Bull Environ Contam Toxicol (2009) 82:133–136
DOI 10.1007/s00128-008-9555-x

Facile and Rapid Determination of Contamination in Sulphur Pesticide Formulations by Liquid Chromatography–Tandem Mass Spectrometry

George Panos Balayiannis · Helen Anagnostopoulos · Irene Kellidou

Received: 11 March 2008 / Accepted: 27 August 2008 / Published online: 17 September 2008

Abstract Up to 7% of pesticide products in the European market are illegal, counterfeit or of poor quality. As a result, regulatory authorities are required to determine a wide variety of impurities in many different pesticide formulations. A simple, rapid and exceptionally reliable LC-MS-MS method for the determination of carbendazim residual quantities in sulphur formulations, used in organic agriculture, has been developed and validated. Linearity of response was established ($R^2 = 0.9997$) over a wide range of concentrations (0.01–2 μg/mL). Recovery ranged from 94% to 101%. LOD and LOQ were 0.003 μg/mL and 0.005 μg/mL, respectively, and lack of interference was confirmed.

Keywords Pesticides · Impurities · Organic agriculture · LC-MS-MS

A significant amount (5–7%) of all pesticide products circulated in the European market is either of low quality, counterfeit or illegally traded (ECPA 2006a, b; 2007). The issue is becoming more and more important as the number of incidents is increasing rapidly and becoming a threat for crop protection, consumer health and the environment. Important economic and reputation damage is also caused to farmers as well as the crop protection industry. In the past 3 years, regulatory authorities are more and more often required to confirm quality problems concerning such products.

In Greece, the problem is growing and according to the latest estimations as much as 20% of the market is now dominated by illegal or counterfeit pesticides, particularly in areas of the country near the borders (Naftemporiki 2007).

The Laboratory of Chemical Control of Pesticides of the Benaki Phytopathological Institute is the official regulatory authority for the quality control of pesticides in the Greek market. In the past few years the number of complaints for pesticide formulations that have caused damage to cultivations or have proved ineffective has risen sharply. These cases were investigated and in the majority of them it was confirmed that the formulations were either of low quality or counterfeit, containing reduced amounts of active ingredients, unauthorized active substances and/or other harmful impurities (Benaki Phytopathological Institute, Laboratory of Chemical Control of Pesticides, unpublished results 2002–2007).

In 2005, greenhouse organic cucumbers that had been treated exclusively with sulphur were found to contain residues of carbendazim. Investigation indicated that the source of contamination originated from the sulphur formulation. The complexity of the matrix, the basicity of the benzimidazole moiety of carbendazim and the increased reactivity of sulphur were a major problem and a challenge for the analysis of the samples. A new sensitive and exceptionally specific method was developed. It is extremely rapid (5 min total analysis time), simple (no use of buffers, acidic or basic solutions) and very accurate over a wide range of concentrations (0.01–2 μg/mL).

Materials and Methods

Standard carbendazim (99.7% w/w) was kindly supplied by Bayer CropScience (Monheim am Rhein, Germany). Blank (i.e. carbendazim-free) sulphur formulation was supplied

G. P. Balayiannis (✉) · H. Anagnostopoulos · I. Kellidou
Laboratory of Chemical Control of Pesticides,
Benaki Phytopathological Institute, Stefanou Delta 8,
151 46 Kifissia, Greece
e-mail: G.Balayiannis@bpi.gr

from the producing company. Solvents were LabScan LC-MS grade (Gliwice, Poland). Nylon syringe filters (0.45 and 0.2 μm) were Macherey-Nagel (Düren, Germany).

A standard stock solution of 10 μg/mL in MeOH was prepared since carbendazim has very low solubility in all organic solvents.

Standard solutions of 0.01, 0.05, 0.1, 0.25, 0.5, 1, and 2 μg/mL were prepared separately, by appropriate dilution of the standard stock solution, in order to establish the linearity of the MS–MS analyzer.

Spiked samples of 0.15 and 1.15 μg/mL nominal concentrations were also prepared from the blank sample.

For sample preparation, 20 g of sulphur formulation were dissolved in 100 mL of MeOH followed by sonication (10 min) and centrifugation (10 min). The supernatant was then filtered through 0.45 and then 0.2 μm nylon syringe filters and analysed.

Sulphur samples, blanks and spiked samples were prepared for analysis in exactly the same way except from the blank sample which was concentrated to half volume by evaporation of the solvent under a gentle stream of nitrogen.

The high-performance liquid chromatograph used was Varian (Walnut Creek, CA, USA), composed of two pumps (Prostar 210), an autosampler (Prostar 420) with a 5 μL sample loop, a column oven (Prostar 510), and a Polaris 3 μm C18-A (50 mm × 2.0 mm i.d., 5 μm particle size) reversed-phase column. A Dell Core 2 Duo personal computer using Varian MSWS software (Version 6.8) was used for acquisition and treatment of data.

The mobile phase was methanol: water 30:70 isocratic, at a flow-rate of 0.2 mL min^{-1}. Each run lasted 5 min. All chromatographic solvents were on-line degassed with a vacuum degasser (Varian Prostar 590). The mass spectrometer used was a Varian 1200 L triple quadrupole MS–MS system fitted with an electrospray ionization (ESI) interface. The ESI interface was operated in the positive ion detection mode. MS–MS experiments were carried out with an argon pressure of approximately 1.0 mTorr in the collision cell.

Calibration of the mass analyser was performed by infusion (20 μL min^{-1}) of 1 μg/mL standard carbendazim. Initially the molecular ion of carbendazim (MH^+:192 mass-to charge ratio, m/z) was monitored. Capillary voltage was optimized to achieve maximum abundance and stability. Next, m/z range from 50 to 192 was scanned for the major product ions. The most abundant and characteristic ion (160 m/z) was chosen for quantitation and two other ions (132 and 105 m/z) were selected for confirmation, excluding any product ions common with other pesticides. Collision energy values for each product ion were optimized (14, 28 and 36 keV for 160, 132 and 105 m/z, respectively). Findings were confirmed by literature (Pizzutti et al. 2007).

ESI interface conditions were: capillary voltage, 5000 V in positive ion (PI) mode; nebulizing gas (N_2) pressure, 50 psi, drying gas (N_2) pressure and temperature, 20 psi and 300°C, respectively; electron multiplier voltage, 1300 V.

During analysis Q1 was set to 192 m/z (MH^+) and Q3 was monitoring 160, 132 and 105 m/z (dwell times of 0.2, 0.3 and 0.5 sec, respectively).

Results and Discussion

Determination of residual quantities of chemical impurities in pesticide formulations presents special difficulties and cannot be resolved by means of trivial residue analysis. A sensitive detector is required in order to trace down the residual amounts of the contaminants. However, the active ingredient (a.i.) of a pesticide formulation (which is present at very high concentration) may cause significant interference or even contamination of the detector.

As mentioned before the majority of cases of low quality pesticide formulations involve either reduced amounts of a.i., or contamination with other active substances and/or harmful impurities.

The usual source of “contamination” with another active ingredient is the line of production and/or packaging (shift from one formulation to another without sufficient cleaning). Limits for this type of cross contamination are clearly defined by US law (EPA 1996), but not yet in EU legislation. However, it is now very important that formulations used in organic crop protection (e.g. sulphur, copper, etc.) do not contain any detectable quantities of non-allowed pesticide. In any other case there is always the possibility of contaminating the organic product, causing a chain of events that will end up to the financial compensation of the organic farmer by the pesticide producer.

Quantitation of carbendazim residues in sulphur proved to be an especially difficult task. In the present study, the official CIPAC method for carbendazim formulations (CIPAC 1998) was applied. Under the acidic conditions of the sample preparation, carbendazim being a weak base gave a side reaction, resulting to the disappearance of its peak in sample chromatograms. Consequently, neutral conditions had to be used throughout sample preparation and analysis. Sample preparation involving simple dilution with MeOH, filtration and analysis using HPLC-DAD (column: Phenomenex Luna, C-18, 250 × 4.6, 5 μm, eluent: 50:50 MeOH/water, Brito et al. 2002; Michel and Buszewski 2004) gave results that could not be acceptable

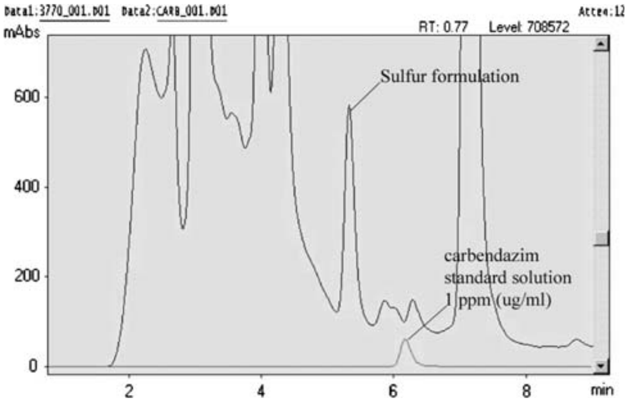

Fig. 1 HPLC-DAD chromatogram of sulphur formulation and carbendazim standard solution (1 μg/mL)

without any further confirmation, due to peak overlapping (Fig. 1).

The solution to the problem was the LC-MS-MS analyzer. Simple LC–MS analysis does not present any advantage compared with other LC detectors (UV, DAD etc.), since in complex matrices (such as sulphur) the molecular ion ($M + H^+$) is often lost in background noise. However, tandem MS has the ability to resolve even co-eluting peaks, by breaking up molecular ions and tracking down specific product ions for each compound. Taking advantage of this, the developed method has excellent linearity of response ($y = 1.08E + 7x - 5.04E + 4$, $r^2 = 0.999705$) over a wide range of concentrations (0.01, 0.05, 0.1, 0.25, 0.5, 1, and 2 μg/mL, Fig. 2).

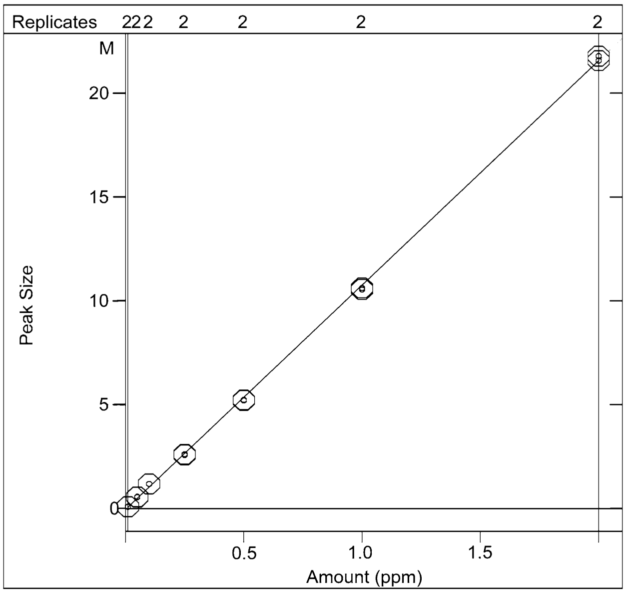

Fig. 2 Linearity of the method over a wide range of concentrations (0.01, 0.05, 0.1, 0.25, 0.5, 1, and 2 μg/mL). $R^2 = 0.999705$, $y = 1.08e + 7x - 5.04e + 4$

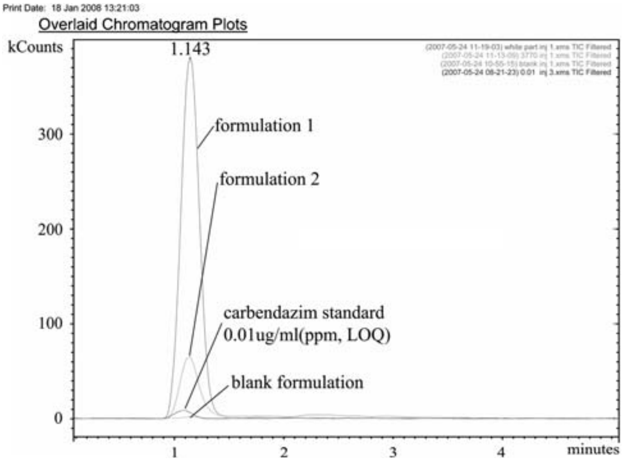

Fig. 3 Representative chromatograms of two formulations, carbendazim standard solution (0.01 μg/mL) and chromatogram blank

Recoveries ranged from 94% to 101% (mean 97.9%, RSD% 5.1, n = 5) for spiked concentrations of 0.15 and 1.15 μg/mL. Lack of interference was confirmed by injection of a blank sample (Fig. 3). The limit of detection (0.003 μg/mL) and the limit of quantification (0.005 μg/mL) were determined from the concentrations of carbendazim that gave a signal-to-noise ratio of 3 and 10, respectively.

In conclusion, the determination of very low concentrations of any unwanted residual substances in pesticide formulations is a task that can be dealt only with the use of sophisticated methods of analysis. In the case of thermolabile and non-volatile compounds, as carbendazim, LC-MS-MS is the method of choice, since it overcomes the selectivity and interference problems of LC-MS and HPLC-DAD methods. It can also be concluded that as far as pesticide formulations used in organic agriculture are concerned, separate production lines need to be used, otherwise the risk of contamination by other active ingredients is very high.

References

Brito NM, Navickiene S, Polese L, Jardim EFG, Abakerli RB, Ribeiro ML (2002) Determination of pesticide residues in coconut water by liquid–liquid extraction and gas chromatography with electron-capture plus thermionic specific detection and solid-phase extraction and high performance liquid chromatography with ultraviolet detection. J Chromatogr A 957:201–209. doi: 10.1016/S0021-9673(02)00351-5

CIPAC (1998) Handbook Vol. H analysis of technical and formulated pesticides. In: Dobrat W, Martijn A (eds) Black Bear Press Ltd, King's Hedges Road, Cambridge, UK

ECPA (2006) news release, 26 April 2006 http://www.ecpa.be/website/page.asp?mi=1&news=15310, reached on 30th January 2008

ECPA (2006) news release, 17 October 2006, http://www.ecpa.be/website/page.asp?mi=1&news=15722

ECPA (2007) news release, 29 March 2007, http://www.ecpa.be/website/page.asp?mi=1&news=16139, reached on 30th January 2008

EPA (1996) Pesticide registration-PR-notice 96-8/31.10.1996

Michel M, Buszewski B (2004) Optimization of a matrix solid-phase dispersion method for the determination analysis of carbendazim residue in plant material. J Chromatogr B 800:309–314

Naftemporiki (2007) Issue of 27-9-2007 p 10

Pizzutti IR, de Kok A, Zanella R, Adaimea MB, Hiemstra M, Wickert C, Prestes OD (2007) Method validation for the analysis of 169 pesticides in soya grain, without clean up, by liquid chromatography–tandem mass spectrometry using positive and negative electrospray ionization. J Chromatogr A 1142(2):123–136