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PREPARATIVE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY AS AN AID IN ORGANIC SYNTHESIS

VERONIKA R. MEYER
Institute of Organic Chemistry, University of Bern, Freiestrasse 3, CH-3012 Bern (Switzerland)

SUMMARY

A preparative high-performance liquid chromatographic (HPLC) system is presented which allows the separation of synthesis products in amounts of several hundred milligrams per injection. It consists of columns of high separation efficiency with microparticulate silica as stationary phase. Standard equipment can be used with minor adaptions. The development of a separation, starting from a thin-layer chromatogram, the preparation of the sample prior to chromatographic separation and some problems arising in preparative HPLC are discussed. Some applications of the method in the field of organic synthesis are presented.

INTRODUCTION

Practical examples of separations by preparative high-performance liquid chromatography (HPLC) are seldom found in the chemical literature, *e.g.*, refs. 1–5. It seems that this particular application of HPLC is either not adequately known or, on the contrary, is in such widespread use that publication is no longer necessary. There is some evidence that the former is true.

The term "preparative HPLC" indicates that fractions are collected from liquid chromatography columns of high separation performance. This implies that the columns are filled with a microparticulate stationary phase of narrow size range. For a given separation problem, the sample capacity of the column is then defined only by the inner diameter of the column.

However, the columns mentioned above and the method itself are both rather expensive. It is, therefore, obvious that preparative HPLC should be used only when other methods of purification, especially open-column liquid chromatography and flash chromatography, fail. This paper deals with some applications of preparative HPLC in the field of organic synthesis. The presented systems allow the separation of the complex reaction mixtures usually encountered in synthetic work. Samples of up to 800 mg can be injected to yield, within a short time, sufficient amounts of pure compounds for spectroscopic structure elucidation, the determination of exact physical data or for further synthesis.

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THEORETICAL

It is the aim of any preparative separation to obtain pure compounds with as little effort as possible. To attain this goal it is necessary to operate the column under a condition of mass or volume overload. Some theoretical discussions of these phenomena are found in the literature[6–17].

Volume overload is much easier to investigate than mass overload. Some formulae for the calculation of the maximum sample volume as a function of column deadvolume, capacity factor, separation factor, resolution and plate number are known[6–9] besides other suggestions[10–12]. It is advantageous to utilize volume overload if the resolution of the interesting peaks is quite high, and if the sample has a poor solubility in the mobile phase. However, in the case of high resolution the separation problem can probably be solved with open-column or flash chromatography[18] and is not a candidate for preparative HPLC. Fortunately, the case of poor sample solubility is quite rare in the field of organic synthesis; it always implies a reduction of the sample throughput per unit time. For these reasons, volume overload is of minor interest in separation systems of high performance.

Mass overload is a complex function of the adsorption behaviour of sample components, capacity factors, separation factors and other parameters. Of course, mass overload begins once the linear part of the adsorption isotherm is exceeded, *i.e.*, with sample amounts surpassing 10^{-4}–10^{-5} g per gram of silica[13,14]. From this point of view, preparative columns are always used under overload conditions. However, in practice, this only becomes a matter of concern at the point where the resolution becomes insufficient due to mass overload. Obviously, this point depends on the parameters mentioned above. Although some investigations in this field have been published[2,6,7,9,15–17], there are no mathematical models (except in refs. 9, 17). It is possible that this problem cannot be solved in a general manner. The behaviour observed under mass overload conditions of the preparative columns used in our laboratory will be published later[19]. It is a matter of course that such conditions can only be studied with very simple test mixtures.

INSTRUMENTATION

General requirements

Preparative HPLC may be performed with standard instrumentation, but needs some adaptions.

Pump. A high flow-rate capability of the pump is necessary. Many pumps are available with preparative pump heads. The Van Deemter curves of the columns used should be known; the flow-rate of the pump used should correspond at least to the Van Deemter optimum.

Sampling device. Any sampling device enabling the injection of samples up to 5 ml can be used. Six-port valves allow the installation of laboratory-made loops of any volume and are very convenient.

Detector. The detector needs to be insensitive. A refractive index detector, perhaps equipped with a preparative cell, is sufficient for all purposes. For samples exhibiting UV absorption, a UV/VIS spectrophotometer with a cell of path length 0.5 mm is preferred. With concentration-sensitive detectors, splitting does not influ-

ence the detector signal, hence it is useless to apply an analytical, sensitive detector together with a splitter.

Extra-column volumes. Extra-column volumes are not critical in preparative HPLC.

Fraction collection. Manual fraction collection is recommended. Overloaded systems are often unstable, and automatic fraction collection provides no advantage other than facilitation of well-known and tested separations of large amounts (more than 10 g of reaction mixture).

Apparatus used

Pump. Altex 100 solvent-metering pump (Altex, Berkeley, CA, U.S.A.) with preparative head. Flow-rate 0–28 ml/min.

Sampling device. Rheodyne 7120 or 7125 syringe-loading sample injector (Rheodyne, Berkeley, CA, U.S.A.). Various loops of 0.1–5 ml were made from 1 mm I.D. (1/16 in. O.D.) steel capillary tube; 1 m of this capillary corresponds to a volume of 0.785 ml. To avoid sample loss the loop should not be filled completely. Due to uneven flow velocity within the capillary a sample loss of more than 10% is inevitable if the loop is filled completely. Losses are negligibly small for loops filled up to only 80% of capacity. Samples are injected by means of cheap glass syringes with Luer lock; a suitable Luer tip needle is available from Rheodyne.

Refractive index detector. RefractoMonitor LDC 1107 (Laboratory Data Control, Riviera Beach, FL, U.S.A.).

UV/VIS detector. Uvikon 720 LC spectrophotometer (Kontron, Zurich, Switzerland) with a preparative cell of path length 0.5 mm. The outlet of the detector consists of a PTFE capillary tube whose length is not critical.

PHASE SYSTEM

For preparative HPLC of organic products, synthesized in our Institute, adsorption chromatography on silica is preferred over reversed-phase chromatography. Due to their medium-polar or non-polar nature, the samples are sparingly soluble in polar mobile phases. Moreover, reaction mixtures often comprise structural isomers whose separation succeeds best on silica. Similar considerations can be found in the work of Rüedi *et al.*[3].

For the mobile phase, a mixture of hexane and *tert.*-butyl methyl ether is often the first choice. The ether acts as a modifier to yield the required elution strength; it is preferred because it does not undergo peroxide formation[20]. When samples of high volatility have to be separated and isolated, a mobile phase with a low boiling point is needed. In these cases, mixtures of pentane and diethyl ether are preferred. If necessary for selectivity or polarity reasons, other solvents are used, *e.g.*, methylene chloride. All mobile phases must be of high optical purity and free of any high boiling residues. HPLC-quality solvents, as offered by many manufacturers of chemicals, are very convenient.

CHROMATOGRAPHIC SYSTEMS

Two types of columns are used in our laboratory for preparative HPLC. They

differ in size and are referred to as either "semi-preparative" or "preparative". The head and base of all columns are flat (with stainless-steel frits) rather than conical, as sometimes recommended, *e.g.*, ref. 14. Gareil and Rosset[21] state that conical ends do not influence the separation if the sample amount exceeds 1 mg per gram of stationary phase.

Semi-preparative column

The column has an I.D. of 10 mm (1/2 in. O.D.) and a length of 25 cm. The stationary phase consists of 5-μm silica (LiChrosorb Si 60, Merck No. 9388). The column is filled in our laboratory at 300 bar with a balanced-density slurry in accordance with our packing method for analytical columns[22]. The typical plate number is 15,000 (measured with nitrobenzene in hexane, avoiding mass or volume overload, but with preparative apparatus). This column is suitable for samples of 5–50 mg per injection. Volume overload occurs if the sample volume exceeds 200 μl[19]. The Van Deemter plot is shown in Fig. 1. As this column can be repacked by ourselves, it is used without a precolumn. Several items have been used occasionally since 1982 and their lifetimes are not yet known.

Preparative column

This is a column of 21.2 mm I.D. (1 in. O.D.) and 25 cm length and is commercially available from Du Pont. The stationary phase consists of 7-μm silica. In the unused state, the plate number is 8000 (guaranteed by the manufacturer and confirmed by our test). After 5 years of regular use, this value dropped to 6800 (measured with nitrobenzene in hexane, avoiding mass or volume overload, but with preparative apparatus). The price of each column is $ 1200.

This column is suited for samples of 100–800 mg per injection. Volume over-

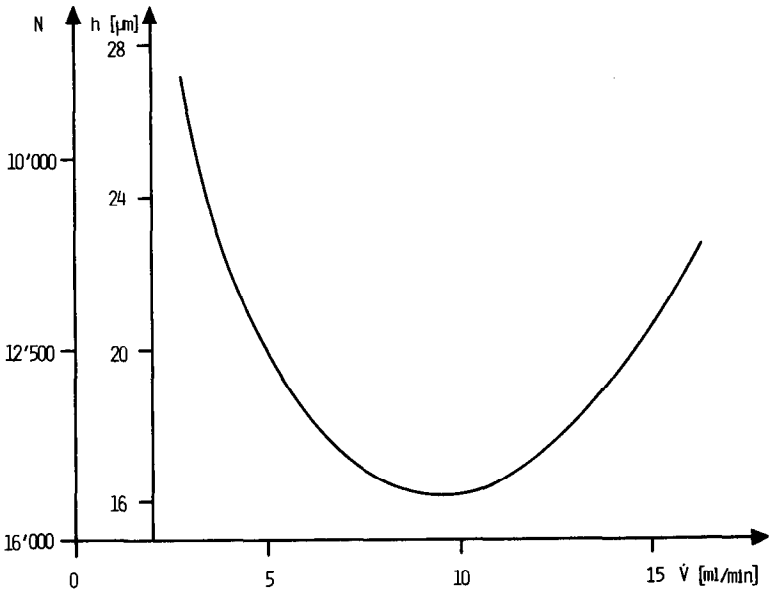

Fig. 1. Van Deemter plot for the semi-preparative column. Sample: nitrobenzene. Mobile phase: hexane.

load occurs if the sample volume exceeds 2 ml[19]. The Van Deemter plot is not known owing to technical problems that arose when this determination was attempted. A plot of minor usefulness, since the axes are unlabelled, was presented by Krusche[2]. Use of the column at a flow-rate of at least 20 ml/min is recommended[23], but we always use it at 14 ml/min.

As this column is very expensive, it should not be used without a precolumn. The precolumn consists of a short column (5 cm × 4.6 mm I.D.) with 1/4-in. fittings and is connected to the preparative column with a short piece of capillary tube. It is dry-filled with coarse silica (25–40 μm) for every 2–5 injections and retains traces of any strongly adsorbed compounds. No influence of the precolumn on the plate number is detectable, *i.e.*, the plate number of the preparative column is identical whether determined with or without the precolumn.

DEVELOPMENT OF A SEPARATION

The development of a separation is always a three-step procedure, which is shown in Fig. 2. This example demonstrates the isolation of pure *tert.*-butylperoxycarbonylbicyclo[2.2.2]octane, a rather simple separation problem.

(a) With thin-layer chromatography (TLC), a mobile phase is evaluated exhibiting a R_F value of *ca.* 0.3. As mentioned previously, a mixture of hexane and *tert.*-butyl methyl ether is often the first choice. Cases calling for the use of ternary mixtures are very rare. If the thin-layer chromatogram indicates sufficient separation, open-column liquid chromatography or flash chromatography[18] should be tried instead of preparative HPLC.

(b) The mobile phase evaluated by TLC is used for an analytical HPLC separation. The column employed in this step must have a high plate number (10,000 plates or more) to allow the number and resolution of peaks present in the sample to be estimated. Special attention must be paid to minor components with insufficient separation from the desired peak. In a preparative separation, they can be eliminated by careful fraction collection.

(c) The chromatogram obtained from a preparative high-performance column is more or less similar to that from an analytical column. Deviations occur mainly from overloading effects. In the example presented three fractions were collected. The first peak yielded 10 mg and was not investigated further. The second peak yielded 68 mg and was identified by NMR spectroscopy as N,N′-dicyclohexylcarbodiimide. The third peak yielded 151 mg of the desired *tert.*-butylperoxycarbonylbicyclo[2.2.2]octane in pure form.

PREPARATION OF THE SAMPLE

Since the columns used in preparative HPLC are very expensive, careful sample preparation is absolutely necessary. The most important step is the purification of the crude reaction mixture by classical methods, *e.g.*, extraction, distillation or crystallization. Moreover, some kind of chromatographic purification should not be omitted. Open-column liquid chromatography or flash chromatography[18] is recommended; in some cases a simple filtration through coarse silica is sufficient. The criterion for adequate purification is the lack of spots at the start of the thin-layer chromatogram.

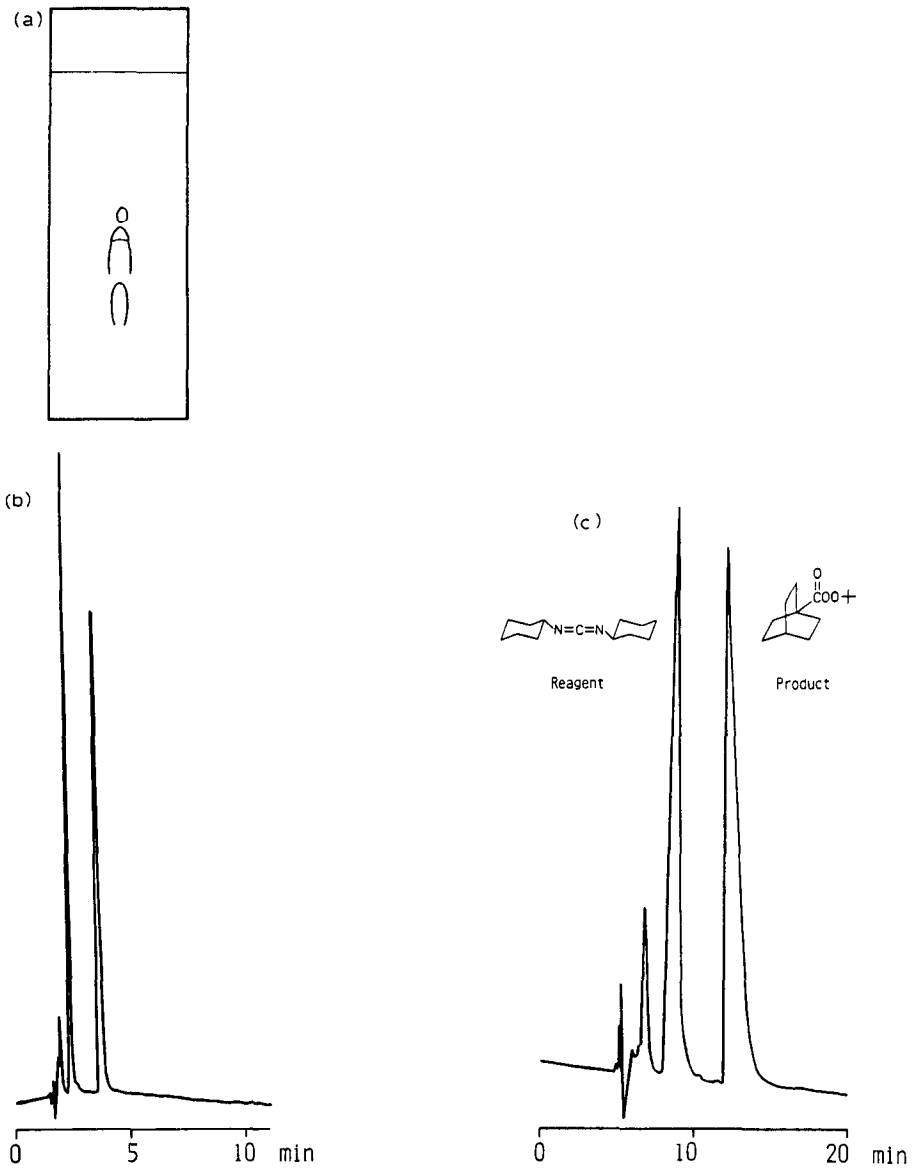

Fig. 2. Development of the preparative separation of *tert.*-butylperoxycarbonylbicyclo[2.2.2]octane. Mobile phase in all cases: hexane–*tert.*-butyl methyl ether (20:1). a, Thin-layer chromatogram on silica. b, Analytical HPLC on a 5-μm silica column (25 cm × 3.2 mm); flow-rate 1 ml/min; refractive index detector. c, Preparative HPLC on a 7-μm silica column (25 cm × 21.2 mm) and a precolumn; sample, 1.5 ml solution containing 340 mg solute; flow-rate 14 ml/min; refractive index detector.

Any sample needs to be diluted prior to injection. Undiluted samples clog the column and give rise to distorted peaks due to local mass overload. A 1:1 dilution is often sufficient. The solvent used for dilution should be the mobile phase itself; in cases of solubility problems a slightly more polar solvent mixture may be used. The

dramatic deterioration of resolution caused by dilution with a solvent of excessive polarity was demonstrated by Wall[16]. In addition, flushing of the column with a mobile phase containing an unsuitable diluent can cause precipitation of the solute either in the loop or in the column.

Filtration of the sample solution, while often unnecessary, is recommended if the solution is turbid and if the column is used without a precolumn.

PROBLEMS IN PREPARATIVE HPLC

Solubility of the sample

Some problems caused by poor solubility of the sample in the mobile phase have been discussed above. Usually, the sample is quite soluble in solvent mixtures suitable for adsorption chromatography. Solubility problems are often induced by impurities. Sometimes the problem can be solved by simple filtration of the turbid solution and removal of the impurities.

Very apolar samples

For samples of extreme apolarity even hexane or pentane is too strong an eluent. An example is the separation of two isomeric dienes, shown in Fig. 3. With a capacity factor of *ca.* 1, the resolution is too poor to allow a preparative separation. It is possible that this and similar problems could be solved by the use of perfluo-

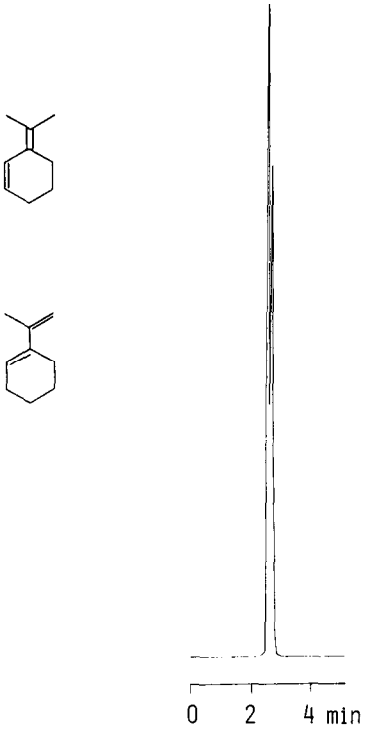

Fig. 3. Analytical separation of two isomeric dienes. Column: 25 cm × 3.2 mm. Stationary phase: 5-μm silica. Mobile phase: 0.5 ml/min pentane containing 0.02% acetonitrile. Detector: UV, 230 nm.

roalkanes as mobile phase[24]. These solvents are less polar than hexane or pentane and thus yield a larger capacity factor. By assuming that the relative retention of the two compounds remains unchanged, their resolution is expected to increase. However, we have no experience with perfluoroalkanes.

Ill-defined peak forms

Overloaded systems often show ill-defined peak forms. In such cases it is difficult or even impossible to judge the true nature of the peaks. It is therefore necessary to collect a great number of fractions, and to identify them by NMR spectroscopy or other methods. An extreme example is shown in Fig. 4. After a careful identification of numerous fractions, the two isomeric benzobarrelenes could be collected in pure form. It is supposed that peak distortion is enhanced when apolar mobile phases are used.

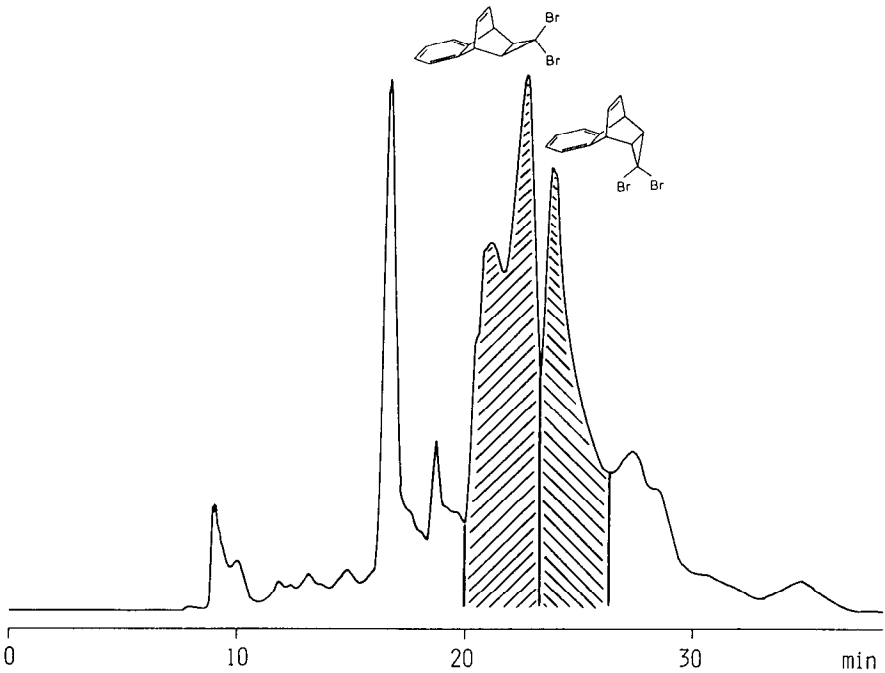

Fig. 4. Preparative separation of *exo-* and *endo*-dibromobenzobarrelenes. Sample: 800 µl solution containing 500 mg solute. Column: 25 cm × 21.2 mm in accordance with Figs. 6 and 8 and a precolumn. Stationary phase: 7-µm silica. Mobile phase: 14 ml/min pentane containing 0.02% acetonitrile. Detector: UV, 254 nm.

Peaks of unknown identity

Peak size not only depends on the relative mass contribution of the individual components in the sample but also on their optical properties (UV absorption at the wavelength chosen or refactive index in relation to the refractive index of the mobile phase). This can lead to confusing effects and to premature discarding of small peaks which were not recognized as the components of interest. As an example, during the

preparative separation of an ester (with low UV absorption), the main peak was collected and two minor peaks were discarded. The collected fraction proved to be urea, a reagent used in the synthesis, while the ester wanted could no longer be isolated from the waste bottle.

Another example of confusing peak size is shown in Fig. 5. In this case, the pheromone wanted could be isolated in pure form as one of the minor peaks.

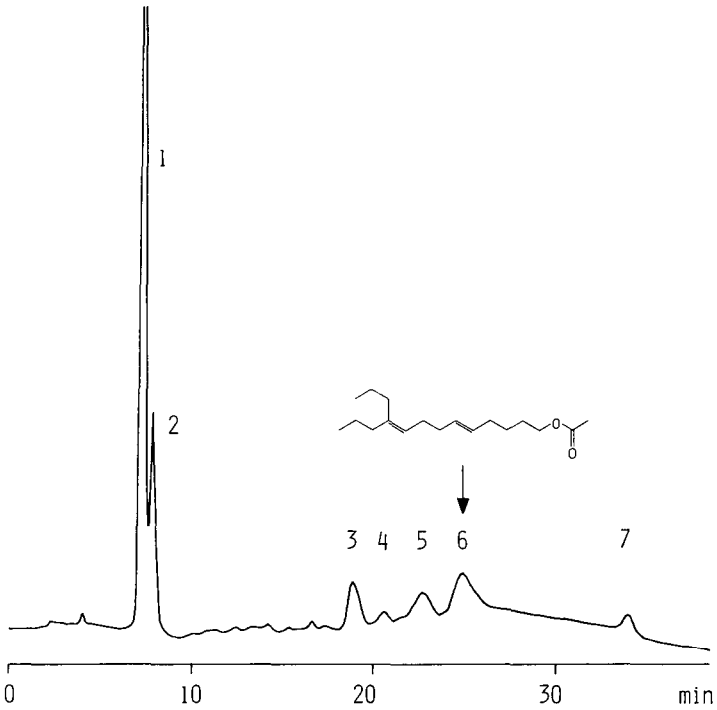

Fig. 5. Preparative separation of a pheromone. Sample: 1 ml solution containing 220 mg solute. Column: 25 cm × 21.2 mm in accordance with Figs. 6 and 8 and a precolumn. Stationary phase: 7-μm silica. Mobile phase: 14 ml/min dichloromethane–hexane (1:1). Detector: refractive index.

EXAMPLES

As a further illustration of the method described in this paper, two additional examples of preparative separations are presented here. The first one is the separation of diethyl ketone with different degrees of bromination (Fig. 6). The total amount of sample injected was 800 mg, but obviously it could have been increased considerably.

The second example is the separation of the photoproducts of *threo*-3-phenylhept-6-en-2-ol[25] (Figs. 7 and 8). The most interesting peak was 2 which could be isolated in pure form. Other pure fractions were 1, 5 and 7, whereas fractions 3, 4

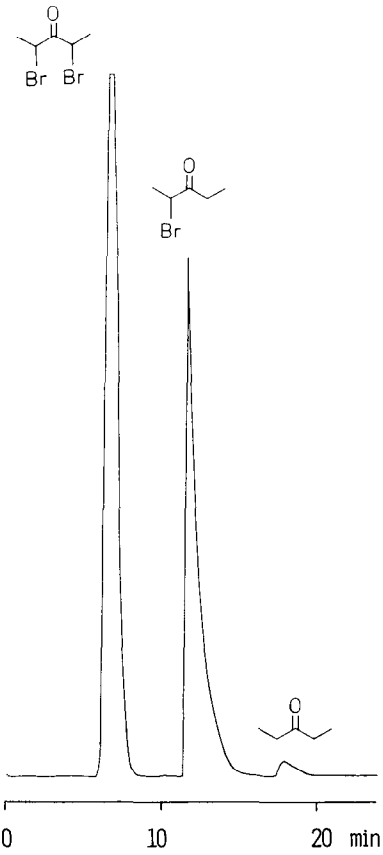

Fig. 6. Preparative separation of brominated diethyl ketone. Sample: 1.6 ml solution containing 800 mg solute. Column: 25 cm × 21.2 mm and a precolumn. Stationary phase: 7-μm silica. Mobile phase: 14 ml/min pentane–diethyl ether (19:1). Detector: UV, 270 nm.

and 6 consisted of mixtures. Photolysis and subsequent preparative HPLC was the only possible way to obtain the desired compound.

Another example of preparative HPLC, the separation and isolation of four nitriles with different positions of a double bond, has been published[26].

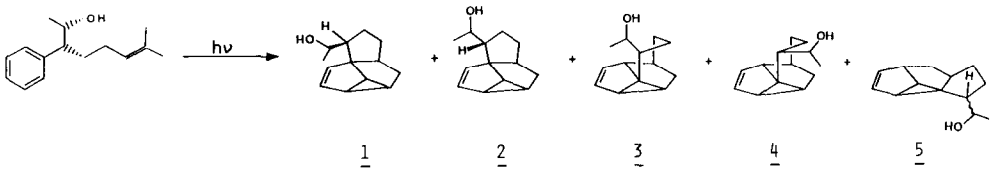

Fig. 7. Photoproducts of *threo*-3-phenylhept-6-en-2-ol.

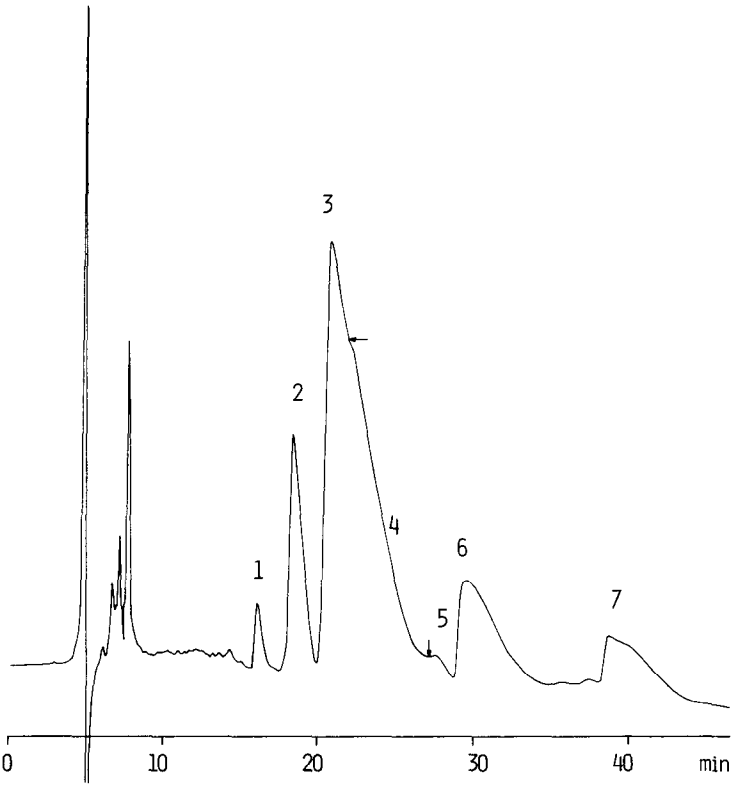

Fig. 8. Preparative separation of the photoproducts of *threo*-3-phenylhept-6-en-2-ol. Sample: 1 ml solution containing 480 mg solute. Column: 25 cm × 21.2 mm and a precolumn. Stationary phase: 7-μm silica. Mobile phase: 14 ml/min hexane–*tert.*-butyl methyl ether (9:1). Detector: refractive index. Fractions: 1, compound 1 (as in Fig. 7); 2, compound 2; 3, compound 3 + some starting material; 4, compound 3 + starting material (2:1 mixture); 5, starting material; 6, compound 4 + starting material and an unknown product; 7, compound 5.

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