Large-scale multi-residue methods for pesticides and their degradation products in food by advanced LC-MS

Amadeo R. Fernández-Alba, Juan F. García-Reyes

Pesticide testing in foodstuffs is a challenging application for mass spectrometry (MS), since it implies simultaneous trace analysis of hundreds of compounds belonging to a many classes, preferably undertaken in one fast run. The appearance and the use of new, more polar pesticides has prompted the use of liquid chromatography electrospray ionization MS (LC-ESI-MS) instead of gas chromatography. Currently, large-scale multi-residue methods (LSMRMs) (i.e. covering over 80 analytes) for pesticide analysis are developed using LC with tandem MS (LC-MS^2) with triple quadrupole mass analyzers or LC with time-of-flight MS (LC-TOF-MS).

The present article provides a review of the most significant MRMs for determining pesticide residues in foodstuffs. We discuss the main features of LC-$MS^2$and LC-TOF-MS instruments, including recently introduced advances in instrumentation. In addition, we describe the advantages and the pitfalls of these methods, together with examples of the application of LC-TOF-MS and LC-MS^2 to large-scale screening, identification and quantitation of pesticides in foodstuffs.

Keywords: Degradation product; Food; Large-scale multi-residue method; Liquid chromatography; Mass spectrometry; Pesticide; Tandem mass spectrometry; Target analysis; Time-of-flight; Trace analysis

Amadeo R. Fernández-Alba*
Pesticide Residue Research Group, Department of Hydrogeology and Analytical Chemistry, University of Almería, 04120 La Cañada de San Urbano, Almería, Spain

Juan F. García-Reyes
Analytical Chemistry Research Group, Department of Physical and Analytical Chemistry, University of Jaén, 23071 Jaén, Spain

*Corresponding author.
Tel.: +34 950 015 034;
Fax: +34 950 014 102;
E-mail: amadeo@ual.es

1. Introduction

Pesticide-residue analysis is of paramount importance not only for the protection of human health but also for international trade and regulatory control. More than 1000 substances active against pests are used worldwide [1]. Probably no other use of chemicals is regulated more extensively than that of pesticides.

The great number of possible residues means that it is necessary to develop multi-residue methods (MRMs) that are as comprehensive as possible in order for official laboratories to exercise effective control [2]. Under this scenario, we should define the term MRM. In the past few years, an MRM typically comprised 10–50 compounds covered in a single run using LC-MS. But, considering the international trade of fruits and vegetables, with different regulations on the use of pesticides for each country, it is clear that the requirements for a control laboratory are increasingly stringent. Thus, we define the term ''large-scale multi-residue method'' (LSMRM) as being when the number of compounds analyzed is very high (i.e. 100–300 compounds). It is important to note that LSMRMs have very often been developed using gas chromatography with mass spectrometry (GC-MS), but not liquid chromatography with MS (LC-MS) [2].

For most pesticide residues and their transformation products, regulatory guidelines set maximum residue levels (MRLs) in food to protect the people from potential negative health effects or contamination. In general, MRLs in European Food Regulations (91/414/EEC) are in the range 0.01–10 mg/kg [3], depending on the commodity-pesticide combination, the lowest level being characteristic of banned or very toxic compounds – because it is considered that this would be the minimum limit of detection (LOD) achievable

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[4]. These regulations are particularly strict in the case of food intended for consumption by infants, as established by Commission Directives 1999/50/EC and 1999/30/EU, which require that baby food contains no detectable levels of pesticide residues (0.01 mg/kg) [5]. These low MRLs have prompted development of more powerful, more sensitive analytical methods [6–8]. These pesticide levels have to be monitored, and, for this type of LSMRM, efficiency must be maximized without sacrificing data quality [9].

For pesticide testing in food, an extraction procedure as comprehensive as the MRM instrumental method is required. Nowadays, there are well-known general extraction procedures based on acetonitrile [10,11], ethyl acetate [12–15] or acetone [16,17] that are very efficient for that purpose. These extraction methodologies are typically very fast, cost effective and easily automated to allow performing some 50 extractions per day in a medium-sized laboratory.

A common practical analytical approach to MRMs applied in many routine laboratories on pesticide-residue testing comprises selecting a list of around 50–100 compounds that are amenable to GC and LC. The decision as to which of these compounds must be included in the analysis is not straightforward. It can be done only by using the established priority list combined with any other information related to agricultural uses in the region. It means that the majority of the low-frequency or misused compounds are not targeted. In that way, false negatives are guaranteed when residues from these lists are present in the sample. In this scenario, it is desirable that the MRM approach implemented in official routine laboratories should be extended as much as possible to those pesticides commonly used in other countries or even to illegal compounds that could be available commercially on the ''black market'' [18]. It is not difficult to find advertisements for a wide range of agrochemicals that are not approved (e.g., isofenphos methyl) or are no longer authorized in the European Union (EU) (or other countries), which can be ordered via the Internet.

From an analytical point of view, this task is difficult to tackle since it involves extending the scope of the MRMs to several hundred chemicals. Moreover, it is difficult to carry out such approaches cost effectively due to the time and the money required when upgrading methods by incorporating new compounds and the management of these standards and solutions, and due to the extra analytical efforts and overall decrease in laboratory throughput that result. It is therefore essential to explore and to evaluate all options in developing efficient LSMRMs.

According to the literature, experience and available data, we can consider LSMRMs for GC-amenable compounds to be extensively developed and efficient using current GC-MS and GC-tandem MS (GC-MS^2) instruments [2,19]. By contrast, we are at the early stages of developing LC-MS technology, so most of the routine methods applied usually targeted a comparatively small number of compounds (e.g., <50), covering only compounds that have not been amenable to GC-MS. This approach has changed, so it is accepted that the new LC-MS instruments can obtain good results in determining several different specific groups of pesticides, including parent compounds and their metabolites along with typical GC-amenable compounds, such as organophosphorous pesticides [20].

There is no doubt that LC-MS^2 with a triple quadrupole (QqQ) analyzer is already a more popular, widely used methodology for pesticide testing in foodstuffs worldwide [6]. Besides, in recent years, the development of new LC-MS systems with time-of-flight (TOF) analyzers has featured convenient quantitation. This technique can generate high specificity without limiting the number of simultaneously observed target compounds. The advantage of a TOF-MS analyzer for screening is the ability to examine a data file for theoretically unlimited number of pesticides (e.g., 300 compounds) with high sensitivity within one run [7,8].

The aim of this article is to review critically the main approaches to development of LSMRMs with LC-MS, mainly LC-MS^2using QqQ instruments (LC-QqQ-MS^2) and LC-MS using time-of-flight instruments (LC-TOF-MS). We review the most significant MRMs for determining pesticide residues in foodstuffs. We discuss the main features of LC-MS^2 and LC-TOF-MS instruments, including new instrumentation recently introduced. In addition, we describe the advantages and the pitfalls of these methods, together with examples of the application of LC-TOF-MS and LC-MS^2 to the large-scale screening, identification and quantitation of pesticides in foodstuffs.

2. Multiresidue methods with LC-MS^2

2.1. Introduction

The introduction of MS^2 instrumentation with atmospheric pressure ionization (API) sources in the past decade has revolutionized trace analyses of chemicals in food and the environment [21]. This coupling combines the advantages of LC and MS for the separation and the unequivocal identification of pesticides at low-mg/kg levels in complex matrices [21,22]. LC-MS^2 methods greatly reduce the need for dedicated clean-up steps, resulting in optimized analysis time and costs, with little chance of false-positive findings. Pesticide analysis by LC-MS^2 is already used in the regulatory area due to its optimum capability in performing multi-residue analyses. Although there have been reports of many LC-MS^2 applications for various pesticides, only a limited number of these studies have described a comprehensive (>75 pesticides) MRM for screening pesticides belonging to