Advantages of a hydrophilic-lipophilic balanced polymeric phase over a standard hydrophobic PS-DVB-phase in Solid-Phase Extraction

T. Gersthagen, Düren/GER, M. Paschert, Düren/GER, C. Schmitz, Düren/GER, H. R. Wollseifen, Düren/GER, M. Roedel, Düren/GER
Dr. T. Gersthagen, tgersthagen@mn-net.com, MACHEREY-NAGEL GmbH & Co. KG, Valencienner Str. 11, 52355 Düren, Germany

Introduction

Solid-phase extraction (SPE) is a fast, cost-effective sample preparation technique for enrichment of analytes and/or for purifying complex samples before their analysis. Classical SPE phases are based on silica. These (bonded) silica phases have several drawbacks (e.g. pH limitation). Polymer technology overcomes some of the disadvantages of silica-based materials [1]. In nowadays polymeric polystyrene–divinylbenzene (PS-DVB) resins are the preferred adsorbents for many applications in the field of clinical chemistry, forensic and toxicology, environmental or food chemistry. Here we present the comparison of a standard hydrophobic PS-DVB-phase with a recently developed hydrophilic-lipophilic balanced polymeric phase (CHROMABOND® HLB).

Results

Comparison of recovery rates of selected drugs in dry and conditioned state

Several drugs of different polarity in serum are applied to a solid phase column in wetted and non-wetted state. Recovery rates show that especially polar analytes like caffeine are adsorbed only very inefficient on a hydrophobic PS-DVB phase. CHROMABOND® HLB can retain even very polar analytes in non-wetted state without a significant loss on recovery.

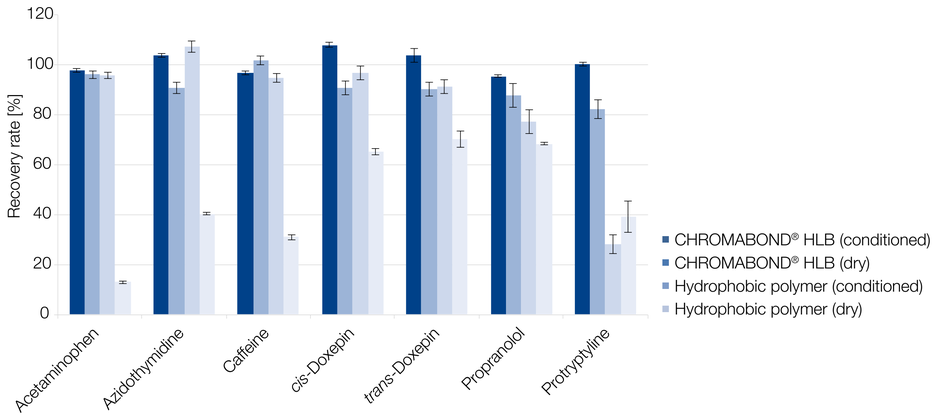

Fig. 1: Comparison of recovery rates of different drugs in serum. SPE-Procedure: column (1 mL, 30 mg) conditioning 1) 1 mL methanol 2) 1 mL H_2O, sample application: 1 mL serum (spiked with 10 µg/mL of each analyte), washing: 2 mL H_2O, drying: 10 min with applied vacuum, elution: 2 mL methanol, evaporation: under nitrogen, reconstitution: in 1 mL H_2O + 0.1 % formic acid. Further analysis: HPLC with EC 150/2 NUCLEODUR® Pyramid C_{18}, 3 µm column, Eluent: A: dest. water + 0.1 % formic acid, B: methanol + 0.1 % formic acid, 30–45 % B in 15 min, Flow rate: 0.3 mL/min, Temperature: 30 °C, Detection: UV, 254 nm, Injection: 5 µL

Comparison of recovery rates in different matrices

To demonstrate the retention ability of both polymeric phases selected analytes from a different range of polarity were compared. After solid phase extraction subsequent analysis by LC-MS/MS followed.

Analytes	Recovery rates CHROMABOND® HLB [%], (n = 5)	Recovery rates hydrophobic PS-DVB phase [%], (n = 5)	log p [2]
Clothianidin	69 ± 6	9 ± 2	0.54
Methomyl	79 ± 5	24 ± 3	0.72
Imidacloprid	93 ± 5	0	0.87
Methabenzthiazuron	73 ± 5	1 ± 0	1.79
Cyanazine	100 ± 10	86 ± 3	1.96
Atrazin	110 ± 18	100 ± 4	2.20
Isoproturon	100 ± 4	97 ± 9	2.57
Propyzamid	85 ± 7	64 ± 10	3.18
Myclobutanil	102 ± 11	76 ± 11	3.66
Pipernonyl-butoxide	101 ± 9	97 ± 4	4.10
Chlorpyrifos	50 ± 5	9 ± 2	4.78

Tab. 1: Comparison of recovery rates of different pesticides in tab water. SPE-Procedure: column (3 mL, 200 mg) conditioning 5 mL methanol, 5 mL dest. water, sample application: 1000 mL tap water (spiked with 50 ng of each analyte), washing: 10 mL dest. water, drying: 5 min with applied vacuum (–15 psi), elution: 6 mL acetonitrile, evaporation: under nitrogen, 40 °C, reconstitution: in 1 mL dest. water – acetonitrile (95:5, v/v), Further analysis: LC-MS/MS with EC 50/2 NUCLEOSHELL® PFP, 2.7 µm column, Eluent: A: dest. water + 0.1 % formic acid, B: acetonitrile + 0.1 % formic acid, 5–95 % B in 15 min, 95 % B for 5 min,95–5 % B in 1 min, 5 % B for 9 min, Flow rate: 0.3 mL/min, Temperature: 40 °C, Detection: MS, Selected Reaction Monitoring (SRM), Injection: 5 µL

Analytes	Recovery rates CHROMABOND® HLB [%], (n = 5)	Recovery rates hydrophobic PS-DVB phase [%], (n = 5)	log p [2]
Atenolol	71 ± 1	64 ± 1	0.43
Sulfamethoxazole	94 ± 2	87 ± 1	0.79
Sulfachloropyridazine	86 ± 2	80 ± 2	0.85
Indapamid	88 ± 3	74 ± 10	3.46
Ketoprofen	84 ± 3	66 ± 3	3.61
Diphenhydramine	88 ± 2	92 ± 3	3.65
Imiprmine	76 ± 3	76 ± 4	4.28
Nortriptylin	77 ± 2	13 ± 2	4.43
Protriptylin	82 ± 1	18 ± 2	4.50
Trimipramin	82 ± 2	64 ± 16	4.76
Amitriptylin	78 ± 1	66 ± 20	4.81

Tab. 2: Comparison of recovery rates of different pharmaceuticals in serum. SPE-Procedure: column (1 mL, 30 mg) conditioning 1 mL methanol, 1 mL dest. water, sample application: 1 mL serum (spiked with 50 ng of each analyte), washing: 1 mL dest. water, drying: 10 min with applied vacuum, elution: 2 mL methanol, evaporation: under nitrogen, 40 °C, reconstitution: in 1 mL dest. water – acetonitrile (95:5, v/v), Further analysis: LC-MS/MS with EC 50/2 NUCLEOSHELL® PFP, 2.7 µm column, Eluent: A: dest. water + 0.1 % formic acid, B: acetonitrile + 0.1 % formic acid, 5–95 % B in 7.5 min, 95 % B for 1 min,95–5 % B in 0.5 min, 5 % B for 5 min, Flow rate: 0.3 mL/min,Temperature: 30 °C, Detection: MS, Selected Reaction Monitoring (SRM), Injection: 5 µL

Enrichment of chloramphenicol with CHROMABOND® HLB in honey

Chloramphenicol is a polar broad-spectrum antibiotic drug. It has several undesirable side effects and is in the EU not used as a drug any-more [3]. For food safety reasons upper limits in food and animal food in the EU apply [4]. The hydrophilic-lipophilic-balanced polymeric phase showed very good recovery rates for chloramphenicol even in a complicated matrix like honey.

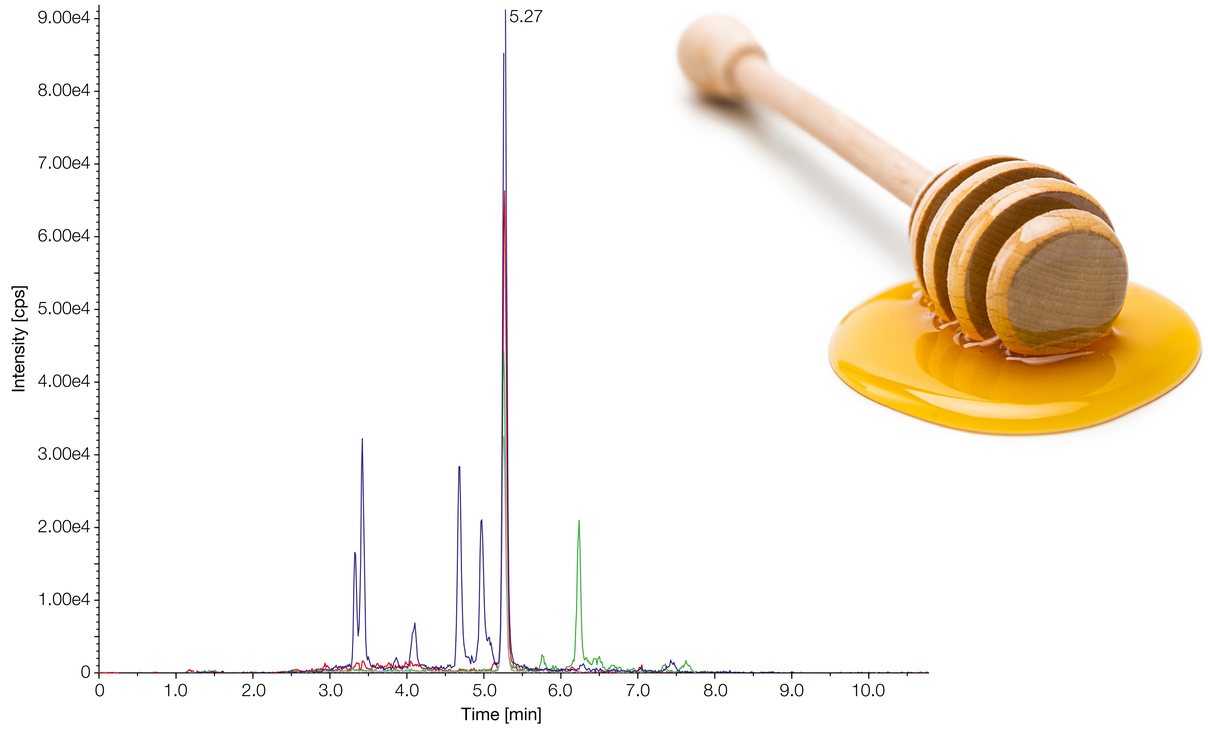

Fig. 2: Chromatogramm of chloramphenicol spiked honey sample, 1 µg/kg.

	Chloramphenicol [%]	Chloramphenicol (internal standard considered) [%]	Chloramphenicol-d5 [%]
Recovery rates with CHROMABOND® HLB	74,6 ± 2,7 (n = 3)	92,9 ± 3,4 (n = 3)	80,3 ± 4,7 (n = 3)

Tab. 3: Recovery rates of chloramphenicol and chloramphenicol-d5 in honey.

Conclusion

The results show that the hydrophilic-lipophilic-balanced polymeric phase CHROMABOND® HLB has several advantages over a hydrophobic PS-DVB phase. The excellent water wettability allows retention of analytes even with dry adsorbent material without a significant loss of recovery. Whereas the hydrophobic polymeric phase shows a dramatic loss of recovery.

Also recovery rates of polar analytes in a polar matrix are better in comparison to the hydrophobic polymeric phase. Mid-polar and hydrophic compounds can be found at least equally. CHROMABOND® HLB is a very good choice for solid phase extraction when enrichment of polar analytes in polar matrices or of analytes with great variety of polarities is needed. Additionally, it can remove several LC-MS/MS interfering matrix molecules for analysis without undesirable matrix effects.

Literature

[1] Solid Phase Extraction Technique – Trends, Opportunities and Applications, Polish J. of Environ. Stud. Vol. 15, No. 5 (2006), 677-690
[2] Calculated online on Chemicalize by ChemAxon
[3] Side-effects of Chloramphenicol and Aureomycin, Br Med J. 1951, 384–2, 388–392
[4] Verordnung (EG) Nr. 470/2009