International Food Research Journal 15(3): 281-286 (2008)

Determination of *para*-dichlorobenzene residues in honey by purge and trap with GCMSD

[1]Lu, C., [2*]Buddhadasa, S., [2]Barone, S. and [1]Bramwell, P.

[1]*School of Applied Sciences, RMIT University, Melbourne, Victoria, Australia*
[2]*National Measurement Institute, Melbourne, Victoria, Australia*

Abstract: *para*-Dichlorobenzene (*p*-DCB) belongs to a group of volatile organic compounds (VOCs) that can contribute towards environment and food contamination. *p*-DCB residues may be found in honey due to its use by beekeepers (during honey combs processing) to avoid wax moth infestation. This residue has been shown to be harmful to humans due to being potentially toxic and carcinogenic. Studies have revealed the presence of *p*-DCB in various foods over a wide concentration range (2-200 µg/kg). A Purge and Trap with GCMSD method for the measurement of *p*-DCB residues in honey was developed in a collaborative partnership between the National Measurement Institute of Australia and the RMIT University to support the Australian honey industry and Australian consumers. This paper outlines the method designed to determine *p*-DCB at concentrations as low as 1 µg/kg in honey. The method was developed and validated using a purge and trap concentrator coupled to a gas chromatograph with a mass selective detector (GC-MSD). The method was validated to cover a range of 1-20 µg/kg *p*-DCB in honey, achieving mean recoveries at 64-77% with a standard deviation range of 3-6%.

Keywords: Honey, *p*-DCB, purge and trap, gas chromatography, mass selective detector

INTRODUCTION

p-DCB, also called paramoth, has been used for many years by beekeepers as an insecticide against the wax moth *Galleria mellonella* and to a lesser extent *Achroia grisella,* during storage of honey comb (Liu *et al.,* 2004; Tananaki *et al.,* 2005). When *p*-DCB is added to honey, it is absorbed by the wax and the honey is protected by eliminating attack from wax moths for up to two years (Botitsi *et al.,* 2006). The devastating effects of these insects are known to beekeepers throughout the world, and understandably, they use *p*-DCB treatment to prevent these occurrences (Erdoğrul, 2007; Rial-Otero, 2007). Studies have shown *p*-DCB does not kill all stages of wax moth and will not clean up established moth infestations (Botitsi *et al.,* 2006). Furthermore its use may lead to the contamination of the honey, making it unsuitable for human consumption (Liu *et al.,* 2004). Study carried out by Tananaki *et al.* (2005) on *p*-DCB was limited to three spike levels ranging from 10-280 µg/kg and a survey which resulted in over 50% of the samples determined to contain levels less than 10 µg/kg. Therefore this study was designed to evaluate the recovery levels of *p*-DCB at the lower end approaching 1 µg/kg which appears to have a higher significance in terms of being present.

Due to a number of requests on an analytical method based on GCMS to determine *p*-DCB at 1 µg/kg by the honey industry, a literature review was carried out to evaluate the current methods. Most methods used for the analysis were broadly based on a VOCs extraction technique followed by an instrumental analysis. As examples, method used by Blasco *et al.* (2004), Erdoğrul (2007), Jiménez *et al.* (1998a), Jiménez *et al.* (1998b) and Rissato *et al.* (2004) for the analysis of VOCs was by solid-phase micro extraction (SPME) followed by GC-Electron Capture Detector (GC-ECD). Yu *et al.* (2004) showed that GC-Flame Photometric Detection (GC-FPD) could also be used for the determination of VOCs. Another method published by Jiménez *et al.* (2000) and Martel and Zeggane (2002) was based on solvent extraction and analysis by High Performance Liquid Chromatography with Photo Diodearray Detection (HPLC-PDA). There were also methods published for VOCs in honey by Bernal *et al.* (1996), Bernal *et al.* (2000), Erdoğrul (2007), Jiménez *et al.* (2002), Martínez *et al.* (2002) and Soria *et al.* (2007) using instruments such as GC-ECD, GC- Flame Ionization Detector (GC-FID) and GC-MSD however *p*-DCB was not included in any of these studies.

The aim of this work was to develop a rapid, sensitive and cost effective procedure to determine the levels of *p*-DCB in honey. A sample of honey

*Corresponding author
Email: *saman.buddhadasa@measurement.gov.au*

was dissolved in Milli-Q water and purged with ultra high purity Helium in a purge and trap concentrator. *p*-DCB residues were efficiently transferred from the aqueous phase to the vapour phase and retained within the trap containing a sorbent material. When purging was complete, the trap was rapidly heated and back-flushed with helium to desorb the volatile chemicals including *p*-DCB into the GC split injector via a heated transfer line. A split injector system was utilised to remove excess purge gas, since the purge-gas volume was greater than that required for the carrier gas. The material was moved into the mass selective detector through the column, where the *p*-DCB was identified, and measured using internal and reference standards. Confirmations of the results were carried out using mass spectral and retention time comparisons of the reference standards against those generated by the samples. Components were quantified using isotopically labeled analogue d_4-*p*-DCB internal standard with a seven-point external calibration curve of *p*-DCB.

MATERIALS AND METHODS

Milli-Q water used for the study was generated by an ultra pure water system (Millipore Corp™, Germany). Standard solutions were stored in 2 ml GC vials (Alltech™, USA). A PTFE stirring bar (Cowie techology™, UK) was provided to homogenise the honey matrix. Purge and trap vials (40 ml) were supplied by Yellow™, NZ.

A concentrated solution of DWM-588 (Ultrascientific™, US), containing *p*-DCB at 2000 µg/ml in methanol was purchased and diluted with methanol to obtain a stock solution of 10 µg/ml. Using volumes of 2, 5, 10, 20, 30, 40 and 50 µl of the 10 mg/l stock solution, calibration standards were prepared at 4, 10, 20, 40, 60, 80 and 100 µg/l in 5.0 ml of Milli-Q water. The isotopically labelled d_4-*p*-DCB, used as the internal standard was supplied by Ultrascientific™, USA with a part number STM-341N (2000 µg/ml in methanol). System monitoring compounds (surrogate standards), were also purchased from Ultrascientific™, STM-330N which contains 4-bromofluorobenzene, dibromofluoromethane and toluene-d_8, at 2000 µg/ml in methanol. These solutions were cooled to a temperature of about <4°C and stored in 2.0 ml GC vials, filled to capacity (to minimise headspace), capped immediately after filling and stored at <4°C in a fridge.

A purge and trap system (EST Analytical™, US) was used for extraction of the target compound from the honey samples. Detection and measurement of the analytes was performed on a HP5890 GC coupled to a HP 5971A quadrupole mass selective detector.

Extraction of p-DCB

A 5.0 g sample of honey taken from a batch of honey previously tested by the method and determined to be free of *p*-DCB at or above 1 µg/kg was accurately weighed (to 2 decimal places) into a 40 ml purge and trap vial. Samples used for recoveries were prepared by spiking appropriate volumes of *p*-DCB standard solution into the honey free of *p*-DCB. The spiked concentrations were 1, 5, 10 and 20 µg/kg each in 5.0 ml of Milli-Q water. Blank honey samples were also prepared by adding only 5.0 ml of Milli-Q water to residue–free honey sample. All spiked samples were analysed in replicates of seven. A stirring bar was inserted into each spiked honey sample vial to improve the purging efficiency. The spiked solutions were homogenized in the vials by vortex mixing for 2-3 minutes, until complete homogenization.

Samples were purged for 11 minutes at 40°C using helium, at a flow rate of 37.5 ml/min into a Vocarb 3000 trap, kept at room temperature in a 5.0 ml sparge tube, at a pressure of 270 kPa and were desorbed for 2 minutes at 250°C. The trap was heated for 5 minutes at 220°C for compounds to desorb. Capillary transfer line and valves were heated at 130°C to avoid volatile compound condensation. The transfer line temperature used in the interface between the purge and trap and the GC was 130°C.

Determination of p-DCB

The GC-MS conditions were set at an injector temperature of 220°C, interface temperature of 250°C, initial temperature of 35°C, with a hold time of 3 minutes. A temperature ramp rate of 10°C/minute was used to reach a final temperature of 240°C and held for 0.5 minute. Quantitative results were read from the computer by MS ChemStation software. Peak purity checks were performed for this compound using the MS ChemStation software.

RESULTS

The purge and trap and GC separation conditions had been established on the basis of the following parameters: satisfactory separation of the analytes, relatively short analysis time and maximum peak area ratio.

Table 1: Validation data for seven replicate spikes showing recoveries of *p*-DCB

Spiking level (µg/kg)	Mean Recovery (%) ± RSD (%)	Relative standard uncertainty (%)
1	77 ± 6	15
5	67 ± 3	9
10	69 ± 5	14
20	64 ± 3	9

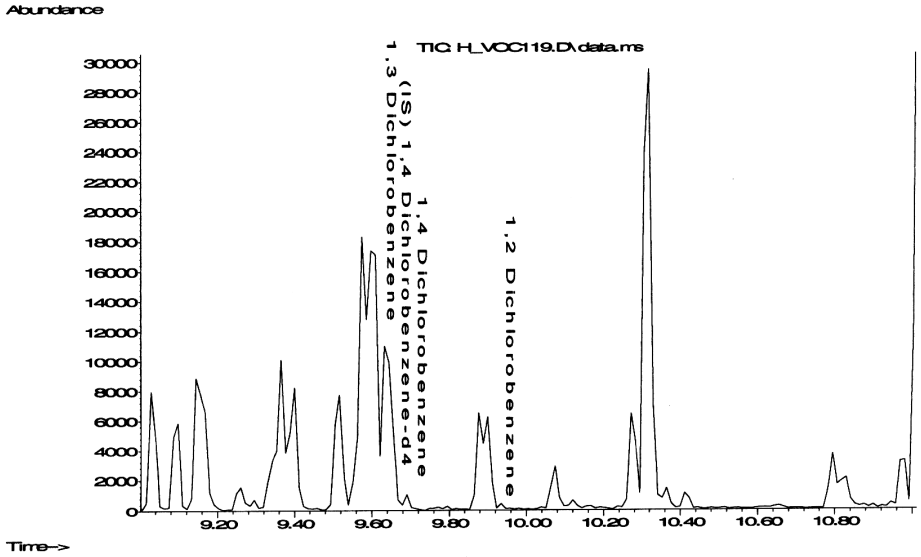

Figure 1: Total ion current (TIC) chromatogram of a honey extract spiked with 1, 2-DCB, 1, 4-DCB (*p*-DCB), 1, 3-DCB d_4-*p*-DCB

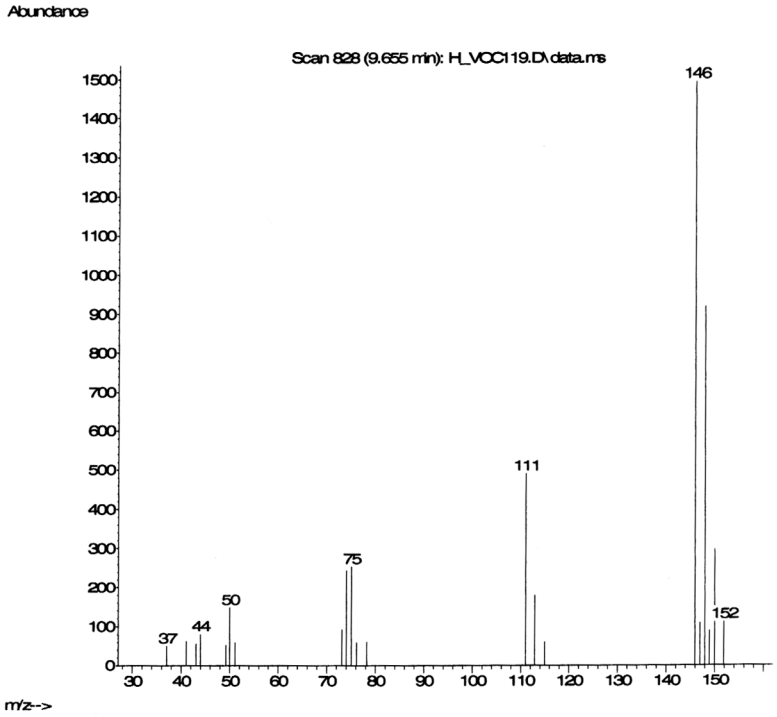

Figure 2: Electron impact mass spectra of *p*-DCB

The chromatograms (Figures 1 and 2) show the ability of the method to respond to *p*-DCB in the sample matrix. The extracted ion confirms chromatogram in Figure 1 indicates the presence of *p*-DCB in the spiked honey sample at 9.66/13.20 minute, the internal standard d_4-*p*-DCB, is also shown at 9.65/13.20 minute to ensure the quantification is robust. An electron impact mass spectrum shown in Figure 2 is to confirm the target compounds, *p*-DCB.

DISCUSSION

Specificity

Specificity was assessed by analysing *p*-DCB honey samples to investigate the presence of potential interferences. No interfering peaks were present in the elution region of *p*-DCB in the GC-MS system. Accuracy was estimated as mean recovery percentage and precision was estimated from repeatability and within laboratory reproducibility conditions in terms of the percentage of relative standard deviations (percent RSD). The confirmation of *p*-DCB was carried out by comparing the electron impact mass spectra of the reference *p*-DCB with the sample mass spectra (Figure 2).

Precision and accuracy

The intra-day precision and accuracy of the method was assessed by spiking *p*-DCB free honey with *p*-DCB at four different concentration levels (1, 5, 10 and 20 µg/kg respectively). Each sample was analysed during the same day in replicates of seven, with d_4-*p*-DCB internal standard. As shown in Table 1, the percent RSD ranging from 3% to 6% for the intra-day and from 4% to 8% for the inter-day calibration indicated a satisfactory precision on an intra-day basis. The recovery data indicates that the accuracy and the precision to be at a satisfactory level. Recoveries ranged from 64-77 percent with an uncertainty range of 9-15 percent. The recoveries were shown to be relatively low due to a number of most likely reasons. *p*-DCB is a volatile compound and needs substantial care to minimize losses at all stages specially prior to spiking into the honey. The purge and trap stage also can influence the recoveries and utmost care was taken to ensure the honey is well dissolved in the Milli-Q water and the purge efficiency using ultra high pure Helium was optimized for most of the volatile organic compounds including *p*-DCB. The fluctuation of the percent RSD and relative standard uncertainty may be due to the operation errors during the spiking and this was regarded to be within the acceptable level for this work. In order to comply with quality control procedures the study included the measurement of blanks, reference standards, recoveries, surrogate and internal standards. To obtain proper statistics, recoveries were carried out in replicates of seven.

Method validation

a. Linear range

The linearity of the method for the target compound was validated. The data were collected for four different spiking levels, ranging from 1 to 20 µg/kg, in the present of internal standard, using seven duplicate analysis for each level. Calibration curve were plotting the analytes to internal standard peak area ratios versus the anaytes concentration for *p*-DCB.

b. Limitation of detection of quantification

The limits of detection and quantification were estimated from the analysis of spiked honey samples and reference standards. The limit of detection for *p*-DCB was 0.2 µg/kg and the limit of quantification was 1 µg/kg. The limit of detection was based on the lowest reference standard used and the limit of quantification was determined using the lowest validated spike of 1 µg/kg.

Based on this study, it was important to include recoveries in each batch honey analysed for *p*-DCB and the results corrected to reflect the recovery levels. The spikes for the recoveries should be carried out in a similar range to those found in samples to ensure the recoverable level based on the natural contamination. It was also recommended that the calibration standards be prepared on a daily basis and the work carried out within the same day to ensure minimum loss of *p*-DCB and all samples needed to be prepared in the laboratory free of volatile solvent vapour to avoid cross contamination.

CONCLUSION

The analytical methodology, purge and trap extraction technique developed for the determination of *p*-DCB in honey is simple, rapid and efficient while GC–MSD analysis in Electron impact mode enables selective and sensitive detection of *p*-DCB. The use of the isotopically labeled d_4-*p*-DCB as internal standard improves the accuracy of quantification of the material. Method validation was performed on GC-MS systems using four different spiking levels at seven replicates of honey samples with low percent RSD between replicates within a given concentration. The method is suitable for the identification and

quantification of *p*-DCB residues in honey over a range of concentrations, and a rigorous quality control protocol used ensures the reliability of the results.

ACKNOWLEDGEMENTS

The authors would like to thank Shyam Kumaran, Neil Menz, Samantha Duong and Dianne Laugher of the National Measurement Institute of Australia, Food Metrology Department in South Melbourne for providing laboratory facilities, technical support, invaluable advice and inspiring guidance for the success of the project.

REFERENCES

Botitsi, E.V., Kormali, P.N., Kontou, S.N., Economou, A. and Tsipi, D.F. 2006. Development and validation of a new analytical method for the determination of 1, 4-dichrobenzene in honey by gas chromatograph-isotope dilution mass spectrometry after steam-distillation. Analytica Chimica Acta 579 (1): 53-60.

Blasco, C., Lino, C.M., Picó, Y., Pena, A., Font, G. and Silveira, M.I.N. 2004. Determination of organochlorine pesticide residues in honey from the central zone of Portugal and the Valencian community of Spain. Journal of Chromatograph A 1049 (1-2): 155-160.

Bernal, J.L., del Nozal, M.J., Rivera, J.M., Jiménez, J.J. and Atienza, J. 1996. Determination of the fungicide vinclozolin in honey and bee larvae by solid-phase and solvent extraction with gas chromatography and electron-capture and mass spectrometric detection. Journal of Chromatograph A 754 (1-2): 507-513.

Bernal, J., Jiménez, J.J., del Nozal, M.J., Higes, M. and Llorente, J. 2000. Gas chromatographic determination of acrinathrine and 3-phenoxybenzaldehyde residues in honey. Journal of Chromatograph A 882 (1-2): 239-243.

Erdoğrul, Ö. 2007. Levels of selected pesticides in honey samples from Kahramanmaras, Turkey. Food Control 18 (7): 866-871.

Jiménez, J.J., Bernal, J.L., del Nozal, M.J., Martín, M.T. and Mayorga, A.L. 1998a. Soild-phase microextraction applied to the analysis of pesticide residues in honey using gas chromatography with electron-capture detection. Journal of Chromatography A 829 (1-2): 269-277.

Jiménez, J.J., Bernal, J.L., del Nozal, M.J., Toribio, L. and Martín, M.T. 1998b. Gas chromatography with electron-capture-phosphorus detection in the analysis of pesticides in honey after elution from a Florisil column. Journal of Chromatography A 823 (1-2): 381-387.

Jiménez, J.J., Bernal, J.L., del Nozal, M.J., Novo, M., Higes, M. and Llorente, J. 2000. Determination of rotenone residues in raw honey by solid-phase extraction and high-performance liquid chromatography. Journal of Chromatograph A 871 (1-2): 67-73.

Jiménez, J.J., Bernal, J.L., Toribio, L., del Nozal, M.J. and Martín, M.T. 2002. Capillary gas chromatography with mass spectrometric and atomic emission detection for characterization and monitoring chlordimeform degradation in honey. Journal of Chromatograph A 946 (1-2): 247-253.

Liu, W., Zheng, M., Wang, D., Xing, Y., Zhao, X., Ma, X. and Qian, Y. 2004. Formation of PCDD/Fs and PCBs in the process of production of 1,4-dichlorobenzene. Chemosphere 57 (10): 1317-1323.

Martel, A.C. and Zeggane, S. 2002. Determination of acaricides in honey by high-performance liquid chromatography with photodiode array detection. Journal of Chromatography A 954 (1-2): 173-180.

Martínez, E., Lacorte, S., Llobet, I., Viana, P., and Barcelo, D. 2002. Multicomponent analysis of volatile organic compounds in water by automated purge and trap coupled to gas chromatograph-mass spectrometry. Journal of Chromatograph A 959 (1-2): 181-190.

Rial-Otero, R., Gaspar, E.M., Moura, I. and Capelo, J.L. 2007. Chromatographic-based methods for pesticide determination in honey: An overview. Journal of Chromatography A 71 (2): 503-514.

Rissato, S.R., Galhiane, M.S., Knoll, F.R.N. and Apon, B.M. 2004. Supercritical fluid extraction for pesticide multiresidue analysis in honey: determination by gas chromatography with electron-capture and mass spectrometry detection. Journal of Chromatography A 1048 (2): 153-159.

Soria, A.C., Martínez-Castro, I. and Sanz, J. 2007. Estimation of recovery by multistep purge and trap gas chromatography-mass spectrometric analysis of honey volatiles. Journal of Chromatography A 1157 (1-2): 430-436.

Tananaki, C., Zotou, A. and Thrasyvoulou, A. 2005. Determination of 1, 2-dibromoethane, 1,4-dichlorobenzene and naphthalene residues in honey by gas chromatograph-mass spectrometry using purge and trap thermal desorption extraction. Journal of Chromatography A 1083 (1-2): 146-152.

Yu, J., Wu, C. and Xing, J. 2004. Development of new solid-phase microextraction fibers by sol-gel technology for the determination of organophosphorus pesticide multiresidues in food. Journal of Chromatography A 1036 (2): 101-111.