

Be(e) wary - determination of neonicotinoid insecticides in honey

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SUMMARY

Neonicotinoids are active substances used in plant protection products to control harmful insects. They are systemic pesticides, which means that they are taken up by the plant and transported through its leaves, flowers, roots, and stems, as well as pollen and nectar. Neonicotinoids affect the central nerve system of insects, leading to eventual paralysis and death [1]. Three honey samples from different sources were analysed for neonicotinoid content according to current regulating guidelines via a fast and simple HPLC method.

INTRODUCTION

Neonicotinoids are one of the most widely used classes of pesticides [2]. Five neonicotinoid insecticides are approved as active substances in the EU for the use in plant protection products, namely clothianidin, imidacloprid, thiamethoxam, acetamiprid, and thiacloprid [1]. They are closely monitored by the European Commission. Because of the potential risk for bees, the use of three of the substances (imidacloprid, clothianidin, thiamethoxam) was restricted in 2013 (see Regulation (EU) No 485/2013) [3]. In April 2018, the European Commission banned these three neonicotinoids for the outdoor use and only the permit for usage in permanent greenhouse remains [4]. For acetamiprid the EFSA established a low risk to bees.

A ban or further restrictions of this substance are neither scientifically nor legally appropriate. The fifth neonicotinoid, thiacloprid, is a candidate for substitution based on its endocrine disrupting properties [3]. In this application clothianidin, thiamethoxam, imidacloprid, and acetamiprid in honey samples are determined referring to the maximum residue levels which are specified in Commission Reg. (EU) 2017/671 [5], Commission Reg. (EU) 491/2014 [6] and Commission Reg. (EU) 2017/626 [7]. Three different honey samples have been tested. One of the samples was the KNAUER honey, produced from a bee colony located in the KNAUER garden. The other ones were commercially available canola honey and fruit blossom honey.

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RESULTS

A reversed phase method was developed where the four neonicotinoids are baseline separated. The method was optimized regarding temperature and gradient slope using DryLab simulation software. A calibration in a range from 0.5 $\mu\text{g/mL}$ to 10 $\mu\text{g/mL}$ was prepared. **Fig 1** shows the separation of a mixed standard at a concentration of 10 $\mu\text{g/mL}$. The calibration showed a good linearity and all correlation coefficients are calculated as $R^2 > 0.9996$. Based on the measurement at a concentration of 0.5 $\mu\text{g/mL}$ the LOD and LOQ were calculated. The calculated values for the single compounds are summarized in **Tab A1** (additional information). Sample preparation was carried out using a citrate-buffered QuEChERS

extraction. The recovery rate including sample preparation was determined at three different levels: LOQ, 2 x LOQ, upper end of calibration. For the compounds following recovery rates were calculated (averaged values over all levels): clothianidin 87 %, thiamethoxam 91 %, imidacloprid 92 % and acetamiprid 95 %. Furthermore, three different honey samples were analyzed regarding neonicotinoids. **Fig 2 to 4** show the chromatograms of the QuEChERS extracted and cleaned samples. In one of three samples residues of clothianidin were detected but they were in the range of limit of detection and hence far below the maximum residue level of 0.05 mg/kg for honey and other apiculture products [8].

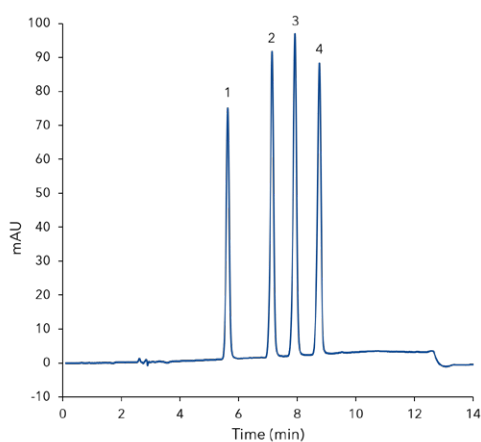


Fig. 1 Chromatogram of mixed standard at 10 $\mu\text{g/mL}$, 1) thiamethoxam, 2) clothianidin, 3) imidacloprid, 4) acetamiprid

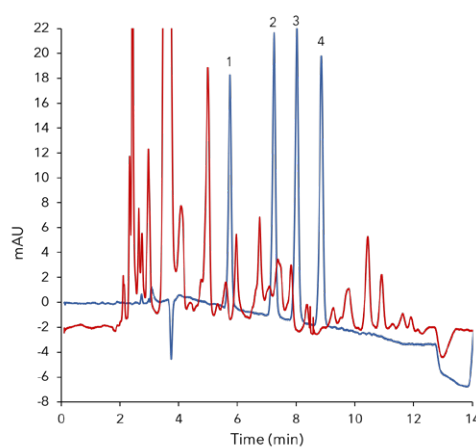


Fig. 2 Overlay of standard at 2.50 $\mu\text{g/mL}$ (blue) and cleaned sample of fruit blossom honey (red)

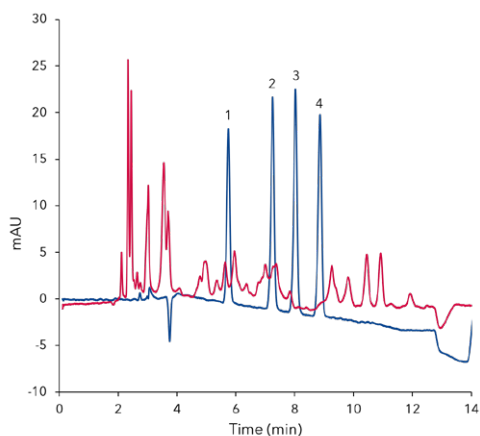


Fig. 3 Overlay of standard at 2.50 $\mu\text{g/mL}$ (blue) and cleaned sample of KNAUER honey (red)

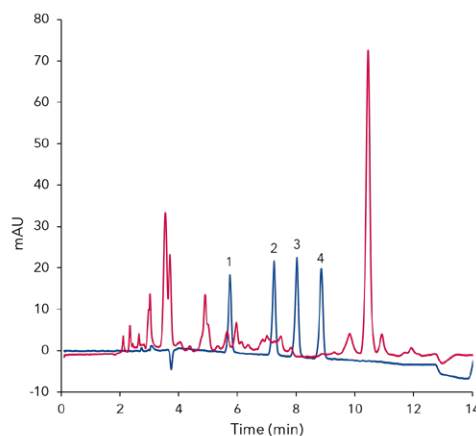


Fig. 4 Overlay of standard at 2.50 $\mu\text{g/mL}$ (blue) and cleaned sample of fruit blossom

MATERIALS AND METHODS

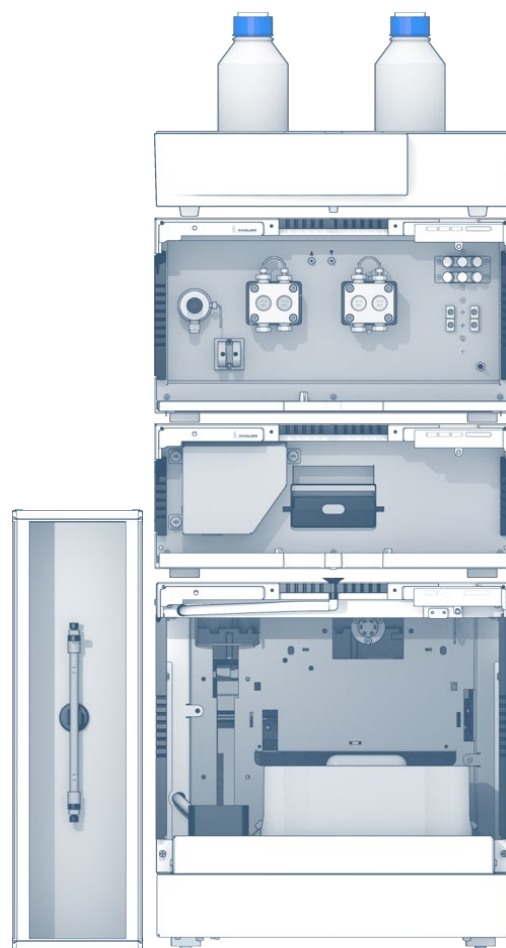
The application was performed on an AZURA HPLC Plus System equipped with an AZURA P 6.1L HPG pump, AZURA CT 2.1 column thermostat, AZURA autosampler AS 6.1L and AZURA DAD 6.1L detector. The mobile phase was a composition of acetonitrile and water, both containing 0.1 % formic acid. The gradient method has a total run time of 15 minutes including equilibration. The flow rate was set to 1 mL/min. Temperature was set to 30 °C and detection was carried out at 260 nm with a data rate of 20 Hz. For the sample preparation BEKOlut QuEChERS Citrate-Kit-01 and PSA-Kit-02 were used. The QuEChERS extraction protocol is described in the additional results section. The used column in a dimension 250 x 4.6 mm ID was filled with Eurospher II 100 5 C18P silica.

REFERENCES

- [1] https://ec.europa.eu/food/plant/pesticides/approval_active_substances/approval_renewal/neonicotinoids_en
- [2] Codling, G., et al. Concentrations of neonicotinoid insecticides in honey, pollen and honey bees (*Apis mellifera* L.) in central Saskatchewan, Canada, *Chemosphere*, Volume 144, 2016, Pages 2321-2328, ISSN 0045-6535, <https://doi.org/10.1016/j.chemosphere.2015.10.135>.
- [3] https://ec.europa.eu/food/plant/pesticides/approval_active_substances/approval_renewal/neonicotinoids_en
- [4] Official Journal of the European Union, L 132, 30 May 2018, <https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=OJ:L:2018:132:TOC>
- [5] Commission Regulation (EU) 2017/671 of 7 April 2017 amending Annex II to Regulation (EC) No 396/2005, <http://data.europa.eu/eli/reg/2017/671/oj>
- [6] Commission Regulation (EU) No 491/2014 of 5 May 2014 amending Annexes II and III to Regulation (EC) No 396/2005, <http://data.europa.eu/eli/reg/2014/491/oj>
- [7] Commission Regulation (EU) 2017/626 of 31 March 2017 amending Annexes II and III to Regulation (EC) No 396/2005, <http://data.europa.eu/eli/reg/2017/626/oj>
- [8] Regulation (EC) No 396/2005 of the European Parliament and of the Council of 23 February 2005, <http://data.europa.eu/eli/reg/2005/396/oj>

CONCLUSION

Using QuEChERS extraction for sample preparation makes the handling of samples very easy and reduces time compared to e.g. solid phase extraction. Fortunately, neither the KNAUER honey nor the other tested samples were contaminated with neonicotinoids. Although banning neonicotinoids for the outside use, monitoring them is still mandatory. The developed method is suitable for quality control of honey or other apiculture products.



ADDITIONAL RESULTS

Tab. A1 LOD and LOQ values for single compounds

| Compound | LOD (S/N=3) in ng/mL | LOQ (S/N=10) in ng/mL |
|--------------|----------------------|-----------------------|
| Thiamthoxam | 52.5 | 175.1 |
| Clothianidin | 48.2 | 160.6 |
| Imidacloprid | 45.8 | 152.5 |
| Acetamiprid | 49.6 | 165.3 |

ADDITIONAL MATERIALS AND METHODS

Tab. A2 Method parameters

| | | | |
|--------------------|--|------------------|-----------|
| Eluent A | d_d H ₂ O + 0.1 % formic acid | | |
| Eluent B | Acetonitrile + 0.1 % formic acid | | |
| Flow rate | 1 mL/min | | |
| Pump program | Time (min) | % A | % B |
| | 0 | 75 | 25 |
| | 8 | 65 | 35 |
| | 10 | 65 | 35 |
| | 10.02 | 75 | 25 |
| | 15 | 75 | 25 |
| Column temperature | 30°C | Injection volume | 5 µL |
| Injection mode | Partial loop | Detection | UV 260 nm |
| Data rate | 20 Hz | Time constant | 0.05 s |

Tab. A3 Sample preparation

| Extraction | |
|------------|--|
| Step 1 | Weigh 10 g of honey sample into a 50 ml falcon tube |
| Step 2 | Add 10 mL of deionized water, shake until honey is dissolved |
| Step 3 | Add 10 mL of acetonitrile |
| Step 4 | Add the contents of the BEKOLut Citrate-Kit-01 and shake for 1 minute |
| Step 5 | Centrifuge samples at 4000 x g for 5 minutes |
| Clean-up | |
| Step 1 | Transfer 3 mL of supernatant into a BEKOLut PSA-Kit-02 dispersive SPE tube |
| Step 2 | Vortex the samples for 30 seconds |
| Step 3 | Centrifuge samples at 4000 x g for 5 minutes |
| Step 4 | Transfer purified supernatant into an appropriate vessel/vial |

Tab. A4 System configuration & data

| Instrument | Description | Article No. |
|-------------------|--|--|
| Pump | AZURA P6.1L, HPG | APH35EA |
| Autosampler | AZURA AS 6.1L | AAA00AA |
| Detector | AZURA DAD 6.1L | ADC11 |
| Flow cell | High Sensitivity KNAUER LightGuide UV Flow Cell Cartridge | AMD59XA |
| Column thermostat | AZURA CT 2.1 | A05852 |
| Column | KNAUER Vertex Plus column, Eurospher II 100-5 C18P, 250 x 4.6 mm ID | 25EE182E2J |
| Software | ClarityChrom 7.4.2 - Workstation, autosampler control included ClarityChrom 7.4.2 - PDA extension | A1670 A1676 |

RELATED KNAUER APPLICATIONS

VFD0161 - Determination of sugars in honey using HILIC separation and RI detection

VFD0169 - Determination of sugars in honey - comparison of refractive index and light scattering detection

VEV0012J - Determination of Carbamate Insecticides by HPLC with post-column derivatization