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HIGH-PERFORMANCE EXCLUSION CHROMATOGRAPHY OF LOW-MOLECULAR-WEIGHT ADDITIVES

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SUMMARY

The use of high-performance exclusion chromatography with small pore-size gels of 8–10-μm particle diameter for the analyses and/or clean-up of a variety of sample types was investigated. In many cases, low molecular weight additives from polymers or oils could be determined directly since the latter were of a sufficiently higher molecular weight to be resolved. In those cases where the additives were unseparated, the exclusion technique served as a clean-up method and these compounds could be further separated by another liquid chromatographic mode, such as reversed phase. The technique was applied to a steroid in a cream base, polyethylene additives, sulfur in a compounded rubber and a pesticide formulation, DDT in butter and additives in vegetable and lubricating oils.

INTRODUCTION

Gel-permeation chromatography (GPC), also known as exclusion chromatography, has long been used as a method for the determination of polymeric molecular weight distribution, for analysis of polymer additives, preparative fractionation and sample clean-up. However, techniques formerly used required long columns and low linear velocities (*i.e.*, flow-rates) in order to achieve the required resolution. Following the development of microparticulate columns for ion-exchange, liquid–solid, and bonded-phase chromatography in the early 1970's, microparticles also became available for the exclusion chromatographic mode[1–4], allowing more rapid chromatographic separations. The GPC microparticles resulted in shorter columns (30–50 cm *vs.* 120 cm), consuming less solvent and permitting more convenient thermostatting.

Relative to other HPLC modes, the beauty of the exclusion technique lies in its simplicity. Often, one merely dissolves the sample in the mobile phase and injects it. In contrast to the other liquid chromatographic (LC) modes, usually all sample components elute between the excluded volume and the total permeation volume, a fixed time (volume) interval. Thus, little operator experience in chromatography is required and the interpretation of the chromatogram is fairly easy. The only decision to be made is in choosing the optimum pore size which can be selected by knowing the molecular weight operating range of the column (or columns) and matching it with the suspected molecular weight (range) of the sample.

The purpose of the present report is to demonstrate the application of high-performance exclusion chromatography to the rapid separation of low molecular weight (less than a thousand) additives in a wide variety of complex matrices. In most cases, the samples, ranging from steroid creams to agricultural sprays, were merely dissolved in tetrahydrofuran (THF) and injected with little or no additional clean-up. Although this form of chromatography is not new, the greatly improved efficiency of the microparticles allows more speed in successful application of the technique.

EXPERIMENTAL

Apparatus

A Varian Model 8510 was used as the liquid chromatograph. It was equipped with a Model 8050 Autosampler with a 10-μl loop, a Varichrom™ variable-wavelength detector connected in series with a Varian refractive index detector which was thermostatted with a water bath.

Columns

Exclusion chromatography was carried out on MicroPak TSK columns (Varian) packed with cross-linked polystyrene of 8–10 μm particle diameter. Technical data for the columns employed can be seen in Table I. The columns were 30 or 50 cm × 8 mm I.D. They were packed in THF and used in that solvent in all experiments.

TABLE I

PROPERTIES OF MICROPAK TSK COLUMNS

PS = based on polystyrene standards.

Name	*Average pore size (Å)*	*Approximate exclusion limit (PS)*	*Operating range (PS)*
MicroPak 1000H	40	10^3	50–1000
MicroPak 2000H	250	10^4	100–8000
MicroPak 3000H	1500	6×10^4	100–60,000

Procedures

In general, a known amount of sample was dissolved or diluted with THF. The solution was then placed in an Autosampler vial and injected via the Autosampler. If necessary, prior to injection the diluted samples were filtered to remove particulate matter.

For those materials where reversed-phase techniques were used, the eluent from the exclusion chromatography system was collected manually in a tapered vial. The fraction was concentrated by gentle evaporation with the aid of a nitrogen stream.

All samples studied were obtained commercially except the following which were gifts: the Captan-Signal™ Sulfur Dust (Stauffer, Richmond, Calif., U.S.A.); acrylonitrile–butadiene copolymer (Goodyear Tire & Rubber, Akton, Ohio, U.S.A.); and the fluocinonide steroid cream and placebo (Syntex, Palo Alto, Calif., U.S.A.).

All solvents were distilled-in-glass from Burdick & Jackson (Muskegon, Mich., U.S.A.).

RESULTS AND DISCUSSION

Steroid cream

Ointments used for treating various skin disorders generally consist of a small amount of active ingredient in a cream base. To control the amount of active ingredient it is necessary to have a sensitive, simple method for its determination. Fig. 1 shows that the placebo cream base gave a relatively simple chromatogram with both ultraviolet (UV) and refractive index (RI) detection. The topical cream containing 0.5% of the steroid fluocinonide gave a unique peak in the UV trace of Fig. 2. Compared to Fig. 1, note that the RI detector did not detect any perceptible difference for this relatively small amount of steroid in the cream. Although unresolved from some incipients in the cream base, selective detection at 254 nm permitted the direct observation of the UV-absorbing steroid.

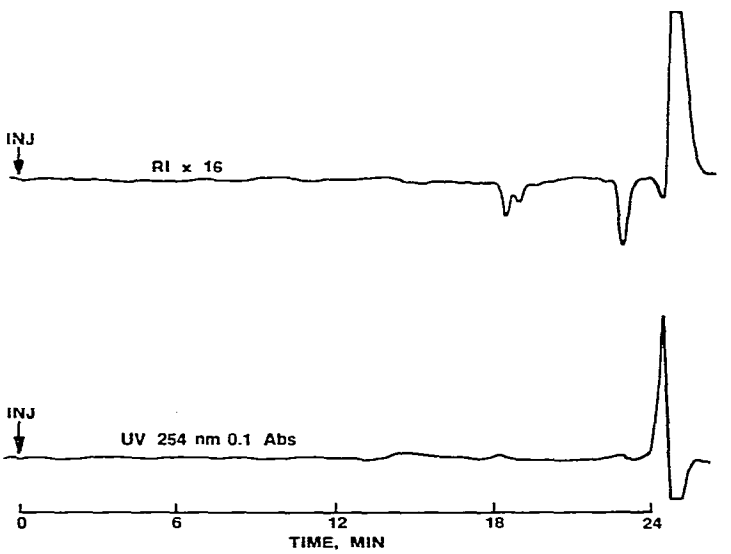

Fig. 1. Exclusion chromatogram of placebo cream. Flow-rate, 0.3 ml/min. Eluent, THF. Column, MicroPak TSK 1000H, 50 cm × 8 mm. Sample, 2 g dissolved in 10 ml THF. Injection, 10 μl.

The usual technique for handling this type of sample is a preliminary extraction followed by reversed-phase chromatography[5]. In this procedure, some cream may be co-extracted and therefore injected onto the column, thus making it necessary to repeatedly "clean" the column to remove the sorbed cream. With exclusion chromatography both extraction and/or column clean-up were unnecessary.

The example clearly showed that high-speed exclusion chromatography combined with specific detection (in this case 254 nm UV) can provide a rapid, sensitive and specific determination of an active ingredient in an inert matrix without the need for preliminary extraction.

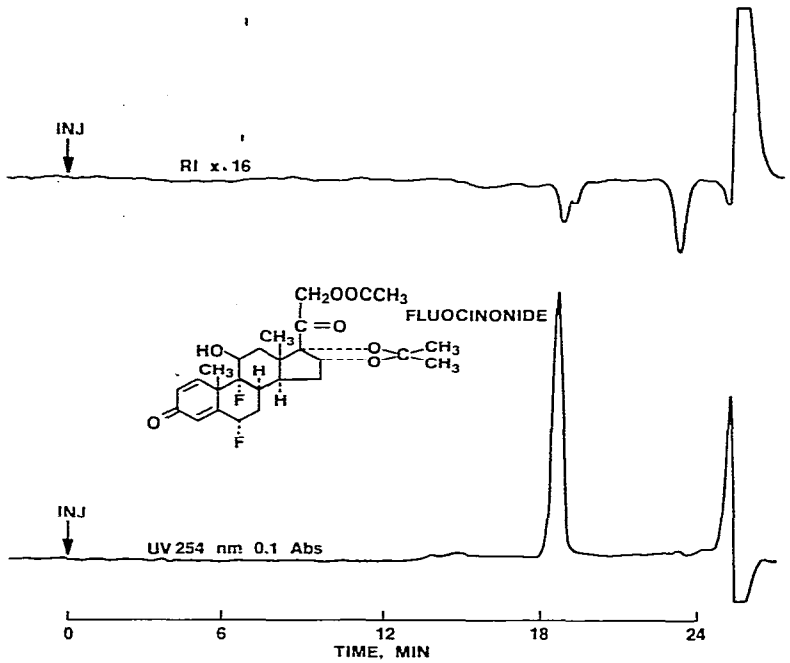

Fig. 2. Exclusion chromatogram of Fluocinonide steroidal cream. Same conditions as Fig. 1 (note RI trace is unchanged).

Elemental sulfur

Elemental sulfur is widely used as an industrial chemical. The largest amount is consumed in the manufacture of sulfuric acid but it also finds use in pulp and paper manufacture, for agricultural fungicides, in rubber vulcanization and for the manufacture of other chemicals and dyes. A number of non-chromatographic methods have been developed for the analysis of elemental sulfur but they suffer from poor sensitivity and/or selectivity. A gas chromatographic (GC) method has been reported[6], but accurate GC analysis of sulfur is difficult and, in addition, elemental sulfur vapor contains a number of different species. The selective retention of sulfur on divinylbenzene–polystyrene packings has been reported by Cassidy[7] but on large-particle gels. Use of microparticulate packings for the separation of elemental sulfur on a multiple column set has recently been reported[8] and the present report is an application of a single MicroPak TSK 1000H column to sulfur analysis in two diverse sample matrices.

Fig. 3 depicts the chromatogram which results when flowers of sulfur, converted to the crystalline form by heat, were dissolved in THF and injected onto a MicroPak TSK 1000H column. This column is normally used for exclusion chromatography but sulfur is retained beyond the total permeation volume due to its selective interaction with the polystyrene (PS) matrix. Other components in a sample may separate by the normal exclusion process. Hence, they will elute before and well away from the sulfur. In addition, at 254 nm, sulfur gives strong absorbance ($\varepsilon \approx 7200$ in THF) with a minimum detectable quantity of less than 10 ng.

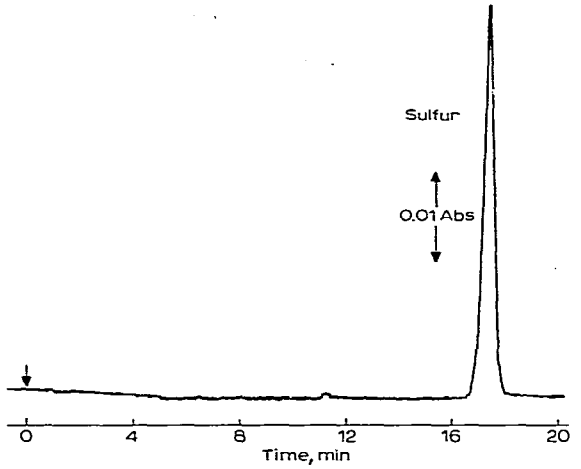

Fig. 3. Exclusion chromatogram of sulfur. Flow-rate, 0.5 ml/min. Concentration, 98.5 μg/ml. Other conditions same as Fig. 1.

To test the usefulness of the technique, a sample of Captan-Signal Sulfur Dust (15% Captan, 50% sulfur, 35% inert ingredients), a product of Stauffer used for preventing powdery mildew and botrytis in grapes, was dissolved in THF with gentle heating and then centrifuged to remove the suspended inert ingredients. The supernatant liquid was injected onto the column, resulting in the chromatogram shown in Fig. 4. The chromatogram yielded two peaks, the first for N-(trichloromethylthio)-4-cyclohexane-1,2-dicarboximide, and the second for sulfur. Since sulfur was in greater proportion and gave a stronger signal, the attenuation was changed between the two peaks.

Next a sample of compounded Chemigum™ (a butadiene–acrylonitrile co-polymer manufactured by Goodyear) was extracted with THF to remove sulfur as

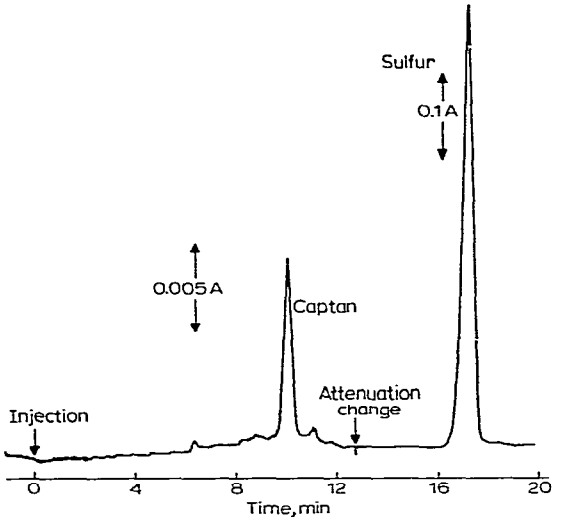

Fig. 4. Exclusion chromatogram of Captan-Signal Sulfur Dust. Concentration, 2 mg/ml. Other conditions same as Fig. 3.

well as other additives, such as antioxidants and accelerators. The THF extract was injected and gave the chromatogram of Fig. 5. The peaks eluting between 5 and 13 min were due to extracted polymer, antioxidant, dibutylphthalate and disulfide accelerators. Sulfur was well resolved and eluted much later than the other compounds, even those of similar molecular weight.

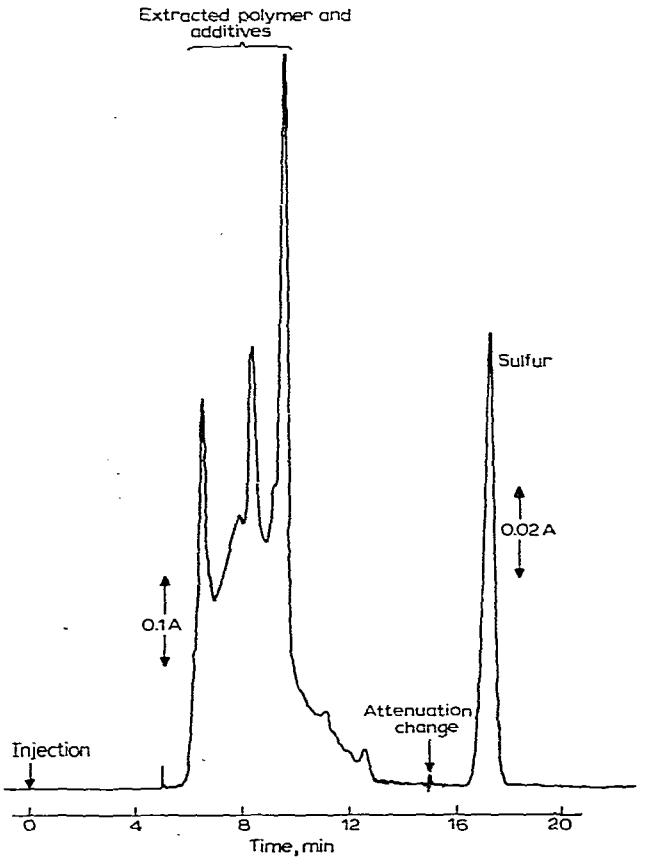

Fig. 5. Exclusion chromatogram of compounded rubber extract. Concentration, 74 mg/ml. Other conditions same as Fig. 3.

Both of these applications demonstrate the usefulness of a single exclusion column for the analysis of sulfur. In addition, Cassidy[7] has shown that the analysis of elemental sulfur in petroleum products and in process water from a heavy-water plant is feasible. The main advantage over other LC techniques is the great selectivity for sulfur compared to other classes of compounds. Most compounds show little or no interaction with the PS matrix when THF is employed as the mobile phase since they operate under a "pure" exclusion mechanism. By proper calibration, elemental sulfur can be quantitatively determined in a wide variety of matrices.

Polyethylene extractables

Polyolefins (*e.g.*, polyethylene, polypropylene) generally contain small amounts

of additives to improve various properties of the final product. These materials are usually one or more antioxidants but may also be compounds such as light absorbing agents or colorants. By-products from the polymerization such as monomers and initiators are often present. Polyolefins are difficult to dissolve in common solvents and at mild temperatures, so recourse is made to extraction techniques to isolate the soluble additives.

First, to demonstrate the potential of the exclusion technique for suspected additives, a number of standards were run on a three-column set. A wavelength of 215 nm was chosen for monitoring since the thiodipropionate antioxidants absorb only at low UV wavelengths. However, even at these wavelengths they display weak extinction coefficients. Fig. 6 shows the separation of six common antioxidants

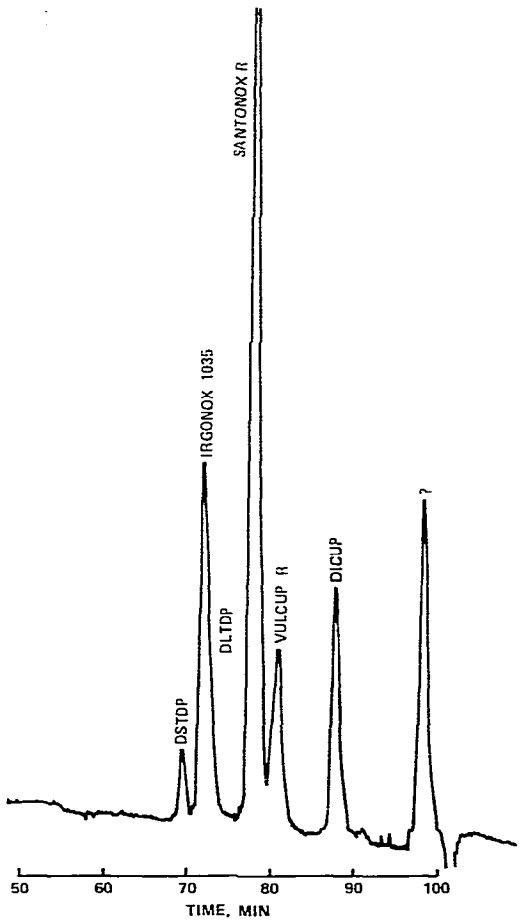

Fig. 6. Exclusion chromatogram of polyethylene additive standards. Columns, 50 cm × 8 mm MicroPak TSK 3000H, 50 cm × 8 mm MicroPak, TSK 2000H and 80 cm × 8 mm MicroPak TSK 1000H. Solvent, THF. Flow-rate, 0.5 ml/min. Detection, 215 nm. Sample concentration, approximately 1 mg/ml. 10-μl injection of DSTDP (distearylthiodipropionate), Irganox 1035 [thiodiethylene-bis-(3,5-di-*tert.*-butyl-4-hydroxy)-hydrocinnamate], DLTDP (dilaurylthiodipropionate), Santonox R [di(2-methyl-4-hydroxy-5-*tert.*-butyl)-phenyl sulfide], Vulcup R [α,α'(bis-*tert.*-butylperoxy)-diisopropylbenzene] and Dicup (dicumylperoxide).

(DSTDP, DLTDP, Irganox and Santonox) and initiators (Vulcup and Dicup) found in commercial polyethylenes, often in combinations. An unknown peak eluting at slightly under 100 min was noted, probably a decomposition product from the peroxide initiators, which are known to be unstable.

Fig. 7 demonstrates the separation of additives obtained from the THF-extraction of a polyethylene film. The antioxidants were well separated from the residual peroxide initiator, Vulcup. Some unidentified minor peaks, presumably lower molecular weight compounds, were observed. These may be other decomposition products of the peroxide, possibly phenols.

This example shows the utility of GPC for analysis of the extractables from polymers with poor solubility in the mobile phase used for the exclusion separation.

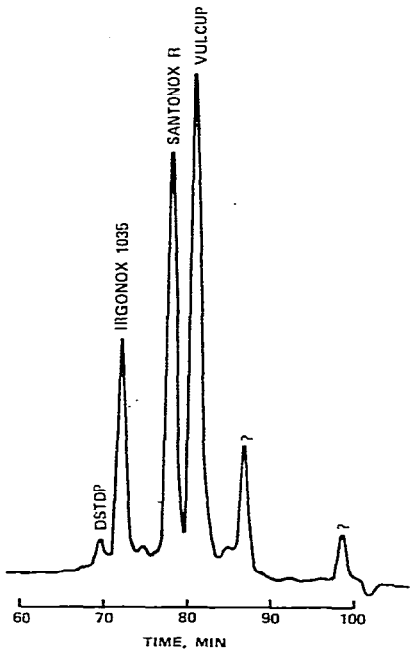

Fig. 7. Exclusion chromatogram of polyethylene extract. 5 g of film were extracted with 100 ml of THF; the extract was concentrated a factor of 20 prior to injection. Other conditions same as Fig. 6.

DDT in butter extract

Although the chlorinated pesticide, *p,p'*-DDT was outlawed several years ago in the United States, due to its persistence it still occasionally appears in unsuspected places. To illustrate the applicability of exclusion chromatography to handle a typical low molecular weight pesticide in a dairy product, a sample of pure butter was deliberately "contaminated" with 100 ppm DDT and carried through a clean-up procedure. First, an exclusion chromatogram of a standard DDT gave an elution volume of 15.2 ml when run on a 250-Å column at a 1-ml/min flow-rate. The unspiked butter dissolved in THF, filtered to remove insoluble matter and chromatographed under identical conditions, gave the fairly uncomplicated chromatogram of Fig. 8. Note the presence of a small peak eluting after the higher molecular weight lipids, etc.,

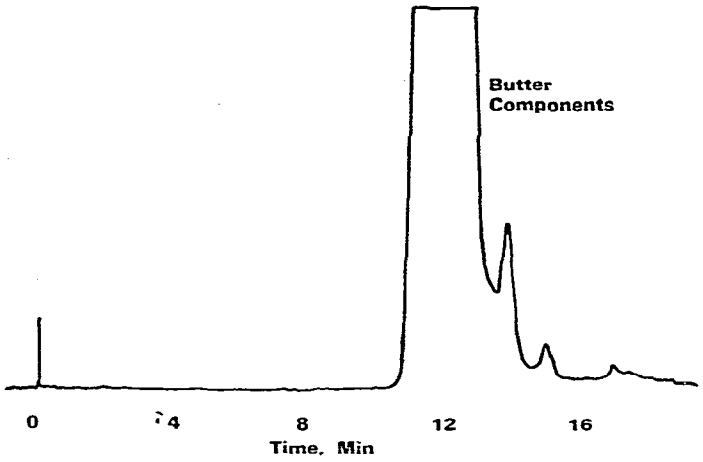

Fig. 8. Exclusion chromatogram of butter extract. Column, 60 cm × 8 mm MicroPak TSK 2000H. Flow-rate, 1 ml/min. Mobile phase THF. Sample concentration, 0.5 g/ml; 10-μl injection. Detector, Varichrom at 215 nm, 0.2 a.u.f.s.

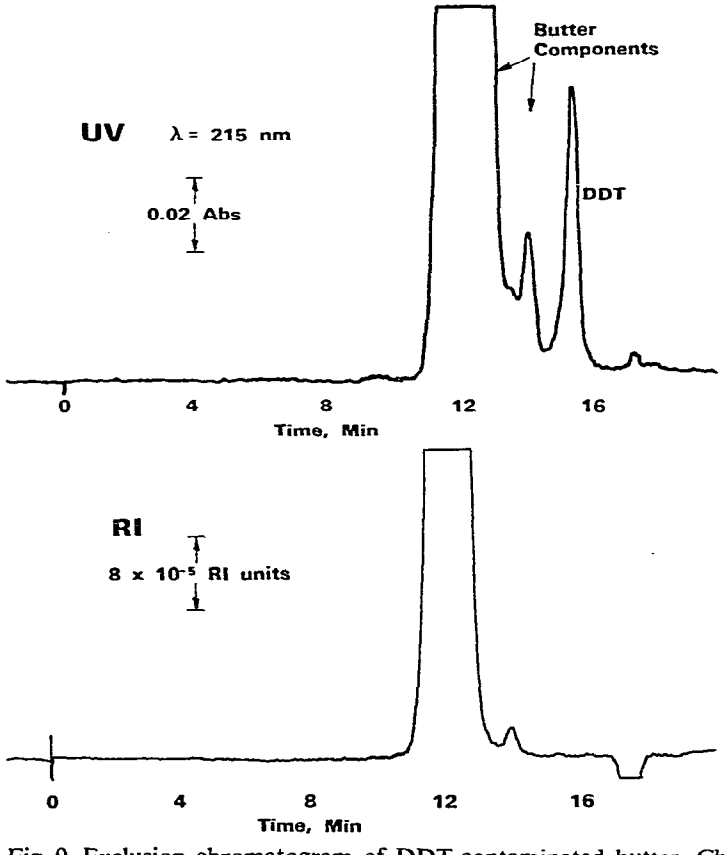

Fig. 9. Exclusion chromatogram of DDT-contaminated butter. Chromatographic conditions same as Fig. 8. Detectors, Varichrom, RI. Note that at 100-ppm concentration the DDT peak is not detected by RI.

at a volume of 15.2 ml. Next, a sample of butter spiked with 100 ppm of DDT was treated similarly and indeed a larger peak at 15.2 ml appeared in the chromatogram as can be seen in Fig. 9. The RI detector gave no response to this small amount of DDT. The fraction eluting between 14.9 and 15.7 ml (800 μl) was collected for both the "control" and "spiked" samples of butter. After a 20-fold concentration (by evaporation) the concentrates were injected onto a reversed-phase column. The resulting high-sensitivity ($\lambda = 210$ nm) gradient chromatogram of the "spiked" sample (Fig. 10) clearly showed a well resolved DDT peak among a large array of extraneous peaks resulting from the THF concentrate and mobile phase (water and acetonitrile) impurities. One rather large unknown peak eluting at just under 12 min was also observed. This peak could have resulted from a small portion of the large lipid tail of Fig. 9 which was collected with the DDT fraction.

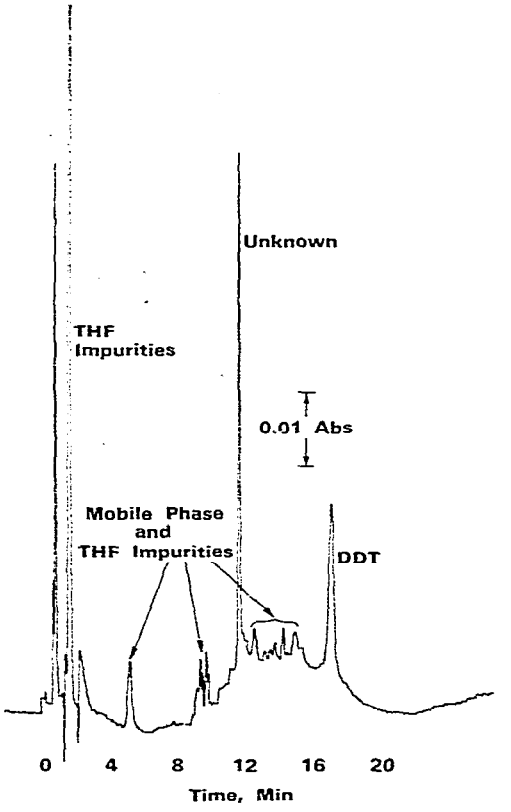

Fig. 10. Reversed-phase separation of DDT fraction isolated by exclusion chromatography. Column, 25 cm × 2.2 mm MicroPak MCH. Flow-rate, 1 ml/min; solvent A, water; solvent B, acetonitrile; gradient, 10–60% solvent B at a rate of 5%/min. Detector, Varichrom at 210 nm.

This experiment did serve to illustrate the ease with which high-performance exclusion chromatography could be used to clean-up a dairy product in only a matter of minutes. Previous illustrations of DDT clean-up on large particle size gels[9–10] required well over an hour and the resultant isolated pesticide was diluted in solvent

volumes ranging from 30 to 70 ml. Here the volume was less than a milliliter, a convenient size for further characterization by GC or LC. In addition, it further illustrates that a combination of exclusion chromatography with reversed-phase chromatography provides a more universal approach to the analysis of a complex mixture[8].

Petroleum and vegetable oil additives

Low levels of additives are added to various types of oils to preserve their lifetime or give them special properties for specific applications. For petroleum products, oxidative inhibitors are required to prevent deterioration at high temperatures and pressures such as will occur in an automobile engine, while for vegetable oils antioxidants are required to avoid rancidity. Most oils are of higher molecular weights than the additives and thus the exclusion technique is ideal for their prefractionation, clean-up, or often, as is the case with high-performance microparticulate columns, for the direct analyses of low molecular weight additives without prior separation.

A lubricating oil, used in aerospace and marine systems, was known to contain two additives, Antioxidant 702 and Tricresylphosphate (TCP), a mixture of isomers used primarily in extreme pressure applications. A method was required to rapidly monitor the oil for these additives present at the 1 and 0.5% levels, respectively. The additives could be separated from each other by exclusion by virtue of their large difference in molecular weight as can be seen in Fig. 11. Fortunately, the oil was of a sufficiently higher molecular weight, as seen in Fig. 12, that it was almost completely resolved from the Antioxidant 702 even though it was present in over 200 times greater concentration. The entire analysis took place in 16 min with no sample clean-up required. Method development took only a short time since it was known in advance that all components would elute prior to the total permeation volume.

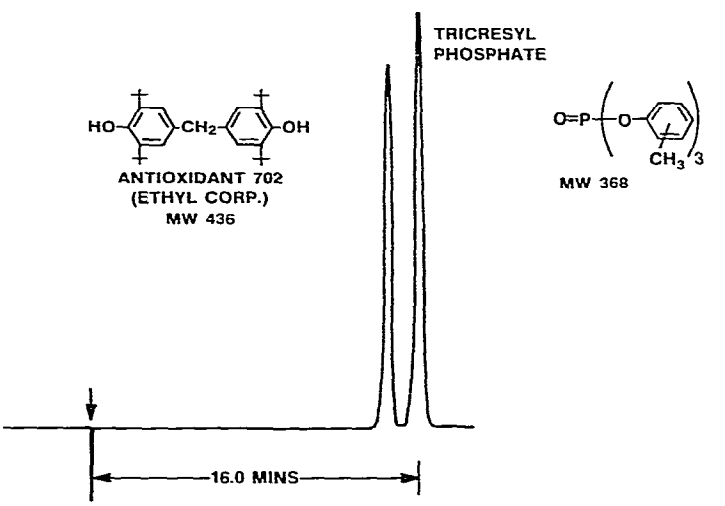

Fig. 11. Exclusion chromatogram of oil additives. Column, 50 cm × 8 mm MicroPak TSK 2000H. Flow-rate, 0.5 ml/min.

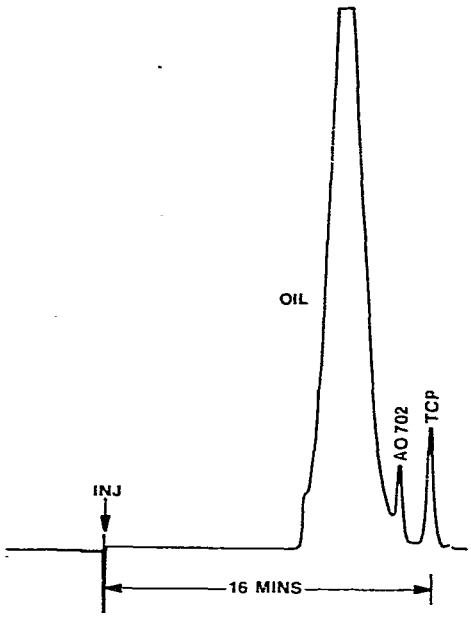

Fig. 12. Exclusion chromatogram of stabilized oil. Conditions same as Fig. 11.

The antioxidants BHT and BHA are approved for food use and are frequently added to vegetable oils and other food products to preserve their freshness. With molecular weights of 220 and 180, respectively, they would be expected to be separated from vegetable oils which consist mainly of triglycerides with molecular weights of over 600 (*i.e.*, fatty acid composition C_{10} and above). Soybean oil is frequently used as an all purpose salad oil. A commercial soybean oil was diluted 1:1 with THF and injected directly onto a MicroPak TSK 2000H column. Fig. 13 shows that the two antioxidants, although themselves unseparated on the exclusion column,were well resolved from the soybean oil incipients.

To confirm further the identity of the peak identified as BHA and BHT in Fig. 13, about 800 μl of the effluent eluting at the detector exit between 15–16 min were collected in a tapered tube. The fraction was concentrated (by evaporation) 20-fold and then injected onto a reversed-phase column using the conditions outlined in Fig. 10. Although not shown, two major peaks, determined to be BHA and BHT by running standards, were obtained, thereby confirming the preliminary peak assignment of the exclusion chromatogram to these antioxidants. Assuming the responses of the two antioxidants were equivalent, the amount of total antioxidant was calculated to be about 0.001% by weight, well below the permitted usage level[11].

CONCLUSION

This study demonstrates that the technique of high-performance exclusion chromatography is ideally suited to the analysis or clean-up of a wide variety of sample types. Often for low molecular weight additives, resolution is sufficient that they can be determined directly by exclusion chromatography. For some samples,

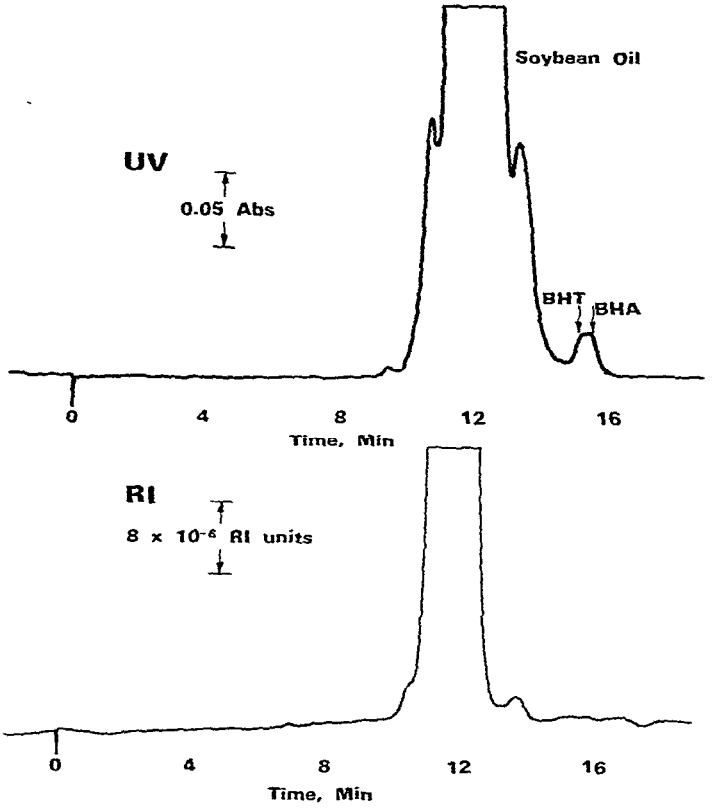

Fig. 13. Exclusion chromatogram of soybean oil. Same conditions as Fig. 8. Sample diluted 1:1 with THF prior to injection.

the technique can be used to provide a prefractionation or clean-up with the actua separation being carried out with a secondary chromatographic method such as reversed-phase LC. For such work, high-performance exclusion columns have the advantage of low peak dilution.

Exclusion columns are uncontaminated by most non-polar compounds since THF, used in this study, is of sufficient solvent power to prevent solute interaction with the polystyrene backbone. Thus, some oils, creams and non-polar polymeric materials which would have a tendency to be strongly retained by many LC columns usually elute from exclusion columns. If such materials are sorbed onto other columns they could affect chromatographic retention. In such cases the sample may need clean-up prior to LC or the column may need clean-up as often as at the conclusion of every run or at least periodically to ensure reproducible retention or column lifetime.

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