Journal of Chromatography A, 1323 (2014) 28–38

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Comparison of different solid-phase-extraction cartridges for a fatty acid cleanup of the ethyl acetate/cyclohexane based multi-pesticide residue method EN 12393

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ARTICLE INFO

Article history:
Received 9 February 2013
Received in revised form 30 October 2013
Accepted 31 October 2013
Available online 11 November 2013

Keywords:
Pesticide analysis
Cleanup
Solid phase extraction
EN 12393
Fatty acids
Matrix effects

ABSTRACT

SPE cartridges of different anion exchange materials and florisil were compared regarding their efficiency to remove free fatty acids from ethylacetate/cyclohexane (1:1) extracts, their elution profiles and recovery rates for 38 representative pesticides, their contribution to an elevated background during gas chromatography–mass spectrometry (GC–MS), and on possible matrix effects caused by the cartridge material itself. From the seven tested cartridges, only Varian PSA (PSA) and Silicycle SiliaPrep Diamine (SPD) were very well able to retain fatty acids from ethylacetate/cyclohexane solutions and provided satisfying recoveries and elution profiles for the tested pesticides. Thus, with both cartridges a fast and simple cleanup was developed and tested with 86 pesticides as well as with EN 12393 GPC extracts of oat flour. The SPE cleanup clearly improved the identification of pesticides and reduced false negative findings due to retention time shifts and superimpositions of quantifier and/or qualifier ions. As compared with dispersive SPE it was shown, that depending on the amount of sorbent the cleanup efficiency was comparable, but recoveries were generally better for cartridge SPE procedures.

1. Introduction

Monitoring pesticides and contaminants in baby food products and the respective ingredients is of great importance for the safety of infants and children. Since babies and toddlers are more vulnerable to pesticides than adults, EU legislation generally sets maximum residue limits (MRL) of 0.01 mg kg^{-1} for foods for infants and young children as well as for infant formulae and follow-on formulae [1,2]. A recent amendment defined even lower MRLs (0.003–0.008 mg kg^{-1}) for specific compounds, so-called banned and restricted pesticides [3,4]. Monitoring and control of pesticide residues at such low levels by multi-residue methods [5] is a great challenge for baby food producers, regulatory agencies and commercial laboratories. Therefore, large sample sizes are almost required to achieve low limits of quantification (LOQ), which, however, simultaneously result in high matrix loads of concentrated extracts, resulting in different matrix effects [6–9]. Although different approaches are well recognized to overcome matrix effects [10], the most promising approach is a thorough and selective cleanup of extracts while obtaining recoveries as close as possible to 100% with good precision, ruggedness, and speed concerning sample throughput. Besides the well known QuEChERS method [11], the most established and well recognized method in Germany is EN 12393 [12–14], developed in the 1980ies and modified in 1995 to substitute dichloromethane by ethylacetate/cyclohexane (1:1) [15]. The method is validated for a wide range of sample matrices (fruits, vegetables, cereals, spices, coffee, tea, cocoa, nuts) and provides an excellent sample concentration, but in terms of solvent and time consumption it has some drawbacks comparing to other methods. However, for baby food or organic food, EN 12393 guarantees robust and confident results at the lower μg kg^{-1} level for about 300 pesticides.

The most exceptional cleanup step of EN 12393 is gel permeation chromatography (GPC), which excellently removes high molecular weight matrix compounds especially fat, while small molecules like fatty acids, natural colourants, sugars, terpenes, and sterols still can be found in the measuring solutions. As preliminary tests and literature showed [8,16,17], the source of matrix effects during gas chromatography–mass spectrometry (GC–MS) can mainly be referred to free fatty acids responsible for retention time shifts, signal suppression, and signal enhancement. Therefore, a further cleanup step is necessary to improve identification and quantification of pesticide residues.

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http://dx.doi.org/10.1016/j.chroma.2013.10.099

Within the present study, different anion exchange SPE cartridges and a florisil cartridge were compared regarding their ability to remove fatty acids from EN 12393 GPC fractions. They were examined involving the breakthrough volume for fatty acids, the recovery and elution profile for selected pesticides as well as their contribution to a higher background and possible matrix effects. The most promising cartridges were selected, and a fast simple cleanup procedure was developed including recovery studies for 86 non polar to semi polar pesticides. Finally, the SPE procedures were applied to an oat sample, which is known for its high amounts of free fatty acids. With the aim of simplification, dispersive procedures were additionally compared with the developed cartridge SPE cleanup.

2. Experimental

2.1. Reagents and materials

2.1.1. Solvents, chemicals

Acetonitrile, methanol (both LiChrosolv, LC–MS grade), acetone, toluene, ethyl acetate and cyclohexane (all SupraSolv for GC analysis), Hexamethyldisilazane (HMDS, puriss p.a. for GC), trifluoroacetic acid (TFA; reagent plus), anhydrous sodium sulphate (Na_2SO_4, pro analysi) and sodium chloride (NaCl, pro analysi) were purchased from Merck KGaA (Darmstadt, Germany). Filter units (0.2 μm) and folded filters (597½) were purchased from Whatman GmbH (Dassel, Germany). The folded filters were extracted with acetone for 2 h. Deionised water was used for sample preparation.

2.1.2. Standards

Certified pesticide standards were used. Crotoxyphos (purity 90.0%) were purchased at ChemService Inc. (West Chester, PA, USA). Fensulfothion (96.0%) were purchased at Sigma–Aldrich Chemie GmbH (Munich, Germany) and Triamiphos (0.1 g L^{-1}) at Honeywell Riedel-de Haën AG (Seelze, Germany). All other pesticide standards were obtained by Dr. Ehrensdorfer GmbH (Augsburg, Germany). They had purities of >92.0%. Stock solutions of 1 g L^{-1} were prepared in toluene and diluted for fortification in ethylacetate/cyclohexane. The fatty acid standards (>93%) were purchased from Sigma–Aldrich Chemie GmbH and stock solutions of 5 g L^{-1} prepared in ethylacetate/cyclohexane (1:1).

2.1.3. Sorbents

BondElut LRC-PSA 500 mg 6-mL cartridges (PSA), BondElut PSA bulk sorbent and BondElut LRC-Florisil 500 mg 6-mL cartridges (Florisil) were purchased from Varian Deutschland GmbH (Darmstadt, Germany). Strata X-AW 33 μm, 200 mg 6-mL cartridges (X-AW) strata Screen-A 55 μm, 70 Å, 200 mg 6-mL cartridges (Screen-A) were obtained from Phenomenex (Aschaffenburg, Germany). OASIS WAX 150 mg 6-mL cartridges (WAX) and OASIS MAX 150 mg 6-mL cartridges (MAX) were from Waters (Eschborn, Germany). SiliaPrep Diamine 500 mg 6-mL cartridges (SPD) and the respective bulk sorbent were obtained from Dichrom GmbH (Marl, Germany).

2.1.4. Matrix

German oat grains, with spelt, were grinded in a laboratory mill (Grindomix GM 200, Retsch GmbH, Haan, Germany) to a fine flour. The absence of pesticide residues was checked at Central Laboratories Friedrichsdorf.

2.2. Gas chromatography–mass spectrometry

2.2.1. GC–MS setup for determination of pesticides

For recovery experiments, matrix-effect calculations and obtaining total ion current chromatograms a combined selected ion monitoring and scan method was used on an Agilent 7890A gas chromatograph, equipped with an Agilent 7683B series injector tower (Agilent Technologies GmbH, Waldbronn, Germany) and a PTV injector (CIS4, Gerstel, Mühlheim an der Ruhr, Germany). The GC system was connected to an Agilent 5975C inert quadrupole MSD. ChemStation software was used for instrumental control and data analysis. The samples were separated on a HP-5 ms (30 m × 0.25 mm i.d., 0.25 μm) column, connected to a HP-5 ms (1.5 m × 0.32 mm, 0.25 μm) pre-column at the inlet end. The temperature programme started with a holding time of 2.0 min at 70 °C and a first ramp with 25 °C min^{-1} to 150 °C directly followed by a second ramp of 3 °C min^{-1} to 200 °C. Then, with 8 °C min^{-1} to 280 °C held for 10 min and ramped again with 35 °C min^{-1} to 325 °C held for 3 min. The transferline temperature was set to 280 °C. The carrier gas was helium and set at constant flow of approx. 2.5 mL min^{-1} using the retention time locking (RTL) programme on the Agilent 7890A; chlorpyriphos-methyl (16.59 min) was the RTL reference substance. The PTV injection parameters were as follows: injection volume: 10 μL; vent time: 0.3 min; vent flow: 200 mL min^{-1}; vent pressure: 3.8 psi; temperature programme: 70 °C for 0.25 min, 720 °C min^{-1} to 250 °C held to the end of GC–MS method; injection liner: Gerstel 1.5 mm i.d., baffled and deactivated glass liners; the quantification was done by using an adequate target ion out of the SIM chromatogram. For confirmation purposes further one or two qualifier ions were consulted. Matrix compounds were identified by automatic comparison of the obtained mass spectra with the spectra of NIST database as well as comparison of retention times of selected matrix compounds (fatty acids).

2.2.2. GC–MS setup for determination of silylated fatty acids

A HP 5890 II Series gas chromatograph equipped with a HP 7673 split/splitless injector and a HP 5971 single-quadrupole MSD (Hewlett-Packard, Palo Alto, CA, USA) was used for quantification of trimethylsilyl derivates of fatty acids (palmitic acid, oleic acid and linoleic acid). The samples were separated on a HP-5 ms (30 m × 0.25 mm i.d., 0.25 μm) column, which was connected to a HP-5 ms pre-column (1.5 m × 0.32 mm, 0.25 μm). Temperature programme: 115 °C for 3 min, 15 °C min^{-1} to 220 °C held for 5 min, 3 °C min^{-1} to 240 °C without holding and ramped again with 25 °C min^{-1} to 320 °C held for 10 min The injection volume was 1 μL with a splitless period of 1.1 min. The inlet temperature was set at 210 °C with a constant helium flow of approx. 1 mL min^{-1}. This system was locked manually to p,p-DDT at 25.25 min. Agilent 2 mm i.d., direct and deactivated glass liners were used. An SIM method was used for quantification.

2.3. Sample preparation

The extraction procedure (10 g oat sample) followed a modified version of DIN EN 12393-2 procedure N [14]. The sample was dispersed with 100 mL deionised water and 200 mL acetone for 2 min by Ultra-Turrax TP18 (IKA Werke GmbH & Co. KG, Staufen, Germany). For liquid–liquid partitioning 35 g NaCl and 100 mL ethylacetate/cyclohexane (1:1) ware added and the sample was homogenized by Ultra-Turrax for 1 min. After separation (approx. 30 min.), the organic layer (200 mL) was dried by passing it through 100 g $NaSO_4$ in a filter. Filter and $NaSO_4$ were rinsed 3-times with 20 mL ethylacetate/cyclohexane (1:1). The combined eluates were collected in graduated 500 mL TurboVap500 tubes with 1.0 mL stem, evaporated to <1 mL by TurboVap500 (Calliper Life Sciences GmbH, Mainz, Germany) and filled up to 1.0 mL with ethylacetate/cyclohexane (1:1). Approximately 10 mL ethylacetate/cyclohexane (1:1) were added, the solution dried by adding a salt mixture (NaCl/Na_2SO_4, 1:1), filtrated through a 0.2 μm filter unit, and finally filled up to 10.0 mL with ethylacetate/cyclohexane (1:1). GPC cleanup subsequently was conducted