ELSEVIER

Journal of Chromatography A, 880 (2000) 113–120

JOURNAL OF CHROMATOGRAPHY A

www.elsevier.com/locate/chroma

Determination of 1-nitropyrene in herbs after selective enrichment by a sol–gel-generated immunoaffinity column

B. Spitzer[a], M. Cichna[a], P. Markl[a], G. Sontag[a,*], D. Knopp[b], R. Niessner[b]

[a]*Institute for Analytical Chemistry, University of Vienna, Waehringerstrasse 38, A-1090 Vienna, Austria*

[b]*Institute of Hydrochemistry and Chemical Balneology, Technical University Munich, Marchioninistrasse 17, D-81377 Munich, Germany*

Abstract

Using the determination of 1-nitropyrene as an example the paper demonstrates the advantages of including a highly selective sol–gel-generated immunoaffinity column in the sequence of clean-up steps necessary to determine haptens in complex matrices. The sol–gel method to immobilise antibodies enlarges the variety of immunoaffinity columns available and leads to mechanically stable columns with constant retention characteristics. The sample preparation scheme proposed combines acetonitrile extraction, size-exclusion and immunoaffinity chromatography. 1-Nitropyrene is then separated by reversed-phase HPLC from interfering compounds and determined after catalytic on-line reduction to the corresponding amine by spectrofluorimetry. Concentrations in the range from 0.1 to 1.4 μg/kg 1-nitropyrene were detected in herbs.

Keywords: Sol–gel immunoaffinity chromatography; Sample preparation; Food analysis; Immunoaffinity chromatography; Nitropyrenes

1. Introduction

Nitrated polycyclic aromatic hydrocarbons (NPAHs) are formed by reactions of polycyclic aromatic hydrocarbons (PAHs) with nitrogen oxides. Air combustion of petrol or diesel or cooking foodstuffs on an open fire generates both components and their reaction forms trace amounts of NPAHs which are released into the atmosphere. They can be inhaled in the gas phase or after adsorption on airborne particulate matter or enter the food chain by condensation on, e.g., plants and vegetables. The amounts of NPAHs entering the atmosphere are relatively small compared to the amounts of PAHs generated. Members of both classes of compounds show mutagenicity in the Ames test and are genotoxic in other test systems [1–3]. Non-substituted PAHs are however mutagenic only after metabolic activation. NPAHs such as nitropyrenes and nitrofluoranthenes are strongly direct acting mutagens. It has been demonstrated that most of the direct-acting mutagenicity of diesel and air particulates is associated with NPAHs some of which belong to the most potent mutagens tested. In a study on the carcinogenic risks of diesel and gasoline engine exhausts the International Agency for Research on Cancer lists these NPAHs as possibly carcinogenic to humans [4]. In order to evaluate the carcinogenic health risks associated with these compounds it is not only necessary to study their toxicity but also to arrive at a realistic picture of human exposure based on analytical data.

*Corresponding author. Tel.: +43-1-4277-52303; fax: +43-1-4277-9523.

E-mail address: gerhard.sontag@univie.ac.at (G. Sontag)

0021-9673/00/$ – see front matter
PII: S0021-9673(99)01311-4

Several analytical methods have already been developed for the determination of NPAHs in environmental and food samples. They make use of the high separation efficiency of capillary gas chromatography combined with detection methods of a varying degree of selectivity. Capillary gas chromatography combined with non-specific determination by flame ionisation [5–8] or more specific detectors like electron-capture [8–10] or nitrogen–phosphorus detectors [11,12] offer increasing degrees of sensitivity and selectivity. The thermal energy analysis (TEA) system [8,13,14] – a complex reaction detection system generating chemiluminescence signals – has the excellent selectivity and sensitivity needed for quantitative analysis in complex matrices in the sub-ng range. Using mass spectrometers as detectors offers the possibility to apply different ionisation techniques like electron impact [12,15], chemical ionisation [16] and positive or negative ion chemical ionisation [10,11,17]. Schuetzle et al. [18] showed that high-resolution mass spectrometry (MS) and tandem mass spectrometry techniques can also be applied for quantification of 1-nitropyrene. Quantitative determinations by mass spectrometry, however, suffer from limitations due to the dependence of the signal size from the ionization mode and from the differences in volatility and thermal instability of different NPAHs. Some NPAHs like 1-nitropyrene partially decomposes during gas chromatographic–mass spectrometric analysis [19] and measurements using mass spectrometry with negative chemical ionisation showed that some compounds can be detected at low concentration while others escape detection even at much higher concentrations [20].

Using high-performance liquid chromatography (HPLC) as separation technique in combination with other selective measurement methods avoids these problems. HPLC columns have lower efficiency than GC columns or capillaries but this is compensated by the increased range of possibilities accessible for optimising the separation of complex mixtures by the selection of a suitable HPLC phase system and/or operation mode. Various detection techniques have been used for the determination of NPAHs in the eluate of HPLC columns [21]. The sensitivity and selectivity of UV detectors [20–23] are insufficient for determination of NPAHs in the ppb and sub-ppb range.

Fluorescence detectors [20,24–29] – which can be applied after the reduction of nitro compounds to their amino analogs –, chemiluminescence detectors [30,31] and electrochemical detectors [32–35] are sensitive and selective enough to determine NPAHs in trace concentrations. The combination of liquid chromatography–mass spectrometry [36] is undoubtedly an attractive technique for identification of nitropolycyclic aromatic hydrocarbons.

The complexity of food sample preparations necessitates the use of elaborate sample preparation schemes which have to be designed to achieve high enrichment factors and the selectivity needed for the detection system without intolerable losses. Such schemes [15,37,38] include several steps like Soxhlet-, ultrasonic- or supercritical fluid extraction, followed in variable sequence by liquid–liquid extraction, solid-phase extraction and gel permeation chromatography. However, in spite of the use of selective detection techniques (GC–MS in the selected ion monitoring mode, HPLC with fluorescence or electrochemical detection) complex chromatograms are obtained making the identification uncertain. These difficulties could explain why there are only a few papers which give quantitative results for the content of 1-nitropyrene in food [12,15,29,38] and their results differ considerably.

The present paper demonstrates the potential of including an immunoaffinity column prepared by immobilising antibodies by the versatile sol–gel method as a very selective step in the sample preparation. The hapten 1-nitropyrene serves as a tracer for NPAH contamination and is determined in herbs by extracting it into acetonitrile. High-molecular-mass matrix components are then removed by gel permeation chromatography before selectively retaining 1-nitropyrene on the immunoaffinity column and determining it after elution and separation of remaining matrix components by HPLC using a reaction detection system which reduces 1-nitropyrene to the corresponding amine before selectively measuring its concentration by fluorescence detection.

2. Experimental

2.1. Instrumentation

The HPLC system consisted of a Hewlett-Packard (Palo Alto, CA, USA) HPLC pump, Model 1050, a

Rheodyne (Cotati, CA, USA) Model 7125 injection valve adapted with a 20-μl loop, a Merck LiChroCART (Darmstadt, Germany) cartridge, 250×3 mm I.D., packed with LiChrospher PAH, the reductor column placed in an oven from C.I.L. (Sainte Foy la Grande, France), a Waters–Millipore (Milford, MA, USA) 470 fluorescence detector and a DECpc $450D_2LP$ computer equipped with a Bischoff (Leonberg, Germany) Mc Dacq software.

Loading, washing and elution of the affinity column was carried out with a low-pressure Econo pump, Model EP-1 from Bio-Rad (Hercules, CA, USA).

2.2. Reagents

Acetonitrile (analytical-reagent grade), methanol (analytical-reagent grade), platinum(II) chloride, rhodium acetate, disodium hydrogenphosphate dihydrate (analytical-reagent grade), sodium dihydrogenphosphate dihydrate (analytical-reagent grade), hydrochloric acid (analytical-reagent grade), tetramethoxysilane and the LiChrospher PAH HPLC column were all purchased from Merck; aluminium oxide (5 μm) from Macherey–Nagel (Düren, Germany) and sodium chloride (''pur.'' grade), ammonia (analytical-reagent grade) and sodium borohydrate from Riedel-de Haen (Seelze, Germany). Triton X-100 was bought from Acros Organics (Geel, Belgium) and 9-nitroanthracene, 2-nitrofluorene, 1-nitropyrene, 3-nitrofluoranthene from Dr. Ehrenstorfer (Augsburg, Germany). Sephadex LH-20 gel permeation column packing material was bought from Pharmacia Biotech (Vienna, Austria).

2.3. Standards, buffer and mobile phases

9-Nitroanthracene, 2-nitrofluorene, 1-nitropyrene, 3-nitrofluoranthene stock solutions of each compound were prepared by dissolving 1 to 4 mg of the compound in 100 ml acetonitrile. The solutions were kept at 4°C in flasks covered with an aluminium foil. Diluted standard solutions of different concentrations were prepared daily from stock solutions by dilution with acetonitrile.

Phosphate-buffered saline (PBS) was prepared by dissolving 12.46 g disodium hydrogenphosphate dihydrate, 1.56 g sodium dihydrogenphosphate dihydrate and 8.5 g sodium chloride in 1 l of bidistilled water.

Mobile phase I consisted of 800 ml methanol and 200 ml bidistilled water, mobile phase II of 900 ml methanol and 100 ml bidistilled water. Each solution was degassed in an ultrasonic bath (Sonorex Super RK 103 H, Bandelin, Berlin, Germany) and then filtered through a 0.45-μm membrane filter from Millipore (Vienna, Austria).

2.4. Sample preparation steps

2.4.1. Gel permeation column

A 100-g amount of Sephadex LH-20 was swollen overnight in methanol and packed in a column (210 mm×40 mm I.D.) by gravity flow. In order to determine the elution volume 9.78 ng 1-nitropyrene in 10 ml methanol was applied and the compound was eluted with methanol collecting increments of 10 ml. The fractions containing 1-nitropyrene (160–260 ml elution volume) were identified by HPLC with fluorescence detection.

2.4.2. Immunoaffinity column

2.4.2.1. Isolation of the IgG fraction from rabbit anti-1-nitropyrene antiserum

The immunoglobulin G (IgG) fraction of a rabbit anti-1-nitropyrene antiserum was isolated by the caprylic acid/ammonium sulfate precipitation procedure of McKinney and Parkinson [39]. The amount of IgG was determined by measuring the UV absorption at 280 nm using the following equation: $\text{IgG (mg/ml)} = A/1.4$

2.4.2.2. Preparation of the immunoaffinity column

A silica sol was prepared by mixing 0.4 ml of 0.04 *M* aqueous hydrochloric acid, 1.5 ml of bidistilled water and 6.8 ml tetramethoxysilane under stirring. The mixture was sonicated under ice-cooling for 30 min. A 2-mg amount of the IgG fraction was mixed with 1 ml PBS. A 1-ml aliquot of the silica sol was added to the ice-cooled antibody solution. Gelation occurred within 2 min, the gel was weighed and the crystallising dish was stored at 4°C for aging. The aging process was stopped when a weight loss of 50% was achieved. The silicate glass was ground in a mortar, 0.64 g of the glass matrix were packed into a 3-ml glass column from Merck and then washed

with 20 ml PBS. The column was stored in PBS at 4°C.

2.4.2.3. Operation of the immunoaffinity column

After pre-conditioning of the column with 20 ml of acetonitrile–bidistilled water (10:90, v/v) the column was loaded by pumping the sample solution consisting of 50 ml acetonitrile–bidistilled water (10:90, v/v) containing the analyte through the column. Then the column was flushed with 20 ml acetonitrile–water (10:90, v/v) to remove non-specifically bound matrix components. The dissociation of the antigen–antibody complexes resulting in the elution of the trapped analyte was carried out with acetonitrile–bidistilled water (40:60, v/v). Subsequently the immunoaffinity column was regenerated with 20 ml PBS and stored in PBS at 4°C. All steps were performed at a flow-rate of 1 ml/min.

2.4.2.4. Determination of the breakthrough capacity

The pre-conditioned column was overloaded with 40 ml of a 9.76 ng/ml solution of 1-nitropyrene in acetonitrile–bidistilled water (10:90, v/v) using a flow-rate of 1 ml/min. In order to determine the breakthrough capacity – defined as the mass of analyte accumulated on the column before the outlet concentration reaches 5% of the initial concentration – 2-ml fractions of the eluate were collected and the concentration of 1-nitropyrene was determined after online reduction by HPLC with fluorescence detection.

2.4.2.5. Determination of the analyte recovery

The pre-conditioned column was loaded with 9.78 ng 1-nitropyrene in 50 ml acetonitrile–bidistilled water (10:90, v/v) with a flow-rate of 1 ml/min. The amount of 1-nitropyrene retained and recovered was determined after elution with 3 ml acetonitrile–bidistilled water (40:60, v/v). This procedure was repeated four times.

2.4.3. Preparation of the silica gel filter columns

Silica gel filter columns were prepared as described above for the immunoaffinity column, but without addition of antibodies. A 0.5-g amount of the silicate gel glass was packed into a 3-ml glass column from Merck. The columns were flushed with 20 ml PBS and stored in PBS at 4°C. Before using a filter column it was conditioned with 20 ml acetonitrile–bidistilled water (10:90, v/v). After a single use the filter column was discarded.

2.5. HPLC separation, reduction and detection

A 20-μl volume of a standard mixture containing 2-nitrofluorene, 9-nitroanthracene, 1-nitropyrene and 3-nitrofluoranthene was injected on the column (LiChrospher PAH) and isocratically separated at room temperature with methanol–water (800:200, v/v) using a flow-rate of 0.4 ml/min. After leaving the separation column NPAHs are reduced to their amino analogues in a reductor column [39] before their detection by fluorescence measurements.

2.5.1. Reductor column

The preparation of the platinum–rhodium catalyst on aluminium oxide is described by Tejada et al. [26]. The material was filled by drypacking into a steel column (100 mm×4.6 mm I.D.) and then compressed with methanol–bidistilled water (1:1, v/v) at a flow-rate of 2 ml/min. To prevent the formation of a void volume at the column head the column was opened several times and the missing material was added. After conditioning the reductor column with the mobile phase (2 ml/min) for 1 h and keeping its temperature at 80°C it was ready for use.

2.5.2. Fluorescence detection

The effluent from the reductor column was monitored by a programmable fluorescence detector at optimal wavelengths (2-aminofluorene: λ_{ex}=263 nm, λ_{em}=504 nm; 9-aminoanthracene: λ_{ex}=285 nm, λ_{em}=370 nm; 1-aminopyrene: λ_{ex}=360 nm, λ_{em}=430 nm, 3-aminofluorene: λ_{ex}=300 nm, λ_{em}=530 nm).

2.6. Analysis of samples

The samples consisted of commercially available dried herbs (basil, chervil, marjoram, oregano, sage) which were purchased at a local market.

2.6.1. Sample preparation

A 10-g amount of the sample was put into a beaker. After adding 40 ml acetonitrile the mixture

was sonicated for 1 h at room temperature. The extract was filtered to remove solid parts. Acetonitrile was evaporated in a rotary evaporator at 20°C (Rotavapor, RE 120, Buechi, Switzerland) and the residue dissolved in 4 ml methanol. This extract was transfered onto the gel permeation column and the compounds were eluted with methanol using a flow-rate of 2 ml/min. The fraction from 160 to 260 ml was collected. The solvent was evaporated and the residue dissolved in 5 ml acetonitrile. This solution was further diluted with 45 ml bidistilled water, filtrated through the conditioned sol–gel filter column applying a slight vacuum (VacMaster, Macherey–Nagel) and pumped with a flow-rate of 1 ml/min through the pre-conditioned immunoaffinity column. Then the column was flushed with 20 ml acetonitrile–water (10:90, v/v) to remove non-specifically bound matrix components, the analyte was eluted with about 3 ml acetonitrile–bidistilled water (40:60, v/v) into a 5-ml flask and filled up with acetonitrile.

2.6.2. Chromatographic separation and detection

A 20-μl volume of the purified sample solution was injected into the chromatographic system and the compounds were separated on the LiChrospher PAH column using mobile phase I or II at a flow-rate of 0.4 ml/min. 1-Nitropyrene was detected (λ_{ex}= 360 nm, λ_{em}=430 nm) after catalytic reduction in the reductor column.

2.6.3. Quantitative determination of 1-nitropyrene

2.6.3.1. Calibration by standard addition

A 200-g amount of oregano was extracted in 800 ml acetonitrile sonicating it for 1 h. The solvent was evaporated in a rotary evaporator and the residue was dissolved in 100 ml methanol. Five-ml aliquots of the unspiked and of three spiked samples (3.2 ng, 6.5 ng, 9.76 ng 1-nitropyrene/5 ml) were purified and chromatographed as described above. The peak heights were measured and plotted against the amount of analyte added. This procedure was repeated three times.

The recovery was determined by dividing the slope of the linear regression line for the standard addition by the slope of the linear regression line of 1-nitropyrene standard solutions in the same concentration range.

2.6.3.2. External calibration

A calibration curve ranging from 1 to 800 μg 1-nitropyrene/l was established and the total amount of 1-nitropyrene in each eluate of the immunoaffinity column was calculated.

3. Results and discussion

The immunoglobulin G fraction of a rabbit 1-nitropyrene antiserum was isolated with a yield of 5 mg IgG/ml of antiserum.

3.1. Performance characteristics of the immunoaffinity column

The absence of non-specific adsorption of 1-nitropyrene on the sol–gel glass matrix was shown by comparing the breakthrough curves obtained with the immunoaffinity column and an empty column packed with a sol–gel matrix without the addition of antibodies. The breakthrough capacity of the immunoaffinity column was 68.5 ng. The recovery of 1-nitropyrene was 96.0% with a standard deviation of 1.1% (n=4).

The sol–gel immunoaffinity column was mechanically stable and did not show changes in specific retention properties after 30 absorption/desorption cycles. In executing the analysis method the ever present danger of clogging by solid particles was prevented by a sol–gel filter column before the immunoaffinity column.

3.2. Development of the sample preparation method

The problems encountered in developing the sample preparation method are discussed below.

The initial extraction of the analyte from a homogenised sample is a critical step in a sample preparation scheme. Using a minimal volume of a solvent compatible with subsequent analysis steps it should rapidly isolate the analyte from interfering matrix components with a high extraction yield. The co-extraction of matrix components leads to two

types of interferences: the solubilisation of matrix components which interfere with the final determination of the analyte and/or the formation of precipitates in subsequent analysis steps resulting in analyte losses.

Initially it was hoped that the highly selective immunoaffinity column could remove all interfering matrix components from the acetonitrile extract, but the acetonitrile concentration is a critical parameter for the operation of the immunoaffinity column. 1-Nitropyrene is not quantitatively retained on columns operated with concentrations higher than 10% (v/v) acetonitrile in bidistilled water. Forty percent (v/v) acetonitrile in bidistilled water elutes 1-nitropyrene retained on the columns. Diluting the sample extract to 10% (v/v) acetonitrile with bidistilled water, however, leads to the precipitation of co-extracted matrix components. Since these components include high-molecular-mass compounds it was decided to use a solvent extraction technique which co-extracts only small amounts of matrix components and remove high-molecular-mass compounds by gel permeation chromatography.

Extracting 1-nitropyrene from herbs (e.g., basil, chervil, marjoram, oregano, sage) by sonication resulted in shorter extraction times and necessitated smaller solvent volumes than Soxhlet extraction. Since Sephadex LH-20 columns swollen in methanol have been used in this laboratory for several months without a deterioration of their performance caused by matrix components they were selected as gel permeation columns. In order to replace acetonitrile the original sample extract was evaporated and the residue taken up in methanol before feeding the solution in the gel permeation column. The fraction of the methanol eluate containing the analyte was collected, the methanol evaporated, the residue dissolved in acetonitrile and diluted to 10% (v/v) acetonitrile with bidistilled water. To extend the life of the immunoaffinity column this solution was filtered through a disposable small filter column packed with ''empty'' sol–gel glass before loading the immunoaffinity column.

The chromatograms shown in Figs. 1 and 2 illustrate the efficiency of the purification of oregano and sage extracts by immunoaffinity chromatography. The change could also be detected by ''unarmed'' senses: coloured solutions with the smell typical for the herbs were applied to the immunoaffinity column, while the fraction of the eluate containing the analyte was colourless with no other smell than the solvent acetonitrile. In addition, the simplification of the matrix achieved made it possible to reduce the analysis time: Using mobile phase I the retention time of 1-nitropyrene was about 30 min. After removal of interfering peaks the 1-nitropyrene peak could be eluted in about half the time by using mobile phase II.

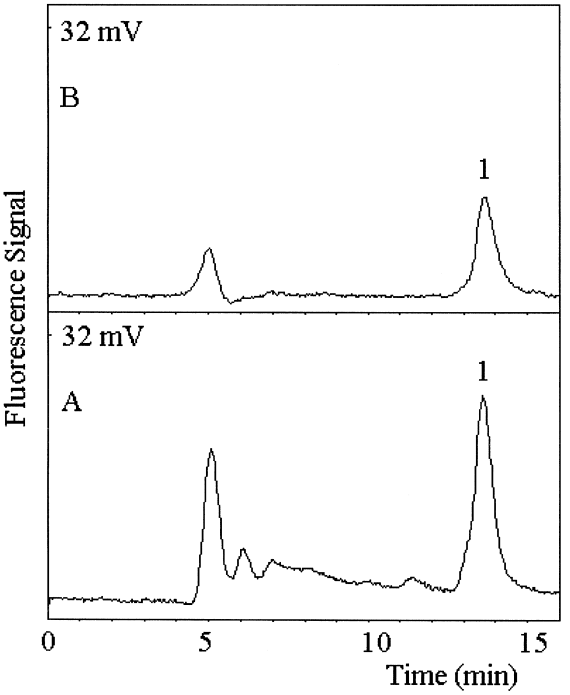

Fig. 1. Chromatograms of an oregano extract (A) before the immunoaffinity column; (B) after the immunoaffinity column; 1: 1-nitropyrene.

3.3. Quantitative determination

In order to calibrate the method in the low-ppb range 20 μl of four different solutions of 1-nitropyrene in the concentration range 0.5 and 8 μg/l were injected. In this concentration range the calibration curve was linear with a regression coefficient $r=0.9947$. The detection limit was found to be 0.3 μg/l ($S/N=3$).

To show the applicability of the sample clean-up

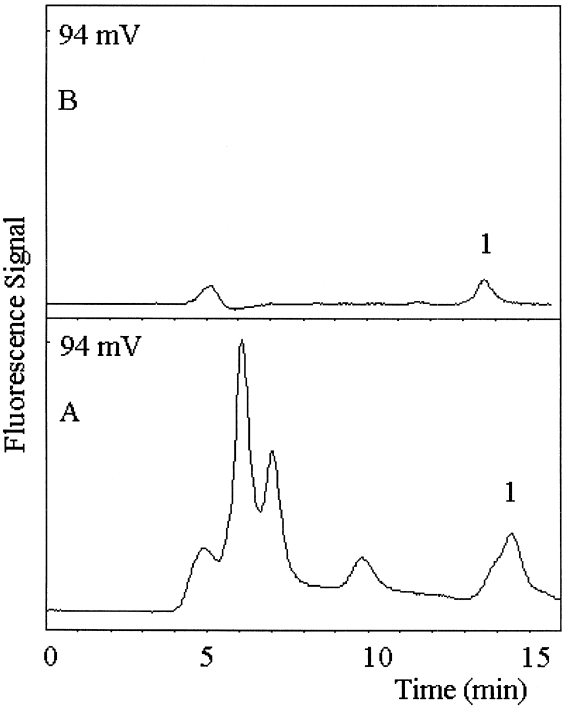

Fig. 2. Chromatograms of a sage extract (A) before the immunoaffinity column; (B) after the immunoaffinity column; 1: 1-nitropyrene.

procedures developed we applied it to the determination of 1-nitropyrene in oregano. Using the standard addition method triplicate analysis were carried out for three spiked and one unspiked samples resulting in a linear calibration line given by $y=705.17x+67.77$ with a regression coefficient of $r=0.9944$ and standard deviation $s_{y,x}=38.7$. The recovery obtained by spiking the solid sample and carrying it through the whole analysis method was 57.6% with a relative standard deviation of 9.8% ($n=3$). The sample contained 0.14 μg 1-nitropyrene/kg oregano (relative standard deviation=0.04, $n=3$). The recovery is due to the complex matrix low but as can be seen in Figs. 1 and 2 the purification of extracts is effective and this is the basis for the reliable determination of 1-nitropyrene.

In addition, the applicability of the method was demonstrated by analysing different matrices obtaining the following results: basil (1.4 μg/kg), chervil (0.1 μg/kg), sage (0.1 μg/kg) and two marjoram samples (0.2 and 0.4 μg/kg).

4. Conclusions

The inclusion of immunoaffinity chromatography in the sample clean-up steps necessary to analyse complex mixtures has frequently been hampered by a lack of appropriate immunoaffinity columns. The sol–gel method opened a new path for the synthesis of immunoaffinity column packing materials by encapsulating antibodies (monoclonal or in the form of isolated IgG fraction from antisera) in the pores of a silica matrix [40–43]. Since any antibody or antiserum fraction can be immobilised by this technique it largely broadens the spectrum of affinity columns available. Compared with other immobilisation techniques it offers a number of advantages. Since it is a mild immobilisation technique the antibodies are neither destroyed or their configuration changed. Since they are dissolved in the liquid contained in the pores access to their paratopes is not restricted. Antibodies therefore largely retain their affinity and specificity to the antigen. The narrow pores of the silica gel matrix exclude large molecules and prevent the loss of encapsulated large antibody molecules from the interior of the pores. Immunoaffinity columns packed with a packing material produced by the sol–gel method are stable since they do not show ''column bleeding'' and are especially suited for the analysis of small molecules (haptens) which are the only ones not excluded from the antibody molecules in the pores.

Using the sol–gel immunoaffinity column for the determination of 1-nitropyrene in herbs it was demonstrated that this type of column can be included in the clean-up of complex food matrix.

Acknowledgements

This work was funded by the ''Fonds zur Förderung der wissenschaftlichen Forschung'' (P. No. 10803-CHE).

References

[1] H.S. Rosenkranz, Mutat. Res. 101 (1982) 1.
[2] H.S. Rosenkranz, R. Mermelstein, Mutat. Res. 114 (1983) 217.

[3] I. Salmeen, A.M. Durisin, Th.J. Prater, T. Riley, D. Schuetzle, Mutat. Res. 104 (1982) 17.
[4] Diesel and Gasoline Engine Exhausts and Some Nitroarenes, IARC Monographs on Evaluation of Carcinogenic Risk to Humans, IARC, Lyon, 1989.
[5] S.L. Kopczynski, Int. J. Environ. Anal. Chem. 30 (1987) 1.
[6] M.T. Galceran, E. Moyano, J. Chromatogr. 607 (1992) 287.
[7] R. Niles, Y.L. Tan, Anal. Chim. Acta 221 (1989) 53.
[8] R.M. Campbell, M.L. Lee, Anal. Chem. 56 (1984) 1026.
[9] D.L. LaCourse, T.E. Jensen, Anal. Chem. 58 (1986) 1894.
[10] D.S. Douce, M.R. Clench, M. Cooke, J. Wang, J. Chromatogr. A 786 (1997) 275.
[11] A. Liberti, P. Ciccioli, A. Cecinato, E. Brancaleoni, C. DiPalo, J. High Resolut. Chromatogr. Chromatogr. Commun. 7 (1984) 389.
[12] S. Schlemitz, W. Pfannhauser, Z. Lebensm. Unters. Forsch. 203 (1996) 61.
[13] B.A. Tomkins, R.S. Brazell, M.E. Roth, V.H. Ostrum, Anal. Chem. 56 (1984) 781.
[14] W.C. Yu, D.H. Fine, K.S. Chiu, K. Biemann, Anal. Chem. 56 (1984) 1158.
[15] S. Schlemitz, W. Pfannhauser, Z. Lebensm. Unters. Forsch. 205 (1997) 305.
[16] T. Negishi, M. Nakano, K. Yanai, Ch.H. Kim, M. Fukushima, Environ. Pollut. 50 (1988) 279.
[17] H. Stray, S. Mano, A. Mikalsen, M. Oehme, J. High Resolut. Chromatogr. Chromatogr. Commun. 7 (1984) 74.
[18] D. Schuetzle, T.L. Riley, T.J. Prater, T.M. Harvey, D.F. Hunt, Anal. Chem. 54 (1982) 265.
[19] J.A. Sweetman, F.W. Karasek, D. Schuetzle, J. Chromatogr. 247 (1982) 245.
[20] J. Cvacka, J. Barek, A.G. Fogg, J.C. Moreira, J. Zima, Analyst 123 (1998) 9R.
[21] T.-Y. Liu, A. Robbat Jr., J. Chromatogr. 539 (1991) 1.
[22] P.P. Fu, Y. Zhang, Y.-L. Mao, L.S. Von Tungeln, Y. Kim, H. Jung, M.-J. Jun, J. Chromatogr. 642 (1993) 107.
[23] W. Lindner, W. Posch, O.S. Wolfbeis, P. Tritthart, Chromatographia 20 (1985) 213.
[24] E. Veigl, W. Posch, W. Lindner, P. Tritthart, Chromatographia 38 (1994) 199.
[25] T. Kinouchi, H. Tsutsui, Y. Ohnishi, Mutat. Res. 171 (1986) 105.
[26] S.B. Tejada, R.B. Zweidinger, J.E. Sigsby Jr., Anal. Chem. 58 (1986) 1827.
[27] W.A. Mc Crehan, W.E. May, S.D. Yang, B.A. Benner Jr., Anal. Chem. 60 (1988) 194.
[28] T. Kamiura, T. Kawayara, M. Tanaka, T. Nakadoi, Anal. Chim. Acta 254 (1991) 27.
[29] S. Schlemitz, W. Pfannhauser, Food Addit. Contam. 13 (1996) 969.
[30] A. Robbat, N.P. Corso, T.-Y. Liu, Anal. Chem 60 (1988) 173.
[31] H. Li, R. Westerholm, J. Chromatogr. A 664 (1994) 177.
[32] S.M. Rappaport, Z.L. Jin, X.B. Xu, J. Chromatogr. 240 (1982) 145.
[33] Z. Jin, St.M. Rappaport, Anal. Chem. 55 (1983) 1778.
[34] M.T. Galceran, E. Moyano, Talanta 40 (1993) 615.
[35] M. Murayama, P.K. Dasgupta, Anal. Chem. 68 (1996) 1226.
[36] L. Bonfanti, M. Careri, A. Mangia, P. Manini, M. Maspero, J. Chromatogr. A 728 (1996) 359.
[37] B.K. Larsson, H. Pyysalo, M. Sauri, Z. Lebensm. Unters. Forsch. 187 (1988) 546.
[38] M.J. Dennis, R.C. Massey, D.J. McWeeny, M.E. Knowles, Food Addit. Contam. 1 (1984) 29.
[39] M.M. Mc Kinney, A. Parkinson, J. Immunol. Methods 96 (1987) 271.
[40] J. Zuehlke, D. Knopp, R. Niessner, Fresenius J. Anal. Chem. 352 (1995) 654.
[41] M. Cichna, D. Knopp, R. Niessner, Anal. Chim. Acta 339 (1997) 241.
[42] A. Turniansky, D. Avnir, A. Bronshtein, N. Aharonson, M. Altstein, J. Sol–Gel Sci. Technol. 7 (1996) 135.
[43] A. Bronshtein, N. Aharonson, D. Avnir, A. Turniansky, M. Altstein, Chem. Mater. 9 (1997) 2632.