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Analytical Methods

LC/ESI–MS/MS method for the identification and quantification of spinosad residues in olive oils

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ABSTRACT

This paper reports the use of liquid chromatography/electrospray ionisation tandem mass spectrometry (LC/ESI-MS/MS) method for the identification and quantification of residues of the natural insect control agent Spinosad in olive oils. The method determines the active ingredients Spinosyns A and D and two minor metabolites Spinosyns B and K without laborious sample treatment. All four analytes are determined simultaneously in a single injection using positive electrospray ionisation LC–MS with multiple reaction monitoring (MRM). For the quantitative analysis of samples an external calibration curve was built. The calibration curves for each analyte were linear in the concentration range 20–500 ng/mL with a correlation coefficient ranging between 0.995 and 0.999. Results from spike and recovery experiments at levels of 100 and 200 ng/mL gave mean recoveries ranging from 87–116% with satisfactory precision (relative standard deviation (RSD) from 1–8%). The excellent selectivity and sensitivity allows quantification and identification of low levels of Spinosad in olive oils (limits of quantification (LOQs) 0.004–0.073).

1. Introduction

Modern conventional agricultural production depends heavily on the use of pesticides and their residues can persist to the harvest stage, making possible the contamination of the final product (Benincasa et al., 2006; Benincasa et al., 2008; Caravita et al., 2007). Environmental safety, human health and rural development are the main goals of European Community Agricultural Policy. The quality and security of foodstuffs including the absence of chemical residues is the aim of Italian Agricultural Policy (Iannotta, Belfiore, Monardo et al., 2007). Both European Union and the Codex Alimentarius Commission of the Food and Agriculture Organization of the United Nations have established maximum pesticide residue limits (MRLs). Organic farming, regulated by the EEC Regulation No. 2092/91 and its updates recently reformulated, is an agricultural management system based on a variety of farming practices aimed at promoting sustainable agricultural development (Iannotta, Belfiore, Noce, Scalercio, & Vizzarri, 2007). The key-point is to minimise pesticide inputs in agro-ecosystems with increasing selectivity of target pests favouring pest control strategies aimed at safeguarding the environment. Organic farming regulation allows exclusively the use of plant defence products listed in annex II B previously authorised by the Member State, according to the Council Directive (Dir 91/414/EEC).

In order to control Tephritid fruit flies several active substances listed in annex II B have been evaluated (Iannotta, Belfiore, Brandmayer, Noce, & Scalercio, 2007; Mayoral, Benuzzi, & Ladurner, 2006; Mazor & Erez, 2004). Many substances showing insecticidal properties in conjunction with selective bait attractants have been tested and utilised for Tephritid pest control (Braham, Pasqualini, & Ncira, 2007; Jang, Holler, Moses, Salvato, & Fraser, 2007; Montiel Bueno & Jones, 2002). Among them Spinosad has been applied to over 200 different crops in order to control many pests (Bret, Larson, Schoonover, Sparks, & Thompson, 1997; Thompson, Dutton, & Sparks, 2000) and, in particular, many fruit flies belonging to the Tephritid family (Burns, Harris, Moreno, & Eger, 2001; Vargas, Miller, & Prokopy, 2002; Wang et al., 2005). It has also been recently utilised in olive groves in order to perform *Bactrocera oleae* control (Collier & Van Steenwyk, 2003; Rice, 2000).

Spinosad is an aerobic fermentation product of the naturally occurring soil bacterium actinomycete, *Saccharopolyspora spinosa* (Mertz & Yao, 1990) whose mode of action is associated with the excitation of the insect nervous system by uniquely altering the function of nicotinic and GABA-gated ion channels (Salgado, 1998). Spinosyns are the active ingredients in an insect control agent produced by fermentation of *S. spinosa*. They are macrolides consisting of a 21-carbon, tetracyclic lactone, to which are attached two deoxysugars: tri-*O*-methylated rhamnose and forosamine.

The most active components of the Spinosyn family of compounds are Spinosyns A and D; other congeners have different levels of methylation and are significantly less active (Madduri et al., 2001). Analytical methods were needed to determine the

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magnitude of residues in the fruit and processed commodities treated with Spinosad. Residue methods have been previously reported for Spinosad in cottonseed and processed cottonseed commodities, in soil, sediment, water, leafy vegetables, peppers, tomatoes, meat, milk, cream, and eggs (West & Turner, 1998). Thus, the following work is presented for the identification and quantification of Spinosyns A, D, B and K in olive oils by using liquid chromatography/electrospray ionisation tandem mass spectrometry (LC/ESI–MS/MS) method.

2. Materials and methods

2.1. Chemicals

Certified standard of Spinosad (purity 90.4%) was obtained from Dow AgroSciences LLC (Indianapolis); acetonitrile and formic acid were of LC/MS grade and were supplied from Sigma–Aldrich (Riedel-de Haën, Laborchemikalien, Seelze); aqueous solution were prepared using ultrapure water, with a resistivity of 18.2 MΩ cm, obtained from a Milli-Q plus system (Millipore, Bedford, MA, USA).

2.2. Treatments performed on olive plants

Applications of Spintor Fly (GF-130) (1 L/5 L of water) were carried out weekly from the end of August until the end of October during the year crop 2008 by utilising a MeterJet™ spray gun (Spraying Systems Co.®, Wheaton, Illinois), connected to a manual backpack sprayer. This spray gun delivers a precisely metered volume of spray at low pressure, with a nozzle producing drops with a diameter of millimetres. The solution was applied on the southern side of the tree canopy, on a spot area of 40 cm. On each tree 25 mL of the solution were sprayed in order to apply 0.24 g/ha of Spinosad. In order to evaluate the field persistence of Spinosad, olive samples (1 kg) were randomly collected immediately after the field application of the product on treated trees, after 2 days and then after 1 week. A control sample was collected from untreated trees. Drupes of treated samples were picked up only from the sprayed spot area. The olive oil samples utilised for recovery tests and to build the calibration curve were free from pesticide.

2.3. Olive sampling and oil extraction

The olive samples came from a farm placed in Terranova da Sibari (CS) (39°39′0″N 16°20′0″E; altitude: 313 m above sea level; area: 43.06 Km^2; population: 5262; density: 122,20 inhabitants per Km^2), located in the southern Italian region Calabria. Due to its excellent geographical position, between the famous mountains of Pollino and the Ionian Sea, Terranova da Sibari is sheltered and has a warm climate. Annual rainfall is not less than 80 mm and average air humidity of 65%.

Olive oils were obtained from handpicked olives of Cassanese cultivar from the olive trees where applications of Spintor Fly (GF-130) were performed as previously described. Olives were collected at the end of September (immediately after the field application of the product on treated trees, after 2 days and after 1 week). The oil was obtained by crushing 1 kg of olives using Oliomio 50 hammer mill (Toscana Enologica Mori, Val di Pesa (FI)). The oil was separated by centrifugation after 20 min of malaxation.

2.4. Preparation of standard solutions

A standard stock solution was prepared dissolving Spinosad reference material in acetonitrile. Aliquots of this solution were further diluted with water/acetonitrile/0.1% formic acid (70:30) to obtain calibration standards at concentrations range between 20–500 ng/mL. Fortification standard solutions for the determination of the recovery were prepared directly from the stock standard solution.

2.5. Preparation of matrix-matched standards and extraction of samples

Fortified olive oil recovery samples were prepared by adding 200 μL of the appropriate fortification standard solution into 2 g of olive oil. The sample was shaken for a minute to dissolve the residue in the oil. Acetonitrile (10 mL) was then added to the oil and centrifuged at 4000 rpm for 20 min. Before remixing of the oil with the solvent occured, a portion of the upper layer was taken and properly diluted with water/acetonitrile/0.1% formic acid (70:30) in order to obtain fortification standards at concentrations of 15, 30, 50, 100, 300 and 500 ng/mL.

3. Instrumentation

3.1. Mass spectrometry

Sample analyses were performed using a MSD Sciex Applied Biosystem API 4000 Q-Trap mass spectrometer. The LC–MS was operated in the positive ion mode using multiple reaction monitoring (MRM) of the transition m/z 718.1 → m/z 128.1 (Spinosyns B); m/z 718.1 → m/z 142.2 (Spinosyns K); m/z 732.1→ m/z 142.2 (Spinosyn A) and m/z 746.2 → m/z 142.2 (Spinosyn D). The experimental conditions were as follow: ionspray voltage (IS) 5500 V; curtain gas 30 psi; temperature 500 °C; ion source gas(1) 40 psi; ion source gas(2) 50 psi; collision gas thickness (CAD) medium; entrance potential (EP) 7 V. Declustering potential and collision energy were optimised for each transition monitored.

3.2. High performance liquid chromatography (HPLC)

HPLC was performed using an Agilent Technologies 1200 series liquid chromatography system equipped with G1379B degasser, G1312A pump, and G1329A autosampler. The analytes were separated on a Polaris C18-A HPLC column [3 μm particle size, 50 mm length and 2 mm i.d. (Varian Inc., USA)] at a flow rate of 300 μL/min and an injection volume of 10 μL. The elution program was as follows: at the start 90% solvent A (0,1% aqueous formic acid) and 10% solvent B (acetonitrile); the percentage of solvent B was linearly increased to 100% in 4.0 min, hold for 1 min and ramped to original composition in 2 min. The total elution time was 10 min per injection.

4. Method validation

For recovery experiments two different volumes of standard solution were added to 2 g of olive oil in a centrifuge bottle and extracted as previously described in the experimental section in order to obtain fortification standards at concentrations of 100 and 200 ng/mL. The extraction was performed three times per each fortification level. The sample data was processed by the external standard technique using matrix-matched calibration. The limit of quantification (LOQ) was defined as the amount equivalent to ten times the method noise, which included the instrument noise and background signal contributed by the matrix blank. Calibration graphs of the peak area of the MS/MS transitions selected for quantification versus theoretical pesticide concentration were constructed using a least-squares linear regression analysis. In order to evaluate possible matrix effects, slopes of the calibration graphs obtained in each case were compared with those obtained with the standards in solvent.