High Resolution and Ultra Trace Analysis of Pesticides Using Silica Monoliths

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One of the challenges in daily lab life is the analysis of pollutants in complex samples such as food and beverages. In this context pesticides are a dominant group which is applied for increased productivity and yield in agriculture.

Pesticides are effective components for fighting pests on plants which are subsequently being used for the production of our daily food. Residuals of pesticides would seriously affect consumer's health, therefore, food and beverages have to be analysed carefully with respect to the type and amount of these pollutants every day. Fast and simple HPLC methods are needed for the identification and quantification of pesticides in matrix containing samples.

Here we describe the chromatographic separation of 32 pesticides using a monolithic silica HPLC column (Chromolith®) and compare it with the separation on one of the newest core shell HPLC columns. Furthermore, the quantitative analysis of pesticides in food samples is demonstrated utilising a typical sample preparation procedure and a monolithic silica capillary in combination with LC-MS.

Experimental

Materials and methods

The HPLC systems used were a Dionex Ultimate 3000 nano and a Dionex Ultimate 3000 (both from Thermo Scientific, Waltham, MA, USA) including Chromolith® HighResolution RP-18 endcapped 100-4.6mm analytical monolithic silica column and Chromolith® CapRod® RP-18 endcapped 150-0.1mm analytical monolithic silica capillary column (both Merck Millipore, Darmstadt, Germany). A UV detector was operated at 254nm and the data acquisition was performed with Chromeleon software.

A Bruker Esquire 3000plus mass spectrometer with an ion trap and a nano-electrospray ionisation (ESI) source operated in positive mode was utilised with an m/z scan in the range of 200 – 290.

Sample preparation

For the preparation of the kidney sample 1 g of porcine kidney was homogenised and spiked with 2mL of pesticide stock solution. After vortexing and centrifugation the supernatant was decanted off and acetonitrile evaporated. Followed by a typical SPE procedure with a LiChrolut RP-18e 500mg cartridge (Merck Millipore), analytes were eluted with 5mL acetonitrile. Then water was added, organic solvent evaporated and filtered utilising a 0.45µm syringe filter (Merck Millipore). Blank samples were prepared using an identical procedure.

Results and Discussion

Silica monoliths (Chromolith® columns) have been proven to be very efficient HPLC columns for the qualitative and quantitative analysis of complex sample mixtures including pesticides in food [1,2]. Chromolith® columns consist of one piece of a porous silica rod with a bimodal pore structure providing macro- or throughpores and mesopores in the silica skeleton *(Figure 1)* [3].

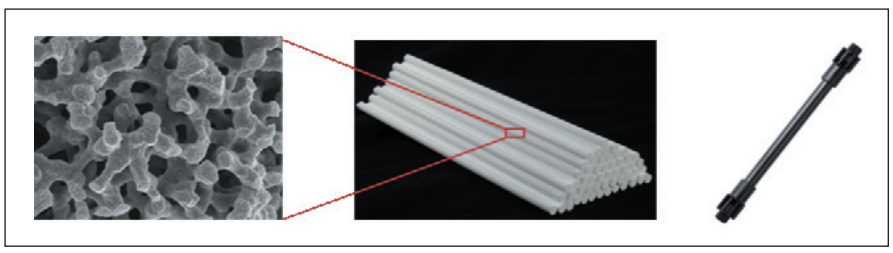

Figure 1. Images of bare monolithic silica rods (centre) and a cladded, ready to use column (right). The SEM image (left) shows the porous structure of a Chromolith® column.

The macropores offer a high permeability and respectively low column backpressure, thus allowing a fast operation of the columns. On the other hand the mesopores provide a high surface area. This is needed for a sufficient selectivity and resolution in order to separate components of similar chemical structure.

Recently, a second generation of silica monoliths has been developed with a macropore size of ca. 1.1µm, a mesopore size of 15nm and a corresponding specific surface area of 250 m2/g. These Chromolith® HR columns display separation efficiencies up to 180.000N/m and a corresponding column backpressure of 70 bar (1000 psi).

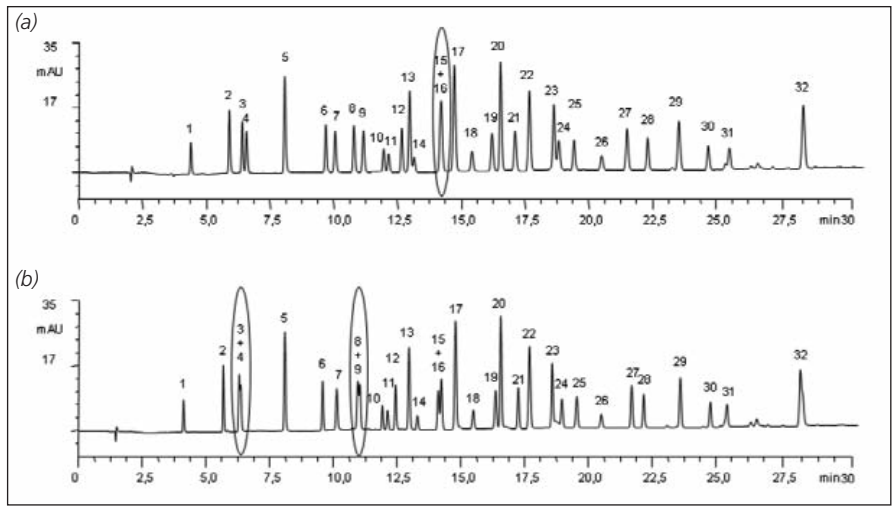

Figure 2. LC-UV chromatogram of a mixture of 32 pesticides separated on Chromolith® HighResolution RP-18 endcapped 100-4.6mm. Mobile phase A: ACN, B: 10mM NH4COOH, gradient: 10% A to 75% A in 30 min. Flow rate: 1mL/min. Detection: UV 254nm. Temperature: 35°C. Injection volume: 0.1µL.

Figure 2a describes the chromatographic separation of 32 different pesticides on Chromolith® HR in less than 30 minutes. Most of the components are baseline separated; only two (15: Isoproturon, 16: Diuron) could not be separated under the chosen eluent conditions. The resolution power Rs (Rs = [α-1] [k/k+1] N1/2) of a HPLC column is the key issue for separating complex mixtures. It is determined by the selectivity α, the separation efficiency N and the capacity factor k (retention). For comparison, the resolution power of a new core shell HPLC column was studied utilising the same pesticide mixture *(Figure 2b)*. Despite the fact that core shell materials possess very high separation efficiencies, two peak pairs (3/4: Deethylatrazine/Chloridazon and 8/9: Cyanazine/Desethylterbutylazine) could not be separated. This can be explained by the column selectivity, determined by factors such as surface area and type of surface modification of the stationary phase material.

Selectivity and resolution of a given HPLC column are inherent properties essential for the differentiation of analytes with similar chemical structure. On the other hand sensitivity is very important to determine even traces of compounds such as pesticides in food in a quantitative manner. Monolithic silica capillaries (Chromolith® CapRod®) are available with small internal diameters (e.g. 100µm) and offer high sensitivities needed for trace analyses. Because of their low consumption of eluent they are ideally suited for the combination with MS detection.

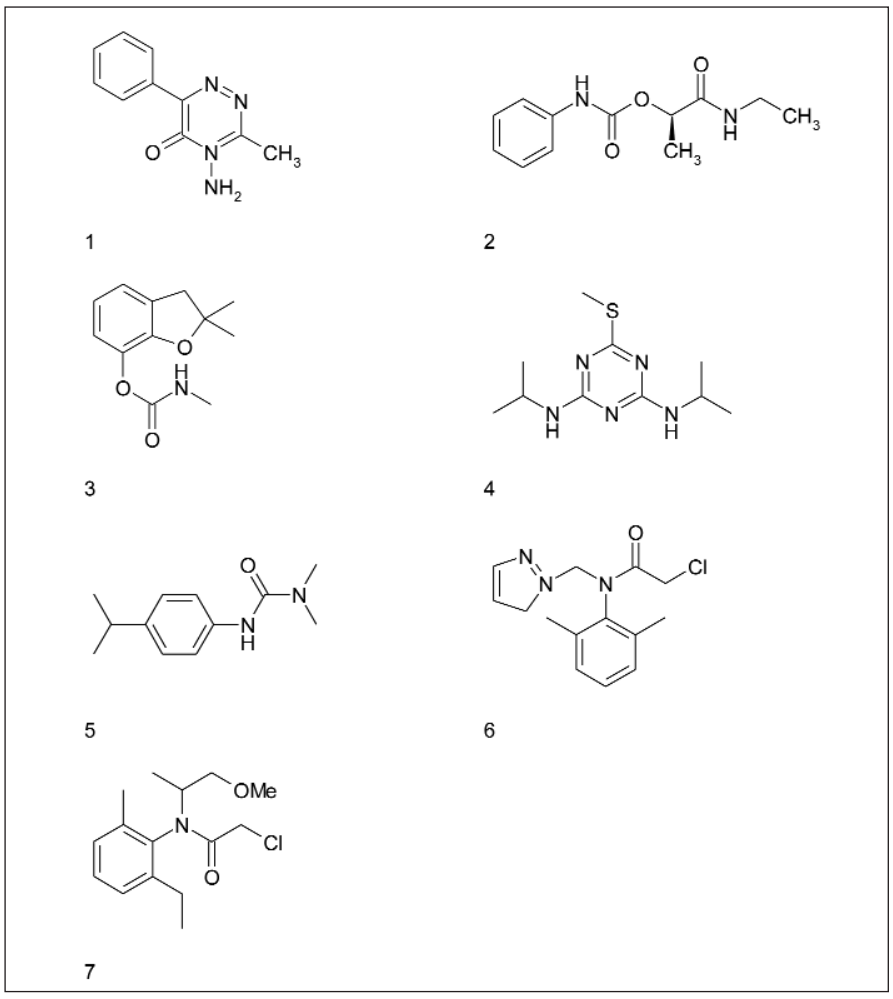

Figure 3. Structural formulas of pesticides utilised for the spiking of porcine kidney. 1 Metramitron, 2 Carbetamide, 3 Carbofuran, 4 Prometryne, 5 Isoproturon, 6 Metazachlor, 7 Metolachlor.

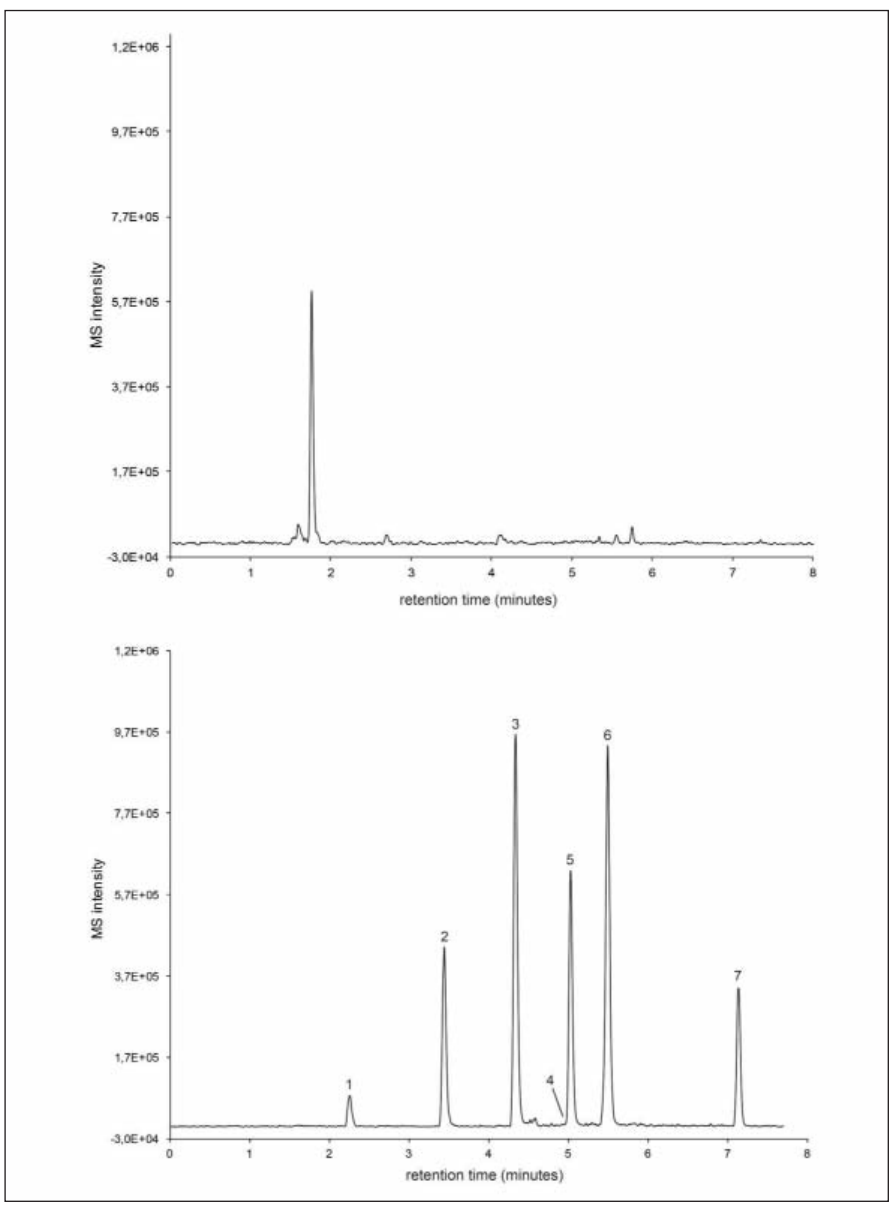

Figure 4. LC-MS base peak chromatogram (BPC) of seven pesticides spiked in porcine kidney and separated on a Chromolith® CapRod® RP-18 endcapped 150-0.1mm monolithic silica capillary column (m/z 200-290). Top: Blank sample (undiluted), bottom: spiked sample (diluted 1:100). Mobile phase A: water + 0.1% formic acid, mobile phase B: acetonitrile + 0.1% formic acid; gradient: 20% B to 80% B in 10 min. For peak annotations see Table I.

Porcine kidney as a complex food matrix was spiked with a mixture of seven pesticides *(see Figure 3)* and after a typical sample preparation procedure the residue was analysed utilising a monolithic silica capillary.

The LC-MS run of this sample as well as of a blank are shown in *Figure 4*. All peaks are very sharp and elute baseline separated. Prometryne was not recovered after sample preparation, presumably due to strong interaction with matrix components (for comparison see also chromatogram in *Figure 5*). No matrix components are visible in the chromatogram of the spiked sample, which was diluted by a factor of 1:100 prior to analysis. The undiluted blank sample displays some peaks, which can be attributed to the matrix.

Calibration curves were prepared for the most hydrophilic and most hydrophobic compounds of the pesticide mixture (metamitron, MM and metazachlor, MC, respectively). A typical chromatogram with a run time of approximately seven minutes utilised for this work is show in *Figure 5* (top). The linear range of the curve was determined to be in between 0.2 – 5.0 pg (0.16 – 3.33 ng/µL, MM) and 0.6 – 5.9 pg (0.40 – 3.93 ng/µL, MC). The limit of detection (LOD) for the equipment used was calculated to be very low 0.24 pg (0.16 ng/µL, MM) and 0.59 pg (0.40 ng/µL, MC). All data is displayed in *Figure 5* (bottom).

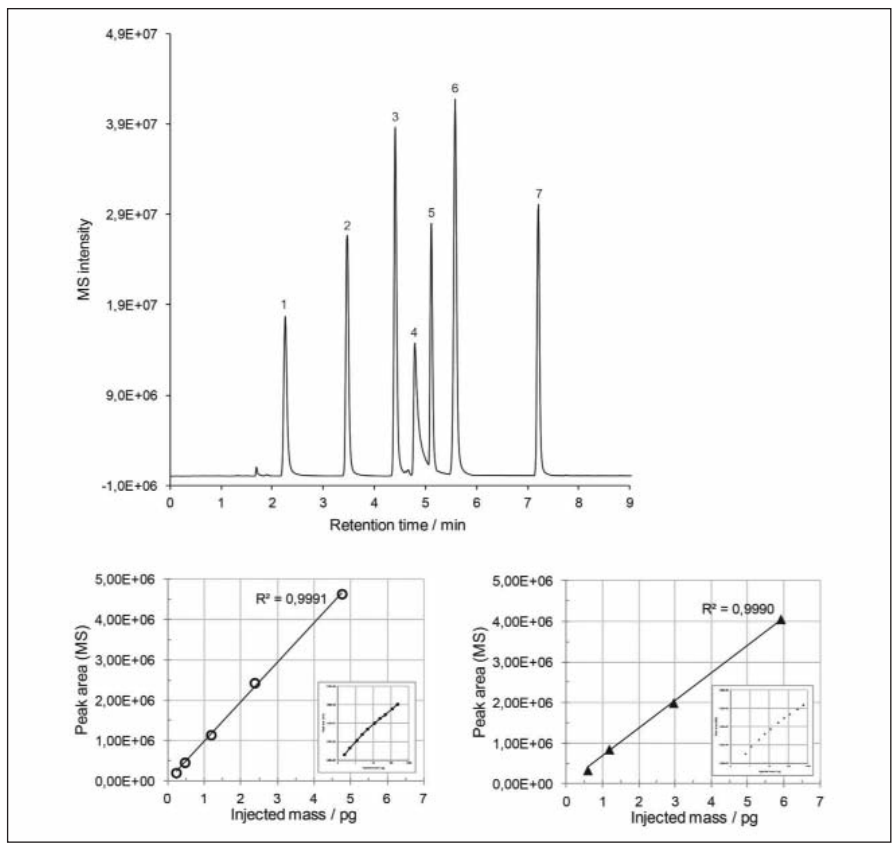

Figure 5: Top: LC-MS BPC displaying the separation of the stock solution of seven pesticides on a Chromolith® CapRod® RP-18 endcapped 150-0.1mm monolithic silica capillary column. These chromatograms were utilised for the preparation of a calibration curve and the determination of the limit of detection for both metamitron and metolachlor (m/z 200-290). Mobile phase A: water + 0.1% formic acid, mobile phase B: acetonitrile + 0.1% formic acid; gradient: 20% B to 80% B in 10 min. For peak annotations see Table I. Bottom: Calibration curves for both metamitron (open circles, left) and metolachlor (closed triangles, right). The insets show the complete concentration range covered while the large diagrams display the linear regions of the curves. The limits of detection for the setup utilised are 0.24 pg (0.16ng/µL) for metamitron and 0.59 pg (0.40ng/µL) for metolachlor.

Table 1. Stock solution of seven pesticides: Peak number, substance and substance class, activity, corresponding monoisotopic mass and concentration of analytes.

Peak no.	Substance	Substance class	activity	Monoisotopic mass / g/Mol	concentration / µg/mL
1	Metamitron	triazinone	herbicide	202.1	159
2	Carbetamide	carbamate	herbicide	236.1	487
3	Carbofuran	carbamate	acarizide, insekticide, nematicide	221.1	548
4	Prometryne	triazine	herbicide	241.1	60
5	Isoproturon	phenylurea	algicide, herbicide	206.1	159
6	Metazachlor	chloracetanilide, pyrazole	herbicide	277.1	796
7	Metolachlor	chloracetanilide	herbicide	283.1	395

Summary and Conclusions

The separation of a mixture of 32 pesticides could be achieved in a very short time on a new type of monolithic silica column (Chromolith® HR). Selectivity and resolution was slightly better as compared to the separation on one of the newest core shell materials. Furthermore, the quantitative determination of traces of pesticides in food was achieved on a monolithic silica capillary column with a small internal diameter of 100µm coupled to a mass spectrometer.

Literature

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3. K. Cabrera, J. Sep. Sci 2004, 27, 843-852.

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