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Miniaturized multiresidue method for determination of 267 pesticides, their metabolites and polychlorinated biphenyls in low mass beebread samples by liquid and gas chromatography coupled with tandem mass spectrometry

Tomasz Kiljanek *, Alicja Niewiadowska, Marta Małysiak, Andrzej Posyniak

Department of Pharmacology and Toxicology, National Veterinary Research Institute, Aleja Partyzantów 57, 24-100, Puławy, Poland

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ABSTRACT

Current work presents developed and validated miniaturized method for residue analysis of 261 pesticides and their metabolites as well as 6 congeners of non-dioxin like polychlorinated biphenyls (ndl-PCB) in a very low mass beebread sample. Sample preparation is based on modified QuEChERS protocol with all steps miniaturized to enable multiresidue analysis of sample with extremely low weight. Sample of beebread (0.3 g) was extracted with 1 mL of acetonitrile containing 5% formic acid and ammonium formate salt were added, then extract was subjected to clean-up by freezing and two-step dispersive solid phase extraction (dSPE) with a Supel QuE Verde sorbents (Supelclean ENVI-Carb Y; Supelclean PSA; Z-Sep+; magnesium sulfate). After 1st step dSPE a portion of extract was analyzed by liquid chromatography tandem mass spectrometry (LC-MS/MS) for 200 pesticide residues. Remaining extract was subjected to 2nd step dSPE clean-up by another Supel QuE Verde and then after concentration and solvent exchange it was analyzed by gas chromatography tandem mass spectrometry (GC-MS/MS) for another 61 pesticide and 6 ndl-PCB residues. Method enables determination of residues of 101 insecticides, 72 herbicides, 67 fungicides, 10 acaricides, 6 growth regulators, 5 veterinary drugs and 6 ndl-PCB's. Particular attention was paid to the pesticides being active substances of plant protection products recommended for the protection of winter oilseed rape and apple orchards which during their blooming periods are one of the most attractive sources of food for pollinators and could serve as representatives of other economically important crops. Method was validated according to the Guidance document SANTE/12682/2019 at six concentration levels from 0.001 to 0.5 mg kg^{-1}. The analysis of beebread samples spiked at the level of 0.01 mg kg^{-1}showed mean recovery (trueness) value of about 98% and RSDr (precision) below 20%. The small weight of the sample did not adversely affect the limits of quantification and 75% of analytes could be quantified at least at concentration of 0.005 mg kg^{-1}. Developed mini-method was tested in the analysis of beebread samples, each extracted from individual cell of honeycomb. It is the first time when analyses at single comb cell level were possible.

1. Introduction

Beebread is pollen collected by foraging bees, mixed with nectar and honey bee secretions and stored in comb cells. Foraging bees can overcome a distance of 3 km from hive or even further to collect pollen which is then transformed into beebread. Beebread is the bees' main source of proteins, minerals, fats and other substances [1]. Beebread is an essential source of food not only for honeybees, but also for bumblebees and solitary bees which store pollen in their nests. The main contaminants of pollen are pesticides [1]. Exposure through contaminated pollen is considered pivotal as it presents the highest risk of pesticide exposure across all bee species [2]. In consequence contaminated beebread could be one of the main sources of dietary exposure of bees to pesticides. Adult nurse honeybee consume up to 12 mg of pollen per day, an adult solitary bee consumes 10.2 mg of pollen per day whilst an adult bumblebee consumes 30.3 mg of pollen per day [3]. Long-lived winter bees can consume even 240 mg of beebread over 120 days winter period [4]. Bumblebee queen consumes 279.2 mg of pollen through six day

* Corresponding author.
E-mail address: tomasz.kiljanek@piwet.pulawy.pl (T. Kiljanek).

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period of her life [3]. However, the actual risk that multiple pesticide residues might pose to non-target bee species is difficult to assess due to the lack of clear evidence of their actual concentrations [2].

Assessment and understanding of bee's exposure to pesticides is essential for a protection of bee health. Two sources of exposure should be taken primarily into account: pesticides used in agriculture as plant protection products, and pesticides intentionally introduced into hives by beekeepers as varroacides. Wide range of applied substances is the reason for a development of multi-residue method with wide scope of pesticides analyzed in beebread. Appropriate analytical methods should overcome the difficulties related not only to complexity of the matrix, potential interferences, high number of compounds and low levels of their concentration but also low sample weight, especially in case of pollen stored by bumblebees and solitary bees. Beebread has so far been insufficiently analyzed despite its ability to store pesticides for long periods of time [5]. There is also a need for the validated detection methods during monitoring and exposure assessment [6]. The range of analyzed compounds should be expanded, especially to fungicides and herbicides [2].

QuEChERS is nowadays the most universal sample preparation method, that allows simultaneous determination of a large number of pesticides in a sample [7]. QuEChERS method was already used in studies in which level of beebread contamination with pesticides was studied. The range of analytes was very diverse, from neonicotinoids analysis during field studies [8,9] through honeybee exposure assessment with selected pesticides [10–12] up to the analysis of a wide scope of compounds [13–15]. Analysis of a wide range of pesticides is possible when both LC-MS/MS and GC-MS/MS techniques are used, however few such methods have been published until now [14,16]. Analysis of sample with two different chromatographic instruments requires sufficient sample quantity in order to prepare two extracts of one sample or even to conduct two separate sample preparations. In each case, the constraints of the extraction steps, purification, or the need to concentrate the extracts should be taken into account. Already used methods of analysing pesticides in beebread requires sample portions from 1.5 up to 10 g weight [14,17]. Multi-residue method in which beebread sample is analyzed by both LC-MS/MS and GC-MS/MS enable analysis of 112 pesticides in 2 g sample [16] or 322 veterinary drugs in 10 g sample [14]. The analysis of pesticides in beebread is a compromise between the range of the analyzed compounds and sensitivity of their determinations and the amount of material available for sample preparation. Even in case of honeybee studies beebread availability was sometimes limited, especially in case of colony collapse disorder (CCD) affected honeybee colonies [4,18]. Amount of beebread in honeybee hive is however huge in comparison to quantity of pollen stored in Bombus or Osmia nest.

The aim of this study was to develop and validate miniaturized analytical method for a determination of multiple pesticides in as low as possible weight of beebread or pollen store sample which can be analyzed by both LC-MS/MS and GC-MS/MS with lowest limits of quantification. Particular attention was carried out for pesticides which are the active substances of products listed in recommendations for the protection of winter oilseed rape and apple orchards. These two economically important crops during their blooming periods are one of the most attractive sources of pollen and nectar for pollinators. Such a miniaturized and multiresidue method will be next used in the first pan-European quantification of the exposure of pesticides to managed and wild bees.

2. Materials and method

2.1. Reagents

Certified pesticide standards (purity 94–99%) were obtained from Dr. Ehrenstorfer brand of LGC Standards (Augsburg, Germany), Toronto Research Chemicals (Toronto, Canada) and Sigma Aldrich brand of Merck (Seelze, Germany). Individual stock solutions of pesticides at concentrations of 250–1500 μg/mL were prepared in acetone, acetonitrile, methanol or dimethylformamide and stored in amber screw-capped glass vial at a temperature below −18 °C. Mixed standard solutions for validation and calibration were prepared by appropriate dilutions of stock standard solutions with acetonitrile. Internal standard spiking solution at concentrations of 0.5–50 μg/mL (imidacloprid-d_4, acetamiprid-d_3, carbendazim-d_3, clothianidin-d_3, thiamethoxam-d_4, chlorpyrifos-d_{10} and deltamethrin-d_5) in acetonitrile was also prepared.

Ultra Resi-Analyzed and LC-MS purity grade acetonitrile, acetone, n-hexane and methanol were supplied by J.T. Baker brand of Avantor Performance Materials (Deventer, The Netherlands). Dimethylformamide, ammonium formate, formic acid and Supel™ QuE Verde mini tube with sorbents (Supelclean™ ENVI-Carb™ Y, 10 mg; Supelclean™ PSA, 50 mg; Z-Sep+, 60 mg; magnesium sulfate, 150 mg) for clean-up step were obtained from Sigma Aldrich (Bellefonte, PA, USA). Deionized water was obtained by Milli-Q Plus system from Merc Millipore (Billerica, Ma, USA).

2.2. Sample preparation

A 0.3 g of beebread sample was weighted into a 5 mL centrifuge tube and a 10 μL of internal standard spiking solution was added. Then 2 glass beads and 0.7 mL of deionized water was added and sample was shaken in a MiniG mechanical disrupter with vertically movement platform (SPEX Sample Prep, Metuchen, NJ, USA) for 3 min. Then extraction was carried out with the use of 1 mL of 5% formic acid solution in acetonitrile and once more shaking. Afterwards 0.5 g ammonium formate salt for partitioning was added and the sample was shaken again. The sample was also centrifuged at 3500 rpm, −12 °C for 20 min. Subsequently supernatant was transferred into 5 mL centrifuge tube and freeze out at −45 °C for 40 min and afterwards centrifuged for 10 min. Then supernatant was transferred into 2 mL Supel™ QuE Verde mini tube for 1st step dSPE clean-up. The sample was shaken for 10 min and centrifuged using the same conditions as before. Afterwards 0.08 mL of extract was transferred into Nanosep MF centrifugal device with Bio-Inert membrane 0.2 μm (Pall, United States), filtered and transferred into injection vial with 0.1 mL insert for LC-MS/MS analysis. Remaining part of extract was transferred into another Supel™ QuE Verde mini tube for 2nd step clean-up, and the extract was shaken and centrifuged like in 1st step. Afterwards known volume of extract was evaporated to dryness under gentle stream of nitrogen and dissolved in 8-times less volume of hexane, filtered with Nanosep MF centrifugal device with Bio-Inert membrane 0.2 μm (Pall, United States) and transferred into injection vial with micro-insert for GC-MS/MS analysis. Scheme of miniaturized sample preparation procedure is presented in Fig. 1.

2.3. Analysis

2.3.1. LC-MS/MS

In this work Agilent series 1260 HPLC system equipped with a G4225A degasser, G1312A pump, G1367E autosampler, and G1330 thermostat was used (Waldronn, Germany). Chromatographic separation was performed on Luna 3 μm Phenyl-Hexyl 150 × 2.0 mm column (Phenomenex, Torrance, NJ, USA), with the use of water with 5 mM ammonium formate (pH = 6.0, adjusted by formic acid) and acetonitrile mobile phases. The flow rate was 400 μL min^{-1} and column was thermostated at the temperature of 50 °C. Gradient elution was applied with 95% water mobile phase initially hold for 1 min, decreased to 5% in 26 min and hold for 6.5 min, then increased to 95% and hold constant till the end of analysis. The injection volume was 2 μL. Total time of LC analysis was 40 min.

In this study AB Sciex QTRAP® 6500 LC–MS/MS system (Framingham, MA, USA) with Turbo Spray Ion Drive with positive and negative ionisation was used for the mass spectrometric analysis. The ion spray voltage was set 4500 V and −4500 V for positive and negative ionisation respectively. Source temperature was set at 550 °C. Nitrogen was used as

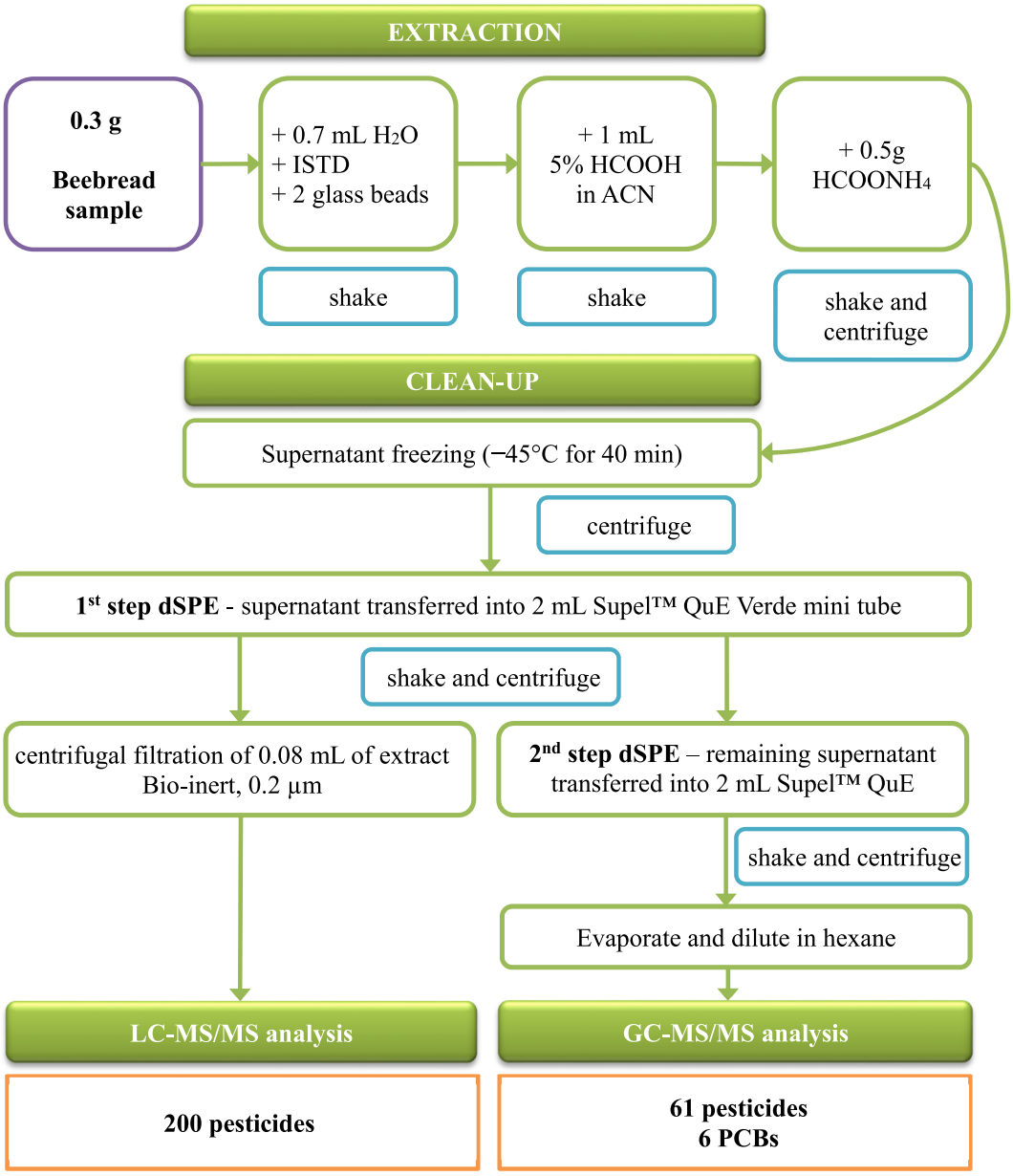

Fig. 1. Diagram of a miniaturized method of beebread samples analysis.

a curtain gas (45 psi), collision gas (medium), and ion source gases: nebuliser gas (50 psi) and heater gas (40 psi). Analyst 1.6.2 software was used to control LC−MS/MS system and for data acquisition. Quantitative and qualitative analysis was done with MultiQuant software version 3.0 based on two most intensive precursor ion-product ion MRM transitions. The values of the LC–MS/MS optimised parameters for each MRM transition are shown in Supplementary materials in Table A.1. Procedural standard calibration was used for calibration.

2.3.2. GC-MS/MS

The GC-MS/MS system was equipped with an Agilent gas chromatograph 7890A+ (Palo Alto, CA, USA), autosampler series 7693 B, split/splitless injector in pulsed splitless mode and tandem mass spectrometry detector 7000 B with electron impact type ionisation source. Chromatographic separation was performed on a HP-5 MS UI capillary column (30 m × 0.25 mm ID, 0.25 µm, Agilent Technologies, USA) with helium in constant flow rate 0.95 mL min^{-1} as a carrier gas. The injection volume was 1 µL. Oven temperature program was set as follows: initial temperature of 80 °C hold for 1 min, increased to 200 °C at 40 °C min^{-1}, increased to 210 °C at 2.3 °C min^{-1} and hold 5 min, increased to 266 °C at 3 °C min^{-1} and then finally increased to 320 °C at 10 °C min^{-1}. Analysis run time was 43 min. Other operating conditions were as follows: inlet temperature – 295 °C, transfer line temperature – 325 °C, source temperature – 300 °C, temperature MS1 and MS2 quadrupoles – 150 °C, collision gas (N_2) – flow 1.5 mL min^{-1}, quench gas (He) – flow 2.25 mL min^{-1}. GC-MS/MS system was controlled by Mass Hunter software version B.07.01. Quantitative and qualitative analysis was done also with Mass Hunter software based on two most intensive precursor ion-product ion MRM transitions. The values of the GC–MS/MS optimised parameters for each MRM transition are shown in Supplementary materials in Table A.2. Procedural standard calibration was used for calibration.

3. Method validation

Validation experiment was carried out according to requirements of SANTE/12682/2019 guidance to evaluate linearity, matrix effect, limit of quantification, specificity, trueness and precision [19]. The beebread

samples free from pesticide residues were used as blank, to spike aliquots for validation studies and to prepare procedural standard calibration. Linearity was determined at six concentration levels between 0.001 and 0.5 mg kg^{-1} by preparation of the procedural standards in duplicate. Matrix effect was examined by comparison of the slope received for procedural standard calibration curve and solvent standard calibration curve. The limit of quantification (LOQ) was the lowest concentration of compound that can be quantified with acceptable trueness and precision. The limit of detection (LOD) was the concentration at which the analyte could be detected and it corresponds to one-third of the LOQ value, provided that the signal-to-noise ratio was higher than or equal to 3. Beebread samples spiked with pesticides at the levels of 0.001, 0.005, 0.01, 0.05, 0.1 and 0.5 mg kg^{-1} with five replications for each level were used to evaluate trueness and precision.

3.1. Real samples

Developed and validated mini-method has been used in the analysis of 22 beebread samples taken out from a piece of honeycomb of 5 × 8 cm size. Each beebread sample was taken out from individual cell of honeycomb and each represents different cell. Samples weight ranges from 0.19 to 0.25 g that is less than default 0.3 g, which was individually compensated by addition of proportionally less volume of internal standards solution. Routine recovery checks with 0.2 g sample weigh spiked with pesticides at LOQ levels done in each batch of analyses meet the criteria of SANTE/12682/2019 document, ensuring quality of the results [19].

4. Results and discussion

4.1. Method development

The developed method is a modification of the QuEChERS method which enables adaptation of almost all stages of sample preparation procedure to specific needs resulting from the properties of the material to be tested, including its quantity. Current method enables analysis of samples weighing 0.3 g, which is few times less in comparison to already published methods of beebread analysis. Such a small analytical sample weight allows the analysis of samples taken not only from honeybees but also from other bee species, including solitary bees. Substantial improvements or advantages of developed method over existing methods were summarized in Table 1. Until now 2 g beebread sample was a minimum weight in case of both LC-MS/MS and GC-MS/MS multiresidue analysis [16]. Most methods required 2 – 5 g sample of beebread for the analysis of pesticide residues [8,21,22]. Already published micro-QuEChERS method to analyse insecticide residues in guttation fluid by LC-MS/MS requires 1 g of sample [23]. Other scaled down QuEChERS method enable analysis of 20 neonicotinoids and fungicides in 100 mg pollen sample [24].

As a consequence of miniaturized sample weight, also all subsequent stages of the analysis were miniaturized. Volume of extraction solution was reduced to 1 mL only. Mini tubes with sorbents were used for clean-up. Micro centrifugal filters enable filtering of the extract with a volume of several dozen microliters and minimize its losses during this step.

At the QuEChERS extraction step there is a choice between the uses of buffered or non-buffered version. In this study formate buffering was used during the extraction of pesticides from beebread matrix. Acetonitrile with formic acid and ammonium formate salt ensures appropriate extraction conditions of acidic and basic compounds. Many pesticides permitted to use as plant protection products are compounds with acid or base properties and thus require specific conditions of analysis. Our previous experience with the analysis of beebread has indicated the need to optimize the method in terms of the lowest possible contamination of mass spectrometers, especially GC-MS/MS, thus formate buffering was chosen. Formate buffer approach was successfully applied at the QuEChERS extraction step in the analysis of acidic and basic compounds in other matrices with mass spectrometry instead of magnesium sulfate and other non-volatile compounds for salting out use [25]. This is the first time when formate buffer approach was used in beebread analysis. Acetate buffered extraction conditions were the most widely used so far [10,11,21,22,26,27]. Citrate-buffered version of QuEChERS were used in much less number of beebread studies but among them are methods with both LC-MS/MS and GC-MS/MS analysis [8,14,16]. Extraction without buffering were used for the pesticide residue analysis in recently stored pollen taken from combs [12].

QuEChERS protocol enables different modifications of the clean-up step. In order to remove as much of the matrix components as possible before the dSPE step, the extract was first frozen at −45 °C for 40 min. In the freezing step, approximately 20% of the co-extractive materials present in the extract were removed. Freezing out was already used as beebread extract clean-up step [10,26]. Selection of optimal sorbents for dSPE is one of the most important modifications. Sorbents should enable the disposal of interfering substances from beebread matrix without the loss of analytes. In current study a ready-made mixture of clean-up sorbents – Supel QuE Verde was used. QuE Verde consists of Supelclean ENVI-Carb Y, Supelclean PSA, Z-Sep + sorbents and magnesium sulfate. This mixture has a composition that corresponds to that used by us in the analysis of bees (PSA with Z-Sep+) [28] but with addition of ENVI-Carb Y sorbent which is a form of graphitized carbon black (GCB) sorbent. PSA together with Z-Sep+ and ENVI-Carb Y sorbents provides the best possible clean-up of challenging matrix such as beebread. Supel QuE Verde was successfully used in the analysis of matrices with a high content of pigments or fats [29]. Beebread is a complex and difficult to

Table 1
Comparison of the developed method with the methods used so far in pesticide residue analysis of beebread samples in terms of the characteristics of individual stages of the analysis.

Characteristic of the method	Developed method	Existing methods
sample weight	0.3 g	1.5 g [17] 2 g [10,16,21,22,26] 3 g [11,13,21] 5 g [8,12,27] 10 g [14,15]
extraction	formate buffered QuEChERS	non-buffered QuEChERS [12] citrate buffered QuEChERS [8,14,16] acetate buffered QuEChERS [10,11,21,22,26,27] methods other than QuEChERS [15,17]
clean-up	freezing and dSPE + dSPE with Que Verde	freezing out [10,26] dSPE with PSA [10,16,26] dSPE with PSA and C18 [8,12,14,27] dSPE with PSA, C18 and GCB [22] SPE [15] SPE + SPE [11,17] dSPE + SPE [21]
instrumental analysis	LC-MS/MS and GC-MS/MS	LC-MS/MS [8,10–12,17,26,27] GC-MS/MS [20] LC-MS/MS and GC-MS [15] LC-MS/MS and GC-MS/MS [14,16]
number of analyzed compounds	261 pesticides + 6 ndl-PCBs	5 neonicotinoids [8,17] 13 neonicotinoids and pyrethroids [10,26] 25 pesticides [11] 63 pesticides [12] 93 pesticides [15] 112 pesticides [16] 173 pesticides [13] 200 pesticides [21] 322 veterinary drug residues [14]

analyse matrix containing various components like different proteins, carbohydrates, lipids and pigments. So far, no other method has used QuE Verde to clean-up beebread extracts. Previously published methods for dSPE clean-up of beebread samples use mainly PSA sorbent alone [10,16,26] or in mixture with C18 [8,12,14,27]. Till now only one method use GCB together with PSA and C18 for dSPE clean-up of beebread samples [22]. When other than QuEChERS extraction protocol were utilized then solid phase extraction (SPE) were used instead of dSPE for clean-up of beebread extracts [15]. Due to a complexity of beebread matrix some authors use two sequential SPE columns for clean-up [9,11] or use SPE as a further purification step following a dSPE [21]. So far, however, no one has used methods in which two dSPE were used successively for beebread clean-up. Such dual-dSPE clean-up was introduced as novel concept of clean-up in fish tissue analysis by GC-MS/MS [30]. Our experience shows that one step dSPE does not provide a satisfactory clean-up of the beebread extract for GC-MS/MS analysis and regular maintenance of the system was needed. A single dSPE removes approximately 50% of the co-extractive materials present in the extract after the freezing step. Too many co-extractive material still remain in the final extract when only one step dSPE was applied, which was observed as partial co-elution of MRM peaks of p,p'-DDD and o,p'-DDT and resulted as contamination of liner in the injector. In order to overcome this difficulties two-step dSPE with QuE Verde mini tube was incorporated for beebread clean-up. The second dSPE step removes approximately 85% of the co-extractive materials which remains in the extract after the first dSPE step. The entire procedure of extraction, freezing, and double dSPE removes approximately 99.8% of the matrix. Such sequential dSPE clean-up positively influenced GC analysis and LOQs of analytes without negative influence on planar pesticides, however, analysis of some LC amenable analytes was impossible. Double QuE Verde clean-up resulted in a lack of recovery of a number of LC compounds with acidic or basic properties such as bromoxynil, DMPF, MCPA, mesotrione, nitenpyram, propamocarb, propoxycarbazone sodium, prothioconazole, spiroxamine, sulcotrione and tembotrione. Finally beebread extract after freezing and 1st step dSPE with QuE Verde was subjected to LC analysis whilst after 2nd step dSPE with QuE Verde was subjected to GC.

In order to analyse 267 pesticides and contaminants in beebread it was necessary to use two high sensitive and selective techniques, both LC-MS/MS and GC-MS/MS. MRM transitions and MS/MS instrument parameters were optimised separately for each LC amenable compound: declustering potential (DP), collision energy (CE) and collision cell exit potential (CXP). Ion source parameters such as source temperature, capillary voltage and both GS1, GS2 gas flows were also optimised. In GC-MS/MS analysis precursor ion, product ions, collision energy (CE) and dwell time for each analyte were optimised.

The developed method is the first one which enables both LC-MS/MS and GC-MS/MS analysis of 261 pesticide residues and 6 congeners of ndl-PCBs in a sample as small as 0.3 g of beebread. This method enables analysis of pesticides from various category of use: 101 insecticides, 72 herbicides, 67 fungicides, 10 acaricides, 6 growth regulators and 5 veterinary drugs. List of compounds analyzed in beebread together with their category of use, pesticide approval status under Regulation (EC) No 1107/2009 [31] and inclusion in program for integrated pest management for apple trees or winter oilseed rape [32,33] and technique of instrumental analysis are listed in Supplementary materials in Table A.3.

5. Method validation

To confirm that the developed method of pesticide residue determination in beebread samples is suitable for its intended use the initial full validation according to SANTE/12682/2019 guidance was carried out. Validation results are listed in Table 2. Data obtained during the validation process meet the criteria of the SANTE document [19].

Developed method is sensitive and enables analysis at low concentration levels. The LOQ values have been established as follows: 0.001 mg kg^{-1} for 105, 0.005 mg kg^{-1} for 96, 0.01 mg kg^{-1} for 31, 0.05 mg kg^{-1} for 31 and 0.1 mg kg^{-1} for 4 compounds respectively. The LOD levels were in the range of 0.0003–0.033 mg kg^{-1}.

All studied compounds demonstrated good linearity up to the level of 0.5 mg kg^{-1}. Deviation of back-calculated concentration from true concentration was within the range ±20%. Obtained values of correlation coefficient (R^2) were higher than 0.99 for 74% of analytes and higher than 0.98 for all analytes.

Trueness and precision were evaluated using average recovery and repeatability (RSDr) calculated for each spike level tested. The majority of compounds (97%) showed recovery values within the recommended range of 70–120%. For some compounds the recovery rate was outside the recommended range. In 30 cases, recovery was below 70% and in 13 cases it was above 120%, but the RSDr values did not exceeded 20%. All compounds showed satisfactory precision and RSDr values did not exceeded 20% in all cases.

Matrix effect was negligible for majority of analytes, 52% of compounds showed matrix effect in the acceptable range ($-20\% < ME < 20\%$). Signal suppression was demonstrated for 33% of analyzed compounds, and enhancement for 15% of compounds. Procedural standard calibration was used to overcome and compensate matrix effects.

Developed method enables reliable and sensitive pesticide residue analysis in beebread samples. Results of beebread analysis allow assessing the oral exposure of bees to pesticides, including toxicity assessment on bee larvae.

5.1. Real samples

In order to show applicability and usefulness of developed miniaturized method 22 samples of beebread taken from adjacent cells of honeycomb piece were analyzed. Detailed results of each individual sample analysis are presented in Fig. 2. Diversity in pesticide contamination of beebread from adjacent comb cells was shown. Only one beebread sample was free from pesticide residues whilst others contained up to nine pesticides simultaneously. Three pesticides as median were detected in beebread samples. In total 14 pesticides were detected: azoxystrobin, boscalid, carbendazim, chlorpyrifos, cyprodinil, difenoconazole, dimethoate, fludioxonil, fluopyram, DMF, DMPF, pyraclostrobin, pyrimethanil and tau-fluvalinate. MRM chromatograms of individual beebread samples analyzed by GC-MS/MS and LC-MS/MS are shown on Fig. A.1 in Supplementary materials. Concentrations of determined pesticides are generally low but variability between individual cells is high. Diversity in single cells in terms of number of pesticides as well as in their concentrations could be especially important in case of most toxic pesticides, with great chronic oral toxicity.

To the best of our knowledge it is the first time when pesticide residue analyses at single comb cell level of resolution were possible. The aspect of the differentiation of pesticide residues in beebread from neighbouring cells has not been studied so far. The percentage of samples containing residues is high, but in line with already published data which showed that the extent of beebread contamination with pesticides could vary from 27% to even 100% of positive samples [4,34]. The results for the individual pesticides are consistent with the results of other authors. Tau-fluvalinate, DMF and chlorpyrifos were one of the most often determined pesticides in fresh stored pollen samples from Spain [12]. Similarly to our results DMPF was detected much less than DMF [12]. Chlorpyrifos or tau-fluvalinate were detected in 86% of tested beebread samples from normal honeybee colonies and 91% or 100% of samples from CCD-affected colonies, respectively [18]. Residues of fungicides like carbendazim, cyprodinil, fludioxonil, pyrimethanil, pyraclostrobin or azoxystrobin has also been previously determined in beebread samples [12,16,34].

Beebread samples should be first homogenated and analyzed by methods characterised by low limits of quantifications, whenever it is possible.

Table 2
List of compounds analyzed in beebread by LC-MS/MS or GC-MS/MS together with results of validation process (LOD, LOQ, linearity, recovery, precision and matrix effect).

Compound	LOD (mg/kg)	LOQ (mg/kg)	Technique	Recovery, % (RSDr, %)						ME, %
				0.001 mg kg^{-1}	0.005 mg kg^{-1}	0.01 mg kg^{-1}	0.05 mg kg^{-1}	0.1 mg kg^{-1}	0.5 mg kg^{-1}	
1-Naphthylacetamide (1-NAD)	0.0017	0.005	LC-MS/MS		74 (14)	101 (7)	94 (7)	104 (9)	90 (10)	−25
2,4-D	0.017	0.05	LC-MS/MS				83 (5)	114 (9)	73 (15)	−82
6-chloro-4-hydroxy-3-phenyl-pyridazine	0.0003	0.001	LC-MS/MS	116 (8)	89 (10)	89 (11)	89 (7)	103 (8)	85 (10)	−23
6-hydroxy bentazone	0.0003	0.001	LC-MS/MS	100 (10)	90 (19)	86 (14)	80 (4)	109 (8)	65 (11)	78
Acequinocyl	0.033	0.1	LC-MS/MS					120 (4)	108 (10)	16
Acetamiprid	0.0003	0.001	LC-MS/MS	110 (16)	100 (18)	94 (5)	87 (8)	105 (6)	91 (14)	27
Acetochlor	0.0017	0.005	LC-MS/MS		94 (17)	95 (16)	81 (11)	119 (15)	71 (7)	−6
Acrinathrin	0.017	0.05	LC-MS/MS				104 (18)	106 (14)	78 (20)	16
Aldrin	0.0003	0.001	GC-MS/MS	104 (14)	94 (11)	110 (12)	97 (8)	74 (16)	86 (8)	18
alpha-Endosulfan	0.0003	0.001	GC-MS/MS	106 (18)	85 (17)	92 (6)	80 (9)	108 (10)	69 (4)	−2
alpha-HCH	0.0003	0.001	GC-MS/MS	112 (16)	89 (8)	105 (7)	92 (9)	91 (17)	96 (8)	14
Amidosulfuron	0.0003	0.001	LC-MS/MS	66 (17)	99 (19)	131 (8)	138 (7)	118 (8)	106 (7)	−22
Asulam	0.0017	0.005	LC-MS/MS		96 (14)	88 (16)	71 (13)	107 (8)	82 (12)	−43
Azinphos-ethyl	0.0003	0.001	GC-MS/MS	87 (10)	80 (6)	109 (5)	119 (12)	102 (10)	77 (9)	161
Azinphos-methyl	0.0003	0.001	GC-MS/MS	84 (14)	75 (4)	121 (9)	130 (14)	107 (16)	75 (6)	116
Azoxystrobin	0.0003	0.001	LC-MS/MS	70 (20)	103 (11)	104 (13)	78 (6)	104 (18)	92 (15)	8
Bentazone	0.0003	0.001	LC-MS/MS	100 (12)	70 (15)	78 (15)	74 (7)	118 (8)	79 (8)	−17
beta-Endosulfan	0.0003	0.001	GC-MS/MS	106 (20)	85 (18)	88 (4)	84 (8)	110 (13)	78 (6)	56
beta-HCH	0.0003	0.001	GC-MS/MS	76 (17)	88 (11)	105 (12)	93 (17)	122 (18)	82 (9)	29
Bifenazate	0.0017	0.005	LC-MS/MS		109 (9)	97 (16)	71 (19)	101 (7)	82 (14)	−3
Bifenox	0.0017	0.005	GC-MS/MS		96 (20)	115 (7)	99 (9)	103 (7)	86 (11)	17
Bifenthrin	0.0003	0.001	GC-MS/MS	120 (10)	86 (12)	92 (11)	97 (9)	105 (7)	97 (15)	−53
Bixafen	0.0017	0.005	LC-MS/MS		84 (17)	104 (4)	98 (10)	115 (8)	72 (9)	6
Boscalid	0.0003	0.001	LC-MS/MS	80 (18)	104 (8)	101 (15)	72 (7)	114 (6)	72 (11)	5
Bromopropylate	0.0017	0.005	GC-MS/MS		110 (10)	118 (19)	84 (19)	76 (17)	72 (13)	64
Bromoxynil	0.017	0.05	LC-MS/MS				95 (6)	120 (7)	69 (5)	−38
Bupirimate	0.0003	0.001	LC-MS/MS	102 (14)	94 (10)	105 (12)	95 (7)	117 (6)	88 (8)	4
Carbaryl	0.0003	0.001	LC-MS/MS	96 (6)	85 (12)	98 (12)	97 (11)	100 (10)	101 (11)	0
Carbendazim	0.0003	0.001	LC-MS/MS	64 (9)	80 (14)	104 (6)	94 (8)	95 (2)	98 (8)	17
Carbetamide	0.0017	0.005	LC-MS/MS		84 (13)	96 (14)	85 (5)	109 (6)	88 (10)	−7
Carboxin	0.0003	0.001	LC-MS/MS	92 (19)	81 (15)	103 (14)	91 (10)	107 (6)	85 (7)	−14
Carfentrazone-ethyl	0.0017	0.005	LC-MS/MS		97 (14)	104 (9)	76 (10)	100 (8)	85 (8)	−5
Chlorantraniliprole	0.0003	0.001	LC-MS/MS	120 (16)	96 (18)	108 (11)	86 (13)	104 (11)	84 (18)	−25
Chlorfenvinphos	0.0017	0.005	GC-MS/MS		110 (9)	108 (9)	92 (16)	121 (17)	75 (5)	−21
Chloridazon	0.0003	0.001	LC-MS/MS	110 (17)	97 (15)	100 (15)	93 (6)	104 (8)	85 (8)	3
Chlorothalonil	0.0033	0.01	GC-MS/MS			85 (10)	74 (5)	108 (15)	93 (7)	−21
Chlorotoluron	0.0003	0.001	LC-MS/MS	104 (15)	88 (12)	85 (8)	86 (6)	107 (9)	78 (15)	−2
Chlorpropham	0.0003	0.001	GC-MS/MS	77 (16)	82 (6)	110 (4)	106 (10)	108 (10)	97 (5)	24
Chlorpyrifos	0.0017	0.005	GC-MS/MS		97 (8)	85 (10)	92 (7)	106 (6)	95 (4)	5
Chlorpyrifos-methyl	0.0017	0.005	GC-MS/MS		94 (11)	108 (10)	105 (6)	101 (6)	97 (4)	15
Chlorsulfuron	0.0003	0.001	LC-MS/MS	104 (18)	106 (15)	107 (5)	126 (6)	106 (9)	77 (10)	−16
cis-Chlordane	0.0017	0.005	GC-MS/MS		76 (16)	102 (17)	86 (14)	110 (9)	77 (10)	−27
cis-Heptachlor epoxide	0.0017	0.005	GC-MS/MS		88 (16)	99 (12)	96 (10)	111 (3)	102 (5)	−10
Clethodim	0.0017	0.005	LC-MS/MS		100 (16)	109 (8)	89 (9)	96 (8)	55 (16)	−9
Clofentezine	0.0017	0.005	LC-MS/MS		109 (9)	108 (5)	85 (14)	95 (10)	73 (14)	17
Clomazone	0.0003	0.001	LC-MS/MS	90 (18)	102 (10)	97 (8)	91 (10)	108 (16)	88 (6)	14
Clothianidin	0.0017	0.005	LC-MS/MS		96 (13)	83 (13)	92 (6)	91 (16)	82 (13)	36
Coumaphos	0.0017	0.005	LC-MS/MS		88 (19)	94 (7)	88 (9)	100 (12)	77 (9)	35
Cyantraniliprole	0.0017	0.005	LC-MS/MS		87 (11)	106 (14)	77 (12)	104 (14)	100 (12)	−13
Cyazofamid	0.0017	0.005	LC-MS/MS		96 (14)	79 (14)	73 (6)	103 (7)	71 (11)	9
Cycloxydim	0.0017	0.005	LC-MS/MS		109 (10)	121 (10)	97 (8)	104 (13)	83 (10)	−18
Cyflufenamid	0.0017	0.005	LC-MS/MS		102 (18)	77 (17)	62 (9)	112 (7)	73 (9)	−1
Cyfluthrin (sum of isomers)	0.0003	0.001		97 (13)	75 (11)	93 (4)	102 (10)	103 (10)	88 (5)	270

(*continued on next page*)

Table 2 (*continued*)

Compound	LOD (mg/kg)	LOQ (mg/kg)	Technique	Recovery, % (RSDr, %)						ME, %
				0.001 mg kg^{-1}	0.005 mg kg^{-1}	0.01 mg kg^{-1}	0.05 mg kg^{-1}	0.1 mg kg^{-1}	0.5 mg kg^{-1}	
			GC-MS/MS							
Cymiazol	0.0017	0.005	GC-MS/MS		83 (18)	95 (18)	89 (12)	100 (12)	81 (4)	−82
Cymoxanil	0.0017	0.005	LC-MS/MS		83 (17)	76 (8)	85 (9)	100 (8)	80 (13)	−11
Cypermethrin (sum of isomers)	0.0003	0.001	GC-MS/MS	118 (11)	76 (20)	96 (17)	99 (5)	100 (8)	92 (7)	−29
Cyproconazole	0.0017	0.005	LC-MS/MS		106 (15)	103 (6)	83 (14)	108 (10)	100 (8)	−51
Cyprodinil	0.0017	0.005	LC-MS/MS		83 (13)	96 (7)	105 (4)	111 (12)	81 (7)	−36
Deltamethrin	0.0017	0.005	GC-MS/MS		80 (10)	94 (10)	97 (6)	100 (7)	101 (11)	−26
Desmedipham	0.0017	0.005	LC-MS/MS		109 (14)	114 (7)	109 (9)	93 (9)	96 (7)	10
Diazinon	0.0017	0.005	GC-MS/MS		109 (11)	95 (15)	85 (14)	94 (5)	84 (4)	15
Dichlorprop-P	0.033	0.1	LC-MS/MS					107 (14)	69 (11)	−62
Dieldrin	0.0003	0.001	GC-MS/MS	85 (15)	94 (16)	102 (14)	92 (7)	106 (5)	89 (6)	20
Difenoconazole	0.0003	0.001	LC-MS/MS	84 (18)	111 (20)	85 (14)	74 (5)	107 (5)	78 (8)	−20
Diflubenzuron	0.0033	0.01	LC-MS/MS			79 (19)	65 (8)	119 (5)	70 (4)	−8
Diflufenican	0.0003	0.001	LC-MS/MS	108 (8)	72 (17)	83 (16)	81 (8)	119 (10)	76 (6)	14
Dimethachlor	0.0003	0.001	LC-MS/MS	114 (12)	98 (10)	90 (6)	90 (8)	110 (11)	81 (8)	4
Dimethoate	0.0003	0.001	LC-MS/MS	110 (17)	93 (9)	91 (8)	84 (6)	109 (9)	84 (6)	12
Dimethomorph	0.0003	0.001	LC-MS/MS	96 (9)	106 (7)	91 (17)	77 (4)	115 (7)	84 (16)	−3
Dimoxystrobin	0.0003	0.001	LC-MS/MS	80 (18)	98 (13)	111 (14)	100 (12)	114 (13)	106 (17)	15
Dithianon	0.017	0.05	LC-MS/MS				102 (9)	101 (15)	84 (2)	23
Dodine	0.033	0.1	LC-MS/MS					108 (11)	90 (15)	−90
Endosulfan sulfate	0.0003	0.001	GC-MS/MS	103 (12)	93 (18)	86 (11)	87 (10)	113 (12)	73 (6)	81
Endrin	0.0003	0.001	GC-MS/MS	120 (20)	89 (15)	100 (3)	92 (10)	107 (6)	95 (7)	66
Epoxiconazole	0.0033	0.01	LC-MS/MS			108 (5)	106 (8)	118 (10)	78 (10)	−20
Esfenvalerate (Fenvalerate)	0.0003	0.001	GC-MS/MS	120 (10)	82 (10)	93 (16)	95 (5)	97 (7)	88 (6)	−7
Ethametsulfuron-methyl	0.017	0.05	LC-MS/MS				109 (8)	110 (14)	86 (17)	2
Ethofumesate	0.017	0.05	LC-MS/MS				86 (8)	118 (12)	94 (17)	−23
Ethoprophos	0.0017	0.005	LC-MS/MS		118 (6)	111 (14)	89 (6)	109 (2)	84 (9)	−11
Etofenprox	0.0017	0.005	GC-MS/MS		76 (9)	100 (7)	96 (4)	98 (10)	77 (6)	93
Etoxazole	0.0003	0.001	LC-MS/MS	104 (9)	89 (10)	87 (16)	70 (8)	107 (6)	71 (11)	−15
Famoxadone	0.0003	0.001	GC-MS/MS	103 (16)	92 (7)	99 (8)	101 (20)	118 (9)	99 (13)	481
Fenazaquin	0.0017	0.005	GC-MS/MS		95 (12)	106 (5)	97 (7)	94 (12)	72 (7)	−48
Fenbuconazole	0.0017	0.005	LC-MS/MS		112 (8)	116 (12)	83 (6)	96 (4)	77 (12)	9
Fenhexamid	0.0033	0.01	LC-MS/MS			111 (13)	82 (10)	96 (12)	83 (8)	−26
Fenitrothion	0.0017	0.005	GC-MS/MS		85 (6)	108 (11)	100 (3)	102 (17)	108 (10)	13
Fenoxaprop-P-ethyl	0.0033	0.01	LC-MS/MS			99 (18)	88 (9)	109 (8)	80 (13)	−31
Fenoxycarb	0.0033	0.01	LC-MS/MS			105 (19)	93 (14)	110 (10)	82 (5)	4
Fenpropidin	0.0003	0.001	LC-MS/MS	108 (8)	82 (15)	100 (16)	86 (10)	82 (13)	81 (14)	−60
Fenpropimorph	0.0003	0.001	LC-MS/MS	112 (8)	87 (20)	90 (18)	77 (11)	118 (5)	77 (16)	−70
Fenpyroximate	0.0003	0.001	LC-MS/MS	114 (5)	95 (15)	76 (19)	61 (7)	108 (10)	76 (9)	−2
Fenthion	0.0017	0.005	LC-MS/MS		84 (1)	88 (12)	75 (3)	101 (3)	71 (6)	−23
Fenthion-sulfone	0.017	0.05	LC-MS/MS				112 (13)	108 (15)	75 (17)	−18
Fenthion-sulfoxide	0.0033	0.01	LC-MS/MS			106 (7)	84 (3)	89 (9)	84 (6)	3
Fipronil	0.0003	0.001	LC-MS/MS	94 (10)	100 (8)	82 (8)	71 (5)	117 (10)	72 (10)	9
Fipronil-carboxamide	0.0003	0.001	LC-MS/MS	92 (12)	62 (10)	92 (6)	64 (10)	–	–	−3
Fipronil-desulfinyl	0.0003	0.001	LC-MS/MS	96 (9)	82 (14)	108 (13)	78 (8)	–	–	23
Fipronil-sulfide	0.0003	0.001	LC-MS/MS	100 (14)	74 (20)	81 (11)	78 (5)	101 (8)	71 (9)	5
Fipronil-sulfone	0.0003	0.001	LC-MS/MS	92 (14)	117 (14)	73 (10)	71 (10)	120 (8)	71 (7)	4
Flazasulfuron	0.0003	0.001	LC-MS/MS	86 (16)	107 (7)	110 (12)	114 (12)	104 (8)	94 (9)	−5
Flonicamid	0.0033	0.01	LC-MS/MS			120 (13)	98 (7)	99 (11)	76 (12)	7
Florasulam	0.0003	0.001	LC-MS/MS	108 (8)	103 (6)	113 (10)	113 (8)	103 (12)	83 (12)	6
Fluazifop-P-butyl	0.0003	0.001	LC-MS/MS	96 (12)	84 (16)	89 (9)	84 (6)	116 (7)	81 (9)	−12
Fluazinam	0.0003	0.001	LC-MS/MS	96 (12)	109 (18)	77 (18)	68 (6)	115 (4)	62 (18)	−10
Fludioxonil	0.0017	0.005	LC-MS/MS		113 (8)	94 (12)	80 (6)	116 (8)	105 (6)	20
Flufenacet	0.0003	0.001	LC-MS/MS	82 (10)	95 (19)	100 (12)	95 (5)	101 (4)	91 (18)	2
Fluopyram	0.0003	0.001	LC-MS/MS	84 (14)	90 (12)	86 (11)	75 (12)	107 (7)	84 (4)	10
Flupyradifurone	0.0017	0.005	LC-MS/MS		94 (18)	105 (10)	70 (11)	106 (13)	77 (8)	2
Fluquinconazole	0.0017	0.005	LC-MS/MS		81 (15)	83 (9)	78 (10)	94 (7)	80 (11)	−6
Flurochloridone	0.0017	0.005	LC-MS/MS		88 (20)	111 (16)	75 (18)	108 (19)	67 (17)	15
Fluroxypyr	0.017	0.05	LC-MS/MS				69 (13)	120 (7)	75 (8)	−62
Fluroxypyr-meptyl	0.017	0.05	LC-MS/MS				102 (14)	113 (14)	80 (17)	−12

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Table 2 (*continued*)

Compound	LOD (mg/kg)	LOQ (mg/kg)	Technique	Recovery, % (RSDr, %)						ME, %
				0.001 mg kg^{-1}	0.005 mg kg^{-1}	0.01 mg kg^{-1}	0.05 mg kg^{-1}	0.1 mg kg^{-1}	0.5 mg kg^{-1}	
Flurprimidol	0.033	0.1	GC-MS/MS					103 (11)	72 (12)	148
Flusilazole	0.0003	0.001	LC-MS/MS	100 (16)	91 (16)	100 (10)	101 (8)	112 (8)	103 (9)	−14
Flutriafol	0.017	0.05	LC-MS/MS				90 (11)	110 (6)	76 (12)	−35
Fluxapyroxad	0.0003	0.001	LC-MS/MS	72 (18)	92 (9)	97 (8)	96 (7)	112 (11)	96 (9)	−4
Foramsulfuron	0.0017	0.005	LC-MS/MS		97 (17)	91 (4)	94 (10)	101 (6)	98 (10)	−34
Gibberellin A4	0.017	0.05	LC-MS/MS				76 (14)	116 (15)	74 (16)	−31
HCB	0.0017	0.005	GC-MS/MS		89 (14)	122 (10)	97 (18)	94 (32)	89 (20)	−77
Heptachlor	0.0003	0.001	GC-MS/MS	118 (16)	89 (8)	106 (10)	96 (9)	101 (18)	93 (6)	35
Heptenophos	0.0003	0.001	GC-MS/MS	108 (10)	88 (20)	85 (8)	93 (15)	96 (16)	100 (9)	32
Hexythiazox	0.0003	0.001	LC-MS/MS	88 (15)	82 (14)	83 (14)	84 (12)	105 (10)	71 (9)	−10
Hymexazol	0.017	0.05	LC-MS/MS				105 (6)	113 (5)	86 (12)	−50
Imazalil	0.0033	0.01	LC-MS/MS			90 (18)	86 (14)	77 (14)	72 (8)	−64
Imidacloprid	0.0003	0.001	LC-MS/MS	102 (14)	113 (14)	111 (12)	120 (18)	96 (16)	80 (6)	6
Imidacloprid-olefin	0.0003	0.001	LC-MS/MS	114 (16)	128 (19)	105 (17)	94 (16)	84 (9)	100 (19)	−21
Imidacloprid-urea	0.0017	0.005	LC-MS/MS		110 (17)	114 (7)	75 (19)	87 (19)	113 (8)	−62
Indolylbutyric acid (IBA)	0.017	0.05	LC-MS/MS				110 (14)	95 (12)	73 (14)	−4
Indoxacarb	0.0017	0.005	LC-MS/MS		84 (10)	97 (15)	83 (4)	113 (12)	106 (4)	0
Iodosulfuron-methyl-sodium	0.0017	0.005	LC-MS/MS		88 (16)	114 (12)	103 (11)	114 (7)	95 (10)	−32
Ipconazole	0.0017	0.005	LC-MS/MS		97 (16)	110 (8)	106 (8)	106 (5)	68 (6)	−22
Iprodione	0.017	0.05	LC-MS/MS				76 (14)	109 (18)	77 (13)	0
Isoproturon	0.0017	0.005	LC-MS/MS		94 (13)	86 (10)	90 (7)	104 (7)	84 (6)	−6
Isopyrazam	0.0003	0.001	LC-MS/MS	102 (8)	95 (18)	115 (6)	109 (7)	97 (11)	82 (9)	−5
Isoxaflutole	0.017	0.05	LC-MS/MS				111 (5)	100 (5)	91 (12)	−60
Kresoxim-methyl	0.0003	0.001	GC-MS/MS	89 (18)	87 (6)	108 (16)	87 (11)	111 (7)	84 (7)	19
lambda-Cyhalothrin	0.0003	0.001	GC-MS/MS	95 (19)	77 (11)	86 (7)	91 (6)	98 (11)	72 (2)	224
Lenacil	0.0003	0.001	LC-MS/MS	84 (18)	95 (18)	81 (7)	81 (10)	100 (8)	87 (8)	−30
Lindane	0.0003	0.001	GC-MS/MS	116 (5)	92 (10)	121 (10)	101 (15)	77 (18)	78 (7)	3
Linuron	0.0017	0.005	LC-MS/MS		82 (17)	99 (8)	99 (14)	96 (14)	79 (12)	3
Malathion	0.0017	0.005	GC-MS/MS		82 (6)	100 (10)	106 (4)	116 (10)	93 (6)	39
Mandipropamid	0.0017	0.005	LC-MS/MS		72 (10)	95 (14)	92 (9)	109 (13)	89 (15)	17
MCPA	0.017	0.05	LC-MS/MS				75 (2)	120 (11)	69 (17)	−70
MCPB	0.017	0.05	LC-MS/MS				88 (13)	102 (14)	60 (16)	11
Mecoprop-P	0.017	0.05	LC-MS/MS				102 (7)	120 (6)	70 (10)	−32
Mepanipyrim	0.0017	0.005	LC-MS/MS		92 (18)	99 (13)	86 (12)	100 (9)	76 (10)	−14
Mesosulfuron-methyl	0.0017	0.005	LC-MS/MS		116 (15)	97 (8)	96 (8)	112 (8)	76 (12)	17
Mesotrione	0.0017	0.005	LC-MS/MS		86 (10)	112 (12)	100 (16)	116 (9)	72 (8)	−4
Metaflumizone	0.0017	0.005	LC-MS/MS		71 (12)	103 (15)	73 (7)	111 (8)	66 (16)	30
Metalaxyl-M (Metalaxyl)	0.0017	0.005	LC-MS/MS		69 (19)	90 (12)	89 (12)	112 (5)	96 (5)	12
Metamitron	0.0033	0.01	LC-MS/MS			116 (6)	117 (2)	117 (5)	96 (4)	−23
Metazachlor	0.0033	0.01	LC-MS/MS			102 (4)	99 (11)	104 (8)	82 (10)	3
Metconazole	0.0017	0.005	LC-MS/MS		103 (8)	112 (13)	104 (7)	105 (11)	98 (7)	−41
Methidathion	0.0003	0.001	GC-MS/MS	108 (12)	82 (8)	93 (10)	102 (3)	113 (15)	77 (4)	50
Methiocarb	0.0017	0.005	LC-MS/MS		90 (8)	90 (6)	88 (11)	107 (10)	73 (12)	−2
Methiocarb sulfoxide	0.0003	0.001	LC-MS/MS	108 (8)	93 (16)	82 (10)	97 (12)	106 (13)	77 (9)	−7
Methiocarb sulfone	0.0017	0.005	LC-MS/MS		78 (15)	99 (15)	85 (12)	115 (9)	89 (6)	10
Methoxychlor	0.0033	0.01	GC-MS/MS			101 (10)	89 (9)	92 (7)	95 (8)	44
Methoxyfenozide	0.017	0.05	LC-MS/MS				122 (14)	102 (18)	80 (20)	−21
Metrafenone	0.0003	0.001	LC-MS/MS	84 (11)	90 (15)	83 (9)	78 (9)	113 (12)	85 (13)	3
Metribuzin	0.0017	0.005	LC-MS/MS		81 (4)	96 (11)	82 (8)	92 (11)	78 (10)	−53
Metsulfuron-methyl	0.0003	0.001	LC-MS/MS	114 (8)	106 (13)	112 (7)	118 (6)	101 (11)	72 (13)	−15
Mevinphos	0.0017	0.005	LC-MS/MS		94 (10)	100 (18)	90 (6)	106 (8)	82 (12)	23
Myclobutanil	0.0017	0.005	LC-MS/MS		90 (15)	115 (5)	92 (6)	117 (10)	82 (10)	12
N-2,4-Dimethylphenyl-formamide (DMF)	0.0017	0.005	LC-MS/MS		68 (17)	95 (8)	87 (10)	117 (5)	89 (12)	−14
N-2,4-Dimethylphenyl-N′-methylformamide (DMPF)	0.0017	0.005	LC-MS/MS		90 (19)	83 (13)	89 (9)	78 (7)	114 (17)	−77
Napropamide	0.0003	0.001	LC-MS/MS	92 (18)	91 (18)	104 (12)	99 (7)	113 (10)	85 (7)	−5
Nicosulfuron	0.0003	0.001	LC-MS/MS	92 (12)	78 (12)	92 (7)	91 (9)	117 (6)	73 (14)	−35
Nitenpyram	0.0033	0.01	LC-MS/MS			108 (18)	115 (9)	119 (10)	96 (5)	−70
Novaluron	0.0033	0.01	LC-MS/MS			101 (16)	74 (19)	109 (11)	58 (18)	29
Omethoate	0.0033	0.01	LC-MS/MS			96 (8)	88 (6)	109 (7)	80 (7)	−21
Oxychlordane	0.0003	0.001	GC-MS/MS	92 (14)	84 (10)	97 (10)	77 (6)	85 (8)	88 (4)	43

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Table 2 (*continued*)

Compound	LOD (mg/kg)	LOQ (mg/kg)	Technique	Recovery, % (RSDr, %)						ME, %
				0.001 mg kg^{-1}	0.005 mg kg^{-1}	0.01 mg kg^{-1}	0.05 mg kg^{-1}	0.1 mg kg^{-1}	0.5 mg kg^{-1}	
Oxyfluorfen	0.017	0.05	LC-MS/MS				97 (13)	97 (12)	65 (17)	−28
o,p'-DDT	0.0003	0.001	GC-MS/MS	97 (12)	84 (11)	89 (15)	93 (5)	100 (10)	90 (9)	10
p,p'-DDD	0.0003	0.001	GC-MS/MS	106 (11)	86 (16)	90 (17)	89 (6)	109 (15)	73 (5)	28
p,p'-DDE	0.0003	0.001	GC-MS/MS	100 (18)	77 (18)	87 (14)	88 (8)	100 (11)	76 (8)	−9
p,p'-DDT	0.0003	0.001	GC-MS/MS	112 (4)	87 (8)	92 (6)	92 (5)	105 (3)	100 (6)	−5
Paclobutrazol	0.0033	0.01	LC-MS/MS			98 (6)	94 (9)	111 (6)	89 (10)	−41
Parathion-ethyl	0.0003	0.001	GC-MS/MS	81 (17)	71 (16)	95 (5)	104 (6)	75 (11)	83 (6)	8
Parathion-methyl	0.0003	0.001	GC-MS/MS	120 (10)	89 (6)	102 (13)	94 (7)	108 (6)	88 (4)	57
PCB 101	0.0003	0.001	GC-MS/MS	89 (13)	84 (20)	86 (9)	79 (5)	105 (12)	83 (6)	−41
PCB 138	0.0003	0.001	GC-MS/MS	97 (10)	81 (19)	75 (10)	73 (14)	107 (15)	85 (11)	−53
PCB 153	0.0003	0.001	GC-MS/MS	86 (12)	81 (7)	100 (12)	87 (7)	109 (10)	89 (5)	−64
PCB 180	0.0003	0.001	GC-MS/MS	96 (16)	88 (19)	91 (7)	84 (9)	104 (15)	77 (6)	−65
PCB 28	0.0003	0.001	GC-MS/MS	104 (5)	85 (7)	103 (7)	89 (6)	92 (12)	95 (10)	−30
PCB 52	0.0003	0.001	GC-MS/MS	97 (3)	86 (9)	94 (6)	84 (8)	98 (4)	88 (4)	−25
Penconazole	0.0033	0.01	LC-MS/MS			95 (11)	85 (12)	115 (10)	102 (6)	−30
Pencycuron	0.0003	0.001	LC-MS/MS	76 (12)	86 (9)	91 (6)	88 (6)	115 (5)	82 (4)	5
Pendimethalin	0.0003	0.001	GC-MS/MS	107 (19)	76 (8)	97 (15)	97 (6)	109 (3)	117 (3)	16
Penthiopyrad	0.0003	0.001	LC-MS/MS	114 (10)	97 (14)	103 (7)	102 (6)	103 (9)	89 (10)	7
Permethrin (sum of isomers)	0.0003	0.001	GC-MS/MS	111 (17)	86 (10)	98 (7)	100 (7)	101 (5)	86 (11)	36
Pethoxamid	0.0017	0.005	LC-MS/MS		101 (5)	98 (8)	102 (8)	106 (6)	88 (8)	1
Phenmedipham	0.0017	0.005	LC-MS/MS		80 (11)	76 (12)	72 (8)	110 (12)	77 (19)	2
Phosalone	0.0003	0.001	GC-MS/MS	81 (13)	68 (11)	104 (7)	108 (12)	102 (8)	77 (6)	114
Phosmet	0.017	0.05	LC-MS/MS				103 (14)	120 (10)	134 (20)	−18
Phoxim	0.0033	0.01	LC-MS/MS			110 (5)	93 (7)	114 (19)	78 (10)	1
Picoxystrobin	0.0033	0.01	LC-MS/MS			101 (13)	75 (43)	96 (18)	98 (20)	−6
Pirimicarb	0.0017	0.005	LC-MS/MS		101 (12)	98 (9)	91 (6)	102 (11)	77 (7)	−5
Pirimicarb-desmethyl	0.0003	0.001	LC-MS/MS	70 (10)	81 (9)	91 (13)	89 (10)	119 (7)	81 (5)	−22
Pirimiphos-ethyl	0.0017	0.005	LC-MS/MS		117 (14)	96 (10)	100 (10)	106 (14)	87 (8)	−18
Pirimiphos-methyl	0.0017	0.005	LC-MS/MS		73 (16)	90 (7)	85 (6)	106 (10)	85 (11)	−2
Prochloraz	0.0017	0.005	LC-MS/MS		98 (13)	95 (18)	87 (11)	112 (19)	108 (13)	−34
Profenofos	0.017	0.05	GC-MS/MS				75 (14)	113 (18)	111 (18)	−15
Propamocarb	0.0017	0.005	LC-MS/MS		93 (19)	95 (15)	78 (17)	112 (17)	77 (11)	−79
Propaquizafop	0.0017	0.005	LC-MS/MS		85 (12)	87 (13)	83 (10)	119 (5)	68 (7)	−17
Propargite	0.0017	0.005	LC-MS/MS		100 (7)	102 (20)	122 (13)	109 (10)	98 (14)	−16
Propiconazole	0.0017	0.005	LC-MS/MS		111 (11)	111 (11)	84 (9)	94 (6)	66 (11)	−15
Propoxur	0.0017	0.005	LC-MS/MS		98 (9)	97 (15)	91 (7)	99 (10)	91 (17)	10
Propoxycarbazone-sodium	0.0033	0.01	LC-MS/MS			112 (16)	129 (20)	119 (13)	74 (15)	−74
Propyzamide	0.0017	0.005	LC-MS/MS		70 (12)	94 (18)	86 (16)	113 (7)	89 (8)	0
Proquinazid	0.0017	0.005	LC-MS/MS		93 (17)	88 (9)	85 (7)	107 (8)	75 (6)	−17
Prosulfocarb	0.0017	0.005	LC-MS/MS		95 (13)	85 (10)	83 (10)	120 (11)	80 (7)	−4
Prothioconazole	0.017	0.05	LC-MS/MS				89 (15)	114 (10)	66 (7)	−2
Prothioconazole-desthio	0.0033	0.01	LC-MS/MS			105 (6)	98 (14)	110 (14)	82 (8)	−55
Pymetrozine	0.0033	0.01	LC-MS/MS			110 (10)	111 (8)	120 (4)	117 (17)	−77
Pyraclostrobin	0.0003	0.001	LC-MS/MS	100 (7)	86 (15)	87 (8)	81 (5)	114 (8)	84 (6)	9
Pyrazophos	0.0003	0.001	GC-MS/MS	118 (17)	90 (17)	77 (20)	93 (15)	88 (14)	94 (15)	−57
Pyridate	0.0033	0.01	LC-MS/MS			83 (20)	86 (6)	131 (5)	82 (5)	−24
Pyrimethanil	0.0017	0.005	LC-MS/MS		91 (12)	94 (6)	92 (7)	112 (7)	82 (16)	−23
Pyriproxyfen	0.0017	0.005	LC-MS/MS		92 (20)	98 (18)	80 (13)	101 (15)	70 (16)	−10
Quinmerac	0.017	0.05	LC-MS/MS				74 (16)	117 (9)	88 (11)	−96
Quinoclamine	0.0017	0.005	LC-MS/MS		74 (6)	112 (13)	85 (7)	104 (9)	76 (6)	−50
Quinoxyfen	0.0033	0.01	LC-MS/MS			102 (20)	86 (10)	100 (5)	46 (16)	−29
Quizalofop-P-ethyl	0.0003	0.001	LC-MS/MS	76 (20)	114 (19)	77 (11)	77 (6)	114 (6)	76 (16)	−23
Quizalofop-P-tefuryl	0.0017	0.005	LC-MS/MS		94 (13)	116 (19)	73 (13)	–	–	−40
Resmethrin	0.0033	0.01	GC-MS/MS			76 (20)	83 (17)	108 (18)	74 (11)	47
Rimsulfuron	0.0017	0.005	LC-MS/MS		82 (16)	121 (5)	124 (8)	113 (6)	83 (5)	−9

(*continued on next page*)

Table 2 (*continued*)

Compound	LOD (mg/kg)	LOQ (mg/kg)	Technique	Recovery, % (RSDr, %)						ME, %
				0.001 mg kg^{-1}	0.005 mg kg^{-1}	0.01 mg kg^{-1}	0.05 mg kg^{-1}	0.1 mg kg^{-1}	0.5 mg kg^{-1}	
Silthiofam	0.0017	0.005	LC-MS/MS		87 (19)	101 (8)	92 (5)	111 (8)	92 (9)	16
S-Metolachlor	0.0003	0.001	LC-MS/MS	104 (11)	86 (19)	97 (11)	83 (8)	106 (8)	85 (11)	2
Spinosad (mix of Spinosyn A & D)	0.0017	0.005	LC-MS/MS		92 (19)	86 (20)	79 (20)	103 (11)	118 (19)	−50
Spirodiclofen	0.0003	0.001	GC-MS/MS	94 (16)	85 (10)	105 (20)	86 (8)	101 (19)	113 (10)	90
Spirotetramat	0.0003	0.001	LC-MS/MS	106 (14)	125 (19)	113 (20)	60 (8)	117 (5)	78 (13)	0
Spirotetramat-enol	0.0017	0.005	LC-MS/MS		98 (9)	113 (7)	95 (3)	111 (9)	78 (8)	−11
Spirotetramat-enol glucoside	0.017	0.05	LC-MS/MS				122 (12)	83 (13)	74 (20)	−90
Spirotetramat-keto hydroxy	0.0033	0.01	LC-MS/MS			106 (13)	96 (9)	93 (14)	76 (6)	−34
Spiroxamine	0.0017	0.005	LC-MS/MS		82 (15)	108 (20)	86 (16)	90 (17)	78 (9)	−75
Sulcotrione	0.0033	0.01	LC-MS/MS			102 (9)	110 (11)	120 (3)	82 (13)	−78
Sulfosulfuron	0.0017	0.005	LC-MS/MS		70 (19)	123 (13)	134 (8)	104 (14)	74 (9)	−16
Sulfoxaflor	0.017	0.05	LC-MS/MS				117 (11)	86 (9)	91 (20)	−61
tau-Fluvalinate	0.0003	0.001	GC-MS/MS	111 (18)	96 (15)	101 (17)	96 (3)	95 (4)	109 (9)	254
Tebuconazole	0.0017	0.005	LC-MS/MS		96 (13)	101 (9)	104 (5)	116 (7)	80 (8)	−41
Tebufenozide	0.0033	0.01	LC-MS/MS			120 (9)	99 (12)	80 (18)	96 (20)	14
Tebufenpyrad	0.0017	0.005	LC-MS/MS		87 (19)	111 (11)	113 (9)	106 (6)	77 (17)	20
Teflubenzuron	0.0017	0.005	LC-MS/MS		70 (12)	98 (9)	84 (11)	109 (14)	86 (12)	13
Tefluthrin	0.0003	0.001	GC-MS/MS	108 (8)	80 (5)	105 (9)	99 (2)	94 (2)	79 (6)	59
Tembotrione	0.017	0.05	LC-MS/MS				110 (10)	126 (9)	97 (13)	−54
Tepraloxydim	0.017	0.05	LC-MS/MS				95 (10)	98 (11)	99 (10)	12
Terbuthylazine	0.0017	0.005	LC-MS/MS		102 (14)	92 (5)	90 (7)	109 (7)	80 (9)	−20
Tetraconazole	0.0017	0.005	LC-MS/MS		85 (8)	107 (13)	70 (11)	114 (7)	76 (10)	−1
Tetramethrin	0.0017	0.005	GC-MS/MS		103 (9)	104 (12)	105 (4)	113 (7)	103 (15)	−29
Thiacloprid	0.0003	0.001	LC-MS/MS	114 (5)	100 (9)	100 (13)	95 (5)	111 (5)	114 (4)	3
Thiacloprid-amide	0.0017	0.005	LC-MS/MS		94 (8)	107 (13)	103 (7)	109 (7)	85 (12)	−36
Thiamethoxam	0.0017	0.005	LC-MS/MS		89 (11)	110 (13)	94 (12)	104 (10)	100 (4)	25
Thifensulfuron-methyl	0.0003	0.001	LC-MS/MS	106 (16)	105 (20)	120 (8)	102 (11)	113 (9)	95 (12)	−25
Thiophanate-methyl	0.0003	0.001	LC-MS/MS	106 (11)	88 (12)	96 (9)	98 (12)	106 (11)	78 (7)	−34
Tralkoxydim	0.0017	0.005	LC-MS/MS		84 (19)	111 (12)	103 (5)	107 (6)	68 (11)	−10
trans-Chlordane	0.0003	0.001	GC-MS/MS	104 (11)	84 (16)	96 (11)	89 (8)	112 (4)	86 (6)	−19
trans-Heptachlor epoxide	0.0033	0.01	GC-MS/MS			83 (7)	87 (7)	105 (5)	87 (4)	37
Triadimefon	0.0017	0.005	LC-MS/MS		105 (7)	100 (14)	91 (10)	119 (8)	75 (8)	13
Triadimenol	0.017	0.05	LC-MS/MS				83 (10)	116 (6)	84 (12)	−46
Triazophos	0.0003	0.001	GC-MS/MS	95 (17)	81 (15)	88 (9)	99 (8)	115 (12)	90 (6)	67
Tribenuron-methyl	0.0033	0.01	LC-MS/MS			105 (13)	140 (20)	120 (13)	59 (19)	−46
Trifloxystrobin	0.0003	0.001	LC-MS/MS	96 (16)	93 (20)	89 (9)	80 (8)	107 (8)	73 (15)	−13
Triflusulfuron-methyl	0.017	0.05	LC-MS/MS				91 (17)	83 (15)	62 (13)	−35
Trinexapac-ethyl	0.017	0.05	LC-MS/MS				80 (13)	118 (10)	107 (9)	−60
Triticonazole	0.0033	0.01	LC-MS/MS			108 (8)	94 (7)	115 (7)	69 (4)	−48
Vinclozolin	0.0003	0.001	GC-MS/MS	110 (9)	75 (5)	106 (12)	101 (8)	111 (8)	84 (5)	32

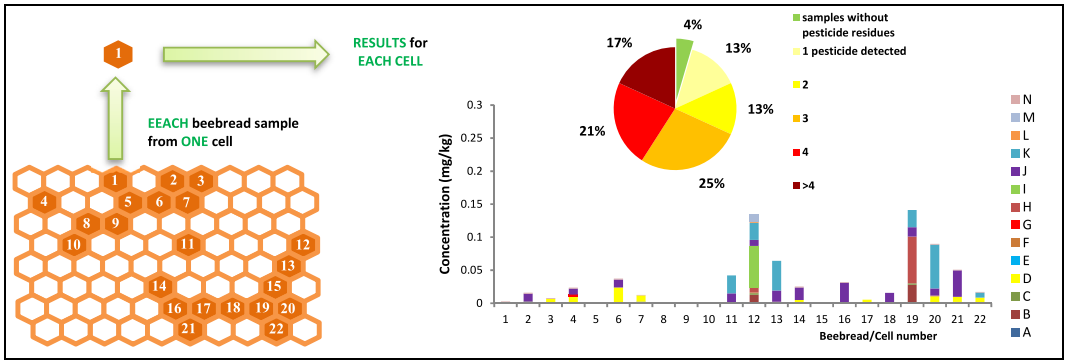

Fig. 2. Results of analysis of beebread samples taken out from single cells of honeycomb. The number of residues found simultaneously in the sample and the concentrations of the determined pesticides: azoxystrobin (A), boscalid (B), carbendazim (C), chlorpyrifos (D), cyprodinil (E), difenoconazole (F), dimethoate (G), fludioxonil (H), fluopyram (I), DMF (J), DMPF (K), pyraclostrobin (L), pyrimethanil (M), tau-fluvalinate (N).

6. Conclusion

The developed method to the best of our knowledge is the first miniaturized method of beebread analysis with a sample as low as 0.3 g weight. Despite a very small sample quantity method allows determination of 267 substances in each sample and both LC-MS/MS and GC-MS/MS analysis. This validated method enable analysis of active substances of plant protection products such as insecticides, fungicides, herbicides, acaricides, growth regulators, veterinary medicinal products and their metabolites as well as ndl-PCBs. For the first time formate buffered approach and QuE Verde sorbents were used in beebread sample preparations. Developed protocol of two-step sequential dSPE gave excellent clean-up of challenging beebread matrix, rich in proteins, carbohydrates, lipids and pigments. Method usefulness to pesticide residue analysis of beebread from single honeycomb cells was shown. The developed method enables analysis on an unprecedented miniaturized scale and with a very wide range of analyzed substances, which makes it a great tool in the assessment of bees' exposure to chemicals.

Credit author statement

Tomasz Kiljanek: Conceptualization, Methodology, Investigation, Validation, Writing – Original Draft, Writing –Reviewing and Editing, Visualization, Supervision, Project administration, Funding acquisition. **Alicja Niewiadowska:** Writing –Reviewing and Editing, Supervision. **Marta Gaweł:** Validation, Resources. **Andrzej Posyniak:** Writing –Reviewing and Editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.talanta.2021.122721.

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