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NON-AQUEOUS SIZE-EXCLUSION CHROMATOGRAPHY COUPLED ON-LINE TO REVERSED-PHASE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

INTERFACE DEVELOPMENT AND APPLICATIONS TO THE ANALYSIS OF LOW-MOLECULAR-WEIGHT CONTAMINANTS AND ADDITIVES IN FOODS[a]

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SUMMARY

An interface has been developed which permits the on-line coupling of size-exclusion chromatography in tetrahydrofuran with aqueous reversed-phase high-performance liquid chromatography. The interface isolates the required size exclusion chromatography fraction and dilutes it with water to ensure reconcentration of analytes on the reversed-phase column prior to gradient elution. Operational parameters and the influence of analyte polarity have been examined in detail. A predictive system is presented for determining the applicability of the system to any analyte, based on solute retention times on an ODS phase eluted with a methanol–water gradient. The method is illustrated with examples of direct analyses of crude lipid extracts from a snack product for 2,6-di-*tert*.-4-methylphenol and from chocolate for dibutyl phthalate. Detection limits of *ca.* 0.5 mg/kg have been achieved.

INTRODUCTION

Few trace-level contaminants may be determined directly in foodstuffs. They must usually be separated from the bulk of the food prior to analysis by relatively non-selective methods such as solvent extraction. The majority of measurements are made using chromatographic techniques and the complexity of food extracts places severe demands upon the performance of chromatography columns. Even where

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immunological methods are employed, sample preparation requirements are often stringent in order to avoid matrix effects.

Davis and Giddings[1] have estimated that a column efficiency of *ca.* 200 000 theoretical plates is required to give a 90% probability (at a resolution, R_s, of 1.0) that on chromatography of a sample containing 20 randomly chosen compounds, individual peaks will be due to a single component. Martin *et al.*[2,3] have presented an analysis which indicates that the separation problem is even more severe. In contrast, the efficiency of a typical high-performance liquid chromatography (HPLC) column is 10 000–20 000 plates and for capillary gas chromatographic (GC) columns, 50 000–100 000 plates. Some analytes possess properties permitting selective detection (by electron capture or fluorescence for example), while proper choice of chromatographic phase selectivity may also alleviate the problem. Nevertheless, in most cases extensive cleanup of food extracts is necessary in order to achieve an acceptable degree of confidence in the identification of the target compound.

Current methods of cleanup often require a laborious series of solvent partition and low-resolution chromatography stages. The time assigned to cleanup in many cases accounts for 80–90% of the entire analysis and this is an unacceptable diversion of manpower from other more productive work. It is not unusual for a batch of six samples to represent one day's throughput for a skilled analyst. In addition, manual processing of samples is a tedious task which however requires constant attention to detail in order to achieve consistent results. Thus there is a demand for the automation of sample cleanup.

The application of sequential chromatographic stages (multi-dimensional chromatography) to resolve components of complex mixtures is well known. The method is often carried out off-line but this is not desirable in routine analysis. On-line multi-dimensional separation is in principle simple, but practical considerations have limited its utility. Two (or more) columns of differing selectivity are connected via a switching valve. The analyte peak from the first column is diverted onto the second under conditions chosen such that the analyte is strongly retained. Step or gradient elution of the second column separates the analyte from compounds co-eluting from the initial column. Many HPLC instruments provide timed events capable of controlling external devices, facilitating the automation of column switching methods.

The choice of chromatographic modes to couple depends to some extent upon the nature of the sample and in particular the solubility of target compounds. However, if optimum results are to be obtained, it is important to select modes which have different underlying mechanisms. Reversed-phase HPLC and size-exclusion chromatography (SEC) offer complementary advantages. Reversed-phase chromatography depends upon enthalpic differences between solutes. Its selectivity may be varied between fairly wide limits by suitable choice of conditions but column capacity is relatively limited. SEC is highly predictable —all solutes elute within one column volume — and thus automation is facilitated. Because it is non-enthalpic and does not involve interactions of solutes with column packings, sample loadings can be relatively high. However, separation efficiency is limited. The closely related technique of gel permeation chromatography (GPC) has been applied in selected areas of lipid cleanup[4,5], notably for organochlorine and organophosphorus pesticides. An automated system was devised by Tindle and Stalling[6] and this is commercially available. Recently Hopper and Griffitt described[7] an accessory to this system which automat-

ically concentrates the eluent fraction containing the desired compound into a GC autosampler vial ready for immediate injection.

On-line column switching is facile if the eluent employed with the first column is non-eluting for the analyte when on the second column. Thus it is a simple matter to combine aqueous SEC with reversed phase HPLC[8–12]. The only interface required is a standard high-pressure valve; most common injection valves may be employed and electric or pneumatic actuators are readily obtainable. However, many analytes and residues have limited solubility in water, restricting the utility of this method.

The critical aspect of any column switching method is the refocusing of analyte at the head of the second column. HPLC peaks are typically 0.5–1.0 ml in volume, which, unless reconcentrated on transfer, would give rise to an inferior performance on the second column. When the two modes of chromatography employed are incompatible in the sense that the mobile phase for the primary separation is a strong eluent in the second, the only general method available for their on-line combination entails heart-cutting. In this approach, the volume of analyte peak transferred is restricted in order to limit degradation of second column performance. However, sensitivity is often unacceptably reduced. Johnson *et al.*[13] employed heart cutting between SEC in tetrahydrofuran (THF) and reversed-phase chromatography to determine the pesticide malathion on tomato plants, but with a detection limit of 200 mg/kg.

This communication describes a system for combining on-line non-aqueous SEC with reversed-phase chromatography which achieves detection limits (with phthalates, for example) of less than 1 mg/kg.

EXPERIMENTAL

Apparatus and materials

The system consisted of a Waters (Millipore, Harlow, U.K.) Model 6000A pump; a Gilson (Anachem, Luton, U.K.) Model 231–401 diluter and autosampler fitted with a 500-μl loop; an LKB (Milton Keynes, U.K.) Model 2150 pump; a Rheodyne Model 7010 valve injector fitted with a 5-ml loop and pneumatically operated actuator; a Gilson gradient chromatograph, Model 702, controlled by an Apple IIe microcomputer with external events module 501; a Spectra-Physics Model SP8773 UV detector or Perkin-Elmer (Beaconsfield, U.K.) LS 4 fluorescence detector; and a Trivector (Sandy, U.K.) Trilab 2000 data station. Other components used included a zero dead volume "T" (Valco); a precolumn filter containing a 2-μm frit (Upchurch); and a detector outlet pressure restrictor set at about 2 bar (Upchurch).

A high-performance poly(styrene–divinylbenzene) size-exclusion column, 100 Å PLgel, (5 μm, 300 × 7.7 mm), was obtained from Polymer Labs. (Church Stretton, U.K.). Spherisorb ODS reversed-phase columns (250 × 4.9 mm, 5 μm), were from Hichrom (Reading, U.K.). All organic solvents used were of HPLC quality, from Rathburn Chemicals (Walkerburn, U.K.). HPLC grade water was purchased from Fisons (Loughborough, U.K.). Food samples were purchased locally from normal retail outlets.

Lipid extraction

A weighed amount of food was homogenised in 100 ml acetone–hexane (1:1

v/v). Where required, samples were spiked before homogenisation and left to stand overnight. The residue was filtered and re-extracted with a further 100-ml aliquot of the same solvent. The combined filtrates were dried over sodium sulphate and solvent removed by rotary evaporation. The mass of fat obtained was recorded and the lipid stored at −18°C until required. Lipid residues were dissolved in toluene for coupled column analysis.

Chromatography

Size exclusion. Samples dissolved in toluene were placed in the autosampler and 100-μl aliquots injected by partial loop fill. For all analytes an autosampler cycle time of 50 min was selected to allow for completion of the remainder of the analysis. As mobile phase THF was used at 1.0 ml/min.

Interface conditions. During SEC fractionation of the crude lipid extract, water was pumped into the "tee" union at the outlet of the SEC column (Fig. 1) at a constant 4.0 ml/min. At a time dependant upon the SEC retention of the target compound, the interface valve was switched to load the trapping loop (initially filled

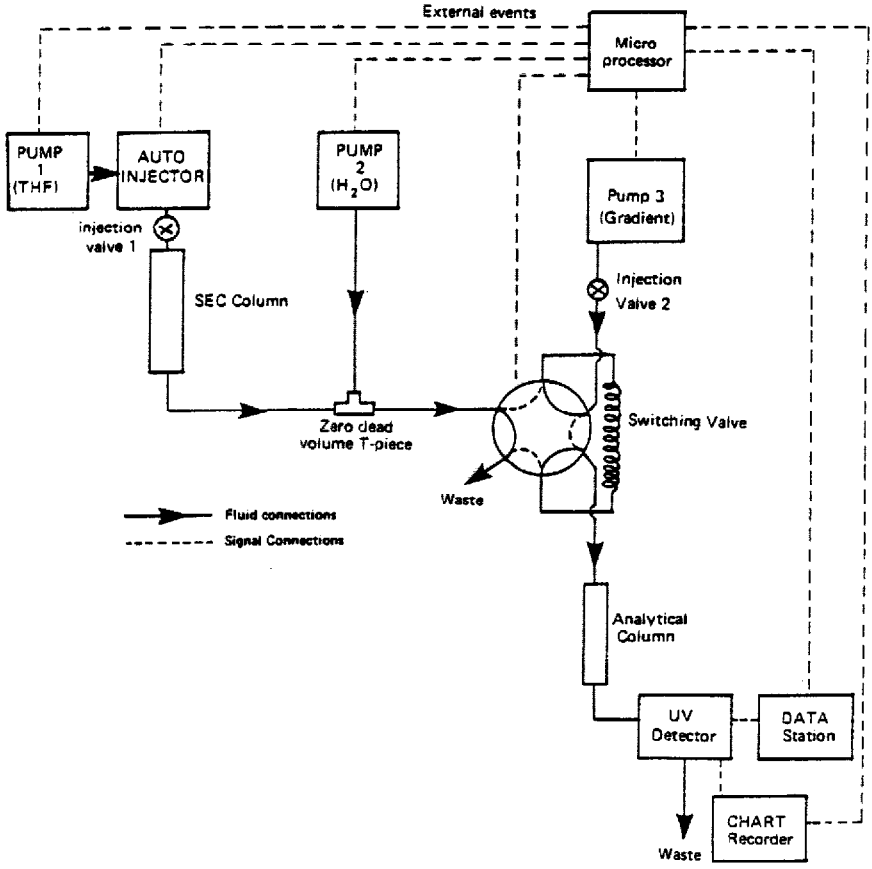

Fig. 1. Diagrammatic representation of the coupled LC–LC system.

with HPLC quality water) with the premixed analyte peak, now in THF–water. After a set collection time (1.00 min for all applications reported) the interface valve was actuated to inject the trapped diluted SEC peak onto the reversed-phase column, which had been pre-equilibrated with water during the SEC separation.

Reversed phase. Four loop volumes of water were used to flush the sample onto the reversed-phase column. The loop was then switched out of line and a linear gradient initiated, running up to 100% acetonitrile over 20 min. After 5 min hold at 100% acetonitrile, a rapid (2 min) reversed gradient was carried out, and the column re-equilibrated with water for a further 5 min.

RESULTS AND DISCUSSION

System development

The optimum eluents for non-aqueous SEC have solubility parameters close to that of the gel employed. Although surface-modified silica may be used, it has several drawbacks; incomplete surface coverage, or loss of bonded material on use, will lead to exposure of active silanols with consequent potential selective adsorption of sample components. The restricted range of pore sizes, and particularly the low pore volumes available with narrow pore silicas, further limits work with small molecules. Thus cross-linked polystyrene is preferred for the separation of a wide range of lipophilic organic compounds and for this gel effective solvents include toluene, chloroform and THF. All these are strong eluents for reversed-phase chromatography and cannot be injected directly in volumes of 0.5–1.0 ml, typical of peak widths on standard 7.7-mm internal diameter high-performance SEC columns. Non-aqueous SEC cannot therefore be coupled directly with reversed-phase chromatography.

One possible on-line interface methold would utilise some form of transport device to remove the SEC mobile phase and replace it with water or other non-eluting solvent before injection onto the reversed-phase column. This would be essential with an SEC eluent immiscible with water, but mechanical devices of this kind are expensive and often present difficulties in operation. An alternative approach involves diluting the SEC peak with a weak solvent, prior to injection onto a reversed-phase column, so that the mixture is non-eluting for the analyte on the chosen packing. The analyte may then be reconcentrated from the diluted peak by adsorption onto the head of the reversed-phase column and subsequently efficiently separated by gradient elution. In this case the SEC mobile phase must be miscible with water, and THF is the only practicable candidate. The major problem with THF as eluent is its poor stability. This was overcome by not transferring THF from the containers it was supplied in and by maintaining it in darkness under a constant low purge of helium. In addition samples for analysis were dissolved in toluene, rather than THF. It was found that interferences accumulated rapidly in samples stored in THF. Toluene is a good solvent for lipids, is highly compatible with the SEC gel and because of its small size is well resolved from all common analytes.

The potential of the proposed method of interfacing was shown in earlier work[4], where phthalates in 400 μl acetonitrile were reconcentrated after passive dilution of the sample during loading into a 2.0-ml sample loop filled with water. The valve was plumbed so that during injection the sample passed through the loop in the same direction as on loading, instead of being swept backwards as in the standard

configuration. Sufficient mixing was achieved simply by the axial dispersion caused by injection. However, with THF as eluent and analytes more polar than phthalates, poor peak profiles were obtained. This arose as a direct result of inadequate mixing on-line. Similar samples mixed off-line gave excellent peak shapes. Preliminary experiments with loops filled with ballotini to promote mixing were abandoned because of the low free volume fraction of the loop; a packed column of 690 × 4.6 mm would have been required to obtain a 5-ml sample volume.

Thus the interface shown in Fig. 1 was devised. The required SEC peak is diluted with water introduced from another pump via a "tee" immediately following the SEC column outlet and the mixture is trapped in a switching valve with a suitable large volume loop. The SEC peak dilution ratio may be altered as required by varying the THF and water pump flow-rates. The trapping loop was employed to avoid putting the SEC column under excessive pressure when loading the diluted analyte peak on to the reversed-phase column. One problem with polystyrene SEC columns is the relative softness of the gel, which restricts the maximum acceptable pressure drop across the type of column employed in this work to about 100 bar. Direct coupling of the columns would be more flexible, permitting microprocessor control over the volume of SEC peak transferred simply by altering the valve switching times. To achieve this at a constant dilution ratio in the current system, it is necessary to install a trapping loop of a different size. This was not considered a significant problem because the automated apparatus is designed for long runs of analyses of the same kind.

Water quality is a major constraint on the sensitivity achievable with this method. A considerable volume of water (up to 30 ml) is pumped through the reversed phase column between analyses, and many impurities will be concentrated at the head of the column in a manner similar to that required for the analyte. Early experiments using water from a commercial purification system showed an unacceptable level of impurities. HPLC grade water from several commercial suppliers was evaluated by inspection of a blank gradient chromatogram with detection at 254 nm. Water quality varied significantly between suppliers, but the water selected for use was essentially free from interferences.

Pump flow-rate stability is critical to successful implementation of a column switching method based on timed events. The performance of the HPLC pumps employed was monitored and shown to be satisfactory. At least 50 measurements were taken during each test period (1 h) at regular intervals over two years. Typical coefficients of variation (C.V.) for THF and water pumps (1 ml/min and 4 ml/min respectively) were 0.7 and 0.9%; there was little change with time. This gives an indication of short-term fluctuations of pump flow, over time periods of less than 1 min. A more important indication of pump stability was obtained by monitoring analyte retention times. The C.V.s for the peak maxima retention times of dibutyl phthalate, diethylstilboestrol and 1-phenylpropan-2-ol on the SEC column were found to fall within the range 0.14–0.31% (n = 12).

SEC columns containing packings with pore sizes of 50 and 100 Å were compared for their resolution from lipids of representative small solutes. The 50-Å column gave inadequate separation and was therefore not evaluated further. A single 300-mm, 100-Å column was used for all the work reported here. One potential limitation of the proposed method is the high resolution of these columns, which may result in resolution of individual members of analyte families thereby restricting group

analyses. However, analyte homologues varying by 2–3 methylene groups would co-elute within the 60-s trapping period for SEC peaks employed during the evaluation of the technique. This is an unnecessarily wide window for single compounds, as shown by peak area data. Peaks from dibutyl phthalate standards were integrated over selected intervals and the collection times required to obtain 95% and 98% recoveries found to be 23 and 28 s. Thus on this basis the collection window could usefully be reduced to 40 s. Obviously the narrower this window, the fewer potential interferences will be transferred to the reversed phase column. However, some allowance has to be made for pump flow rate variations and for sample viscosity effects.

One major application of the reported system is the direct analysis of lipophilic analytes in co-extracted fat. The SEC retention times of small molecules decrease in the presence of high concentrations of lipids through the effect of viscous drag[4]. Both the concentration and type of lipid has an effect on viscosity, as shown in Fig. 2, which plots phthalate recovery against sample dilution ratio for a range of spiked fats and oils.

For any given analyte, the detection limit attainable is a function of mass loading onto the SEC column and of the extent of interferences in the final reversed-phase chromatogram. Increasing the injection volume for a given mass of lipid reduces the effect of viscosity on analyte elution times but broadens the analyte SEC peak by the additional volume. Fig. 3 shows dibutyl phthalate peak areas against volume of injected spiked vegetable oil, diluted 1:16 with toluene, with constant switching times. No more than 400 μl of this sample was acceptable, equivalent to a lipid injection of *ca.* 25 mg. Additional experiments using direct fluorescence detection of the eluent from the SEC column (to overcome the high UV background from olive oil) with a sample consisting of zearalenone spiked into diluted olive oil also indicated that a maximum of 25 mg oil (100 μl of a 1:4 solution) could be injected without a reduction in retention time for co-injected analytes. In neat olive oil the retention time was reduced by 18 s, representing a 30% loss of solute with the peak collection window of

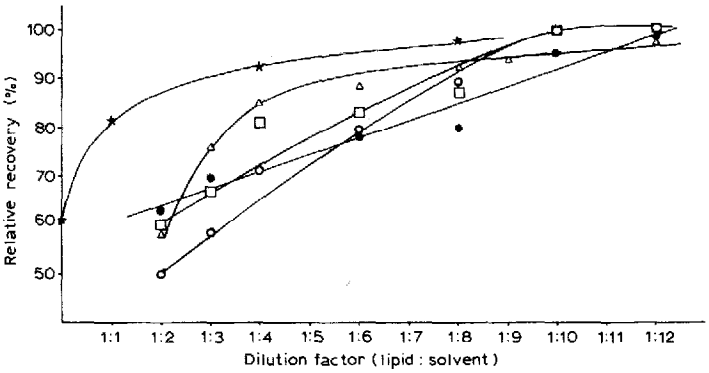

Fig. 2. Comparison of recovery of DBP with lipid concentration for a range of lipid types. (□) Crisp, (○) chicken, (●) beef, (△) chocolate, (★) olive oil. Where required lipid was extracted as described in Experimental. Analysis was carried out as described in Experimental. Chromatographic conditions: columns, PLgel, 100 Å, 5 μm, 300 × 7.9 mm and Spherisorb ODS, 5 μm, 250 × 4.9 mm. Detection at 254 nm. SEC eluent, THF at 1 ml min^{-1}. Dilution water, 4 ml min^{-1}. RPC mobile phase at 1 ml min^{-1}; 0–20 min, 100% H_2O; 20–30 min, 0–100% acetonitrile; 30–50 min, 100% acetonitrile.

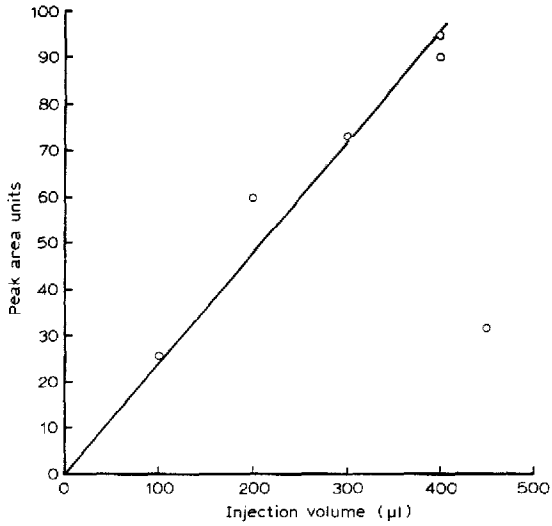

Fig. 3. Effect of sample volume on recovery of dibutyl phthalate in vegetable oil. Chromatographic conditions as in Fig. 2.

60 s set according to the SEC retention time for zearalenone standards injected in the absence of oil. For dibutyl phthalate added to vegetable oil a detection limit of *ca.* 0.2 mg/kg could be attained at a signal-to-noise ratio of 3:1. The sensitivity achievable with samples will be discussed below.

Prediction of analysis conditions

The extent of dilution with water required for the SEC peak to be retained on the reversed-phase column is an important system parameter. The holding loop flushing time is a major element of the overall analysis time and the volume of water required for purging could be a constraint upon achievable sensitivity depending upon its purity. Thus an attempt was made to predict the dilution ratio required for any given analyte.

The important parameter for solute reconcentration is its retention on the reversed phase column in a mobile phase consisting of water and THF. The problem reduces to calculation of the water–THF composition just permitting acceptable reconcentration. It was anticipated that for any given reversed phase column, there would be a constant limiting analyte capacity ratio, below which reconcentration would not be possible. Determination of this unknown minimum capacity ratio was carried out empirically. Three test solutes covering a moderate polarity range (dibutyl phthalate, diethylstilboestrol and 1-phenylpropan-2-ol) were chromatographed on the reversed-phase column in a number of isocratic acetonitrile–water mobile phases giving rise to capacity ratios of between 0.5 and 20 and graphs of capacity ratio *versus* mobile phase acetonitrile content prepared. The same compounds were also chromatographed on the coupled column system using interface loops varying in size from 2.5 to 5 ml. Comparison of the coupled column chromatograms obtained under these conditions with those from standards injected directly onto the Spherisorb column

showed whether reconcentration had taken place. This indicated the maximum THF concentration permitting concentration. The simple solvent transfer rule[14]:

$$\varphi_{CH_3CN} = \varphi_{THF} \cdot \delta_{CH_3CN}/\delta_{THF} \quad (1)$$

where φ_{THF}, δ_{THF}, φ_{CH_3CN}, δ_{CH_3CN} are the mole fractions and solubility parameters of THF and acetonitrile respectively, was then applied to calculate the acetonitrile content of a water–acetonitrile mixture having the same solvent strength. Interpolation of this value on the graph previously prepared gave the equivalent isocratic acetonitrile–water capacity ratio. For all three test compounds it was found that a capacity ratio of *ca.* 2.5 indicated reconcentration. It is therefore necessary only to determine experimentally the composition of the isocratic acetonitrile–water mixture giving a capacity ratio of 2.5 for any analyte, and, using the solvent transfer rules, to translate this into an equivalent THF concentration and thus to a water dilution factor.

The process of prediction was taken one step further to avoid the need to carry out repetitive isocratic retention experiments for each required analyte. Berridge[15] has published a BASIC computer program (based on the calculations of Dolan *et al.*[16]) which calculates the isocratic mobile phase composition yielding the same retention time as that found experimentally when the same column is eluted with a gradient of $6.6/t_0$ % methanol per min.

Application of this program and eqn. 1 indicated that any solute giving under the specified conditions a gradient retention time of greater than 19 min should have on the same column a capacity factor of > 2.5 in an aqueous mobile phase containing 20% THF. Reconcentration on the coupled column system would occur with a loop volume no greater than 5 ml. This result was tested by comparison of retention data on the specified methanol gradient with the effectiveness of reconcentration on the coupled column system for a further ten compounds. These included phenol (adequate reconcentration) and sulphadimidine, orcinol and caffeine (inadequate). It was concluded that a retention time in excess of 22 min on the standard gradient was a more reliable predictor of reconcentration. The three compounds for which reconcentration failed are readily water soluble and are therefore good candidates for coupled aqueous SEC–reversed-phase chromatography. It is probable therefore that any analyte of limited water solubility would be suitable for separation on the coupled column system described here.

Applications

Samples of extruded potato snack products containing 2,6-di-*tert.*-butyl-4-methylphenol (BHT) and of chocolate confectionary products containing dibutyl phthalate were analysed by the method described. Coupled column chromatograms of standards and samples are shown in Figs. 4 and 5. It can be seen that the cleanup achieved is adequate for determination of these compounds at about 0.5 mg/kg (blank samples were not available) and that the major limitation on sensitivity is the presence of co-extractives. Approaches to achieving better resolution could be to use a longer SEC column of the same or different pore size, to add a flushable pre-column between the SEC and reversed-phase columns, or to switch the analyte from the reversed-phase column onto a third column with different selectivity. A more realistic alternative may be to enhance overall selectivity by incorporating post-column deri-

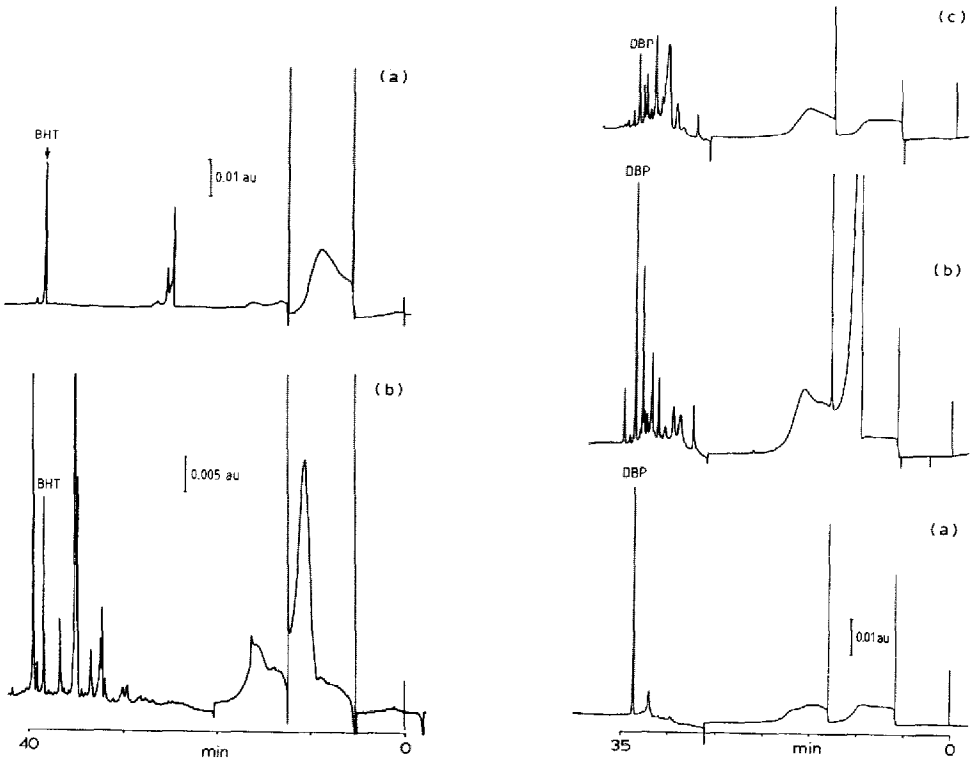

Fig. 4. Analysis of BHT in solvent and in a reformed snack product on the coupled LC–LC system. Sample: (a) 1.0 μg BHT in 100 μl toluene, (b) 25 μl extracted lipid and 75 μl toluene. Analysis was carried out as described in Experimental. Columns as in Fig. 2, detection at 280 nm. Gradient: 0–15 min, 100% H_2O; 15–17 min, 0–20% acetonitrile; 17–30 min, 20–80% acetonitrile; 30–35 min, 80–100% acetonitrile; 35–40 min, 100% acetonitrile.

Fig. 5. Analysis of DBP in solvent and in chocolate extract on the coupled LC–LC system. Sample: (a) 0.15 μg DBP in 100 μl toluene, (b) chocolate bar extract (25 μl) in 100 μl toluene, (c) chocolate sweet extract (25 μl) in 100 μl toluene. Chocolate extractions were carried out as described in Experimental. Analysis was carried out as described in Experimental. Chromatographic conditions as in Fig. 2.

vatisation for fluorescence detection. Pre-column derivatisation could create SEC resolution problems due to the increased size of the analyte.

CONCLUSIONS

It has been shown that non-aqueous SEC and reversed-phase chromatography may be coupled for the determination in crude lipid extracts of analytes having a polarity equal or greater to that of phenol with, for the examples shown, detection limits of about 0.5 mg/kg. Maximum lipid loadings on the SEC column employed were examined in detail and found to be *ca.* 25 mg. There was a dependency of the preferred sample concentration/volume conditions on the nature of the lipid.

REFERENCES

1 J. M. Davis and J. C. Giddings, *Anal. Chem.*, 55 (1983) 418.
2 M. Martin, D. P. Herman and G. Guiochon, *Anal. Chem.*, 58 (1986) 2200.
3 M. Martin, D. P. Herman and G. Guiochon, *Anal. Chem.*, 59 (1987) 384.
4 M. J. Shepherd, in J. Gilbert (Editor), *Analysis of Food Contaminants*, Elsevier Applied Science, London, 1984, p. 1.
5 M. J. Shepherd, *Int. Anal.*, 2 (1988) 14.
6 R. C. Tindle and D. L. Stalling, *Anal. Chem.*, 44 (1972) 1768.
7 M. L. Hopper and K. R. Griffitt, *J. Assoc. Off. Anal. Chem.*, 70 (1987) 724.
8 R. E. Majors and T. V. Alfredson, in J. F. Lawrence (Editor), *Trace Analysis*, Vol. 2, Academic Press, London, 1982, p. 111.
9 F. Erni and R. W. Frei, *J. Chromatogr.*, 149 (1978) 561.
10 J. A. Apffel, T. V. Alfredson and R. E. Majors, *J. Chromatogr.*, 206 (1981) 43.
11 J. C. Kraak, K. Kuiper and E. Ruyter, *Int. J. Environ. Anal. Chem.*, 25 (1986) 209.
12 H. Takahagi, K. Inoue and M. Horiguchi, *J. Chromatogr.*, 352 (1986) 369.
13 E. L. Johnson, R. J. Gloor and R. E. Majors, *J. Chromatogr.*, 149 (1978) 571.
14 P. J. Schoenmakers, H. A. H. Billiet and L. de Galan, *J. Chromatogr.*, 205 (1981) 13.
15 J. C. Berridge, *Techniques for the Automated Optimisation of HPLC Separations*, Wiley, Chichester, 1986.
16 J. W. Dolan, J. R. Gant and L. R. Snyder, *J. Chromatogr.*, 165 (1979) 31.