Determination of 20 neonicotinoid insecticides and their metabolites in infant foods by a modified QuEChERS method combined with HPLC-MS/MS

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Abstract

A rapid, effective, and reliable method for the simultaneous detection of 20 neonicotinoids and their metabolites in infant foods has been developed using liquid chromatography-tandem mass spectrometry (HPLC-MS/MS). To improve the accuracy and precision of the method, different extraction solvents, extraction methods, and adsorbents were evaluated to achieve a better recovery and clean-up effect. Under optimized conditions, the samples were extracted with acetic acid acidified acetonitrile/ethyl acetate by ultrasonication, and then were cleaned with reduced graphene oxide@Fe_3O_4 (rGO@Fe_3O_4) and primary and secondary amine (PSA) through a QuEChERS step. A matrix-matched calibration method was applied for quantification. In three different food matrixes (vegetable & fruit cookies, grain rice cereals, and vegetable purees), all the target compounds showed good linearity, both with values of r^2 > 0.99. The average recovery of all neonicotinoids ranges from 68.0%~106.5% (vegetable & fruit cookies), 70.4%~105.4% (grain rice cereals), and 71.9%~107.4% (vegetable purees). Relative standard deviations were all < 15% for intraday and interday precision. The values of limit of detection and limit of quantification were, respectively, ranging from 0.02-0.35 μg kg^{-1} and 0.1-1.0 μg kg^{-1}. The presented method was applied to the analysis of real samples.

Introduction

Neonicotinoids generally contain three chemical structures: N-cyanoamidines, N-nitroguanidines, and nitromethylenes[1]. They are often applied as seed treatments, soil application, and foliar spraying to translocate to all plant organs and thus avoid insect damage[1–3]. Since their invention in the mid-1990s, neonicotinoids have quickly become widely used, accounting for 25% of the world's pesticide market[4]. Even though neonicotinoids provide positive results from a pest control perspective, their residues' adverse ecological, environmental, and public health effects in pollen, nectar, crops, fruits, and vegetables should also be reviewed. Recent studies have indicated that neonicotinoids can affect the acetylcholine levels of honey bees, resulting in paralysis, loss of orientation and flight ability, and possibly even death[5,6]. Due to their systemic mode of action, approximately 73% of pollen and honey collected from beehives contained at least one neonicotinoid[7]. Furthermore, neonicotinoids are highly stable in water and soil and cannot be washed off before consumption, potentially endangering human health[3,8–10]. A lack of toxicological data makes it difficult to fully assess how neonicotinoids affect human health. Still, mammalian studies have shown that neonicotinoids can severely damage the central nervous system and adversely affect reproduction[10–16]. It is worth noting that some neonicotinoid metabolites are even more toxic than their parent compounds[10,15–17]. With the widespread use of toxic neonicotinoids and their cumulative effects, their deleterious effects on infant brain development deserve special attention[18]. An FDA dietary study clearly stated that considerable levels of neonicotinoids were detected about 6–31% frequency among commercial infant foods[19]. Therefore, it is critical to establish reliable analytical methods to detect neonicotinoids and their metabolites in commercialized infant food.

Several analytical methodologies are commonly employed to detect neonicotinoid residues in food. These include high-performance liquid chromatography with ultraviolet detection (HPLC-UV)[20–23], high-performance liquid chromatography-mass spectrometry (HPLC-MS)[24–27], ion chromatography (IC)[28], gas chromatography (GC)[29], capillary electrophoresis (CE)[30–32], and non-chromatographic methods[33–37]. Overall, among these methods, HPLC-MS is the most commonly used, based on its reliability, sensitivity, and selectivity. While instrument performance is positively correlated with the accuracy of results, sample pre-treatment techniques have a much more major impact. It is no exaggeration to say that the performance of the analytical instrument will be determined by whether or not it can be fully utilized. Traditional sample pre-treatment methods, such as liquid-liquid extraction (LLE)[38], solid-phase extraction (SPE)[39–41], etc., are considered less environmentally friendly due to their high consumption of organic solvents and lengthy operations, and gradually being replaced by the QuEChERS (Quick, Easy, Cheap, Efficient, Rugged and Safe) method[42–44].

Although numerous studies have been conducted on neonicotinoid residues in adult foods, little attention has been paid to infant foods. This article aimed to develop a simple, rapid, and efficient QuEChERS method combined with HPLC-MS/MS to detect neonicotinoids as well as their metabolites in infant foods. As far as we know, the present study is the first to simultaneous determination of 20 neonicotinoid insecticides and their metabolites in infant foods. Various conditions for sample pre-treatment, chromatographic separations, and mass spectrometric detection were investigated and optimized. This established method was further validated and applied to real samples.

Materials And Methods

Materials and Chemicals

The analytical standards of 20 neonicotinoids and their metabolites (Fig 1), including IMI (≥99.8%), CYC (≥92.7%), THX (≥99.0%), CLO (≥99.8%), and FLO (≥99.0%), were purchased from Dr. Ehrenstorfer Gmbh (Germany); 5-OH-IMI (≥99.7%), DN (≥99.0%), UF (≥99.0%), and TFNG (≥99.7%) from A ChemTek (USA); 6-CHL (≥99.0%), ACE (≥99.2%), DM-ACE (≥99.5%), THA (≥99.4%), IMIT (≥98.0%), DM-CLO (≥98.2%), and DNT (≥99.9%) from Tan-Mo Technology (China); SUL (≥99.0%) and TFNA-AM (≥99.8%) from CATO Research Chemicals (USA); IM-1-4 (≥98.0%) AltaScientific (China). Sorbents, such as Neutral alumina (Alumina-N) was obtained from Kermel (China); Graphitized carbon black (GCB), C18 (ODS), Primary and secondary amine (PSA), and aminopropyl (-NH_2) from Biocomma (China); Silica mesoporous SBA-15 (Pore size 6-13 nm), and reduced graphene oxide@Fe_3O_4 (rGO@Fe_3O_4) from XFNANO (China); Multi-walled carbon nanotubes (MWCNT) (≥99%) from Tanfeng Tech (China); Captiva EMR-Lipid from Agilent (USA). HPLC grade formic acid, acetonitrile, acetic acid, methanol, and ethyl acetate were acquired from Merk (USA). Deionized water was produced using a Milli-Q purification system (Millipore, USA). Infant foods, including vegetable & fruit cookies, grain rice cereals, and vegetable purees, were sourced from a local supermarket.

Standard Solutions

Individual standard solutions of 20 neonicotinoids and their metabolites were prepared by separately dissolving the technical grade materials in methanol. Mixed standard solutions containing each target compound for this study were prepared in a mixture of appropriate amounts of the individual stock solutions

with 10% aqueous acetonitrile (containing 0.1% formic acid). A series of working solutions (mixed standard solutions and matrix-matched standard solutions) were prepared at the concentration of 0.1, 0.2, 0.5, 1, 2, 5, 10, 20, 50, 100, 150 ug L^{-1}. All the stock and working standard solutions were stored at -20 ℃ until further use.

Sample Preparation

The representative sample's aliquot (4.0 g) was initially weighed and transferred into a 50 mL poly-propylene centrifuge tube. Then, it was dissolved with 4 mL of deionized water and extracted with 20 mL of acetonitrile/ethyl acetate acidified with 0.1% acetic acid (50/50, v/v). Each extraction process should be vortexed for 30 s and ultrasonicated for 10 min and then centrifuged at 5000 rpm for 10 min. Subsequently, 10 mL of the upper extract was collected, transferred into a 15 mL glass tube, and concentrated to dryness under a gentle stream of nitrogen. The residues were then redissolved with 1 mL of 10% aqueous acetonitrile (containing 0.1% formic acid), and treated with 40 mg PSA, 30 mg rGO@Fe_3O_4 powder, vortexed 1 min. Take the upper transparent layer to pass through a 0.22 um nylon membrane for HPLC-MS/MS analysis.

LC-MS/MS Analysis

A 2040C HPLC (Shimadzu, Japan) coupled with an 8045 Triple Quadrupole mass spectrometer (Shimadzu, Japan) was used for sample analysis. Chromatographic separation was performed at 30 ℃ on an InertSustain AQ-C18 column (2.1 mm × 100 mm, 3.0 um, Shimadzu, Japan), in which the mobile phase consisted of 5 mM ammonium acetate and 0.1% formic acid in water (mobile phase A) and 0.1% formic acid in acetonitrile (mobile phase B). The following gradient program was developed by applying 5 uL injection volume and 0.3 mL/min flow rate. Starting with 0-10% B in 0.2 min, 10-35% B in 2.8 min, 35-75% B in 4 min, re-equilibration at 10% B for 0.5 min, and held at 10 % B in 4.5 min.

The MS/MS detection was performed in multiple reaction monitoring (MRM) mode with positive ESI. Data collection was monitored using the LabSolution Insight software (5.91), and the optimized source parameters were as follows:

• interface voltage of 4 kV

• desolvation line temperature of 250 ℃

• heat block temperature of 400 ℃

• nebulizer gas (nitrogen) flow at 3 L/min

• drying gas (air) flow at 10 L/min

• heating gas (nitrogen) flow at 10 L/min

Results And Discussion

Optimization of HPLC-MS/MS conditions

Multi-residue insecticides detection in complex matrices requires effective chromatographic separation and sensitive mass spectrometric detection. In this regard, instrument parameters should be optimized to enhance selectivity and sensitivity.

A direct infusion of standard solutions of individual and mixed neonicotinoids was used to tune the mass spectrometry for each insecticide. The mass spectrometer was operated in MRM (multiple reaction monitoring) mode to select the most abundant m/z value. It was found that the signals of the 20 neonicotinoid insecticides were more than five times higher in the ESI positive ion mode compared to the ESI negative ion mode. Therefore, the protonated molecular ion $(M+H)^+$ was determined for each analyte and used as a precursor ion. A pair of product ions with the highest abundance and stability were selected for confirmation, while the ion with the highest intensity was used for quantification. The optimized MS^2 parameters are shown in Table 1.

Table 1
Mass spectrometry parameters for the analysis of 20 neonicotinoids and their metabolites

Analyte	Precursor ion (m/z)	Product ion (m/z)	CE(V)	Retention time (min)
IMI	256.1	209.1*,175.1	-14, -17	6.25
5-OH-IMI	272.1	225.1*,134.1	-15, -41	5.42
6-CHL	158.0	122.0*,51.1	-21, -36	6.74
NTP	271.1	126.0*,225.0	-26, -16	4.77
ACE	223.1	126.1*,56.1	-22, -15	6.40
DM-ACE	209.1	126.0*,90.1	-17, -33	5.95
IM-1-4	157.1	126.1*,73.0	-15, -44	1.82
THA	253.0	126.1*,99.0	-20, -43	7.09
IMIT	262.0	181.1*,122.1	-17, -30	6.43
CYC	323.1	151.1*,276.1	-22, -15	5.56
THX	292.0	211.1*,181.1	-11, -23	5.41
CLO	250.0	169.0*,131.9	-10, -14	5.91
DM-CLO	236.0	132.0*, 113.0	-13, -26	5.63
DNT	203.1	129.1*,113.1	-12, -10	3.75
DN	158.1	102.1*,57.1	-17, -25	1.51
UF	159.1	102.1*,67.1	-13, -18	2.12
SUL	278.1	174.0*,154.0	-8, -28	7.15
FLO	230.1	203.0*,173.8	-16, -19	7.29
TFNG	249.0	148.0*,128.0	-33, -35	4.74
TFNA-AM	191.0	98.1*,73.1	-30, -31	3.93
* Quantitation ion				

The column type, mobile phase, and gradient elution program are the main factors influencing chromatographic separation. Neonicotinoids are stable and hydrophilic under acidic conditions[45]. Therefore, adding formic acid to the mobile phase will improve the corresponding intensity of the target peaks. As such, a water- and acid-resistant column, InertSustain AQ-C18, was chosen. Then, three sizes of columns (a. 2.1 mm × 50 mm; b. 2.1 mm × 100 mm; c. 2.1 mm × 150 mm) were tested for separation efficiency of the neonicotinoids. According to the results, the retention time of (a) was too short, causing the neonicotinoids to barely separate from the impurities. In (c), the retention time is too long, which is time-consuming. Alternative (b) satisfies both separation efficiency and time-saving requirements and is preferred. Additionally, the influence of the mobile phase composition on the mass spectrometry signal is evaluated. It was found that adding a small amount of ammonium acetate to the aqueous acidic mobile phase could greatly enhance neonicotinoids signals and decrease noises. And for the organic mobile phase in terms of sensitivity, acetonitrile provides sharper peaks and a higher signal-to-noise ratio than methanol. Altogether, a InertSustain AQ-C18 (2.1 mm × 100 mm) column plus mobile phases (A (H_2O + 0.1% formic acid + 5 mM ammonium acetate) and B (acetonitrile + 0.1% formic acid)) were employed for chromatographic separation.

Optimization of the extraction solvent

Three different solvents and six solvent combinations were examined to obtain satisfactory recoveries of 20 neonicotinoids and their metabolites in vegetable & fruit cookies. The experiment was conducted as described in sample preparation section, but without a clean-up step. As shown in Fig. 2a, average recoveries of 20 neonicotinoids obtained with methanol ranged from 27.0%-69.7%. As for acetonitrile, they were from 41.0%-85.4%. Ethyl acetate provides the data of 20.7%-99.4%. Thus, it seems that acetonitrile and ethyl acetate are better than methanol for most of the analyte. To further select the best extraction solvent, six different combinations were experimented. The combination of solvents improved the analytes' recovery compared to a single solvent, especially the acetonitrile/ethyl acetate mixture (Fig. 2b). In total, the average recovery of the 20 neonicotinoids at acetonitrile/ethyl acetate (V/V) = 1:1 varied from 47.4–90.8%, with relatively favorable results. It is worth mentioning that adding acid to the extraction solvent improves the analytes' recovery. With 0.1% addition, acetic acid was more effective than formic acid (about 8.2% or more for 6-CHL, IM-1-4, THX, and TFNG). Finally, a solvent mixture (acetonitrile/ethyl acetate (V: V) = 1:1) with the addition of 0.01% acetic acid was used as the extraction method to perform the analysis.

Optimization of the extraction method

In this study, under the conditions of acetonitrile-ethyl acetate (1:1, v/v) (with 0.01% acetic acid) as an extractant, the effects of different extraction method (vortex, oscillation and ultrasonic) were compared. Figure 3 reveals that vortex and ultrasonic were superior to oscillation of the three preparation procedures. To accomplish a relatively good extraction, two minutes of vortexing and ten minutes of sonicating were sufficient, and extending the extraction duration did

not increase the recovery of neonicotinoids. In terms of multi-sample processing, the ultrasonic extraction method appears to be more favorable. After ten minutes of sonication, the average recovery of the 20 neonicotinoid insecticides ranged from 52.1–92.2% (Fig. 3).

Evaluation of different sorbents for dispersive solid-phase extraction clean-up in the QuEChERS method

Developing a simultaneous multi-class residue analysis approach requires the extraction and cleaning of pesticides with various physicochemical properties under the same conditions. For this purpose, a modified QuEChERS approach was used in this work since the SPE method could not identify both the 20 neonicotinoids and their metabolites simultaneously. Given the breadth of available sorbents as alternatives to the clean-up step, this study examined the adsorption capability of nine commonly used sorbents (including Alumina-N, GCB, C18, PSA, -NH_2, EMR-Lipid, rGO@Fe_3O_4, MWCNT, and SBA-15) for 20 neonicotinoids and their metabolites. It was found that GCB, MWCNT, and SBA-15 were not suitable for clean-up sorbent because of the severe adsorption of certain neonicotinoid insecticides. Figure 4 shows the average recoveries of 20 analytes in blank vegetable & fruit cookies after being treated with 50 mg of the rest six sorbents (Alumina-N, C18, PSA, -NH_2, EMR-Lipid, and rGO@Fe_3O_4), respectively. Clearly, rGO@Fe_3O_4 shows the best clean-up effect for all the 20 analytes and provides recoveries from 82.7%-103.4%. It is, however, not very practical to use rGO@Fe_3O_4 alone for pigment purification, and the addition of PSA can overcome this difficulty. Using PSA plus rGO@Fe_3O_4, the mass sensitivity of 20 analytes to be measured was improved by 15%. Different doses (20 mg, 30 mg, 40 mg, 50 mg, and 60 mg) were compared based on the color shades of vegetable & fruit cookie extracts (1 mL) after PSA treatment. With an increasing dosage of PSA, the color of the solution became lighter, and once the dosage reached 40 mg, it was no longer changing. As a result, the PSA amount was set to 40 mg, and various dosage of rGO@Fe_3O_4 (20 mg, 30 mg, 40 mg, 50 mg, and 60 mg) were added to form a clean-up combination to optimize the purification method. As shown in Fig. 5, when the rGO@Fe_3O_4 amount was increased from 20 mg to 30 mg, there was a slight increase in the recoveries of the 20 analytes. In comparison, increasing the amount from 30 mg to 60 mg resulted in an insignificant difference. In summary, PSA + rGO@Fe_3O_4 (40 mg + 30 mg) was chosen as the sorbent for the QuEChERS method, and the average recoveries of the 20 analytes ranged between 78.2% and 99.1%.

Matrix Effects (ME)

The slope of the solvent calibration curve and the matrix-matched blank extract calibration curve were used to determine the ME, according to the equation: ME (%) = [(slope of matrix-matched calibration curve - slope of solvent standard calibration curve) / slope of solvent standard calibration curve] × 100. This study evaluated the matrix effects of 20 neonicotinoids and their metabolites in three common infant foods (vegetable & fruit cookies, grain rice cereals, and vegetable purees). As illustrated in Fig. 6, the highest ME value was assigned to grain rice cereals, followed by cookies and the smallest puree. By and large, most insecticides demonstrated strong matrix enhancement (> 25%), with IMIT and DN even exceeding 200%. In order to compensate ME, matrix-matched calibration standards were used for quantification.

Linearity, Limit of Detection (LOD), and Limit of Quantification (LOQ)

A matrix-matched standard solution (grain rice cereals) was used to test the linearity of each insecticide. Calibration curves were constructed by plotting the insecticide/IS peak area ratios against the concentration of the corresponding calibration standards at several different levels (0.1–150 ug L^{-1}) (6 replicates per level). For all 20 analytes, regression lines with coefficient of determination (r^2) above 0.99 were obtained as shown in Table 2. LODs and LOQs of the present method correspond to the signal-to-noise ratios of 3 and 10, respectively.

Table 2
Linear range, correlation coefficient, matrix-matched calibration curves, LOD and LOQ of 20 insecticides.

Analytes	Linear range ($\mu g\ L^{-1}$)	Calibration equation	(r^2)	LOD ($\mu g\ kg^{-1}$)	LOQ ($\mu g\ kg^{-1}$)
IMI	0.10–150	$y = 6.48814\times10^4x + 2.04375\times10^5$	0.99697	0.03	0.10
5-OH-IMI	0.10–20	$y = 3.06136\times10^4x + 2.13584\times10^5$	0.99977	0.03	0.10
6-CHL	0.50–150	$y = 4.68328\times10^3x$-4.32262×10^3	0.99966	0.15	0.50
NTP	0.10–150	$y = 1.35791\times10^5x + 1.38557\times10^5$	0.99968	0.05	0.10
ACE	0.10–150	$y = 2.46311\times10^5x + 1.04398\times10^5$	0.99989	0.05	0.10
DM-ACE	0.10–150	$y = 2.96158\times10^4x + 5.10213\times10^4$	0.99909	0.04	0.10
IM-1-4	0.10–150	$y = 1.19841\times10^5x + 1.6017\times10^5$	0.99927	0.03	0.10
THA	0.10–150	$y = 1.91302\times10^5x + 8.89287\times10^4$	0.99994	0.04	0.10
IMIT	0.20–150	$y = 1.81392\times10^4x + 8.30912\times10^4$	0.99759	0.06	0.20
CYC	0.10–150	$y = 7.72841\times10^4x + 1.03523\times10^5$	0.99936	0.03	0.10
THX	0.10–150	$y = 1.94394\times10^5x + 3.01757\times10^5$	0.99947	0.05	0.10
CLO	0.20–150	$y = 1.35816\times10^4x + 6.51004\times10^4$	0.99872	0.06	0.20
DM-CLO	0.10–50	$y = 2.36747\times10^3x$+1.61315×10^4	0.9995	0.03	0.10
DNT	0.10–150	$y = 2.21956\times10^5x + 3.32085\times10^4$	0.99997	0.04	0.10
DN	0.10–150	$y = 1.54079\times10^5x + 4.75588\times10^5$	0.99498	0.03	0.10
UF	0.10–150	$y = 3.47059\times10^5x + 2.47365\times10^5$	0.99983	0.02	0.10
SUL	0.20–150	$y = 1.04821\times10^4x + 5.87326\times10^3$	0.99954	0.05	0.20
FLO	0.50–150	$y = 1.45297\times10^5x + 1.49268\times10^5$	0.99956	0.15	0.50
TFNG	0.50–150	$y = 4.64025\times10^3x + 1.6581\times10^4$	0.99771	0.10	0.30
TFNA-AM	1.0-150	$y = 4.74677\times10^3x$ -2.01129×10^3	0.99992	0.35	1.0

Accuracy and Precision

A recovery rate experiment was conducted to test the method's accuracy. Blank samples were spiked with three levels (1.0, 10, and 100 μg kg^{-1}) following 6 replications to determine the recovery rate. The method's precision was calculated as the relative standard deviation (RSD) of the six samples spiked at three concentration levels. As can be seen in Table 3, the intraday average recovery of all pesticide ranges from 64.2%~108.7% (vegetable & fruit cookies), 69.1%~106.5% (grain rice cereals), and 71.0%~109.8% (vegetable purees). The interday recoveries were from 68.6%~105.5% (vegetable & fruit cookies), 70.7%~106.5% (grain rice cereals), and 73.0%~108.7% (vegetable purees) (Table 4). Good repeatability is demonstrated with RSD < 15.0% for all analytes (Tables 3 and 4). The results mentioned above indicate the method's simplicity, efficiency, and reliability, as it can be used to identify 20 neonicotinoids and their metabolites in infant foods simultaneously. Typical multiple reaction monitoring (MRM) chromatograms of a spiked sample are shown in Fig. 7.

Table 3
Recovery rates of all target compounds spiked in three different matrices at three different concentrations (Intraday n = 6)

Analyte	vegetable & fruit cookies						grain rice cereals						vegetable purees		
	1.0 μg kg^{-1}		10 μg kg^{-1}		100 μg kg^{-1}		1.0 μg kg^{-1}		10 μg kg^{-1}		100 μg kg^{-1}		1.0 μg kg^{-1}		10
	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Rec (%)
IMI	73.9	12.7	98.0	5.2	102.0	6.1	80.6	7.3	99.7	6.9	99.0	6.5	82.7	9.3	96.
5-OH-IMI	78.6	6.5	78.0	12.2	97.5	6.2	77.9	8.6	85.9	9.7	88.8	9.4	79.7	11.9	86.
6-CHL	74.8	7.3	103.9	3.9	99.4	6.2	69.1	13.1	85.8	11.6	96.5	6.7	71.0	12.7	83.
NTP	75.9	11.2	97.4	7.5	91.3	5.7	77.6	10.1	87.1	8.2	99.5	4.3	81.1	8.5	82.
ACE	64.2	8.4	89.8	3.5	92.8	7.0	80.0	6.8	81.6	9.5	92.5	7.7	83.9	6.1	81.
DM-ACE	75.9	11.3	99.9	7.2	108.7	4.5	91.1	8.4	75.2	7.6	94.8	9.2	89.1	7.2	75.
IM-1-4	77.1	13.3	83.4	4.5	93.1	5.6	73.2	9.7	71.5	14.6	93.3	6.9	81.1	11.3	77.
THA	76.1	11.2	86.1	6.6	98.9	6.7	88.1	9.2	100.4	8.5	91.4	5.4	90.2	9.5	92.
IMIT	70.0	11.9	93.6	6.5	96.1	6.8	74.4	10.2	105.2	9.9	100.7	5.0	79.7	11.1	100
CYC	79.5	10.6	93.2	7.4	105.9	6.7	83.7	10.1	104.3	6.8	93.0	6.2	91.0	6.3	101
THX	75.9	10.6	72.7	11.0	98.1	3.4	87.9	10.1	76.0	7.5	101.2	7.7	94.5	8.7	77.
CLO	69.7	13.4	99.3	7.0	98.5	6.1	79.8	11.8	104.7	8.5	100.7	2.5	81.7	10.9	106
DM-CLO	76.2	11.7	101.2	5.2	104.3	4.8	89.1	5.1	72.1	11.2	100.2	7.8	91.6	10.9	72.
DNT	84.1	8.9	72.8	8.5	92.6	5.1	72.2	10.2	72.5	6.8	106.5	8.3	77.8	9.4	76.
DN	81.8	10.1	91.4	7.3	94.3	8.4	80.6	11.1	82.2	11.1	90.0	7.1	82.6	9.0	71.
UF	64.3	14.6	87.5	10.0	99.3	6.6	76.8	8.1	99.0	7.6	102.6	5.6	83.5	8.9	100
SUL	79.2	8.1	75.6	7.3	78.8	10.7	78.7	4.5	73.3	12.2	95.5	4.7	74.5	11.0	89.
FLO	72.9	13.3	96.8	5.6	99.5	8.8	85.2	8.7	97.7	9.4	98.7	4.4	93.9	6.3	82.
TFNG	72.8	6.0	108.0	6.1	94.9	7.8	75.2	6.8	88.1	9.2	101.0	7.0	82.6	8.9	82.
TFNA-AM	70.1	13.7	71.2	10.2	94.8	8.8	84.4	3.6	78.9	9.9	98.7	7.4	88.4	9.4	87.

Table 4
Recovery rates of all target compounds spiked in three different matrices at three different concentrations (Interday n = 6)

Analyte	vegetable & fruit cookies						grain rice cereals						vegetable purees		
	1.0 μg kg^{-1}		10 μg kg^{-1}		100 μg kg^{-1}		1.0 μg kg^{-1}		10 μg kg^{-1}		100 μg kg^{-1}		1.0 μg kg^{-1}		10
	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Rec (%)
IMI	72.3	12.7	99.8	6.4	104.3	5.6	78.2	9.8	95.4	7.8	96.8	8.7	82.7	9.2	93.
5-OH-IMI	78.9	9.7	76.0	9.1	97.1	6.1	79.1	8.5	89.1	7.9	90.7	6.6	80.4	8.6	86.
6-CHL	82.0	11.6	102.5	6.0	97.6	6.4	70.7	10.8	86.7	9.3	95.6	5.3	73.0	10.3	84.
NTP	77.7	8.9	96.8	6.4	88.0	6.6	77.8	10.1	84.7	8.8	98.8	5.2	82.4	9.4	80.
ACE	69.7	12.5	85.3	7.6	90.5	6.7	83.8	7.7	83.0	7.9	89.5	7.2	90.0	9.2	79.
DM-ACE	77.1	9.5	105.5	6.0	104.9	9.9	89.5	7.6	78.4	9.2	95.7	7.2	93.2	8.1	75.
IM-1-4	73.8	9.6	86.4	7.1	94.5	7.0	72.8	10.2	73.9	10.9	97.8	7.0	76.2	11.0	81.
THA	77.2	10.3	85.5	7.5	94.9	7.6	89.1	9.8	98.6	7.0	96.4	10.5	94.4	7.5	96.
IMIT	72.4	9.7	91.7	6.7	95.7	10.1	74.4	8.1	106.5	7.3	99.1	5.6	74.6	9.9	103
CYC	83.0	12.1	91.5	7.3	104.5	5.9	82.7	8.7	103.1	8.1	90.3	6.1	85.7	8.2	101
THX	74.8	9.1	74.9	9.4	94.6	5.7	84.1	8.4	77.7	9.2	100.7	6.0	89.8	8.8	79.
CLO	68.6	11.3	98.2	6.7	98.5	5.9	80.6	10.0	102.8	7.2	103.9	6.1	87.0	9.3	103
DM-CLO	81.7	9.7	97.2	6.3	102.2	10.4	90.8	7.4	73.8	10.2	98.7	6.8	93.1	10.1	71.
DNT	83.9	9.6	74.7	10.4	95.7	6.9	73.1	10.8	73.3	10.5	103.5	6.4	78.9	9.6	73.
DN	79.4	11.0	94.2	6.3	92.6	6.8	80.3	8.7	80.5	10.6	91.3	6.9	86.1	8.3	73.
UF	70.1	10.9	88.4	6.7	100.0	7.7	80.8	9.6	97.5	7.7	100.1	5.7	85.2	12.0	98.
SUL	80.5	7.9	73.3	8.2	81.3	8.6	74.7	9.6	72.5	11.5	95.7	6.0	77.1	9.3	84.
FLO	71.4	9.7	96.8	6.1	100.7	6.6	90.1	9.1	91.1	8.3	99.5	5.6	92.3	6.8	86.
TFNG	72.5	9.3	101.5	6.2	98.1	7.3	74.7	8.8	84.6	9.4	99.0	6.8	79.6	10.6	82.
TFNA-AM	73.9	10.4	73.3	8.2	96.6	6.5	85.8	7.2	81.7	9.1	97.1	6.5	87.2	8.3	84.

Analysis of real samples

Although China does not yet have a national standard detection method for neonicotinoid pesticides in infant foods, the national standard method for neonicotinoid pesticides in fruits and vegetables (GB/T 20769 – 2008) would be an ideal control method for comparison with ours[46]. The established method and GB/T 20769 – 2008 method were applied to test fifteen samples of infant foods collected from local supermarkets (5 kinds of cookies, 5 kinds of cereals, and 5 kinds of purees) in order to ascertain the accuracy of the method. Both methods have detected acetamiprid in one cookie sample and thiamethoxam in one puree sample. As shown in Table 5, each sample was tested 6 replicates, no significant difference can be found between the results of the two methods. This indicates our method's sensitivity and stability are good and that it can be used for real sample detection.

Table 5
Results of neonicotinoids determination in infant foods measured by GB method and proposed method

Matrix/analytes	Method	Sample (μg/kg)						Average value	RSD
		S1	S2	S3	S4	S5	S6	(μg/kg)	(%)
cookies /ACE	GB/T 20769 – 2008	4.76	4.66	4.56	4.66	4.61	4.55	4.63	1.68
	Proposed method	4.76	4.63	4.49	4.57	4.66	4.41	4.59	2.71
cereals /THX	GB/T 20769 – 2008	5.37	5.36	5.41	5.30	5.62	5.69	5.46	2.86
	Proposed method	5.57	5.64	5.56	5.35	5.69	5.65	5.58	2.17

Conclusion

A modified QuEChERS method was developed for simultaneously determine 20 neonicotinoids and their metabolites in infant foods. Nine commonly used sorbents were evaluated for their impact on ME. We found that rGO@Fe_3O_4 hardly interacts with neonicotinoids, but it does a good job of adsorbing impurities in the matrix. When combined with PSA, mass spectrometry is significantly more sensitive, as pigment interference is eliminated. After optimizing the mass spectrometry parameters, extraction solvent, and extraction method, the present method was able to produce good recovery and precision for all target compounds. The developed method was applied for real sample determination, and some neonicotinoid pesticides were found to be detectable, implying that safety test for infant foods should be taken seriously.

Declarations

Author Contributions Liqiang Guo and Kai Li contributed equally to this work. Project management: Kai Li; experiment and analysis: Liqiang Guo, Jinling Zhang, Guoning Tian, Ke Wang, Yajing Li; writing manuscript: Kai Li; revising: Kai Li; final approval: all authors.

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Data Availability All data generated or analyzed during this study are included in this published article.

Ethical Approval This article does not contain any studies with human participants or animals performed by the authors.

Informed Consent Informed consent is not applicable.

Conflicts of Interest The authors declare no competing interests.

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Figures

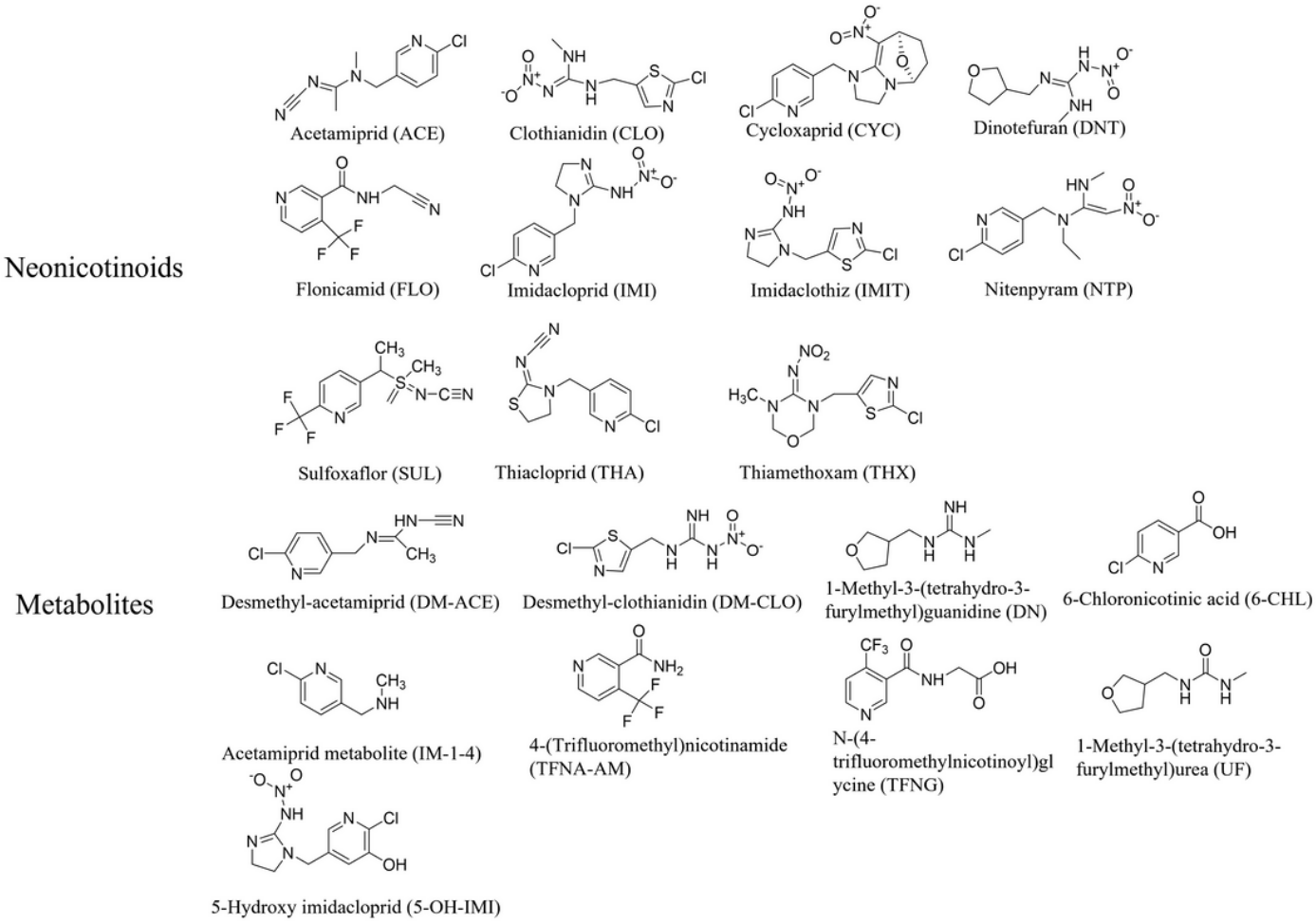

Figure 1

Chemical structures of 20 neonicotinoids and their metabolites.

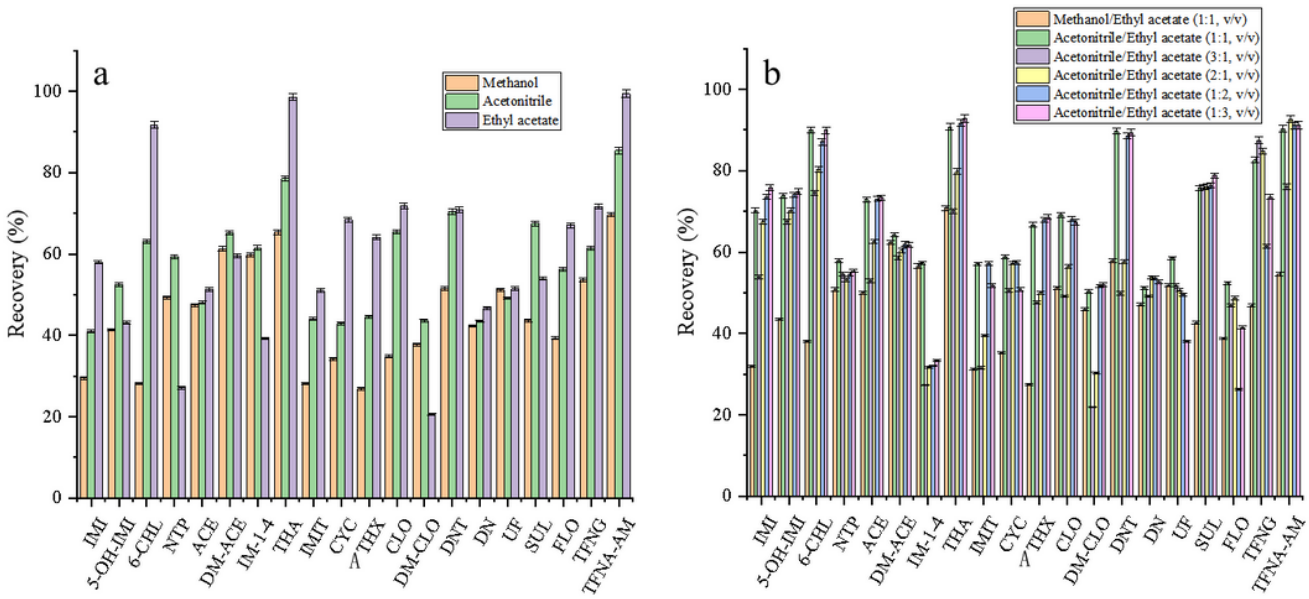

Figure 2

Comparison of recoveries for all analytes with different extraction solvents (a. single solvent; b. mixture).

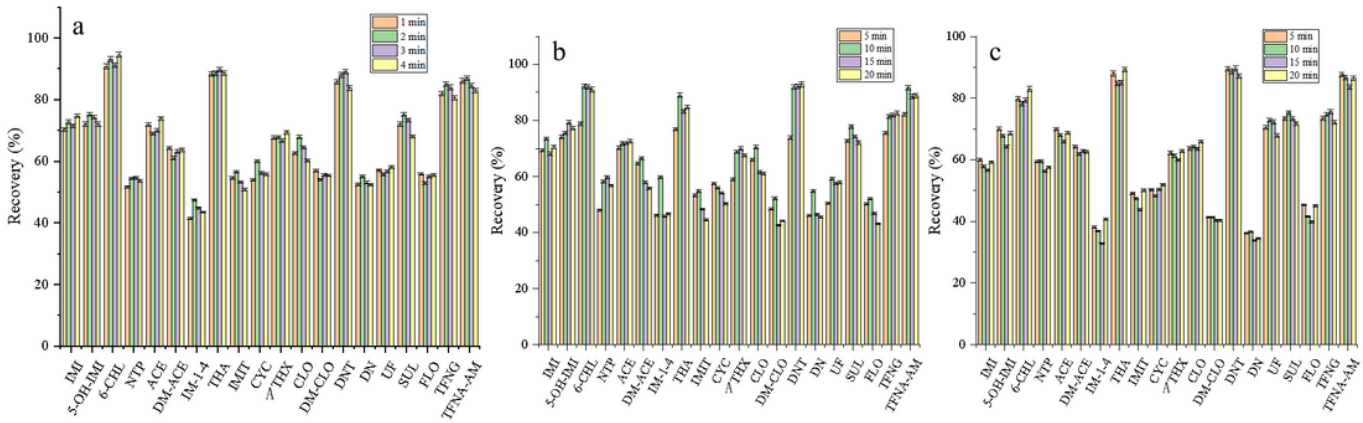

Figure 3

Comparison of recoveries for all analytes with different extraction methods (a. vortex; b. sonication; c. oscillation).

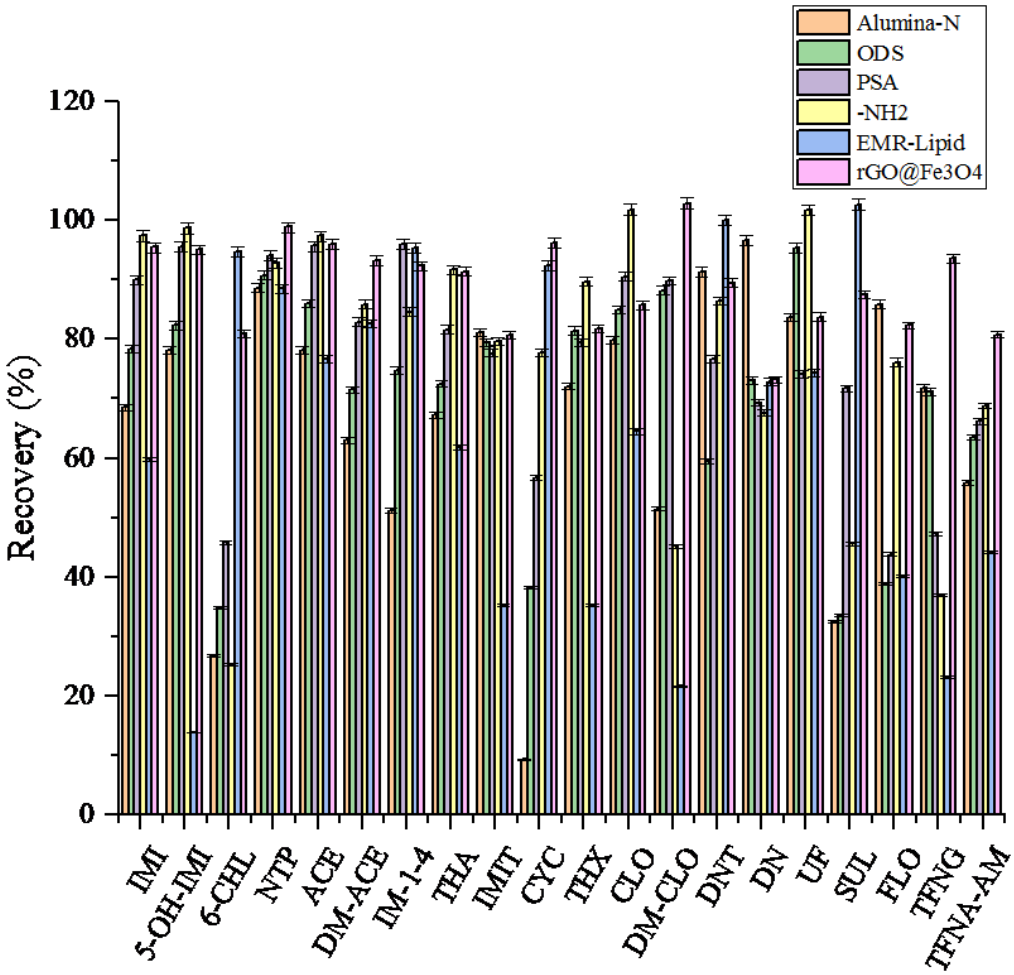

Figure 4

Comparison of recoveries for all analytes treated with different QuEChERS sorbents.

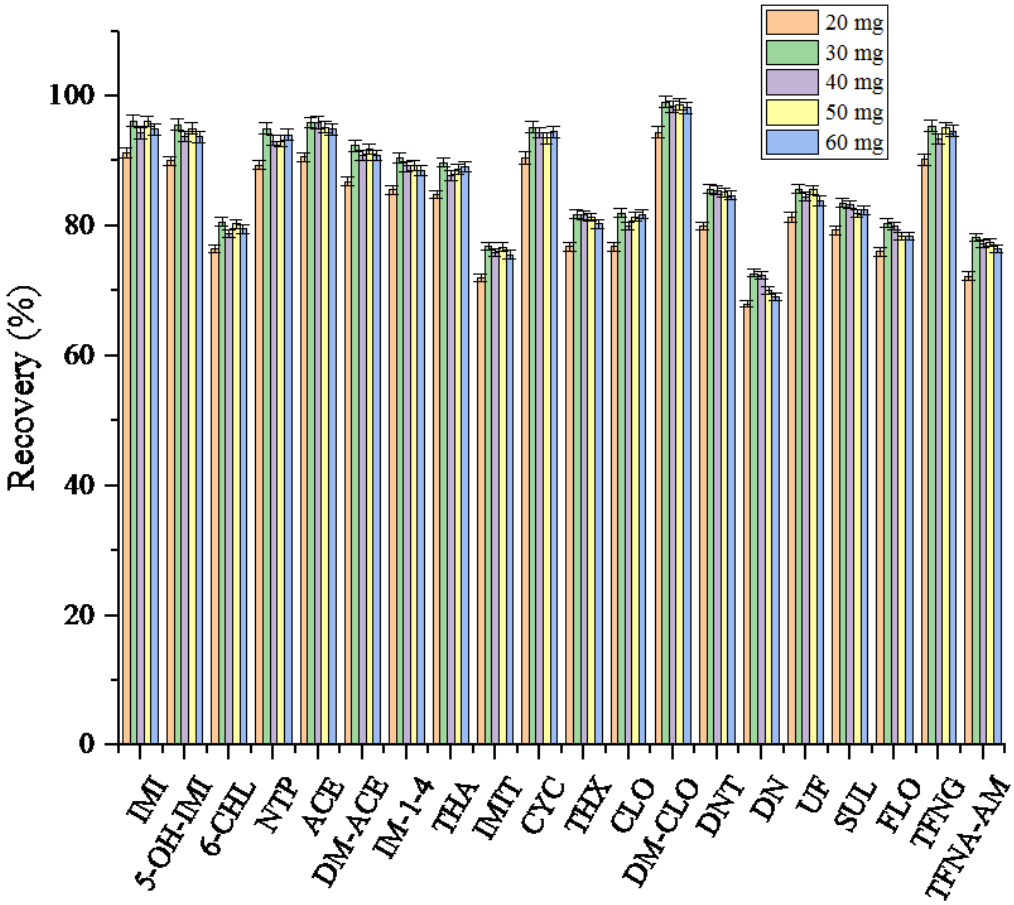

Figure 5

Comparison of recoveries for all analytes treated with PSA (40 mg) plus different amount of rGO@Fe_3O_4 (20 mg, 30 mg, 40 mg, 50 mg and 60 mg).

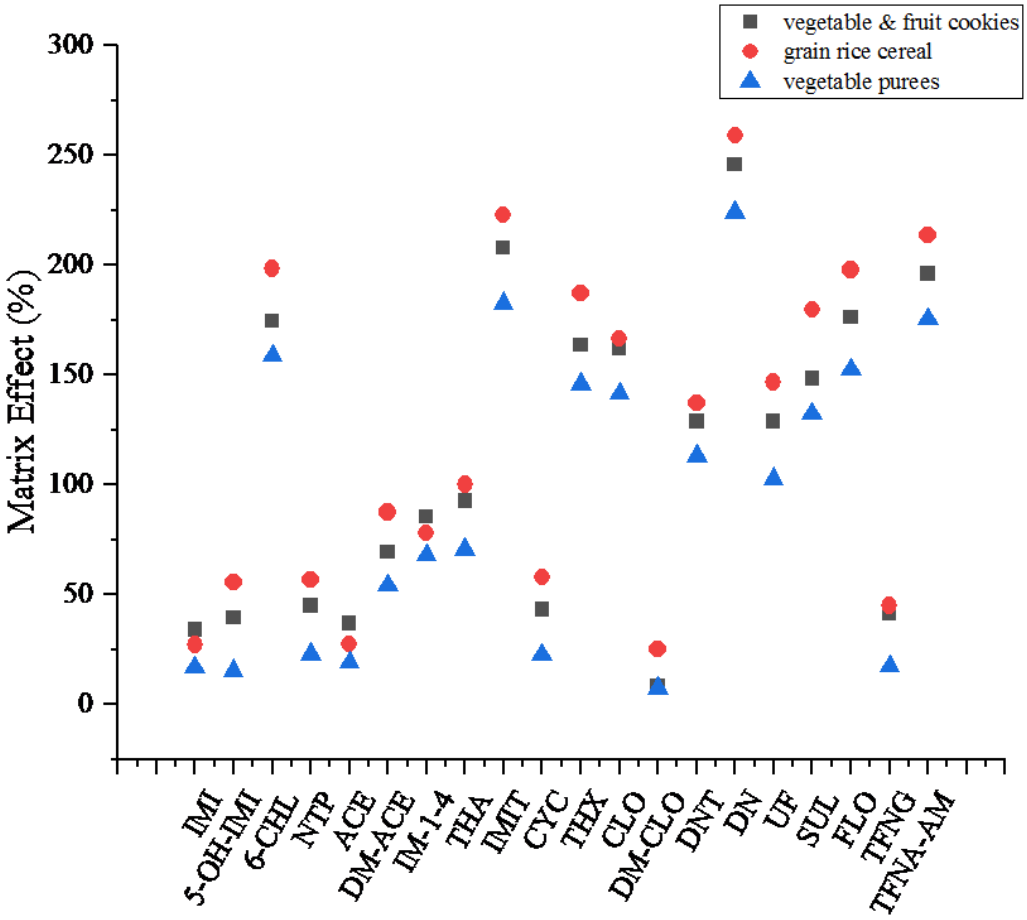

Figure 6

Matrix effect of 20 neonicotinoids and their metabolites in infant foods.

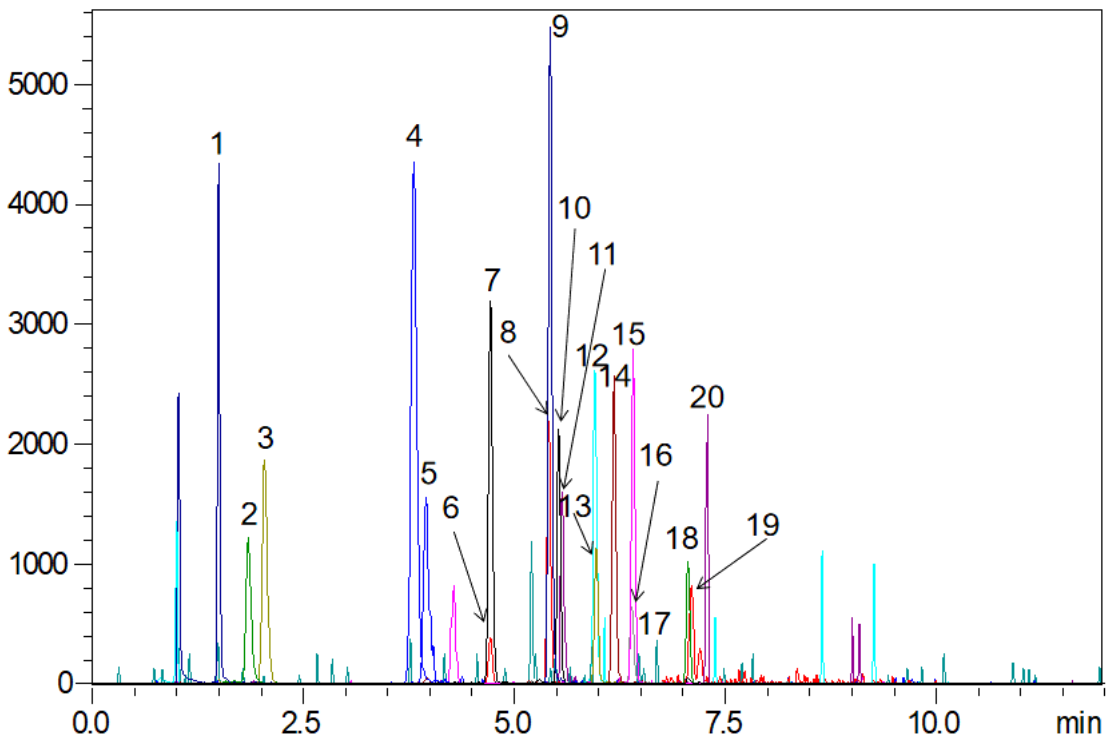

Figure 7

Chromatograms of 20 kinds of neonicotinoid pesticides and its metabolites in vegetable & fruit cookies（1.0 μg kg^{-1}）(1-20 (DN, IM-1-4, UF, DNT, TFNA-AM, TFNG, NTP, THX , 5-OH-IMI, CYC, DM-CLO, CLO, DM-ACE, IMI, ACE, IMIT, 6-CHL, THA, SUL, FLO))