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Validation of a qualitative screening method for pesticides in fruits and vegetables by gas chromatography quadrupole-time of flight mass spectrometry with atmospheric pressure chemical ionization

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HIGHLIGHTS

- Applicability of GC-(APCI)QTOF MS as new tool for wide-scope screening of pesticides in fruits and vegetables demonstrated.
- Validation of screening method according to SANCO/12571/2013.
- Detection of the pesticides based on the presence of M+·/MH+ in most cases.
- Screening detection limit 0.01 mg kg^{-1} for 77% of the pesticides investigated.
- Successful identification at 0.01 mg kg^{-1} for 70% of the pesticides/matrix combinations.

GRAPHICAL ABSTRACT

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ABSTRACT

A wide-scope screening method was developed for the detection of pesticides in fruit and vegetables. The method was based on gas chromatography coupled to a hybrid quadrupole time-of-flight mass spectrometer with an atmospheric pressure chemical ionization source (GC-(APCI)QTOF MS). A non-target acquisition was performed through two alternating scan events: one at low collision energy and another at a higher collision energy ramp (MS^E). In this way, both protonated molecule and/or molecular ion together with fragment ions were obtained in a single run. Validation was performed according to SANCO/12571/2013 by analysing 20 samples (10 different commodities in duplicate), fortified with a test set of 132 pesticides at 0.01, 0.05 and 0.20 mg kg^{-1}. For screening, the detection was based on one diagnostic ion (in most cases the protonated molecule). Overall, at the 0.01 mg kg^{-1} level, 89% of the 2620 fortifications made were detected. The screening detection limit for individual pesticides was 0.01 mg kg^{-1} for 77% of the pesticides investigated. The possibilities for identification according to the SANCO criteria, requiring two ions with a mass accuracy $\leq \pm 5$ ppm and an ion-ratio deviation $\leq \pm 30\%$, were investigated. At the 0.01 mg kg^{-1} level, identification was possible for 70% of the pesticides detected during screening. This increased to 87% and 93% at the 0.05 and 0.20 mg kg^{-1} level, respectively. Insufficient sensitivity for the second ion was the main reason for the inability to identify detected pesticides, followed by deviations in mass accuracy and ion ratios.

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1. Introduction

It is widely known that pesticides are commonly used in agriculture to control pests and improve yields of crops. Monitoring pesticide residues is crucial, as it assures that applications are made according to the proposed good agricultural practices and the product is safe for the consumer. The high number of different active substances used, together with the low concentration levels admitted by the legislation, makes that pesticide residue analysis is a challenging task. In the past decade numerous papers on multi-residue methods based on chromatography with tandem mass spectrometry (LC–MS/MS and GC–MS/MS) have appeared and these techniques have been implemented in many routine laboratories. This approach involves a targeted measurement and is typically limited to 100–300 pesticides which is much lower than the number of pesticides that might be present in the samples. For this reason, there is a need for wide-scope methods able to detect a larger number of compounds at low level in complex sample matrices in an efficient and effective way.

Chromatography combined with full scan mass spectrometry is ideally suited for simultaneous detection of very high numbers of pesticides. The first routine applications were based on GC with electron ionisation (EI) and low resolution single quadrupole [1–3] or ion trap MS [4]. However, the sensitivity and selectivity, especially with more generic sample preparation approaches such as QuEChERS, were not always sufficient. To improve this, comprehensive two dimensional chromatography with low resolution TOF-MS (GCxGC-TOF-MS) has been applied [5] and, alternatively, GC with high resolution TOF-MS [6–10] and QTOF MS [11,12]. Although the latter approach was more straightforward in terms of data handling, the dynamic range of the early high resolution TOF generations was poor and the resolving power rather limited (approx. 8000 FWHM at m/z 100–400). Meanwhile in LC the possibilities for full scan MS detection rapidly improved. New TOF and orbitrap mass analyzers, interfaced to LC through atmospheric pressure ionisation, were introduced that outperformed the dedicated EI-high resolution TOFs for GC. As a result of this, and the fact that many pesticides are LC–MS amenable, the research focus in the past years seemed to have shifted towards LC-full scan approaches [13–19]. However, not all pesticides are LC amenable and to cover all pesticides GC is still needed. Furthermore, many pesticides are both LC and GC amenable which makes GC–MS and LC–MS valuable complementary techniques.

One of the possible solutions to advance in the possibilities of GC-full scan HRMS is a new atmospheric pressure chemical ionization (APCI) source that allows coupling GC to analyzers designed for LC. APCI is a very promising alternative source to couple GC with a range of high-end mass spectrometers (MS/MS, TOF, QTOF) providing highly sensitive and selective detection. Its use has been recently reported in GC–MS based methods for pesticide residue analysis in fruit and vegetables, employing triple quadrupole MS/MS [20–22] or hybrid QTOF [23,24]. GC-(APCI) TOF MS has also been used in other fields, for example for impurity identification in pharmaceutical development, profiling of phenolic compounds in oil, metabolic fingerprinting and profiling, or coupled with comprehensive two-dimensional GC for flame retardants and plasticizers [25–28]. A few works have reported the use of quadrupole time of flight (QTOF) MS to investigate substances in acrylic adhesives of food packaging materials, or polycyclic and nitro-polycyclic aromatic hydrocarbons in mosses [29,30].

Apart from providing access to a range of existing MS systems initially developed for LC–MS, APCI results in much less fragmentation compared to electron ionization (EI). As widely known, EI has been the most frequently used ionization source in GC–MS methods, and rather strong fragmentation of the molecule typically occurs during ionisation. As a result, the most diagnostic ion of the analyte -the molecular ion-, is often lost and similar spectra may be obtained for analogues of certain analyte classes. The use of EI reference spectra of target analytes is required for detection and identification. In addition, retention times to distinguish between structural analogues are essential. Although EI-high resolution/accurate mass MS has strong potential for elucidation of unknowns, the absence of a (quasi) molecular ion is a serious drawback [31].

The aim of this work is to evaluate the performance of GC-(APCI)QTOF MS for screening of pesticide residues in fruits and vegetables. This has been done according to the SANCO/12571/2013 protocol for validation of screening methods [32] using a test set of 132 pesticides. The screening detection limit has been established for each pesticide by testing the method for a group of different fruit and vegetable commodities ($n = 20$). In addition, the possibility for identification according to the SANCO criteria has been evaluated.

2. Experimental

2.1. Reagents

Reference standards of pesticides were purchased from Dr. Ehrenstorfer (Augsburg, Germany). Stock solutions (around 500 μg mL^{-1}) were prepared by dissolving solid reference standards in acetone and stored in a freezer at −20 °C. Working solutions were prepared by diluting stock solutions in acetone for sample fortification and diluting in hexane for injection in the chromatographic system.

Acetone (pesticide residue analysis quality), hexane (ultra trace quality), acetonitrile (reagent grade), toluene (for GC residue analysis), formic acid (HCOOH, content >98%) and glacial acetic acid were purchased from Scharlab (Barcelona, Spain). HPLC-grade water was obtained by purifying demineralized water in a Milli-Q Gradient A10 system (Millipore, Bedford, MA, USA). Anhydrous magnesium sulfate (extra pure) and anhydrous sodium acetate (reagent grade) were purchased from Scharlab. The QuEChERS commercial products, 2 mL micro centrifuge tubes for dispersive solid phase extraction (d-SPE) containing 50 mg primary secondary amine (PSA) and, 150 mg anhydrous magnesium sulfate and 150 mg C18 were purchased from Teknokroma (Barcelona, Spain).

2.2. Samples

For this work 10 different fruit and vegetable commodities were selected. Apple, cucumber, lettuce, tomato, pepper and carrot samples were obtained from a local organic food store in Castellón (Spain). The other matrices (grape, orange, strawberry and cauliflower) were samples taken from untreated plots from GLP field residue trials. Fruits and vegetables were homogenised using a grinder. For the validation, samples were fortified by addition of a mix solution of 132 pesticides to 10 g portions of the homogenized material. This was done for each matrix in duplicate, at three levels (0.01, 0.05 and 0.20 mg kg^{-1}). This way, a total number of 20 samples were analyzed at each fortification level.

2.3. Sample preparation

The extraction procedure was carried out following an acetate-buffered version of the QuEChERS method with some modifications, as was applied in a previous study in our laboratory [9]. Additionally, formic acid was added to the final extracts to help stabilizing pesticides that can be degraded in acetonitrile under the basic conditions of the QuEChERS clean-up [33].