



ISSN: 0976-3031

Available Online at <http://www.recentscientific.com>

CODEN: IJRSFP (USA)

International Journal of Recent Scientific Research
Vol. 8, Issue, 9, pp. 20301-20307, September, 2017

**International Journal of
Recent Scientific
Research**

DOI: 10.24327/IJRSR

Research Article

DETERMINATION OF OXIDATION BYPRODUCTS OF CARBARYL IN HONEY BY LIQUID CHROMATOGRAPHY-MASS SPECTROMETRY

Prasanth, J¹., Vincy M. V^{2*} and Brilliant R³

¹Department of Environmental Sciences, Bharathiar University,
Coimbatore, Tamil Nadu

²Department of Zoology, St. Berchmans College, Changanacherry, Kerala

³Department of Environmental Sciences, St. John's College, Anchal, Kerala

DOI: <http://dx.doi.org/10.24327/ijrsr.2017.0809.0868>

ARTICLE INFO

Article History:

Received 16th June, 2017

Received in revised form 25th
July, 2017

Accepted 23rd August, 2017

Published online 28th September, 2017

Key Words:

Carbaryl; LC-MS/MS, degradation products, fragmentation pattern

ABSTRACT

Honey is a 'natural product' and EU regulates honey under the Council Directive 2001/110/EC. According to the European Union (EU); the food Codex Alimentarius and various other international honey standards - honey stipulates a pure product that does not allow for the addition of any other substance. Honey bees fly two to three miles from their hive to find sources of food (nectar and pollen). They will usually forage during daylight. Pollen and nectar are collected from certain flowering plants, carried back to the hive, stored in wax cells, and used as a protein-rich food source for the colony members. Bees may be killed while foraging on blooming plants that have been treated by certain pesticides. The greatest hazard, however, is from insecticides, such as carbaryl (Sevin), Methyl parathion (Pennacp-M) and others that may be unintentionally carried with pollen back to the hive. Some of these pesticides are not stable in the environment or metabolised to different forms and this is probably the main reason why they were detected rarely and at lower concentrations in beehive products. Therefore, identification and confirmation of metabolites of pesticides in honey matrix are also equally relevant. Mass spectrometry technique based on nominal mass is proposed for the screening and identification of metabolites while high resolution mass spectrometry can be effectively used for confirmation of identified metabolites. This study intended to design a workflow in order to identify and confirm major metabolites/degradation products of pesticides used/ significant in apiculture namely carbaryl in honey matrix. By Nominal mass and accurate mass LC-MS/MS, 3 degradation products of carbaryl is identified and their structural relationship with carbaryl is established by comparing the fragmentation pattern.

Copyright © Prasanth, J., Vincy M. V and Brilliant R, 2017, this is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution and reproduction in any medium, provided the original work is properly cited.

INTRODUCTION

Carbamate insecticides, such as carbaryl (1-naphthyl-N-methylcarbamate), are highly toxic, have a wide range of activity, and comprise a major portion of pesticides used in the agriculture industry. The ester bond between N-methyl carbamic acid and 1-naphthol is responsible for carbaryl toxicity (Soriana *et al.*, 2001). The increasing use of carbamate pesticides poses a risk to apiculture and human environment. Carbamate pesticides began to replace organochlorine such as DDT and organo phosphorous pesticides, due to their low environmental persistence and low toxic effects on mammals (Fernandez *et al.*, 2000; Petropoulou *et al.*, 2006). Carbamates are competitive inhibitors of neuronal nicotinic acetylcholine receptors and acetylcholinesterase (Zhang *et al.*, 2006; Smulders *et al.*, 2003). Thus, it is necessary to quantify

their residue amounts in foods to prevent harmful effects on animals, human and the environment (Abad *et al.*, 1999; Rawn *et al.*, 2006; Rawn *et al.*, 2006; Cabras *et al.*, 1992; Inthavong and Bordet, 2002).

Honey, a natural product of bees, is composed mainly of monosaccharides and oligosaccharides, totaling 77%, with glucose and fructose having average contents of 30% and 38%, respectively (Kujawski and Namiesnik, 2008). In many types of honeys, over 300 substances belonging to several chemical groups have been identified, such as phenolic acids, flavonoids and amino acids (Pyrzynska and Biesaga, 2009; Yao *et al.*, 2004; Hermosín *et al.*, 2003). Honey composition is highly influenced by the types of flowers used by the bees as well as regional and climatic conditions (Ajlouni and Sujirapinyokul, 2010). Quality of honey is directly related to its floral origin

*Corresponding author: Vincy M. V

Department of Zoology, St. Berchmans College, Changanacherry, Kerala

and the region of production (Bogdanov, 2006). Its quality control is performed to monitor residues (Luo *et al.*, 2004). Residues of pesticides have been found in apian products (Pirard *et al.*, 2007; Ravoet *et al.*, 2015); thus it is convenient to develop methods to evaluate their presence. Pesticides protect agricultural crops, but over use and in correct use can pose risks to human health and the environment (Caldas *et al.*, 2009; Zanella *et al.*, 2008). Even if small amounts of pesticide residues remain in the food supply, they constitute a potential risk for the human health because of their sub-acute and chronic toxicity (Mukherjee, 2009; Rissato *et al.*, 2006). According to the Directive 96/23/EC, it is necessary to research *N*-methylcarbamate pesticide residues in honey (Anonymous, 1996).

The application of MS in combination with chromatography [GC or liquid chromatography (LC)] has been well recognized as the “gold standard” for both quantification and semi quantitative screening of food contaminants, such as pesticides (Alder *et al.*, 2006). Although GC-MS continues to be used in the analyses of volatile, moderate to non-polar small molecules (e.g. PCBs, dioxins, other halogenated aromatic compounds and many pesticides), recent developments in both LC and MS have resulted in very powerful instrumentation for sensitive and selective determination of other more polar or ionic contaminants at trace levels in food (Malik *et al.*, 2010; O’Mahony *et al.*, 2013) including veterinary medicines (Le Bizec *et al.*, 2009; Bogialli and Di Corcia, 2009), pesticides (Fernandez-Alba *et al.*, 2008; Botitsi *et al.*, 2011), toxins (Suzuki and Quilliam, 2011; Capriotti *et al.*, 2012) and so-called “emerging contaminants” (Farre and Barcelo, 2013). Holcapek *et al.* (2012) recently reviewed developments in LC-MS over the past decade including a helpful overview of the different mass analyzers available, many of which have been applied to the analysis of food contaminants by LC-MS (Grimalt *et al.*, 2010; Kaufmann *et al.*, 2010; 2011).

Totti *et al.* (2006) developed a method based on matrix solid phase dispersion (MSPD) using C18 as dispersant and dichloromethane-methanol as eluent and liquid chromatography-atmospheric pressure chemical ionization-mass spectrometry (LC-APCI-MS) for the simultaneous determination of imidacloprid, 6-chloronicotinic acid, adicarb, adicarb sulfoxide and adicarb sulfone in honey bees. Screening with LC-MS-Q-Orbitrap or LC-MS-Q-ToF gives the analyst the possibility to get a fast overview of the status of samples. These systems allow obtaining accurate mass spectra and, in addition, MS/MS experiments as in triple quadrupole (QQQ) instruments. The most important difference to classical LC-MS/MS (QQQ) is that the number of analytes is not limited, in principle (Lippold *et al.*, 2016). In the present pilot study, a comprehensive residue screening of honey samples were performed in order to determine the forced degradation of carbaryl and its degradation products followed by analysis with nominal and accurate mass LC-MS/MS system.

MATERIALS

Chemicals and Reagents

Standard solution of carbaryl (1-Naphthyl-N-methyl carbamate) Pestanal analytical grade standard (CAS no. 63-25-2) was procured from Sigma Aldrich. Working standard solution of concentration 10 mg/l of carbaryl was prepared by

diluting the stock in Acetonitrile. Working standard solution is stored in the refrigerator at 4°C and has a stability of at least 3 months.

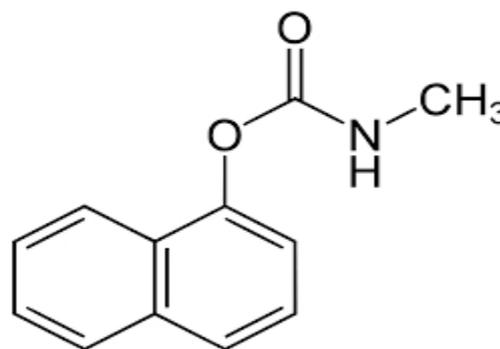


Fig 1 Structure of carbaryl

Ammonium hydroxide, 30% ACS reagent and 1M HCl, Ammonium acetate, reagent grade were purchased from Sigma Aldrich. 50% H₂O₂ solution was acquired from Vetec. LCMS grade Acetonitrile, Chromasolv was procured from Fluka.

Sample collection and preparation

Honey samples were collected from natural sources, teak and mango plantations of Wayanad district, Kerala, India. This sample is locally collected by the tribal community of Wayanad. The honey samples mainly consisted of Larger Bee Honey. Sample extraction and clean up were performed as proposed by Rissato *et al.* (2006) and Pittella (2009). Sample collected has light brown color and was very viscous in nature, but found no cane sugar or jaggery when kept for settling.

Forced degradation

Weighed about 1.0 g of honey sample into a 10 ml of volumetric flask and made up to the volume with deionized water. 1.0 ml of the made up solution was transferred to a 25 ml volumetric flask. The sample was spiked at 100 ppb level with 0.1 ml of 10 ppm carbaryl standard solution. 400 µl of 1N HCl and 400 µl of 50% H₂O₂ were added to moisten the sample. The flask was kept on benchtop for 4 hours in ice cold conditions. After 4 hours, the sample was neutralized with 400 µl of 5N ammonium hydroxide. It is then made up to the volume with Acetonitrile. A blank solution was also prepared by keeping all similar conditions without spiking with carbaryl. Sample was then filtered through a 0.45 µm syringe filter and transferred to 2 ml HPLC vial for injection to LCMS system.

Instrumentation

Chromatographic conditions

An Agilent 1290 infinity UHPLC system installed with a reverse phase C18 column (Ascentis express column of dimension 15 x 4.6 mm, 2.7 µm) procured from Suppelco was used for the separation of various components of the prepared sample. 10 mM ammonium acetate in deionized water was used as Mobile phase A and Acetonitrile as Mobile phase B. A gradient elution with a flow rate of 1 ml/minute was set up for the elution. Time 0 minutes to 7 minutes the mobile phase B ratio changes from 7% to 45%. At 15 minutes % B increased to 62%. From 17.5 minutes and 20 minutes the % B was kept constant at 85%. Composition changed to (90:10) at 25 minutes. In 30 minutes, the composition changed to the initial

composition. Column was kept at a constant temperature of 45°C. 10.0 µl was injected to the LCMS system for analysis.

Mass spectrometric parameters

Agilent triple quad 6470 LCMS system coupled to Agilent UHPLC 1290 infinity II was used for collecting the initial data. Triple quad system provided the nominal mass data. Fragmentation pattern of carbaryl was generated by optimizing the collision energy. A Fragmentor voltage of 100 V and collision energy of 12.0 V was used for generation of parent mass scan and product ion scan in 6470 QQQ LCMS system. Each major fragments of carbaryl was taken into account and performed precursor ion scan in order to identify the structurally related compounds or metabolites of carbaryl. Further analysis was carried out by high resolution accurate mass 6530 QTOF system from Agilent technologies. Both MS systems were operated in electrospray ionization mode with positive polarity. Mass range of TOF MS was kept between 100 Da to 1200 Da whereas the TOF MS/MS mass range was kept from 50 Da to 1200 Da. Three different collision energies were kept for the fragmentation in QTOF System. Simultaneous on the fly acquisition of both TOF MS and TOF MS/MS data are acquired by the Auto MS/MS option available at MassHunter acquisition software for QTOF system. MassHunter Qualitative software has the capability to provide both average fragmentation patterns of all three collision energies and with individual collision energy. Agilent High resolution mass spectrometry 6530 QTOF system was utilized in order to confirm the mass and possible elemental composition of identified masses corresponds to UV chromatogram. All the potential compounds were identified by the powerful data mining option "Find by Auto MS/MS" available with MassHunter Qualitative software.

RESULTS AND DISCUSSION

Identification of degradation products of carbaryl

Honeybees are known to be very sensitive to even a single exposure of carbamate (Akca et al., 2009; Hardstone and Scott, 2010). UV analysis at 254 nm was conducted and found many UV peaks other than the peak of the carbaryl. Initial scanning of masses in the sample was performed by Parent ion scan (MS2 scan) of the sample. Additional data was generated with the help of precursor ion scan and the neutral loss scan of the triple quad instrument and found structurally related compounds. Identification of the accurate masses of these compounds was conducted with the help of high resolution mass spectrometry.

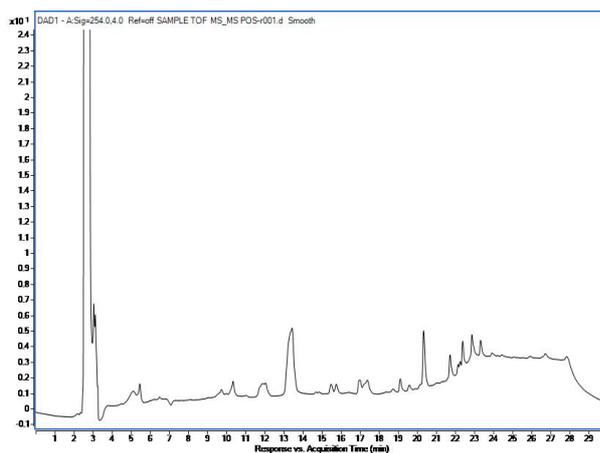


Fig 2 description is 'UV profile of honey sample having degraded compound, Carbaryl

The preliminary scan showed three major m/z other than carbaryl in ESI POS ionization mode. Carbaryl mass was accurately measured as m/z: 202.0864 with a mass accuracy of 0.99 ppm. The extracted ion chromatogram of carbaryl showed that the compound was eluting at 15.795 minutes. Fragmentation pattern of carbaryl was simultaneously generated by Auto MS/MS workflow (Fig. 2)

Based on accurate mass, isotopic pattern with its abundance and spacing, possible elemental composition was calculated with the help of Molecular Feature Correlator (MFE) feature of Agilent MassHunter Qualitative software. The predicted elemental composition is found to be the actual molecular formula of carbaryl with an overall score of 97.27 which provided confidence in the accuracy of mass analysis. (Fig.3, Fig.4).

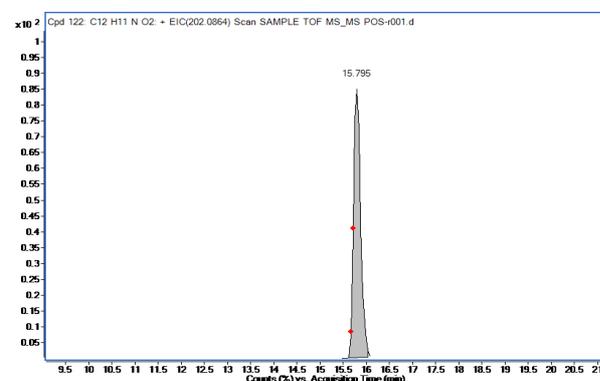


Fig 3 description is Extraction ion chromatogram of Carbaryl showing elution at 15.796 min

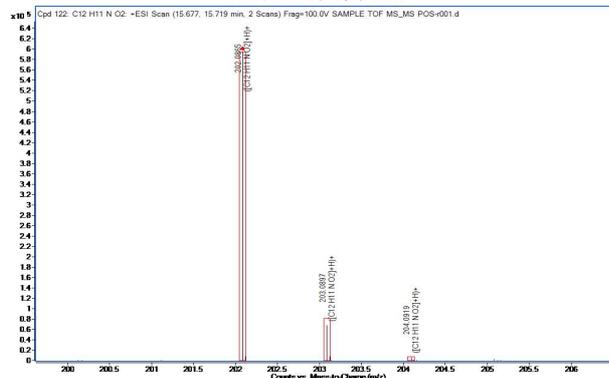
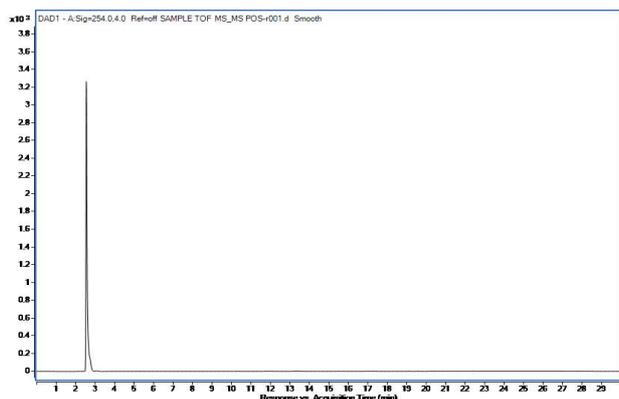


Fig 4 Isotopic distribution of carbaryl

Fragmentation pattern of carbaryl was generated at three different collision energies however, collision energy 12 found to be the optimum for carbaryl molecule. The major fragments of carbaryl were m/z: 145.0644, 127.0534, 117.0696, 115.0554 and 58.0294 at this collision energy (Fig 5).

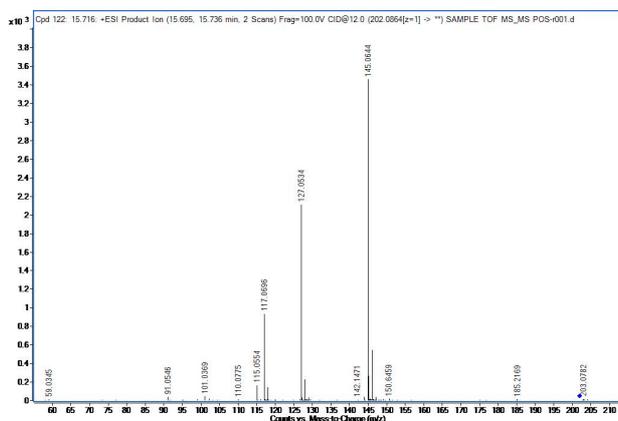


Fig 5 Fragmentation pattern of carbaryl

The possible formula and structures of parent molecule, carbaryl and its fragments were predicted with the help of Molecular structure correlator (MSC) feature of MassHunter software. Based on the accurate mass of both TOF MS and TOF MS/MS, MSC facilitate the data base search in various databases such as Chemspider, Pubchem, Metlin etc. MSC also correlate the experimental fragmentation pattern of carbaryl and the Insilco fragments generated from the mol file of carbaryl. The structure of fragments m/z: 145.0644, 127.0534, and 117.0696 predicted by MSC are given below (Fig 6).

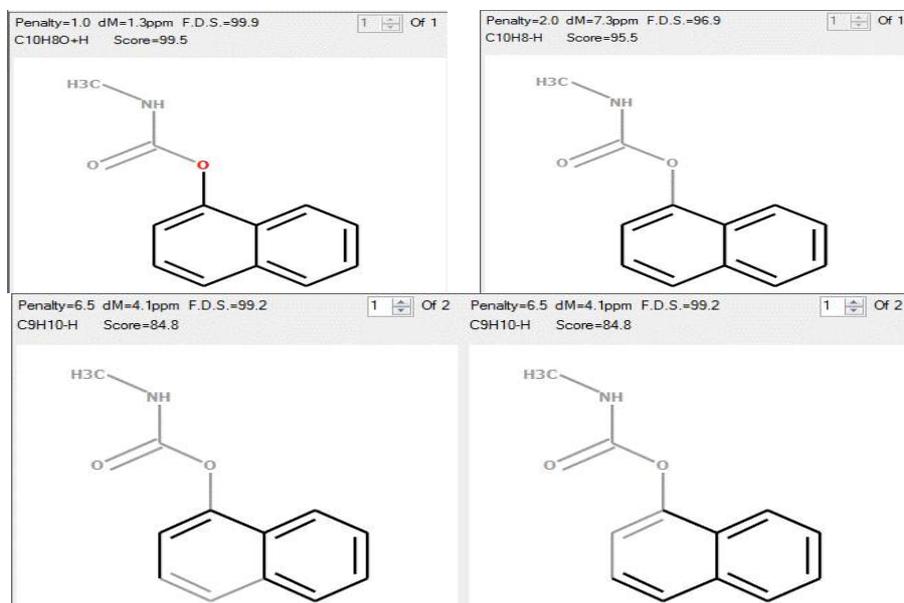


Fig 6 Structure of fragments predicted by MSC

Best	Formula	Score	Mass	Mass (MFG)	Diff (ppm)	Diff (abs. ppm)	Diff (mDa)	RT	RT Available (DB)	RT (DB)
•	C ₆ H ₈ O ₄	84.66	144.0422	144.0423	0.23	0.23	0.03	2.712		
Species	Ion Formula	m/z	Height	Score (MFG)	Score (MS)	Score (mass)	Score (iso. abund)	Score (iso. spacing)		
	(M+H) ⁺	C ₆ H ₉ O ₄	145.04...	199663.4	84.66	99.7	99.99	99.72	99.09	
m/z	m/z (Calc)	Diff (ppm)	Diff (mDa)	Height	Height (Calc)	Height %	Height % (Calc)	Height Sum %	Height Sum % (Calc)	
145.04...	145.0495	0.51	0.1	199663.4	198824.3	100	100	100	93.2	92.8
146.05...	146.053	0.58	0.1	12564.5	13411.3	6.3	6.7	5.9	6.3	

Fig 7 Details of C₆H₈O₄, its elemental composition and its mass accuracy

One structure each was predicted for first two fragments where as two possible structures were predicted for the rest of the fragment, m/z: 117.0696. The predicted formula of the fragment 145.0664 was C₁₀H₈O-H. The elemental composition C₁₀H₈-H was proposed for fragment m/z: 127.0534. For fragment m/z: 117.0696, the possible elemental composition is C₉H₁₀-H.

Degradation products

Preliminary scan revealed that there was m/z: 145.0, 163.0 and 104.1 as major masses other than the carbaryl mass. Accurate masses of the same m/z were found to be 145.0495, 163.0601 and 104.1072 measured by QTOF. The extracted ion chromatogram of all these three masses were showed that they are eluting between 2.5 minutes at 2.7 minutes. The elemental composition of C₆H₈O₄ was predicted for m/z: 145.0495 with an overall score of 84.66. The mass accuracy for this measurement was 0.23 ppm (Fig.7).

The red boxes in the mass spectrum represents in the theoretical isotopic pattern whereas the black line in the middle of this theoretical isotopic pattern represents the measured mass. Measured isotopic intensity and isotopic spacing are also found to be matching with the theoretical values (Fig.8, Fig.9).

A hydrolytic degradation mechanism has been proposed by Sogorb *et al.* (2002). According to this pathway, carbaryl reacts with tyrosine residues on rabbit serum albumin molecules to yield 1-naphthol and carbamylated rabbit serum albumin. Water molecules that attack the carbamylated complex, releasing carbamic acid and free enzymes, the latter of which was subjected to a new catalytic cycle.

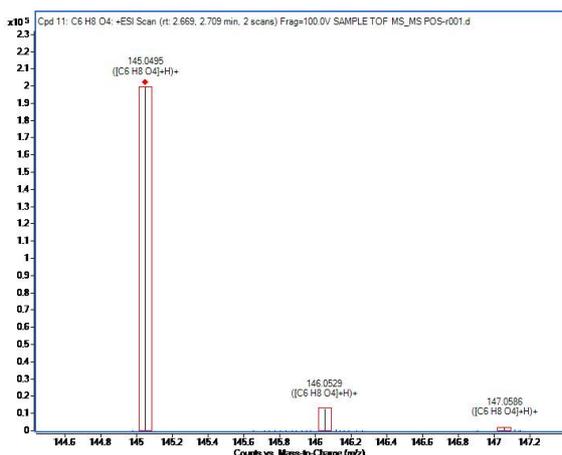


Fig 8 Isotopic pattern of $C_6H_8O_4$

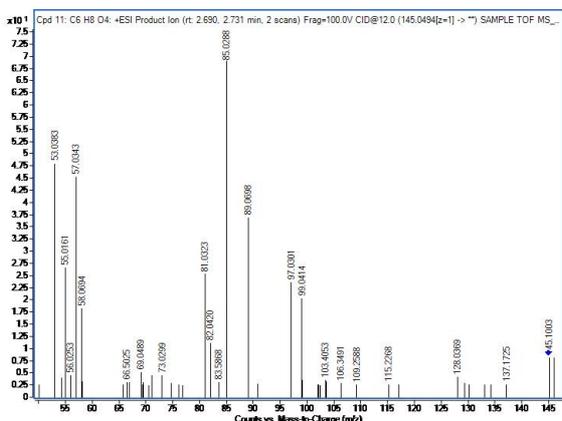


Fig 9 Fragmentation pattern of $C_6H_8O_4$

The elemental composition of $C_6H_{10}O_5$ was predicted for m/z: 163.0601 with an overall score of 91.72. The mass accuracy for this measurement was 0.13 ppm (Fig. 10, Fig. 11, Fig. 12).

Best	Formula	Score	Mass	Mass (MFG)	Diff (ppm)	Diff (abs. ppm)	Diff (mDa)	RT	ID Source	Score (MFG)	DBE
1	$C_6H_{10}O_5$	91.72	162.0528	162.0528	0.13	0.13	0.02	2.633	MFG	91.72	0
Species											
	Ion Formula	m/z	Score (MFG)	Score (MS)	Score (mass)	Height (%)	Height (Calc)	Height (%)	Height (Sum %)	Height (Sum % (Calc))	
	(M+H)	$C_6H_{11}O_5$	163.06	590419.6	91.72	99.47	100	98.18	99.95		
	m/z	m/z (Calc)	Diff (ppm)	Height (%)	Height (Calc)	Height (%)	Height (%) (Calc)	Height Sum %	Height Sum % (Calc)		
	163.06	163.0601	0.09	0	590419.6	582732.6	100	93.8	92.6		
	164.06	164.0635	0.02	0.1	33411.1	39863.2	5.7	6.8	5.3	6.3	
	165.06	165.0648	0.12	0	5697.8	7132.6	1	1.2	0.9	1.1	

Fig 10 Details of $C_6H_{10}O_5$, its elemental composition and its mass accuracy

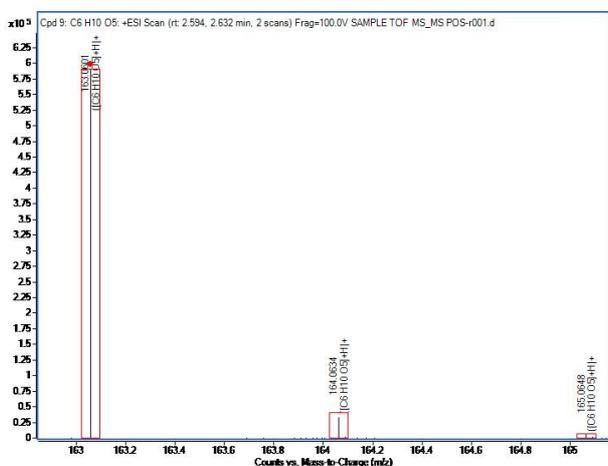


Fig 11 Isotopic pattern of $C_6H_{10}O_5$

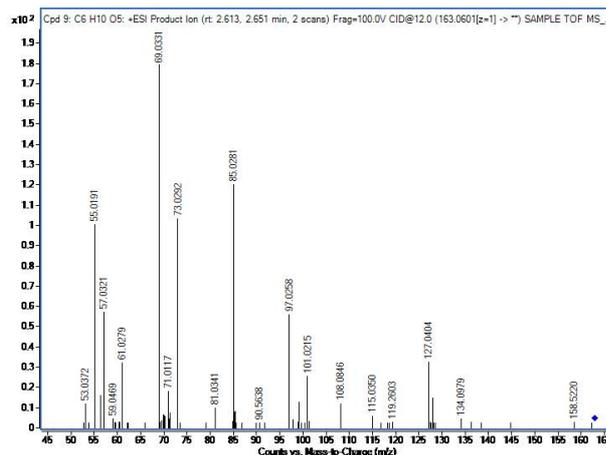


Fig 12 Fragmentation pattern of $C_6H_{10}O_5$

The elemental composition of $C_5H_{13}NO$ was predicted for mass 103.0999 with an overall score of 96.47. The mass accuracy for this measurement was 2.16 ppm. Isotopes were also shown good matching with the theoretical values (Fig.13, Fig.14, Fig.15).

Best	Formula	Score	Mass	Mass (MFG)	Diff (ppm)	Diff (abs. ppm)	Diff (mDa)	RT	ID Source	Score (MFG)	DBE
1	$C_5H_{13}NO$	96.47	103.0999	103.0997	-2.16	-2.16	-0.22	2.768	MFG	96.47	0
Species											
	Ion Formula	m/z	Score (MFG)	Score (MS)	Score (mass)	Height (%)	Height (Calc)	Height (%)	Height (Sum %)	Height (Sum % (Calc))	
	(M+H)	$C_5H_{14}NO$	104.10	310996.5	96.47	99.39	99.26	99.1	99.9		
	m/z	m/z (Calc)	Diff (ppm)	Height (%)	Height (Calc)	Height (%)	Height (%) (Calc)	Height Sum %	Height Sum % (Calc)		
	104.10	104.107	-2.15	-0.2	310996.9	308752.9	100	100	94.7	94.1	
	105.11	105.11	-2.57	-0.3	16097.7	18439.7	5.2	6	4.9	5.6	

Fig 13 Details of $C_5H_{13}NO$, its elemental composition and its mass accuracy

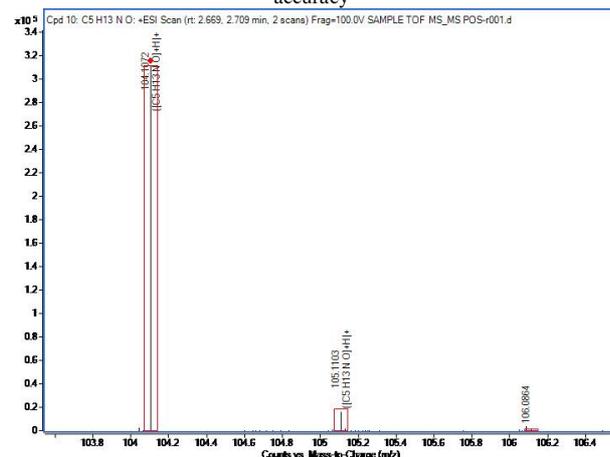


Fig 14 Isotopic pattern of $C_5H_{13}NO$

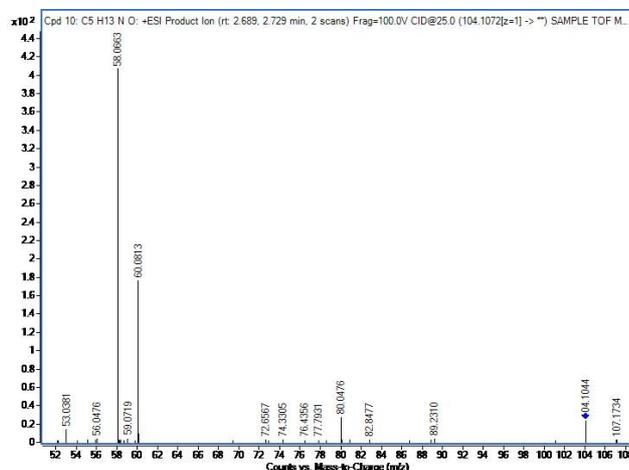


Fig 15 Fragmentation pattern of $C_5H_{13}NO$

It is observed from the fragmentation pattern of the three identified degradation products that some of the fragments of these degradation products were matching with fragments of carbaryl and hence they were structurally related compounds. For example, fragmentation pattern of 145.0495 included m/z:115, which was one of the fragments of carbaryl. Similar way, fragmentation pattern of 104.1072 contained 58.0 and fragmentation pattern of m/z: 163.0601 had 127.0 & 145.0 as fragments.

CONCLUSION

The increasing use of carbamate pesticides poses a risk to apiculture and human environment (Koc *et al.*, 2008). Carbaryl and its degradation products were studied by forced degradation followed by analysis with nominal and accurate mass LC-MS/MS system. Both organophosphorus and carbamate insecticides are known to inhibit cholinesterase, the enzyme that hydrolyses the neurotransmitter acetylcholine (Sultatos, 2005). Honeybees are known to be very sensitive to even a single exposure of carbamate (Akca *et al.*, 2009; Hardstone and Scott, 2010) and organophosphorus insecticides (Hardstone and Scott, 2010; Abrol and Andotra, 2003), which result in high mortality within 24 h after exposure. The LD₅₀ for topical exposure to dimethoate, chlorpyrifos, and carbaryl in honeybees were determined to be 22.4, 35.4, and 42.8 vg per bee (Abrol and Andotra, 2003), which corresponded to approximately 862, 1362, and 1646 µg kg⁻¹ in bee food, respectively. Possible elemental composition of masses identified were calculated by considering the accurate masses of parent masses and its fragments, isotopic pattern, intensity of isotopes and isotopic spacing. Structural relationship of carbaryl and its degraded products were established by comparing fragmentation patterns as they have common fragments.

References

- Abad, A., Moreno, M. J., Pelegri, R., Martinez, M. I., Saez, A., Gamon, M. and Montaya, A. (1999). Determination of carbaryl, carbofuran and methiocarb in cucumbers and strawberries by monoclonal enzyme immunoassays and high performance liquid chromatography with fluorescence detection. An analytical comparison. *J. Chromatogr. A*, 833(1): 3-12.
- Abrol, D. P. and Andotra, R. S. (2003). Relative toxicity of some insecticides to *Apis mellifera* L. *J. Asia Pac. Entomol.*, 6(2) 235-237.
- Ajlouni, S. and Sujirapinyokul, P. (2010). Hydroxymethylfurfuraldehyde and amylase contents in Australian honey. *Food Chem.*, 119(3): 1000–1005.
- Akca, I., Tuncer, C., Guler, A. and Saruhan, I. (2009). Residual toxicity of 8 different insecticides on honey bee (*Apis mellifera*, Hymenoptera: Apidae). *J. Anim. Vet. Adv.*, 8(3): 436–440.
- Alder, L., Greulich, K., Kempe, G. Vieth, B. (2006). Residue analysis of 500 high priority pesticides: better by GC-MS or LC-MS/MS? *Mass Spectrom. Rev.*, 25(6): 838–865.
- Anonymous. 1996. Council Directive 96/23/EC of 29 April 1996. On measures to monitor certain substances and residues thereof in live animals and animal products and repealing Directives 85/358/EEC and 86/469/EEC and Decisions 89/187/EEC and 91/664/EEC. No: L 125/11. 23.05.1996.
- Bogdanov, S. (2006). Contaminants of bee products, *Apidologie*, 37(1): 1-18.
- Bogialli, S. and Di Corcia, A. (2009). Recent applications of liquid chromatography-mass spectrometry to residue analysis of antimicrobials in food of animal origin. *Anal. Bioanal. Chem.*, 395(4): 947–966.
- Botitsi, H. V., Garbis, S. D., Economou, A. and Tsipi, D. F. (2011). Current mass spectrometry strategies for the analysis of pesticides and their metabolites in food and water matrices. *Mass Spectrom. Rev.*, 30(5): 907–939.
- Cabras, P., Tuberoso, C., Melis, M. and Martini, G. (1992). Multiresidue method for pesticide determination in wine by high-performance liquid chromatography. *J. Agric. Food Chem.*, 40(5): 817-819.
- Caldas, S. S., Demoliner, A. and Primel, E. G. (2009). Validation of a method using solid phase extraction and liquid chromatography for the determination of pesticide residues in groundwaters. *J. Braz. Chem. Soc.*, 20(1): 125–132.
- Capriotti, A. L., Caruso, G., Cavaliere, C., Foglia, P., Samperi, R. and Lagana, A. (2012). Multiclass mycotoxin analysis in food, environmental and biological matrices with chromatography/mass spectrometry. *Mass Spectrom. Rev.*, 31(6): 466-503.
- Farre, M. and Barcelo, D. (2013). Analysis of emerging contaminants in food. *Trends Anal. Chem.*, 43: 240–253.
- Fernandez-Alba, A. R. and Garcia-Reyes, J. F. (2008). Large-scale multi-residue methods for pesticides and their degradation products in food by advanced LC-MS. *Trends Anal. Chem.*, 27(11): 973-990.
- Fernandez, J. M., Vazquez, P. P. and Vidal, J.L.M. (2000). Analysis of *N*-methylcarbamate pesticides insecticides and some of their main metabolites in urine with liquid chromatography using diode array detection and electrospray mass spectrometry. *Anal. Chim. Acta.*, 412(1-2): 131-139.
- Grimalt, S., Sancho, J. V., Pozo, O. J. and Hernandez, F. (2010). Quantification, confirmation and screening capability of UHPLC coupled to triple quadrupole and hybrid quadrupole time-of-flight mass spectrometry in pesticide residue analysis. *J. Mass Spectrom.*, 45(4): 421-436.
- Hardstone, M. C. and Scott, J. G. (2010). Is *Apis mellifera* more sensitive to insecticides than other insects? *Pest Manag. Sci.*, 66(11): 1171-1180.
- Hermosin, I., Chicón, R. M. and Cabezudo, M. D. (2003). Free amino acid composition and botanical origin of honey. *Food Chem.* 83(2): 263-268.
- Holcapek, M., Jirasko, R. and Lisa, M. (2012). Recent developments in liquid chromatography-mass spectrometry and related techniques. *J. Chromatogr. A*, 1259: 3-15.
- Inthavong, D. and Bordet, F. (2002). Multiresidue analytical method for carbamate pesticides in meat products analysis by LC/DAD and by LC/ACPI/MS/MS. 4th E.P.R.W. May 28-31, Roma, Italy.
- Kaufmann, A., Butcher, P., Maden, K., Walker, S. and Widmer, M. (2010). Comprehensive comparison of liquid chromatography selectivity as provided by two types of liquid chromatography detectors (high resolution mass spectrometry and tandem mass spectrometry): “Where is the crossover point? *Anal. Chim. Acta*, 673(1): 60-72.

- Kaufmann, A., Butcher, P., Maden, K., Walker, S. and Widmer, M. (2011). Quantitative and confirmative performance of liquid chromatography coupled to high-resolution mass spectrometry compared to tandem mass spectrometry. *Rapid Commun. Mass Spectrom.*, 25(7): 979-992.
- Koc, F., Yigit, Y., Das, Y. K., Gurel, Y. and Yarali, C. (2008). Determination of aldicarb, propoxur, carbofuran, carbaryl and methiocarb residues in honey by HPLC with post-column derivatization and fluorescence detection after elution from a florisil column. *J Food Dru Anal.*, 16(3): 39-45.
- Kujawski, M. W. and Namiesnik, J. (2008). Challenges in preparing honey samples for chromatographic determination of contaminants and trace residues. *Trends Anal. Chem.*, 27(9): 785-793.
- Le Bizec, B., Pinel, G. and Antignac, J. P. (2009). Options for veterinary drug analysis using mass spectrometry. *J. Chromatogr. A*, 1216(46): 8016-8034.
- Lippold, R., Hardebusch, B., Bäuerle, F., Heizmann, J., Radykewicz, T., Scheibner, O. and Bromirski, M. (2016). Screening for pesticides residues in honey applying LC-Q-ToF MS and LC-Q-Orbitrap MS. <http://eprw2016.com/wp-content/uploads/2016/06/Ralf-Lippold1-honey.pdf>
- Luo, X. B. Chen, B. and Yao, S. Z. (2004). High-performance liquid chromatography with electrospray mass spectrometry for rapid and sensitive determination of sanguinarine and chelerythrine in exogenously contaminated honey. *Chromatographia*, 60(5): 347-351.
- O'Mahony, J., Clarke, L., Whelan, M., Kennedy, R. O., Lehotay, S. J. and Danaher, M. (2013). The use of ultra-high pressure liquid chromatography with tandem mass spectrometric detection in the analysis of agrochemical residues and mycotoxins in food – Challenges and applications. *J. Chromatogr. A*, 1292: 83-95.
- Malik, A. K., Blasco, C. and Pico, Y. (2010). Liquid chromatography-mass spectrometry in food safety, Liquid chromatography-mass spectrometry in food safety. *J. Chromatogr. A*, 1217(25): 4018-4040.
- Mukherjee, I. (2009). Determination of pesticide residues in honey samples. *Bull. Environ. Contam. Toxicol.*, 83: 818-821.
- Petropoulou, S. S. E., Gikas, E., Tsarbopoulos, A. and Siskos, P. A. (2006). Gas chromatographic-tandem mass spectrometric method for the quantitation of carbofuran, carbaryl and their main metabolites in applicators' urine. *J. Chromatogr. A* 1108(1): 99-110.
- Pirard, C., Widart, J., Nguyen, B. K., Deleuze, C., Heudt, L., Haubruge, E., De Pauw, E. and Focant, J. F. (2007). Development and validation of a multi-residue method for pesticide determination in honey using on-column liquid-liquid extraction and liquid chromatography-tandem mass spectrometry. *J. Chromatogr. A* 1152(1-2): 116-123.
- Pittella, C. M. (2009). Determinação de resíduos de agrotóxicos em mel de abelhas (*Apis* sp) por cromatografia gasosa acoplada à espectrometria de massas. Dissertation, Universidade Federal de Minas Gerais.
- Pyrzyska, K. and Biesaga, M. (2009). Analysis of phenolic acids and flavonoids in honey. *Trends Anal. Chem.*, 28(7): 893-902.
- Ravoet, J., Reybroeck, W. and de Graaf, D. C. (2015). Pesticides for apicultural and/or agricultural application found in Belgian honey bee wax combs. *Bull Environ Contam Toxicol.*, 84: 543-548.
- Rawn, D. F. K., Roscoe, V., Krakalovich, T. and Hanson, C. (2006). N-methyl carbamate concentrations and dietary intake estimates for apple and grape juices available on the retail market in Canada. *Food Addit. Contam.*, 21(6): 553-563.
- Rawn, D. F. K., Roscoe, V., Trelka, Y., Hanson, C., Krakalovich, T. and Dabeka, R. W. (2006). N-methyl carbamate pesticide residues in conventional and organic infant foods available on the Canadian retail market, 2001-03. *Food Addit. Contam.*, 23(7): 651-659.
- Rissato, S. R., Galhiane, M. S., Knoll, F. R. N., deAndrade, R. M. B. and deAlmeida, M. V. (2006). Multiresidue method for monitoring environmental contamination by pesticides in the Bauru region (SP) using honey as bioindicator. *Quim. Nova.*, 29(5): 950-955.
- Soriana, J. M., Jimenez, B. and Molto, J.C. (2001). Analysis of carbamate pesticides and their metabolites in water by solid phase extraction and liquid chromatography: a review. *Crit. Rev. Anal. Chem.*, 31(1): 19-52.
- Smulders, C. J., Bueters, T. J., Van Kleef, R. G. and Vijverberg, H. P. (2003). Selective effects of carbamate pesticides on rat neuronal nicotinic acetylcholine receptors and rat brain acetylcholinesterase. *Toxicol. Appl. Pharmacol.*, 193(2): 139-146.
- Sogorb, M. A., Carrera, V., Benabent, M. and Vilanova, E. (2002). Rabbit serum albumin hydrolyzes the carbamate carbaryl. *Chem. Res. Toxicol.*, 15(4): 520-526.
- Sultatos, L. G. (2005). Interactions of organophosphorus and carbamate compounds with cholinesterases. In: R.C. Gupta, (ed.) Toxicology of organophosphate & carbamate compounds, Academic, Burlington, pp. 209-218.
- Suzuki, T. and Quilliam, M. A. (2011). LC-MS/MS analysis of diarrhetic shellfish poisoning (DSP) toxins, okadaic acid and dinophysistoxin analogues, and other lipophilic toxins. *Anal. Sci.*, 27(5): 571-584.
- Totti, S., Fernandez, M., Ghini, S., Pico, Y., Fini, F., Manes, J. and Girotti, S. (2006). Application of matrix solid phase dispersion to the determination of imidacloprid, carbaryl, aldicarb, and their main metabolites in honeybees by liquid chromatography-mass spectrometry detection. *Talanta*, 69(3): 724-729.
- Yao, L., Jiang, Y., D'Arcy, B., Singanusong, R., Datta, N., Caffin, N. and Raymont, K. (2004). Quantitative high-performance liquid chromatography analyses of flavonoids in Australian Eucalyptus honeys. *J. Agric. Food Chem.*, 52(2): 210-214.
- Zanella, R., Primel, E. G., Gonc-alves, F. F., Martins, M. L., Adaime, M. B., Marchesan, E. and Machado, S. L O. (2008). Study of the degradation of the herbicide clomazone in distilled and in irrigated rice field waters using HPLC-DAD and GC-MS. *J. Braz. Chem. Soc.*, 19(5): 987-995.
- Zhang, J. and Lee, H. K. (2006). Application of liquid phase microextraction and on-column derivatization combined with gas chromatography-mass spectrometry to the determination of carbamate pesticides. *J. Chromatogr. A*, 1117(1): 31-37.