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Determination of 1,2-dibromoethane, 1,4-dichlorobenzene and naphthalene residues in honey by gas chromatography–mass spectrometry using purge and trap thermal desorption extraction

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Abstract

A highly sensitive method for the determination of 1,2-dibromoethane, 1,2-dichlorobenzene and naphthalene residues in honey was developed, using gas chromatography–mass spectrometry combined with a purge and trap thermal desorption system as the extraction technique. Optimal conditions for isolation and separation were established and calibration curves were constructed. Linearity was held between 2.4 and 300 $\mu g\,kg^{-1}$ honey for 1,2-dibromoethane, 0.5 and 300 $\mu g\,kg^{-1}$ for 1,4-dichlorobenzene and 0.125 and 3000 $\mu g\,kg^{-1}$ for naphthalene. The detection limits were found to be 0.8, 0.15 and 0.05 $\mu g\,kg^{-1}$ honey for 1,2-dibromoethane, 1,4-dichlorobenzene and naphthalene, respectively. The method was applied to the analysis of 25 Greek honey samples. 1,2-Dibromoethane was not found in the majority of the samples, while only one sample was found to contain both 1,4-dichlorobenzene and naphthalene residues at concentrations exceeding 10 $\mu g\,kg^{-1}$.

Keywords: Honey; 1,2-Dibromoethane; 1,4-Dichlorobenzene; Naphthalene; Gas chromatography–mass spectrometry; Purge and trap

1. Introduction

Larvae of the wax moth *Galleria mellonella* and to a lesser extent *Achroia grisella* attack the honey combs during storage and can even damage the wooden frames in which they hang. The devastating activity of these insects is known to beekeepers the world over. Smaller enterprises must control the infestation as best as they can, using fumigants. Several chemical fumigants effectively used in the past are methyl bromide, ethylene dibromide or 1,2-dibromoethane (EDB) and 1,4-dichlorobenzene (PDCB). Although EDB provided a very effective answer, it has long been banned, as it is a severe carcinogen and readily absorbed by beeswax and honey. Its replacement phosphine is particularly ineffective when the storage rooms are not well sealed. In developing countries control is attempted by treating the empty combs with sulphur dioxide and/or naphthalene balls. Both methods are relatively ineffective and furthermore the second method poses a potential health hazard.

Of the aforementioned antiparasitics, PDCB does not kill all stages of wax moth and will not clean up a severe case of moths already established. It remains only a preventative. Furthermore its use leads to residues in honey and wax. While residues of up to approximately 0.002 $mg\,kg^{-1}$ honey may result from the use of precontaminated wax, residues of more than 0.01 $mg\,kg^{-1}$ indicate the use of PDCB in one's own beekeeping. In Switzerland, a country with one of the highest bee population densities, positive findings by the cantonal laboratories in 1999 led to the establishment of a "Swiss tolerance level" of 0.01 $mg\,kg^{-1}$ for PDCB in honey [1], however, worldwide there is no "maximum residue limit" (MRL) for honey. The use of naphthalene, as a moth control agent, in relation to residues in wax and honey is probably

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similarly problematic as for PDCB. For EDB an action level of 30 μg kg^{-1} in honey has been set, which represents the limit at, or above which, the US Food and Drug Administration Agency (FDA) will take legal action to remove products from the market [2].

The potential health hazards of PDCB, EDB and naphthalene and their difficulty to be removed from the wax, make imperative the control of their residues in honey. Although a number of papers have been published for the determination of residues of antibiotics [3–6] and acaricides in honey [7–12], there is only a limited number of publications related to the determination of PDCB and naphthalene [13,14], while to our best knowledge no research has been conducted on EDB. Usually, the analysis of acaricides and antibiotics is carried out by means of gas- [7,10,11,13,14] and liquid chromatography [3–6,8,9,12] and sample clean-up is based on liquid–liquid extraction (LLE) [6,11,12], solid-phase extraction (SPE) [3–5,8,9,11], solid-phase microextraction (SPME) [7,14] and headspace extraction [10,13,14]. In this work is presented for the first time the simultaneous determination of PDCB, EDB and naphthalene residues in honey, using a purge and trap-gas chromatography–mass spectrometry (P&T-GC–MS) system. After development and validation, the method was applied to the analysis of 25 samples of honey produced in Greece. We confined ourselves to investigate the presence of the parameters referred to above, merely to domestic honeys, aiming to screen Greek honeys for residues of antiparasitics.

2. Experimental

2.1. Reagents

All the reagents used for the assay were of analytical-reagent grade (>99%). EDB was purchased from Dr. Ehrenstorfer (Augsburg, Germany), PDCB was purchased from Riedel-de Haën (Seelze, Germany) and naphthalene was purchased from BDH (Pool, UK). Stock solutions of these compounds were prepared in GC-grade acetone, obtained from Merck (Darmstadt, Germany), at a concentration of 1200 mg l^{-1} and were stored at −18 °C. Styrene from Aldrich (Steinheim, Germany) was used as the internal standard and its stock solution was prepared in acetone at a concentration of 90 mg l^{-1}. Dilute solutions of each compound were prepared daily by serially diluting the stock solutions with acetone. The water (Pestanal grade), that was used in all experiments, was obtained from Riedel-de Haën (Seelze, Germany).

2.2. Apparatus

A purge and trap system, model 4560, O.I. Analytical (College Station, Texas, USA) was used for the purging of analytes from the liquid honey samples and their subsequent trapping on a preconditioned glass-lined stainless steel desorption tube (GLT), containing the porous polymer Tenax TA (100 mg). The desorbed compounds were separated on a HP-5MS (Agilent) fused silica capillary column (30 m × 0.25 mm I.D., 0.25 μm film thickness). Detection and identification of the analytes was performed on an Agilent, model 6890, gas chromatograph attached to an Agilent 5973 mass spectrometer (Palo Alto, CA, USA).

2.3. Extraction

The samples were preheated in the 25 ml purge and trap glass test tube, at 40 °C (2 min), using the heater blanket around the tube and the regulated temperature controller of the purge and trap device. This heating of the sample served to reduce the viscosity of the honey to permit better purging of the liquid sample for subsequent trapping on the adsorbent trap. Extraction of the analytes and adsorption onto the Tenax resin was carried out by He purging (sparge gas) at 40 ml min^{-1} (40 min), keeping the sample temperature at 40 °C. A dry-purge step followed by blowing He through the trap at 40 ml min^{-1} (2 min) and heating the trap at 100 °C (2 min). The purpose of the dry purge was to reduce the water vapour condensation on the adsorbent trap, which is caused by the high relative humidity of the sparge gas as it exits the apparatus. Moisture condensation on the Tenax resin will result in reduced trapping efficiency. Desorption was performed by raising the trap temperature to 180 °C (6 min) and subsequent transfer of the analytes to the GC column was carried out by keeping the temperature of the transfer line at 100 °C (2 min). Helium was blown through the trap and transfer line at 40 ml min^{-1} (6 min). Finally, the trap temperature was raised to 200 °C in order to remove any contamination. Table 1 shows in detail the operating conditions of the purge and trap system.

2.4. Gas chromatographic–mass spectrometric analysis

The thermally desorbed compounds were conducted via the transfer line to the split–splitless type injector and injected onto the GC column in the split-mode, at a split ratio of 1:10. Separation was performed under the following conditions: injector temperature: 220 °C; column temperature: 40 °C (5 min), at 1 °C min^{-1} to 55 °C, at 10 °C min^{-1} to 120 °C and at 20 °C min^{-1} to 280 °C (5 min); He at 1 ml min^{-1}; MS conditions: interface temperature: 280 °C;

Table 1
Operating conditions of the purge and trap system

Steps	Temperature (°C)	Heating time (min)	He passing time (min)	He flow-rate (ml min^{-1})
Pre-heat	40[a]	2[a]	–	–
Purge	40[a]	40[a]	40[a]	40[a]
Dry-purge	100[b]	2[b]	2[b]	40[b]
Desorption	180[b], 100[c]	2[b]	6[b,c]	40[b,c]
Bake	200[b]	8[b]	8[b]	40[b]

[a] For the sample.
[b] For the trap.
[c] For the transfer line.