QuEChERS methodology combined with high performance liquid chromatography-UV/DAD for the determination of common pesticides in honey

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Abstract
Over the past few decades, honey products have been found to contain residues of a variety of contaminants. Among them pesticides residues resulting from treatments applied either inside beehives or in the agricultural environment have become an issue of great awareness. In this work, a modified QuEChERS (quick, easy, cheap, effective, rugged and safe) extraction method is proposed for the simultaneous quantification of amitraz, bromopropylate, coumaphos and tau-fluvalinate, which are the most frequently used insecticides to control varroatosis and ascospherosis in hives. Analyses were performed by high performance liquid chromatography on a C18 reversed-phase column with UV/diode array detection (HPLC-UV/DAD). An isocratic elution system was used with acetonitrile-water (80:20 v/v) containing 0,01 M acetic acid as the mobile phase while the selected compounds, amitraz, bromopropylate, coumaphos and τ-fluvalinate were detected at 249, 233, 313 and 254 nm, respectively. Overall recovery rates from honey samples ranged from 78% (bromopropylate) to 103% (amitraz), with correlation coefficients >0,99 in all cases. The proposed methodology was successfully applied to the analysis of 19 commercial honey samples.
Keywords: Honey, QuEChERS, HPLC-UV/DAD, insecticides, residues.

1. Introduction

It is known that honey bee populations are in declining health in Europe and North America (Mutinelli et al., 2010, Seitz et al., 2016). It is likely that these colony losses result from a combination of multiple factors, including diminished wildflower diversity and habitat, exposure to pesticides, and numerous diseases and parasites (Williamson et al., 2014). Nowadays, the parasitic mite Varroa destructor is considered one of the main concerns for apiculture worldwide (Rosenkranz et al., 2010). To this day, the most common practice to control Varroa is the use of in-hive acaricides (Ruffinengo et al., 2014, Mullin et al., 2010). Despite the often efficient Varroa control promoted by these chemicals, innumerous side effects have been observed. Two acaricides in particular, tau-fluvalinate and coumaphos, were ubiquitously prevalent in colonies and are frequently found at high concentrations (Mullin et al., 2010). Since the half-life of tau-fluvalinate and coumaphos is 5 years in wax (Bogdanov 2004), these pesticides can easily accumulate in colonies to reach unsafe levels (Mullin et al., 2010, Williamson et al., 2014). Therefore, in the present study a method based on QuEChERS and analysis by high performance liquid chromatography on a C18 reversed-phase column with UV/ diode array detection (HPLC-UV/DAD) for the determination of amitraz, bromopropylate, coumaphos and tau-fluvalinate, was used.

2. Materials and Methods

2.1. Chemicals and Materials

All compounds were obtained from Sigma-Aldrich with purity higher or equal than 97% (St. Quentin Fallavier, France), amitraz 98.8%, bromopropylate 99.2%, coumaphos 99% except tau-fluvalinate 93.4%. Stock standard solutions of each compound at 1000 mg L^{-1} were prepared in methanol and stored at -18 ^{o}C. A mixture of these standards at 10 mg L^{-1}, prepared in methanol, stored at -18 ^{o}C was stable for at least 6 months. LC-MS methanol was obtained from Fluka (Sigma-Aldrich). The water used was purified by a Milli-Q water system (Millipore, France). “Citrate QuEChERS kits” were obtained from Agilent Technologies: salts are packaged separately and consist in 4 g of anhydrous $MgSO_4$, 1 g of sodium chloride, 1 g sodium citrate dihydrate and 500 mg of disodium citrate sesquihydrate. Fifteen mL centrifuge tubes of PSA were purchased from Carlo-Erba: PSA tubes contain 150 mg of anhydrous $MgSO_4$, 25 mg of PSA bonded silica.

2.2. Collection of honey samples

Various Greece commercial honeys from 8 different botanical origins (thyme, conifers, fir, orange, citrus, pine tree, herbs and multi-flowers) were purchased. These 8 honey types were used to validate the methods and to check the robustness of the methods.
A total of 22 different honey samples (including samples from organic production) were analyzed. These

samples were purchased from different local markets (Ioannina, Greece) and all of them were analyzed following the procedure described in section 2.3. Those samples showing the absence of the target compounds were used as blank samples during the optimization and validation of the method. In order to avoid errors and ensure the reliability of the results, an internal control was carried out. This internal control based on the use of a blank extract that eliminated false positives caused by a contamination in the extraction procedure.

2.3. Sample preparation procedure

The protocol for the sample preparation was designed for a mass of 5 g of honey. The samples were mixed with 10 mL water and 10 mL acetonitrile, and vortexed. Next, the extraction salts (4 g MgSO4, 1 g NaCl, 1 g sodium citrate dihydrate and 0,5 g disodium citrate sesquihydrate) were added to the samples. The mixtures were vigorously shaken for 3 min and then centrifuged for 5 min at 4,000×g. The supernatant (2 mL) was recovered, and purification salts (0,15 g MgSO4 and 0,025 g PSA) were added. After vigorous shaking for 3 min, the mixture was centrifuged for 5 min at 4,000×g. The supernatant (2 mL) was recovered and evaporated to dryness under nitrogen. Finally, re-disolved in 1 mL methanol for HPLC-UV/DAD (inject 20 μL) analysis.

3. Results and Discussion

Linearity range was checked by an eight calibration curve (10-1000 ppb) by fortifying commercial honeys with standard solutions. It is noteworthy that all honeys were checked for an absence of contamination with the considered pesticides. The LOD ranged from 10 to 20 μg kg^{-1} and the LOQ ranged from 35 to 65 μg kg^{-1}. The recoveries calculated using the matrix-matched calibration curves were in the range of 78-103%. These results showed that the method was very sensitive and enabled the detection and quantification of all of the substances investigated in the honey that have toxicological relevance at very low concentrations. The developed analytical procedure was applied to pesticide residues determination in 22 honey samples. The most commonly found pesticides were coumaphos, followed by amitraz. The residue contaminations were correlated to agriculture and apiculture practices and the pollution may pose a threat to human health.

4. Conclusions

The proposed analytical method was proved to be very simple, rapid, consuming small amount of organic solvent, and consequently producing little hazardous waste. Due to high accuracy and precision, the method may be easily implemented in routine determinations of pesticide residues in honeys.

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