

High capacity sample cleaning for pesticide residue analysis using the example of different matrices

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Summary

Sample cleanup according to EN 12393, EN 1528 and US EPA SW-846 Method 3640A and other similar methods are time-consuming and solvent-intensive procedures. All these methods are based on a GPC separation with polystyrene gel in glass columns with diameters from 10 to 40 mm and a length between 300 and 600 mm. With the widely used Bio-Beads SX-3 gel, a separation with dichloromethane or a mixture of cyclohexane/ethyl acetate takes more than approx. 45 minutes. Due to its different pore volume, the new EuroClean GPC gel offers much higher efficiency and separation according to the listed methods can be optimized to 30 min using a shorter column. Long-term validation of the EuroClean material has proven its robustness and applicability for sample cleaning of pesticide residues. Two typical but very different sample matrices were applied and separated repeatedly in order to ensure suitability for environmental and food laboratories. With the improvement in GPC analysis run times, it is now possible to start two complete sequences of 15 samples each working day with the automated KNAUER GPC Cleanup Unit 6500. By using a shorter column bed length, solvent savings in the range of 30 % are possible.

Introduction

The GPC cleanup method for environment and food analysis is a powerful way to increase the limit of detection of multi-pesticide residues and other trace impurities. The procedure is in the majority of cases the same: extraction of a food or environmental sample is done with organic solvents. After separating the organic fractions, the high molecular weight compounds that would disturb the subsequent sub-trace analysis are removed via a GPC cleanup step. The GPC column packing materials typically have a molecular mass cut off of approx. 2 kDa. Since the impurities to be monitored only have molecular masses up to 400 Da, the undesired high molecular mass matrix elutes before the desired compounds.

Approx. 50 g of dry polystyrene-based GPC packing material is required to clean 5 to 10 ml of highly concentrated sample extracts. After swelling the packing material with an organic solvent such as THF, dichloromethane or ethyl acetate, the packing material is filled into a glass column of approx. 600 mm length and 25 mm inner diameter. Studies of long-term behaviour were done using a column inner diameter of only 9 mm to conserve solvent. The first column was filled with the widely-used and well documented[1-3] Bio-Beads SX-3 gel. Column performance was tested according to the US EPA 3640A method. The second column was filled with the new EuroClean GPC-material and tested in the same manner.

Results

The new EuroClean column packing material showed a significantly higher resolution compared to the Bio-Beads column (see Fig. 1). This result offers the potential benefits to reduce column length, run time and solvent consumption. At first, we used a mixture of cyclohexane/dichloromethane (85:15) as the eluent and applied an olive oil sample. A rapid decrease of column performance was apparent after a few sample runs. We concluded that this eluent is not recommendable for EuroClean because the enlarged pore volume of this material is sensitive to nonpolar solvent compositions. Dichloromethane showed stable column performance under the conditions listed for olive oil samples according to US EPA SW-846 Method 3640A over 25 runs (see Fig. 2). Environmental samples from mud and sediment of seawater lagoons were tested according to EPA 3510C /EPA 3550C and showed a very stable separation performance over 25 runs (see Fig. 3).

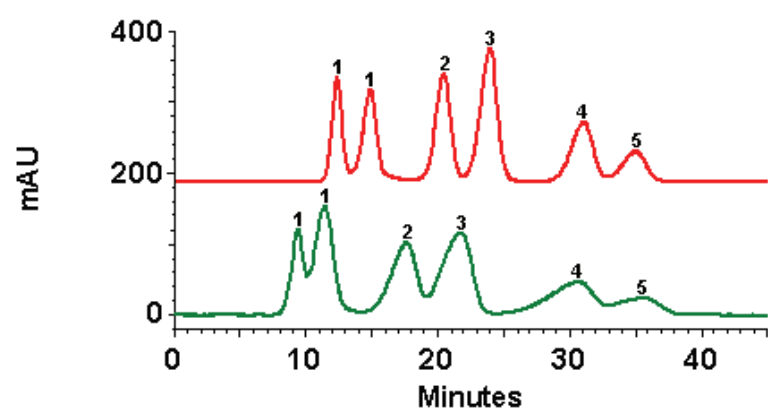


Fig. 1 Standard test chromatogram in CH_2Cl_2 with Bio-Beads (green) and EuroClean (red), 1= corn oil, 2= bis-(2-ethylhexyl)phthalate, 3= methoxychlor, 4= perylene, 5= sulfur

Method parameters

The GPC cleanup steps were performed using a KNAUER GPC Cleanup Unit 6500 equipped with Smartline Pump 1000 and 200 μl sample loops.

Cleanup method

Column	glass column 700 x 9 mm
Packing material A	BioBeads SX-3, 600 mm bed length
Packing material B	EuroClean, 580 mm bed length
Eluent	Cyclohexane/dichloromethane (85:15), dichloromethane
Flow rate	1 ml/min
Back pressure (Bio-Beads)	6 bar
Backpressure (EuroClean)	4-5 bar
Injection volume	200 μl
Temperature	Ambient
Detection	UV at 254 nm (2 Hz); 3 mm flow cell
Analysis time	45 min

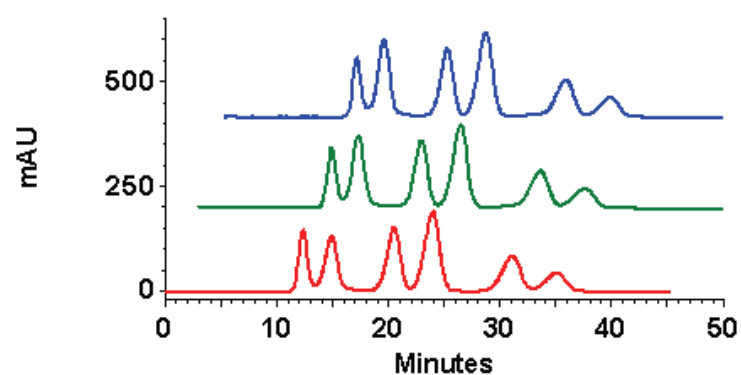


Fig. 2 Standard test in CH_2Cl_2 with fresh EuroClean column (red), after 12 runs (green) and after 25 runs (blue) using an environment sample extract according to EPA 3510C 1996 and EPA 3550C 2007

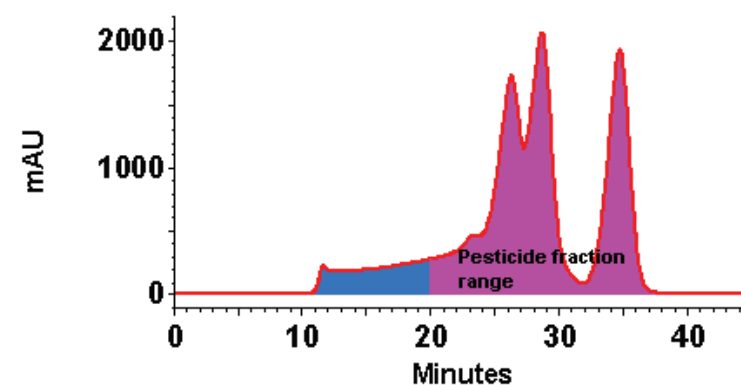


Fig. 3 Sample run in CH_2Cl_2 of a mud extract according to EPA3550C 2007

Conclusion

The new EuroClean GPC sample cleanup packing material produces significantly higher resolution values for the separation of multi-pesticide residues in different matrices using dichloromethane as eluent. This implicates that with a reduced EuroClean column bed length, a dramatically shorter run time and less solvent consumption can be realized. During the validation procedure it became evident that nonpolar solvent compositions are not recommendable for this kind of sample cleaning.

- References**
- [1] Chamberlain, S. J. *Analyst* **1990**, 115; 1161 – 1165.
 - [2] Di Muccio, A.; Ausilie, A.; Versori, L.; Camoni, I.; Dammmanco, T.; Gannibetti, L.; Santillo, A. and Versori, F. *Analyst* **1990**, 115; 1167 – 1169.
 - [3] Guardia-Rubio, M.; Fernandez-De Condova, M. L.; Ayovby-Canada, M. J. And Ruiz-Medina, A. J. *Chromatogr. A* **2006** Volume 1108; 231 – 239.