A New GC-MS Method for the Determination of p-Dichlorobenzene in Honey

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Introduction

Para-dichlorobenzene (1,4-dichlorobenzene, p-DCB) is a fumigant insecticide used by beekeepers worldwide to control the greater wax moth, Galleria mellonella, during the storage of beeswax combs. Such use is, however, contrary to "good beekeeping practice", as it leads to residues in honey and wax. Because of its toxicity and possible carcinogenic effects (NIOSH, 2000), use of p-DCB should be avoided.

To our knowledge, no publications are available on the p-DCB content of commercial honey, except for a summary on the p-DCB levels found in the German market from 1997 (Wallner, 1997) and an analysis of p-DCB residues carried out on Swiss retail market honey samples by the cantonal food control authorities in 1997-2002 (Bogdanov, 2004).

Due to the new EEC regulation (EC No 396/2005, 2005) which specifies maximum residue levels (MRLs) of pesticides in or on food and feed of plant and animal origin, a maximum concentration of 0.01 mg p-DCB /kg honey has been applied.

Investigations of honeys from some European countries (including Greece) showed that a relatively high percentage of samples analysed contained p-DCB in concentrations higher than the applied MRLs. There exists therefore a need for regular checking and quality control of honey, using an analytical method with a detection limit below 1/10 of the MRL values applied.

Materials and Methods

Collection of samples.

Samples were purchased from the Greek market in February 2005.

Materials

Methanol analytical grade was purchased from Merck. All other chemicals were trace analysis picograde purchased from Promochem (Germany). The isotopic labeled p-DCB d4 and chlorobenzene d5 for the preparation of internal and injection standard solutions respectively, were purchased from Cambridge Isotope Laboratories (USA). Non-labeled dichlorobenzene isomers were purchased from ChemService (USA). Basic alumina, purchased from MP Biomedicals Germany GmbH, was activated overnight in an oven at 200 ^{0}C.

Extraction

5 g of honey were weighed in a 50-mL falcon tube and dissolved in 10 mL of water. 10 mL of methanol and 100ml p-DCB d4 (50 mg/ml) internal standard solution were added and the samples were vortexed for 1 min. Extraction steps with 10 ml of diethyl-ether and 10 ml of petroleum-ether followed and the etheric layer was separated by centrifugation and collected in a spherical vial. After the addition of hexane, the samples were subjected to controlled concentration for the evaporation of ether.

Clean up

The hexane fraction obtained, containing the p-DCB, was brought onto a glass column (length 30 cm, 8 mm I.D.) plugged with glass wool and packed with 5 g of basic alumina. The p-dichlorobenzene was eluted with 50 mL of an

hexane/dichloromethane mixture (1:1 v/v). Finally the eluate was evaporated to a final volume of 0.5 mL and 10 µl of injection standard, containing 50 ng/ml of chlorobenzene d5, were added.

Instrumental analysis

The quantification of p-dichlorobenzene was performed by GC-MS on an ion trap mass spectrometer (Polaris ThermoFinnigan) coupled to a Trace GC gas chromatograph and equipped with an AS2000 autosampler (ThermoFinnigan). The quantification was carried out by the isotopic dilution method.

Results and Discussion.

GC-Mass spectrometry

The concentrated honey clean up extract was analyzed by GC-MS using EI in positive mode. The mass spectrum of p-DCB exhibits intense peaks at 146 m/z $(M-H)^+$,at 111.1 m/z $(M-Cl)^+$ and at 75.1 m/z $(M-2Cl)^+$. The unique isotope pattern 3:1 or 3:2 due to the presence of one or two chlorine atoms was evident from the M+2 satellite signals (figure 1).

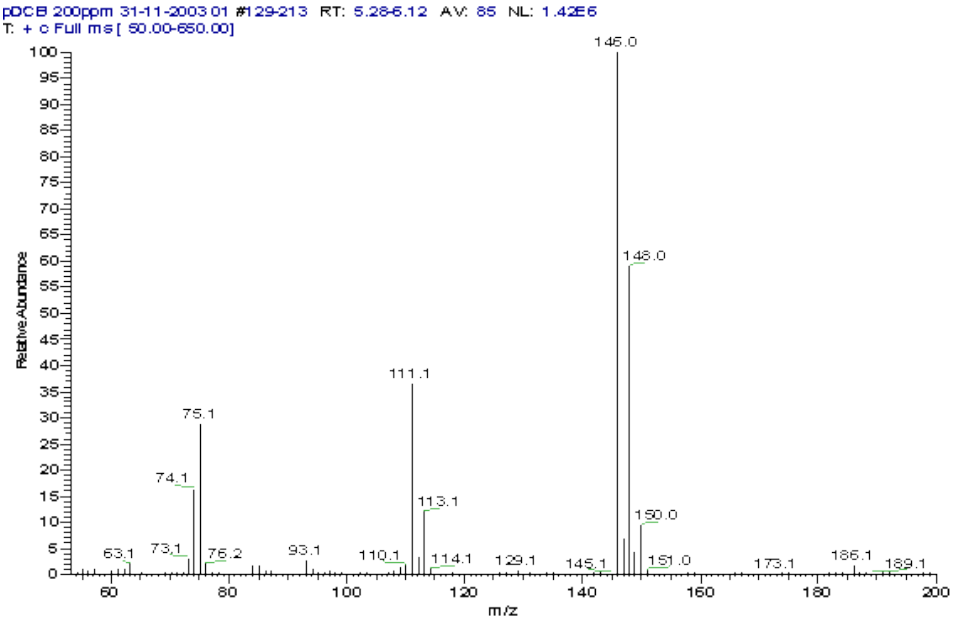

Figure 1. MS spectrum of p-DCB, EI positive mode, 70eV.

Quantification

The quantification of the p-DCB was performed by GC-MS in the SIM mode using p-DCB d4 as internal standard. The analytical system repeatability was established by 10 replicate injections of a standard solution at 100 ng/ml and the RSD value of the peak areas was 5%. The proposed method was validated for its linearity, precision, accuracy and sensitivity. A 4 level calibration curve was constructed at 20, 100, 200 and 400 ng/ml (figure 2). The calibration curve was expressed by

$Y=0.000170399+0.00121802*X$ with correlation coefficient $R^2=0.9995$.

Precision and accuracy was checked using a residue free honey sample spiked at 10 ng/g. The sample was analyzed 10 times and the concentration was calculated from the calibration curve. The mean value was calculated at 9.4 ng/g with RSD 13%. The detection limit of the method was estimated in terms of baseline noise. The LOD was defined as the p-DCB concentration yielding signal with signal to noise ratio 3:1 and estimated at 0.8 ng/g.

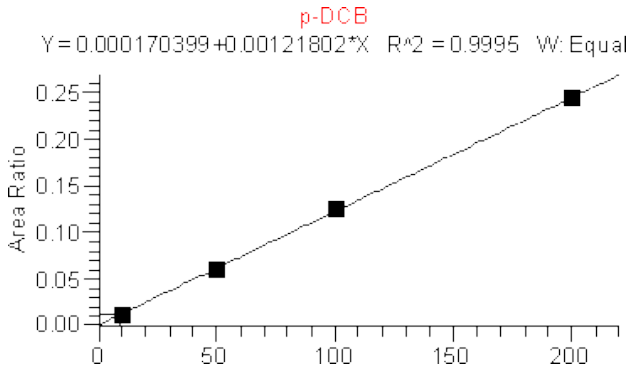

Figure 2. Calibration curve of p-DCB.

Application of the method

The performance of the proposed method was tested on five honey samples collected from the Greek market. In three of them p-DCB was not detected, in one sample p-DCB concentration was found at 8.5±2.6 ng/g and the last one was found at 45.1 ng/g.

References

1. Charriere, J D; Imdorf, A (1999) Protection of honey combs from wax moth damage. American Bee Journal 139 (8): 627–630.

2. US Department of Labor: NIOSH/OSHA/DOE (2000) Health Guidelines for p-dichlorobenzene. Occupational Safety & Health Administration, 200 Constitution Avenue, NW, Washington, DC 20210, USA.

3. S Bogdanov, V Kilchenmann, K Seiler, H Pfefferli, TH Frey, B Roux, P Wenk and J Noser Residues of para-dichlorobenzene in honey and beeswax. Journal of Apicultural Research 43(1): 14–16 (2004)

4. European Council Regulation (EC) No 396/2005 of 23 February 2005

5. Wallner, K (1997) The actual beeswax quality in foundations from the market. Apidologie 28(3–4): 168–171.