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Review

Supercritical fluid chromatography–proton nuclear magnetic resonance spectroscopy coupling[1]

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Abstract

The direct on-line coupling between supercritical fluid chromatography (SFC) and supercritical fluid extraction (SFE) and proton high-field nuclear magnetic resonance (NMR) spectroscopy is described. The resolution of the ^{1}H NMR spectra obtained in SFC–NMR and SFE–NMR coupling experiments under continuous-flow conditions approaches the resolution of conventionally recorded NMR spectra. Due to the supercritical measuring conditions the proton spin-lattice relaxation times T_1 are increased two times at least. A pressure gradient applied for separation purposes results in an highfield shift of ^{1}H NMR signals. SFC–NMR separations performed with plastifiers, acrylates and vitamins show the broad application range of the technique. SFE–NMR investigations have been performed with natural products as well as with polymers. © 1997 Elsevier Science B.V.

Keywords: Reviews; Nuclear magnetic resonance spectroscopy; Detection, SFC; Vitamins; Acrylates; Phthalates

Contents

[1]Dedicated to Professor Anton Rieker on the occasion of his 65th birthday.

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1. Introduction

The hyphenation between high-performance liquid chromatography and nuclear magnetic resonance spectroscopy is now a well accepted analytical technique in academia and industry [1–14]. A broad number of applications is outlined in recent review articles [9,11,12,14] demonstrating the enormous power of combining a separation technique together with a structure elucidation method. Apart from the possibilities of structural assignments, the advantage of direct coupling chromatographic separation methods with ^{1}H NMR spectroscopy is the timesaving nature of this approach. While in traditional off-line separations, different fractions have to be collected and then prepared for the NMR experiment, in on-line experiments separation and detection happen almost simultaneously.

Nevertheless, one remaining problem arises from the use of non-deuterated proton-containing HPLC solvents, i.e. the suppression of the solvent signal, which imposes the search for proton-free solvents. One possibility to get rid of all the problems encountered with solvent signals is to use a separation technique employing non-protonated solvents. In principle this technique could be gas chromatography with NMR detection in the gaseous state. Thus on-line coupling experiments of GC–NMR have been performed by analyzing a mixture of terpenoic compounds with boiling points as high as 473 K [15,16]. Because a phase transfer to the gaseous state is not suitable for sensitive compounds this approach was not further developed. Alternative separation methods could be either adsorption chromatography with halogenated solvents or supercritical fluid chromatography with supercritical CO_2. Adsorption chromatography–NMR coupling experiments have been conducted in the early days of LC–NMR [15] but did not find wide application. SFC has been considered as a niche separation technique for a long time but through the introduction of commercial SFC instruments with packed columns it gained more attention recently. Due to the enhanced diffusion in the supercritical state SFC separations can very often be performed within a 10-min time frame. There is an ongoing debate about the practical use of supercritical fluid chromatography against gas chromatography and high-performance liquid chromatography. Wheras a lot of chromatographers are sure that all existing separation problems may be solved by the use of GC and HPLC, other chromatographers still believe in the benefits of supercritical separation and detection [17–19]. At least one advantage of SFC and SFE is their hyphenation possibility with NMR detection. Because the mobile phase, supercritical CO_2, does not contain any proton solvent signal the whole ^{1}H NMR spectral range can be used for structural identification purposes [23].

2. Design of NMR probes suitable for the registration of NMR spectra in the supercritical state

To fit the requirements of acquiring NMR spectra in the supercritical state special pressure-stable continuous flow probes had to be developed. Due to the phase diagram of CO_2, NMR detection has to be performed at temperatures higher than 305 K and pressures higher than 73 bar.

In contrast to conventional NMR measurements within a rotating cylindrical NMR tube, in continuous-flow NMR spectroscopy a continuous passage of flowing nuclei through the detection cell is taking place. This leads to a destinct residence time τ which is dependent upon the ratio detection volume/flow-rate effecting the spin–lattice and the spin–spin relaxation times in the flowing state: $1/T_{1\ \mathrm{flow}} = 1/T_{1\ \mathrm{static}} + 1/\tau$ and $1/T_{2\ \mathrm{flow}} = 1/T_{2\ \mathrm{static}} + 1/\tau$ [5,24]. As a general rule, the residence time τ in the flow cell during an on-line separation experiment should be longer than 5 s leading to a tolerable line width broadening of 0.2 s. By applying a flow-rate of 1 ml/min for a routine SFC separation with detection

volumes higher than 120 μl no substancial flow broadening occurs.

A gain in sensitivity may be obtained if Boltzmann distribution is established before the nuclei enter the flow cell [24,25]. This prepolarization of nuclei can be accomplished if the effluent in the chromatographic column is allowed to spend a certain time in the magnetic field, which should be three times longer than the static T_1 value. Thus a direct insertion of the chromatographic column within the magnet or the use of a premagnetization coil [24,25] within the probe would be the matter of choice.

There exist several designs of high pressure NMR cells for supercritical fluids [20–22], but Dorn et al. [23] developed the first approach for the NMR on-line monitoring of chromatographic separations under supercritical conditions.

Fig. 1 shows the diagram of a SFC–NMR probe developed by Dorn et al. [23] capable of operating at pressures up to 100 bar and temperatures up to 373 K. The main feature of this pioneering design is the combination of a premagnetization volume for the equilibration of the Boltzmann magnetization together with a pressure-stable flow cell. A 0.25 inch O.D. alumina ceramic tube used for the observation cell was connected to the stainless steel tubing of the equilibration coil (1-ml internal volume) with brass Swagelok reducing fittings, the brass ferrules were replaced with a graphite/Vespel blended ferrule. Thus easy interchange of ceramic cells with different internal diameter tubes could be performed. Detection volumes range between 20–120 μl, a ceramic tube with an internal diameter of 1.6 mm resulting in a detection volume of 20 μl was used for most SFC–NMR experiments. Rapid and stable temperature equilibration of the flow cell is obtained by air flow-rates in the order of 20 ml/min.

With the described experimental design, a static NMR resolution of 1.4 Hz could be obtained, whereas under continuous-flow separation conditions of 2.0 ml/min, signal line widths in the order of 4–5 Hz were observed.

An alternative approach is shown in Fig. 2 [26].

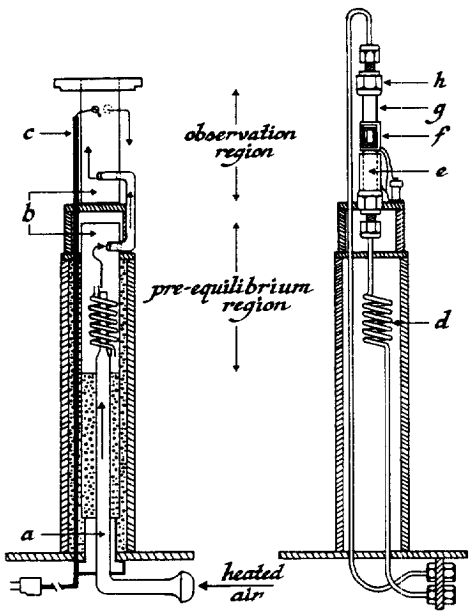

Fig. 1. Schematic diagram of a flow probe for use in directly coupled SFC–NMR: (a) insulated glass transfer line, (b) glass inserts; (c) Cu/constantin thermocouple, (d) stainless steel equilibrium coil, (e) brass shield, (f) Helmholtz coil, (g) ceramic flow cell, (h) brass Swagelok fitting.

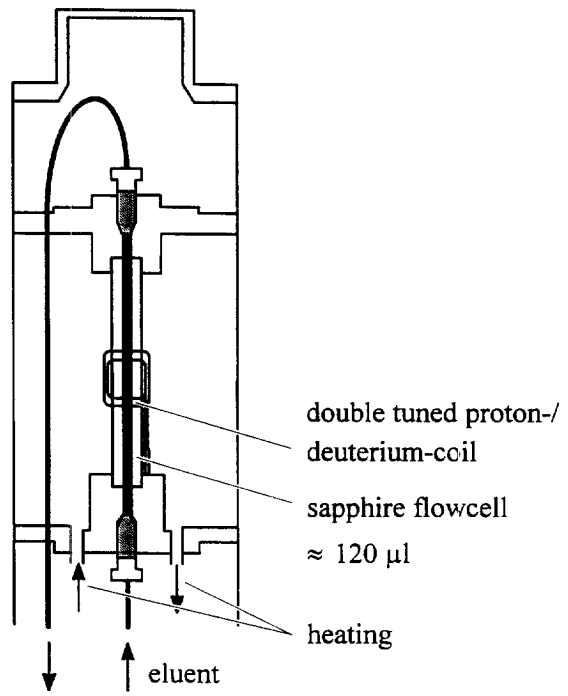

Fig. 2. Schematic diagram of a continuous-flow ^{1}H NMR probe suitable for supercritical state detection.

This design is derived from continuous-flow probes used for liquid state detection [1,24]. The inner glass tube of the originating HPLC–NMR probe is substituted with a sapphire tube (O.D. 5 mm, I.D. 3 mm, detector volume 120 μl) whereas the PEEK capillaries used in the HPLC–NMR probe are replaced by Titan tubings. A double tuned proton–deuterium coil is directly fixed to the sapphire flow cell. The whole arrangement is centered in the glassware of a conventional probe body, in which a thermocouple is inserted, allowing the execution of well-defined temperature-dependent measurements.

The non-rotation of the sapphire detection cell together with the ratio between the detector volume and the employed flow-rate determine the resulting NMR signal line widths. Whereas with rotation of the NMR tube in a conventionally NMR probe, the signal line width of chloroform at the height of the ^{13}C satellites in degassed acetone-d_6 is about 3–4 Hz, SFC continuous-flow probes show values in the order of 15–18 Hz in the liquid and in the supercritical state (Fig. 3). This hump test also indicates that there is no change in signal line width in the ^{1}H NMR spectra in the liquid and the supercritical state.

3. Experimental set-up for SFC–NMR on-line coupling

As an example for coupling an NMR spectrometer with a SFC separation system, a feasible experimental set-up is outlined in Fig. 4. The SFC system is located at a distance of 2 to 2.5 m besides the cryomagnet. Here, the chromatographic system is assembled from commercially available GC and HPLC components. The pump heads of the CO_2 pump are cooled to approximately 275 K with a cryostat. CO_2 is supplied from a gas cylinder with diptube and is cooled through 2 m of steel capillary positioned in the cryostat. Column heating is performed with a GC oven. To maintain supercritical conditions in the NMR detection cell, a back pressure regulator is connected to the outlet of the NMR flow-cell. The back pressure regulator together with

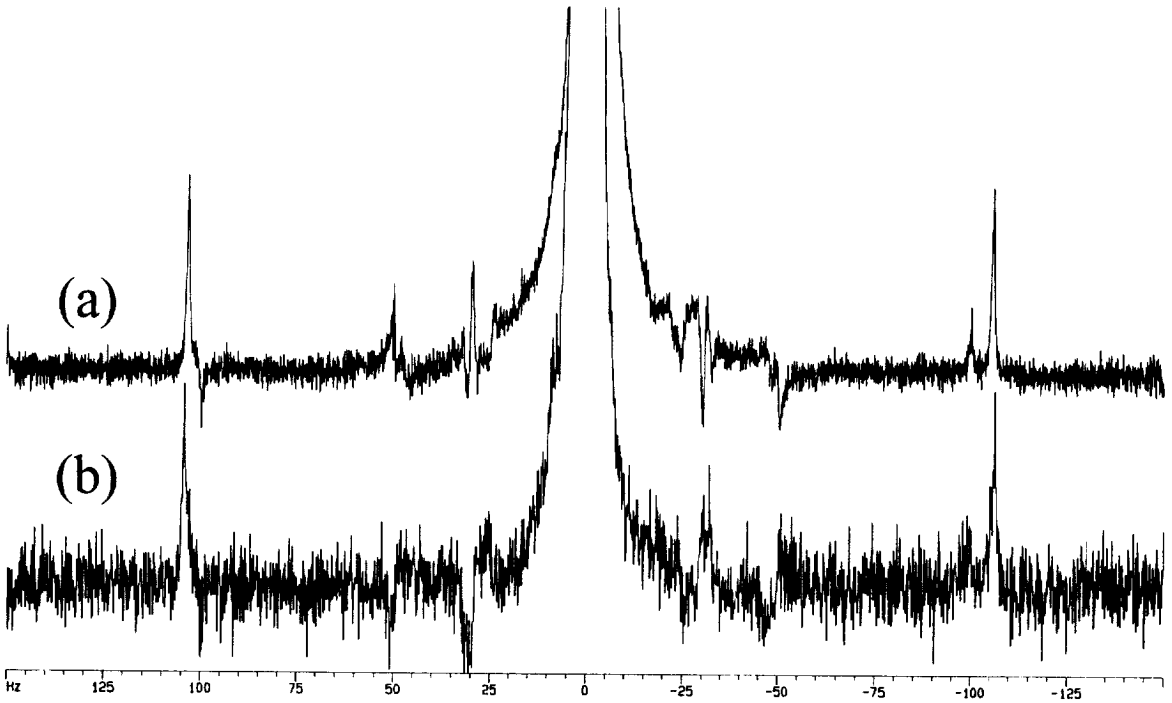

Fig. 3. Comparison of hump tests (400 MHz) under HPLC (a) and SFC (b) conditions.

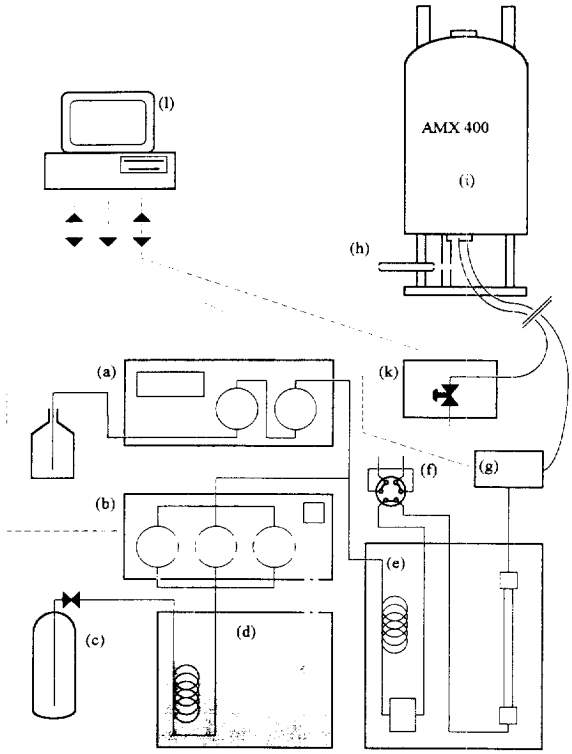

Fig. 4. Experimental arrangement for recording NMR spectra under supercritical conditions: (a) modifier pump, (b) CO_2 pump, (c) cryostat for precooling of CO_2 and pump heads, (d) CO_2 cylinder with dip tube, (e) GC oven with mixing chamber and steel capillary, (f) injection valve, (g) UV detector, (h) air supply for NMR probe heating, (i) NMR instrument with SFC probe, (k) back-pressure regulator, (l) personal computer for control of the chromatographic system.

the whole chromatographic separation system is controlled with a personal computer by a special program for analytical purposes.

3.1. NMR spectroscopy in the supercritical state

In accordance with the hump test spectra shown in Fig. 3, the stopped-flow ^1H NMR spectrum of ethylbenze (Fig. 5) recorded in supercritical CO_2 at a temperature of 323 K and a pressure of 161 bar proves that no degradation in resolution in the supercritical state can be observed. Because NMR signal line widths are reciprocal to the spin–spin relaxation time T_2, it is evident that T_2 for protons does not change dramatically in the supercritical state. This is not the case with respect to the spin–lattice relaxation time T_1.

^1H-T_1 values of benzyl-*n*-butylphthalate have been determined in the supercritical state (250 bar, 321 K), using the inversion recovery method with the standard ($180°-\tau-90°-T$) sequence. Values in the order of 20 s result for the aromatic protons, whereas methyl group protons together with the protons of the ester and methylene group exhibit a T_1 value of 8 s (Fig. 6). These values are two to three times higher than the corresponding values in the liquid state at the same temperature (see Fig. 6). This phenomenon can be explained by the decreased viscosity in the supercritical compared to the liquid state.

Separations in the supercritical state are very often performed with a pressure gradient. Fig. 7 shows the contour plot of the proton chemical shift of chloroform in the supercritical CO_2, (horizontal axis) versus the applied pressure (vertical axis). Within a time increment of 12 s, a pressure gradient starting at 90 bar up to 244 bar at a temperature of 325 K was applied. It is evident that due to the increasing density with increasing pressure the ^1H NMR signals shift to higher field. This behavior necessitates that only modest pressure changes can be performed during the acquisition of ^1H NMR spectra, otherwise a severe signal line broadening would result. Taking into account a pressure gradient from 115 bar to 180 bar within 20 min, actually used during a separation, up to 16 transients can be co-added introducing negligible line broadening.

The drift of the signal, as it can be seen in the contour plot, is in a first estimation proportional to the calculated density of the CO_2. The two steps are introduced by imperfect temperature control during the first experiments.

4. SFC–NMR applications

Supercritical fluid separations are performed using a pressure gradient and often by adding a polar modifier (e.g. methanol) to the supercritical eluent. At low pressures (<100 bar) supercritical CO_2 has the solvating power of aliphatic hydrocarbons, at higher pressures (about 400 bar) its solvating power is similar to dichloromethane.

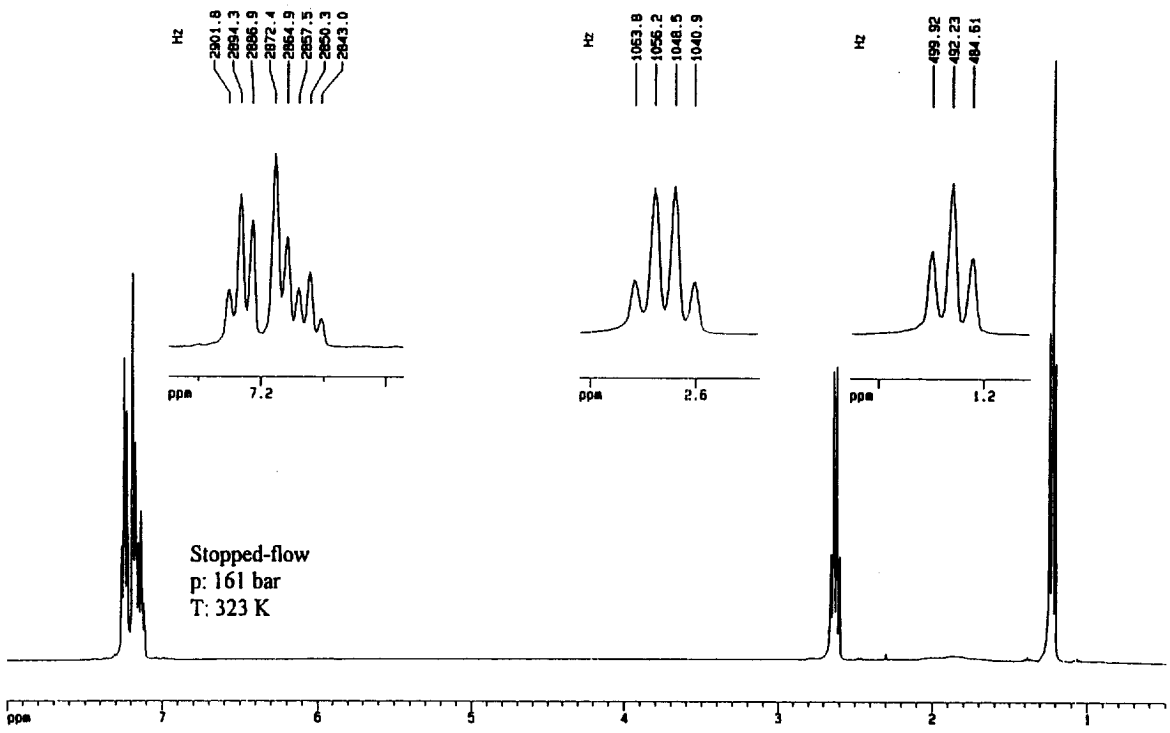

Fig. 5. ^{1}H NMR spectrum of ethylbenzene (400 MHz) in supercritical carbon dioxide.

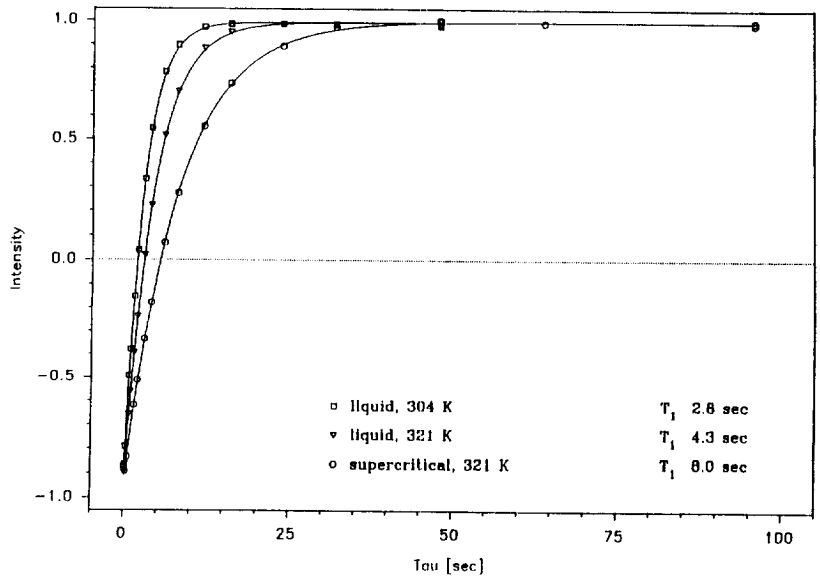

Fig. 6. T_1 values (400 MHz) of the methyl protons of benzyl-*n*-butylphthalate in the liquid state at two different temperatures and in the supercritical state.

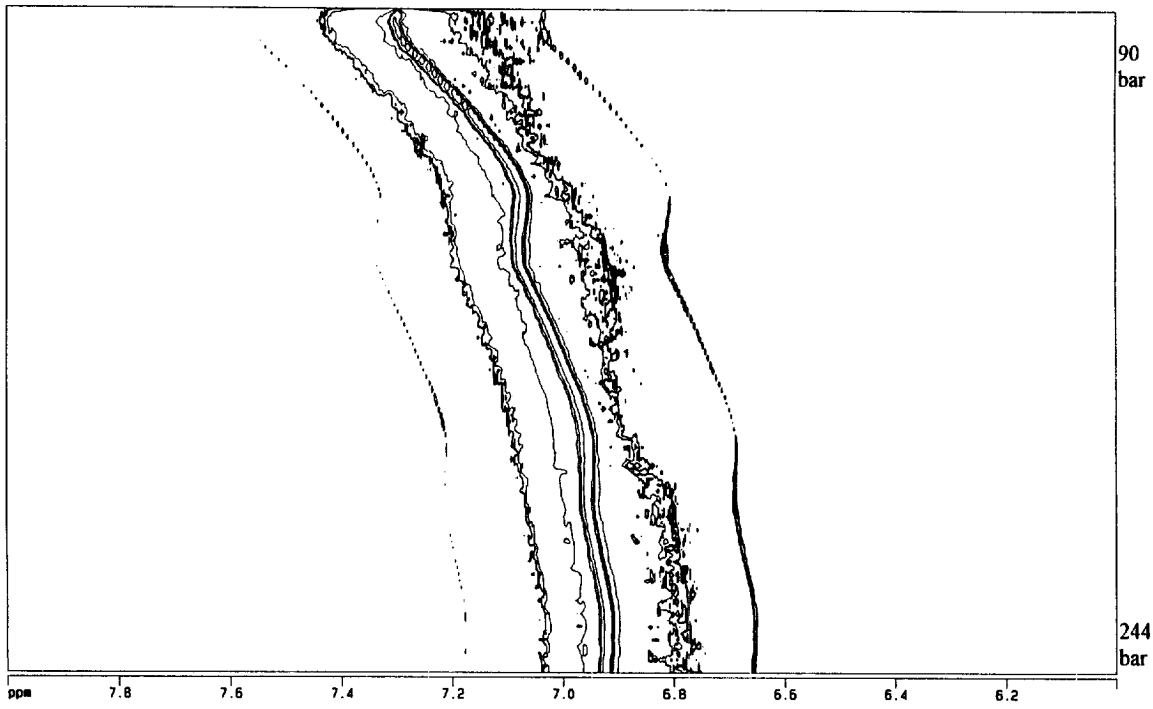

Fig. 7. Contour plot of the signal of chloroform in the supercritical state at a pressure gradient from 90 to 244 bar.

4.1. Hydrocarbons

The first practical example of an on-line SFC– ^{1}H NMR separation was recorded by Dorn et al. [23]. Since up to 90% of a fuel is aliphatic, SFC–NMR on-line analysis is the matter of choice for separation and identification. Fig. 8 shows the SFC– ^{1}H NMR elution profile of a fuel mixture, consisting of isooctane (2,2,4-trimethylpentane), *n*-hexane, *n*-nonane, dodecane and *n*-hexadecane. The SFC separation was accomplished with at a flow-rate of 2.0 ml/min, a C_{18} 250×4.6 mm column, at an isobaric

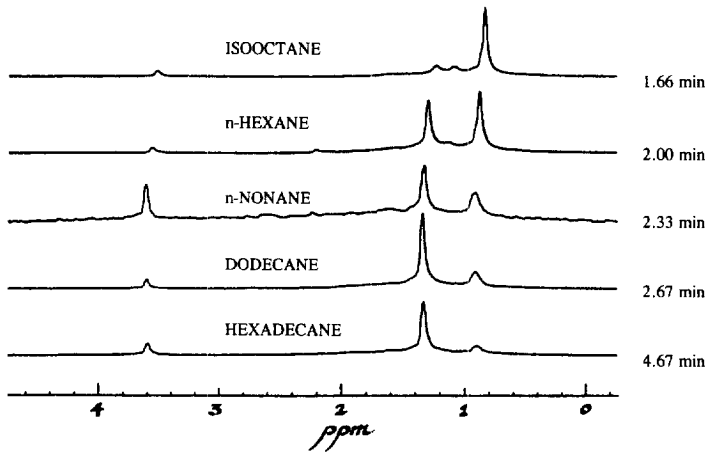

Fig. 8. SFC ^{1}H NMR elution profile (200 MHz) of a fuel model mixture.

pressure of 100 bar and a temperature of 323 K using CO_2 with 1% (w/w) CD_3CN as solvent. Each NMR spectrum shown (2 K points taken over a 2000 Hz bandwidth) consists of 20 coadded transients at an acquisition time of 1 s per transient. The total separation occured within only 5 min. The first eluting isooctane can be easily identified by the methylene to methyl signal ratio, which is increasing in the spectra of the later eluting compounds. Thus, longer elution times are consistent with an increasing chain length of the separated hydrocarbon.

4.2. Phthalates

4.2.1. Continuous-flow measurements

Fig. 9 shows the contour plot of a separation of five plastifiers recorded at a 400 MHz spectrometer with a 120-μl flow cell [26]. The separation was carried out in supercritical CO_2 with a bonded C_8 stationary phase at a flow-rate of 2.0 ml/min employing a linear pressure gradient from 115 to 180 bar in 20 min at a temperature of 353 K. Besides an impurity resonance at 1.25 ppm, the whole spectral range is not obscured from any solvent signals. Fig. 10 shows the corresponding continuous-flow ^1H NMR spectra of all separated compounds and Fig. 11 displays the expanded ^1H NMR spectrum of diallylphthalate. Even small long-range coupling constants in the order of 1.4 Hz can be determined. Thus it is possible to obtain high-resolution ^1H NMR spectra with the experimental arrangement outlined in Fig. 4. On the other hand, the drawbacks already described must be taken into account in the acquisition process of SFC–NMR spectra. One is the increase in spin-lattice relaxation times T_1 due to the reduced viscosity of the supercritical mobile phase. The other is the fact that with increasing pressure the susceptibility of

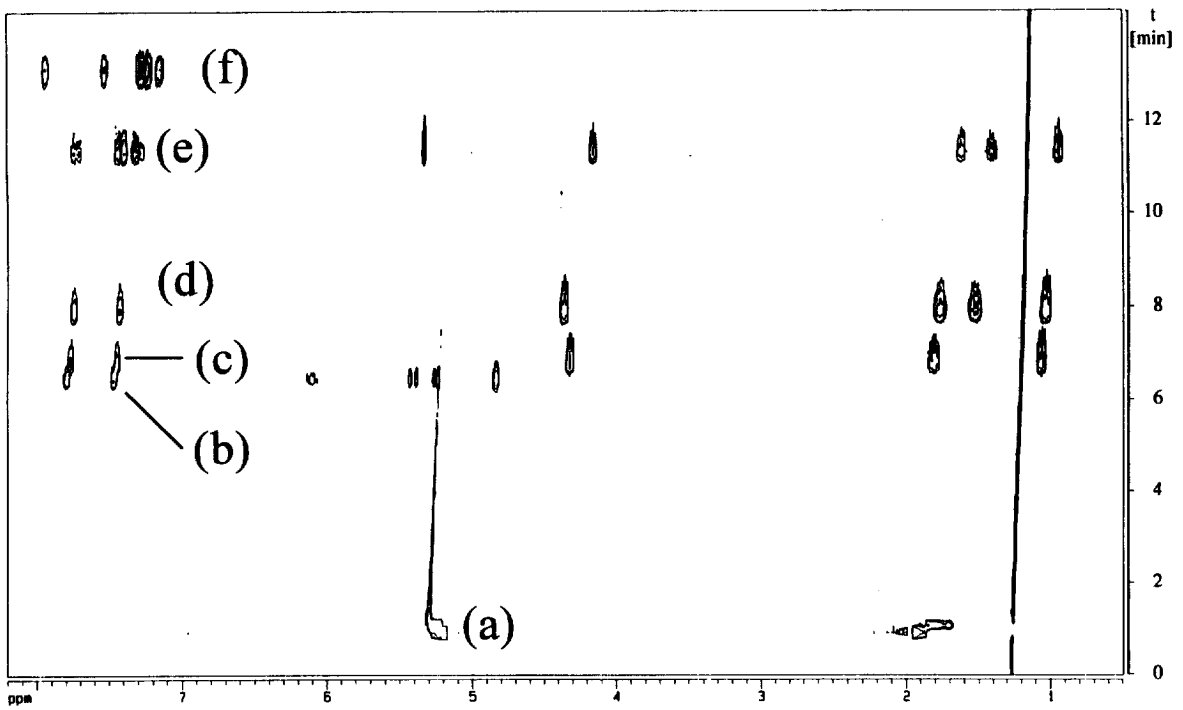

Fig. 9. ^1H NMR chromatogram (contour plot, 400 MHz) of a SFC separation of 5 plastifiers in supercritical CO_2: (a) CH_2Cl_2, (b) diallylphthalate, (c) di-*n*-propyl-phthalate, (d) di-*n*-butylphthalate, (e) benzyl-*n*-butylphthalate, (f) diphenylphthalate.

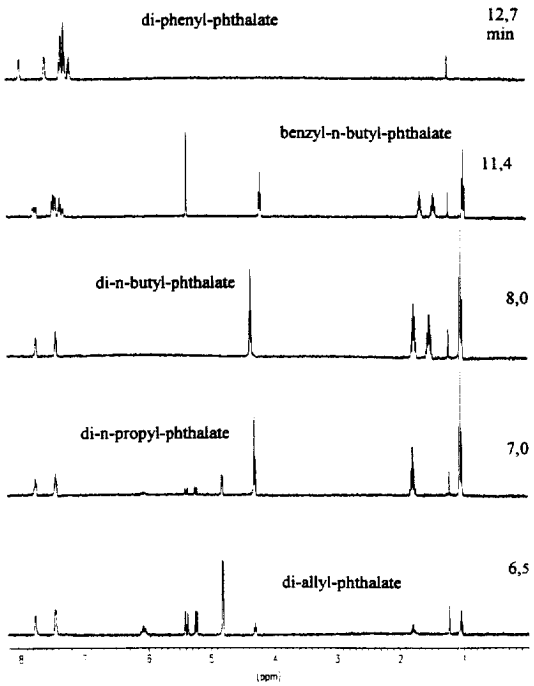

Fig. 10. Continuous-flow ^{1}H NMR spectra (400 MHz) of 5 plastifiers recorded during a separation in supercritical CO_2.

the supercritical CO_2 changes causing a high-field shift of NMR signals.

4.2.2. Stopped-flow measurements

Signal assignment in routine NMR spectroscopy is usually faciliated by the application of two-dimensional assignment techniques such as homonuclear proton–proton shift correlated spectroscopy (^{1}H^{1}H COSY) [27].

Fig. 12 shows the contour plot of one constituent of the phthalate separation. Here the dead volume between the UV detector and the SFC flow cell was determined before separation. After an adequate delay after the occurence of the UV maximum of benzyl-*n*-butylphthalate in the UV detector, the SFC separation was stopped and the two-dimensional acquisition was started. The pressure proved to be stable for several hours, which was sufficient for the acquisition of the two-dimensional COSY spectrum. Despite the intense signal of undeuterated methanol, which was used as a modifier in the SFC separation, the ^{1}H NMR signal connectivities are clearly visible. The corresponding cross peaks in the aromatic system and the aliphatic part of the butyl chain are indicated in the contour plot. This example proves that even under the exotic supercritical fluid conditions, elaborate two-dimensional assignment techniques can be used.

4.3. Acrylates

In an on-line SFC–NMR separation employing a pressure gradient up to 16 transients can be coadded without affecting the NMR resolution. In contrast for isobaric separations, the minimum number of transients is only determined by the concentration of the separated compound. Fig. 13 shows the contour plot of the separation of four acrylates in supercritical CO_2 with 1% of methanol-d_4 used as polar modifier [28]. A 4.6×250 mm cyanopropyl column was used with a flow-rate of 1.0 ml/min employing isobaric conditions (80 bar) for the first 7 min of the separation, a succeeding pressure ramp of 20 bar/min and final isobaric conditions at 100 bar. Throughout the separation, the temperature of the oven was kept constant at 333 K. 8 transients per row were coadded resulting in a time resolution of 6.4 s. The residual ^{1}H NMR signals of methanol at 0.8 and 3.45 ppm show the prescribed high field shift during the pressure ramp between retention times of 7–8 min.

At the left vertical axis of the contour plot, a one-dimensional chromatogram is reconstructed by coadding all appearing ^{1}H NMR signals. This NMR mass chromatogram corresponds to a one-dimensional UV or refractive index chromatogram. The second peak consists of methylmethacrylate and ethylacrylate which is evident from the different chemical shifts provided from the on-line experiment. A clear differentiation is available from the chemical shift of the methyl groups of both compounds. The chemical shift at 1.9 ppm indicates the methyl group at the double bond of methylmethacrylate (peak a) whereas the signal at 1.22 ppm is from the terminal methyl group of ethylacrylate (peak b). Thus the second dimension of the SFC–NMR run, provided by the ^{1}H chemical

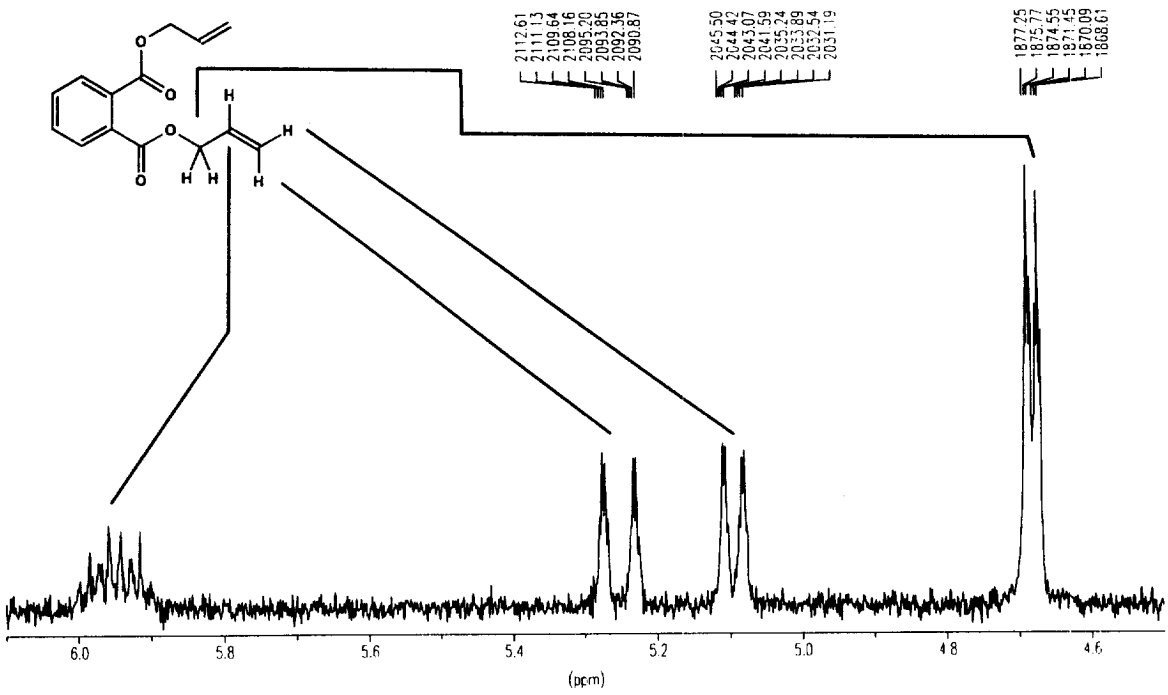

Fig. 11. Continuous-flow ^{1}H NMR spectrum (400 MHz) of diallylphthalate in supercritical CO_2.

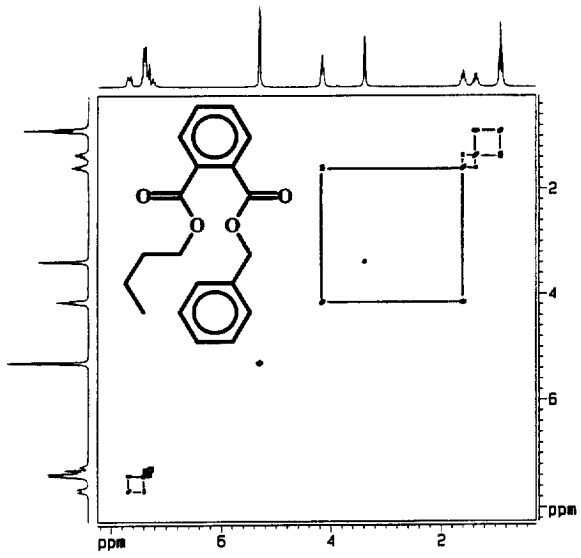

Fig. 12. Contour plot (400 MHz) of a two-dimensional proton–proton shift correlated experiment (COSY 45) of benzyl-*n*-butyl phthalate in supercritical CO_2.

shifts, enables the separation of coeluting compounds.

The spectral quality of the continuous-flow spectra in the supercritical state obtained in an on-line separation is demonstrated in Fig. 14. With the exception that the terminal methyl group signal of *n*-butylacrylate is hidden under the signal of the modifier, the ^{1}H NMR spectrum of Fig. 14 clearly shows that all essential features for structure determination can be derived.

4.4. Vitamin A acetate isomers

Vitamin A and its derivatives are essential for the human organism and play an important role in medical, pharmaceutical and food technologies. Due to the number of conjugated double bonds, there exist several *cis/trans*-isomers which are readily transformed into each other. The biological activities of the isomers differ remarkably, therefore the characterization of the isomers occuring either in biological systems or being formed by technical production is desirable. Therefore all-*trans* vitamin

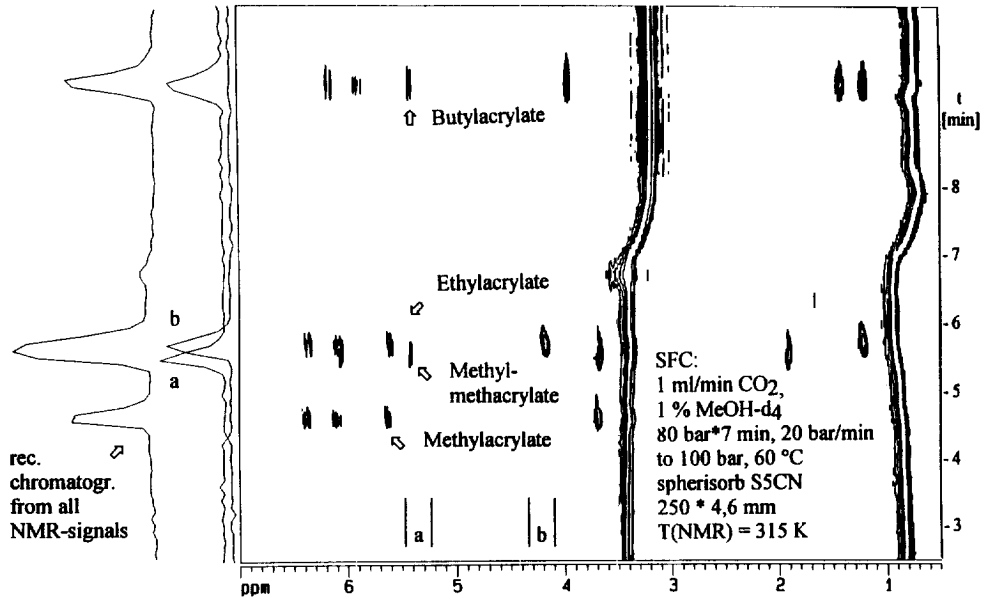

Fig. 13. ^{1}H NMR chromatogram (contour plot, 400 MHz) of a SFC separation of 4 acrylates in supercritical CO_2 with 1% methanol-d_4.

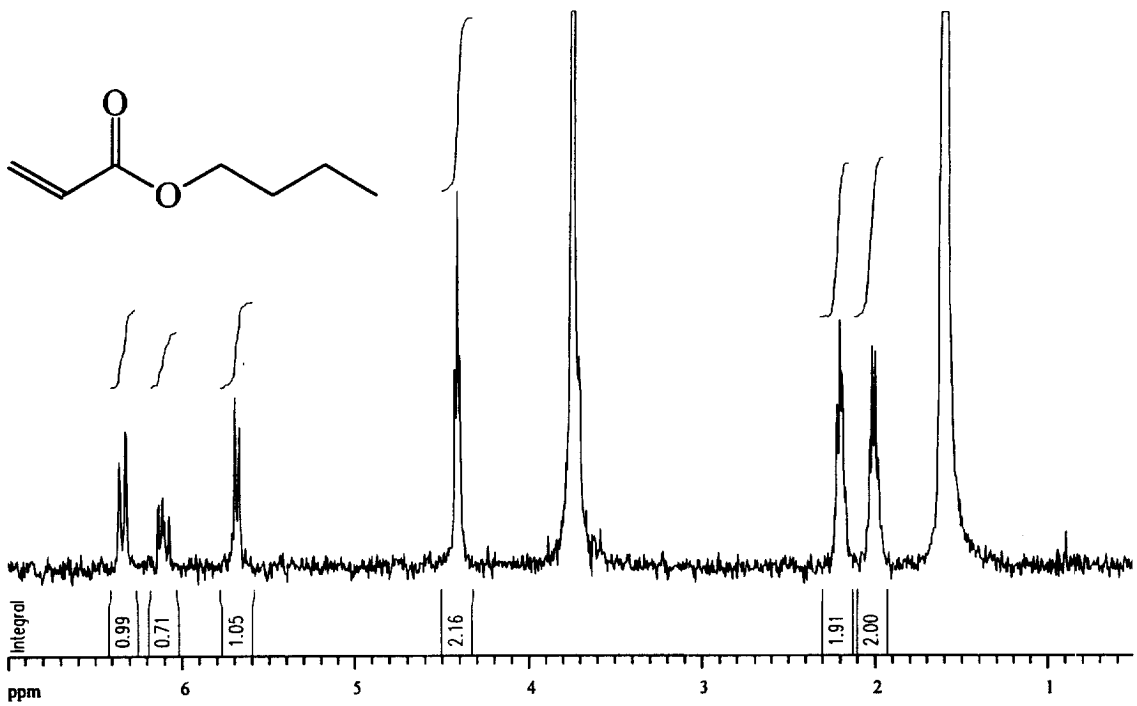

Fig. 14. Continuous-flow ^{1}H NMR spectrum (400 MHz) of *n*-butylacrylate in supercritical CO_2 with 1% methanol-d_4.

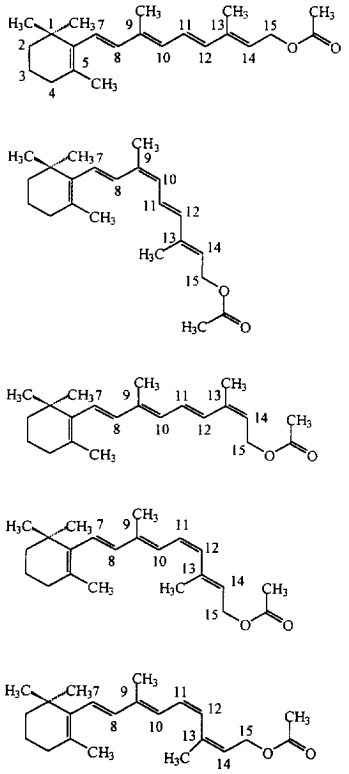

Fig. 15. Structure of the five main isomers of vitamin A acetate.

A acetate together with its four main *cis/trans* isomers (structures shown in Fig. 15) is an ideal system for the demonstration of the capabilities of the SFC–NMR separation–detection technique [29]. The suitability of HPLC ^{1}NMR coupling for structural assignment of vitamin A acetate isomers has been reported previously [30], although *n*-heptane has been used as solvent, which is not ideal from the NMR spectroscopic point of view.

A mixture of vitamin A acetate *cis/trans* isomers was separated with a C_{18} column under isobaric conditions at 333 K at a flow-rate of 0.65 ml/min using CO_2 as eluent [29]. 20 μl of a 30% (m/v) solution of thermally isomerized vitamin A acetate in $CDCl_3$ was injected. 16 transients have been accumulated defining a time resolution of 17.7 s. In an overall acquisition time of 37.5 min, 128 spectra were recorded. The time interval between 10 and 24 min is shown in the contour plot in Fig. 16. By the first evaluation, four groups of ^{1}H NMR signals which are clearly separated by their different elution times can be distinguished. For the following discussion, one-dimensional proton spectra were generated from the on-line plot which have been labeled as fraction 1 to 4. To improve the signal/noise ratio, all NMR spectra belonging to the same SFC peak have been coadded.

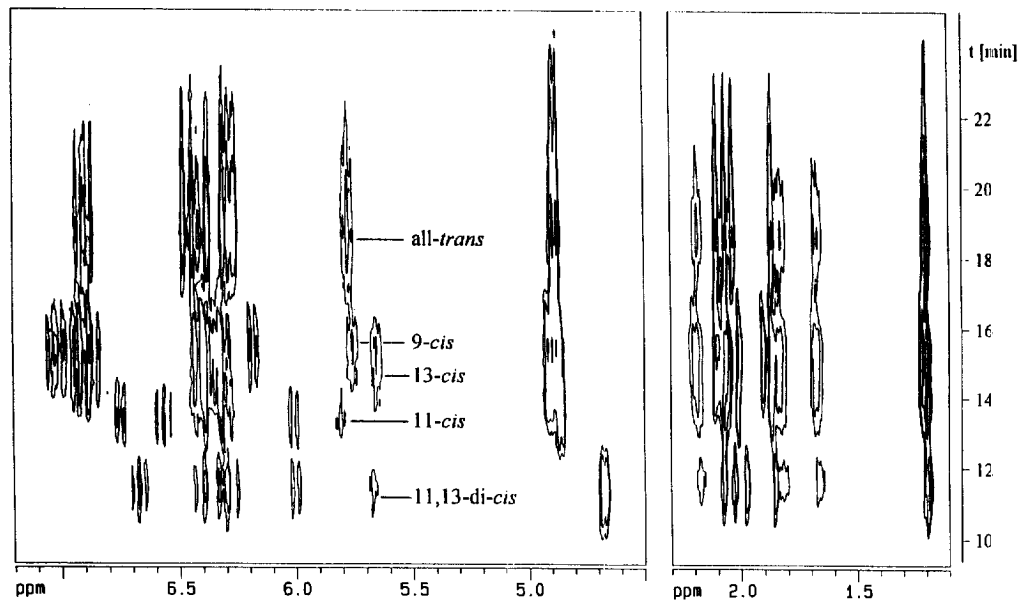

Fig. 16. ^{1}H NMR chromatogram (contour plot, 400 MHz) of a SFC separation of 5 vitamin A acetate isomers in supercritical CO_2.

Analysis of the olefinic part of the spectra leads to the assignment of the 11,13-di-*cis*, 11-*cis* and all-*trans* isomer to the fraction 1, 2 and 4, respectively (Fig. 17). To emphasize the improvement in information content of the spectra in contrast to the spectra acquired in *n*-heptane, the aliphatic region of the resulting spectra is depicted in Fig. 17. All the signals that could be assigned are indicated. It is evident that the structural differences of the *cis*/*trans*-isomers also show a considerable diagnostic effect on the chemical shifts of the aliphatic protons.

A close inspection of the spectrum of the third fraction reveals that it consists of two compounds. At the chemical shift of 4.9 ppm two different sets of methylene protons are apparent, exhibiting typical doublets for protons at C-15. The existence of two coeluting compounds is further proved by the analysis of the aliphatic region shown in Fig. 17.

There are two different signals of methyl groups at the C-1 atom of the ionone ring at 1.19 ppm. Around 2 ppm seven singuletts of methyl groups can be found. This can be explained with partly overlapping signals of four remaining methyl groups of two nearly coeluting isomers. For a more detailed determination the time dependance of the intensities of the ^{1}H NMR signals has been observed. All signals which are not overlapping at the spectroscopic axis can be separated into two groups according to the varying position of their maximum of intensity on the chromatographic axis, i.e. into signals belonging to an earlier and later eluting component. This leads to the identification of the 13-*cis* (#) and 9-*cis* isomer (*) respectively.

The advantage of using SFC as chromatographic method is depicted in Fig. 18. In the spectrum acquired during a HPLC ^{1}H NMR on-line run with *n*-heptane as solvent, the aliphatic region between 0.6 and 1.5 ppm is covered by the remaining signal of the *n*-heptane or distorted by the solvent suppression technique. Although the signals of the methyl groups at 1.7–1.8 ppm are observable, severe distortions from the presaturation of the solvent signals lead to deviations of the intensity ratios. Therefore the region that cannot be observed without distor-

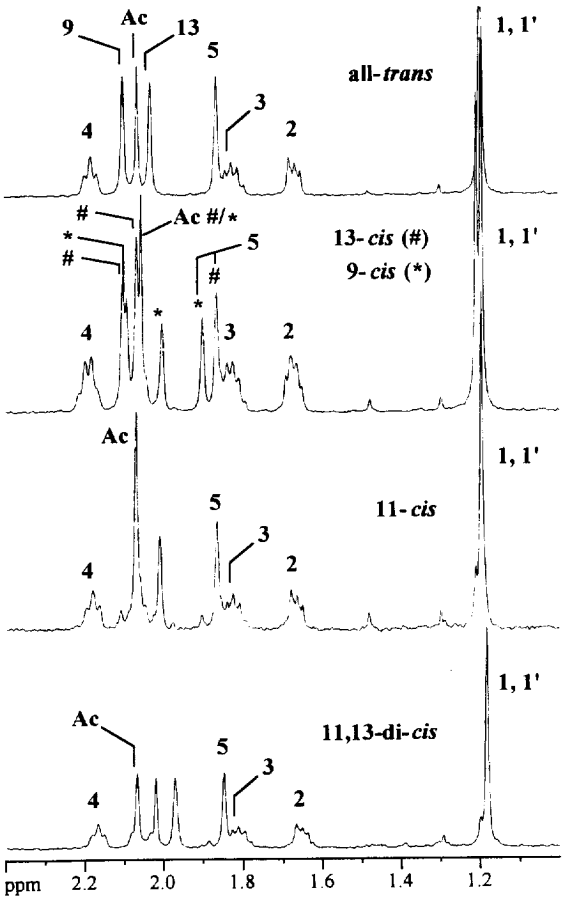

Fig. 17. Aliphatic region of the continuous-flow ^{1}H NMR spectra (400 MHz) of 5 vitamin A acetate isomers recorded during a separation in supercritical CO_2.

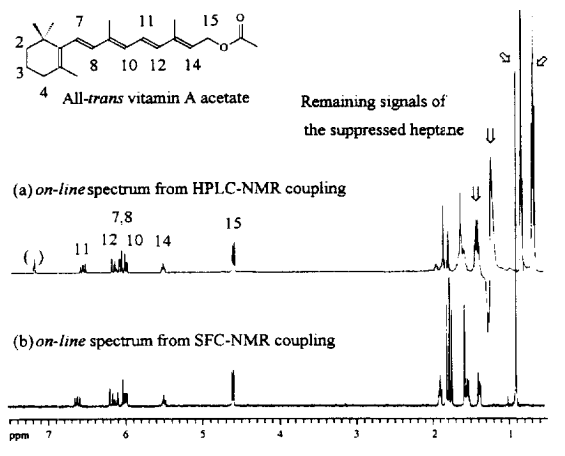

Fig. 18. Comparison of the continuous-flow ^{1}H NMR spectrum of all-trans vitamin A acetate: (a) recorded during a HPLC–NMR separation (b) recorded during a SFC–NMR separation.

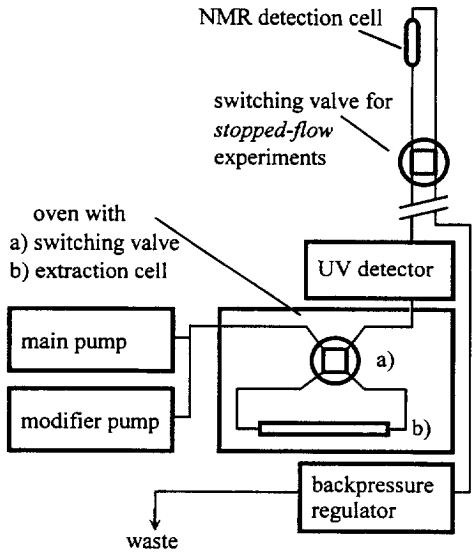

Fig. 19. Experimental setup for the coupling of SFE and NMR.

tions amounts to almost 2 ppm. In contrast, no such distortions occur in the spectra collected on-line during the SFC separation. A linewidth of 1.7 Hz is observed, no extensive baseline correction or further data processing is necessary.

5. SFE–NMR on-line coupling

Extraction with supercritical CO_2 is a technical process with increasing importance. It provides a mild rapid technique of extraction of low or medium polar substances. Supercritical CO_2 is used for supercritical fluid extraction (SFE) in important technical processes such as the decaffeination of coffee and the extraction of hops as well as the extraction of naturally occuring compounds from biomaterials. As a lot of applications are performed in pharmaceutical, polymer, environmental and nutritional fields direct on-line SFE–NMR would be an ideal tool to monitor the extraction process. For the on-line SFE–NMR experiment the apparatus shown in Fig. 19 was used. As 'main pump' served a Hewlett–Packard supercritical fluid chromatograph G1205A. Analytical HPLC columns (4.6×125 mm) were used as extraction cells. The continuous-flow NMR cell was connected between the column outlet and the back pressure regulator.

5.1. Coffee

In Fig. 20, the contour plot of an extraction of 820 mg of roasted coffee is shown [31]. The contour plot

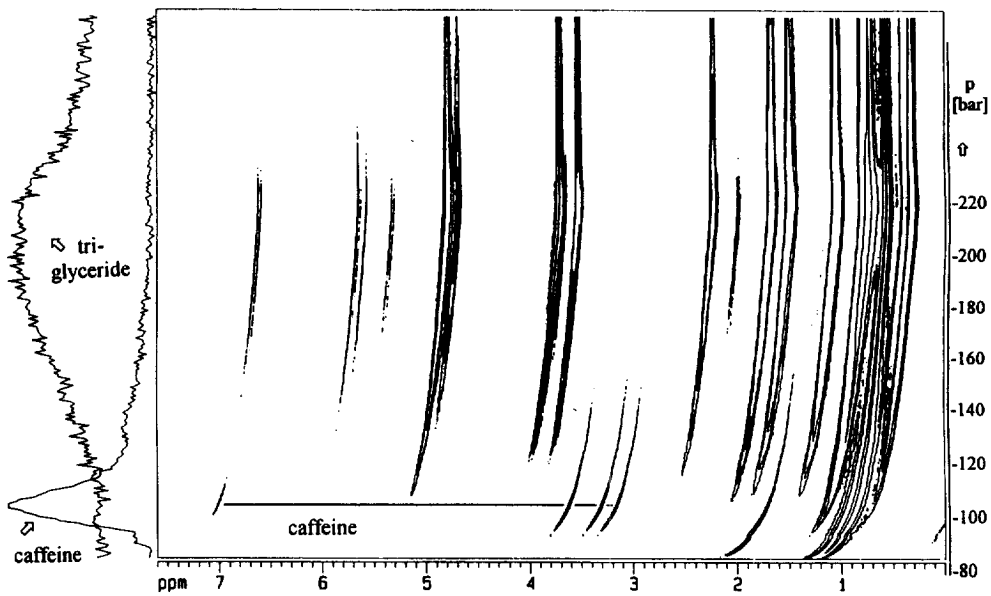

Fig. 20. Extraction of roasted coffee using a linear pressure gradient from 85 to 220 bar.

is similar to the contour plot shown in Fig. 7, where the horizontal axis represents the proton chemical shifts and the vertical axis, the pressure. A linear pressure gradient from 85 to 220 bar within 55 min and a CO_2 flow of 1 ml/min at a temperature of 317 K was applied. One can clearly see the caffeine which is extracted at a pressure of 105 bar as indicated in the contour plot. What also is conspicuous is the upfield shift of the proton signals caused by the increasing density.

The projections shown at the vertical axis of the contour plot are generated by summing up the intensities of the NMR signals of the indicated compound, and therefore represent an extraction profile. The NMR spectrum corresponding to a pressure of 105 bar is shown in Fig. 21 and allows the identification of the caffeine.

At higher pressure other components are extracted over a very wide range of pressure. This is surely a complex mixture of triglycerides and other ingredients of the coffee, which are not separated by their solubility. The spectrum corresponding to the maximum of the extraction profile at 200 bar is shown in Fig. 22. The enlarged section shows the proton pattern corresponding to the outlined glycerol subunit.

The examination of the contour plot of the extraction process shows that the sample composition at the extraction profile between 120 and 220 bar is nearly the same (Fig. 20). It reaches its maximum at approx. 200 bar. This extraction profile can be explained by different, chemically similar substances which show no substantial difference in the NMR-spectrum. Also the extraction of the same compound from different sites in the matrix may lead to a broader extraction profile.

5.2. Pepper

The extraction of black pepper was performed with a pressure gradient from 71 to 294 bar and a CO_2 flow of 0.5 ml/min at a temperature of 317 K [31]. The extraction was stopped at 294 bar and the stopped-flow proton spectrum depicted in Fig. 23 was recorded. The extracted compound could be identified as piperine. Also a two-dimensional proton–proton shift correlated spectrum (COSY45) was recorded during 38 min, shown in Fig. 24. The connectivities are indicated. In this special case with the background information available for the treated sample, the extracted compound could be already identified by the proton NMR spectrum. In other experiments where this might not be the case, only with the additional information provided by two-

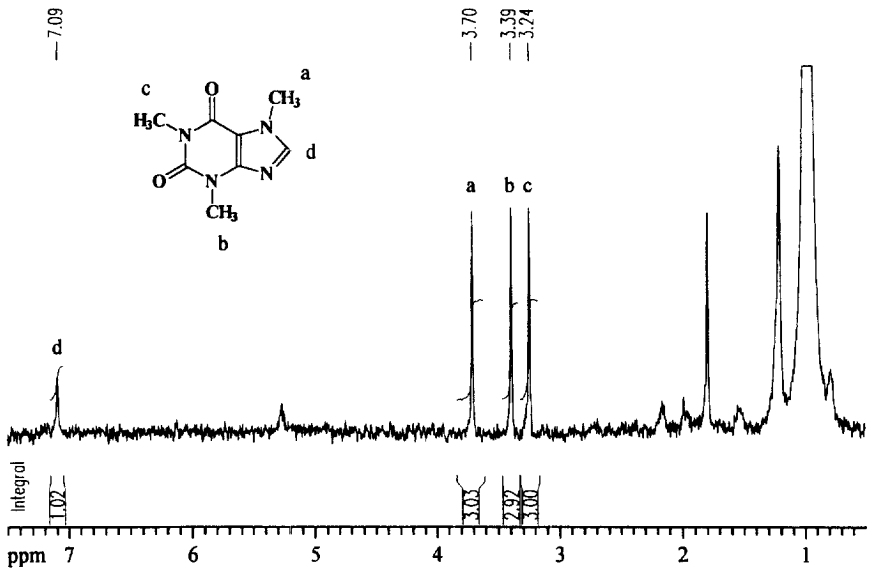

Fig. 21. Continuous-flow ^1H NMR spectrum (400 MHz) of caffeine extracted from coffee with supercritical CO_2.

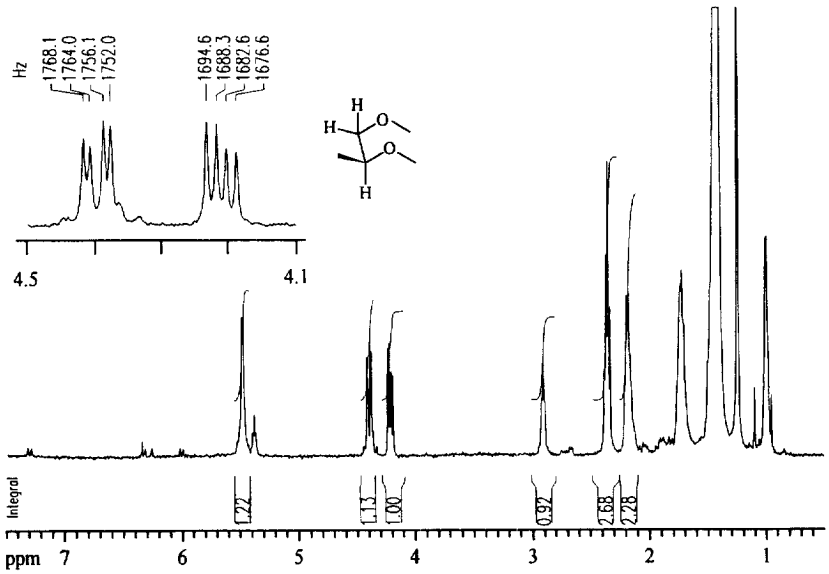

Fig. 22. Continuous-flow ^{1}H NMR spectrum (400 MHz) of an extracted mixture from coffee with supercritical CO_2.

dimensional assignment techniques would an identification be possible.

5.3. Plastifier from PVC

A PVC tube of 2.25 mm O.D. and 0.3 mm I.D. was cut into pieces of approximately 5-mm length. The PVC pieces were packed into a HPLC column which was closed with conventional frits on both sides. The extraction cell was kept at a temperature of 315 K with a CO_2 flow of 1.0 ml/min. For the extraction, a linear density gradient from 0.27 g/ml

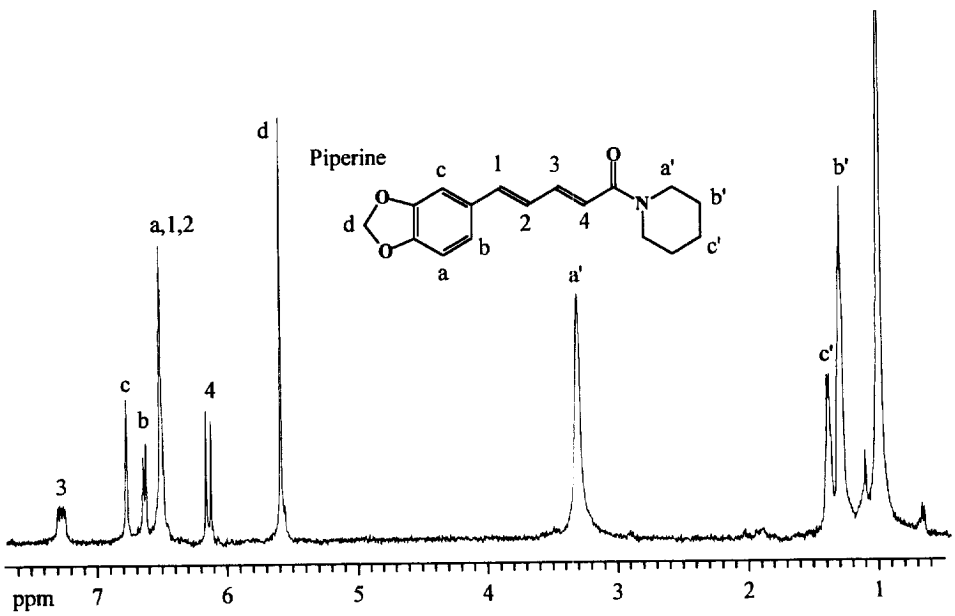

Fig. 23. Continuous-flow ^{1}H NMR spectrum (400 MHz) of piperine extracted from pepper with supercritical CO_2.

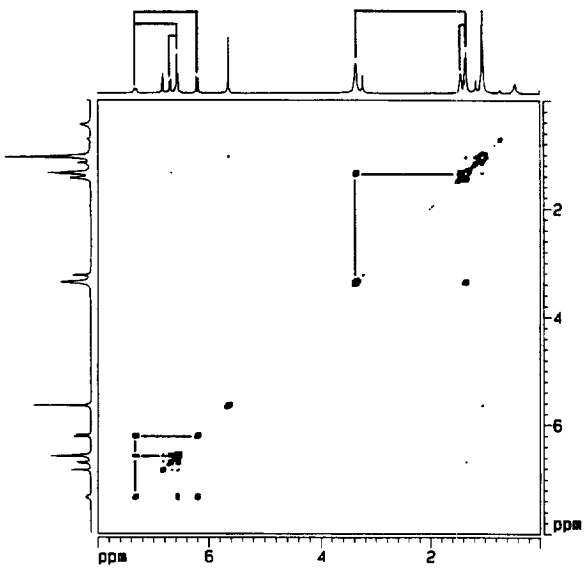

Fig. 24. Contour plot (400 MHz) of a ^{1}H homo-nuclear shift correlated experiment (COSY 45) of piperine in supercritical CO_2.

to 0.9 g/ml within 50 min was applied. With the employed temperature of 315 K, a starting pressure of 79 bar and an end pressure of 317 bar was necessary.

An amount of 644 mg of the PVC tube was placed in the extraction cell. The weight reduction after extraction was 119 mg, corresponding to 18.5%.

Fig. 25 shows the reconstruction of the extraction profile. Here, the aromatic proton signals between 7.2 and 8.2 ppm are summed up and compared to the integral of the ester protons at 4.42 ppm. Both profiles fit well and display the relative concentration of the extracted phthalate as a function of the proceeding extraction. It can be seen, that after a threshold density of 0.26 g/ml, the extraction starts and a maximum is reached at 0.56 g/ml. After that, the concentration decays to a constant value even with increasing density. At the end of the extraction, when the final conditions are kept isobaric, the concentration decays as a linear function.

At the final extraction density of 0.9 g/ml (315 bar), the valve at the SFC probe was closed to switch to stopped-flow conditions. A proton spectrum was acquired under static conditions which is shown in Fig. 26. The structure of the extracted phthalate could be assigned to bis(2-ethylhexyl)-phthalate.

6. Conclusions

The direct coupling of supercritical fluid chromatography and proton Nuclear Magnetic Resonance spectroscopy is feasible. The advantage of NMR detection in the supercritical state is the absence of ^{1}H NMR signals of solvents and impurities, the drawbacks of this hyphenated technique are the pressure dependence of NMR signals and the increased spin-lattice relaxation times. Despite these problems the spectral quality obtained together with

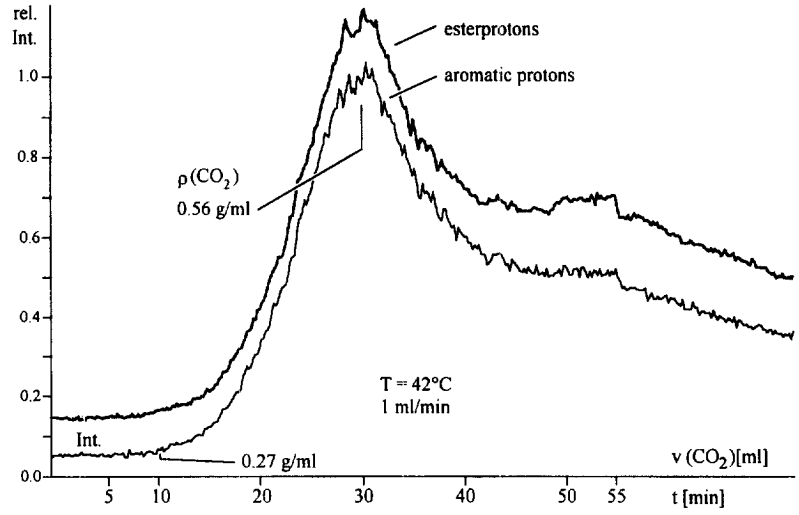

Fig. 25. Extraction profile of a phthalate from PVC.

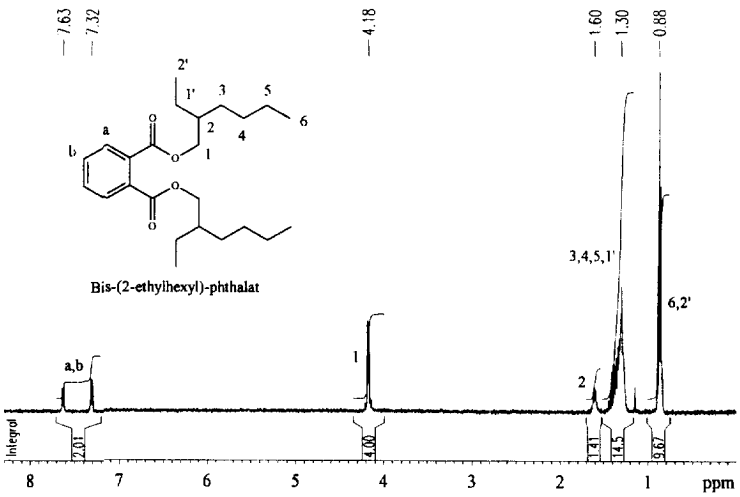

Fig. 26. Stopped-flow ^{1}H NMR spectrum (400 MHz) of an extracted phthalate from PVC with supercritical CO_2.

the capability to acquire two-dimensional NMR spectra may bring SFC–NMR-coupling to an established hyphenated technique. Furthermore the enormous application power of supercritical fluid extraction in combination with ^{1}H NMR detection will lead to new structural elucidation pathways in the search for new, natural occuring compounds which may be used in medical therapy.

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