

Short communication

Supercritical fluid chromatography–mass spectrometry of thyme extracts (*Thymus vulgaris* L.)¹

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Abstract

Capillary supercritical fluid chromatography–mass spectrometry (SFC–MS) has been applied to the analysis of essential oils and commercial supercritical carbon dioxide extracts of thyme (*Thymus vulgaris* L.). A new SFC–MS interface and an injection technique using a retention gap have been applied successfully to separate and identify components of the extracts. © 1997 Elsevier Science B.V.

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1. Introduction

The introduction of capillary columns has improved the separation efficiency of supercritical fluid chromatography (SFC). The linkage of such a SFC device with a mass spectrometer (SFC–MS) enables in most cases a reliable identification of the separated components. However, due to the interface, which has to be used in SFC–MS couplings, part of the separation efficiency of the chromatographic system is lost especially when compared to SFC separations with flame ionization detection [1–7].

Several interfaces for SFC–MS have been described in literature in the last 15 years [1–6]. Using the recently developed interface [8] based on a heat-pipe principle with a stable temperature ($\pm 1^\circ\text{C}$), supercritical conditions can be maintained within the

full length of the interface, which is an advantage in comparison to the formerly used Lee SFC–MS interface. By means of this interface, SFC–MS coupling was considerably improved.

A further problem is the detection of minor constituents, due to the limited injection volume and the high amount of carbon dioxide restricting the sensitivity of the mass spectrometer [8]. The installation of a retention gap allowed the injection of a larger sample volume [9] on the analytical column, as a result of a better separation of the used solvent from the components being analysed, improving their MS identification.

2. Experimental

2.1. Chemicals

Carbon dioxide SFC/SFE grade was obtained from Messer-Griesheim (Duisburg, Germany) and

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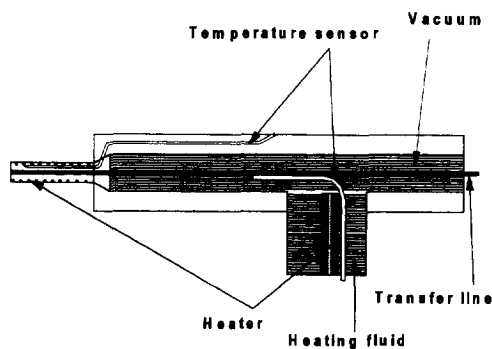


Fig. 1. The SFC-MS interface.

n-hexane (analytical-reagent grade) from Merck (Darmstadt, Germany).

2.2. Samples

Commercial selective and total supercritical carbon dioxide extracts of thyme (*Thymus vulgaris* L.) were obtained from Flavex (Rehlingen, Germany). Essential oil of thyme (*Thymus vulgaris* L.) was from Kaders (Hamburg, Germany).

2.3. SFC-MS equipment

Supercritical separations were carried out using a supercritical fluid chromatograph Dionex series 600 (Idstein, Germany) equipped with a pneumatically-controlled loop injector from VICI AG Valco Europe (Schenkon, Switzerland) with a 0.2- μ l loop, and a

heatable SFC-MS interface (Fig. 1) from Mplus (Bremen, Germany), a SFC-MS frit restrictor from Dionex. MS was conducted on a Finnigan MAT (Bremen, Germany) Model 4500 quadrupole mass spectrometer with 70 eV. The multiplier was set at 1.3 kV and the source temperature was 120°C; scans (1.14 scans per second) from m/z 45 upward to eliminate the huge CO₂ peak at m/z 44.

A 4.40-m uncoated deactivated fused-silica capillary from BGB-Analytik (Rothenfluh, Switzerland) was used as a retention gap (Fig. 2). A 0.4-m section of this capillary was operated at room temperature (subcritical condition) and 4 m at 100°C oven temperature (supercritical condition). The separation column was a 10 m \times 50 μ m I.D. SB-Methyl-100 fused-silica capillary with 0.25 μ m film thickness from Dionex.

The SFC conditions were: oven temperature 100°C isothermal; injector temperature 25°C; injection time: essential oil and selective extract (neat) 100 ms, selective extract dilution (50%) in *n*-hexane 200 ms and total extract dilution (10%) in *n*-hexane 1 s; pressure program: 1 min 80 bar, increase of 3 bar/min to 150 bar, then increase of 20 bar/min to 200 bar, and then isobar; transfer line 100°C, and restrictor 225°C.

3. Results and discussion

By means of the applied retention gap (Fig. 2) and the newly developed SFC-MS interface (Fig. 1), the separation efficiency and the sensitivity of a SFC-

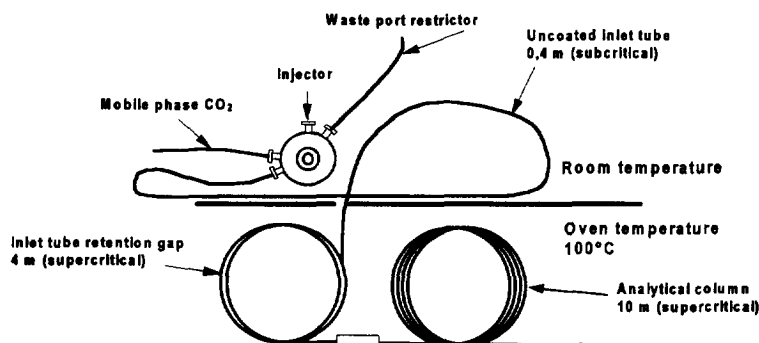


Fig. 2. The applied retention gap.

MS-coupling could be improved considerably in comparison to devices used before [8]. The applied retention gap allowed to inject the tenfold volume. Most of the solvent *n*-hexane could be separated on this retention gap (cf. Fig. 3a,b). The sensitivity of the base peak of *p*-cymene (m/z 119) increases from 188 ion counts obtained by injection without retention gap (Fig. 4a) to 1608 ion counts obtained by injection with retention gap (Fig. 4b). The new technique was suitable for the analysis of carbon dioxide extracts as well as essential oils from aromatic plants. In Fig. 5a and b, the reconstructed ion current (RIC) SFC–MS chromatogram of the carbon dioxide thyme extract dissolved in *n*-hexane is illustrated. The investigation of extracts from *Thymus vulgaris* revealed the presence of *p*-cymene and thymol as main constituents and besides other terpenoids, carvacrol methyl ether as less than 1% of the extract (Fig. 5a,b). The depicted mass spectra

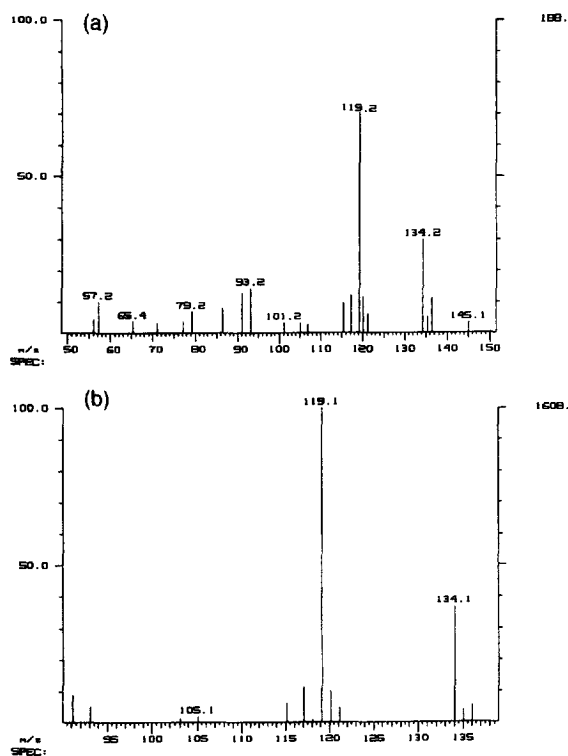


Fig. 4. (a) MS of *p*-cymene obtained by injection without retention gap. (b) MS of *p*-cymene obtained by injection with retention gap.

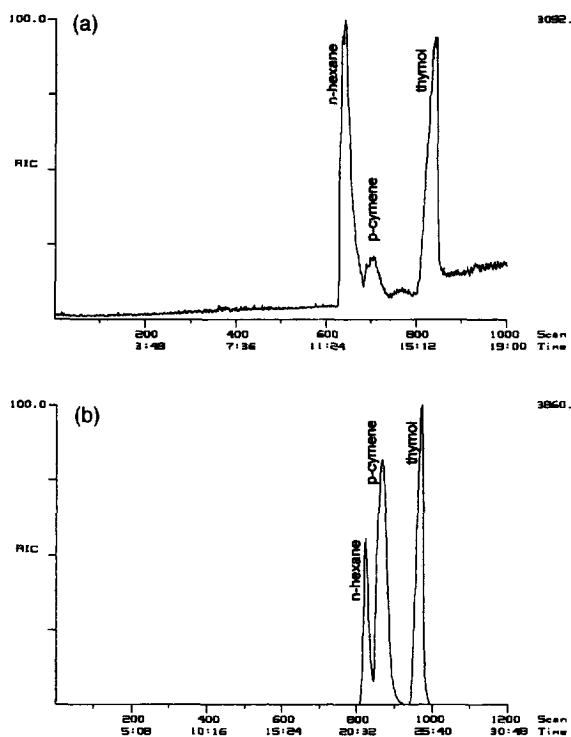


Fig. 3. (a) RIC SFC–MS chromatogram of thyme extract dissolved in *n*-hexane (10%) obtained by injection without retention gap. (b) RIC SFC–MS chromatogram of thyme extract dissolved in *n*-hexane (10%) obtained by injection with retention gap.

(Fig. 4b, Fig. 6a,b) are in good agreement with spectra obtained by GC–electron impact ionization (EI) MS from reference compounds and libraries. The commercially available MS data bases can be used, therefore, for the identification of components from a SFC–MS run. The high abundance of the carbon dioxide peak at m/z 44 generally limits the lower mass range in SFC–MS to approximately 45 u. For this reason, EI spectra obtained from SFC–MS experiments show less information than the respective GC–EI–MS spectra.

The separation of the monoterpene hydrocarbons was very poor (Fig. 5a,b), while oxygenated monoterpenes and sesquiterpene hydrocarbons could be separated sufficiently. In addition, numerous spectra of high-boiling components were obtained, exhibiting an advantage of SFC–MS when compared to GC–MS. Non-volatile constituents of carbon dioxide extracts could be eluted by SFC in contrast to GC without difficulties. The viscous total extract of

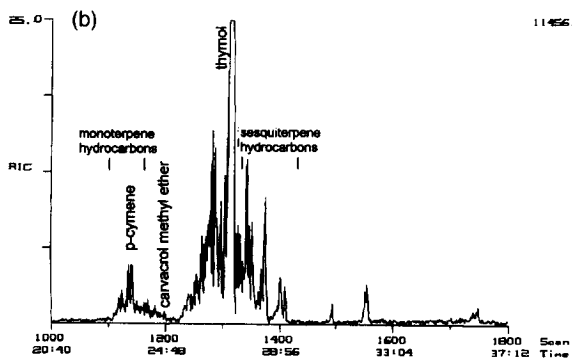
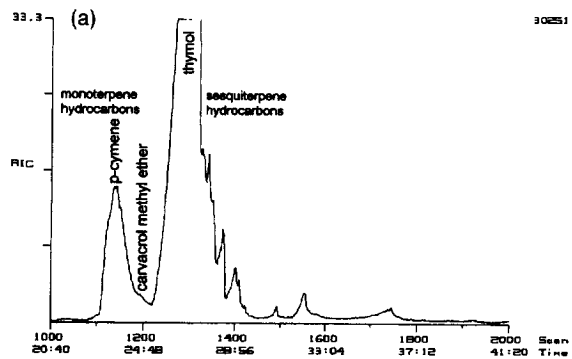


Fig. 5. (a) RIC SFC-MS chromatogram of thyme extract dissolved in *n*-hexane (50%) obtained by injection with retention gap. (b) RIC SFC-MS chromatogram (enhanced) of thyme extract dissolved in *n*-hexane (50%) obtained by injection with retention gap.

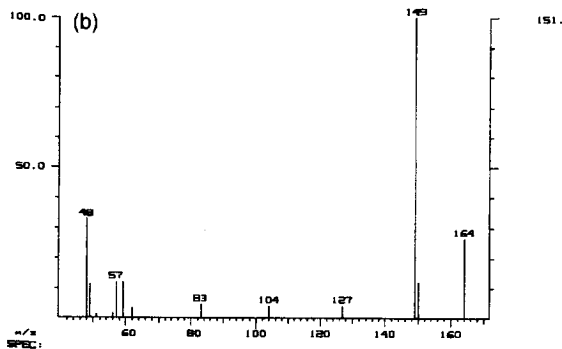
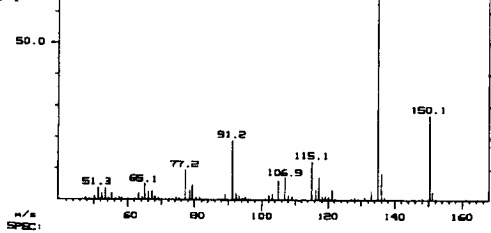


Fig. 6. (a) MS of thymol. (b) MS of carvacrol methyl ether.

thyme had to be injected after dilution dissolved in a suitable solvent, e.g. *n*-hexane. The undesired modifier effect of the applied solvent could be reduced by the use of the retention gap.

4. Conclusion

Optimization of the formerly applied SFC-MS technique [8] using a retention gap improved the separation from the solvent and the identification of components. The modified SFC-MS technique was successfully applied to investigate essential oils and carbon dioxide extracts of thyme.

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