Accurate determination of selected pesticides in soya beans by liquid chromatography coupled to isotope dilution mass spectrometry

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ABSTRACT

A sensitive, accurate and simple liquid chromatography coupled with mass spectrometry method for the determination of 10 selected pesticides in soya beans has been developed and validated. The method is intended for use during the characterization of selected pesticides in a reference material. In this process, high accuracy and appropriate uncertainty levels associated to the analytical measurements are of utmost importance. The analytical procedure is based on sample extraction by the use of a modified QuEChERS (quick, easy, cheap, effective, rugged, safe) extraction and subsequent clean-up of the extract with C_{18}, PSA and Florisil. Analytes were separated on a C_{18} column using gradient elution with water–methanol/2.5 mM ammonium acetate mobile phase, and finally identified and quantified by triple quadrupole mass spectrometry in the multiple reaction monitoring mode (MRM). Reliable and accurate quantification of the analytes was achieved by means of stable isotope-labelled analogues employed as internal standards (IS) and calibration with pure substance solutions containing both, the isotopically labelled and native compounds. Exceptions were made for thiodicarb and malaoxon where the isotopically labelled congeners were not commercially available at the time of analysis. For the quantification of those compounds methomyl-$^{13}C_2^{15}N$ and malathion-D10 were used respectively. The method was validated according to the general principles covered by DG SANCO guidelines. However, validation criteria were set more stringently. Mean recoveries were in the range of 86–103% with RSDs lower than 8.1%. Repeatability and intermediate precision were in the range of 3.9–7.6% and 1.9–8.7% respectively. LODs were theoretically estimated and experimentally confirmed to be in the range 0.001–0.005 mg kg^{-1} in the matrix, while LOQs established as the lowest spiking mass fractionation level were in the range 0.01–0.05 mg kg^{-1}. The method reliably identifies and quantifies the selected pesticides in soya beans at appropriate uncertainty levels, making it suitable for the characterization of candidate reference materials.

1. Introduction

Pesticides comprise a large number of compounds, with extremely diverse physico-chemical properties. They are applied to crops at various stages of cultivation to provide protection against pests and during post-harvest, to prolong storage life and preserve quality. The use of pesticides may however generate residues which involve a risk for both the environment and human health. In order to guarantee food and feed safety and to regulate international trade, the European Union has established maximum residue limits (MRLs) to minimize the presence of pesticide residue levels in different food stuffs [1].

Suitable analytical methodology for monitoring purposes is required to avoid human exposure to pesticide residues through food. In Europe, official control and commercial laboratories involved in the pesticides residues field need to deal with large amounts of analyses as well as fulfilling the requirements for assuring the quality of their results, especially in relation to accreditation according to ISO 17025 [2]. To cope with thousands of possible individual pesticide-matrix combinations, multi-residue methods are needed. Nevertheless, these methods require full validation. Aware of the related difficulties, the EU Directorate General for Health and Consumers (SANCO) produced guidelines for method validation and quality control procedures for pesticide residues in food and feed, which have been recently revised [3]. For each individual pesticide residue,

the acceptability criteria set for the validation parameters includes a method repeatability threshold of 20% and recovery in the range 70–120%. Although these conditions are acceptable for the application of routine analyses in control laboratories, more stringent accuracy criteria are needed for measurement procedures used to assign values to a certified reference material in order to be suitable for quality control purposes.

The guidance document on pesticide residue analytical methods, published by the Organisation for Economic Co-operation and Development (OECD) [4], establishes that food commodities are classified in groups according to their mayor composition. Full validation is needed for a representative commodity and extension of the scope to other matrices from the same group requires demonstrating that the method performance for the new matrix is not affected. In this study soya beans are selected as representative matrix for a high oil content commodity in line with the classification for vegetables, fruits and cereals proposed by SANCO.

Accurate determination of pesticides in fatty food matrices, such as soya beans, remains a challenge due to the complexity of the matrix and low mass fraction levels to be dealt with. Chromatographic techniques, such as gas chromatography (GC) or liquid chromatography (LC) coupled to tandem mass spectrometry (MS/MS), are nowadays commonly applied techniques for routine pesticide determination as they provide satisfactory selectivity and sensitivity [5–7]. LC is preferred over GC in the case of either thermolabile or newly developed pesticides, usually having high polarity and low volatility [8–10]. Regardless of the chromatographic technique chosen, a clean-up step is usually required after extraction, particularly when dealing with complex matrices in order to eliminate matrix effects caused by co-extracted compounds. The matrix effects observed are generally suppression or enhancement of the detector response due to co-eluting matrix constituents [11], interferences in the determination of the analyte of interest, or even shortening of the lifetime of the analytical equipment. These effects can seriously affect quantification at trace levels and concern both, GC and LC, coupled to MS detection. In addition to the removal of matrix constituents by specific dedicated clean-up protocols and chromatographic separations, other strategies could be simultaneously applied in order to reduce matrix effects to an acceptable level. The most applied approaches are external matrix matched calibration, calibration by standard addition, using analogue internal standard or stable isotopically labelled standards [12,13]. The latter appears to be the most robust and efficient approach [11]. Isotope dilution mass spectrometry (IDMS) is based on the addition of isotopically labelled analogues of the analytes to the sample, which are considered the ideal internal standards as they are expected to show identical behaviour to the target analyte in sample pre-treatment (provided isotopic equilibrium can be reached before extraction), chromatographic separation as well as in the compound ionization.

The predominant sample treatment applied for pesticide determination in fatty vegetables is based on liquid extraction with organic solvent, followed by clean-up with solid phase extraction (SPE), gel permeation chromatography (GPC) or low-temperature fat precipitation [14,6,15]. Other procedures include microwave-assisted extraction (MAE), supercritical fluid extraction (SFE), matrix solid phase dispersion (MSPD) or solid phase micro-extraction, although they have been applied with limited success [16]. Moreover the so called QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) has been increasingly used in the last years due to its simplicity, minimum number of sample processing steps and effectiveness for cleaning-up complex samples [17–19]. The technique was originally introduced for the extraction of pesticides from food matrices and has also been applied for other compounds, such as mycotoxins, plant toxins and veterinary drugs [20–22]. It

comprises first an extraction of pesticides from the matrix with acetonitrile, then a salting out step which involves a phase separation of aqueous and organic phases, and a subsequent clean-up of the extract by dispersive SPE (d-SPE).

This study includes the development and validation of a method for pesticide determination in soya beans by LC-MS/MS, with the primary focus to reach a suitable level of measurement uncertainty. Rather than an extensive list, a restricted range of pesticides was targeted including methomyl, imidacloprid, carbendazim, malaoxon, thiodicarb, malathion, iprodione, tebuconazole, diazinon and chlorpyrifos. The pesticides were chosen by paying attention to a number of aspects such as the physico-chemical properties, representation of different pesticide families, the analytical challenges related to their analysis, their frequency of use or the commercial availability of the isotopically labelled analogous. In order to improve the cleanliness of the sample extracts the QuEChERS method was adapted for the selected pesticides and matrix. In addition, accurate quantification of all considered compounds was achieved due to the application of IDMS and calibration with pure substance solutions containing both, the isotopically labelled and native compounds.

2. Experimental

2.1. Reagents and materials

Calibration substances of methomyl (99.5%), imidacloprid (99.0%), malaoxon (98.0%), thiodicarb (99.0%) were obtained from Dr. Ehrenstorfer (Augsburg, Germany). Carbendazim (98.0%), malathion (97.2%), iprodione (99.3%), tebuconazole (99.6%), diazinon (98.3%) and chlorpyrifos (99.5%) were purchased from Pestanal©, Sigma-Aldrich Co St. Louis (Mo, USA).

Isotopically labelled compounds used as internal standards were purchased either as neat crystals when available, or in solution. Carbendazim-d_4 (benzimidazole 4,5,6,7-d_4, 99.3 at% D) and iprodione-d_5 (3,5-dichlorophenyl-2,4,6-d_3; hydantoin-5,5-d_2, 98 at% D) were obtained from CDN Isotopes, Quebec, Canada. Methomyl-$^{13}C_2^{15}N$ (acetohydroxamate-$^{13}C_2$; ^{15}N, 99.0 at% C and 98 at% N, 100 ng μL^{-1} in acetone) was purchased from Cambridge Isotopes laboratories, Inc., Andover, MA, USA. Malation-d_{10} (diethyl D10, liquid, 99.0%), diazinon-d_{10} (diethyl D10, liquid, 97.5%), chlorpyrifos-d_{10} (diethyl D10, neat crystal, 98.0%), tebuconazole-d_6 (ethylene D4, methylene D2, 100 ng μL^{-1} in acetone, 96.5%) were obtained from Dr. Ehrenstorfer (Augsburg, Germany).

Acetonitrile (MeCN) and methanol (MeOH) of LC-MS grade were purchased from Merck KGaA (Darmstadt, Germany), and deionised water (> 18 MΩ cm) was obtained from a Milli-Q system (Millipore, Brussels, Belgium). Formic acid (98%), ammonium formate and acetate (≥ 99%) as well as NaCl were from Fluka, Sigma-Aldrich NV/SA (Bornem, Belgium), acetic acid (≥ 99%) and MgSO$_4$ were from Sigma-Aldrich NV/SA (Bornem, Belgium) and primary–secondary amine (PSA), C18 as well as LC-Florisil® sorbents (100 g, bulk) were provided by Supelco (Bellefonte, PA, USA).

2.2. Preparation of calibration solutions

Individual stock solutions of both native and isotopically labelled pesticides were prepared gravimetrically at the mass fraction of approximately 1 mg g^{-1} in MeCN, with the exception of carbendazim, a compound much less soluble, which was prepared at the mass fraction of approximately 0.1 mg g^{-1} in MeOH. The solutions were kept in capped amber vials at −20 °C until use. Two intermediate solutions, one containing a mixture for the native and another a mixture of the isotopically labelled compounds, were prepared at mass fraction levels in the range