QuEChERS-based method

for neonicotinoid insecticides determination in honey from the Argentine livestock-agricultural area

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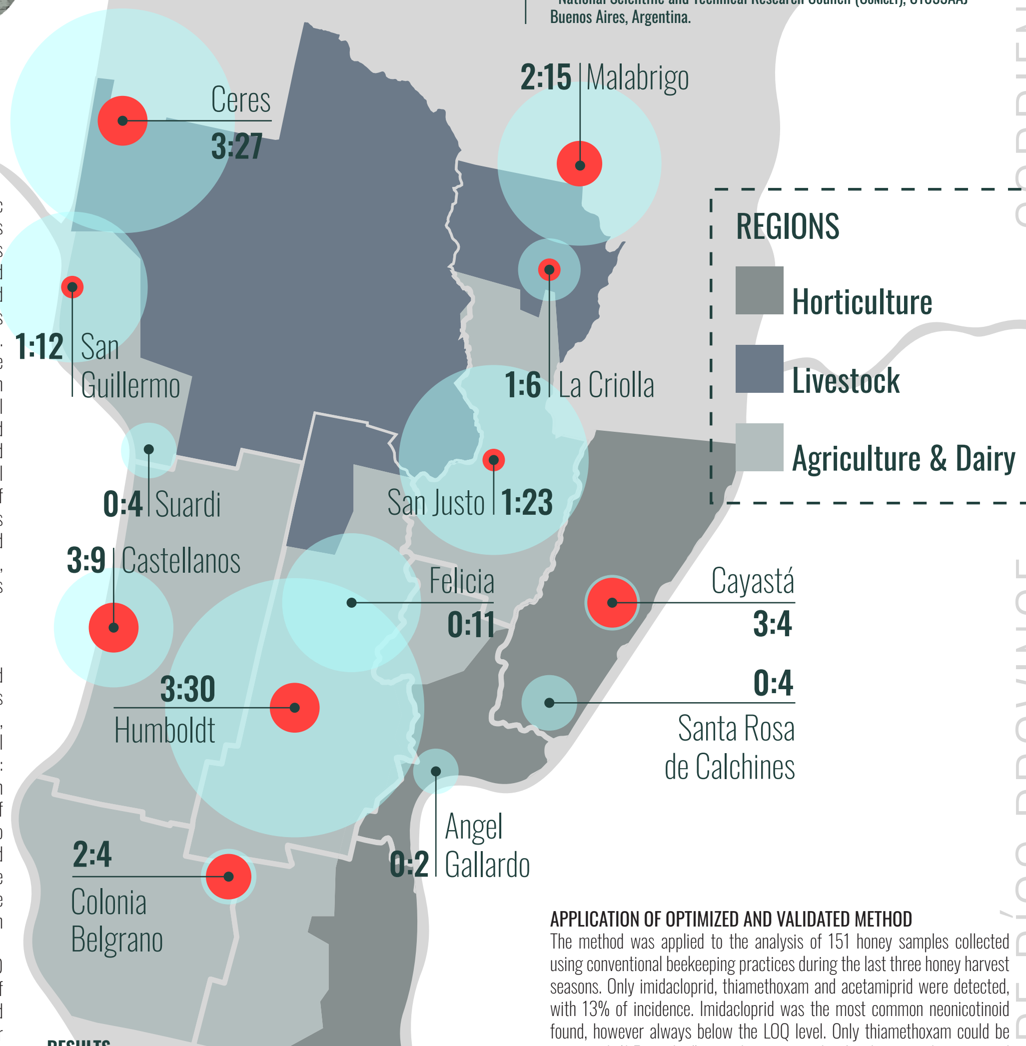

INTRODUCTION_

Due to the great versatility of neonicotinoid applications for the systemic protection of crops against pests and the great target specificity towards invertebrates, this kind of insecticide is widely used nowadays. Several studies have shown that neonicotinoids translocate to the nectar and pollen of treated plants, which represents a potential risk to pollinators, and could also end up in honey produced by bees. Thus, a QuEChERS-based methodology was optimized to evaluate the content of neonicotinoid residue in honey samples. A total of 151 samples from apiaries located in the central strip of Santa Fe province and 28 samples from the local market were analyzed. Although in this area three ecotypes are recognized: the plain zone, in which natural and implanted pastures prevail; the forest area, with arboreal, shrub and herbaceous species; and the coastal area, bordering the Paraná River and its tributaries, with varied vegetation and horticultural production (central figure); Santa Fe stands out for being placed in the most fertile region of Argentina and plays a leading role in agriculture and livestock. This was reflected in the palynological test, where 27 samples were randomly selected and submitted to that analysis, resulting in each sample monofloral honey, with a 77% predominance of clover honey[1]. Interestingly, one honey was composed of 50% soybean pollen (Glycine max L.).

MATERIALS AND METHODS_

For the analytical residue procedure, a two-step experimental design method was carried out to determine the optimal extraction and cleanup conditions for eight neonicotinoids (acetamiprid, clothianidin, dinotefuran, flonicamid, imidacloprid, nitenpyram, thiacloprid and thiamethoxam). Firstly, a two-level fractional design was carried out considering seven independent variables: temperature, pH and time for the sample soaking stage, the concentration of magnesium sulfate and sodium chloride to improve the extraction of the analytes, as well as the amount of C18 and PSA sorbents, were also investigated. After the screening stage, a Box-Behnken design was performed composed of the three significant variables: soaking time and pH, and the amount of PSA. The factorial design methodology combined with the surface response analysis allowed to optimize the process for the efficient extraction of neonicotinoid compounds from honey.

Briefly, the final methodology was comprised of 5 g of sample soaked in 10 mL of Milli-Q water for 40 – 45 min. Followed by the addition of 10 mL of acetonitrile and the mix by vortex for 30 s. The partitioning step was carried out by adding salts of $MgSO_4$ and NaCl (2 and 1 g, respectively). The sugar content of the honey facilitated the saturation of the aqueous phase and allowed the partition using less salt compared to the QuEChERS methodology applied for other matrixes. The tubes were shaken by hand for 2 min and centrifuged for 5 min at 2500 rpm. The dSPE was provided by adding 300 mg of $MgSO_4$, 50 mg of PSA and 100 mg of C18 to 2 mL of the organic layer. The tubes were shaken by hand for 1 min and centrifuged for 5 min at 2500 rpm. The extracts were evaporated to dryness, reconstituted with an aqueous phase plus internal standard, and finally injected in a UHPLC-MS/MS system.

UHPLC System: Waters Acquity UPLC®
Mobile phase A (water): 2% methanol and 5 mM ammonium formate with 0.1% formic acid
Mobile phase B (methanol): 5 mM ammonium formate with 0.1% formic acid
Mode: gradient
Flow: 0.35 mL/min
Injection volume: 10 µL
Column: Acquity UPLC®BEH C18 Shield (100 × 2.1 mm × 1.7 µm)
Column temperature: 40 °C

Waters TQD System
Function: MRM ESI+
Source temp.: 120 °C
Capillary voltage: 2.5 kV
Cone gas flow: 150 L/h
Desolvation gas flow: 700 L/h
Desolvation temp.: 380 °C

Software: MassLynx 4.1

Compound	Precursor (m/z)	CV (V)	Dwell (s)	Fragment I (m/z)	CE (eV)	Fragment II (m/z)	CE (eV)
acetamiprid	223	28	0.02	126	20	56	15
clothianidin	250	18	0.03	169	12	132	18
dinotefuran	203	20	0.05	157	8	129	12
flonicamid	230	30	0.05	203	18	148	25
imidacloprid	256.1	25	0.05	209.1	15	175.1	17
nitenpyram	271.1	25	0.05	224.9	12	125.9	25
thiacloprid	253	35	0.02	126	20	90	40
thiamethoxam	292	22	0.05	132	22	181	12
bendiocarb	224.1	20	0.05	167	8	109	18

Quantitation (precursor $[M+H]^+$ > fragment I) and confirmation transitions (precursor $[M+H]^+$ > fragment II), Cone Voltage (CV) and Collision Energy (CE). Bendiocarb was used as an internal standard (ISTD).

RESULTS_

EXPERIMENTAL DESIGN AND MULTIPLE RESPONSE OPTIMIZATION[2]

From the screening step, three factors were identified with significant effects on the responses: the soaking time and pH, and the PSA amount. Subsequently, a Box-Behnken design was applied to evaluate the significance of these variables by using the desirability function, which allows the optimization of multiple responses by fitting the experimental data (area of each analyte relative to the ISTD) in an appropriate model to generate a response surface. Seven kinds of adjusted models, including linear model, linear with interaction factors and quadratic, were obtained and the responses of the less sensitive neonicotinoids were maximized. The suggested optimal conditions were then experimentally corroborated and used for the development of the analytical method.

VALIDATION

Afterward, the proposed method was validated following the SANTE 12682/2019 guidance document[3]. With satisfactory results: recoveries between 79-120% (RSD ≤ 20%) for the three spiked levels 0.25, 1 and 5 µg kg^{-1}; LOQs of 0.25 µg kg^{-1} to acetamiprid and thiacloprid and 1 µg kg^{-1} for the remaining neonicotinoids.

Neonicotinoid	Linear range (µg kg^{-1})	LOQ (µg kg^{-1})	Recovery rate (%) LOQ level (n=5) (RSD)	Recovery rate (%) 4-5 x LOQ level (n=5) (RSD)	ME (%)
acetamiprid	0.25 – 2	0.25	120 (17)	120 (7)	-12
clothianidin	1 – 10	1.00	111 (13)	113 (7)	-11
dinotefuran	1 – 10	1.00	97 (15)	98 (12)	13
flonicamid	1 – 10	1.00	116 (10)	118 (9)	-25
imidacloprid	1 – 10	1.00	111 (10)	98 (4)	25
nitenpyram	1 – 10	1.00	79 (20)	118 (5)	26
thiacloprid	0.25 – 2	0.25	117 (15)	114 (9)	24
thiamethoxam	1 – 10	1.00	113 (11)	95 (5)	8

Method performance and validation: range of linearity (5 levels), limits of quantitation (LOQ), recoveries (%) and repeatability through relative standard deviation (RSD) and matrix effect (ME%).

APPLICATION OF OPTIMIZED AND VALIDATED METHOD

The method was applied to the analysis of 151 honey samples collected using conventional beekeeping practices during the last three honey harvest seasons. Only imidacloprid, thiamethoxam and acetamiprid were detected, with 13% of incidence. Imidacloprid was the most common neonicotinoid found, however always below the LOQ level. Only thiamethoxam could be quantified (1.5 µg kg^{-1}) in only one sample. Another sample presented imidacloprid plus thiamethoxam residues but <LOQ.

In a commercial honey, was detected clothianidin residues. Since thiamethoxam is transformed to clothianidin in soil, insects and plants this result may be due to the application of either neonicotinoid.

CONCLUSION_

Through the design of experiments, it was possible to reduce the consumption of salts and sorbents during the processing of the samples. Both heating and acidification of the soak solution were not necessary. Acidification, perhaps due to the natural acidity of honey, with pH values ranging from 3.4 to 6.1 [4]. Acceptable recovery performance and low matrix effect were achieved. Although neonicotinoid concentrations were low (<1.5 µg kg^{-1}), suggesting no human risks, they may pose a potential risk to honeybees through chronic exposure.

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