Food Chemistry 260 (2018) 213–220

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Analytical Methods

Quantification of folpet and phthalimide in food by gas chromatography and mass spectrometry: Overcoming potential analytical artefacts

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ARTICLE INFO

Keywords:
Folpet
Phthalimide
Modified QuEChERS
Gas chromatography
Mass spectrometry
Negative chemical ionization

ABSTRACT

Accurate quantification of folpet is problematic because it degrades into phthalimide during sample preparation and analysis by gas chromatography (GC). Thus, EU regulation was recently modified to include phthalimide in the folpet residue definition. However, recent studies have shown that phthalimide could also be generated from different sources, which could lead to an overestimation of the phthalimide content and therefore to false positives. GC coupled with either negative chemical ionisation and single quadrupole mass spectrometry, or electron ionisation with triple quadrupole mass spectrometry (GC-EI-MS/MS), were evaluated for the determination of folpet and phthalimide in food. Both methods were validated in 4 different matrices namely apple puree, rice flour, raspberry puree and infant formula. Better selectivity and precision were obtained with GC-EI-MS/MS. Negligible amounts of phthalimide was found in blank matrices, and validation results met the SANTE/11813/2017 criteria in all matrices at the LOQ concentration levels by using GC-EI-MS/MS.

1. Introduction

Folpet (*N*-trichloromethyl-thio-phthalimide) is a contact fungicide extensively applied in a wide variety of fruits to prevent diseases connected with the presence of mildew, grey mould, spoilage fungi and wood rot fungi (Tomlin,1997). Folpet inhibits many oxidative enzymes, carboxylases and enzymes involved with phosphate metabolism and citrate synthesis (European Food Safety Agency). It is listed as sensitiser and strong irritants of eyes, skin and respiratory system (Raina-Fulton, 2014). Folpet is metabolised in primary crops into phthalimide, while it is degraded into phthalimide under high temperature or basic pH conditions (European Food Safety Agency). Following the European Food Safety Authority (EFSA) reasoned opinion (European Food Safety Agency), the EU authorities modified the definition of the folpet residue to include its main metabolite/degradation product phthalimide by means of the EU Regulation 2016/156 (European Commission, 2016), which entered in force in August 2016. This new regulation ensures that the amount of phthalimide generated either as folpet metabolite in primary crops, or as folpet degradation product in food processing and/or during analysis in the laboratory was taken into account. However, some studies have recently pointed out the potential generation of phthalimide from sources different than folpet breakdown, as for instance the reaction of phthalic anhydride and a nitrogen source molecule (Relana, 2016, 2017). Phthalic anhydride is a ubiquitous compound used in resins, paintings, plastics (PVC), etc. and it is known to react with urea or ammonia at high temperature. This reaction could take place during food processing (i.e. drying) or analysis (i.e. sample preparation or gas chromatography (GC) injection). Obviously, this might cause an analytical artefact for phthalimide that may lead to an overestimation of its amount, and therefore to false positive results for folpet if the current EU regulation is considered (folpet reported as the sum of folpet and phthalimide). In order to minimise the contribution of external sources of phthalimide, it was proposed to report results as sum of folpet and phthalimide only when folpet is detected (Relana, 2016, 2017). However, this approach could lead to false negatives if folpet is completely degraded to phthalimide or if the analytical method used is not able to detect traces of folpet with enough sensitivity. In order to monitor folpet residues properly according to the new EU regulation, a reliable analytical method, able to avoid any analytical artefact for phthalimide, was needed.

GC coupled to either mass spectrometry (MS) or electron-capture detection (ECD) are the techniques of choice for the determination of folpet in food matrices (European Union Reference Laboratory-Single Residue Method, 2017; Montes, Rodríguez, Ramil, Rubí and Cela, 2009; Raina-Fulton, 2014; Zang et al., 2008). In routine laboratories, MS and especially triple quadrupole -MS (MS/MS) was preferred because of its higher capabilities in terms of universality, selectivity and sensitivity. Folpet has a tri-halo group (tri-choro), therefore a priori it is expected to give excellent response and selectivity using negative chemical ionisation (NCI) (Bailey and Belzer, 2007; Barreda et al., 2006; Pizzutti

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https://doi.org/10.1016/j.foodchem.2018.04.002
Received 29 November 2017; Received in revised form 23 March 2018; Accepted 1 April 2018
Available online 03 April 2018
0308-8146/

et al., 2012). Dedicated methods for the simultaneous determination of folpet and phthalimide are currently available (European Union Reference Laboratory-Single Residue Method, 2017), although current trends were to include them within the scope of multiresidue methods, normally using GC-EI-MS/MS (Páleníková, Martínez-Domínguez, Javier Arrebola, Hrouzková and Garrido Frenich, 2015) and less frequently GC-NCI-MS (European Union Reference Laboratory-Single Residue Method, 2017; Pizzutti et al., 2012). Liquid chromatography (LC) coupled to MS could be an alternative to GC–MS for the simultaneous quantification of folpet and phthalimide, however those analytes are not easily amenable by this technique (European Union Reference Laboratory-Single Residue Method, 2017; Raina-Fulton, 2014), and did not yield fragmentation in negative ionisation mode using a a triple quadrupole as shown in previous studies (Badoud, Ernest, Hammel, and Huertas-Pérez, 2018; Berthet et al., 2011). Recently, a method based on LC-atmospheric pressure chemical ionization (APCI)-high resolution MS (HRMS) was developed in our laboratory for the simultaneous determination of folpet and phthalimide in different food matrices (Badoud et al., 2018). The method allowed the reliable quantification of folpet and phthalimide at low concentration levels, by avoiding the potential analytical artefact produced by the hot GC-injector previously described. However, GC continues to be the technique of choice for routine determination of these analytes. On the other hand, special attention should be paid to sample comminution and preparation as folpet can degrades during this stage of the analytical method. It is highly sensitive to basic pH and temperature. The so-called QuEChERS (standing for Quick, Easy, Cheap, Effective, Rugged and Safe) is by far the most applied methodology for pesticides multiresidue determination, and it has been already standardised (European Committee for Standardization, 2008). In the case of folpet (and other sensitive pesticides) decreasing the pH during the first extraction, avoiding any clean-up with primary-secondary amine (PSA) and re-acidifying final MeCN extracts is absolutely essential to minimise its degradation (European Union Reference Laboratory-Single Residue Method, 2017; Badoud et al., 2018).

The aim of this study was the development and validation of two analytical methods for the quantification of folpet and phthalimide. Two platforms, based on GC-NCI-MS and GC-EI-MS/MS, were selected according to the current common practices in routine laboratories in order to check the reliability of quantification of folpet and phthalimide at low concentration levels. Sample preparation was based on a modified QuEChERS procedure, which was previously optimised in our laboratory with the aim of avoiding phthalimide contamination and degradation of folpet (Badoud et al., 2018). Validation was performed according to SANTE/11813/2017guidelines (European Commission, 2017) for the two analytes in apple puree, raspberry puree, rice flour with a target limit of quantification (LOQ) of 10 µg/kg, and for infant formula with a target LOQ of 30 µg/kg.

2. Experimental

2.1. Chemicals and materials

Individual standards of folpet (99.9%), phthalimide (99.9%) and folpet-d4 (99.8%), were purchased from Sigma-Aldrich (Buchs, Switzerland). Phthalimide-d4 (99.0%) was obtained from CDN Isotopes (Pointe-Claire, Quebec, Canada).

Ethyl acetate for residue and pesticide analysis grade was obtained from Acros organics (Geel Belgium). Acetone and *n*-hexane (Suprasolv® grade) as well as MeCN and water (Lichrosolv®, LC-MS grade), formic acid 98–100% (Lichropur®, for LC-MS) and NaCl (Emsure®, analysis grade) used during sample preparation were obtained from Merck (Darmstadt, Germany), whereas anhydrous magnesium sulphate ($MgSO_4$) was obtained from Sigma-Aldrich.

2.1.1. Chemicals and materials decontamination

Before use, all materials and reagents required for sample preparation were treated as described elsewhere (Badoud et al., 2018). Materials were thoroughly rinsed with a mixture of 1:1, v/v acetone and *n*-hexane. Ceramic homogenisers were previously washed with a mixture 1:1, v/v water and methanol. $MgSO_4$ was heated at 550 °C overnight (at least 12 h) and further stored in a glass bottle.

2.2. Preparation of calibration standards

All solutions were prepared by dilution with a mixture of acetone and *n*-hexane 1:1, v/v and stored in glass tubes at −20 °C before use. Individual stock solutions of both native and isotopically labelled compounds were prepared at the concentration of 1000 mg/L, and were kept for no longer than 1 year. Two intermediate working solutions, one containing a mixture of the native and another containing a mixture of the isotopically labelled compounds, were prepared at the concentration of 10 mg/L. These solutions were further diluted 10 times to obtain working solutions at the concentration of 1 mg/L. Intermediate and diluted working solutions were kept in the freezer for no longer than 3 months.

2.3. GC-NCI-MS and GC-EI-MS/MS analysis

The GC-NCI-MS system consisted of an Agilent 7890A GC (Agilent Technologies, Santa Clara, CA, USA) linked to an Agilent 5975C MSD equipped with a chemical ionisation source operating in negative mode, with methane as chemical reagent (Carbagas, Gümligen, Switzerland). For the GC-EI-MS/MS analysis an Agilent 7890B GC was linked to an Agilent 7010 triple quadrupole equipped with an electronic ionisation source (70 eV).

For both platforms, sample extracts were injected using a 7693A autosampler (Agilent Technologies) with a 10 µL syringe, into an Agilent split/split-less injector equipped with a single tapper liner (78.5 × 6.5 × 4.0 mm, Supelco, Buchs, Switzerland). The injection of sample extract (2 µL) into the chromatographic system was performed in the pulsed splitless mode. The injector temperature was constantly held at 250 °C while a pulse pressure of 25 psi was applied for 1 min. Subsequently, the injector was purged during 1 min at a flow rate of 50 mL/min. GC separations were achieved using two Agilent J&W VF-5MS capillary columns (15 m × 0.25 mm × 0.25 µm film thickness), connected by means of a purged ultimate union (Agilent Technologies) in order to perform backflush of low volatile material and reduce the contamination of the analytical column and ionisation source. In addition, a precolumn (J&W VF-5MS capillary column, 2 m × 0.25 mm × 0.25 µm film thickness) was connected to the first analytical column. Helium 99.9997% (Carbagas, Lausanne, Switzerland) was used as carrier gas under constant flow rate of 1.4 and 1.5 mL/min in the first and second column, respectively. The oven temperature was programmed as follows: 60 °C held for 1 min, ramped to 170 °C at 40 °C/min and then to 310 °C at 10 °C/min which was finally held for 1.2 min. After the run (18.9 min) the oven temperature was set at 325 °C and a backflush pressure was held at 50 psi for 3 min. Total run time was 21.9 min. The analytical method was retention time locked using folpet as a locking compound.

For the GC-NCI-MS, the auxiliary temperature was 310 °C, and both the ion source and the quadrupole temperatures were set at 150 °C. Each target compound was monitored in the selective ion monitoring mode (SIM) according to its retention time, with one quantifier ion and a second qualifier ion (Supplementary Table S1).

For the GC-EI-MS/MS, the auxiliary temperature was 280 °C, and the MS parameters were set as follows: electron ionisation energy was 70 eV, the ion source temperature was 280 °C, and the quadrupoles temperatures were 150 °C for both of them. Each target compound was monitored in the multiple reactions monitoring (MRM) mode using time segments, with two transitions per compound, used for both