is solid no additional step was necessary to separate it before the GC analysis.


*Keywords:* Pyrethrins

## 1. Introduction

Pyrethrins are the natural, insecticide acting components of *Chrysanthemum cinerariaefolium*. There are three esters of the chrysanthemum acid, cinerin I, jasmolin I and pyrethrin I, and three esters of the pyrethrum acid, cinerin II, jasmolin II and pyrethrin II. Solvent extracts of *Chrysanthemum cinerariaefolium* contain as main components pyrethrin I and II. Such extracts are applied as indoor insecticides.

Gas chromatographic analysis of these substances is difficult, because pyrethrin I and II with their *cis*-pentadienyl moiety undergo thermal isomerisation to form isopyrethrin I and II at temperatures above 200°C [1–4]. This temperature cannot be avoided either in split/splitless injection systems or in the elution from capillary columns. The result is a continuous conversion to the isopyrethrins on the column. This leads to broad plateaus behind the peaks of pyrethrin I and II which can not be integrated correctly (Fig. 1). The only way to reduce the thermal conversion is by the use of very short thin film columns combined with an on-column injection system [3]. With such columns the capacity and the separation performance, however, will be insufficiant for the analysis of real samples. A further problem is that the single components are not available as standard substances, because the synthesis of the single components is more expensive than their extraction from the flowers. This, however, also means that the natural pyrethrins will only appear together. A determination of the sum of the pyrethrins is therefore sufficient. For this reason the analysis of crude pyrethrum extracts is accomplished

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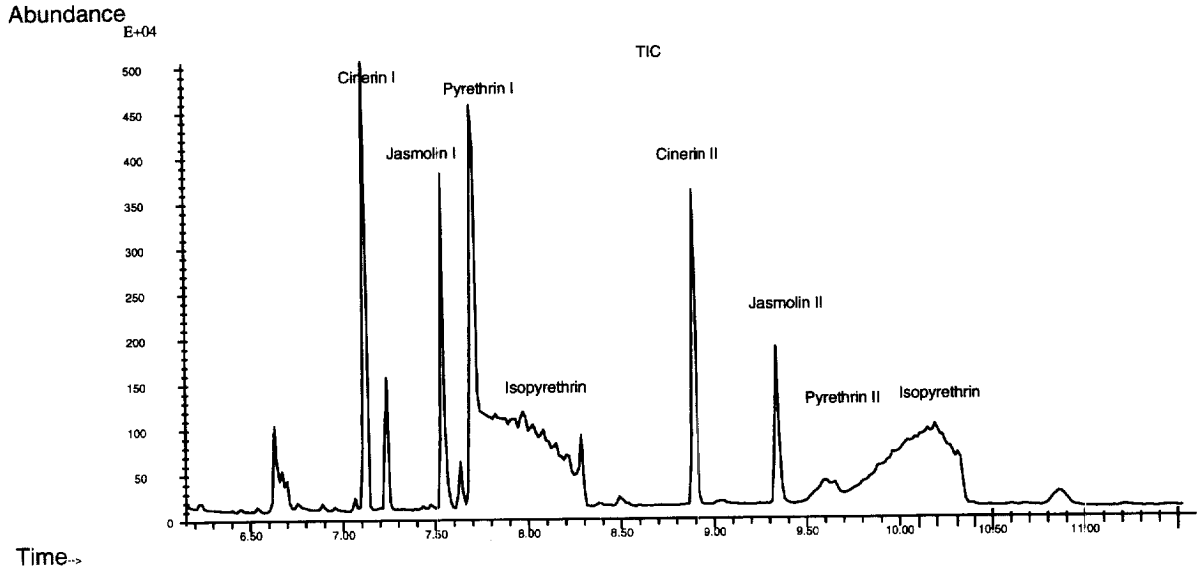


Fig. 1. GC–MS total ion chromatogram of the pyrethrins in pyrethrum.

by saponification and following titration of the resulting acids [5].

To solve the problem of the thermal conversion in GC analysis, derivatisation of the pyrethrins was attempted. In [6] the determination of 2,4-diphenylhydrazones was described. Another evaluation saponificated the pyrethrins into their acids. After separation of the fatty acids, the acids were esterified to their methyl esters [7]. Both methods require a lot of chemical work before the GC analysis. This comprises of several steps and the handling of relatively large volumes. For the determination of the amount of pyrethrins in plant extracts this is no problem. In trace analysis of indoor samples this will decrease the accuracy of the determination. Our objective was to discover if it was possible to derivate the pyrethrins directly in an extraction cell of a supercritical fluid extraction (SFE) system in such a way that the extract can be analysed by GC directly.

One possibility, to convert the pyrethrins in one step, is acidic-catalysed transesterification. Normally acids used, such as sulfuric acid or HCl, disturb the direct determination by GC. The use of acidic alumina as a solid catalyst can solve this problem.

In this study, the reaction of pyrethrins under supercritical conditions in carbon dioxide in the presence of methanol and acidic alumina was determined.

## 2. Experimental

### 2.1. Chemicals

Pyrethrum extract, seasand and acidic alumina for chromatography were obtained from Fluka Chemie AG, Buchs, Switzerland.

Methanol was self-distilled and purified over a carbon black, silicagel column.

Toluene for residue analysis was purchased from Rathburn, Walkerburn, UK.

Carbon dioxide, SFE-grade was obtained from Air Products, Allentown PA, USA.

Chrysanthemum acid was purchased from Sigma, St. Louis, USA.

### 2.2. Instrumentation

#### 2.2.1. SFE apparatus

A self-built apparatus was used containing the following parts. The pump was a suprex prepmaster pump, the oven a GC oven from Delsi Nermag, Argenteuille, France. As a variable restrictor, a Vari

Flow valve from Suprex, Duisburg, Germany, was heated over a metal block with heating cartridges. The temperature was controlled by a Eurotherm, Hilden, Germany, regulator. To flush the restrictor a Knauer, Berlin, Germany, HPLC pump 64 was used. The restrictor outlet leads to the bottom of a 50-ml two-necked pointed flask. On the second neck a dewar cooler was installed as described in [8], with its outlet connected to the hood by a hose.

### 2.3. Extraction

A 3-ml extraction cell was half-filled with purified seasand. Pyrethrum extract was spiked on this sand. A layer of acidic alumina was placed at the outlet of the cell. After the addition of 100 μl of methanol, the cell was closed and installed into the SFE system. The first extraction step was a static step at a temperature of 250°C or 270°C for 60 or 30 min to dissolve the pyrethrins and give them time to react. The next static step at 70°C for 15 min was to equilibrate the system to the dynamic extraction temperature. The final step was the dynamic extraction for 30 min at 70°C with a flow of 1 ml/min. For the whole extraction, the pressure was 40 MPa. Analytes were collected in toluene.

Extracts were analysed on a Hewlett–Packard, Waldbronn, Germany, 6890 gas chromatograph with a 6890 mass selective detector and a 6890 autosampler. The chromatographic conditions in Fig. 1 for the GC–MS separation were as follows: column HP5, 30 m×0.25 mm I.D. 0.25-μm film thickness, 70°C to 190°C (at 40 C°/min) to 275°C (at 15 C°/min), constant flow 1 ml/min. The split ratio was 1:100. Carrier gas was helium 4.6 which was purified with a Megasorb (Messer Griesheim, Germany) gas-purifying system to 7.0 quality. For the following chromatograms, the temperature program starts with 50°C held for 1 min raising the temperature at a rate of 10 C°/min to 275°C final temperature which was held for 7 min. Injection mode was splitless with an injector temperature of 280°C. Injection volume was 1 μl, fast injected. Carrier gas flow was constant, 1 ml/min.

### 2.4. Identification

Identification was accomplished with a HP 6890 Mass Selective Detector. Pyrethrins were identified by comparison of their mass spectra with [9]. Chrysanthemum acid and its methyl ester were identified by comparison with authentic material and spectra from SPECINFO online databank [10].

## 3. Results and discussion

To give a better comprehensibility of the GC–MS, only the basic ions of the chrysanthemates ($m/z$ 123) and of the pyrethrates ($m/z$ 107) are shown. These fragments originate from the acid component of the molecule. Due to fragmentation in the ion source displaying the selected ion, 107 and 123 will be sufficient to identify the chrysanthemates ($m/z$ 123), pyrethrates ($m/z$ 107), the acids as well as the methyl esters and the pyrethrins. Identification of chrysanthemic acid, pyrethric acid, and their methyl ester was achieved from their mass spectra.

Through thermal treatment, pyrethrin I and II were to be converted into their isopyrethrins. This process was also to be irreversible, because the double bonds after the tautomerisation are conjugated to the chain double bonds. It turned out, however, that through thermal treatment large amounts of the ester broke down to its acids. In the presence of methanol, chrysanthemum acid and pyrethrum acid as well as their methyl esters were formed from the natural pyrethrins. At a temperature of 250°C, the expected reaction products were observed but the reaction is not complete (Fig. 2a). Reaction of the pyrethrins with methanol in the presence of acidic alumina at temperatures of up to 150°C is insufficient. The reaction rates were below 10%. For better comparison with Fig. 2a, acidic alumina as catalyst was added to the extraction cell at 250°C. Holding all other conditions the same, Fig. 2b shows the effect of the added catalyst. Instead of reacting to the corresponding acids, mostly the methyl esters were formed. But neither reaction is quantitative. Reaction with a catalyst at 250°C for 1 h converted approximately 90% of the pyrethrins into their corresponding methyl esters. Raising the temperature to 270°C yielded quantitative conversion of the pyrethrins (Fig. 2c). There is only a small amount of chrysanthemic acid left, which probably depends upon the water in the reaction cell. The amount of

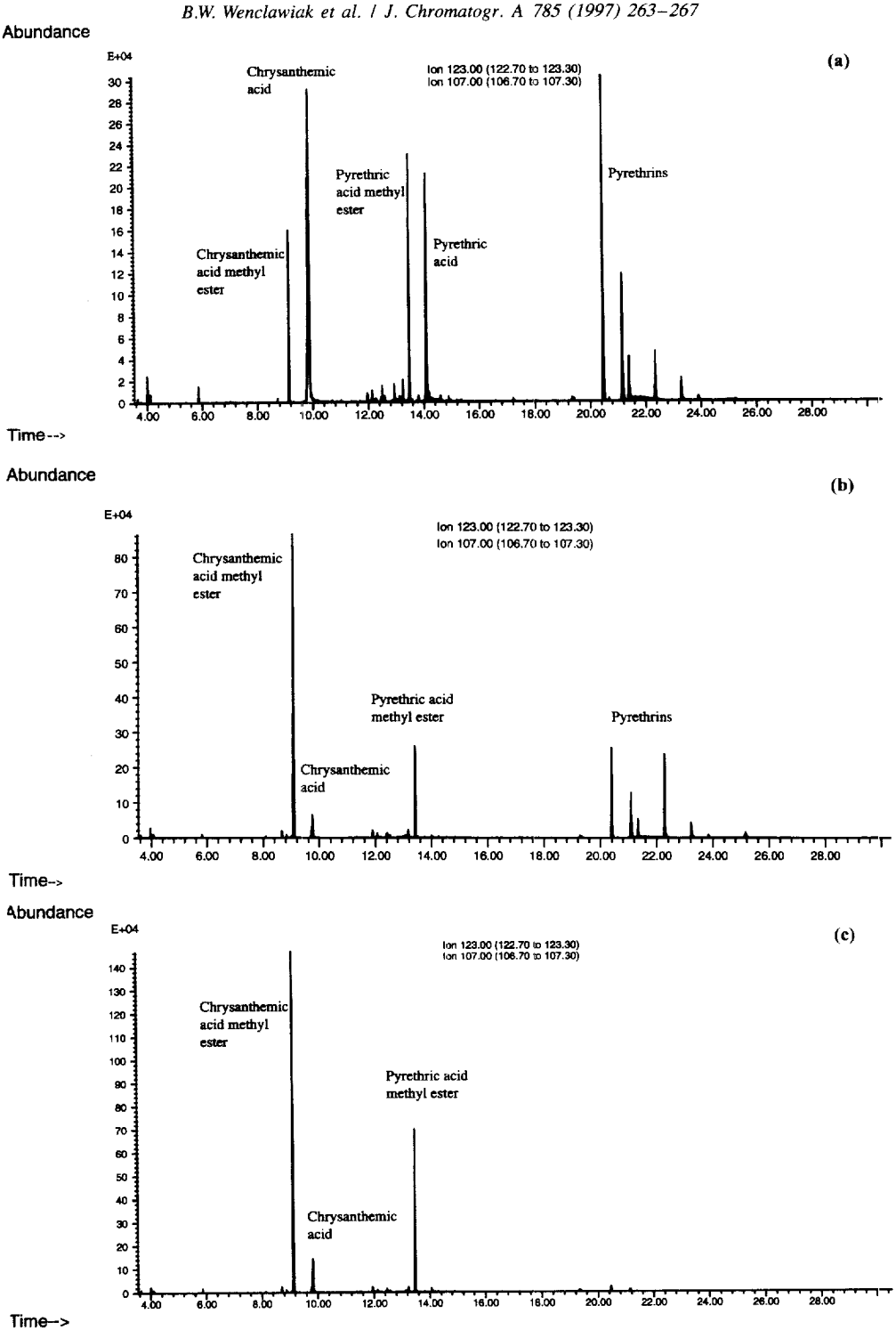


Fig. 2. (a) GC–MS extracted ion chromatogram of pyrethrum after treatment at 250°C for 1 h; (b) GC–MS extracted ion chromatogram of pyrethrum after treatment at 250°C for 1 h in the presence of acidic alumina; (c) GC–MS extracted ion chromatogram of pyrethrum after treatment at 270°C for 1 h in the presence of acidic alumina.

Table 1
Rate of conversion of the natural pyrethrins to chrysanthemic acid (CA) or methyl ester (CAME) at different reaction times

| Reaction time (min) | Sum of CAME and CA (%) | Sum of Pyrethrin I (%) |
|---|---|---|
| 10 | 64.9 | 35.1 |
| 20 | 75.6 | 24.4 |
| 30 | >98 | <2 |
| 60 | >98 | <2 |

the remaining acid can also be quantitated and added to the total amount of the chrysanthemates.

So far the reaction was almost complete within 1 h. At 270°C reaction temperature, the relative standard deviation (R.S.D.) for 1-h reaction time was 16% ($n=3$) for the whole procedure.

Decreasing the reaction time to 30 min did not reduce the conversion rate of the pyrethrins. For these experiments the R.S.D. was 14% ($n=3$). Reaction times of less than 30 min (10 and 20 min) were also evaluated but the reaction of the chrysanthemates was not complete (Table 1).

## 4. Conclusion

The reproducible conversion of the natural pyrethrins into their methyl ester is one possibility for an exact total determination. Nevertheless, a differentiation in chrysanthemates and pyrethrates is possible. Such a method inherits the advantage of fast chromatography and exact quantification. Because the thermal conversion occurs on the alcohol side of the ester molecule which is replaced by the methyl group by transesterification, the separation and quantification is no longer a problem. Quantification with standard substances will also be possible, because the synthesis of the chrysanthemum acid methyl ester and of the pyrethrum acid methyl ester is relatively easy to perform.

Currently, the procedure reported is being studied for use as an analytical method. Additionaly, pyrethroids, which can also form chrysanthemum acid methyl esters are currently being examined.

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