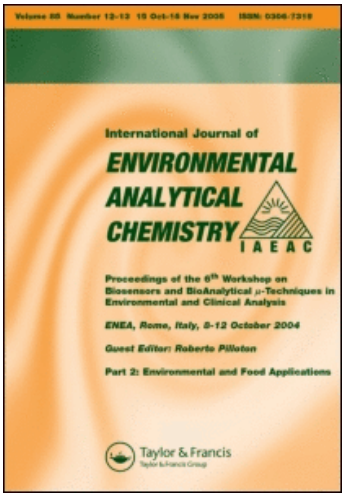

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Determination of bromopropylate in grapes by gas chromatography: evaluation of two analytical methods

Themistokles D. Lekkas[a]; Anastasia D. Nikolaou[a]
[a] Water and Air Quality Laboratory, Department of Environmental Studies, University of the Aegean, University Hill, 81100 Mytilene, Greece

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Determination of bromopropylate in grapes by gas chromatography: evaluation of two analytical methods

THEMISTOKLES D. LEKKAS and ANASTASIA D. NIKOLAOU*

Water and Air Quality Laboratory, Department of Environmental Studies, University of the Aegean, University Hill, 81100 Mytilene, Greece

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Two gas chromatography methods were developed for the determination of the acaricide bromopropylate in grapes. The first included chromatographic separation with a DB-1 column and electron capture detection (GC-ECD), while the second utilized chromatographic separation with a DB-5MS column and mass spectrometry detection (GC-MSD). Sample preparation was the same for both methods and included liquid–liquid extraction with dichloromethane and diethylether, centrifugation, and evaporation. The sample preparation and analysis steps were optimized. The evaluation of the methods by analysis of standard solutions of bromopropylate in hexane gave satisfactory results. The detection limits were $0.02\,mg\,kg^{-1}$ for the GC-ECD method and $0.03\,mg\,kg^{-1}$ for the CG-MS method, and the corresponding quantification limits were 0.04, and $0.06\,mg\,kg^{-1}$, respectively. However, the analysis of spiked grape samples showed that the GC-ECD method could be problematic for the particular substrate. In the cases of very low concentrations of bromopropylate, elevated results were obtained, because of a co-eluting peak. This problem did not exist with the GC-MSD method, which revealed that the co-eluting peak results from the hydrolysis of bromopropylate and corresponds to one of its metabolites. In conclusion, the GC-MSD method proved to be the most reliable for the determination of bromopropylate in grapes, while the degradation of this pesticide in the particular substrate is a subject of interest for further scientific investigation.

Keywords: Bromopropylate; Grapes; Gas chromatography; Mass spectrometry

1. Introduction

The extended use of a variety of pesticides during the last decades has led to a major increase and improvements in agricultural production. However, the occurrence of pesticide residues in fruit and vegetables has drawn significant scientific attention, because of their toxic effects in humans and the environment. Many of these compounds have been regulated by the European Union [1–2]. In parallel, a large number of studies have focused on the development of analytical methods for the determination of trace amounts of pesticides residues in fruit and vegetables [3–8]. Approximately 500 compounds have been registered as pesticides and pesticide

*Corresponding author. Fax: +30-2251036209. Email: nnikol@aegean.gr

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metabolites worldwide, 300 of which are amenable to gas chromatography (GC), which is the most frequently used technique for their determination, followed by high-performance liquid chromatography (HPLC) [4, 8].

Typically, most GC methods use electron capture detector (ECD) for halogenated pesticides, because of its high sensitivity to halogenated compounds. However, the selectivity of this detector can be poor, because many compounds containing electron-attracting functional groups may also produce a response, creating problems with the accurate determination of the compounds of interest. A step forward in overcoming these analytical difficulties has been the increasing use of the mass spectrometer detector (MSD), initially as a confirmation technique and, as MSD instrumentation technology provides solutions for more accurate measurements, also for routine measurements. The MSD is characterized by a high selectivity, since it has the ability to identify mass fragments corresponding to the compounds of interest, thus revealing their identity. The opportunity to scan for specific fragments (SIM mode) makes MSD methods applicable also for routine analysis with maximum sensitivity [3].

During this study, both GC-ECD and GC-MSD methods have been developed and applied for the determination of the pesticide bromopropylate in grapes. The chemical structure of bromopropylate (Neoron) is presented in figure 1. This compound belongs to the chemical category of acaricides. It is a crystal solid, with a molecular weight of 428.14, melting point of 77°C, vapour pressure of 5.1×10^{-8} mm Hg at 20°C, and water solubility of $< 5\,\mathrm{mg\,L^{-1}}$ at 20°C. The LD50 (orally in rats) is $5000\,\mathrm{mg\,kg^{-1}}$.

The application of bromopropylate in grape cultivations in Greece, particularly in the area of Korinthia, has raised concerns for the quality of the product, taking into account the maximum residue level (MRL) of $0.05\,\mathrm{mg\,kg^{-1}}$ established recently for bromopropylate in fruit by the 2002/79/EC Directive [2]. To the authors' knowledge, measurements of bromopropylate in grapes have not been previously reported in the literature. Analytical methods have been developed and applied for the determination of bromopropylate in other substrates (water, peaches, honey, beeswax) [5–10], while other acaricides have been determined in wine and must, where matrix effects were observed with an ECD detector [11]. The objective of this study was to develop a simple and sensitive GC method that can be readily applied for the determination of bromopropylate in grapes.

bromopropylate

IUPAC: isopropyl 4,4′-dibromobenzilate

STRUCTURE:

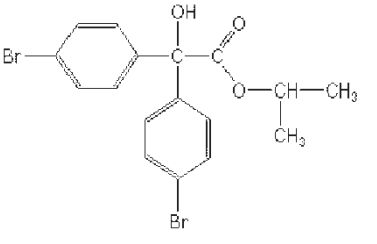

Figure 1. Chemical structure of bromopropylate (Neoron) (Source: Compendium of Pesticides Common Names, Alan Wood, UK. http://www.hclrss.demon.co.uk/ last access August 2005).

2. Experimental

The selection of analytical methods to be used for bromopropylate determination was based on the review articles by Torres *et al.* [3] and Van der Hoff and Van Zoonen [4] regarding the sample preparation and techniques for the detection of acaricides in fruit. During development of the method, the articles by Fernandez-Alba *et al.* [5], Liapis *et al.* [6], Korta *et al.* [9] and Bernal *et al.* [12] were taken into account.

2.1. *Reagents and samples*

Bromopropylate standard (purity >98%) was purchased from ChemService, and acetone, dichloromethane, diethylether, pentane, toluene, *n*-hexane (for organic trace analysis) from Merck. Grapes were collected on 26 and 27 August 2003 from cultivations in areas Souliou and Zeygolatio in Korinthia. Bromopropylate had been applied to these cultivations at the end of July and at the beginning of August 2003. Samples were also collected from cultivation where bromopropylate was not applied, in order to validate the method. These samples were used as blank samples, and for the preparation of bromopropylate standards for matrix-matched calibration.

2.2. *Sample preparation*

The optimization of sample preparation is described in the next section. The optimized procedure applied for sample preparation was as follows: grape samples were blended in a Minipimer Braun blender. Blended sample portions (2×30 g) were used for each analysis. To each 30 g portion, 15 mL of acetone was added, as recommended in the literature due to several advantages including high effectiveness and low toxicity and cost [3, 7], and centrifugation was performed for 5 min at 2000 rpm. Afterwards, 15 mL of dichloromethane and 10 mL of diethylether were added, and centrifugation was performed again for 15 min at 2000 rpm. The two extracts (2×25 mL) were combined, filtered through filter paper, concentrated to dryness using a water bath at 60°C (under a fume hood), and redissolved in 5 mL *n*-pentane/toluene solution 90 : 10. One microlitre of the final extract was injected into the gas chromatograph.

2.3. *Optimization of sample preparation*

2.3.1. Extraction solvents and solvent mixtures. Experimental tests were performed for the optimization of the sample preparation step. Initially, liquid–liquid extraction with 25 mL of dichloromethane was performed, without the addition of diethylether. However, this resulted in a poorer layer separation than when a mixture of dichloromethane–diethylether was used. On the other hand, substitution of dichloromethane with diethylether only, because the latter is less toxic, unfortunately resulted in significantly lower chromatographic responses. The same was true with a dichloromethane–hexane mixture instead of dichloromethane–diethylether.

2.3.2. Sonication step. The addition of a sonication step before the centrifugation (sample placed for 5–20 min in an ultrasonic bath after the addition of acetone) did not lead to any improvements in the chromatographic results.

2.3.3. Centrifugation time. The effect of centrifugation time was tested for both centrifugation steps employed; the times tested ranged from 5 to 30 min. For the first centrifugation step, an increase in the centrifugation time did not have any effect on the responses, so a 5 min interval was selected to minimize sample preparation time. For the second centrifugation step, an increase in the responses and better layer separation were observed with a time increase from 5 to 15 min, while afterwards the results remained the same, and so an interval of 15 min was selected.

2.3.4. Final solvent. The final solvent used for the dissolution of the dried extract was also tested. Hexane, pentane, and toluene were used, resulting in similar responses, which were slightly enhanced by the use of the pentane/toluene mixture.

2.4. *Sample analysis*

The optimized analytical conditions for the GC-ECD and the GC-MSD methods are presented in tables 1 and 2, respectively. Other conditions studied included splitless injection or purge valve on at 0.5 min, as well as different oven-temperature programmes. The splitless injection and the purge valve on at 0.5 min resulted in a higher baseline and subsequently reduced sensitivity, while the different oven-temperature programmes resulted in a longer analysis time without enhancing the chromatographic responses.

3. Results and discussion

3.1. *Evaluation of the methods*

The analysis of standard solutions of bromopropylate in hexane and in spiked grape samples with both methods resulted in linear calibration curves ($R^2 > 0.99$ in all cases) (figure 2), and had a good repeatability and similar detection limits, comparable

Table 1. Instrumentation and analytical conditions of the GC-ECD method.

GC-ECD system	GC—HP5890 series II GC—^{63}Ni ECD
Column	Capillary DB-1, 10 m × 0.53 mm i.d. × 0.25 μm film thickness
Injection type	Split/splitless, purge valve on after 1 min
Injector temperature	200°C
Carrier gas (He) flow	1 mL min^{-1}
Oven-temperature programme	120°C (3 min); rate 5°C min^{-1} to 260°C (8 min)
Detector temperature	300°C

Table 2. Instrumentation and analytical conditions of the GC-MS method.

GC-MS system	GC—MS HP5890 series II GC—HP5971 MS
Column	Capillary DB5-MS, 30 m × 0.32 mm i.d. × 0.25 μm film thickness
Injection type	Split/splitless, purge valve on after 1 min
Injector temperature	200°C
Carrier gas (He) flow	1 mL min^{-1}
Oven-temperature programme	60°C (1 min); rate 30°C min^{-1} to 180°C; rate 1.5°C min^{-1} to 280°C
Solvent delay	15 min
Transfer-line temperature	280°C
Acquisition mode	SIM
EMV	2200
Ions monitored	341, 185, 157

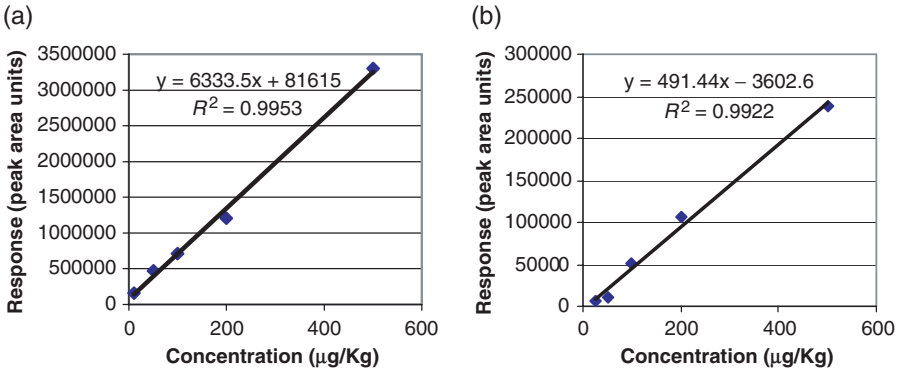

Figure 2. (a) GC-ECD and (b) GC-MSD calibration curves with spiked grape samples.

Table 3. Precision and accuracy data for the GC-ECD and GC-MSD methods.

Concentration spiked (μg/kg)	Concentration measured (μg/kg) ($N = 3$) 1	2	3	Average	Recovery %	RSD%	Detection limit (mg/kg)
GC-ECD method							
10	12.9	14.0	11.9	12.9	129.4	8.4	0.02
50	62.1	65.7	61.2	63.0	126.0	3.8	
100	99.8	106.5	99.2	101.8	101.8	4.0	
200	177.3	201.8	177.3	185.5	92.7	7.6	
500	507.8	508.6	471.3	495.9	99.2	4.3	
GC-MSD method							
25	12.8	10.8	10.5	11.4	45.6	11.1	0.03
50	24.4	28.6	27.0	26.7	53.4	8.0	
100	102.4	98.6	102.1	100.9	100.9	2.3	
200	218.1	195.4	203.8	205.8	102.9	5.6	
500	483.7	490.6	510.9	495.1	99.0	2.9	

with those reported in the literature using GC and HPLC [5, 8]. The bromopropylate standard solutions were stored in a refrigerator at 4°C. They are known to be stable at least for 3 months, and during the present study they have been used within a time interval of 20 days. Samples were spiked by adding appropriate volumes of bromopropylate standard solutions to the blended grape samples.

Recovery tests were performed for the two methods, by analysis of three different spiked grape samples at five concentration levels. The detection limits were determined by analyses of five replicates of a spiked sample at low concentration (0.025 mg kg^{-1} for the GC-ECD method and 0.05 mg kg^{-1} for the GC-MSD method), and were calculated as $3 \times SD$, where SD is the standard deviation of the five measurements. The results are presented in table 3. Recoveries higher than 100% were observed for the lowest concentrations tested with the ECD method, which indicate the presence of an interfering peak, the influence of which becomes more apparent with decreasing concentration of bromopropylate. On the contrary, with the GC-MSD method, in which by using the SIM mode only the particular mass fragments corresponding to bromopropylate are scanned, this phenomenon was not observed, and the recoveries decreased with decreasing concentrations, as is normally the case during the GC determination of most organic compounds.

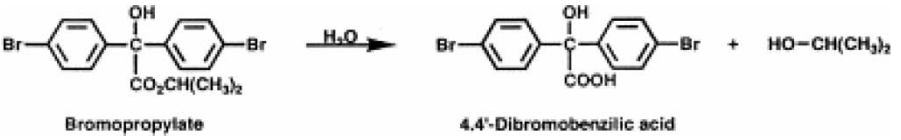

Figure 3. Degradation of bromopropylate (Source: Ref. [10]).

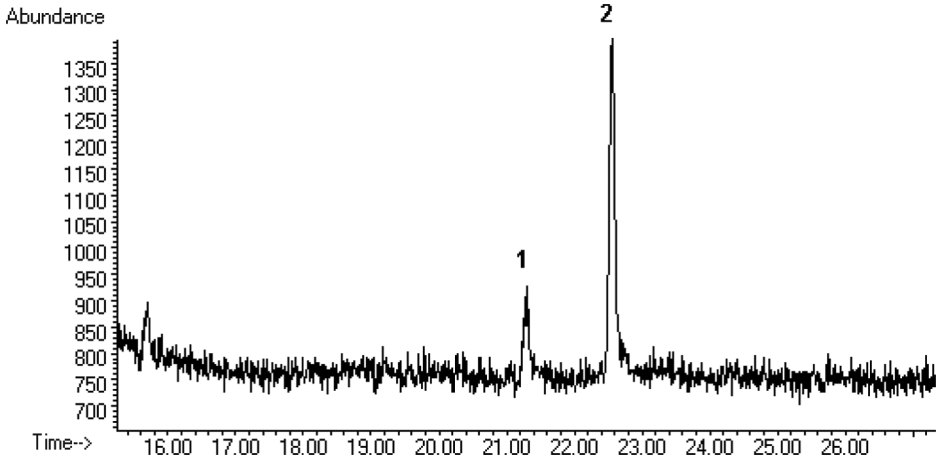

Figure 4. GC-MS chromatogram of a grape sample spiked with $0.05\,mg\,kg^{-1}$ bromopropylate. (1) 4,4′-dibromobenzilic acid (ions 340, 183, 157). (2) Bromopropylate (ions 341, 185, 157).

Degradation of bromopropylate due to ester hydrolysis producing acid and alcohol has been documented in water and in soil [10, 12], and the degradation product 4,4′-dibromobenzilic acid has been identified during previous research [10] (figure 3). The GC-MSD method described above was used to examine the presence of 4,4′-dibromobenzilic acid (ions 340, 183, 157) in the samples. The results confirmed the existence of the particular ions in the grape samples (figure 4) and explained the co-elution of the two peaks with the GC-ECD method, because of the very close retention times of the two peaks. The GC retention times reported by Corta *et al.* [10] were 13.77 and 13.98 min for 4,4′-dibromobenzilic acid and bromopropylate, respectively, using a column with dimensions $25\,m \times 0.32\,mm$. Since the column used in the present study was $10\,m \times 0.53\,mm$, the co-elution of bromopropylate and its metabolite is possible, although a lower rate of oven-temperature increase was applied. The use of a longer column or adoption of an additional cleanup step for the extract (C18 cartridges or gel permeation chromatography) [3] could overcome this problem, but since other matrix effects have been reported with the ECD as well [11], the application of the GC-MSD method seems to be more reliable in any case, if there is the opportunity.

3.2. *Analysis of grape samples*

The developed methods were applied to 10 grape samples collected from the areas Souliou and Zeygolatio of Korinthia, as described in section 2. The calibration was

performed in grape substrate, which was free of bromopropylate, as confirmed by analysis in both GC-ECD and GC-MSD methods.

During all measurements, the GC-MSD method gave significantly lower results (three times lower on average) than those of the GC-ECD method. According to the GC-ECD measurements, the concentrations of bromopropylate in the grape samples ranged from 0.06 to 0.2 mg kg^{-1}, which presents a 'false alarm' regarding the quality of the grape samples according to the 79/2002/EEC Directive, while according to the GC-MS measurements of the same samples, the concentrations ranged from not detectable (< 0.03 mg kg^{-1}) to 0.06 mg kg^{-1}, which indicates the marginal presence of the acaricide in two of the samples analysed (0.06 mg kg^{-1} for both samples).

The differences between the two methods probably occur due to co-elution of other substances, i.e. bromopropylate degradation products, which are determined along with the bromopropylate peak with the GC-ECD method. Therefore, the GC-MSD method seems to be the most reliable for the particular substrate, because of its high selectivity, and can be readily applied, without the need for any extract cleanup step. Unfortunately, no certified standard was available during the present study, to quantify 4,4-dibromobenzilic acid, the metabolite of bromopropylate which was present in the analysed samples. The fate of bromopropylate and metabolites in grape substrate is an issue of interest for further investigation.

4. Conclusions

Two gas chromatography methods (GC-ECD and GC-MSD) were developed for the determination of bromopropylate in grapes. Experimental tests were performed for the optimization of the sample preparation step, which was the same for both methods, and included liquid–liquid extraction with dichloromethane and diethylether, centrifugation, and evaporation. Evaluation of the methods using spiked grape samples and application to real samples showed that the GC-ECD method was problematic for the particular substrate, giving elevated results due to co-elution of other compounds, which, as shown by the GC-MSD method, could be degradation products of bromopropylate. In conclusion, the GC-MSD method proved to be the most reliable for the determination of bromopropylate in grapes. The fate of bromopropylate and its degradation products in grapes and in the environment is a subject of scientific interest for further investigation.

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