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USE OF TWO SIMULTANEOUS DETECTORS IN CAPILLARY SUPERCRITICAL FLUID CHROMATOGRAPHY

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SUMMARY

A simple splitter device was incorporated into a capillary supercritical fluid chromatographic system to allow simultaneous detection with both a universal detector (flame ionization detector) and a selective detector (ultraviolet–visible absorbance detector). The technique is quantitative and reproducible while maintaining the high resolution normally obtainable with a capillary column. The advantage of using two detectors simultaneously is demonstrated. The technique enables the analyst to obtain additional information in the analysis of complex matrices.

INTRODUCTION

Supercritical fluid chromatography (SFC) is a separation technique that is gaining wide acceptance for many applications. Among the attributes of SFC, especially capillary column SFC, are a high separation efficiency[1–3], applicability to thermally labile molecules[4,5] and multi-detector compatibility[6–12]. The high separation efficiency is attributed to the properties of the supercritical fluid employed, which has gas-like diffusivity and liquid-like solvating characteristics. These mobile phase properties, combined with advantages realized by utilizing open-tubular wall-coated capillaries as columns, produce a separation technique with a high resolution similar to that of capillary gas chromatography (GC) and an applicability potentially comparable to that of high-performance liquid chromatography (HPLC). The disadvantage of using small-volume fused-silica capillary columns is that the post- and pre-column dead volume constraints are severe. Detector dead volume constraints are particularly serious when conventional optical detection methods are employed with capillary SFC[13,14].

Carbon dioxide is the most commonly used fluid in SFC because it is supercritical at moderate temperature (*ca.* 35°C) and pressure (*ca.* 75 atm), is non-toxic and is inexpensive to obtain in high purity. These moderate supercritical conditions allow the chromatographer to analyze thermally labile species, such as carbamate

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pesticides[15], without the decomposition that accompanies GC. In addition, many non-volatile species that are not amenable to GC or do not not have UV-absorbing chromophores, and are thus poor candidates for LC, can be chromatographed and detected using capillary SFC systems with a flame ionization detector[16,17]. As decompression can be performed either before or after UV detection, various fluid phase detectors are also applicable in SFC[12–14,18].

Multi-detector compatibility is certainly one of the key attributes of SFC, especially capillary SFC. As illustated by Later *et al.*[12], UV–VIS, flame ionization (FID), Fourier transform infrared (FT-IR) and mass spectrometry (MS) are among the detection techniques that have been applied to SFC analysis. Other commonly used GC and LC detection methods used in SFC include nitrogen–phosphorus detection (NPD)[19], scanning fluorescence detection[18], photodiode array-based scanning UV–VIS detection[20] and dual flame photometric detection (FPD)[21].

There are several advantages in using two or more detectors simultaneously, including (1) a reduced analysis time as a result of obtaining two chromatograms of the same component in a single run and (2) detection yielding two forms of information[22] (universal, *e.g.*, in FID, and specific, *e.g.*, in UV detection) for elucidating the identity of solutes found in complex matrices. So far, no report has appeared that describes the use of FID and UV detection simultaneously. Raynor *et al.*[23] employed a splitter to monitor FID while diverting a portion of the sample to a potassium bromides window for subsequent scanning and spectral analysis with an IR microscope. Levy *et al.*[24] attached a flame ionization and a UV detector in series for the detection of the effluent from a 4.6-mm I.D. packed column in SFC. Finally, a low-dead-volume T-piece has been used in capillary SFC for simultaneous detection by FID and MS[25].

We describe here a splitting device that is very simple and allows simultaneous detection with two detectors in capillary SFC. Detection limits, chromatographic performance and analytical applications are discussed.

EXPERIMENTAL

Apparatus

The experiments were performed on a Model 501 supercritical fluid chromatographic system (Lee Scientific, Salt Lake City, UT, U.S.A.). This system was equipped with a Model 501 UV detector especially designed for capillary SFC[14]. The flame ionization detector was not modified and was operated as prescribed by the instrument manufacturer. In all instances, SFC-gade carbon dioxide (Scott Speciality Gases, Plumsteadville, PA, U.S.A.) was used as the mobile phase. The columns employed were 3 m × 50 μm I.D., SB-Methyl-50 and 10 m × 100 μm I.D. SB-Methyl-100 (Lee Scientific), each with a 0.25-μm film. Split injections were made with a 200-nl internal loop air-actuated Valco (Houston, TX, U.S.A.) injection valve. The injection time was 2 s.

Our splitter is a modification of the SGE (Austin, TX, U.S.A.) Model VSOS-1:1, made especially for the high pressures and low dead volumes utilized in capillary SFC. The transfer lines were varied as described under Results and Discussion, but in most instances the FID transfer line consisted of an 18 cm × 50 μm I.D. frit restrictor (Lee Scientific) and the UV transfer line was a portion of deactivated fused-silica capillary tubing. These transfer lines were connected with a two hole graphite–Vespel ferrule. In all experiments the UV restrictor was mounted after the UV detector flow-cell cuvette.

Chemicals

All chemicals were of analytical-reagent grade or better. Test and calibration solutions were prepared in dichloromethane. Calibration and height equivalent to a theoretical plate (HETP) test solutions consisted of *n*-hexadecane (C_{16}) and *n*-tetracosane (C_{24}) and the polycyclic aromatic hydrocarbon (PAH) species anthracene and pyrene. A number of dilutions of the stock solution were prepared in order to construct calibration graphs.

RESULTS AND DISCUSSION

A calibration graph was constructed for the four test solutes, anthacene, pyrene, C_{16} and C_{24}, with FID and for anthracene and pyrene with UV–VIS detection. The results are summarized in Table I. Both calibration graphs were linear ($r > 0.99$) over at least three orders of magnitude with detection limits of *ca.* 5 ppm for UV–VIS detection and *ca.* 1000 ppm for FID. No decrease in linearity was observed with the highest concentration solution investigated, but it is expected that chromatographic overloading will limit the upper end of the concentration range suitable for this system. As indicated in Table I, the detection limits are only modest, but the concentration (mass) detection limits are fairly good. The mass sensitivity enhancement is expected as predicted by theory for micro-separation schemes and observed experimentally[26–28]. It was found that the splitting ratio is primarily dependent on the amount of restriction used at each detector. By controlling this flow parameter it is possible to vary the relative amount of analyte sent to each of the two detectors.

The detection limits obtained in the simultaneous mode are comparable to, but slightly higher than, those found in the operation of each detector individually. A direct comparison is difficult for the UV detector as different columns of different diameters were used in this study, which was aimed at developing a capillary UV–SFC interface. As peak height was used as an indicator of response in both stand-alone UV experiments[14] and the present experiments, changes in the chromatographic conditions could contribute significantly to differences in the observed detection limits. Ideally, an integrator should be employed so that chromatographic band-broadening

TABLE I

LIMITS OF DETECTION FOR UV–VIS DETECTION AND FID

Conditions: injection, 200 nl before split; injection splitting ratio, 7:1; UV–VIS flow-rate, 3.3 × FID flow-rate; FID at 350°C, UV–VIS detection at 254 nm.

Solute	*Detection limit**	
	UV–VIS	*FID*
Anthracene	4 ppm, 90 pg**	15 ppm, 90 pg**
Pyrene	10 ppm, 200 pg**	10 ppm, 60 pg**
C_{16}	–	10 ppm, 60 pg**
C_{24}	–	8 ppm, 50 pg**

* Detection limits are two standard deviations larger than the noise in the background signal.

** On-column mass detection limits compensated for injection split and split flows.

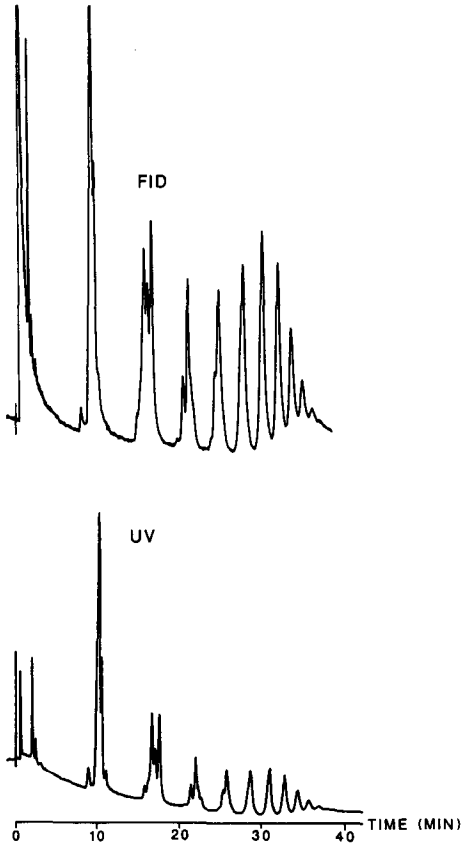

Fig. 1. Chromatograms of phenylhydropolysiloxane. Column temperature, 120°C. The UV trace was obtained at 210 nm. The FID trace was obtained at an attenuation of 2^6 and a detector temperature of 400°C.

contributions to a decrease in detection limits are minimized. The FID mass detection limits compare well with those suggested by the instrument manufacturers, who report an on-column detection limit for *n*-alkanes in the range 50–100 pg. In general, the concentration and mass detection limits are good and the simultaneous detection interface is suitable for most normal capillary SFC applications. Concentration detection limits could possibly be improved upon by careful control of the split flow and by an increase in the column loadability.

When such a device is incorporated into a capillary-based SFC system it is important to know whether the introduction of a post-column detector volume will contribute significantly to chromatographic band broadening. To answer this question we determined HETP values for the *n*-alkane and PAH test mixture used for calibration. This test solution contained solutes that gave k' values from 0.46 for C_{16} to 3.2 for C_{24} under the chromatographic conditions used. The reduced plate height (H) values for the system varied from 0.52 to 1.35 μm. Although the H values for UV detection are slightly lower than those for FID (the volume of the UV detector is less

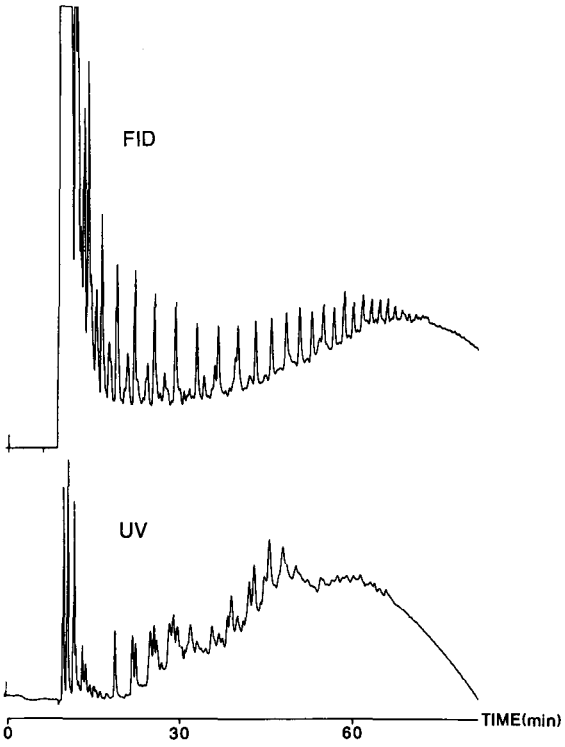

Fig. 2. Chromatogams of a Levelland oil/separation oil. Column temperature, 100°C. UV detection at 254 nm and employing baseline compensation. The FID trace was obtained at an attenuation of 2^6 and a detector temperature of 415°C.

than 60 nl), the H values for the two detectors compare well. Values obtained with the 100-μm I.D. column in the manufacturing test (*ca.* 3000 plates/m) and those obtained with the simultaneous detection method (*ca.* 2900 plates/m) show that the interface device contributes little to post-column dispersion. That high chromatographic efficiency is retained is further illustated by the chromatograms presented in this paper.

A few comments should be made concerning the practical aspects of this experiment. First, because the SFC system operates at high pressure, it is important to ensure that no leaks exist in the system. Any such leaks could result in a loss of pressure before the detectors and a subsequent decrease in the density of the mobile phase. Such a decrease would result in a solubility change, causing a possible loss of chromatographic resolution. Second, because the system is based on a capillary column, pre- and post-column dead-volume constraints are severe, and dead volumes must be kept to the minimum. Finally, a comment should be made about the use of 50-μm I.D. columns. These are the narrowest commercially available separation columns and have very small volumes even when very long. We were unsuccessful with a short piece (3 m) of 50-μm I.D. column, but we believe that the simultaneous detection interface could be used with 10- or 20-m columns, as their larger volumes impose less severe dead-volume constraints. The best results are expected with 100-μm I.D. capillary columns.

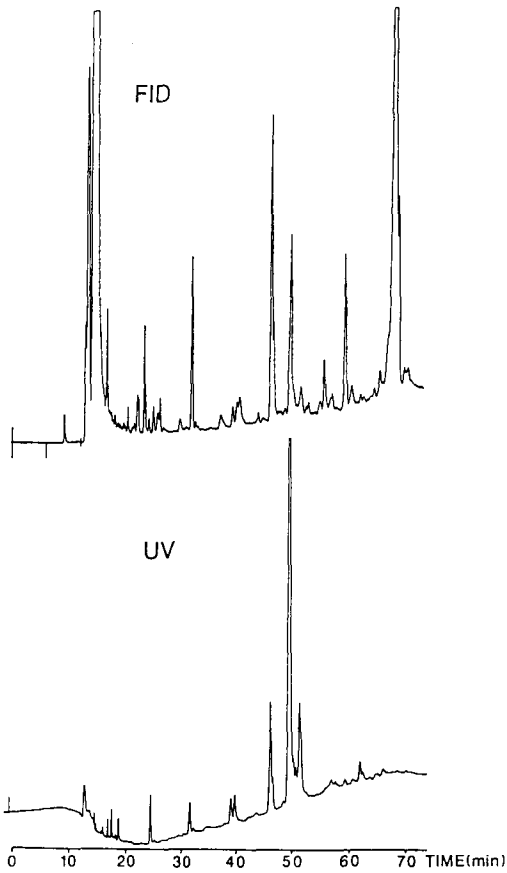

Fig. 3. Chromatogram of a neat sample of a cold-pressed grapefruit oil. Column temperature, 100°C. UV trace at 254 nm. The FID trace was obtained at an attenuation of 2^6 and a detector temperature of 415°C.

Fig. 1 shows the FID and UV capillary chromatograms of a polyphenylhydrosiloxane with the dual-detector split interface. The chromatogram illustrates the effectiveness of transfer of the higher molecular weight oligomers. If the concentration of the sample or the splitting ratio were modified so that the UV detector received more of the sample, the UV trace would be almost identical with that obtained using FID.

Figs. 2 and 3 further illustate the advantages gained by employing two detection methods simultaneously for the analysis of a complex mixture. The oil sample shown in Fig. 2 is a complex mixture of aliphatic and aromatic hydrocarbons. The FID trace alone shows the *n*-alkane distribution, as expected, but gives no selective information about the UV-absorbing species in the sample. Close comparison of the retention times obtained with UV detection and FID shows that there are unresolved peaks, or closely eluted peaks, that are detected by both the flame ionization detector (universal) and the UV detector (selective at 254 nm). These compounds probably correspond to species other than *n*-alkanes and are most likely the aromatic or polycyclic species expected in this type of oil. In addition, the simplified UV trace gives information that is

unattainable with FID alone, and the FID trace represents virtually all organic compounds present. Of particular interest is the formation gained with respect to the early eluting species. As illustrated in Fig. 2, there are at least three major compounds that absorb strongly at 254 nm. They would not be detected if only FID were employed. These components are significant in concentration and certainly contribute substantially to the chemical nature of the sample.

A third example of the utility of the simultaneous detection method is shown in the analysis of a grapefruit oil. Note the excellent chromatographic performance illustrated in Fig. 3. In this sample, the advantage of the two-detector approach is shown by the absence of the major component seen in the UV trace which is eluted late in the FID trace. The presence and concentration of the component eluted mid-way in the UV trace suggests that this species is an aromatic component characteristic of the sample.

In this paper we have illustrated that an interface device, based on a splitter, can be employed in capillary SFC with simultaneous FID and UV–VIS detection. Chromatographic performance is maintained, and the relative amount sent to each detector can be quantified. Calibration for each of the detectors remains linear, while the splitting ratio is controlled by setting the relative restriction used at each flow channel. This simultaneous measurement allows the analyst to obtain additional information, which facilitates the elucidation of complex matrices. We are currently investigating other detector combinations for selective and universal detection.

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