DEVELOPMENT AND VALIDATION OF A NEW METHOD FOR THE SIMULTANEOUS DETERMINATION OF SPINETORAM J AND L IN HONEY FROM DIFFERENT BOTANICAL ORIGINS EMPLOYING SOLID-PHASE EXTRACTION WITH A POLYMERIC SORBENT AND LIQUID CHROMATOGRAPHY COUPLED TO QUADRUPOLE TIME-OF-FLIGHT MASS SPECTROMETRY

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Abstract

The objective of this study was to propose a novel method to determine residues of the bio-insecticide spinetoram, which is a mixture of two components (spinetoram J and L), in honey from multifloral, rosemary and heather botanical origins; liquid chromatography coupled to quadrupole time-of-flight mass spectrometry was the technique employed. An efficient sample treatment (recoveries between 82% and 95%) involving a solid-phase extraction with a polymeric sorbent has been recommended, and no matrix effect was observed. Chromatographic analysis (4 min) was performed in reverse phase mode by using a fused-core column (Kinetex® EVO C_{18}) with acetonitrile and ammonium formate as the mobile phase components, which was applied in isocratic elution mode. Method was validated according to the current European legislation. Not only was it selective, but it also displayed a wide linear range, good precision (relative standard deviation values lower than 9%) and sensitivity (low limits of detection (spinetoram J, 0.1-0.3 μg/kg; spinetoram L, 0.1-0.2 μg/kg) and quantification (spinetoram J, 0.3-1.2 μg/kg; spinetoram L, 0.4-0.7 μg/kg)). Several honey samples were analyzed with this method and no spinetoram residues were found above the limits of detection.

Keywords: Food analysis; Honey; LC-Q-TOF/MS; Method validation; Polymeric sorbents; Spinetoram; Insecticides; Solid-phase extraction.

1. Introduction

Honey is a natural product of great value on account of its nutritional properties and therapeutic applications, and the increase in its consumption over the last few years (Ares, Valverde, Bernal, Toribio, Nozal, & Bernal, 2017; Campone et al., 2019; Seraglio et al., 2019; Valverde, Ibáñez, Bernal, Nozal, Hernández, & Bernal, 2018) has made it one of the most widely consumed bee products. However, during this period residues of contaminants, such as antibiotics and insecticides, have been detected in honey samples from different countries (Ares et al., 2017). Spinetoram is a semi-synthetic bio-insecticide that is a mixture of two components, 3'-O-ethyl-5, 6-dihydro-spinosyn J (major component) and 3'-O-ethyl spinosyn L (minor component), the common names of which are spinetoram J and L, respectively (see Supplementary Material, Figure 1S; Park et al., 2012; Rumbos, Dutton, & Athanassiou, 2018; Zhang, Li, & Lamusi, 2019a), and it is predominantly employed to control the insect orders Lepidoptera, Diptera, and Thysanoptera (Zhang et al., 2019b). As a derivative of a compound of natural origin, spinetoram implies less environmental risk than many systemic and/or synthetic insecticides (DeAmicis et al., 2011), but the consumption of foods, like honey, containing spinetoram can still have an adverse effect on the consumer's health (Zhao et al., 2015). It must be remarked that no study has so far been published to quantify spinetoram in honey or other bee products, although an ion mobility-based method has been recently published in which the screening detection limits of 280 pesticides in several food matrices, including spinetoram J, L and honey, were determined. (Bauer, Kuballa, Rohn, Jantzen, & Luetjohann, 2018; see Supplementary Material, Table 1S). However, sample treatment (quick, easy, cheap, effective, rugged and safe; QuEChERS), separation and detection conditions were not specifically optimized for any pesticide or food matrix, as it was employed an official

method for determining pesticides in vegetables. Nevertheless, spinetoram is currently registered for several major and specialty crops in many countries around the world, as for example, it has shown promising results as an alternative to traditional grain protectants (Andrić, Kljajić, Pražić Golić, Trdan, Bohinc, & Bodroža Solarov, 2019; Rumbos et al., 2018). It has also been evaluated as a substitute for other insecticides, like neonicotinoids, to control resistant thrips (D´Ambrosio, Huseth, & Kennedy, 2018). Subsequently, several methods have been proposed to determine spinetoram in other food matrices, mainly grains and vegetables (Fu et al., 2017; Hengel, 2011; Liu et al., 2011; Kamel et al., 2010; Ko et al., 2016; Kim, Yang, Jin, Yu, Youn, & Lim, 2017; Malhat, 2013; Malhat & Abdallah, 2019; Park et al., 2012; Quian, Wu, Lü, & Li, 2014; Vassilakos, Athanassiou, & Tsiropoulos, 2015a, 2015b; Zhang et al., 2019c; Zhao et al., 2015; see Supplementary Material, Table 1S). A solvent extraction with dichloromethane (Liu et al., 2011), acetonitrile (Hengel, 2011) or a mixture of acetonitrile and triethylamine (Kamel et al., 2010), followed by a solid-phase extraction (SPE) with C_{18} and florisil (Kamel et al., 2010), polymeric (Hengel, 2011) or NH_2 cartridges (Liu et al., 2011) was often employed. However, sample preparation methods based on QuEChERS were mainly proposed (see Supplementary Material, Table 1S). Extraction with acetonitrile and a dispersive SPE clean-up step were used in all cases, although the amount and number of reagents required in each specific case differed. Solvent extraction with an evaporation or clean-up step, and micro-liquid-liquid extraction (MLLE) were also employed in some of the above-mentioned studies. As can be also seen in Table 1S (see Supplementary Material), liquid chromatography (LC) in reverse phase mode using columns with a C_{18} stationary phase was the technique of choice for determining spinetoram in food matrices; meanwhile, mass spectrometry

(MS), and especially tandem mass spectrometers (MS/MS), has been predominantly selected for the detection of spinetoram (see Supplementary Material, Table 1S).

Thus, we sought to develop a specific analytical methodology, by means of liquid chromatography coupled to quadrupole time-of-flight mass spectrometry (LC-Q-TOF/MS), to determine simultaneously spinetoram J and L in honeys from multifloral, rosemary and heather botanical origins (multifloral, rosemary and heather). We have optimized the sample treatment with the aim of providing good recoveries, minimizing the potential matrix effect in all cases. and avoiding potential stability problems observed during sample extraction when applying a multi-residue approach (Grimalt, & Dehouck, 2016). To the best of our knowledge, this is the first study in which spinetoram J and L has been specifically determined in this matrix. In addition, we decided to test honey samples from different botanical origins in order to select the most appropriate sample treatment in each case, as their different chemical composition may strongly affect the determination of both compounds. Further aims of the study focused on validating the proposed method, as well as analyzing different honey samples from various Spanish regions.

2. Materials and methods

2.1. Reagents and materials

Spinetoram (Det. Purity 95%; 80% spinetoram J, 20% spinetoram L) was purchased from Dr. Ehrenstorfer GmBH (Augsburg, Germany). Methanol and acetonitrile (LC grade) were obtained from Panreac (Barcelona, Spain). Ammonium hydroxide, ammonium formate, and formic acid were supplied by Sigma Aldrich Chemie Gbmh (Steinheim, Germany). An ultrasonic bath (J.P. Selecta S.A., Barcelona, Spain), Strata®

C18-E (3 mL with 500 mg of sorbent) and Strata® X (3 mL with 600 mg of sorbent) SPE cartridges (Phenomenex, Torrance, CA, USA), a BRAND® Transferpette® S pipette (500-5000 μL; BRAND GMBH + CO KG, Wertheim, Germany), and a 10-port Visiprep vacuum manifold (Supelco, Bellefonte, PA, USA), were used for the extractions. Nylon syringe filters (17 mm, 0.45 μm) were purchased from Nalgene (Rochester, NY, USA), and ultrapure water was obtained using Milipore Mili-RO plus and Mili-Q systems (Bedford, MA, USA).

2.2. Standards

Standard (matrix-free) stock solutions of spinetoram J and L were prepared by dissolving different amounts of the spinetoram standard accurately weighed (12.5 mg for spinetoram J; 50 mg for spinetoram L) in 10 mL of acetonitrile (≈ 1000 mg/L). These solutions were further diluted with acetonitrile in order to prepare the intermediate and calibration matrix-free standards. It must be specified that as both compounds are present in different amounts in the standard (see Subsection 2.1), different solutions were prepared in order to obtain ones with the same concentrations for both compounds. Honey samples (5 g multifloral and rosemary; 3 g heather), in which the absence of spinetoram had been previously confirmed by means of LC-Q-TOF/MS (blank samples), were spiked with variable amounts of spinetoram before (BF samples) or after (AF samples) sample treatment to prepare the matrix-matched standards. The spiking of the BF samples was done similarly to Jovanov et al. (2014) in order to assure that the analytes were bound to the honey matrix. Briefly, representative portions of the blank honeys were weighed and transferred to a crystallizer where they were homogeneously spiked with the working standard solutions. The mixtures were then stirred with a glass rod to assist the homogenization and left to equilibrate

overnight prior to further analysis. Meanwhile, AF samples were prepared by spiking blank honey samples, which were previously treated with the proposed sample treatment, with working standard solutions that were added to the elution solvent. These matrix-matched standards were used for validation (spiked samples (low, medium, high) and calibration curves), and sample treatment studies. It must be specified that three replicates for each botanical origin, which were injected three times, were prepared for all the above-mentioned studies. Each spiked sample was prepared with 5 g (rosemary and multifloral) or 3 g (heather) of blank honey samples spiked with three different concentrations of spinetoram J and L within the linear range. These were as follows: low-LOQ (see Table 1); medium- 40 µg/kg; high- 160 µg/kg (rosemary and multifloral), 533 µg/kg (heather). All solutions were stored in glass containers in darkness at -20ºC (stock) or 4ºC (working and matrix-matched), and they remained stable for over two weeks

2.3. Sample procurement and treatment

2.3.1. Samples

Eighteen honey samples from different regions of Spain, in which spinetoram treatment had been employed on certain crops, were kindly donated by the Center for Agroenvironmetal and Apicultural Investigation-CIAPA (Marchamalo, Guadalajara, Spain). They were selected according to their different botanical origin, different color (light honey: multifloral and rosemary; dark honey: heather) and composition. Their botanical origin was confirmed by melissopalynological analysis, and corresponded to: rosemary, *Rosmarinus officinalis* (six samples); multifloral (six samples); and heather, *Erica spp* (six samples). In order to homogenize each of these samples, they were individually stirred with a glass rod, and subsequently stored in different tubes in

darkness at 4ºC until analysis. In this study, three replicates (sub-samples) of each honey sample, which were injected in triplicate, were examined for determining potential residues of spinetoram J and L.

2.3.2. Sample treatment

Briefly, 5 g (rosemary and multifloral) or 3 g (heather) homogenized honey sample was diluted in 10 mL of water, and the resulting solution was loaded onto a Strata® X SPE cartridge (previously conditioned with 5 mL of methanol and 5 mL of water) at about 1 mL/min by means of vacuum. The SPE cartridge was then washed with 10 mL of a mixture of acetonitrile and water (10:90, v/v); the rinse was discarded, and after 5 min of drying time the analytes were eluted with 2 mL (rosemary and multifloral) and 4 mL (heather) of acetonitrile, which were measured with a calibrated pippete, the resulting solution being passed through a syringe filter. Figure 1 outlines the steps of the procedure used during the present study.

2.4. LC-Q-TOF/MS system

An Acquity™ LC system (ACQUITY, Waters, Milford, MA, USA) and a Q-TOF/MS spectrometer (maXis impact, Bruker Daltonik, Bremen, Germany) were coupled through an electrospray (ESI) interface, which was operated in the positive mode ionization mode.

2.4.1. LC conditions

A Kinetex® EVO fused-core type column (C_{18}, 50 × 2.1 mm, 2.6 µm, 100 Å) was employed for LC analysis, and this was protected by a Kinetex® EVO C_{18} guard column. Both were acquired from Phenomenex (Torrance, CA, USA). Mobile phase was composed of ammonium formate 10 mM in water (pH 6.4) and acetonitrile (35:65, v/v) applied at a

flow rate of 0.5 mL/min in isocratic elution mode; meanwhile, injection volume and column temperature were set at 5 μL and 25ºC, respectively (see Figure 1).

2.4.2. Q-TOF/MS conditions

The optimal conditions were set as follows after several experiments (flow injection analysis in infusion mode, 80 μL/min) were conducted: capillary voltage, 4000 V; drying gas (nitrogen) flow, 10 L/min; drying gas (nitrogen) temperature, 250ºC; nebulizer pressure, 4 bars. Spectra were acquired in a mass range of mass/charge (*m/z*) 80–1000. The *m/z* scale of the mass spectra was calibrated daily by infusing a sodium formate and sodium acetate mixture. Compounds showed intense $[M+H]^+$ (precursor ions) on their full-scan spectra, which were selected as precursors to obtain product ions for Q-TOF/MS analyses, which were carried out by using an isolation width of 10 *m/z* and a collision energy of 45 eV.

3. Results and discussion

3.1. Optimization of the sample treatment

We decided to test the suitability of SPE as sample treatment for several reasons: i) strength of the scientific literature (Hengel, 2011; Liu et al., 2011; Kamel et al., 2010; Valverde et al., 2018); ii) SPE reduces matrix interferences in complex samples like honey; iii) SPE complies with important principles of green analytical chemistry (Gałuszka, Migaszewski, & Namieśnik, 2013). Optimization was carried out for the most relevant parameters/steps of the SPE procedure by analyzing spiked blank honey samples of the three different botanical origins in all cases (see Subsection 2.2). As a result our experience (Ares et al., 2017; Sánchez-Hernández, Hernández-Domínguez, Martín, Nozal, Higes, & Bernal, 2016; Valverde et al., 2018) and previous publications (Hengel, 2011; Kamel et al., 2010), we felt it appropriate to select the optimal SPE sorbent by considering the suitability

of polymeric (Strata® X) and C_{18} (Strata® C_{18}-E) sorbents to extract spinetoram J and L from honey samples. Moreover, on the basis of several preliminary experiments, it was observed that Strata® X sorbent provided higher recovery values (75%-85%) than the Strata® C_{18}-E (60%-73%; see Supplementary Material, Table 2S), which could be tentatively explained by an incomplete elution of the analytes when using the Strata® C_{18}-E cartridges, and also fewer matrix compounds were observed in the chromatograms. This determined our choice of this sorbent to optimize spinetoram extraction. We then decided on the amount of honey (1-10 g) and the volume of water (5-15 mL) to dissolve it, which was selected based on previous studies (Ares et al., 2017). These tests were performed by injecting blank and BF samples from the three different botanical origins spiked at low concentration level (LOQ; see Table 1), and it was used an ultrasound to facilitate dissolution of the honey. An analysis of the results (data not shown) allowed us to deduce that, for light honey samples (multifloral and rosemary), 5.0 g of honey and 10 mL of water were the optimal values, as they provided the highest signal to noise (S/N) ratio for maximum sensitivity; meanwhile, it was observed that in the case of dark honey samples (heather), amounts in excess of 3 grams dissolved in 10 mL offered the best S/N values. This difference in the amount of honey selected for dark honeys could be explained by the different composition (more complex) in relation to light honeys, as honey composition is closely related to its color (Kaczmarek, Muzolf-Panek, Tomaszwewska-Gras, & Konieczny, 2019). Prior to the diluted sample being loaded onto the SPE cartridges, 5 mL of methanol and water applied sequentially were used to precondition the cartridge. As honey is a complex matrix, we studied the possibility of applying a washing solution capable of eluting major components of the matrix possibly retained in the cartridge, as these can later interfere in chromatographic analysis. Several water and acetonitrile mixtures (90:10, 80:20, 70:30, v/v) and varying volumes (5-15 mL) were tested for

optimizing this SPE step. A study was also made of the possible loss of analytes in this phase by collecting and analyzing the washing liquid by LC-Q-TOF/MS. A 10 mL of a 90:10 (v/v) water and acetonitrile mixture was selected as good recovery rates (> 80%; see Supplementary Material, Figures 2S and 3S) and fewer matrix interferences were observed. Although the highest recoveries values were always obtained when using 5 mL of the selected mixture (see Supplementary Material, Figure 3S), we chose to select 10 mL, in spite of slightly lower recoveries, as this volume provided for better sample clean-up and subsequently cleaner chromatograms. Optimal drying times for the cartridges were also assayed (5-15 min), and no significant differences in the recoveries and chromatograms were observed for times above 5 min (data not shown). Acetonitrile was selected as the elution solvent since it is the most commonly used compound most used for extracting spinetoram from food matrices (see Supplementary Material, Table 1S). Different amounts of acetonitrile (2, 4 and 6 mL) to elute the analytes from the cartridges were tested with the aim of selecting the optimal volume, and it was observed that when analyzing multifloral and rosemary honey samples 2 mL was sufficient to obtain recovery values of over 80% for both compounds, as greater volumes did not significantly affect extraction efficiency (see Supplementary Material, Table 3S); in the case of heather honeys, however, it was necessary to increase the amount of acetonitrile (4 mL) to determine spinetoram J and L (see Supplementary Material, Table 3S). Meanwhile, for heightened concentration of the samples, we considered the possibility of removing some of the elution solvent by using a nitrogen stream at 25ºC; yet, as a significant loss of the analytes (≈ 10%; data not shown) was observed in all cases, we decided not to concentrate the sample.

The overall performance of the proposed sample treatment has demonstrated that it can be considered efficient and relatively rapid. Good recovery values were obtained at all times (82%-95%; see Subsection 3.3.6 and Table 2); the matrix had no significant effect on ionization of the compounds studied for the different types of honey analyzed (responses between 84% and 94%; see Subsection 3.3.3 and Table 2); and the overall procedure time was shorter than 30 minutes. This sample treatment is also environment-friendly in terms of organic solvent consumption (≤ 10 mL), the absence of an evaporation phase, and the number of steps and reagents required. In addition, it is also important to remark that this procedure was optimized for both components of spinetoram, not only for spinetoram J (major component), as it was done in some of the previous studies. Despite good recovery values obtained with SPE procedures for other food matrices (see Supplementary Material, Table 1S), extra steps/instrumentation (including a previous solvent extraction and evaporation in all cases) and larger amounts of reagents were required, and it was also observed a significant matrix effect. Regarding other sample treatments employed for determining spinetoram in food samples, including the screening approach in honey (see Supplementary Material, Table 1S), QuEChERS methods have predominated, and, as in the case of SPE, good extraction efficiencies were achieved, but more steps/instrumentation, reagents and volumes of organic solvents were employed in most of these procedures. However, unlike SPE procedures, the matrix effect was not significant in certain QuEChERS-based studies. The absence of matrix effect was also observed when using MLLE (Malhat & Abdallah, 2019), which can be considered as an efficient alternative to SPE and QuEChERS. Finally, it must be also commented that it was not possible to perform a comparison with the previous study in honey (Bauer et al., 2018) due to the absence of precision, recovery and matrix effect results.

3.2. LC-Q-TOF/MS optimization

3.2.1. LC

Preliminary experiments were conducted by using two columns that provided good results when analyzing pesticides in honey (Gemini® C_{18}, 50 x 2.0 mm, 3 µm, 110 Å, Ares et al., 2017; Kinetex® EVO C_{18}, 50 × 2.1 mm, 2.6 µm, 100 Å, Valverde et al., 2018), the mobile phase being a mixture of acetonitrile and ammonium formate (10 mM) in water, which was selected after a review of the related literature (see Introduction). It must be mentioned that matrix-free and matrix-matched standards at medium concentration level (40 µg/kg; see Subsection 2.2) and for the three different honey botanical origins were analyzed. The Kinetex® EVO column was selected as it provided overall better chromatography with a shorter run time (data not shown). Experiments were conducted in which matrix-free and matrix-matched standards were injected with diverse mobile phases composed of acetonitrile and ammonium formate (10 mM) in water, with variable flow rates (0.3-0.7, mL/min), ammonium formate concentrations (1-20 mM) and pH (3.0-7.5), temperature (25ºC-45ºC) and injection volume (2-10 µL). The aim was to elute spinetoram J and L rapidly whilst preventing co-elution between them and with matrix components, and at the same time obtaining the best chromatographic peak shape. The pH of the mobile phase had a significant impact on the retention of both compounds, which could be related with their physicochemical characteristics, such as pKa (see Supplementary Material, Figure 1S). This finding was to be expected, as it is well known that for separating ionic or ionizable compounds, such as spinetoram J and L, variations in the mobile phase pH may easily lead to dramatic variations in selectivity (Schoenmakers, van Molle, Hayes, & Uunk, 1991), depending on the physico-chemical properties of the analytes. Several tests were performed by modifying the pH of the original 10 mM ammonium formate

(6.4) by adding formic acid (0.1%, v/v) or ammonium hydroxide to achieve pH values ranging between 3.0 and 7.5. It was observed that by increasing the pH of the ammonium formate to 7.5 the analytes were retained unnecessarily; meanwhile, at pH lower than 5.0 it was not possible to obtain sufficient separation between spinetoram J and L. We decided, then, to use the original 10 mM ammonium formate (pH 6.4), as this would provide the best overall chromatographic performance. Under optimal chromatographic conditions (see Subsection 2.4), both compounds eluted in less than 2 minutes (see Figure 2; see Supplementary Material, Figures 4S and 5S) with an overall run time of 4 minutes; this, according to the existing literature, is the fastest proposal to date for spinetoram analysis in food (see Supplementary Material, Table 1S).

3.2.2. Q-TOF/MS

ESI in positive mode was selected to perform the experiments, both on the strength of preliminary tests and reports in the existing literature (see Supplementary Material, Table 1S). To establish optimal conditions, several experiments (flow injection analysis) were conducted for choosing the most appropriate parameters and achieving maximum sensitivity; these involved analyzing matrix-free and matrix matched standards. Final settings are summarized in Subsection 2.4.2. Spinetoram J and L showed an intense $[M+H]^+$ (precursor ions) on their full-scan spectra (*m/z* 748.5173-spinetoram J; 760.5145-spinetoram L; see Figure 3; see Supplementary Material, Figure 6S), which were used by generating extracted ion chromatograms (EIC) for each compound to obtain the maximum sensitivity for quantitative analysis. Moreover, significant fragments (product ions) obtained from the precursor ions for each spinetoram (142.1246 and 203.1346; see Figure 3; see Supplementary Material, Figure 6S) were selected for Q-TOF/MS analyses to confirm their presence in honey. These ions were commonly employed in previous studies (see Supplementary Material, Table 1S).

3.3. Method validation

Validation of the method was based on the Eurachem Guidelines (EURACHEM, 2014), the current European legislation for pesticide residue analysis in foods (European Commission Directorate-General for Health and Food Safety, 2017). The spiking procedure and the number of replicates and injections for each study have been described in Subsection 2.2.

3.3.1. Selectivity

To evaluate the selectivity of the proposed method, a set of unspiked blank honey samples from each botanical origin were injected and the results were compared with those obtained for spiked samples. No chromatographic interference was observed at analytes retention times in any case (see Figure 2; see Supplementary Material, Figures 4S and 5S). In addition, it was observed a high similarity between the LC-Q-TOF/MS spectra of both compounds in matrix-free and matrix-matched standards (see Figure 3; see Supplementary Material, Figure 6S).

3.3.2. Limits of detection and quantification

The limits of detection (LODs) and quantification (LOQs) were determined by the injection of a number of blank samples measurement noise at the elution times for spinetoram J and L, and comparing this response (mean values) with the signal (peak heights) of both analytes at low concentration levels. The LODs and LOQs were estimated to be three and ten times the S/N ratio, respectively. The sensitivity achieved (LODs, 0.1-0.3 µg/kg; LOQs, 0.3-1.2 µg/kg; see Table 1) is more than enough to fulfil European legislation (MRL, 50 µg/kg; European Union Pesticide Database, 2019). In addition, the obtained LOQs are much lower that most of the values reported in previous publications (see Supplementary Material, Table 1S).

3.3.3. Matrix effect

The responses (spinetoram J and L areas) obtained in matrix-free and matrix-matched standards, which were spiked after treatment (AF samples) were compared. The spinetoram J and L responses at the three concentration levels assayed ranged from 84 to 94% (see Table 2); this implies according to the criteria of the European Commission for pesticide residue analysis (± 20% of signal suppression or enhancement; European Commission Directorate-General for Health and Food Safety, 2017) that matrix effect does not need to be addressed in calibration. To confirm this finding the slopes from standard and matrix-matched calibration curves were contrasted (see Table 1), and it was found that in all cases overlapping occurred at the confidence intervals. Therefore, it was concluded that the matrix did not significantly affect ESI ionization of the analytes.

3.3.4. Linearity

Reference standard in solvent (matrix-free) calibration curves were used to quantify spinetoram J and L in honey due to the absence of a significant matrix effect. The analytical range was between LOQs (see Table 1) and 400 µg/L, which correspond to LOQ and 160 µg/kg (multifloral and rosemary honeys) or LOQ and 533 µg/kg (heather honeys) in samples, in line with the proposed sample treatment and unit conversion. Calibration curves were constructed by plotting the signal on the *y*-axis (analyte peak area) against the analyte concentration on the *x*-axis. The graphs obtained in all the calibration curves were straight lines, with coefficient of the determination values (R^2) higher than 0.99 in all cases (see Table 1). Finally, the deviation of back-calculation concentration from true concentration was lower than 20% (data not shown; European Commission Directorate-General for Health and Food Safety, 2017).

3.3.5. Precision

Precision, which was expressed as relative standard deviation (%RSD), experiments were performed concurrently by repeated sample analysis using BF samples, either on the same day (intra-day precision, European Commission Directorate-General for Health and Food Safety, 2017; repeatability, EURACHEM, 2014), or over three consecutive days (inter-day precision, European Commission Directorate-General for Health and Food Safety, 2017; partial reproducibility, EURACHEM, 2014). The obtained %RSD values were lower than 9% in all cases (see Table 3), which are consistent with the current European legislation (%RSD ≤ 20; European Commission Directorate-General for Health and Food Safety, 2017), and it is similar or lower that most of the values reported in previous studies in which spinetoram was determined in food samples (see Supplementary Material, Table 1S).

3.3.6. Trueness

This was evaluated by the mean recoveries (as a measure of trueness), calculated by comparing the measured concentrations in BF samples (see Subsection 2.2), and theoretical concentrations. Mean recoveries ranged from 82% to 95% (%RSD < 8%) in all cases (see Table 2), which complied with the current European legislation (recovery percentages between 70% and 120%; %RSD ≤ 20; European Commission Directorate-General for Health and Food Safety, 2017), and are comparable to previous works (see Supplementary Material, Table 1S).

3.3.7. Uncertainty

The combined method uncertainty (%U) was determined in all cases by the uncertainty of bias ($\%U_{bias}$) combined with that of precision ($\%U_P$), based on the equations summarized in Table 4S (see Supplementary Material; European Accreditation Laboratory Committee, 2013). It must be mentioned that due to the absence of specific-reference certified material or an official analysis method for determining spinetoram in

honey, recovery studies were used to indicate the level of bias, as recommended in the EURACHEM (2014) guidelines. After an examination of the results (see Supplementary Material, Table 5S), it can be concluded that there was a significant variation of the $\%U_{bias}$ and $\%U_P$ values, and subsequently of the %U (< 13% in all cases), depending on the compound, spiking level and botanical origin of the honey. Although in all cases, the $\%U_P$ values are much higher than $\%U_{bias}$, and consequently, it can be identified as major contributor to the combined method's uncertainty (%U).

3.4. Application of the method

No residues above the LODs of spinetoram J and L were found in any of the analyzed samples (see Subsection 2.3.1), which is a good result from and environmental and health point of view. Perhaps this could be explained by reasons such as a potential lack of stability in honey or the physical-chemical characteristics of the compounds (see Supplementary Material, Figure 1S). As regards the issue of stability, despite the fact that we searched for the respective N-demethyl and N-formyl metabolites (Fu et al., 2017; Park et al., 2012), none were detected. The absence of spinetoram residues in honey samples is consistent with the results of the previously mentioned screening study performed for 280 pesticides in honey and several other food matrices (Bauer et al., 2018), and with published data relating to the analysis of other bio-insecticides of the same family, spinosad, in honey (Bargańska, Konieczka, & Namieśnik, 2018; Bargańska, Ślebioda, & Namieśnik, 2013; Gómez-Pérez, Plaza-Bolaños, Romero-González, Martínez-Vidal, & Garrido-Frenich, 2012; Farooqi, Hasan, Akhtar, Arshad, Aslam, & Rafay, 2017; Ueno et al., 2011). Residues of spinosad have been reported in only two publications (Bargańska et al., 2013, 2018) at a rate of less than 12% of the analyzed honey samples at trace levels (< 22 μg/kg).

4. Conclusions

A novel LC-Q-TOF/MS method has been developed and validated for determining spinetoram in honey. A selective and efficient sample treatment based on SPE with polymeric cartridges has been proposed. However, due to the different physical-chemical characteristics of the honey depending on its botanical origin it has been necessary to make slight variations in the SPE proposal for heather honeys (amount of sample, elution volume). No matrix effect on ionization for spinetoram J and L was found for the different honeys analyzed; this implies that they may be quantified with matrix-free solutions, which is an additional advantage of the proposed sample treatment. Separation was achieved in 4 minutes by means of a fused-core column (Kinetex® EVO C_{18}) with an optimized mobile phase applied in isocratic elution mode. The proposed method was fully validated according to current European legislation and the LOQs obtained are much lower than the existing MRL. Several samples were analyzed with the proposed method and no residues of both compounds were detected in any of them. To conclude, this method has not only allowed a rapid determination (< 40 min including sample treatment and LC-Q-TOF/MS analysis), but has also demonstrated some of the advantages of developing specific approaches instead of multi-residue methods, such as the absence of matrix effect or greater precision and extraction efficiency.

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Declaration of interest

None

Abbreviations

AF, samples spiked after sample treatment; **BF**, samples spiked before sample treatment; **EIC**, extracted ion chromatogram; **ESI**, electrospray ionization; MLLE, micro-liquid-liquid extraction; **MRL**, maximum residue level; ***m/z***, mass-to-charge; **Q-TOF/MS**, quadrupole time-of-flight mass spectrometry; **QuEChERS,** quick, easy, cheap, effective, rugged and safe; **RSD,** relative standard deviation; **S/N**, signal to noise.

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Figure captions

Figure 1.- Analytical procedure work-up flow chart.

Figure 2.- Representative LC-Q-TOF/MS chromatograms (EIC in positive mode using the quantification ions; see Subsection 3.2.2) obtained from: (**A**) non-spiked and spiked (40 μg/kg of spinetoram J) multifloral honey sample; (**B**) non-spiked and spiked (10 μg/kg of spinetoram L) multifloral honey sample. The LC-Q-TOF/MS conditions are summarized in Subsection 2.4.

Figure 3.- Full scan ESI-Q-TOF/MS spectra of spinetoram J and L in: (**A**) matrix-free standard; (**B**) multifloral matrix-matched standard. The LC-Q-TOF/MS conditions are summarized in Subsection 2.4.

Figure 1

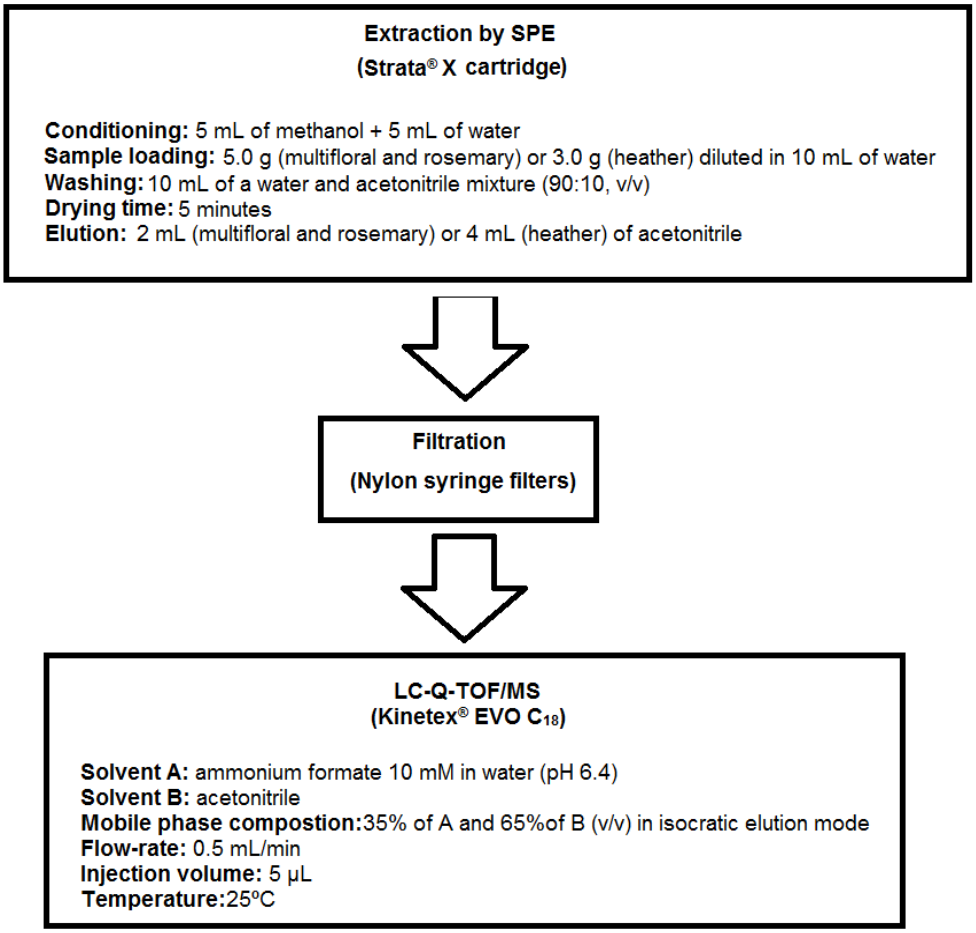
Extraction by SPE
(Strata® X cartridge)
Conditioning: 5 mL of methanol + 5 mL of water
Sample loading: 5.0 g (multifloral and rosemary) or 3.0 g (heather) diluted in 10 mL of water
Washing: 10 mL of a water and acetonitrile mixture (90:10, v/v)
Drying time: 5 minutes
Elution: 2 mL (multifloral and rosemary) or 4 mL (heather) of acetonitrile
Filtration
(Nylon syringe filters)
LC-Q-TOF/MS
(Kinetex® EVO C18)
Solvent A: ammonium formate 10 mM in water (pH 6.4)
Solvent B: acetonitrile
Mobile phase compostion: 35% of A and 65%of B (v/v) in isocratic elution mode
Flow-rate: 0.5 mL/min
Injection volume: 5 µL
Temperature: 25ºC

Figure 2

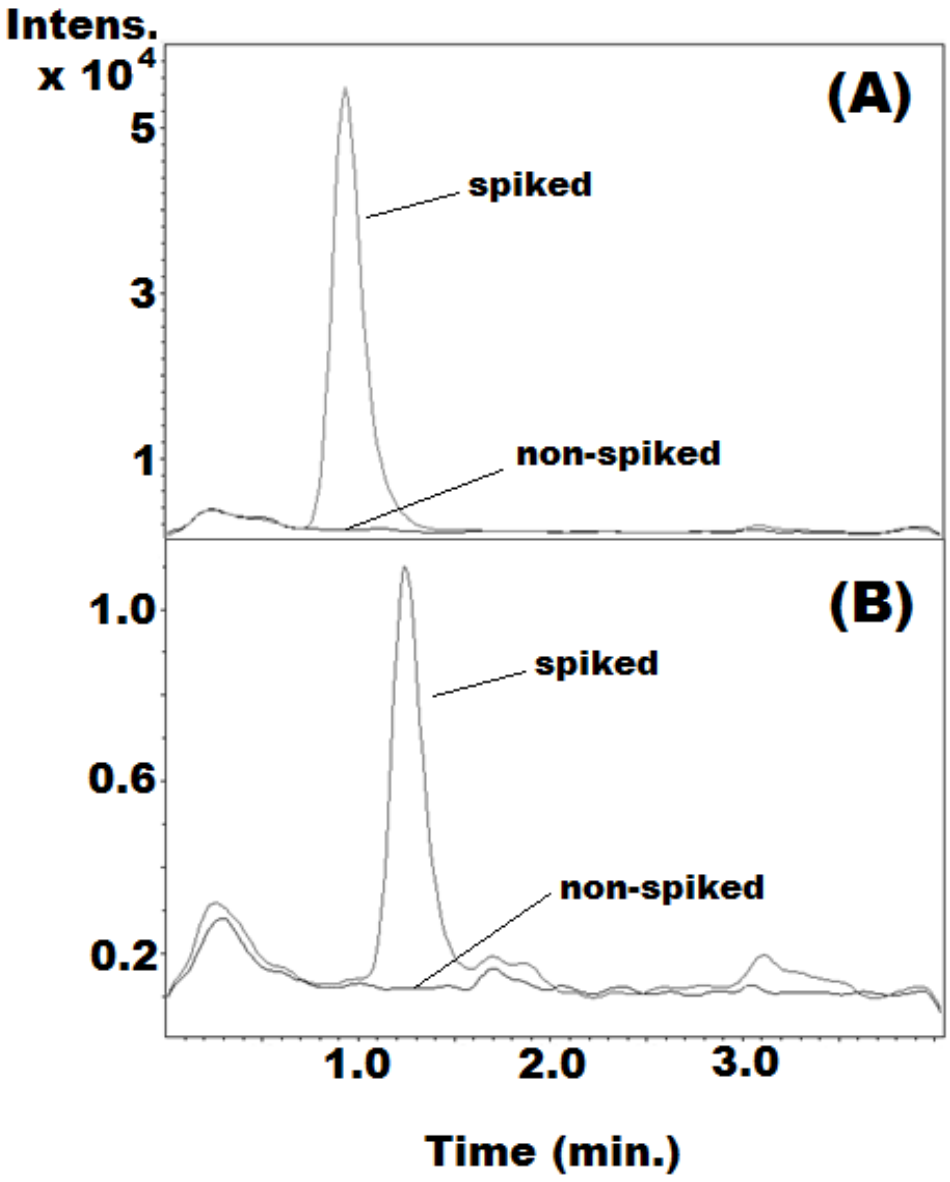
Intens.
x 10^4
5
3
1
(A)
spiked
non-spiked
1.0
0.6
0.2
(B)
spiked
non-spiked
1.0
2.0
3.0
Time (min.)

Figure 3

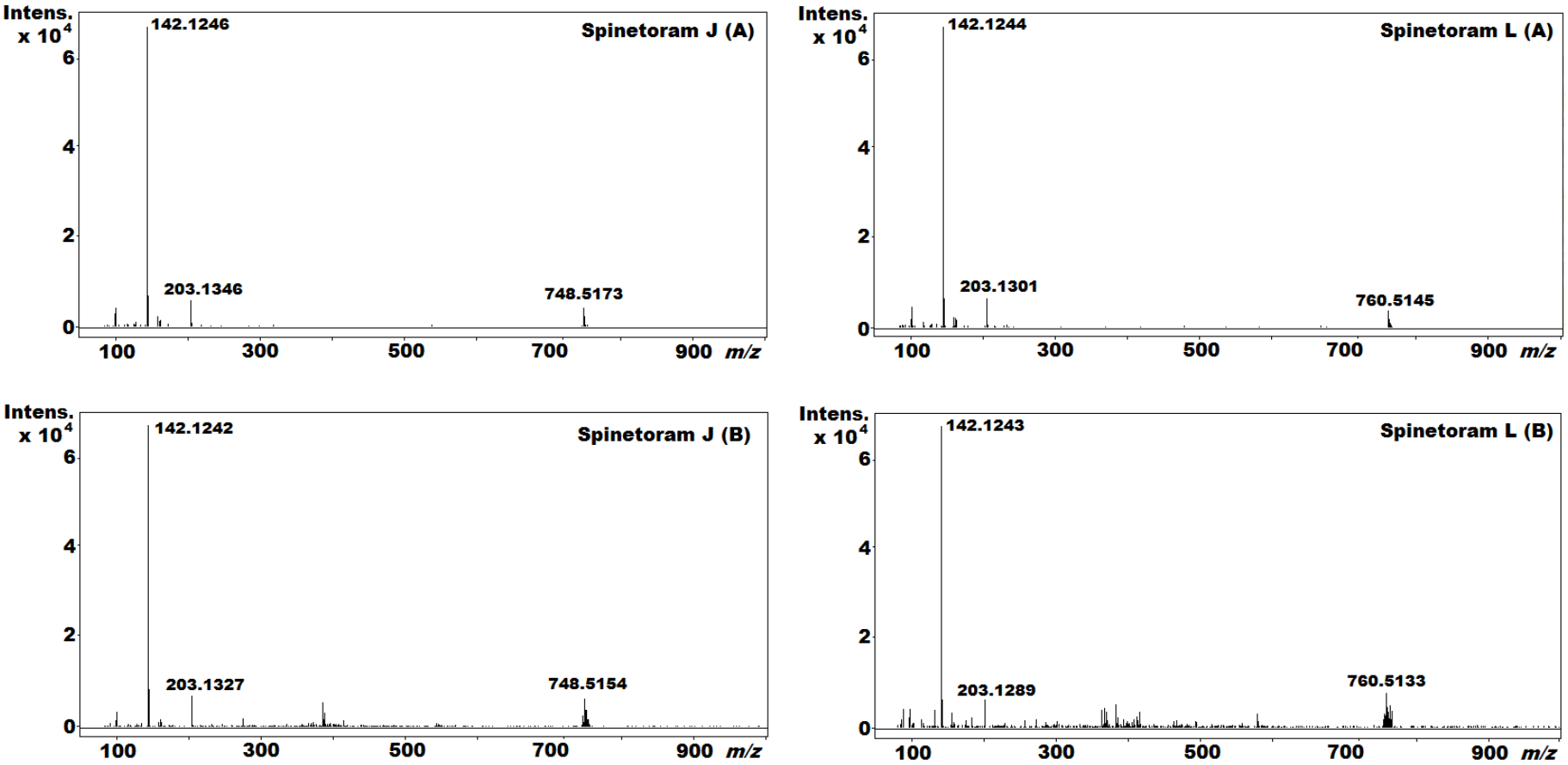
Intens.
x 10^4
6
4
2
0
142.1246
203.1346
748.5173
Spinetoram J (A)
100
300
500
700
900
m/z
Intens.
x 10^4
6
4
2
0
142.1244
203.1301
760.5145
Spinetoram L (A)
100
300
500
700
900
m/z
Intens.
x 10^4
6
4
2
0
142.1242
203.1327
748.5154
Spinetoram J (B)
100
300
500
700
900
m/z
Intens.
x 10^4
6
4
2
0
142.1243
203.1289
760.5133
Spinetoram L (B)
100
300
500
700
900
m/z

Table 1.- Calibration curve data, LOD and LOQ values.

	Compound	Calibration curve[A]	Slope confidence intervals	R^2	LOD[B] (µg/kg)	LOQ[B] (µg/kg)
Multifloral (LOQ-160 µg/kg)[A]	**Spinetoram J**	Matrix-free	34696 ± 1897	0.9996	0.1	0.3
		Matrix-matched	32150 ± 2258	0.9980		
	Spinetoram L	Matrix-free	32667 ± 2506	0.9988	0.1	0.4
		Matrix-matched	28420 ± 2484	0.9972		
Rosemary (LOQ-160 µg/kg)[A]	**Spinetoram J**	Matrix-free	34696 ± 1897	0.9996	0.1	0.4
		Matrix-matched	31226 ± 2332	0.9994		
	Spinetoram L	Matrix-free	32667 ± 2506	0.9988	0.1	0.4
		Matrix-matched	28093 ± 2645	0.9987		
Heather (LOQ-533 µg/kg)[A]	**Spinetoram J**	Matrix-free	34696 ± 1897	0.9996	0.3	1.2
		Matrix-matched	31575 ± 1958	0.9991		
	Spinetoram L	Matrix-free	32667 ± 2506	0.9988	0.2	0.7
		Matrix-matched	27866 ± 2640	0.9974		

[A]Concentrations are the same in the matrix-free and matrix-matched standards according to the proposed sample treatment and the unit conversion.
[B]LOD and LOQ values were calculated in matrix (honey).

Table 2.- Evaluation of the efficiency (recoveries) of the sample treatment and the matrix effect (comparison of responses). Data obtained as described in subsections 3.3.3 and 3.3.6.

Spiked sample		Evaluation of the sample treatment			Evaluation of the matrix effect		
		Mean (%) ± RSD (%)			Mean (%) ± RSD (%)		
		Low	Medium	High	Low	Medium	High
Multifloral	Spinetoram J	87 ± 5	89 ± 5	92 ± 6	92 ± 5	90 ± 6	94 ± 5
	Spinetoram L	88 ± 6	83 ± 6	87 ± 6	89 ± 6	84 ± 6	87 ± 5
Rosemary	Spinetoram J	95 ± 6	91 ± 6	93 ± 5	90 ± 4	87 ± 7	92 ± 6
	Spinetoram L	86 ± 7	90 ± 7	91 ± 4	87 ± 6	88 ± 5	84 ± 5
Heather	Spinetoram J	87 ± 5	83 ± 5	86 ± 6	88 ± 7	93 ± 5	90 ± 4
	Spinetoram L	82 ± 5	86 ± 7	83 ± 6	86 ± 5	87 ± 6	84 ± 5

Low- LOQ (see Table 1); **Medium-** 40 µg/kg; **High-** 160 µg/kg for multifloral and rosemary; 533 µg/kg for heather**.**

Table 3.- Summary of precision studies (%RSD) for the spinetoram J (S-J) and L (S-L) determination in spiked blank honey samples.

	Spiking level	Multifloral		Rosemary		Heather	
		S-J	S-L	S-J	S-L	S-J	S-L
Intraday precision (repeatability)	**Low**	7	8	6	6	7	6
	Medium	7	6	5	7	6	5
	High	7	8	5	4	8	6
Interday precision (partial reproducibility)	**Low**	7	6	6	5	7	6
	Medium	6	8	6	8	7	7
	High	7	7	4	5	7	6

Low- LOQ (see Table 1); **Medium-** 40 µg/kg; **High-** 160 µg/kg for multifloral and rosemary; 533 µg/kg for heather**.**

Supplementary Material

DEVELOPMENT AND VALIDATION OF A NEW METHOD FOR THE SIMULTANEOUS DETERMINATION OF SPINETORAM J AND L IN HONEY FROM DIFFERENT BOTANICAL ORIGINS EMPLOYING SOLID-PHASE EXTRACTION WITH A POLYMERIC SORBENT AND LIQUID CHROMATOGRAPHY COUPLED TO QUADRUPOLE TIME-OF-FLIGHT MASS SPECTROMETRY

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Table 1S.-Comparison of the proposed method with other LC-based procedures for determining spinetoram in food matrices.

Matrix (number of analytes)	Sample treatment (time)	Reagents (g, mL[O])	Matrix Effect[A]	Recoveries[A] (precision,%RSD)	LOQs (µg/Kg)[A]	System (IM, SP, time)	Ref.
Twenty food matrices including honey (280)	QuEChERS[B] (≈ 15 min)	6.8 g, 10 mL of ACN	NS	NS	NS	UPLC-MS/MS (ESI+, C_{18}, 17 min)	**(Bauer et al., 2018)**
Rice (4)[M]	SE + clean-up (> 40 min)	1.1 g, 40 mL of ACN	NS	79%-88% (< 9%)	4-16	LC-MS/MS (ESI+, C_{18}, 9 min)	**(Fu et al., 2017)**
Dried hops (29)	SE + SPE (polymeric) + EV (> 35 min)	15 mL of ACN	Yes	86%-106% (< 5%)	50	LC-MS/MS (ESI+, C_{18}, 14 min)	**(Hengel, 2017)**
Milk, fruits and vegetables (22)[J]	SE + SPE (C_{18} and florisil) + EV (> 35 min)	20 g, 100 mL of 1% TEA in ACN	Yes	74%-103% (< 26)	1-3 (LOD)	UPLC-MS/MS (ESI+, C_{18}, 17 min)	**(Kamel et al., 2010)**
Paprika leaf (4)	QuEChERS[B] (> 30 min)	0.2 g, 10 mL of ACN	Yes	88%-95% (< 3%)	250	LC-MS (ESI+, C_{18}, > 12 min)	**(Kim et al., 2017)**
Livestock (2)	SE + clean-up (> 40 min)	0.5 g, > 40 mL of ACN	NS	81%-107% (< 10)	10	LC-MS/MS (ESI+, C_{18}, 16 min)	**(Ko et al., 2016)**
Vegetable crops (2)	SE + SPE (NH_2) + EV (> 40 min)	> 150 mL of DCM	NS	86%-99% (< 7%)	30	LC-UV, LC-MS/MS (ESI+, C_{18}, 20 min)	**(Liu et al., 2011)**
Tomato (2)	QuEChERS[B] (≈ 30 min)	5.7 g, > 10 mL of 1% TEA in ACN	No	88%-99% (< 13%)	40	LC-PDA (C_{18}, 25 min)	**(Malhat, 2013)**
Green onion (2)	MLLE (> NS)	4 mL of DCM, 1 mL of ACN	No	81%-94%[J] (< 11%)[J]	10[J]	LC-MS/MS (ESI+, C_{18}, 20 min)	**(Malhat & Abdallah, 2019)**

[A]:data related only to spinetoram J and L; [B]: including a clean-up dSPE step; [J]: including only spinetoram J; [M]: including spinetoram metabolites (demethyl and formyl metabolites); [O]: organic solvent; **ACN,** acetonitrile; **DCM,** dichloromethane; **dSPE,** dispersive SPE; **ESI,** Electrospray ionization; **EV**, evaporation; **MLLE,** micro-liquid-liquid extraction; **MS/MS**, tandem mass spectrometry; **NS**, not specified**; PDA,** photodiode array detector; **QuEChERS**, quick, easy, cheap, effective, rugged and safe; **SD,** sample dilution; **SE**, solvent extraction; **TEA,** triethylamine.

Table 1S.- Continued.

Matrix (number of analytes)	Sample treatment (time)	Reagents (g, mL[O])	Matrix Effect[A]	Recoveries[A] (precision,%RSD)	LOQs (µg/Kg)[A]	System (IM, SP, time)	Ref.
Amaranth and parsley (6)[M]	QuEChERS[B] (NP)	7.7 g, 20 mL of ACN	No	74%-113% (< 15%)	30	LC-MS/MS (ESI+, C_{18}, 15 min)	**(Park et al., 2012)**
Cowpea (6)	QuEChERS[B] (> 40 min)	11.1 g, > 20 mL of ACN	NS	73%-109% (< 21%)	NS	UPLC-MS/MS (ESI+, C_{18}, 7 min)	**(Quian et al., 2016)**
Wheat (2)[J]	SE + EV (> 180 min)	32 mL (2 mL of methanol)	No	79%-108% (8%)	NS	LC-UV (C_{18}, 20 min)	**(Vassilakos et al., 2015a)**
Seven grain commodities (1)[J]	SE + EV (> 120 min)	32 mL (2 mL of methanol)	No	NS	NS	LC-UV (C_{18}, 20 min)	**(Vassilakos et al., 2015b)**
Plants- and animal-type foods (2)	QuEChERS[B] (> 45 min)	5.1 g, > 10 mL of ACN	Yes	80%-112% (< 13%)	0.1-0.4	UHPLC-MS/MS (ESI+, C_{18}, 8 min)	**(Zhang et al., 2019c)**
Rice (2)	QuEChERS[B] (> 35 min)	5.7 g, > 20 mL of ACN	No	87%-96% (< 20%)	1	UPLC-MS/MS (ESI+, C_{18}, 5 min)	**(Zhao et al., 2015)**
Honey (2)	SD + SPE (polymeric) (< 30 min)	≤ 10 mL (5 mL of methanol, ≤ 5 mL of ACN)	No	82%-94% (< 9%)	0.3-1.2	LC-Q-TOF/MS (ESI+, C_{18}, 4 min)	**Present study**

[A]:data related only to spinetoram J and L; [B]: including a clean-up dSPE step; [J]: including only spinetoram J; [M]: including spinetoram metabolites (demethyl and formyl metabolites); [O]: organic solvent; **ACN,** acetonitrile; **DCM,** dichloromethane; **dSPE,** dispersive SPE; **ESI,** Electrospray ionization; **EV**, evaporation; **MLLE,** micro-liquid-liquid extraction; **MS/MS**, tandem mass spectrometry; **NS**, not specified**; PDA,** photodiode array detector; **QuEChERS**, quick, easy, cheap, effective, rugged and safe; **SD,** sample dilution; **SE**, solvent extraction; **TEA,** triethylamine.

Table 2S.- Recoveries (mean ± %RSD; three replicates that were injected in triplicate) obtained after testing different SPE sorbents with spiked blank honey samples at medium concentration level (40 µg/kg) with spinetoram J (S-J) and L (S-L).

SPE Sorbent	Compound	Multifloral	Rosemary	Heather
Strata® X	**S-J**	83 ± 6	85 ± 5	80 ± 5
	S-L	77 ± 7	80 ± 6	75 ± 7
Strata® C_{18}-E	**S-J**	67 ± 5	73 ± 7	62 ± 6
	S-L	60 ± 6	71 ± 5	64 ± 7

Table 3S.- Recoveries (mean ± %RSD; three replicates that were injected in triplicate) obtained after testing different elution volumes of acetonitrile (2, 4 and 6 mL) with spiked blank honey samples at medium concentration level (40 µg/kg) with spinetoram J (S-J) and L (S-L).

	Compound	2 mL	4 mL	6 mL
Multifloral	**S-J**	89 ± 5	91 ± 6	93 ± 7
	S-L	83 ± 6	84 ± 7	87 ± 6
Rosemary	**S-J**	91 ± 6	93 ± 7	94 ± 6
	S-L	90 ± 7	91 ± 6	93 ± 7
Heather	**S-J**	73 ± 5	83 ± 5	85 ± 5
	S-L	75 ± 6	86 ± 7	89 ± 6

Table 4S.- Equations employed for measurement uncertainty. Reprinted from Food Chemistry, 266, Valverde, S., Ibáñez, M., Bernal, J. L., Nozal, M. J., Hernández, F., & Bernal, J., Development and validation of ultra high performance-liquid chromatography-tandem mass spectrometry based methods for the determination of neonicotinoid insecticides in honey, 215-222, Copyright (2018), with permission from Elsevier.

Uncertainty of repeatability (U_{rep})	$U_{rep}=\frac{S_{rep}}{\sqrt{n}}$
Uncertainty of repeatability (%U_{rep})	$\%U_{rep} = 100 \times U_{rep}$
Uncertainty inter-days ($U_{inter\text{-}days}$)	$U_{inter-days} = \sqrt{\frac{\bar{x} - RV}{n}}$
Uncertainty of precision (%U_P)	$\%U_P = \sqrt{\%U_{rep}^2 + \%U_{inter-days}^2}$
Relative error (%RE)	$\%RE = 100\ (\bar{x} - RV)$
Uncertainty typical of the measure (U_m)	$Um_= \sqrt{\sum_{i=1}^{N} u_i^2}$
Uncertainty of reference value (U_{RV})	$U_{RV} = \frac{Um}{k}$
Uncertainty of bias (U_{bias})	$U_{bias} = \sqrt{U_{RV}^2 + \frac{U_{rep}^2}{n}}$
Uncertainty of bias (%U_{bias})	$\%U_{Bias} = 100 \times U_{Bias}$
Uncertainty of the method (%U)	$\%U = \sqrt{\%U_P^2 + \%U_{bias}^2}$

k, coverage factor (= 2; 95% of confidence); **n,** number of replicates (n = 6); **RV,** reference value (spiked levels); **u_i,** uncertainty typical of the different contributions; **S_{rep},** standard deviation of repeatability; $\bar{x}$, average recoveries.

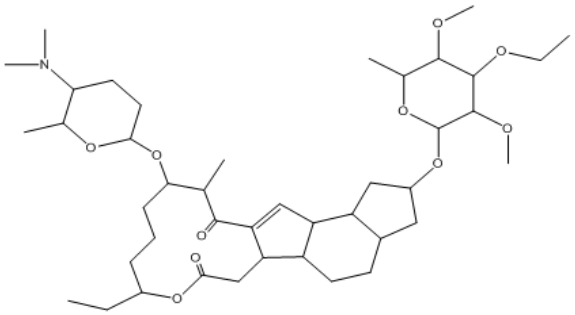
Table 5S.-Principal components of the uncertainty of method validation for spinetoram J (S-J) and L (S-L), which was calculated with spiked samples at three different concentrations (low, medium and high).

	Multifloral						**Rosemary**						**Heather**					
	S-J			**S-L**			**S-J**			**S-L**			**S-J**			**S-L**		
	Low	**Medium**	**High**	**Low**	**Medium**	**High**	**Low**	**Medium**	**High**	**Low**	**Medium**	**High**	**Low**	**Medium**	**High**	**Low**	**Medium**	**High**
$\%U_P$	5.1	5.6	7.9	7.0	11	7.7	8.9	8.6	9.9	8.6	4.9	10	8.8	6.9	8.2	12	8.4	8.5
$\%U_{Bias}$	0.085	0.10	0.076	0.030	0.025	0.030	0.076	0.094	0.077	0.030	0.025	0.030	0.10	0.094	0.12	0.030	0.025	0.030
%U	5.1	5.6	7.9	7.0	11	7.7	8.9	8.6	9.9	8.6	4.9	10	8.8	6.9	8.2	12	8.4	8.5

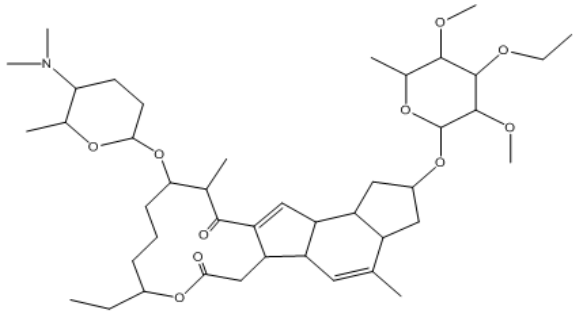
Low- LOQ (see Table 1); **Medium-** 40 µg/kg; **High-** 160 µg/kg for multifloral and rosemary; 533 µg/kg for heather**.**

$\%U_P$, uncertainty of precision (repeatability and inter-day precision); $\%U_{bias}$, uncertainty of the bias; **%U,** combined uncertainty of the method.

Figure 1S.- Main physical-chemical characteristics of spinetoram J and L.



Spinetoram J



Spinetoram L

	Spinetoram J	Spinetoram L
Molecular formula	$C_{42}H_{69}NO_{10}$	$C_{43}H_{69}NO_{10}$
Molecular weight (g/mol)	748.011	760.022
Exact mass (g/mol)	747.492	759.492
Melting point (20°C)	143.4	70.8
Solubility in water (20°C; mg/L)	10 (unbuffered) 423 (pH 5) 11.3 (pH 7) 6.27 (pH 10)	31.9 (unbuffered) 1630 (pH 5) 46.7 (pH 7) 0.706 (pH 10)
Relative Density (~ 20°C ; g/cm³)	1.1495	1.1807
Vapor pressure (Pa; 20°C)	5.3×10^{-5}	2.1×10^{-5}
Octanol-water partition coefficient (20°C)	2.44 (pH 5) 4.09 (pH 7) 4.22 (pH 9)	2.94 (pH 5) 4.49 (pH 7) 4.82 (pH 9
Photolysis DT50 (25°C; days)	0.5	0.3
Dissociation constant (25°C; pKa)	7.86	7.59

Figure 2S.- Evaluation of the extraction efficiency (recoveries) obtained after testing 10 mL of different water and acetonitrile mixtures with spiked blank honey samples for different botanical origins (multifloral, rosemary and heather) at medium concentration level (40 µg/kg). Data represent the mean of three replicates injected in triplicate ± the relative standard deviation of the mean (error bars).

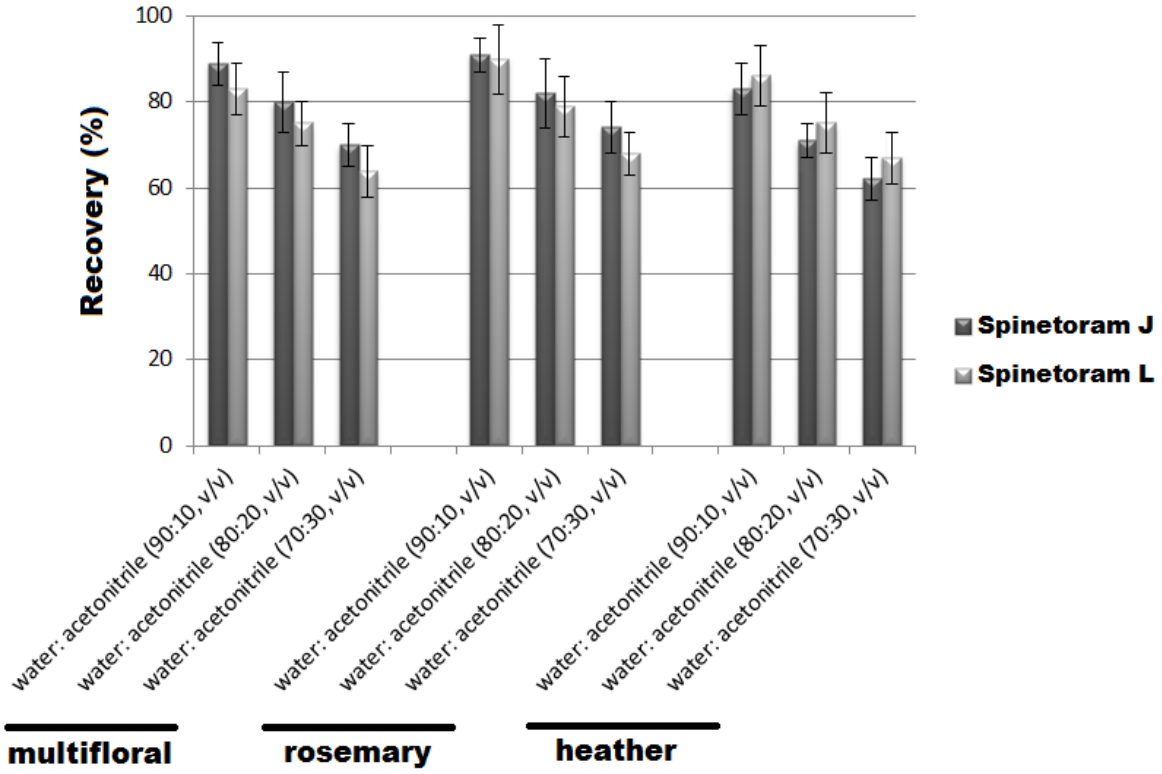


Figure 3S.- Evaluation of the extraction efficiency (recoveries) obtained after testing different volumes of a (90:10, v/v) water and acetonitrile mixture with spiked blank honey samples for different botanical origins (multifloral, rosemary and heather) at medium concentration level (40 µg/kg). Data represent the mean of three replicates injected in triplicate ± the relative standard deviation of the mean (error bars).

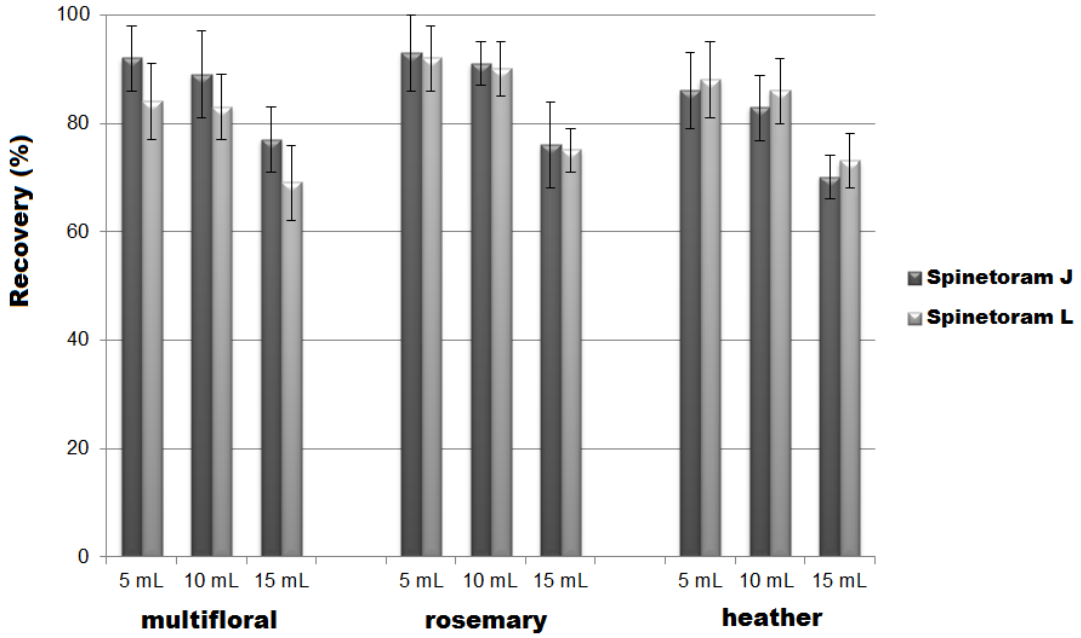


Figure 4S.- Representative LC-Q-TOF/MS chromatograms (EIC in positive mode using the quantification ions; see subsection 3.2.2) obtained from: (**A**) non-spiked and spiked (40 µg/kg of spinetoram J) heather honey sample; (**B**) non-spiked and spiked (10 µg/kg of spinetoram L) heather honey sample. The LC-Q-TOF/MS conditions are summarized in subsection 2.4.

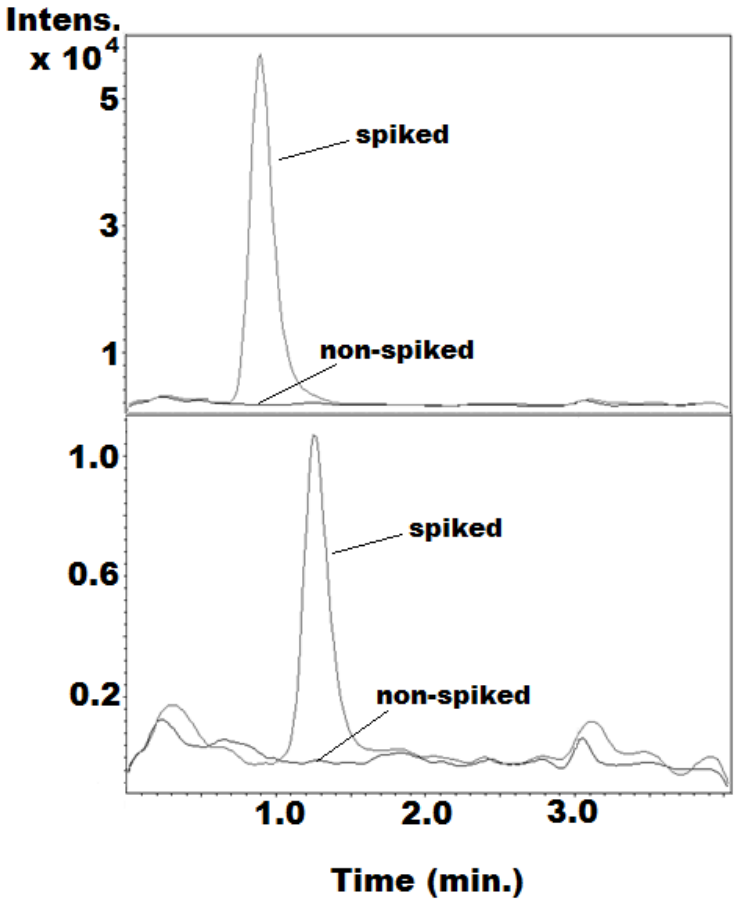


Figure 5S.- Representative LC-Q-TOF/MS chromatograms (EIC in positive mode using the quantification ions; see subsection 3.2.2) obtained from: (**A**) non-spiked and spiked (40 µg/kg of spinetoram J) rosemary honey sample; (**B**) non-spiked and spiked (10 µg/kg of spinetoram L) rosemary honey sample. The LC-Q-TOF/MS conditions are summarized in subsection 2.4.

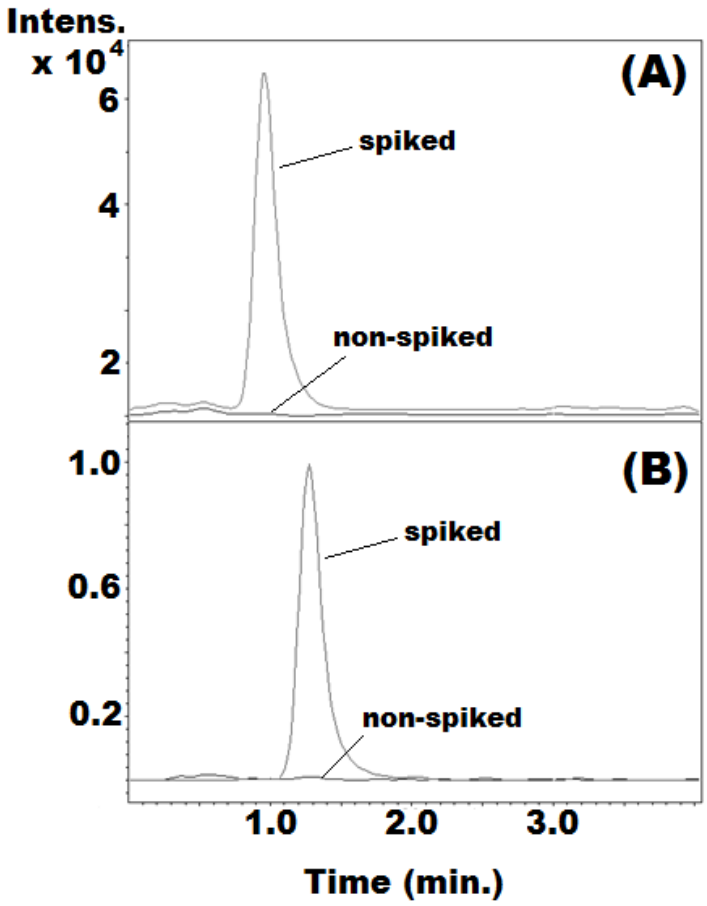


Figure 6S.- Full scan Q-TOF/MS spectra of spinetoram J and L in: (**A**) spiked heather honey sample; (**B**) spiked rosemary honey sample. The LC-Q-TOF/MS conditions are summarized in subsection 2.4.