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Study of the effects of operational parameters on multiresidue pesticide analysis by LC–MS/MS

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ABSTRACT

In this paper, the influence of several operational parameters on a well established multiresidue LC–MS/MS method has been studied in relation to the analysis of 150 pesticides commonly present in vegetable samples. The operational parameters investigated are: (i) the influence of different modifiers (0.1% formic acid; 5 mM ammonium formiate; 5 mM ammonium acetate in aqueous phase) – both on the retention time and on the analytical response of the studied compounds; (ii) the effect of the analytical column's temperature on the retention time and on the analytical response of the pesticides investigated; (iii) the effects of co-elution in mixture containing 150 pesticides and, additionally, (iv) the carrying out of a study about the common transitions obtained by LC–MS/MS. Various common transitions were found among the 150 pesticides, but there were only two problematic cases, the pairs diuron–fluometuron and prometryn–terbutryn, which have common scanned transitions and have very close retention times. The use of ammonium salts as modifier instead of formic acid reports enhancement or suppression of the response depending on the pesticides. No great influence on the retention time or on the response of the pesticides and commodities studied was observed with relation to the column temperature. Two different columns: an HPLC (5 μm particle size) and an UHPLC analytical column (1.8 μm particle size) have been used. As was expected, shorter run times and lower peak width was achieved with the UHPLC column.

In this paper, the effect of the compounds on each other in the MS analysis when the number of co-eluting compounds is quite high is also described. Mainly small suppression or enhancement co-elution effect was observed, but some particular pesticides presented high sensitivity (>±60% effect) when they elute together with others. This is an important factor and it has to be taken into account when performing multiresidue pesticide analysis.

1. Introduction

Pesticides are widely used chemicals in agricultural practice, not only during cultivation but also in post-harvest storage. Various organizations have set stringent regulatory controls on pesticide use in order to minimize exposure on the general population to pesticide residues in food. The great variety of applied pesticides both within European Union countries (EU) and outside of the EU, as well as the arrival of new plant protectors and chemicals, has needed an ever-expanding list of pesticides along with their accompanying maximum residue limits (MRLs). The list of MRLs for a wide variety of commodities and pesticides is updated from time to time and is part of the EU Plant Protection Products Directive (1107/2009 EEC) [1], which is the update of the former directive (91/414/EEC) [2].

Multiresidue analysis, which determines a range of multi-class pesticides as wide as possible, is the primary need for food control laboratories. This is because the great variety of products applied following the different agricultural practices in fruit and vegetable and the international trade.

Regarding the analytical methods, there is no doubt that liquid chromatography–mass spectrometry (LC–MS) currently competes with gas chromatography–mass spectrometry (GC–MS) for the status of the reference technique in this field [3–5]. There are different mass analyzers that can enhance tandem mass spectrometry (MS/MS) capabilities, such as quadrupole ion-trap (QLIT), triple quadrupole (QqQ) and quadrupole time-of-flight (QTOF) – each one has different features [6]. The main advantage of QqQ instruments is their very good quantitative capabilities and their great sensitivity in the selected reaction monitoring (SRM) mode, in addition

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to the capability of simultaneously selecting multiple transitions [7–9]

The low EU MRLs have encouraged the development of more sensitive analytical methods to meet the requirements of complex samples. Therefore, sensitive and reliable confirmatory methods are required to monitor pesticide residues in foods. In this sense, liquid chromatography/tandem mass spectrometry (LC–MS/MS) with a triple quadrupole in selected reactions monitoring (SRM) mode has become, to date, the most widely used technique for the monitoring and the quantification of pesticides in food, as reported extensively in the literature [3,10–18]

The most common stationary phases in the analysis of pesticides in food by HPLC are C8 and C18 with a particle size from 3 μm to 5 μm and a column length from 10 cm to 25 cm. Flow rate, injection volume and run time among others depend on the characteristics of the column and especially on the number of the measurable pesticides. The common flow rates used for these types of columns in pesticide residue analysis are between 0.2 and 0.6 mL min^{-1}. The flow rate is naturally limited by the maximum pressure tolerance of the column or the pump used. The total run time, depending on the number of compounds to be analyzed, typically varies between 15 and 35 min. The injection volume varies from 5 to 20 μL [19–22] – set to achieve a compromise by taking into account two criteria; (i) to introduce the highest volume to ensure adequate sensibility and (ii) to inject the lowest volume to protect the column and the system from any extracts, especially from dirty samples.

Nowadays, there is a clear trend in decreasing the diameter and the particle size of the chromatographic columns in order to reduce the run time and/or enhance resolution.

Over recent years, liquid chromatography–mass spectrometry (LC–MS) and liquid chromatography–mass spectrometry in tandem (LC–MS/MS) have experienced impressive progress, both in terms of technology development and application. More recently, alternative strategies have been developed to obtain increased efficiency, together with short analysis times by using 1.7 μm porous stationary phases, mobile phases at high linear velocities and instrumentation that operates at high pressures (ca. 15,000 psi). This new technology has been commonly called ultra performance liquid chromatography (UPLC, trademark of the Waters company) and rapid resolution liquid chromatography (RRLC, trademark of the Agilent company) [23,24], that is why we call this technique ultra high performance liquid chromatography (UHPLC) to distinguish this procedure from conventional HPLC.

In order to increase the speed of chromatographic separation in HPLC, different strategies based on the increase of the mobile phase flow rate, faster gradients, the use of short columns and/or the use of normal-sized columns with smaller particles size (<2 μm) can be considered. The use of shorter columns, with 1.8 μm particle size, provides faster analysis and improves resolution compared to columns of 3.5 or 5.0 μm particle size. It might be, for instance, a good alternative when the laboratory equipment is not provided with a UHPLC system: its additional resolving capability makes it a powerful technology for those laboratories which do not have UHPLC systems [25,26].

For the chromatographic separation, not only the analytical column but also the mobile phase is of great importance. Two type of eluent are typically used as mobile phase: the most common being pure water (or high content water) eluent as the aqueous phase and methanol or acetonitrile as the organic phase [11,27–29].

Additives and buffers are used in LC mobile phases to improve sensitivity, resolution and reproducibility. Chemical properties and concentration of the additive, as well as pH, have a significant effect on analytes response in ESI. Unfortunately, many of the additives and buffers commonly used in LC are not compatible with ESI/MS. In general, non-volatile buffers such as phosphate and borate tend to cause increased background signal suppression, and rapid contamination of the ion source resulting in decreased sensitivity and stability. Although various volatile additives have been employed in LC–(ESI+)MS, the most common modifiers employed in the analysis of pesticides in food samples are: formic acid (0.01–0.2%) [20,23,28,30–32]; ammonium formiate (2–10 mmol L^{-1}) [24,33,34] and ammonium acetate (1–20 mmol L^{-1}) [12,14,15,23,35]. The addition of the modifier can be performed in both eluent or only in the aqueous phase depending on the analyst preference.

These parameters – and others like column temperature during the chromatographic analysis and the number of compounds included in a mixture – are parameters that must be carefully investigated in order to control all variables that may influence the effectiveness of a multiresidue method. And as well, these are in some cases system dependent and therefore difficult to extrapolate from one method/system to other.

In this paper, the influence of the main operational parameters commented above in an established LC–MS/MS method has been studied. This work is linked to our previous study about the measurement of 160 pesticides by LC–MS/MS [36]. As it was explained in that publication, 10 pesticides were insensitive and/or problematic in detection thus we followed the study with the rest of 150 pesticides. Generally speaking food control laboratories apply very similar extraction and analytical procedures but in many cases there is not clear evidence about how those differences can affect the efficiency of the analysis. Obviously it is expected that pro and contras can be appear with the selection of different parameters but only a good balance can be done if the adequate technical information is available. Consequently, the aim of this work is to evaluate a range of operational-technical parameters those commonly affect the efficiency of the LC–MS/MS measurement in multiresidue pesticide analysis to serve useful information for routine analysis.

2. Experimental

2.1. Chemicals and reagents

Pesticide analytical standards were purchased from Dr. Ehrenstorfer (Ausburg, Germany) and Riedel-de-Haën (Seelze, Germany). HPLC-grade acetonitrile and methanol were obtained from Merck (Darmstadt, Germany). Formic acid, anhydrous magnesium-sulfate, ammonium formiate and ammonium acetate were obtained from Fluka (Buchs, Switzerland). Acetic acid was purchased from Merck (Darmstadt, Germany), and sodium acetate 3-hydrate from Panreac (Barcelona, Spain). PSA (primary–secondary amine) was obtained from Supelco. A Milli-Q-Plus ultrapure water system from Millipore (Milford, MA) was used throughout the study to obtain the HPLC-grade water used during the analyses.

2.2. Pesticide solutions

Individual stock solutions (1000–4000 μg mL^{-1}) were prepared in pure organic solvent depending on their solubility and stored in the dark at −18 °C. When the pesticide was not easily soluble in acetonitrile, 10% of dimethyl sulfoxide (DMSO) was added. After preparation, all the data concerning the preparation of the solution were recorded on a Register Form, on which the weight of the container was recorded as a quality control measure. Each time that the solutions were used, they were equilibrated to room temperature and weighed to check for losses caused by evaporation. If the weight had changed, the differences observed were taken into account when calculating the new concentrations.

Mixtures of pesticides containing medium number (15 compounds) and large number (150 compounds) of compounds were prepared in acetonitrile, containing 10 μg mL^{-1} of each pesticide.