Multi-Residue Confirmation of Pesticides in Honey using Solid Supported Liquid Extraction

Application Note

Authors

C Pirard
Mass Spectrometry Laboratory (C.A.R.T)
University of Liege

Introduction

In recent years, several Belgian beekeepers were asked to shut down their hives due to withering of the hive. The cause of the withering was unknown. A multi-factoral study was initiated, which included a multi-class pesticide residue study from honey, to determine if pesticides were the cause of the decline. The extensive distribution of pesticides causes bees that have been fed on contaminated blossoms to transfer pesticide residues into honey. Multi-residue confirmation methods to identify and quantify widely used pesticides, which could have been the source of the bees decline, needed to be developed. Previously published papers already report determination methods for pesticides in honey, however, most of them analyze only one or two pesticide groups, such as organochlorine or organophosphorous residues. This application note shows the development and validation of 17 pesticides and metabolites of different chemical classes:

Insecticides: Carbofuran (Ca), Methiocarb (Mh), Pirimicarb (Pi), Dimethoate (Dm), Fipronil (Fi), Imidacloprid (Im)

Herbicides: Amidosulfuron (Am), Rimsulfuron (Ri), Atrazine (At), Simazine (Si), Chlorotoluron (Ch), Linuron (Li), Isoxaflutole (Is), Metosulam (Mo)

Fungicides: Diethofencarb (De)

Metabolites: Methiocarb sulfoxide (Mhs), 2-Hydroxytertbutylazine (TOH)

The application described here is based on solid supported liquid-liquid extraction method (SLE) followed by LC/MS/MS. The results are compared to data from standard liquid-liquid extraction (LLE) to check extraction efficiency and appropriateness of the SLE method. More detailed information on the method development and validation has been published previously[1].

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Chem Elut - the solid support

The solid support consists of specially processed, wide-pore, diatomaceous earth packed into clean polypropylene cartridges. The aqueous sample is applied to the dry Chem Elut sorbent. The sample is distributed as a thin film over the chemically inert support, which acts as a stationary phase. Subsequently, elution takes place using immiscible organic solvents. The lipophilic substances are extracted from the aqueous into the organic phase, while the aqueous phase remains on the Chem Elut sorbent. A phase-separation filter is incorporated into the cartridge as a safeguard to ensure that organic eluents remain uncontaminated by aqueous matrix. The extraction on Chem Elut is carried out with gravity only - no vacuum is required (Figure 1).

Sample Preparation and Clean Up

SLE procedure on 5 mL Chem Elut cartridge (part number 12198006)

Method:

1. Spike 1 g honey sample with 20 µL of surrogate standard solution (concentrations listed in Figure 2).

2. Mix with 1.25 mL water and 2.5 mL acetone.

3. Add 1.25 mL of NaCl solution (20 g/100 mL).

4. Apply sample to Chem Elut cartridge by gravity flow.

5. Allow 15 mins for complete adsorption to take place.

6. Elute twice with 10 mL ethyl acetate.

7. Evaporate at 30 °C.

8. Reconstitute with 200 µL acetonitrile/water (10/90).

9. Inject 20 µL into LC/MS/MS.

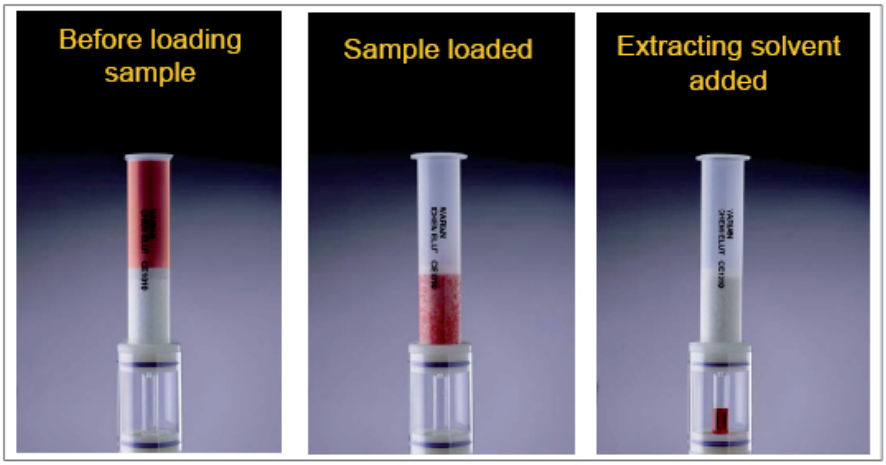


Figure 1. Solid Supported Extraction on Chem Elut cartridges

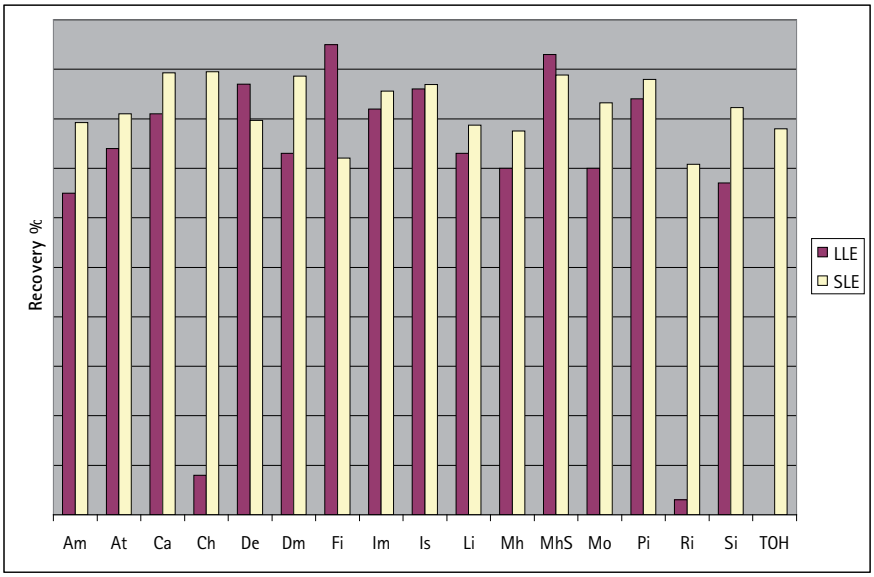
Analysis Conditions

The method is based on HPLC coupled to mass spectrometry (MS) operating in tandem mode (MS/MS) according to EU advice 2002/657/EC [2].

Column:	Polaris C18-A 3 µm, 2.0 x 150 mm (part number A2001150X020)
Temperature:	40 °C
Mobile Phase A:	Water + 0.1% acetic acid
Mobile Phase B:	Acetonitrile + 0.1% acetic acid
Linear Gradient Conditions:	held 10% B for 1 min, to 80% B in 14 mins, to 100% B in 2 mins, held 100% B for 2 mins
Flow Rate:	0.4 mL/min

Results and Discussion

Figure 2 shows the comparison between recoveries obtained after SLE on Chem Elut and classical LLE. The LLE was performed by mixing 1 g honey with 2 mL water and 6.5 mL acetonitrile for 30 mins. After centrifugation the organic layer is evaporated to 100 µL and 100 µL of water is added prior to LC/MS. The comparison shows that the Chem Elut extraction provides similar or even higher extraction efficiency than LLE for most compounds. The greatest advantage of SLE is that the SLE technique avoids emulsion formation, which is standard in LLE, significantly easing the extraction procedure. Key advantages of Chem Elut cartridges are their ease of use and the wide range of compounds that can be extracted efficiently.



Pesticide (ng/mL):
Am 0.4, At 0.4, Ca 0.4, Ch 20.0, De 2.0, Dm 2.0, Fi 10.0, Im 2.0, Is 2.0, Li 2.0, Mh 10.0, MhS 20.0, Mo 2.0, Pi 0.4, Ri 0.4, Si 2.0, TOH 1.0

Figure 2. Recovery comparison of pesticides between solid supported liquid-liquid extraction (SLE) on Chem Elut and classical liquid-liquid extraction (LLE)

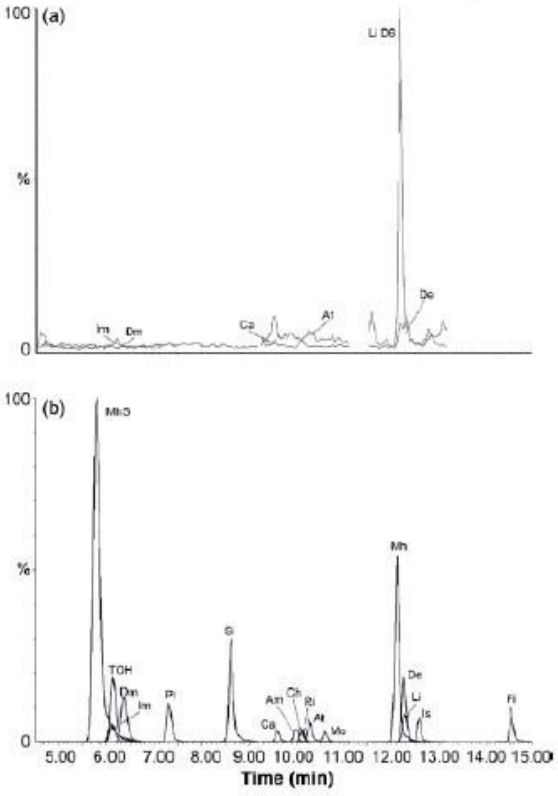


Figure 3. Chromatogram obtained for a honey blank matrix (a) and for a methanolic standard solution (b) using the Polaris C18-A column. Concentrations between 0.4 ng/mL and 20 ng/mL

Figure 3 shows the chromatogram of a methanolic standard solution with pesticide concentration between 0.4 and 20 ng/mL and a blank honey matrix. The total analysis time was 23 mins. The Polaris C18-A column is based on ultra-pure silica with a polar group placed between the primary C18 chain and the silica surface. The resultant packing material contains a surface, which is easily "wetted" with polar eluents and shows unique selectivity for a broad range of chemically different compounds. Furthermore, the polar group shields reactive silanols from polar silanophilic compounds, which improves peak symmetry and minimizes the "collapse" of the C18 chains in high-aqueous eluents. Seventeen pesticides were separated by optimizing the LC gradient, and the co-eluted pesticides with different masses were identified using MRM mode.

Conclusion

A rapid, reliable, time- and resource-saving analytical method is reported for the measurement of 17 pesticides of different chemical classes used in apiculture or in the surrounding agriculture in the context of a bee mortality study. The multi-residue analytical procedure developed in this study was based on a solid supported liquid-liquid extraction step using diatomaceous earth as inert solid support. Extracts were analyzed without further purification by LC/MS/MS in ESI mode. The SLE with Chem Elut cartridges has proven to be efficient for a wide range of pesticides, nearly independent of their polarity.

References

1. C. Pirard, J. Widart, B.K. Nguyen, C. Deleuze, L. Heudt, E. Haubruge, E. De Pauw, J.-F. Focant; Development and validation of a multi-residue method for pesticide determination in honey using on-column liquid-liquid extraction and liquid chromatography tandem mass spectrometry; Journal of Chromatography A. 1152, 116-123, (2007)

2. Commission Decision 2002/657/EC, Off. J. Eur. Commun, 12 August 2002

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