

Short Communication

Analysis of fullerenes by reversed-phase high-performance liquid chromatography

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(First received June 21st, 1993; revised manuscript received August 11th, 1993)

ABSTRACT

Chromatographic separation of fullerenes was achieved with a conventional reversed-phase analytical system. A tetrahydrofuran–acetonitrile mobile phase (60:40) (1.0 ml/min) used with a 25 cm ODS column separates C_{60} and C_{70} with a resolution of 6.4 and an analysis time of 11.4 min. This method is convenient and offers sufficient resolving power to be applied to the analysis of a variety of fullerenes and fullerene reaction product mixtures. The mobile phase allows use of the sensitive ultraviolet absorbance bands of fullerenes in detection. The method is compared with other reversed-phase methods for analysis of fullerene mixtures.

INTRODUCTION

Efforts to develop chromatographic methods to separate fullerenes (C_{60} and C_{70}) have focused on developing preparatory methods which utilize mobile phase mixtures with high solubilities for the compounds, principally mixtures of hexane with methylene chloride, benzene or toluene [1–5]. Good preparatory separations of C_{60} and C_{70} have been obtained with stationary phases such as dinitroanilinopropyl silica [1], tripodal 2,4-dinitrophenyl ether octyl silica [2,3], phenylglycine chiral stationary phase [6] and polystyrene–divinylbenzene [7]. These stationary

phases are not commonly used by chromatographers, and an analytical separation method utilizing conventional chromatographic stationary and mobile phases, especially using the common octadecylsilane (ODS) column, can be more useful to researchers interested in various aspects of the chemistry of the fullerenes.

C_{60} and C_{70} have been resolved on ODS columns with mobile phases of toluene with isopropanol or methanol [8–10]. Absorption by toluene prevents use of the sensitive ultraviolet absorbance region in detecting the eluates. Diack *et al.* [11] have obtained baseline resolution of C_{60} and C_{70} using hexane with an ODS column. Improvement of this separation to allow resolution of fullerene adducts or reaction mixture impurities is not straightforward due to

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the immiscibility of commonly used reversed-phase modifiers in hexane. Jinno *et al.* [9] have obtained better resolution with hexane using a monomeric ODS column (Develosil ODS-5). Reversed-phase chromatography of fullerenes using methylene chloride–acetonitrile mobile phase has been investigated [12,13]. This mobile phase produces good resolution of these and other fullerenes on both Whatman Partisil 5 analytical and Vydac 201TP510 preparatory columns. Methylene chloride is an inhalation irritant, a mutagen and an animal carcinogen [14], and as a chlorinated hydrocarbon disposal of this solvent is strictly regulated, hence its use should be avoided when possible.

We have found that excellent resolution of C_{60} and C_{70} can be obtained with ODS stationary phase and tetrahydrofuran (THF)–acetonitrile (ACN) mobile phase. This mobile phase sufficiently solubilizes C_{60} and C_{70} to allow injection of reasonable quantities of the compounds for analysis, and the ability to detect low concentrations of these and other fullerenes in reaction mixtures is enhanced as strong UV absorbance bands of the compounds can be monitored. Reversed-phase chromatography of C_{60} and C_{70} in methylene chloride with this mobile phase is compared with chromatography using hexane, isopropanol–toluene and other mobile phases.

MATERIALS AND METHODS

Chromatograms were obtained with a Perkin-Elmer Series 4 HPLC chromatograph equipped with a Hitachi 100-10 detector. A Phenomenex Spherex 5 C_{18} column (250 mm \times 4.6 mm) was used in obtaining all chromatograms for which chromatographic parameters are reported. A Hewlett-Packard 1090M chromatograph equipped with a diode array UV–visible absorbance detector and a HP ODS Hypersil 5 μ m column (200 mm \times 4.6 mm) was also used.

Unretained time was determined by injection of 1 M sodium nitrate solution [15]. A mobile phase flow-rate of 1.0 ml/min was used throughout.

Absorbance of the compounds was monitored at 254 except in the case of the isopropanol–

toluene mobile phases, with which absorbance was monitored at 330 nm. Identities of the C_{60} and C_{70} chromatographic peaks were confirmed by comparing spectra of the peaks obtained with the diode array detector with published UV–visible spectra [16].

A portion of a mixture of C_{60} and C_{70} (Mer Labs., Tucson, AZ, USA), was allowed to equilibrate at room temperature with methylene chloride in a sealed vial for several weeks, then filtered through a 0.5- μ m pore diameter PTFE filter (Corning) prior to injection. All mobile phases were either ACS reagent grade or HPLC grade (Fisher Scientific).

RESULTS AND DISCUSSION

Tetrahydrofuran–acetonitrile

With the Spherex ODS column, a mobile phase mixture of THF–ACN (80:20, v/v) produces near-baseline resolution of C_{60} and C_{70} , with capacity factor (k') values of 0.47 and 0.62 for the two compounds, respectively. A 60:40 mixture of these solvents produces a resolution of 6.4, with k' values of 2.2 and 3.8 and plate numbers of 3300 and 5300 for C_{60} and C_{70} , respectively (Fig. 1a). (See Table I for a comparison of this and other reversed-phase separations of C_{60} and C_{70} .) Several unidentified mix-

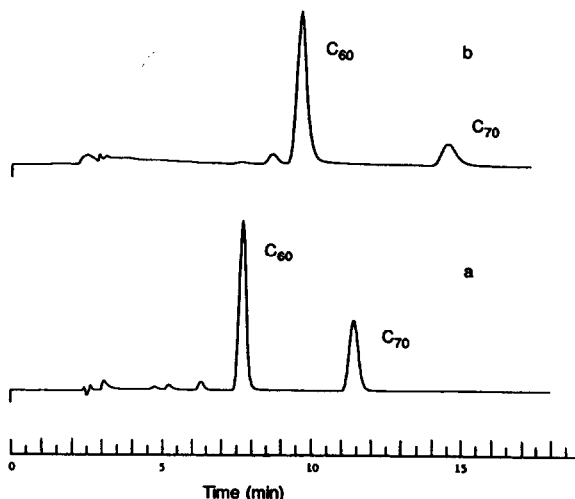


Fig. 1. Separation of C_{60} and C_{70} with an ODS column and (a) THF–ACN (60:40) (254 nm detection) and (b) isopropanol–toluene (75:25) (330 nm detection).

TABLE I
REVERSED-PHASE CHROMATOGRAPHIC SEPARATIONS OF C₆₀ AND C₇₀

Resolution values are estimated from published chromatograms.

Mobile phase	Retention time of C ₇₀ (min)	Resolution ^a	Ref.
Isopropanol–toluene (60:40)	9.3	3.4	8
Isopropanol–toluene (75:25)	14.5	6.1	Present work
Hexane	5.6	1.7	11
Hexane (monomeric ODS)	9.2	5.1	9
Methylene chloride–ACN (67.5:32.5)	20	10	12
THF–ACN (60:40)	11.4	6.4	Present work

^a Of C₆₀ from C₇₀.

ture components eluted before C₆₀ with the THF–ACN mixture.

Slight fronting of both the fullerene peaks is observed on injection of 35 μ l of the methylene chloride sample. This fronting becomes more apparent with higher injection volumes, though similar retention times and reasonable peak profiles are obtained on injection of 75 μ l sample.

A THF–ACN (50:50) mixture gives even greater resolution, 9.7, with no peak distortion at low injection volumes. Reproducible elution of the compounds is not obtained with a 40:60 mixture, probably reflecting diminished solubility of the compounds in this mixture.

Isopropanol–toluene

Isopropanol–toluene mobile phases are effective in resolving C₆₀ and C₇₀. The 60:40 mobile phase mixture used by Meier and Selegue [8] produces a resolution of only 2.4 with our column, less than that reported for the Waters Novapak column. An isopropanol–toluene (75:25) mixture yields a resolution of 6.1, with *k'* values of 2.9 and 4.9 for C₆₀ and C₇₀. This separation is not as efficient as that obtained with the THF–ACN (60:40) mixture, which produces narrower peaks and better resolution with an analysis time (for elution of C₇₀) of 12

min as compared with 15 min with the isopropanol–toluene mixture (Fig. 1b). As with the THF–ACN mobile phase, slight peak fronting is observed with the isopropanol–toluene (75:25) mixture on injection of 35 μ l sample. Impurity peaks eluting before C₆₀ are not as well resolved from C₆₀ as those apparent in the THF–ACN chromatogram (Fig. 1a).

Other mobile phases

A resolution of 1.9, with *k'* values of 0.87 and 1.2 for C₆₀ and C₇₀, is obtained with hexane mobile phase, in agreement with the results of Diack *et al.* [11].

A THF–water (80:20) mixture rapidly elutes and partially resolves C₆₀ and C₇₀. Increasing water in the mixture to 30% separates the components, but produces pronounced peak splitting, indicating a lack of solubility of the compounds at the higher water content.

Baseline resolution of the two compounds is obtained with a THF–methanol (70:30) mobile phase. C₆₀ and C₇₀ are not eluted in a reproducible manner when the THF–methanol mixture ratio is adjusted to the same mixture polarity (*P'*) [17] as the THF–ACN (60:40) mixture, a 34.5:65.5 mixture. Again, it is probable that the chromatographic failure is due to poor solubility of the compounds in the mixture.

CONCLUSIONS

Analysis of fullerenes can be achieved by conventional reverse phase chromatography with THF–ACN mobile phase. The efficiency of separation with this system, coupled with the good solubility of C₆₀ and C₇₀ in this mixture and the ability to detect the compounds utilizing strong absorbance bands in the ultraviolet spectral region, can facilitate analysis of mixtures of a variety of fullerenes and fullerene products.

ACKNOWLEDGEMENTS

This research was sponsored in part by the National Science Foundation Visiting Faculty Analytical Research Associate Program at the University of Tennessee at Knoxville. The C₆₀/C₇₀ sample was supplied by R.N. Compton, UTK/Oak Ridge National Laboratory.

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