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Evaluation and validation of an accurate mass screening method for the analysis of pesticides in fruits and vegetables using liquid chromatography–quadrupole-time of flight–mass spectrometry with automated detection

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ABSTRACT

This study reports the development and validation of a screening method for the detection of pesticides in 11 different fruit and vegetable commodities. The method was based on ultra performance liquid chromatography–quadrupole-time of flight–mass spectrometry (UPLC–QTOF–MS). The objective was to validate the method in accordance with the SANCO guidance document (12571/2013) on analytical quality control and validation procedures for pesticide residues analysis in food and feed. Samples were spiked with 199 pesticides, each at two different concentrations (0.01 and 0.05 mg kg^{-1}) and extracted using the QuEChERS approach. Extracts were analysed by UPLC–QTOF–MS using generic acquisition parameters. Automated detection and data filtering were performed using the UNIFI^{TM} software and the peaks detected evaluated against a proprietary scientific library containing information for 504 pesticides. The results obtained using different data processing parameters were evaluated for 4378 pesticide/commodities combinations at 0.01 and 0.05 mg kg^{-1}. Using mass accuracy (±5 ppm) with retention time (±0.2 min) and a low response threshold (100 counts) the validated Screening Detection Limits (SDLs) were 0.01 mg kg^{-1} and 0.05 mg kg^{-1} for 57% and 79% of the compounds tested, respectively, with an average of 10 false detects per sample analysis. Excluding the most complex matrices (onion and leek) the detection rates increased to 69% and 87%, respectively. The use of additional parameters such as isotopic pattern and fragmentation information further reduced the number of false detects but compromised the detection rates, particularly at lower residue concentrations. The challenges associated with the validation and subsequent implementation of a pesticide multi-residue screening method are also discussed.

1. Introduction

Pesticide residues in foods are high on the list of consumer concerns and consequently laboratories are under ever increasing pressure to screen samples for as many pesticides as possible in a single analysis within an appropriate timescale and at reasonable cost. Most routine analyses for the control of pesticide residues rely on the use of targeted approaches based on a combination of gas chromatography–mass spectrometry (GC–MS) and liquid chromatography mass spectrometry (LC–MS). Usually GC–single quadrupole MS using selected ion monitoring (SIM) or GC tandem mass spectrometry (GC–MS/MS) operated in selected reaction monitoring (SRM) and LC–MS/MS in multiple reaction monitoring (MRM) mode. The scope of targeted analysis is limited to a list of compounds (typically a few hundred), usually selected on the basis of their frequency of occurrence, contribution to the residue definition and legislation requirements. A disadvantage of this approach is that pesticides present in the sample, but not included in such a predefined list, will not be detected. These non-detects are essentially false negative results. One possible option to increase the scope of the analysis and decrease the true false negative rate is the use of high-resolution mass spectrometry (HRMS) instrumentation to perform non-targeted acquisition across the mass range of interest, typically *m/z* 50–1200. For cost, time and practical reasons,

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data obtained using non-targeted acquisition is usually evaluated using targeted data processing against a database or a library of typically several hundreds of pesticides [1–5]. Although the interrogation of the data is performed against the list of compounds included in the database or the library, retrospective evaluation is always possible as data for all compounds that have given sufficient detector response would have been acquired. The key to the successful implementation of this approach for routine analysis will be the capability of the data processing software to accurately detect residues at low concentrations with an acceptable level of false negative results as outlined in the EU guidelines [6]. Although there is no requirement in the guidelines regarding the number of false detects, it is desirable to keep their number as low as possible to minimise the time required for additional investigation and hence to improve the analytical efficiency.

Implementation of qualitative screening methods in routine analysis requires the validation of the whole analytical process from analyte extraction to data processing and the determination of the screening detection limit for each individual pesticide [2,7]. The screening detection limit is the lowest concentration for which it has been demonstrated that a certain analyte can be detected (not necessarily meeting unequivocal identification criteria) in at least 95% of the samples (i.e. a false-negative rate of 5% is acceptable). In this study screening detection limits were evaluated for 199 pesticides in fruits and vegetables using ultra performance liquid chromatography–quadrupole-time of flight (UPLC–QTOF)–MS. The number of detects and false detects was assessed using different data processing parameters and tolerances, including mass accuracy and retention time tolerances, response thresholds, adducts, isotopic pattern and fragment ion(s) information.

The difficulties encountered during the validation and the challenges to be addressed for the implementation of HRMS screening methods (optimisation of software parameters, on-going quality control, validity of the generated data, batch to batch reproducibility, periodical re-assessment of the validation, etc.) are discussed.

2. Experimental

2.1. Reagents and standards

Chromasolv LC–MS grade acetonitrile for extractions was obtained from Sigma Aldrich (Steinheim, Germany). Chromasolv UHPLC grade acetonitrile for chromatography and ammonium acetate were purchased from Biosolve (Greyhound Chromatography, Merseyside, UK). Ultrapure water (18.2 MΩ cm) was obtained using a Purelab ultrapure water system (ELGA Purelab, UK). Waters DisQuE™ dispersive sample preparation pouches (CEN method, containing 4 g of $MgSO_4$, 1 g of NaCl, 0.5 g of disodium hydrogencitrate sesquihydrate and 1 g of trisodium citrate dehydrate salts), and 2 mL clean-up tubes containing 150 mg $MgSO_4$ and 50 mg primary secondary amine (PSA) were obtained from Waters (Manchester, UK).

Certified standards of pesticides were purchased from Sigma Aldrich (Gillingham, UK), Dr. Ehrenstorfer (Augsburg, Germany) and Qmx Laboratories (Thaxted, UK). Individual stock solutions (100–1000 μg mL^{-1}) were prepared in methanol, acetonitrile or ethyl acetate. Intermediate mix solutions (5–25 μg mL^{-1}) in methanol containing a variable number of pesticides were prepared and combined to give a final mixture containing 199 pesticides included in the scientific library, mostly at 1 μg mL^{-1}. The actual concentrations for all pesticides are detailed in Table 1. Standard solutions were stored at −20 °C until use. All the 199 pesticides considered in this work are included in the UK national monitoring programme.

2.2. Samples

Ten different fruit and vegetable commodities of varying matrix complexity (apple, broccoli, celery, leek, melon, nectarine, onion, pear, pepper, tomato) belonging to the high water content group [6] and grapes (high acid content) were included in the validation. Samples labelled as organically grown were purchased in local supermarkets, comminuted and stored at −20 °C. Before use the samples were screened (by UPLC–MS/MS) for the presence of any of the target analytes in the spiking mixture but not for all of the compounds in the scientific library. Sub-samples were spiked before extraction with a mixture containing 199 compounds (included in the library) typically at 0.01 and 0.05 mg kg^{-1}. Twenty-nine pesticides were included at higher concentrations reflecting their lower MS/MS response observed during routine monitoring analyses. Non-spiked (blank) samples were also extracted and analysed to assess the number of false detects.

2.3. Sample extraction

Samples were extracted following a QuEChERS-based approach (citrate buffered method). Briefly, 10 mL of acetonitrile were added to 10 g of homogenised sample contained in a polypropylene centrifuge tube and the sample was shaken for 5 min using a mechanical horizontal shaker. A mixture of salts (4 g of $MgSO_4$, 1 g of NaCl, 0.5 g of disodium hydrogencitrate sesquihydrate and 1 g of trisodium citrate dehydrate) was added and the centrifuge tube shaken by hand immediately, to prevent agglomeration of the salts. The sample extraction mixture was shaken for a further 5 min using a mechanical shaker. Following centrifugation (3500 rpm for 3 min), a 1.5 mL aliquot of the supernatant was transferred to a 2 mL dispersive SPE (dSPE) tube containing 150 mg $MgSO_4$ and 50 mg PSA. The dSPE tube was shaken for 30 s and then centrifuged (3500 rpm for 1 min). The supernatant from the dSPE was then solvent exchanged: 1 mL was evaporated to near dryness and resuspended in 1 mL of acetonitrile:water (1:3, v/v). The composition of the final solvent was a compromise selected to avoid precipitation of the pesticides and to achieve acceptable chromatographic peak shapes, especially for the more polar, early eluting pesticides.

PSA sorbent was used to decrease the concentration of matrix co-extractives. PSA can result in partial or even complete removal of acidic pesticides. Nonetheless, several acidic pesticides included in this study were detected consistently at the concentrations assessed. The inclusion of additional acidic pesticides in the scope of the method would require an assessment of the impact of PSA.

Samples were divided in two separate batches, each of which contained 11 different commodities. The two batches were analysed in different days to account for the instrumental day-to-day variation.

2.4. Instrumentation

Pesticides were chromatographed using an ACQUITY BEH C18 column (100 × 2.1 mm, 1.7 μm) from Waters (Milford, MA, USA), thermostatted at 45 °C, using a binary gradient of water (A) and methanol (B) both containing ammonium acetate (10 mM) at a flow rate of 0.45 mL min^{-1}. The mobile phase composition was changed as follows: 98% A (0–0.25 min), 1% A (12.25–13 min), 98% A (13.01–17.00). These conditions were those used by Waters to create the proprietary library used in this study.

Analyses were performed using an ACQUITY UPLC-I-Class coupled to a Xevo G2-S QTOF-MS (Waters). The electrospray source was operated in positive mode at 1 kV and the sample cone voltage set at 25 V. Nitrogen was used as nebuliser (flow rate 50 L h^{-1}, 120 °C) and desolvation gas (flow rate 1000 L h^{-1}, temperature 550 °C). Data was acquired in the range from 50 to 1200 *m/z*. The data