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The robust and sensitive analysis of polar anionic pesticides in baby foods using a novel cartridge clean-up and ion chromatography-triple quadrupole mass spectrometry

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ABSTRACT

Purpose: This presentation describes the development of a new workflow for the robust and sensitive multiresidue analysis of polar anionic pesticides and polar contaminants in ready prepared mixed-fruit baby foods.

Methods: The workflow is based on the Quick Polar Pesticides Extraction (QuPPe) method(1), but with a Solid Phase Extraction (SPE) cartridge clean-up. Pesticides in the cleaned-up extracts were quantified using Ion chromatography-triple quadrupole mass spectrometry (IC-MS/MS).

Results: The recovery and precision for the quantitation of 12 priority analytes (parent pesticides and metabolites) at 5-10 ng/g in baby foods were compliant with the method validation criteria detailed in the EU SANTE/12682/2019 guidelines (2). These results were achieved without the need for Isotopically Labelled Internal Standards (ILIS). Furthermore the removal of matrix co-extractives with the cartridge SPE clean-up substantially improved the robustness of the workflow.

INTRODUCTION

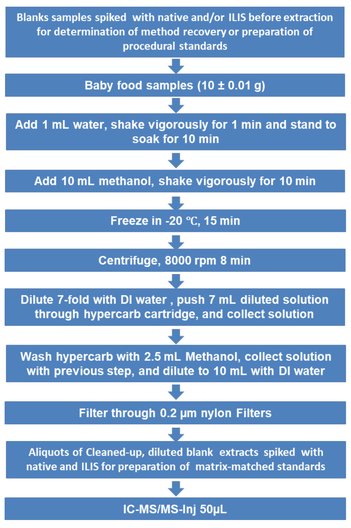
Polar pesticides such as glyphosate and ethephon are widely use in agriculture, while perchlorate is a common contaminant from fertilizers and chlorate is a contaminant from the use of biocides in food processing establishments. Not surprisingly, residues of these and other polar compounds will inevitably occur in food, but these pesticides are monitored less frequently than other classes of pesticides . Primarily, because the physicochemical properties of polar compounds present unique analytical challenges and consequently higher costs of analysis. Because of their high water solubility they are not easily amenable to extraction using methods that involve liquid/liquid partition or clean-up using typical SPE methods which tend to limit the number of polar compounds that can be included in a single method. They are not directly amenable to reversed-phase chromatography so cannot be included in the common multi-residue methods. Hydrophilic Interaction Liquid Interaction Chromatography (HILIC) and Ion Chromatography (IC) provide increased retention of polar compounds compared to reversed–phase chromatography, but the high level of co-extractives often present in non-cleaned extracts can quickly contaminate chromatographic-mass spectrometry systems. In the case of baby foods, additives such as thickening agents create additional challenges. Hence the importance of the workflow described herein and based on the combination of cartridge SPE and IC-MS/MS to overcome these challenges and provide a sensitive and robust method for the analysis of pesticides in baby foods in compliance with the EU SANTE guidelines and EU MRLs .

MATERIALS AND METHODS

Sample Preparation

Baby food (mixed fruit purees) purchased from retail outlets in Beijing, China, were homogenized using a blender. Sub-samples (10 g) were extracted with methanol (10 mL) and then placed in a freezer (15 min) before centrifugation at 8000 rpm, for 8 minutes. The supernatant was diluted 7-fold with ultrapure water. The extract was then and pushed through a Thermo Scientific™ HyperSep™ Hypercarb™ SPE cartridge, 500mg/6mL, stacked on top of a Thermo Scientific™ Titan 3™ Nylon Syringe Filter, 0.2 µm. The eluate was then analyzed using IC-MS/MS. The details of the extraction are shown in the flow diagram (Figure 1). The clean-up allows for a 50 µL injection volume for increased sensitivity.

Figure1. Extraction and clean-up procedure



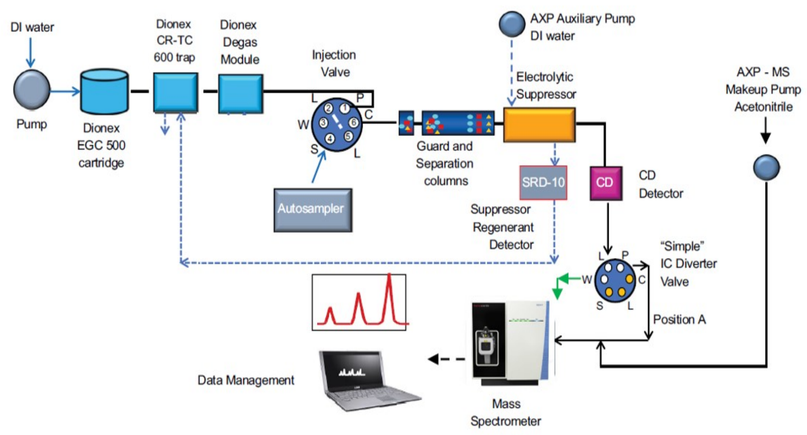
Instrumentation

A Thermo Scientific™ Dionex™ Integrion™ HPIC™ system, fitted with an electrolytic eluent generator and conductivity cell was coupled to a Thermo Scientific™ Dionex™ AS-AP Autosampler and Thermo Scientific™ TSQ Altis™ Triple Quadrupole Mass Spectrometer. Separation was achieved using a Thermo Scientific™ Dionex™ IonPac™ AG19-4µm Guard column (50 mm × 2 mm i.d.) coupled to a Thermo Scientific™ Dionex™ IonPac™ AS19-4µm Analytical (250 mm × 2 mm i.d.) column with elution of polar anionic analytes using a potassium hydroxide gradient. A Thermo Scientific™ Dionex™ ADRS 600 Anion Dynamically Regenerated Suppressor (2 mm), installed after the column, converted the KOH to water before the eluent flow entered a conductivity detector and mass spectrometer which were connected in series. Acetonitrile solvent modifier delivered by an auxiliary pump, via a T junction between the conductivity cell and mass spectrometer, assisted more efficient desolvation in the MS source and typically increased the response of most analytes by 3-4 fold. Data acquisition was performed by selected reaction monitoring (SRM) using pos/neg polarity switching. Most analytes were acquired in the negative mode, but with bialphos and maleic hydrazide acquired in the positive mode. The parameters for best response for each precursor to product ion transition were individually optimized by infusing standards. The injection volume of the cleaned-up extract was 50 µL. All of the individual modules of the system were fully integrated and controlled by a single software for ease of use.

Data Analysis

The system control, data acquisition and data processing was done using Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS), which allows instrument control, method development, quantitative/qualitative analysis, and customizable reporting, all within one package. For data processing, the ion ratio (±30%), retention time (±0.1 min), linearity (>0.99 with residuals < ±20), recovery (70-120%) and precision (≤ 20%) were set as user-defined criteria as per the EU SANTE guidelines. The configuration of the system is illustrated in Figure 2.

Figure 2. Configuration of the integrated IC-MS/MS system

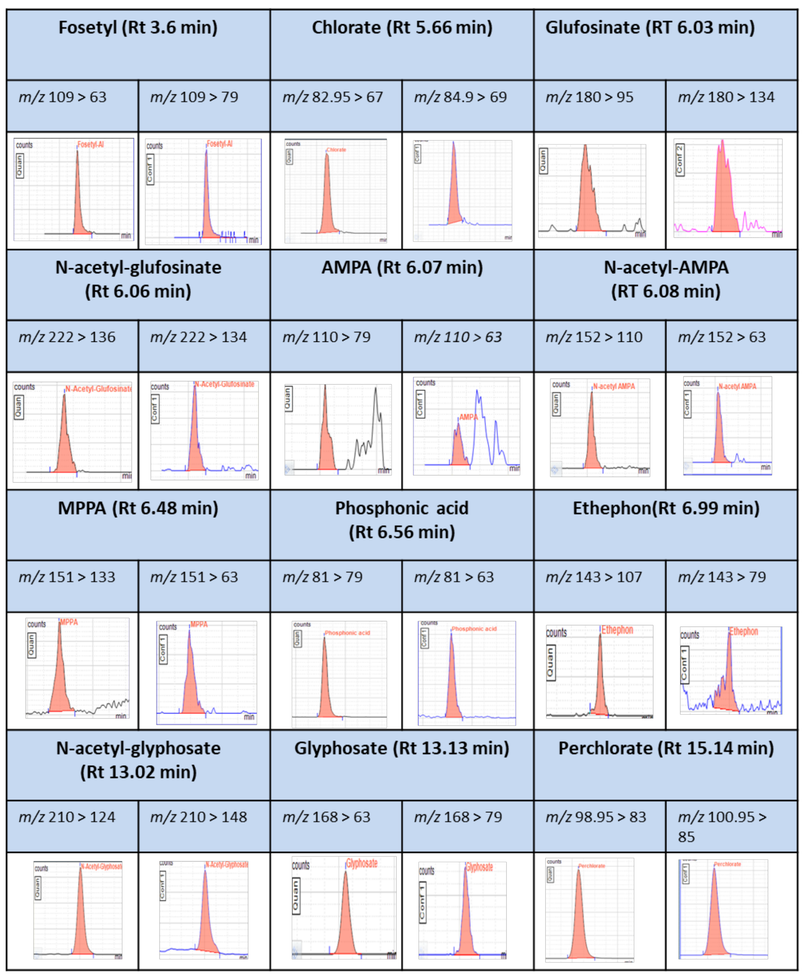


RESULTS

Method performance

The peak shapes and response for the quantifier and qualifier product ions for 12 polar anionic compounds at 0.01 mg/kg in baby food matrix are shown in Figure 3. With the exceptions of AMPA, glufosinate, cyanuric acid and maleic hydrazide, all of the analytes gave a response with sufficient signal: noise for two product ions at 0.0025mg/kg.

Figure 3. Response for quantifier and qualifier product ions for 12 compounds at the 0.01 mg/kg in baby food matrix.



Validation of the work flow.

Because of the analytical challenges with recovery of polar analytes, many laboratories, and especially those in Europe, use Isotopically labelled Internal Standards (ILISs) to correct low recoveries. However, ILIS for all compounds are not readily available in many countries including China. The validation of the workflow was therefore performed using ; seven available ILIS (AMPA, chlorate, perchlorate, ethephon, glyphosate, glufosinate, MPPA), and without ILISs. Matrix-matched calibration and procedural standard calibration [spiked with known concentrations of pesticides before extraction and also known as matrix-extracted calibration (MES)] approaches were also evaluated. The results of validation are shown in Table 1.

Table 1. Recoveries of analytes spiked at 0.005 mg/kg in a baby food matrix

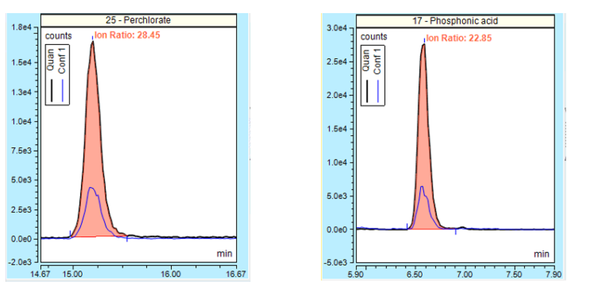
Analytes	Spiked 5 ng/g (glufosinate and cyanuric acid are 10 ng/g: maleic hydrazide is 50 ng/g)							
	MMS no ILIS		MMS + ILIS		Procedural Standards no ILIS		Procedural Standards + ILIS	
	Rec.%	RSD%	Rec.%	RSD%	Rec.%	RSD%	Rec.%	RSD%
Fosetyl-Al	89	2.4	-	-	92	2.3	-	-
Bialphos	84	4.0	-	-	101	3.9	-	-
Glufosinate #	91	10	108	9.0	100	9.7	100	8.8
AMPA	96	18	112	8.0	110	18	115	6.7
HEPA	91	9.4	-	-	117	8.1	-	-
N-acetyl AMPA	84	3.8	-	-	93	3.4	-	-
N-acetyl Glufosinate	91	4.5	-	-	97	4.9	-	-
Chlorate	93	2.4	96	2.1	84	2.7	89	2.2
MPPA	103	8.2	102	6.8	100	8.3	100	6.6
Phosphonic acid	*	*	-	-	*	*	-	-
Ethephon	91	13	98	15	98	11	97	14
Cyanuric acid #	97	13	-	-	84	17	-	-
N-acetyl -glyphosate	83	2.7	-	-	86	3.0	-	-
Glyphosate	77	1.7	109	3.4	81	1.7	104	3.5
Perchlorate	95	2.1	96	2.6	96	2.4	83	2.9
Maleic hydrazide #	78	5.4	-	-	86	5.6	-	-

* Samples 'blanks' contain phosphonic acid so recoveries could not be calculated accurately
# Glufosinate and cyanuric acid were spiked at 0.01 mg/kg and maleic hydrazide at 0.05 mg/kg

Results for all analytes at 0.005 mg/kg, except glufosinate and cyanuric acid (0.01 mg/kg) and maleic hydrazide (0.05 mg/kg) are shown in Table 1. Phosphonic acid (could not be calculated due to a peak in the blank). Recoveries calculated using matrix-matched and procedural standards, both with and without ILIS (where available) were in the range of 83-117 % with %RSDs < 20%. R^2 values were > 0.99 for matrix-matched calibration standards over the range 0.0025 mg/kg to 0.1 mg/kg. All retention times were within ±0.1 minutes and thus results (except phosphonic acid) were fully compliant with the EU SANTE guideline criteria for method validation for pesticide residues.

A high percentage of baby food samples contained residues of perchlorate 0.003- 8.5 mg/kg and phosphonic acid .0.006- 0.04 mg/kg. Examples of residues quantified using standard addition are shown in Figure 4.

Figure 4. Quantitation of 'incurred residues in samples



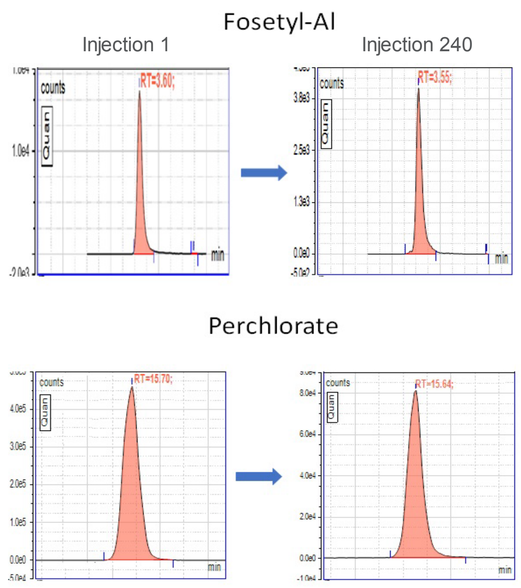
Perchlorate at 0.0007 mg/kg

Phosphonic acid at 0.023 mg/kg

Robustness

The SPE cartridge clean-up substantially improved the robustness of the system. After approximately 240 injections of cleaned-up baby food extracts, the peak shapes, retention times, and the analyte responses remained stable and the mass spectrometer source remained clean demonstrating the robustness of the system. The peak shapes for the first and last eluting compounds, fosetyl-AL and perchlorate respectively, are shown for injection number 1 and injection number 240 in Figure 5.

Figure 5. Stability of responses, peak shapes and retention times after a sequence of 240 injections of baby food.



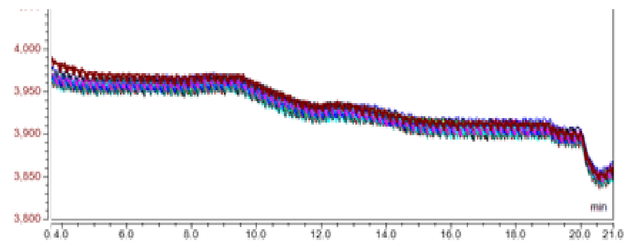
The retention times remained stable during the sequence as shown in Table 2

Table 2. Shift of RT within 240 injections of baby food matrix.

	RT /min (Fosetyl-Al)	RT /min (Perchlorate)
Injection 1	3.60	15.70
Injection 240	3.55	15.64
Shift of RT	0.05	0.06

The system pressure remained stable during the sequence of 240 matrix injections as illustrated in Figure 6. The pressure reading highlighted in red was recorded after injection and the reading in blue after injection 240. This is one of the major benefits of the clean-up. Without the clean-up, matrix co-extractives (possibly thickeners) cause fluctuations in the pressure.

Figure 6. Stability system pressure over a sequence of 240 injections of baby food.



CONCLUSIONS

- The IC-MS/MS fully integrated and automated workflow provides the sensitive robust, reproducible and reliable quantitation and identification of multi-residue polar anionic pesticides in baby food, all in a single analysis.
- The SPE cartridge clean-up method proved very effective at improving the sensitivity and robustness of the method
- The method was validated for most analytes at 0.005 mg/kg with results fully compliant with the EU SANTE validation performance criteria.
- The method described is **Compliant** (with EU SANTE) method performance criteria and MRLs), **Productive** and **Robust**
- Further information on the analysis of polar anionic pesticides is available at www.thermofisher.com/anionicpesticides

REFERENCES

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2. EU SANTE/12682/2019 Guidance document on analytical quality control and method validation procedures for pesticides residues analysis in food and feed, https://ec.europa.eu/food/sites/food/files/plant/docs/pesticides_mrl_guidelines_wrkdoc_2017-11813.pdf (accessed Mar 12, 2019)

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