

Short communication

Analysis of nicotinoid insecticides residues in honey by solid matrix partition clean-up and liquid chromatography–electrospray mass spectrometry

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Abstract

An analytical method for the routine simultaneous determination of four nicotinoid insecticides (acetamiprid, imidacloprid, thiacloprid and thiamethoxam) in commercial multifloral honey was developed. Fortified honey samples, dissolved in water, were cleaned up through Extrelut NT20 column and, finally, insecticides were eluted with dichloromethane. The eluate was evaporated, the residue redissolved in methanol and then analyzed by LC–ESI(+)-MS. Average recoveries of the four analytes were in the range of 76% and 99% at both spiking levels 0.1 and 1.0 mg kg⁻¹. Relative standard deviations (RSDs) were less than 10% for all of the recovery tests. The detection limits (LODs) of the method ranged from 0.01 to 0.1 mg kg⁻¹ for the different insecticides studied. The developed method is linear over the range assayed, 0.5–5.0 µg mL⁻¹, with linear correlation coefficients higher than 0.9993.

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1. Introduction

The increasingly public concern in recent years, about health risk from pesticide residues in the diet, has deeply modified the strategy for crop protection with emphasis on food quality and safety. Unfortunately, honey bees are insects that are greatly affected by insecticides as well as pesticides in general. As a consequence, residues of certain pesticides could appear in apian products, thus it is convenient to evaluate them in order to maintain the characteristics that a natural product such as honey, according to European Union (EU) regulation, should have, mostly because it is traditionally used in child, old and ill people. The maximum concentration of pesticide residues in honey is not included in the Codex Alimentarius [1,2], and the absence of maximum residue limits (MRLs) makes it difficult to ascertain whether a product is safe for consumers. Up

to now, EU legislation has only regulated the MRL for three acaricides in honey [3]. Moreover, the pesticide contents in apian samples serve as a good indication of pesticidal pollution. However, these samples pose substantial analytical problems, due to their complex composition and, particularly, the presence in honey of waxes, pigments and carbohydrates. Many methods have been reported for the analysis of several types of pesticide in honey, such as the acaricides, organophosphorus, carbamate and organochlorine insecticides [4–13].

Neonicotinoid insecticides are a relatively new group of active ingredients with novel modes of action [14]. These insecticides are distributed on large areas of agricultural land, so they could give rise to serious risks for the health and safety of consumer.

Although some papers have been found which deal the determination of nicotinoid residues in vegetables [15–18], no method have been published for simultaneous determination of residues of neonicotinoid insecticides in honey. The goal of the present work is to develop a rapid, sensitive and accurate LC–MS method for determining four nicotinoid insecticides,

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acetamiprid, imidacloprid, thiacloprid and thiamethoxam, in honey samples, following a single extraction with diatomaceous earth material (Extrelut NT20) cartridges. Finally, the proposed procedure was validated [19–23].

2. Experimental

2.1. Reagents, standards and samples

Common names and structures of the four neonicotinoids evaluated here are shown in Fig. 1. Certified pesticide standards (99%) for acetamiprid, imidacloprid, thiacloprid and thiamethoxam were from Riedel-de Haen (Sigma–Aldrich Group, Milano, Italy). HPLC-grade methanol was obtained by Carlo Erba (Milano, Italy). LC-grade water was produced by a Milli-Q water purification system (Millipore, Bedford, MA, USA). Acetic acid (98%) was supplied from BDH Analar (Milano, Italy). Dichloromethane of special grade for pesticide residue analysis was supplied from Merck (Darmstadt, Germany).

Insecticide stock solutions (approximately 1.0 mg mL^{-1}) of individual pesticide standards were prepared by dissolving weighed exactly 50 mg of each analyte in 50 mL of methanol. Standard multicomponent solution ($10 \mu\text{g mL}^{-1}$) was prepared diluting each primary standard solution with methanol and was used for spiking honey, for preparing matrix matched calibration standards in honey blank and for study of the linear dynamic range of the LC–MS method. Matrix matched calibration standards were prepared by adding to extract honey blank samples appropriate volumes of standard working solution at four different levels (0.25, 0.5, 2.5 and $5.0 \mu\text{g mL}^{-1}$) (0.05, 0.1, 0.5 and 1.0 mg kg^{-1}). The standard solutions were stored under refrigerator conditions ($4 \pm 3^\circ\text{C}$) and protected from light.

2.2. Extraction procedure

A 5 g portion of a multifloral commercialized honey sample was weighed in an Erlenmeyer flask, mixed with 20 mL Milli-

Q water. The honey aqueous solution samples unspiked and spiked at two concentration levels (0.1 and 1.0 mg kg^{-1}) were transferred quantitatively on top of an Extrelut-NT 20 cartridges (20 mL, diatomaceous earth material of high pore volume) from Merck (Darmstadt, Germany), Cod No. 1.15069.0001. After the liquid has drained into the cartridge, wait for 10 min in order to obtain an even distribution on the filling material: then a nitrogen flow of 1.0 L min^{-1} was passed for 20 min through the column from bottom to top. The Extrelut-NT20 cartridge was disconnected from the gas line and a $32 \text{ mm} \times 0.70 \text{ mm}$ I.D. Luer Lock needle was attached to the lower tip as a flow restrictor. The column was eluted with five 20 mL portions of dichloromethane. The effluents were collected in a 250 mL round bottom flask, evaporated under vacuum to a small volume at a bath temperature of 40°C and the last solvent traces were then removed by manually rotating the collecting flask. The residue was redissolved with 1.0 mL of methanol and analyzed by LC–ESI–MS. Evaporation of the extracts and reconstitution in low volumes of methanol was necessary in order to reach an adequate preconcentration of pesticides that allowed to obtain low limits of detection (LODs).

2.3. LC and MS conditions

LC–MS was carried out using a Navigator LC–MS (ThermoFinnigan, Milan, Italy). The LC instrument was equipped with a Rheodyne Model 7725 injector. The analytical column was a LichroCart 125–4 Lichrosphere 100 ($5 \mu\text{m}$) (Merck, Darmstadt, Germany). The mobile phase was water (A) and methanol, both acidified with 0.01% acetic acid. The insecticides were separated with the following gradient program: 95% A for 3 min; followed by a linear gradient from 95% A at $t=7$ min to 60% maintaining 60% A 5 min; then by a linear gradient from 60% A at $t=5$ min to 40% maintaining 40% A for 5 min and returning linearly to 95% A in 5 min. The column temperature was 40°C , the flow-rate was 1.0 mL min^{-1} and the injection volume was $20 \mu\text{L}$. The MS system was a quadrupole equipped with an electrospray ionization (ESI) source. The instrument was operated in the positive ionization mode. The operating conditions for ESI were drying gas (nitrogen) flow 10.0 L min^{-1} ; capillary voltage 3500 V; gas temperature 300°C . The fragmentor voltage was kept at 20 V. Flow injection analyses ($10 \mu\text{L}$) were performed for individual pesticide solutions (10 mg L^{-1}) in order to obtain the mass spectral data, from which ions were carefully chosen for analysis in the selected ion monitoring (SIM) mode, using the parameter conditions as above. The external standard method of calibration was used for this analysis. At least seven standard solutions (0, 0.01, 0.02, 0.06, 0.1, 0.2, 0.5 and 1.0 mg L^{-1}) containing all compounds were analyzed by LC–ESI–MS in selected ion mode followed by detection of the signal of the more abundant ions. These ions were identified in full scan mode during the acquisition of the mass spectrum of each insecticide. The injection was performed three times to test the reproducibility. Calibration curves were obtained by plotting peak areas against concentrations of analytes injected.

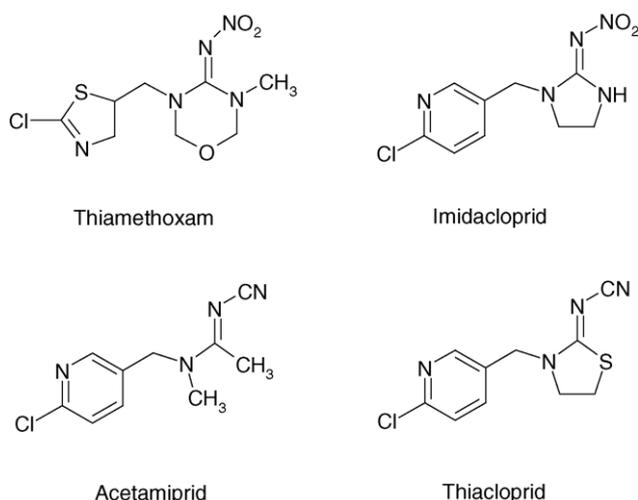


Fig. 1. Names and structures of four neonicotinoids evaluated.

Table 1
Retention times and monitored ions of neonicotinoid insecticides with LC–MS

| Insecticide | Molecular weight | t_R | Monitored ions |
|--------------|------------------|-------|----------------|
| Acetamiprid | 222.7 | 13.05 | 223/225 |
| Imidacloprid | 255.7 | 11.85 | 256/258 |
| Thiacloprid | 252.7 | 14.42 | 253/255 |
| Thiamethoxam | 291.7 | 10.35 | 292/294 |

3. Results and discussion

3.1. LC–MS determination

A gradient system (water and methanol, both acidified with 0.01% acetic acid) was applied to separate four pesticides as independent peaks. Retention times (t_R) were determined individually and are presented in Table 1. Selection of one or two ions for investigation was scheduled according to the following protocols, as detailed in Table 1. The ions used for SIM for each compound gave a strong ion signal with positive mode ESI.

Fig. 2 shows chromatograms of honey sample unspiked and spiked at 1.0 mg kg^{-1} for each insecticide. Chromatograms of spiked honey samples were quite similar to those obtained with the standard solution of pure pesticides. The LC–MS chromatogram of unspiked honey extract shows good baseline stability with no interfering peaks, indicating that the proposed clean up is suitable for the determination of the target analytes. The detector response for all target compounds was linear in the

Table 2
Linear regression data for matrix matched calibration standards

| Insecticide | Slope ^a | Ss ^b | Intercept ^a | Si ^c | r^2 |
|--------------|--------------------|-----------------|------------------------|-----------------|--------|
| Acetamiprid | 1677.29 | 11.7 | −12.65 | 5.9 | 0.9999 |
| Imidacloprid | 455.98 | 0.7 | −0.67 | 0.3 | 0.9999 |
| Thiacloprid | 657.91 | 27.9 | 25.64 | 5.4 | 0.9993 |
| Thiamethoxam | 458.51 | 0.3 | −0.27 | 2.1 | 0.9999 |

^a ($\times 10^3$).

^b Slope standard deviation.

^c Intercept standard deviation.

concentration range $0.01\text{--}1.0 \text{ mg L}^{-1}$ and the correlation coefficients were better than 0.9998.

3.2. Method validation

3.2.1. Linearity

The linearity of a method is a measure of range within which detector response is directly proportional to the concentration of analyte in samples. The calibration was performed by use of matrix-matched calibration standards prepared as described in the experimental section. The linearity of the calibration curves was studied including the origin point. The calibration data obtained for each pesticide in matrix are shown in Table 2. Good linearity of the response was found for all pesticides at concentrations within the tested interval, with linear correlation coefficients higher than 0.9993.

3.2.2. Recovery

Recovery experiments, concerning the four neonicotinoid insecticides, were performed in honey samples, at two fortification levels of 0.1 and 1.0 mg kg^{-1} . The results of a series of six-fold experiments for each fortification level are presented in Table 3. The mean recoveries of honey samples, at the two fortification levels, were between 76% and 99%. It seems that the recovery values were not related to the spiking level.

3.2.3. Precision

The precision of the method was determined by repeatability and reproducibility studies, expressed by the relative standard deviation (RSD). The repeatability RSD_T (intra-assay precision) was measured by comparing standard deviation of the recovery percentages spiked honey samples run the same day. The reproducibility RSD_R (as between-day precision) was determined by analyzing spiked honey samples for four alter-

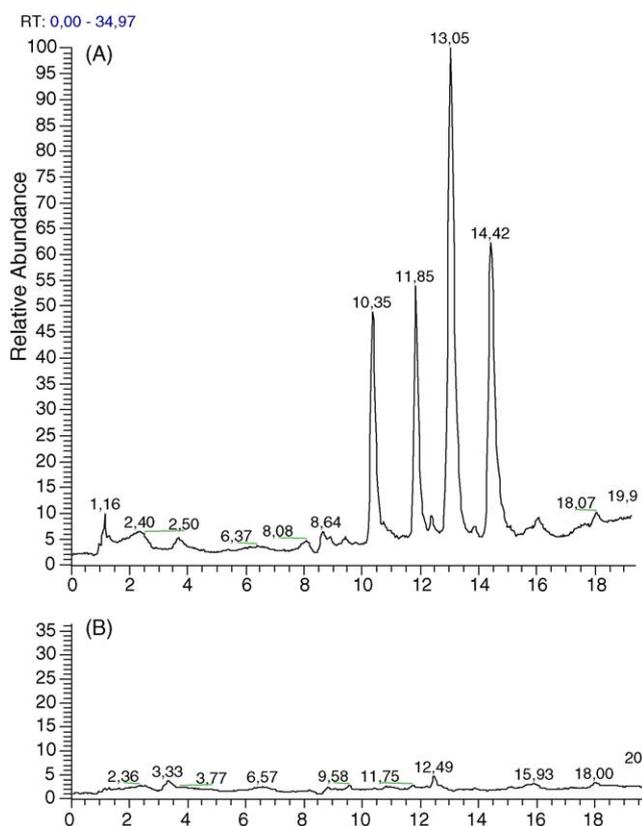


Fig. 2. LC–MS chromatograms obtained from (A) fortified honey sample (spike level 1.0 mg kg^{-1}) and (B) untreated honey control.

Table 3
Recovery (%), repeatability (RSD_T , %) and reproducibility (RSD_R , %) of the insecticides at spiking levels 0.1 and 1.0 mg kg^{-1} ($n = 6$)

| Insecticide | Spike level 0.1 | | | Spike level 1.0 | | |
|--------------|-----------------|-------------|-------------|-----------------|-------------|-------------|
| | Mean (%) | RSD_T (%) | RSD_R (%) | Mean (%) | RSD_T (%) | RSD_R (%) |
| Acetamiprid | 83.9 | 4.1 | 7.5 | 89.6 | 2.2 | 4.5 |
| Imidacloprid | 88.1 | 9.8 | 5.3 | 98.5 | 4.2 | 4.5 |
| Thiacloprid | 76.3 | 8.7 | 6.4 | 91.3 | 6.3 | 7.1 |
| Thiamethoxam | 81.5 | 6.7 | 8.3 | 99.0 | 1.3 | 3.4 |

nate days. Replicated ($n=6$ for each concentration level) samples were all run and the RSD value was calculated for each insecticide. The method was found to be precise ($RSD < 10\%$) for all the compounds studied at both spiking levels (Table 3).

3.2.4. LODs and LOQs

The LODs and LOQs values were calculated from Ordinary Least-Squares Regression Data [21]. This approach consists of using the dispersion characteristics of the regression line of the chromatographic peak area against concentration. When the dispersion characteristics have been calculated the standard deviation of the blank is estimated either by the regression residual standard deviation or by the standard deviation of the intercept. LOD corresponds to the analyte amount for which the area is equal to three times the chosen standard deviation and LOQ corresponds to the analyte amount for which this area is equal to 10 times the standard deviation chosen. The standard deviation chosen to calculate the LODs and LOQs values is the residual standard deviation of the regression line for all insecticides in the analyzed matrix. The LODs and LOQs values obtained were for thiamethoxam 0.01 and 0.04 mg kg⁻¹, for thiacloprid 0.02 and 0.05 mg kg⁻¹, for acetamiprid 0.03 and 0.1 mg kg⁻¹, for imidacloprid 0.1 and 0.3 mg kg⁻¹, respectively.

4. Conclusions

In this paper, an analytical method, for the determination of four neonicotinoid insecticide residues in commercial multifloral honey, is reported. The proposed method involves diluting the honey in water, a clean up step with an SPE Extrelut-NT20 column and analysis by LC–ESI(+)-MS, with acceptable recoveries, repeatabilities and reproducibilities and low detection limits. The selectivity of this technique using selected ion monitoring (SIM) solves problems of coeluting pesticides and avoids most of the coextractive compounds from the honey matrix. In addition, MS detection allows the confirmation of the identity of possible residues. Moreover, the obtained results show that

the diatomaceous earth material (Extrelut) is suitable for the rapid removal of the wax content from the extracted solutions. This proposed analytical procedure is fast, easy to perform and could be utilized for regular monitoring of neonicotinoid pesticide residues in honey matrix.

References

- [1] Codex Alimentarius Draft revised for honey at step 6 of the codex procedure CX 5/19.2. CL 1998/12-6, 1998.
- [2] S. Bogdanov, Mitt. Gebiete Lebensm. Hyg. 90 (1999) 108.
- [3] Council Directive 2001/110/EC of 20 December 2001 relating to honey.
- [4] M. Fernandez, Y. Picò, J. Manes, J. Food Prot. 65 (2002) 1502.
- [5] C. Blasco, M. Fernandez, A. Pena, C. Lino, M.I. Silveira, G. Font, Y. Pico, J. Agric. Food Chem. 51 (2003) 8132.
- [6] A. Herrera, C. Perez-Arquillue, P. Conchello, S. Bayarri, R. Lazaro, C. Yague, A. Arino, Anal. Bioanal. Chem. 381 (2005) 695.
- [7] P. Cabras, M. Melis, L. Spanedda, J. AOAC Int. 76 (1993) 92.
- [8] J.L. Bernal, M.J. del Nozal, L. Toribio, J.J. Jimenez, J. Atienza, J. Chromatogr. A 787 (1997) 129.
- [9] U. Menkissoglu-Spiroudi, G.C. Diamantidis, V.E. Georgiou, A.T. Thrasyvoulou, J. AOAC Int. 83 (2000) 178.
- [10] A.C. Martel, S. Zeggane, J. Chromatogr. A 954 (2002) 173.
- [11] E. Korta, A. Bakkali, L.A. Berrueta, B. Gallo, F. Vicente, J. Chromatogr. A 930 (2001) 21.
- [12] R. Colombo, T. Sauro, Ind. Aliment. 36 (1997) 1151.
- [13] A. Tsigouri, U. Menkissoglu-Spiroudi, A.T. Thrasyvoulou, G.C. Diamantidis, J. AOAC Int. 83 (2000) 1225.
- [14] M. Tomizawa, J.E. Casida, Annu. Rev. Pharmacol. Toxicol. 45 (2005) 247.
- [15] H. Obana, M. Okihashi, K. Akutsu, Y. Kitagawa, S. Hori, J. Agric. Food Chem. 50 (2002) 4464.
- [16] H. Obana, M. Okihashi, K. Akutsu, Y. Kitagawa, S. Hori, J. Agric. Food Chem. 51 (2003) 2501.
- [17] B. Singh, G.D. Foster, S.U. Khan, J. Agric. Food Chem. 52 (2004) 105.
- [18] I. Ferrer, E.M. Thurman, A.R. Fernandez-Alba, Anal. Chem. 77 (2005) 2818.
- [19] R. Causon, J. Chromatogr. B 689 (1997) 175.
- [20] A.R.C. Hill, S.L. Reynolds, Analyst 124 (1999) 953.
- [21] J. Vial, A. Jardy, Anal. Chem. 71 (1999) 2672.
- [22] G.M.F. Pinto, I.C.S.F. Jardim, J. Chromatogr. A 869 (2000) 463.
- [23] Commission Decision 2002/657/EC of 12 August 2002 implementing Council Directive 96/23/EC on the Performance of Analytical Methods and the Interpretation of Results.