# Investigation of some neonicotinoids in honey by LC-MS/MS

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**ABSTRACT**

Honey is a natural substance that is susceptible to contamination by environmental pollutants. The presence of contaminants in honey is an indicator of environmental pollution. Furthermore, it may pose risks to consumer health. This research aimed to optimise a method for the detection of residual quantities of the pesticides acetamiprid (ACE), clothianidin (CLO), imidacloprid (IMI), thiamethoxam (TMX) and thiacloprid (THI) in honey, and subsequently to apply this optimised method to an investigation into the prevalence of neonicotinoidal contamination. The QuEChERS (quick, easy, cheap, effective, robust and safe) method, in conjunction with liquid chromatography-mass spectrometry/mass spectrometry (LC-MS/MS), was employed for the determination of five neonicotinoid in honey. The method was optimized and validated in accordance with European Commission guidelines (2002/657/EC). The method demonstrated a linear correlation with $R^2$ values exceeding 0.99 for all investigated compounds. Mean recoveries ranged between 70% and 110% (99% on average), and relative standard deviations (RSDs) were generally below 20%. The method's CCα (decision limit) and CCβ (detection capability) both ranged from 5 to 20 ng/g and 5-21 ng/g, respectively. Following method validation, the concentrations of IMI, ACE, TMX, CLO, and THI in all honey samples (flower, pine, and chestnut honey) available for retail in Ankara, Türkiye were determined to be <LOD based on the analytical results. It was concluded that the proposed method is usable and advantageous because it is effective, reliable, sensitive, and reproducible and can be used for the simultaneous analysis of more than one analyte in a short time using a few reagents.

## Introduction

Honey has a long history as a natural product processed by bees throughout the world. Honey produced by bees is both a natural and healthy food and is nutritious (14, 30). In general, honey consists of 79% different sugars (38% fructose, 31% glucose, 8% disaccharides, and 2% other sugars) and 17% water. The remaining 4% of honey is a complex matrix containing more than 300 chemical compounds, including enzymes, vitamins, minerals, and amino acids. There are more than 300 monoclonal honey varieties worldwide (acacia, clover, eucalyptus, orange-flower, pine, etc.) that have a unique flavor or stand out with another characteristic (9, 10).

Türkiye is one of the world's richest honey-producing regions, thanks to its geography, climate, and the fact that it produces honey throughout the year. Türkiye is one of the countries with the richest flora in Europe with the presence of approximately 10,000 different plant species, as well as having approximately 75% of the nectar plant species that are important for beekeeping identified in the world. According to the FAO, world honey production report, China has the highest honey production, followed by Türkiye in second place. Almost all (90%) of the global pine honey is produced in Türkiye (3, 9, 17, 26, 28). The production of pine honey is unique among honey types. It is created by the pine cotton bollworm (*Marchalina hellenica*), which lives on the red pine tree (*Pinus brutia*). The pine cotton bollworm takes the protein in the sap that it sucks from the pine and excretes sugary juice from its body. This sweet secretion is collected by honey bees and is transformed into pine honey (1).

Neonicotinoids (NEOs) are crop protection products widely used around the world. Recent scientific studies have reported that they may present a potential health risk. Thus, understanding the amount of NEOs in food products for human consumption is essential. Recently, honey consumption has increased significantly because of its health benefits (4). It is therefore important to monitor NEOs in honey, not only in view of the potential risks to human health and as an indicator of environmental pollution (2). The European Commission (EC) maximum residue levels (MRLs) of neonicotinoid authorized in honey are 50 ng/g for acetamiprid (ACE), imidacloprid (IMI), clothianidin (CLO), and thiamethoxam (TMX) and 200 ng/g for thiacloprid (THI) (Table 1) (7, 12). In 2013, EC seriously limited the use of neonicotinoid pesticides and coated seeds (clothianidin, thiamethoxam and imidacloprid) (5). In April 2018, the European Commission banned these compounds for all outdoor activities (6). The use of CLO, IMI, and TMX was banned by the General Directorate of Food and Control in Türkiye on December 19, 2018 (21).

Several methods have been described for quantifying NEOs in honey using a variety of techniques (LC-MS/MS, GC-MS/MS, UPLC-UV, UPLC-DAD and LC-amperometric detector). Most neonicotinoids are not suitable for Gas chromatography (GC) because they are volatile and non-polar. GC is an analytical technique applicable to gas, liquid, and solid samples (components that are vaporized by heat) (12, 16). Therefore, in this study, LC-MS/MS was used to analyze the extracts obtained from the honey samples.

The complex nature of the honey matrix and the need for nanogram-per-gram measurement necessitates the inclusion of the sample preparation phase in the test procedure. For liquid chromatographic analyses of neonicotinoid pesticide residues, a number of pre-treatment procedures for honey samples have been described. The techniques commonly used as pretreatment procedures include conventional liquid–liquid extraction (LLE), modified QuEChERS, and dispersive liquid–liquid microextraction (DLLME). The QuEChERS method is currently the most universally used and accepted sample preparation method because it requires some chemicals compared with conventional methods, allowing the simultaneous determination of many pesticides (4, 12, 18, 24). In the extraction phase of the honey samples, the QuEChERS method was selected.

To date, a considerable number of studies have been conducted on neonicotinoid in honey on a global scale (22, 19). However, there is a paucity of research on neonicotinoid in honey produced in Türkiye. Given that 80% of Türkiye's honey exports are strained, primarily to the United States and European Union countries, addressing this issue is of particular importance. Furthermore, to the best of our knowledge, no other study on neonicotinoids in honey, either in terms of sample size or sample diversity (especially pine honey), has been published for Türkiye that is as comprehensive as this one.

The aim of this research is to establish a suitable procedure for the analysis of commonly used NEOs (ACE, IMI, THX, CLO and THI) in strained honey samples line with European requirements (Commission Decision 2002/657/EC). The method was subsequently used to examine honey samples from Türkiye, with the objective of evaluating the efficacy of the extant prohibition using LC-MS/MS. The present study on honey sourced from Türkiye represents a significant contribution to the global repository of data on neonicotinoid exposure.

## Materials and Methods

***Chemicals, Reagents, and Solutions:*** Imidacloprid (IMI), thiacloprid (THI), acetamiprid (ACE), thiamethoxam (TMX), clothianidin (CLO), and citric acid trisodium salt dihydrate were purchased from Sigma Aldrich (Germany). All standards had a purity greater than 98%. Acetonitrile (ACN) (LC Purity) and methanol (MeOH) (LC Purity) were obtained from Isolab (Germany). Formic acid was obtained from Merck (USA). Magnesium sulfate was obtained from Sigma-Aldrich (Japan). Sodium chloride (NaCl) was purchased from Sigma-Aldrich (Denmark). Ammonium formate was purchased from Sigma-Aldrich (India). Primary and secondary amino acid (PSA) was obtained from Agilent (USA). Sodium hydrogen citrate dehydrate was purchased from Sigma-Aldrich (Belgium). An Elga 664 (UK) water purification system was used to purify the water.

***Standards:*** Primary standard dilutions ($S_1$) of all analytes were made in 1000 ng/µl of acetonitrile and kept in vials refrigerated at +4 ◦C. Intermediate standard solutions ($S_2$) were further diluted as required in ACN. $S_2$ 100 ng/µl and 10 ng/µl standards were prepared. Working solutions ($S_3$) were obtained by diluting $S_2$ with acetonitrile. For IMI, ACE, TMX, and CLO with a maximum residue limit of 50 ng/g, a mixed $S_3$ of 10 ng/µl was prepared from the $S_2$ of 100 ng/µl. For THI with a maximum residue limit of 200 ng/g, an $S_3$ of 4 ng/µl was prepared from standard $S_2$. These $S_3$'s was used to prepare positive (spike) samples at the 0.5, 1, 1.5, 2, and 5 MRL levels.

***LC–MS/MS Conditions:*** LC-MS/MS analysis was performed using a Shimadzu HPLC instrument (Shimadzu Corporation, Kyoto, Japan). A Shimadzu (8040) triple-quadrupole mass spectrometer was used to link the system. The mass spectrometer (MS) was also fitted with an electrospray ion source. Analyte retention was conducted at 40°C on a Phenomenex Synergi (4 µm Max-RP 80 A 50 x 2mm) LC column. The flow rate and volume

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of injection were set to 0.4 ml $min^{-1}$ and 10 µl respectively. The mobile-phase solvents used were water (0.1% formic acid) solvent A and methanol (MeOH, 5mM ammonium formate) solvent B. Gradient elution program was 0-1 min; 5% B; 1-6 min; 95% B; 6-6.50 (min); 5% B and a 3-min wash at 100% A. The entire chromatography run time was 10 min. The MS parameters were as follows: nebulizing gas stream, 3 L/min; DL heat, 250°C; heating block heat, 400°C; drying gas stream, 15 L/min; and column oven, 40°C.

***Sample Collection:*** A sample of 60 honey (20 each of pine, blossom and chestnut) was obtained from wholesale, retail, and local outlets and offered for consumption in the central districts of Ankara province between June and December 2021. Honey samples were collected according to the sampling procedure outlined in the National Residue Monitoring Program (NRMP) of the Ministry of Agriculture and Forestry, Türkiye (29). Prior to analysis, all samples were stored at room temperature and in darkness.

***Sample Extraction from the Analyses:*** Honey samples were analyzed using a partial modification of the QuEChERS method described by Mrzlikar et al. (23). For sample preparation, a 10-g test solution of honey was placed into a 50 ml centrifuge tube made of polypropylene (Falcon). Ultrapure water 10 mL and acetonitrile 10 mL were added. To this was added (4 g of anhydrous $MgSO_4$, 1 g of NaCl and 1 g of citric acid trisodium salt dihydrate ($C_6H_5Na_3O_7 \cdot {}_2H_2O$) and 0.5 g of disodium hydrogen citrate sesquihydrate ($C_6H_6Na_2O_{7.1.5}H_2O$). The sample was then shaken vigorously for 60 s. Centrifuged at 3000 rcf for 10 min. The supernatant (4 mL) was recovered, and 0.9 g of anhydrous MgSO4 and 0.15 g of primary secondary amino acid sorbent were added. The mixture was vortexed for 30 s and then centrifuged at 3000 rcf for 5 min. Dried under nitrogen ($N_2$) atmosphere at 40 °C. Diluted in 1000 µl methanol/water solvent (20/80). The final extracts were analyzed by LC MS-MS after they had been filtered by passing them through a size 0.22 µm PTFE filter.

***Validation Parameters:*** European requirements (Commission Decision 2002/657/EC) for method performance were followed. The linearity, limit of detection (LOD), limit of quantification (LOQ), decision limit (CCα), detectability (CCβ), accuracy (recovery), precision (repeatability and within-laboratory reproducibility), selectivity, and robustness were evaluated for each NEO (8, 13, 20).

The linearity of the method was verified by constructing calibration curves using spiked blank honey (negative control) samples at concentrations between 25 and 1000 ng/g. The precision and accuracy of the analytical method were evaluated by analyzing spiked honey samples containing IMI, ACE, TMX, CLO, and THI at concentrations of 0.5, 1, and 1.5 times the permitted limit set forth by the European Commission. The method's selectivity was assessed by analyzing a blank honey matrix (n = 10) and verifying the absence of any overlap (signal, peak, etc.) at the point at which the target analytes were expected to elute. LOD and LOQ were calculated using the slope of the calibration curve and the standard deviation (Sd) of the response. European Decision 657/2002/EC recommends two analytical parameters: CCα (the critical alpha concentration at risk alpha, and CCβ, the critical beta concentration at risk) (8). These parameters facilitate the evaluation of the critical concentrations at which the technique can consistently distinguish and quantify a substance while simultaneously considering the inherent variability of the method and the statistical probability of erroneous determination. For substances with maximum residue limits, detection capacity refers to the concentration level at which the method can accurately detect the permissible limit concentrations with a confidence level of 95%. CCα was calculated as the mean measured concentration at the MRL level plus 1.64 times the variance of reproducibility ($SR_{MRL}$) at these concentrations. The calculation for CCβ is derived by summing CCα by 1.64 times the corresponding $SR_{MRL}$, assuming that the $SR_{MRL}$ at the CCα level is equal to that at the MRL level (8).

## Results

***MS/MS Method Development:*** The initial phase involved optimising MS detection conditions. Each compound was injected at 100 ng/g to optimise MS conditions. The instrument was run in the multiple reaction monitoring (mrm) mode. For each analyte, two precursor-to-product ionic passes were monitored. All analytes were analyzed by optimization in ESI mode. IMI, ACE, TMX, CLO, and THI were positively identified. An overview of the precursor and product ions, collision energies (CE), and retention times of each analyte are given in Table 1.

***Chromatographic Conditions Optimization:*** A Phenomenex Synergi (4 µm Max-RP 80 A 50 x 2mm) LC column was used for the chromatographic separation of neonicotinoid. The peak shapes, heights, and retention times of neonicotinoid were determined using this column. To achieve optimal separation of analytes in gradient flow and obtain satisfactory chromatographic separation, the gradient elution program was established as follows: 0-1 min; 5% B; 1-6 min; 95% B; 6-6.50 (min); 5% B and a 3-min wash at 100% A. To determine the flow gradients, the analysis times and peak heights of the chemicals were adjusted under the most favorable conditions. In the mobile phase study, neonicotinoid was

ionized, and the peak shapes, peak heights, and response values obtained were found to be best in mobile phase A (water, 0.1% formic acid) and mobile phase B (methanol, 5 mM ammonium formate). Separation of the five neonicotinoid was completed in a time period of less than 7 mins. The optimized analytical conditions of LC-MS/MS enabled the identification and effective separation of all investigated chemicals with good peak resolution (Figure 1).

***Method Validation:*** Linearities were tested by establishing calibration curves using neonicotinoid-free honey samples (matrix-matched curves) at concentrations within the range allowed by the European Decision for IMI, ACE, TMX, CLO, and THI. Method linearity matrix-matched calibrations (mmc) with triplicate replicates showed that the method was linear, with $R^2$ values >0.99 for all investigated compounds (Figure 2).

**Table 1.** MRM values for neonicotinoids and Maximum residue levels (MRLs) for neonicotinoid pesticides in honey.

| Analyte | Mass | Products Ion | Dwell Time | Polarity | Collision Energy | Retention Time | MRL (ng/g) |
|---|---|---|---|---|---|---|---|
| Acetamiprid | 223 | Q1 126 | 24 | Positive | 23 | 3.592 | 50 |
| | | Q2 56 | 24 | Positive | 17 | 3.592 | |
| Clothianidin | 249 | Q1 169 | 38 | Positive | 12 | 3.349 | 50 |
| | | Q2 131 | 38 | Positive | 16 | 3.349 | |
| Imidacloprid | 256 | Q1 209 | 24 | Positive | 19 | 3.336 | 50 |
| | | Q2 175 | 24 | Positive | 16 | 3.336 | |
| Thiamethoxam | 291 | Q1 211 | 24 | Positive | 13 | 2.952 | 50 |
| | | Q2 181 | 24 | Positive | 24 | 2.952 | |
| Thiacloprid | 252 | Q1 126 | 24 | Positive | 21 | 3.832 | 200 |
| | | Q2 90 | 24 | Positive | 40 | 3.832 | |

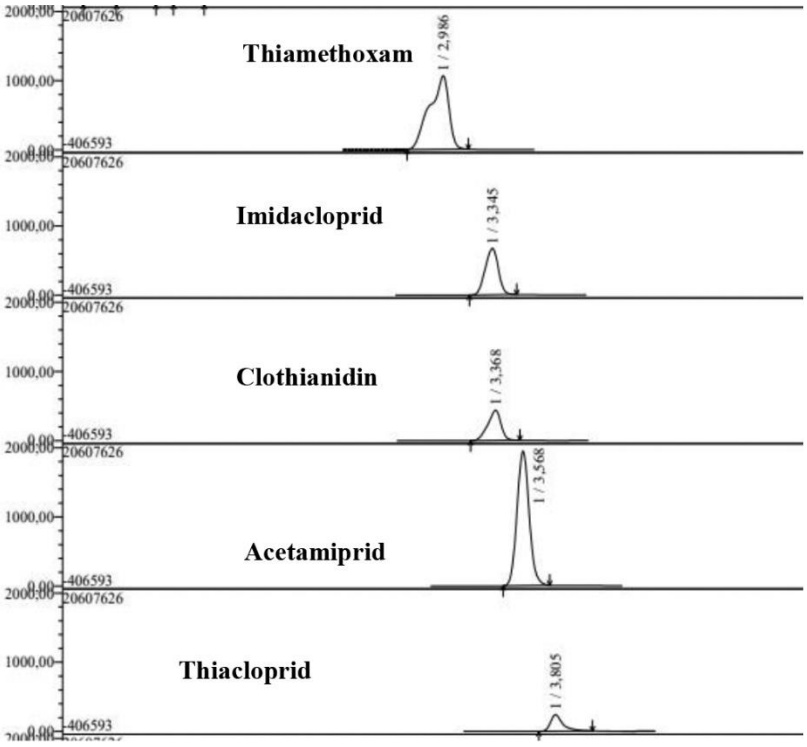
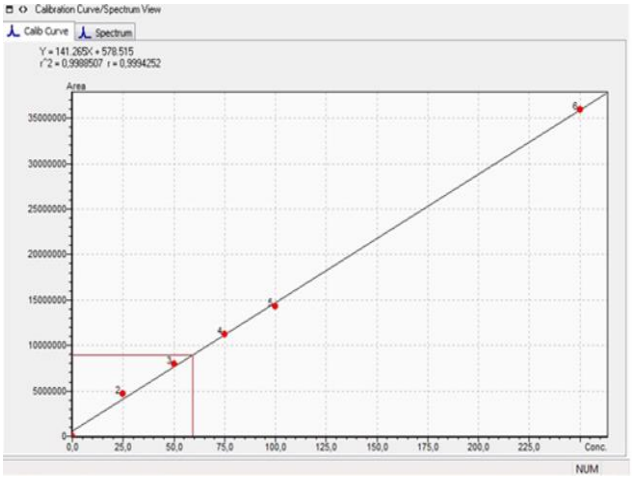
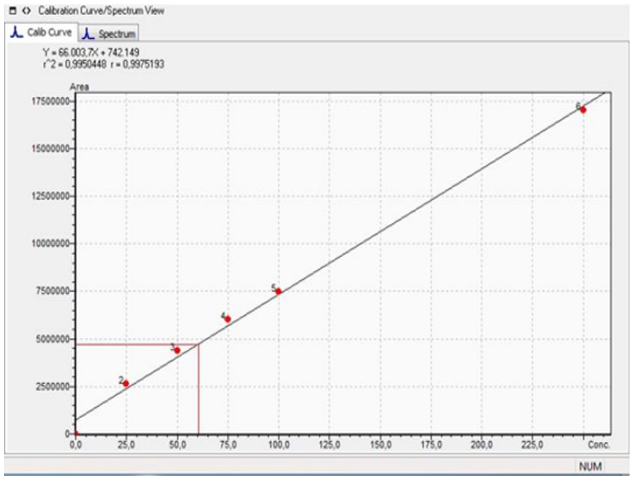



**Figure 1.** LC–MS/MS chromatograms of neonicotinoid.

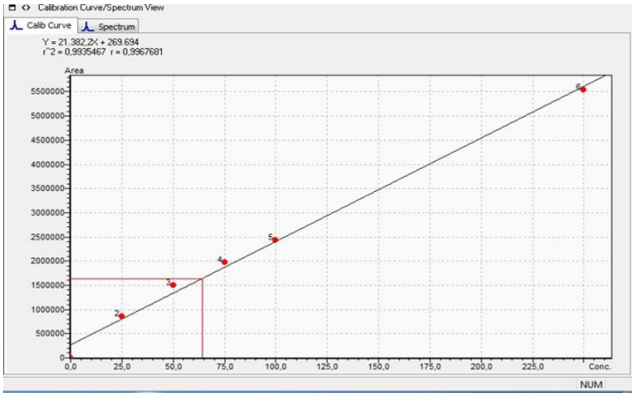
**Acetamiprid**; Correlation coefficient ($R^2$) = 0,9999



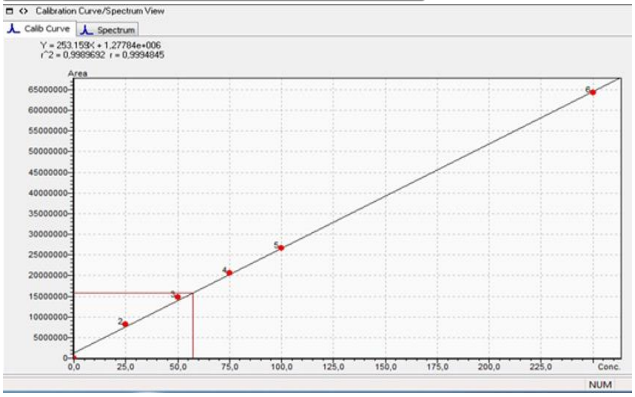

**Imidacloprid**; correlation coefficient ($R^2$) = 0.9950.




**Clothianidin**; Correlation coefficients ($R^2$) =0,9935




**Thiamethoxam**; correlation coefficient ($R^2$) = 0.9994.




**Thiacloprid**; correlation coefficients ($R^2$) =0,9956

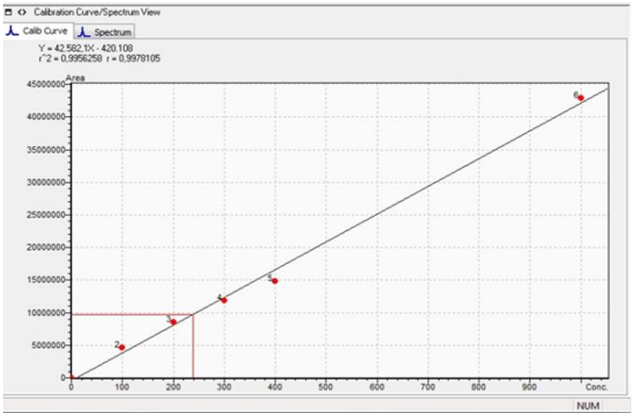



**Figure 2**. Calibration curves of the tested neonicotinoid.

For the purpose of determining LOD and LOQ, spike samples were prepared at concentrations of 5 ng/g for ACE, IMI, CLO, and TMX, and 10 ng/g for THI. These samples were applied to 10 separate empty honey samples using a 6-point calibration line and were measured ten times. The LOD was calculated by multiplying the Sd of the measurements by 3, whereas the LOQ was determined by multiplying the Sd by 10. The observations indicated that the LOD values for ACE, IMI, CLO, TMX, and THI were remarkably low, which can be attributed to the enrichment aspect of the method employed. The results of the analyses are presented in Table 3.

**Table 2.** Method validation data: Percentage recovery (R %), repeatability (RSDr %), and within-laboratory reproducibility ($RSD_R$ %).

| Analyte | Acetamiprid | | | Clothianidin | | | Imidacloprid | | | Thiamethoxam | | | Thiacloprid | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Spike Level (MRL) | 0.5 MRL | 1 MRL | 1.5 MRL | 0.5 MRL | 1 MRL | 1.5 MRL | 0.5 MRL | 1 MRL | 1.5 MRL | 0.5 MRL | 1 MRL | 1.5 MRL | 0.5 MRL | 1 MRL | 1.5 MRL |
| R (%) | 100.6 | 101.8 | 100.1 | 100.9 | 100.5 | 99.1 | 99.4 | 100.6 | 99.5 | 99.1 | 100.5 | 99.7 | 99.6 | 100.0 | 99.0 |
| $RSD_r$ (%) | 3.1 | 3.5 | 3.3 | 3.3 | 2.8 | 2.2 | 3.3 | 1.8 | 1.9 | 3.5 | 2.0 | 1.4 | 3.3 | 2.1 | 1.7 |
| $RSD_R$ (%) | 3.3 | 5.4 | 3.5 | 3.3 | 2.9 | 2.2 | 3.3 | 1.9 | 1.9 | 3.5 | 2.0 | 1.4 | 3.3 | 2.1 | 1.7 |

**Table 3.** Limit of detection (LOD), limit of quantification (LOQ), decision limit (CCα), and detection capability (CCβ) values (ng/g).

| Analyte | Acetamiprid | Clothianidin | Imidacloprid | Thiamethoxam | Thiacloprid |
|---|---|---|---|---|---|
| LOD (Limit of Detection) | 0.6 | 0.6 | 0.7 | 0.9 | 3.8 |
| LOQ (Limit of Quantification) | 2.1 | 2.2 | 2.6 | 3.2 | 12.7 |
| CCα (Decision limit) | 5.4 | 5.2 | 5.1 | 5.1 | 20.2 |
| CCβ (Detection capability) | 5.8 | 5.4 | 5.3 | 5.3 | 20.5 |

The precision and accuracy expressed in the recovery of honey samples was evaluated by analyzing samples containing IMI, ACE, TMX, CLO, and THI at concentrations of 0.5x, 1x, and 1.5x the European Decision limits. For the recovery assays, empty honey samples (10 g) were fortified with the corresponding levels of the $S_3$ mixture of the analytes. Six duplicates per spike stage were analyzed on the same day, and a matrix-matched calibration graph was generated. Each series was run on three different days (54 spiked samples in total) and consisted of one matrix calibration graph and 18 spiked samples. To determine the within-laboratory reproducibility, six replicates for each level of spiking and 3 spikes at each level were produced and analyzed on a different day (a total of 18 spiked samples). The formula was used to calculate the percentage recovery, R%: R% = (C1/C2)*100, where C1 is the level of the test substance in the fortified samples and C2 is the level of the analyte that is added to a "blank" honey. EC Decision 2002/657/EC recommends a recovery of 50-120% for <1 µg/kg, 70-110% for 1-10 µg/kg and 80-110% for >10 µg/kg. Table 2 presents the recoveries of the five analytes within the acceptable range of the precision criteria. Precision was based on two parameters: the repeatability and the within-laboratory reproducibility. From these experiments, the precision (repeatability and intra-laboratory reproducibility expressed as percentage relative standard deviations) was assessed. One-way analysis of variance (ANOVA) was also used to estimate method repeatability and within-laboratory reproducibility. In the ANOVA analysis of variance table, when the criterion Fh ($F_{\text{calculated, Table shows F value}}$) < Fk ($F_{\text{critical value, Table shows F measure value}}$) for each analyte was checked, it was found that the experimental Fh values were lower than the theoretical Fk in all analyses. It was seen that the results came from the same batch. Table 2 presents the data for repeatability (RSDr %) and within-laboratory reproducibility (RSDR %). These results indicate that the methods have good reproducibility.

To complete the validation procedure according to Decision 2002/657/EC, CCα and CCβ were calculated for honey. CCα and CCβ were calculated using the coefficient of variance of reproducibility ($SR_{MRL}$) at the MRL level determined using the ANOVA method in the precision section. The formula was used to calculate the $SR_{MRL} = \sqrt{S_r^2 + S_b^2}$, where $S_r^2$ is the square root of the within-group mean and $S_b^2$ is the between-group standard deviation. If $S_b$ is negative, then it is taken as zero. Decision limits (CCα) were computed as the mean of the measured levels plus 1.64 times the corresponding $SR_{MRL}$. CCβ has been obtained as 1.64 times the decision limit (CCα) plus the relevant $SR_{MRL}$, as follows. The CCα and CCβ levels of the analytes at the MRL level are presented in Table 3.

For the selectivity and robustness parameters, 20 different samples (10 different blank honey matrices and 10 different blank honey mrl spiked matrices) were used**.** It was checked at the point where the objective analytes were expected to elute for any overlap (signal, peak, etc.). The chromatograms of each substance were not affected. In the robustness parameter of the method, matrix robustness and data from experiments performed over a long period by different analysts were evaluated. The specificity parameter data were checked for matrix robustness and reproducibility robustness in the context of in-laboratory reproducibility studies in which experiments were performed by different analysts.

***Application to Real Samples:*** The developed method was used to analyze sixty honey samples. Analyses performed according to Directive 2002/657/EC the concentrations of IMI, ACE, TMX, CLO, and THI in all honey samples (flower, pine, and chestnut honey) available for retail in Ankara, Türkiye were determined to be <LOD based on the analytical results. An example of the chromatograms of the analyzed flower, pine, and chestnut honey is shown in Figure 3.

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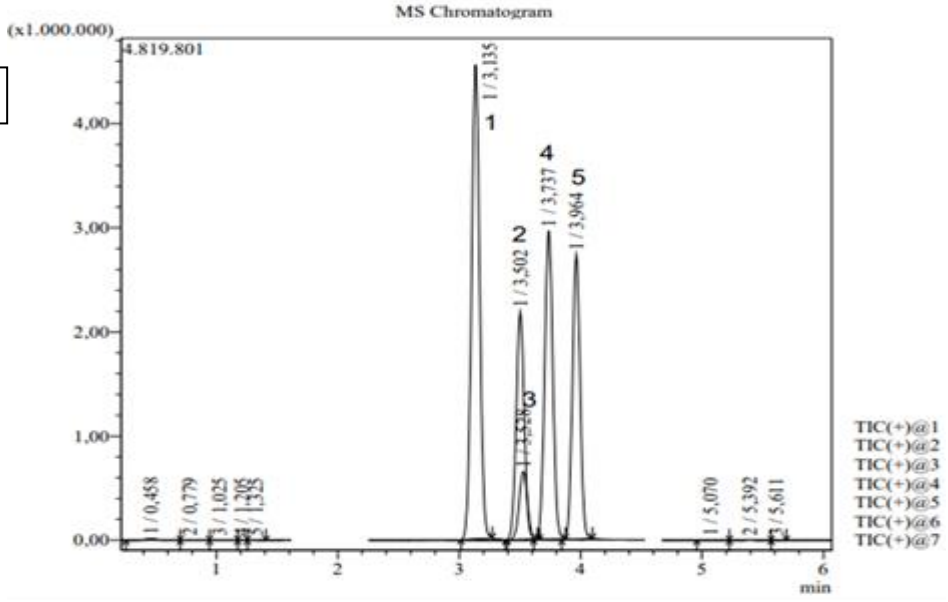
A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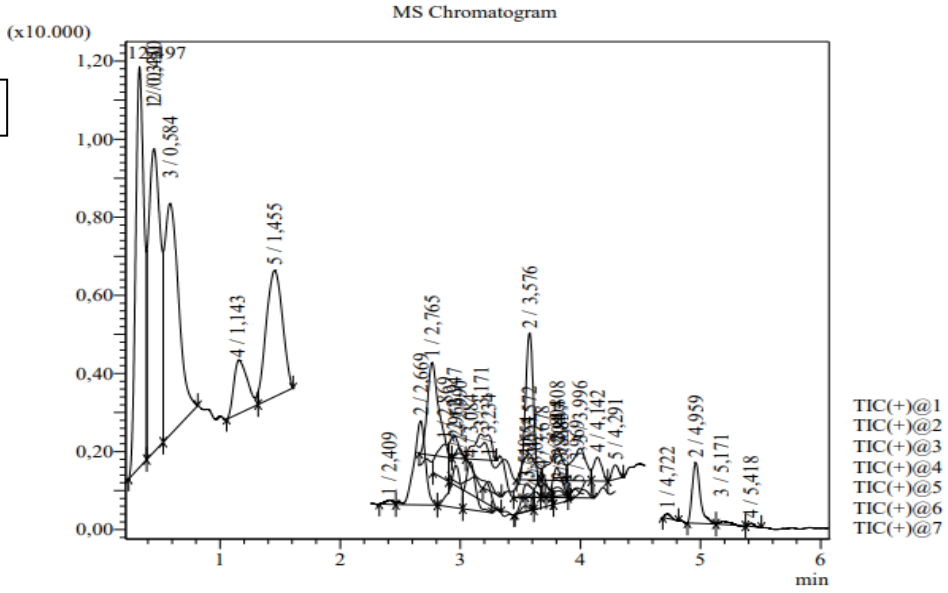
4.819.801
1 / 3,135
2 / 3,502
1 / 3,737
1 / 3,964
1 / 0,458
2 / 0,779
3 / 1,025
1 / 5,070
2 / 5,392
3 / 5,611
TIC(+)@1
TIC(+)@2
TIC(+)@3
TIC(+)@4
TIC(+)@5
TIC(+)@6
TIC(+)@7
min

B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(x10.000)
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5 / 1,455
4 / 1,143
2 / 3,576
1 / 2,765
2 / 2,669
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1 / 4,722
2 / 4,959
3 / 5,171
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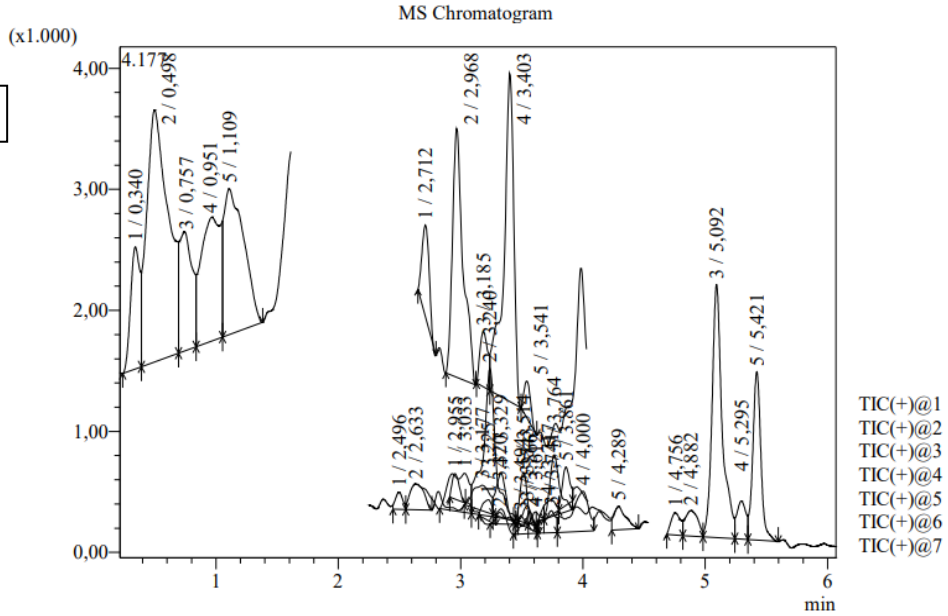
TIC(+)@5
TIC(+)@6
TIC(+)@7
min

C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(x1.000)
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5 / 3,541
3 / 5,092
5 / 5,421
4 / 5,295
1 / 4,756
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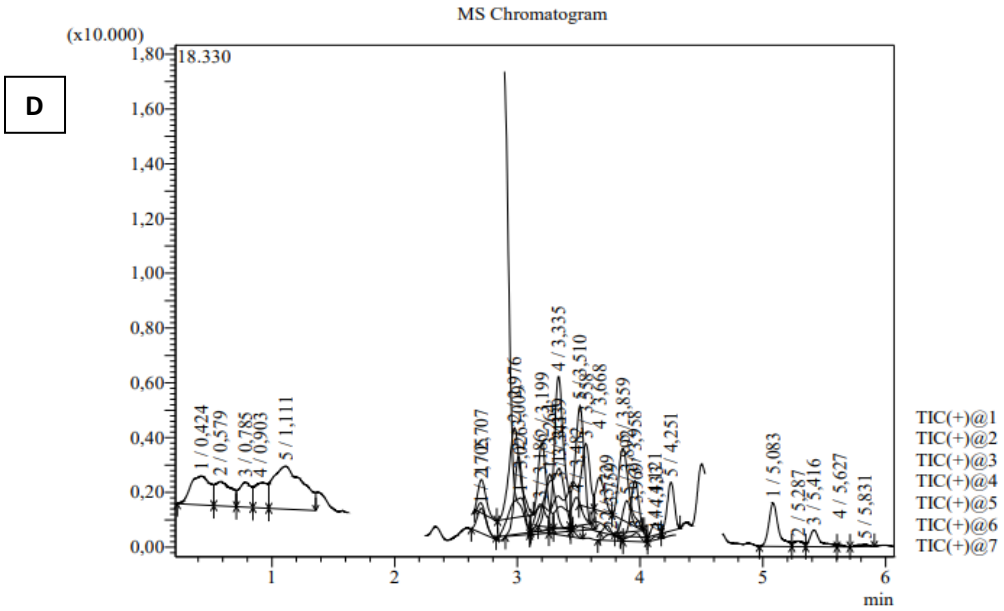


**Figure 3.** LC–MS/MS chromatograms of spiked and negative honey samples as Mass spectra and characteristic fragment pattern chromatograms. A: Honey samples spiked with 50 ng/g for ACE, IMI, CLO, TMX, and 200 ng/g for THI. B: negative pine honey chromatogram, C: negative flower honey chromatogram, D: negative chestnut honey chromatogram. Peaks 1: TMX, 2: IMI, 3: CLO, 4: ACE, 5: THI.

## Discussion and Conclusion

Exposure of honey bees to NEOs can result in pollution of bee products, particularly honey, which is the most widely consumed bee product. This risks public health. Because of its health benefits, honey consumption has increased significantly in recent years (4). It is therefore important to be able to detect these substances in honey, not only because of the potential serious risks to public health, but also because their concentrations may indicate the danger they pose to the environment in general. Ensuring the safety and quality control of honey requires monitoring chemical contaminants in honey and ensuring the absence of toxic residues in the natural product at levels harmful to the consumer (11,27).

Tanner and Czerwenka (27) investigated the residues of three neonicotinoid in 41 honey samples in Austria by LC-MS/MS and revealed the presence of THI (27.4 µg/kg) in 18 samples (22%), ACE (15.2 µg/kg) in 2 samples (5%), and TMX in 1 sample. However, none of these residues exceeded the MRL, and on average, the floral honey samples contained more neo compounds than the wood honey. Although there are slight differences between these reported results and the results obtained from the study, the studies are similar in the sense that no samples exceeded the limit values in both studies. The differences between the analyses may be due to differences in regions and years of analysis and the fact that these products were used during the analysis period.

Song et al. (25), the residues of NEOs in 30 honey samples from various regions of China were investigated using LC MS-MS with anion exchange DPX and LC-MS/MS. They reported that the prevalence of neo pesticides in 30 honey samples ranged from 13% to 33%, and the residues were approximately 11–120 µg/kg, with the maximum levels of dinotefuran, CLO, IMI, and THX exceeding 102 µg/kg The difference between the results obtained in this study and the latest study could be due to the particular extraction methods used and the fact that the use of these products was permitted at the time of the study.

Mrzlikar et al. (23) investigated neonicotinoid residues in 51 honey samples of different plant origins (28 flowers, 15 forests, 5 acacia, 2 lindens, and 1 chestnut) obtained from particular geographical areas of Slovenia between 2014 and 2016 by LC-MS/MS revealed the presence of only THI and ACE. While ACE was detected in 6 samples honey (4 flowers, 1 forest, and 1 linden), it was reported to exceed the LOQ value (2 ng/g) in only one flower sample. THI was reported to be above the LOD in 30 honey samples. The highest level was observed in a flower honey sample (9.6 ng/g) Differences in regions and years of analysis may explain the discrepancy between this study and the results of the current study.

Iplikcioglu et al. (15) investigated the presence of neonicotinoid in 44 strained honey samples obtained from different areas of Türkiye using the LC-MS/Q-TOF method and reported that no neonicotinoid pesticide was found in any of the samples. In this study, analyses were performed by LC-MS/MS, and our findings are consistent with the literature. Although there are slight differences

between these reported results and the results obtained from the study, there is a similarity between the studies in terms of the absence of samples exceeding the limit values in all the study results. It is predicted that slight differences between analyses may be due to differences in regions and years of analysis and the methods and equipment used. Considering all these studies, it is also predicted that although the use of neo pesticides is widespread, the fact that the residue in honey samples is below the limit values may be due to the rapid metabolism of these products by bees.

In this study, adaptation and validation of the IMI, ACE, TMX, CLO, and THI test methods in strained honey were performed. Validation studies were carried out for all neonicotinoid in accordance with the requirements of Directive 2002/657/EC. The advantages of the QuEChERS (cheap, effective, fast, simple, robust and safe) method used in this study were assessed by the use of small amounts of reagents, which allows the analysis of multiple analytes simultaneously in a short time and at a lower cost. In addition, the fact that five different analytes could be analyzed using a single extraction method in this study also highlights the usability of the method. The results suggest that the method employed could be valuable for monitoring the analytes included in the EU residue limit. This was achieved through the use of a highly sensitive and specific LC-MS/MS analytical method developed for detecting neo pesticide residues in honey. The method's usefulness in terms of dissemination is also highlighted. Examination of the honey samples revealed the absence of any residues, indicating the success of the decree enacted in Türkiye in 2018, which prohibited the use of neonicotinoid.

Therefore, the non-detection of neo residues in honey samples is an important result for public health as well as for honey producers and consumers. However, because studies on neonicotinoid in our country are limited in number and region, it is necessary to perform these analyses within a traceable and sustainable plan to reach a definite conclusion in terms of public health.

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## Ethical Statement

This study does not present any ethical concerns. The study was submitted to the General Directorate of Food Control of the Ministry of Agriculture and Forestry of the Republic of Türkiye, and necessary permissions were obtained (27.12.2023/E-71037622-903.03.02.02-12602914).

## Conflict of Interest

The authors declare that they have no conflict of interest.

## Author Contributions

HE; conceptualization, methodology, writing original draft, writing-review & editing, visualization. LA; conceptualization, methodology, writing-review & editing, visualization.

## Data Availability Statement

The data supporting this study's findings are available from the corresponding author upon reasonable request.

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