Comparison of clean-up methods (GPC, QuEChERS) for the analysis of cocoa and cocoa products on selected groups of pesticides and relevant metabolites

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Introduction

In the context of a diploma thesis conducted at our institute, it was attempted to apply the QuEChERS approach to the fat-rich matrices, especially for cocoa products. The examined cocoa powder and cocoa products (chocolate and cocoa butter) had significant differences regarding their fat content (10% - 100%). In fat-containing products, the combination of accelerated solvent extraction (ASE) and gel permeation chromatography (GPC) is usually used to remove the fat matrix. Normally a loss of polar components (Ex. phenoxycarboxylic acids) can be observed here. Another disadvantage compared to QuEChERS is the bigger amount of time needed as well as a higher wastage of solvent. In order to remove the co-extracted fat, different dispersive SPE materials (PSA/C18, Z-Sep+ [Sigma Aldrich] or EMR-Lipid [Agilent Technologies]) were tested. With a view to check the current SANTE [1] requirements for basic validation (recovery 70% -120%, RSDr < 20%) relevant active substances were selected from the large number of different classes of pesticides. Specifically substitute substances were selected from the pesticide classes of phenoxycarboxylic acids, neonicotinoids, sulfoximines, phenyl amides, N-methyl carbamates, tetramic acids, organophosphorous, organochlorine compounds and pyrethroids. These selected active substances (11) and their relevant metabolites (7) covered a wide range of polarity.

Method

Extraction 1/2
- weighted sample: 7,5 g
- 10 ml H_2O
- 15 ml Acetonitril (1vol% acetic acid)

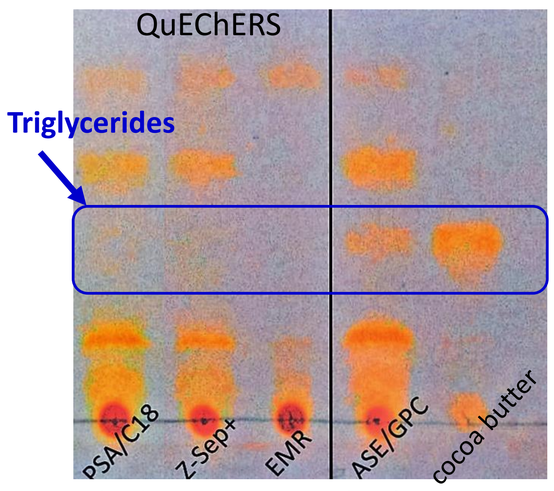
Extraction 2/2
- 10 min shaking
- 6 g $MgSO_4$, 1,5 g NaOAc
- 10 min shaking

Clean-up
- dispers SPE (PSA/C18, Z-Sep+, EMR- Lipid)

Quantification system
- GC-MS/MS
- LC-MS/MS

Results

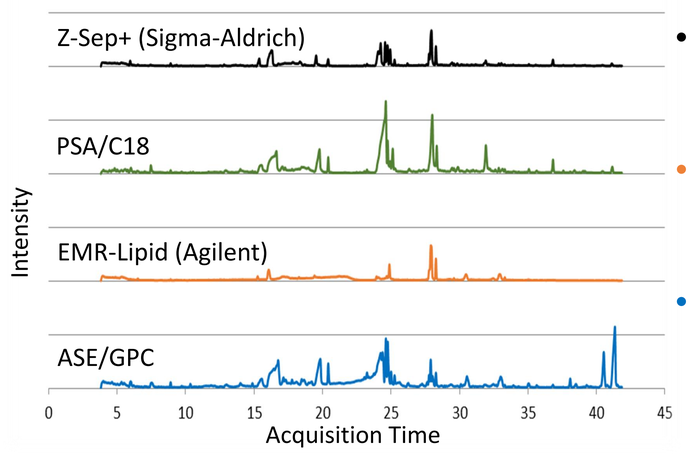
Thin-layer chromatography



- **Reference:** diluted cocoa butter (4 µg/ml) and "ASE/GPC-method"
- **All dispersive SPE materials:** good fat separation
- EMR-Lipid: best fat and fat-soluble-substances separation

Fig. 1 Thin-layer chromatography of cocoa powder : Extraction with QuEChERS followed by Clean-up with PSA/C18, Z-Sep+ and EMR-Lipid, for reference: „ASE/GPC-method" and diluted cocoa butter

GC/MS-Full-Scan-TIC



- **Z-SEP+** (QuEChERS): quit good matrix removal
- EMR-Lipid (QuEChERS): lowest matrix interferences
- ASE/GPC und PSA/C18: worse separation of matrix compounds

Fig. 2 GC/MS-full-scan-TIC of unspiked cocoa powder: "ASE/GPC-method" and QuEChERS followed by Clean-up with PSA/C18, Z-Sep+ and EMR-Lipid

Quantification results

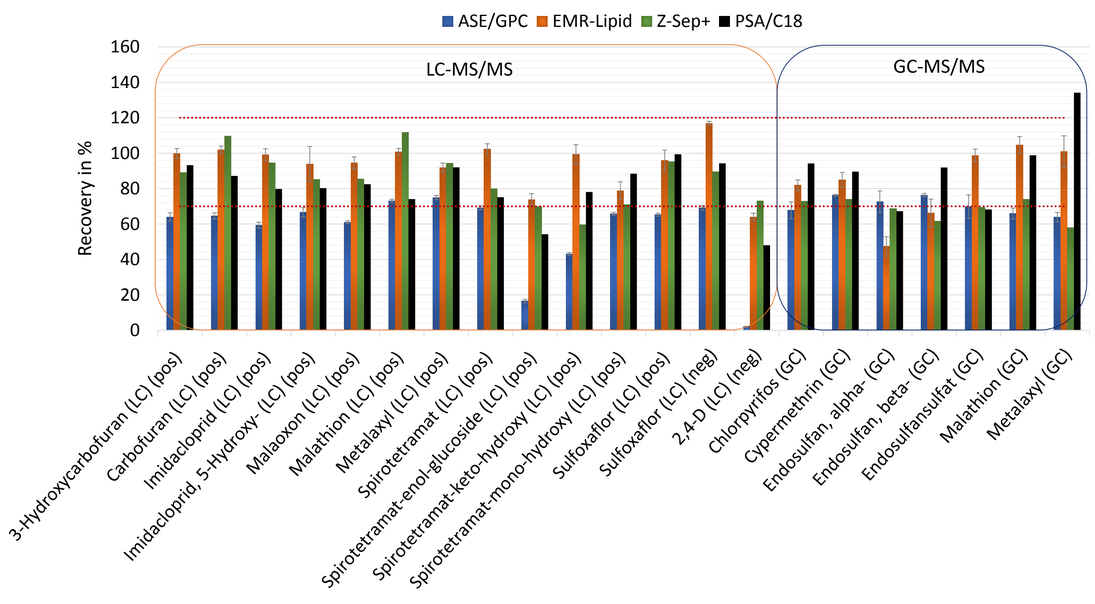


Fig. 3 Quantification results of spiked (0,1 mg/kg) cocoa powder: "ASE/GPC-method" and Extraction with QuEChERS followed by Clean-up with EMR-Lipid, Z-Sep+ and PSA/C18.

Conclusion / Outlook

During the extraction and clean-up by ASE / GPC, as expected, partially insufficient recoveries have been achieved for polar components (Ex. 2,4-D and Spirotetramat-enol-glycoside). In contrast QuEChERS followed by Clean-up with dSPEs, all dispersive SPE materials (PSA/C18, Z-SEP+ and EMR-Lipid) showed acceptable recoveries over the entire area, hereby the material EMR-Lipid had the best fat, fat-soluble-substances and matrix separation (GC/MS-TIC).

Currently the basic validation is performed of all multi methods active substances (including the metabolites) and in particular the compounds mentioned in the ICCO guide [2].

References:
[1] Document No. SANTE/11945/2015 Method Guidance document on analytical quality control and method validation procedures for pesticides residues analysis in food and feed, Supersedes SANCO/12571/2013 Implemented by 01/01/2016.
[2] Bateman R, Pesticide Use in Cocoa A Guide for Training Administrative and Research Staff (Third Edition, 2015), International Cocoa Organization (ICCO)

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