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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.

ion source temperature: 230 °C; quadrupole temperature 150 °C; ionisation: EI +70 eV. Data were acquired and processed with the ChemStation software. Identification of the analytes in the samples was achieved by comparing the mass spectra of unknown peaks with those stored in the US National Institute of Standards and Technology (NIST) and Wiley electronic libraries and was confirmed by spiking the samples with authentic standard compounds.

### 2.5. Procedures

#### 2.5.1. Preparation of standard solutions

A 5-level calibration was carried out by spiking aliquots of residue-free honey with known concentrations of the analytes. The residue-free honey was produced from bee colonies that were transferred to a pine forest in the suburbs of Thessaloniki city and was collected from hives where empty new frames had been placed.

Spiked working standard solutions used for calibration were prepared as follows: aliquots of 10 g of residue-free honey were accurately weighed in clean beakers and then were diluted with 5 g of water on a 4-place decimal balance. The solutions were quantitatively transferred to the 25 ml glass tube of the purge and trap system and spiked with 25 μl volumes of EDB, PDCB and naphthalene standard mixtures, in acetone, at different concentrations. A 25 μl volume of the internal standard styrene (90 mg $l^{-1}$) was also added to each solution. The spiked solutions were homogenized in the tube by vortex mixing for 30 s. A blank sample was also prepared by adding only the internal standard solution (25 μl of the 90 mg $l^{-1}$ solution) to the residue-free honey sample. All standards were analyzed in triplicate.

#### 2.5.2. Preparation of samples

Twenty five honey samples were collected from beekeepers from different areas of Greece and stored in dark glass containers at −18 °C until use. Aliquots of 10 g of each sample were accurately weighed and diluted with 5 g of water. The diluted samples were transferred to the 25 ml glass tube of the purge and trap system, where a 25 μl volume of the internal standard solution (90 mg $l^{-1}$) in acetone was added and the mixtures were homogenized by vortex mixing for 30 s. All samples were analyzed in triplicate.

## 3. Results and discussion

The optimal purge and trap and GC separation conditions were established on the basis of the following parameters: satisfactory separation of the analytes, relatively short analysis time and maximum peak area ratio. The influence of different He flow-rates, temperatures and heating times during the purge and trap steps, as well as the influence of different GC temperature programs on the aforementioned parameters was investigated and optimised.

Under the optimal conditions, the retention times of EDB, PDCB and naphthalene were 5.6, 19.6, and 32.1 min, respectively, as shown in Fig. 1. Peak purity checks were performed for all three compounds using the ChemStation software, and revealed that octanal, an endogenous compound of honey, coelutes with PDCB (Fig. 2b). The fragmentation patterns of the compounds and the internal standard are shown in Fig. 3. The characteristic ions are *m*/*z* 107 for EDB, *m*/*z* 111 and 146 for PDCB, *m*/*z* 128 for naphthalene, and *m*/*z* 78 and 104 for the internal standard.

Quantitative determination of EDB and naphthalene was based on the calculation of peak area ratio relative to internal standard styrene. Due to interference from octanal the determination of PDCB could not be carried out using this approach. In this case, quantification of PDCB was based on sum peak area of its two characteristic fragments (*m*/*z* 111 and 146) versus the sum peak area of the two characteristic fragments (*m*/*z* 78 and 104) of styrene [15].

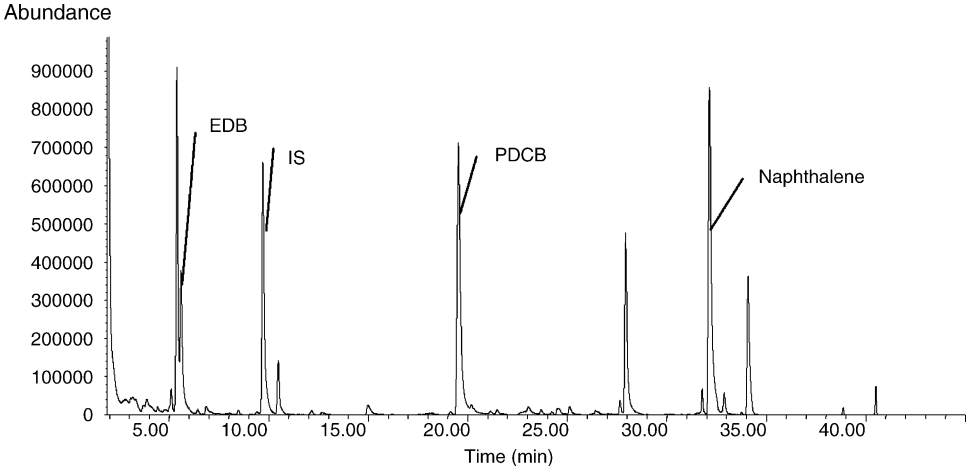


Fig. 1. Mass total ion chromatogram of a standard mixture of 150 μg $kg^{-1}$ honey, with respect to each of EDB, PDCB and naphthalene, in the presence of the internal standard styrene, obtained with chromatographic and extraction conditions as described in Section 2.

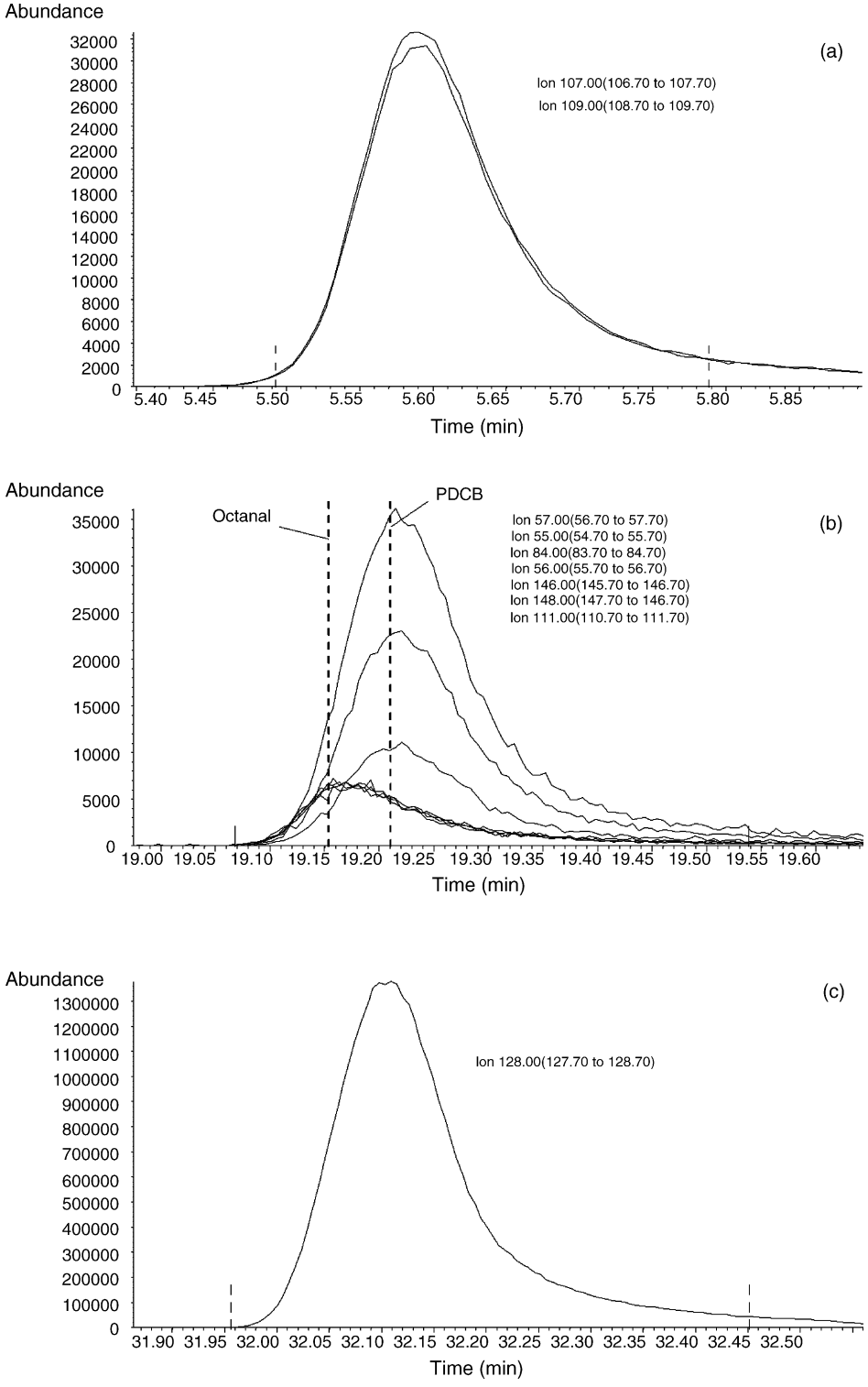


Fig. 2. Peak purity checks of EDB (a), PDCB (b) and naphthalene (c).

### 3.1. Method validation

#### 3.1.1. Linearity

The linearity of the method for each compound assayed was examined. The data were collected for five different concentrations of EDB, PDCB and naphthalene in mixtures, ranging from 2.4 to 300 $\mu g\,kg^{-1}$ honey for EDB, 0.5–300 $\mu g\,kg^{-1}$ for PDCB and 0.125–3000 $\mu g\,kg^{-1}$ for naphthalene, in the presence of internal standard, using triplicate analysis for each mixture. Calibration graphs were

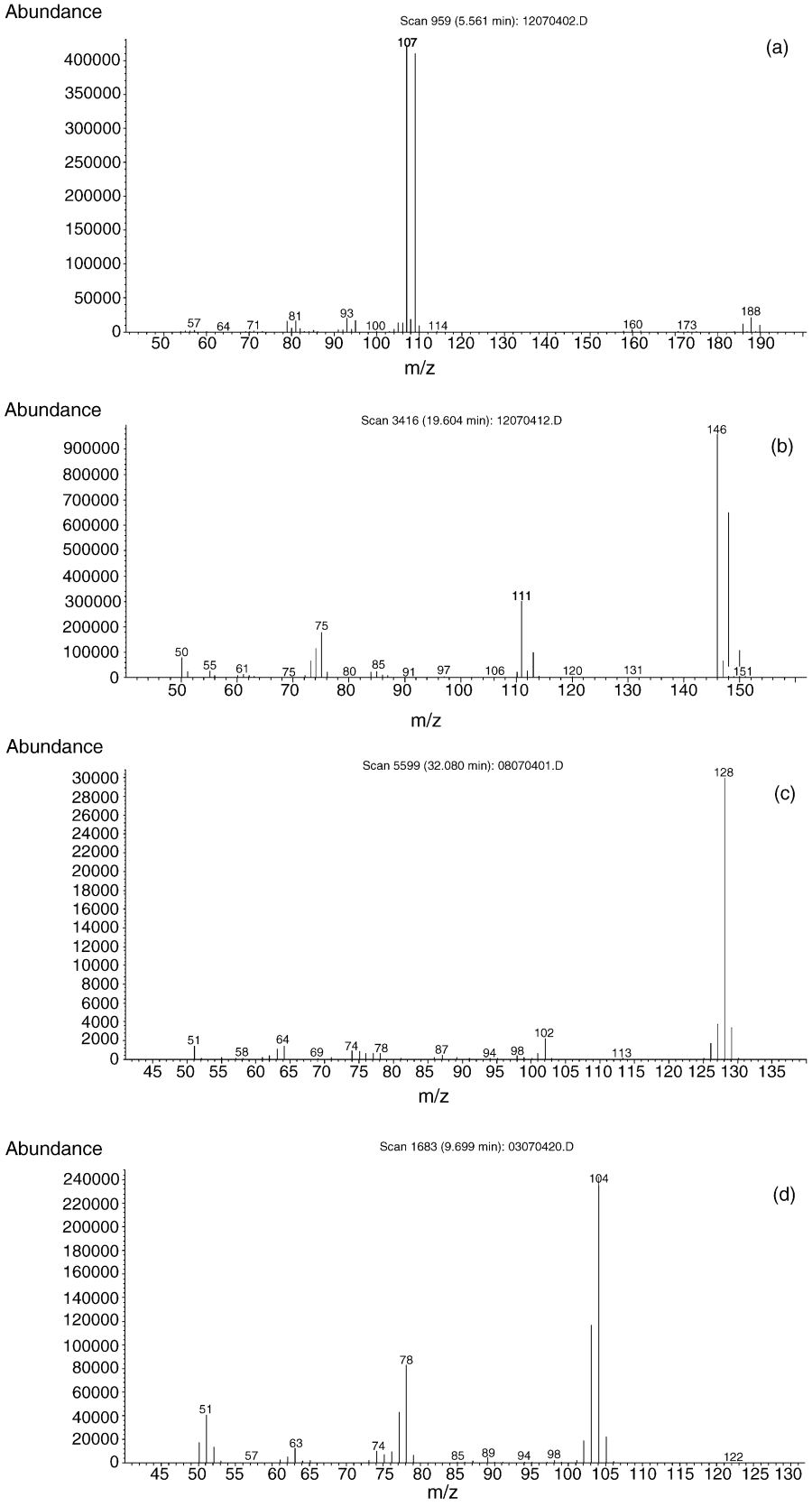


Fig. 3. Ion mass spectra of 150 μg EDB $kg^{-1}$ honey (a), 150 μg PDCB $kg^{-1}$ honey (b), 150 μg naphthalene $kg^{-1}$ honey (c) and 225 μg internal standard $kg^{-1}$ honey (d).

Table 2
Precision and accuracy of the assay

| Compound | Added ($\mu g\,kg^{-1}$ honey) | Found[a] ± SD ($\mu g\,kg^{-1}$ honey) | | RSD (%) | | Recovery (%) | |
|---|---|---|---|---|---|---|---|
| | | WD | BD | WD | BD | WD | BD |
| EDB | 10 | 9.5 ± 0.7 | 9.0 ± 0.7 | 7.4 | 7.8 | 95.0 | 90.0 |
| | 150 | 137 ± 10 | 139 ± 12 | 7.3 | 8.6 | 91.3 | 92.7 |
| | 280 | 240 ± 14 | 233 ± 9 | 5.8 | 3.9 | 85.7 | 83.2 |
| PDCB | 10 | 10.0 ± 1.2 | 11.4 ± 1.6 | 12.0 | 14.0 | 100.0 | 114.0 |
| | 150 | 143 ± 10 | 152 ± 7 | 7.0 | 4.5 | 95.3 | 101.0 |
| | 280 | 233 ± 9 | 239 ± 8 | 3.9 | 3.3 | 83.2 | 85.4 |
| Naphthalene | 10 | 10.6 ± 0.3 | 9.3 ± 0.7 | 2.8 | 7.5 | 106.0 | 93.0 |
| | 150 | 154 ± 9 | 154 ± 7 | 5.8 | 4.5 | 102.7 | 102.7 |
| | 280 | 261 ± 26 | 260 ± 8 | 9.9 | 3.1 | 93.2 | 92.9 |

SD: standard deviation; RSD: relative standard deviation; WD: within-day; and BD: between-day.
[a] Means of values calculated from the regression straight-line equation for five determinations within a day (WD) and three determinations per day over five consecutive days (BD).

constructed by plotting the analyte to internal standard peak-area ratios versus the analyte concentrations for EDB and naphthalene. In the case of PDCB the ratio of the sum peak area of its two characteristic ions versus the sum peak area of the two characteristic ions of the internal standard was used. Linear least squares regression was used to calculate the slope and intercept, with their respective standard deviations, and the correlation coefficient. The regression equation for EDB was: $y = (0.0141 \pm 0.0599) + (0.0163 \pm 0.0004)x$, for PDCB: $y = (0.0026 \pm 0.0661) + (0.0168 \pm 0.0004)x$ and for naphthalene: $y = (0.0020 \pm 0.0230) + (0.0486 \pm 0.0014)x$. The correlation coefficients were 0.9986, 0.9983 and 0.9975 for EDB, PDCB and naphthalene, respectively.

### 3.1.2. Limits of detection and quantification

The limits of detection and quantification were estimated from the analysis of spiked honey. The limits of detection were calculated from the amount of the analyte required to give a signal-to-noise ratio of 3 and were found to be 0.8, 0.15 and 0.05 $\mu g\,kg^{-1}$ honey for EDB, PDCB and naphthalene,

Table 3
Precision of determination in three replicate analyses of Greek honey samples

| Sample number | EDB ($\mu g\,kg^{-1}$ honey) | RSD (%) | PDCB ($\mu g\,kg^{-1}$ honey) | RSD (%) | Naphthalene ($\mu g\,kg^{-1}$ honey) | RSD (%) |
|---|---|---|---|---|---|---|
| 1 | ND | | 26 ± 0.2 | 0.8 | 7.8 ± 1 | 12.8 |
| 2 | ND | | 13 ± 1 | 7.7 | 1.7 ± 0.3 | 17.6 |
| 3 | ND | | 24 ± 1 | 4.2 | 1.8 ± 0.1 | 5.6 |
| 4 | ND | | 42 ± 1 | 2.4 | 0.70 ± 0.1 | 14.3 |
| 5 | ND | | 24 ± 3 | 12.5 | 17 ± 2 | 11.8 |
| 6 | ND | | 7.4 ± 0.1 | 1.4 | 0.80 ± 0.1 | 12.5 |
| 7 | NQ | | 1.8 ± 0.3 | 16.7 | 6.4 ± 0.5 | 7.8 |
| 8 | NQ | | 6.2 ± 0.7 | 11.3 | 4.8 ± 0.4 | 8.3 |
| 9 | ND | | 7.9 ± 1.0 | 12.6 | 2.8 ± 0.4 | 14.3 |
| 10 | ND | | 2.5 ± 0.7 | 12.7 | 0.60 ± 0.1 | 16.7 |
| 11 | ND | | 1.0 ± 0.1 | 10.0 | 0.80 ± 0.1 | 12.5 |
| 12 | ND | | 2.1 ± 0.3 | 14.3 | 1.2 ± 0.04 | 3.3 |
| 13 | ND | | 2.0 ± 0.3 | 15.0 | 0.90 ± 0.1 | 11.1 |
| 14 | ND | | 0.90 ± 0.1 | 11.1 | 1.1 ± 0.2 | 18.2 |
| 15 | ND | | 1.3 ± 0.1 | 7.7 | 1.0 ± 0.2 | 20.0 |
| 16 | ND | | 29 ± 2 | 6.9 | 0.20 ± 0.03 | 15.0 |
| 17 | ND | | 0.60 ± 0.01 | 1.7 | 0.50 ± 0.1 | 20.0 |
| 18 | 75 ± 3 | 4.0 | NQ | | 0.30 ± 0.05 | 16.7 |
| 19 | 12 ± 0.5 | 4.2 | NQ | | NQ | |
| 20 | ND | | 261 ± 4 | 1.5 | 0.16 ± 0.03 | 18.7 |
| 21 | ND | | 186 ± 7 | 3.8 | 0.43 ± 0.03 | 6.9 |
| 22 | ND | | 17 ± 2 | 11.8 | NQ | |
| 23 | ND | | 1.2 ± 0.2 | 16.7 | 0.28 ± 0.05 | 17.9 |
| 24 | ND | | 1.8 ± 0.1 | 5.6 | 0.15 ± 0.01 | 6.7 |
| 25 | ND | | 1.2 ± 0.1 | 8.3 | NQ | |

ND: not detected and NQ: not quantified.

respectively. The limits of quantification were determined with a signal-to-noise ratio of 10 and were found to be 2.4, 0.5 and 0.125 $\mu g\,kg^{-1}$ honey for EDB, PDCB and naphthalene, respectively.

### 3.1.3. Precision and accuracy

The intra-day precision and accuracy of the method was assessed by analyzing five times each of three residue-free honey samples, spiked with standard mixtures of the analytes at low (10 $\mu g\,kg^{-1}$ honey), medium (150 $\mu g\,kg^{-1}$ honey) and high (280 $\mu g\,kg^{-1}$ honey) concentration levels, in the presence of internal standard, during the same day. As shown in Table 2, the relative standard deviations (RSDs) ranged from 2.8% to 12.0% for the intra-day and from 3.1% to 14.0% for the inter-day calibration, indicating a satisfactory precision. A high degree of accuracy was achieved, as estimated by the recovery values, which ranged from 83.2% to 106.0% and from 83.2% to 114.0% for the intra- and inter-day calibration, respectively.

## 3.2. Method application

Twenty five honey samples produced in different areas of Greece were analysed for residues of EDB, PDCB and naphthalene by the method developed herein. Each sample was analysed in triplicate.

The results of the analyses of real samples are summarized in Table 3. As can be seen all samples contained both PDCB and naphthalene residues. EDB was found along with PDCB and naphthalene only in four samples, in two of which its concentration was below the quantification limit of the method, in the third EDB was present at a concentration below the action level set by the American FDA (30 $\mu g\,kg^{-1}$) and only one had an EDB concentration that exceeded 30 $\mu g\,kg^{-1}$. Nine samples were found to be contaminated with a PDCB concentration exceeding the "Swiss tolerance level" of 10 $\mu g\,kg^{-1}$, with two of them containing high concentrations of PDCB (261 and 186 $\mu g\,kg^{-1}$). Naphthalene did not seem to consist a major problem. There was only one sample containing naphthalene as well as PDCB residues, both at concentrations exceeding 10 $\mu g\,kg^{-1}$.

## 4. Conclusions

The presence of EDB, PDCB and naphthalene residues in 25 Greek honey samples was investigated by applying a new analytical method developed in this work. The method permits the simultaneous determination of EDB, PDCB and naphthalene with high sensitivity using gas chromatography–mass spectrometry with purge and trap as the extraction technique. Styrene was used as the internal standard. Accuracy and precision were checked at three concentration levels for each analyte. The recovery values ranged from 83.2% to 106.0% and the RSD values from 2.8% to 12.0%. The detection limits were found to be 0.8, 0.15 and 0.05 $\mu g\,kg^{-1}$ honey for EDB, PDCB and naphthalene, respectively. The results of Greek honey analysis revealed that of the three compounds investigated, only the presence of PDCB residues consisted the main problem in 36% of the samples analysed.

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