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Guidelines for the validation of qualitative multi-residue methods used to detect pesticides in food

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There is a current trend for many laboratories to develop and use qualitative gas chromatography–mass spectrometry (GC-MS) and liquid chromatography-mass spectrometry (LC-MS) based multi-residue methods (MRMs) in order to greatly increase the number of pesticides that they can target. Before these qualitative MRMs can be used for the monitoring of pesticide residues in food, their fitness-for-purpose needs to be established by initial method validation. This paper sets out to assess the performances of two such qualitative MRMs against a set of parameters and criteria that might be suitable for their effective validation. As expected, the ease of detection was often dependent on the particular pesticide/commodity combinations that were targeted, especially at the lowest concentrations tested (0.01 mg/kg). The two examples also clearly demonstrated that the percentage of pesticides detected was dependent on many factors, but particularly on the capabilities of the automated software/library packages and the parameters and threshold settings selected for operation. Another very important consideration was the condition of chromatographic system and detector at the time of analysis. If the system was relatively clean, then the detection rate was much higher than if it had become contaminated over time from previous injections of sample extracts. The parameters and criteria suggested for method validation of qualitative MRMs are aimed at achieving a 95% confidence level of pesticide detection. However, the presence of any pesticide that is 'detected' will need subsequent analysis for quantification and, depending on the qualitative method used, further evidence of identity. © 2012 John Wiley & Sons, Ltd.

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Introduction

Pesticides are substances intended for preventing, destroying, or controlling any pest, including vectors of human or animal disease, unwanted species of plants, or animals causing harm during or otherwise interfering with the production, processing, storage, transportation, or marketing of food, agricultural commodities, wood and wood products, or animal feedstuffs. Maximum residue levels (MRLs) have been set by the Codex Alimentarius Commission,[1] the European Union (EU),[2] and many other countries for the post-registration monitoring and control of pesticide residues in a wide range of different foods, both of plant and animal origin. Reliable analytical data are essential to enforce this legislation and for the assessment of consumer exposure to pesticide residues. Before any analytical method is adopted it must therefore be validated to demonstrate that it is fit-for-purpose. The *Pesticide Manual* [3] contains entries for >1400 pesticides, and Regulation (EC) No. 396/2005[2] lists >152 000 MRLs for pesticides in 380 defined commodities. To deal with these huge numbers of pesticide/commodity combinations, residue laboratories are forced to employ MRMs. Over the past five decades or so, there have been many publications describing different quantitative MRMs based on solvent extraction, chromatographic separation, and determination using gas chromatography (GC) with semi-selective detectors such as the electron-capture detector (ECD), the flame photometric detector (FPD), and the nitrogen phosphorus detector (NPD). In more recent years MS has become the detector of choice, mainly because they have become more affordable and offer multi-analyte detection, adequate sensitivity and greater specificity. In fact, methods based on GC-MS can combine almost all of the pesticides previously analyzed by the different semi-selective into a single MRM. In addition, the coupling of MS with high performance liquid chromatography (HPLC) systems has resulted in a rapid advance in the development of MRMs for pesticides that are not amenable to GC-MS. LC-MS-based methods are complementary to those based on GC-MS and both are required to provide a comprehensive coverage of target pesticides, for both qualitative and quantitative MRMs.

Quantitative MRMs typically use quadrupole technologies, either GC-MS operated in selected ion monitoring mode (SIM) or tandem quadrupoles (MS/MS) operated in selected reaction monitoring mode (SRM), whereas qualitative screening methods are typically based on full-range MS acquisition using full scan quadrupole, time-of-flight (TOF-MS) or ion trap detectors.

For quantitative methods two or more ions, or ion transitions, that are characteristic of each target analyte are selected together with retention times for identification and quantification purposes. Although quantitative target pesticide methods can provide identification of the individual pesticides, only those

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pesticides with the ions pre-programmed into the method will be detected. Hence, any pesticides where the ions have not been pre-programmed into the method will not be detected. By contrast, qualitative methods rarely provide sufficient information to meet quantification criteria but do not require ion masses to be pre-programmed into the acquisition method. For these methods the mass spectrometer is operated in full-scan mode so that a total ion current chromatogram is obtained. All pesticides that have been extracted, chromatographed, and ionized have the potential to be detected, but in practice the scope of the method is limited by the effectiveness of the data processing software package.

Although many MRMs for the determination of pesticide residues in foods have been published, there is a clear preference amongst residue laboratories to employ either acetone,[4] acetonitrile,[5] or ethyl acetate,[6,7] as the extraction solvent. Even when a published method is adopted by a laboratory it is unlikely to be used exactly as it has been described. This is inevitable especially where laboratories do not have access to exactly the same instrumentation. Then, modifications to the method may be necessary to optimize the performance with the instrumentation that is available. Also, technology is rapidly evolving and the latest instruments are likely to provide improved sensitivity and specificity. Consequently it is no longer practical to prescribe in detail a standard MRM that will have longevity of use. This is not an issue as it has been demonstrated that in the hands of experienced analysts, different instruments and different MRMs are capable of producing equivalent results.[8] This not only highlights the importance of the analyst, but also the need for widely accepted guidelines and criteria to be established for the validation of MRMs.

The requirements for validating quantitative MRMs have already been adequately covered in several documents and papers[9–11] and therefore, will not be covered in detail in this paper. Instead the focus will be on the requirements for validation of qualitative MRMs and their role in complementing quantitative MRMs.

The need for robust qualitative methods

The continuous increase in the numbers of target pesticides sought means that it is now quite usual for between 200 and 500 compounds to be included in the scope of quantitative MRMs. The methods inevitably become unwieldy and more time consuming as larger numbers of target pesticides are included in standard solutions that are used for spiking and calibration. For each target pesticide, a calibration curve must be constructed using matrix matched standards to allow recovery values to be calculated from spiked sample extracts and residue concentrations calculated in test sample extracts. Thus, the amount of time spent on processing this data obviously increases as the scope of the method, in terms of numbers of target pesticides, is expanded.

However, the results of monitoring programmes often show that only a fraction of the scope (typically $<$100 pesticides) are regularly found across all of the different food types. Consequently, valuable time and effort is wasted in generating ongoing QC data for compounds that are seldom, if ever, found. Of course there is a risk that residues of these compounds could occur through accidental or deliberate misuse. Thus a more cost-effective approach is required that enables the analyst to be able to detect the possible presence of residues at concentrations prescribed in the relevant MRL regulations. Hence the need for qualitative MRMs that have a broad scope capable of demonstrating the presence and absence of residues, reliably with reduced ongoing QC. When a particular pesticide is detected using a qualitative MRM, a follow-up analysis using a validated quantitative MRM would still be necessary for identification purposes and to accurately determine the residue concentration.

Requirements for efficient qualitative MRMs

In order to increase efficiency, decrease workloads, and reduce the number of target pesticides in the scope of a quantitative MRM, the qualitative MRM should ideally:

- be able to detect $>$200 target analytes at a reporting limit of 0.01 mg/kg in a wide range of foods;
- be rapid and easy to use; and
- use automated software to process the data in such a way that it requires minimal input from the analyst.

In order to obtain as much information as possible to help with analyte identification the mass spectrometer is usually operated in full-scan mode. As for quantitative MRMs both GC and LC instruments have to be used in order to be able to detect both non-polar and polar compounds. The recent introduction of higher mass accuracy and resolution instruments, such as those based on TOF and/or Orbitrap technologies can help to improve specificity to eliminate chemical interference, but generate large amounts of information that require sophisticated automated software for efficient data processing. Crucially, the instrument parameters and software settings need to be carefully and expertly optimized in order to minimize the occurrence of false detects and false negative results and hence the need for time-consuming input from the analyst.

Validation of qualitative methods

Table 1 lists the parameters and criteria that are required for validating quantitative MRMs (taken from the EU SANCO document[9] and proposes which of these are applicable for validating qualitative MRMs.

As already indicated, qualitative methods are useful for detecting the presence of pesticide residues that are seldom likely to occur in samples of food. For such methods the degree of confidence in the detection, at and above, a satisfactory reporting limit (RL) for each targeted analyte, needs to be established during method validation. If an analyte has been sought, but not detected it should be reported as '$<$RL'. Any residues that are detected using a qualitative method should normally trigger further analysis using a validated quantitative method.

For efficiency, it is helpful if the same extraction, clean-up and concentration procedures as used for the quantitative MRM are also used for the qualitative MRM as well as for both GC and LC amenable pesticides. The most important parameters that must be established when validating a qualitative MRM are the selectivity (to avoid high number of false detects) and the LOD (limit of detection). However, it is also recommended that the robustness of the method is checked regularly by reference to changes in the numbers of false-negatives and false-detects in spiked samples analysed with each batch of analyses. The robustness and LOD of the method are largely dependent on the condition of the analytical system (e.g. GC-inlet) and type of samples (e.g. ion suppression in LC-MS). As these will change with time from batch

Table 1. Method validation parameters and criteria.

Parameter	How to address	Criterion	Applicability
Accuracy	Determine mean recovery from spikes	70–120%	Quantitative MRMs only
Linearity	Construct calibration curve	Residuals $< \pm 20\%$	Quantitative MRMs only
LoD	**The lowest concentration where 95% confidence of detection of analyte(s) is achieved**.	**≤ default MRL (0.01 mg/kg)**	**Qualitative MRMs** Quantitative MRMs
LoQ	The lowest concentration at which criteria for accuracy and precision are met.		
Matrix effect	**By comparing detector response from standards made up in solvent with standards made up in sample matrix**	**No criteria. Matrix effects may vary from pesticide to pesticide as well as between samples**	**Qualitative MRMs** and Quantitative MRMs
Precision (RSD_r)	Determine repeatability from replicate spikes analysed in same batch of samples	≤20%	Quantitative MRMs only
*Precision (RSD_R)**	*Determine reproducibility from replicate spikes analysed on different days*	*≤20%*	*Quantitative MRMs only*
Selectivity	**Response should be attributable to the analyte Check for any response in reagent sample matrix blanks**.	<30% LoD <30% LoQ	**Qualitative MRMs** ** Quantitative MRMs
Robustness*	***How often the method fails to meet the criteria that are applicable above***		***Qualitative MRMs** and Quantitative MRMs*

* It is not essential to address these parameters during initial method validation as they can be derived from ongoing QC data generated as the method starts to be used for routine analyses.

** No requirement has been set since any detect by the qualitative method is supposed to be followed up by an additional confirmatory analysis. However, selectivity should be such that the number of false detects is low enough for efficient use in routine practise.

to batch, effective ongoing QC procedures are necessary to support the initial method validation data.

Validation should be focused on assessing the reliability of the method to detect the presence of a targeted analyte at, or above, a specified concentration. The LOD of the method has to be established from spiking experiments using targeted analytes and samples that are representative of the matrix scope of the method. Hence by definition, the LOD will be the lowest spiking concentration at which the target analyte can be detected with a high degree of certainty or confidence. If the analyte can be detected in 95% of the spiked samples (i.e. a 5% false-negative rate) then this might be regarded as acceptable. The LOD for this confidence rate can then be adopted as the RL. There is no necessity to set criteria for the numbers of false detects providing that any samples containing suspect residues are identified and quantified using a validated quantitative method.

As for the validation of quantitative methods, representative commodities from the different commodity groups as specified in the SANCO document[9] may be selected. However, it is recommended that all pesticides that are to be targeted are included in the initial validation. It is also suggested that any initial validation focused on a particular commodity group should involve the analysis of 10 different commodities in duplicate spiked with the target pesticides at the required reporting concentration and the same 10 commodities 'unspiked' to act as 'blanks'. Since many MRLs that are included in the various regulations are default values set at 0.01 mg/kg, then this is likely to be the target RL of the method. The LOD of the method must therefore be at or below the RL.

There are two other important aspects of methodology that are seldom considered during validation, sample processing and extraction efficiency.

Sample processing

Methods are usually validated using laboratory-spiked samples. In practice, the samples are normally spiked after sample preparation has already taken place. Sample processing can have two important effects on the quality of results. Firstly, poorly homogenized samples will mean that truly representative sub-samples cannot be abstracted. This is particularly important with the trend to miniaturize the methods and to use smaller sub-samples. It is recommended that all laboratories should check that the sample processing procedures employed are fit-for-purpose. Secondly, and particularly for fruit and vegetable samples, homogenization at room temperature will disrupt plant cells and release enzymes that can rapidly react and/or degrade certain pesticides.[12] Cryogenic sample processing can reduce both heterogeneity and improve pesticide stability and is therefore recommended for such samples.

Extraction efficiency

During method validation extraction efficiency is normally only tested on laboratory-spiked samples but these cannot properly represent agriculturally incurred residues where the pesticide may 'bind' more strongly to the sample matrix. Extraction efficiency is dependent on a number of factors including the composition of the sample, the polarity of the extraction solvent, temperature, shaking time, physico-chemical properties of the pesticide (e.g. K_{OW}), and the particle size of the sample after processing. Because of the different physico-chemical properties of individual pesticides and the different matrix composition of samples, MRMs will inevitably result in a compromise for some individual pesticides. Method validation should highlight these compromises.

Examples of qualitative analysis and validation data generated for two different qualitative MRMs

The following two examples show data that was generated using qualitative MRMs and serve two purposes:

1. Demonstration of the great potential of qualitative methods to detect pesticides beyond the scope of quantitative methods covering frequently occurring residues.
2. Demonstration of the importance of validation in assessing the performance of the method.

Example 1. Using gas chromatography with a single quadrupole mass spectrometry (GC-MS)

In this example a generic multi-residue extraction and clean-up[13] were adopted. In brief, samples were extracted with acetonitrile and an aliquot then diluted with water. The pesticides were concentrated and cleaned up using two SPE cartridges PS-DVB and diethylaminopropyl in series. Pesticides were finally eluted with ethyl acetate affecting a solvent exchange. From previous experience, it was noted that an extensive clean-up approach was necessary for improved detectability, especially for challenging compounds such as captan and folpet. Sample extracts were injected (PTV) onto a gas chromatograph linked to a single quadrupole mass spectrometer (GC-MS AT 5975) operated in SIM/SCAN mode with retention time locking. The full-scan mass spectra from sample extracts were automatically compared to the spectra contained in a dedicated library by using a commercially available software package: Deconvolution Reporting Software (DRS version 4.0.1).[14] The software library (RTLPEST3.L) contained spectra for 926 compounds, plus retention times (RTs) for use with retention time locking. The software automatically performed data processing, including peak deconvolution, and matched the sample spectra plus RT against library spectra plus RT. A summary report of all the compounds 'detected' was generated including a nominal percentage match. Various parameters had to be set by the analyst including the 'match threshold' (set at 45) and the RT tolerance (set at $\pm$30 s). These settings are critical as if they are set too strict compounds could be missed (false negatives) and if they were set too loose then there would be too many tentative detects that would need further investigation.

Forty batches of fruits and vegetable extracts spiked with 94 pesticides at 4 different concentrations (0.01, 0.02, 0.2 and 0.5 mg/kg) were analyzed at different times over a period of 12 months.

Results

The overall percentage of the pesticides detected at each concentration in the various crops over 12 months is shown in Table 2. The detection capability depended mainly on the concentration and the spectral match threshold set. At the lower concentrations, the match factor had a significant effect on the detection rate. Using a match threshold set at 45, 82% of the pesticides could be detected at 0.01 mg/kg. However, the number of detects in blank samples (either false detects or correct identifications at concentrations below the RL) was considered too high for use in routine practice. Setting the match threshold to 60 improved this situation but also reduced the overall detection rate in the samples spiked at 0.01 mg/kg to 69%. At higher concentrations, the detection rates were improved and less affected by the match factor. These results are in agreement with those reported by others.[15,16] Overall, the majority of the pesticides added were found without any manual analyst intervention which clearly shows the benefit and potential of the method to find pesticides outside of the routine quantitative scope.

Using these data, a retrospective validation following the SANCO/10684/2009 guidance was performed. In contrast with the assessment above, the confidence of detection was now determined at an individual pesticide concentration. The validation guidelines state that, at a specified concentration the pesticide needs to be detected with 95% confidence, or in 38 out of the 40 samples in this example. The results are summarized in Table 3. At higher concentrations (0.2 and 0.5 mg/kg), most of the individual pesticides were detected consistently in the 40 samples over 12 months. At lower concentrations the

Table 2. Overall detection rate of pesticides in various vegetables and fruit using GC full-scan MS with automated library-based detection

Spiking concentration	0.01 mg/kg		0.02 mg/kg		0.2 mg/kg		0.5 mg/kg	
Match threshold	45	**60**	45	**60**	45	**60**	45	**60**
% pesticides detected*	82	**69**	92	**86**	98	**98**	99	**98**

* Percentage of 3760 pesticide/matrix combinations (94 pesticides spiked into extracts of 40 different vegetable and fruit samples, analysed over a period of 12 months).

Table 3. Reliability of detection of individual pesticides in vegetables and fruit using GC full-scan MS with automated library-based detection

Spiking concentration	0.01 mg/kg		0.02 mg/kg		0.2 mg/kg		0.5 mg/kg	
Match threshold	45	**60**	45	**60**	45	**60**	45	**60**
			Numbers of the 94 spiked pesticides detected					
Confidence level								
>**95**%	42	**21**	63	**43**	88	**85**	89	**89**
>90%	49	34	72	57	91	91	93	92
50–90%	37	35	18	31	3	3	1	2
<50%	8	25	4	6	0	0	0	0

number of pesticides meeting the validation criterion decreased rapidly. This could be attributed to a combination of insufficient sensitivity, selectivity, and deficiencies in the automated software. However, if the results from the different batches of the same commodities are studied more carefully then the influence of the matrices is also evident (Table 4). Although some matrices appear to be more problematic than others, the greatest variation was between batches as shown by results obtained for the same commodity. This was almost certainly due to the condition of the instrument at the time of analysis of each batch. Performance was affected by the cleanliness of the GC injector, the GC column and the mass spectrometer at that particular time. This clearly demonstrates the need for regular maintenance procedures and ongoing QC to check the performance of the method from batch to batch.

The data from this example indicate that most of the pesticides investigated can be detected reliably in an automated fashion at 0.2 mg/kg and, based on the observed relationship between concentration and detectability, probably down to 0.1 or 0.05 mg/kg. The *absence* of a pesticide in samples at these concentrations can only be reported for those pesticides that successfully passed initial validation. On the other hand, the *presence* of any pesticide found by the qualitative method, regardless of its validation status, can always be reported provided that a subsequent analysis by a confirmatory (quantitative) method will be performed. It should also be noted that certain pesticides that exhibited high sensitivity were found to be present at concentrations below 0.01 mg/kg when subsequent quantitative analysis was performed.

Example 2. Using ultra high performance liquid chromatography with a time of flight mass spectrometry (UHPLC-TOF-MS)

The qualitative detection of pesticides by comparing GC-MS full-scan data or GC-TOF-MS data against a library of EI mass spectra has been established for a number of years. By contrast, the use of TOF-MS for matching spectra against user-constructed libraries of accurate mass (typically electrospray ionization) and retention time is relatively recent such that the practice and software are evolving rapidly. Therefore, the information provided here should be considered as indicative of the potential capabilities of TOF-MS.

In this example, 50 samples containing 'incurred' residues that had previously been analyzed using a validated quantitative MRM (acetate buffered acetonitrile extraction,[17] no clean-up, UHPLC-MS/MS) were re-analyzed using citrate buffered acetonitrile extraction,[18] no clean-up, but with quantification by UHPLC-MS/MS and screening by UHPLC-TOF-MS (Agilent 1200 series LC system and an Agilent 6230 TOF) at a resolution of 11 000 – 14 000 FWHM across the mass range of interest. In total, the 50 samples were originally found to contain 169 residues including 35 different pesticides at concentrations between 0.01 and 0.78 mg/kg.

The data from TOF-MS were evaluated using a commercially automated software detection system (Agilent MassHunter Qualitative Analysis) to detect, but in this case, not to quantify the residues in the samples. The pesticides were detected by the software, with and without analyst intervention, based on the presence of signals for the exact mass of the main adduct (±5 mDa) at the expected retention time ±30 s stored in a user constructed library of approximately 450 pesticides.

Overall, 93% of the 169 pesticides found by the quantitative LC-MS/MS method were also detected by the qualitative automated UHPLC-TOF-MS method as shown in Table 5. This is in agreement with results obtained by others.[19] Following intervention by the analyst the detection rate increased slightly, mostly because of the improved detection of acetamiprid which gave a poor peak shape. The poor peak shape occurred because of the use of generic non-optimized conditions (injection of neat acetonitrile) and was evident in the initial validation. This facilitated the decision to include acetamiprid in the complementary LC-MS/MS analysis (extracts diluted with water to improve peak shape) thus minimizing the possibility for non-detection of acetamiprid.

Table 4. The influence of matrix and batch to batch variability on the numbers of pesticide residues detected using GC full-scan MS

Matrices	Numbers of pesticides detected* in different batches of samples			
	0.01 mg/kg	0.02 mg/kg	0.2 mg/kg	0.5 mg/kg
Mandarin	**42**, 50, 74	57, 73, 84	83, 92, 93	84, 94, 94
Cabbage	**53**, 66	76, 85	92, 93	91, 94
Pepper	**55**, 58, 64, 79	81, 79, 80, 87	93, 92, 93, 94	93, 94, 94, 94
Lettuce	**57**, 61, 66, 82	81, 80, 84, 86	91, 92, 92, 92	93, 94, 93, 93
Apple	**60**, 72, 77, 81	75, 89, 85, 89	94, 92, 93, 94	94, 93, 93, 94
Strawberry	**60**, 68	81, 83	91, 93	94, 93
Grape	**62**, 64, 74, 75	77, 79, 86, 87	93, 91, 94, 92	93, 90, 94, 94
Chicory	**62**, 74	86, 87	90, 94	90, 94

* Match threshold 60, number of spiked pesticides = 94.

Table 5. UHPLC-TOF-MS qualitative analysis of fruit and vegetable samples containing agriculturally incurred pesticide residues

Matrix (# samples)	Found ≥0.01 mg/kg by UHPLC-MS/MS	Software-based detection	Software-based detection + analyst intervention
Lettuce (N = 10)	30	28	29
Grape (N = 20)	68	63	67
Pear (N = 20)	71	66	67
Total	169	157	163
% of Total	100	93	96

Table 6. Overall UHPLC-TOF-MS detection rate of incurred pesticide residues at different concentrations across different sample matrices

Incurred residue concentration (mg/kg)	0.01	0.02	0.03	0.04	≥0.05
No. of different pesticides previously detected by LC-MS/MS	8	19	13	12	19
No. of target residues detected previously by LC-MS/MS	21	36	18	16	84
No. of residues detected by UHPLC-TOF-MS with automated processing (no analyst intervention)	13	30	18	16	83
No. of residues detected by UHPLC-TOF-MS with analyst intervention	19	32	18	16	84
% automated detection rate UHPLC-TOF-MS versus LC-MS/MS	68	83	100	100	99

Excludes 6 residues of acetamiprid (poor peak shape by UHPLC-TOF-MS) but detected by LC-MS/MS.

The small number of pesticides detected by LC-MS/MS but not detected by TOF-MS were typically due to insufficient detector response, for example, spinosad. The detection rate for 8 different pesticides at 0.01 mg/kg was 68% improving to 83% for 19 different pesticides at 0.02 mg/kg (Table 6). However, caution needs to be exercised when comparing detection rates of different systems using different extracts containing pesticides at the 0.01 mg/kg. For the quantitative LC-MS/MS MRM, a default expanded measurement uncertainty of 50% would mean that the result has an equal chance to fall below or above the reporting limit even using the same MRM. At 0.02 mg/kg the residue should be detected. Pirimicarb (2 samples), cyprodinil, and boscalid were detected automatically at 0.02 mg/kg, but not at 0.01 mg/kg. At 0.03 mg/kg and above, the detection rate was almost 100% for a total of 25 different pesticides across the three different sample types.

The use of a library to filter the data was essential for efficient management of data outputs. Without the use of the library each file typically generated more than 30 000 peaks (compounds). With the library, the number was reduced to detection of a few hits per file. Some of the empirical formulae correspond to possible pesticides not detected by LC-MS/MS (i.e. potential false negatives), but the identities could not be verified because the analytical standards were not available at the time. Similar to the situation with GC-MS, regular cleaning of the system was critical to maintaining good performance, especially when analysing non-cleaned-up extracts.

These data probably represent a best-case scenario using instrumentation that is currently available. Increasing the complexity of the matrix will cause detection rates to decrease and will probably require the use of even more sensitive, higher resolution instruments to be able to produce acceptable data. Nevertheless the results presented in this paper do serve to demonstrate that it should be feasible to start to implement UHPLC-TOF-MS with automated data processing for the routine detection of large numbers of pesticides in less-complex matrices of fruit and vegetables. This is especially the case if action is taken to transfer pesticides giving poor validation results to other methods, in order to minimize the risk of false negative results.

The two examples serve to demonstrate how qualitative MRMs can be utilized to automatically detect pesticides with minimum additional effort and costs. They also show that the performances of qualitative MRMs are limited by a number of important factors that relate directly to the performance of the mass spectrometer and the associated data processing software package:

1. The scope of the method will be limited by the number of target compounds that are included in the software library.
2. The ability of the automated software to be able to match the mass spectrometric and chromatographic information from the sample with the information in the library. The importance of setting optimum thresholds and tolerances is particularly critical.
3. The sensitivity and selectivity of the mass spectrometric detector at the time of analysis.

Qualitative MRMs that use generic extraction and clean-up procedures, and automated library-based detection can be utilized to greatly extend the scope of target pesticides and hence reduce the burden of data processing that is necessary when using quantitative MRMs. However, it is important to validate such methods before use so that their performance and limitations are known. The first examples given in this paper demonstrate that the operating condition of the instrument affects detection capability and highlights the importance of adequate ongoing QC to monitor performance from batch to batch. It also shows that under normal operating conditions a single quadrupole MS linked to a GC-MS with a nominal mass accuracy lacks the specificity and sensitivity to detect many compounds in food matrices at 0.01 mg/kg. Most extracts from food samples will contain only a few different pesticides. A food sample extract that has been spiked with several hundreds of different pesticides for AQC reasons will not reflect this situation. The additional pesticides could potentially cause problems such as signal suppression or enhancement and difficulties with peak deconvolution, not too dissimilar to the effects of complex matrices. More advanced techniques such as GC x GC-TOF-MS[20] or GC with high resolution TOF-MS have the potential to improve this, provided that adequate software tools are available.

The second example utilized UHPLC-TOF-MS as qualitative method. Although not assessed as extensively as the GC-MS method from the first example, the data obtained indicate a similar performance at the lower concentrations. The detectability at 0.01 mg/kg was 68% for the UHPLC-TOF-MS compared with 69% (match threshold = 60) for the GC-MS, and at 0.02 mg/kg it was 83% and 86%, respectively. However, a more fair comparison would require this to be demonstrated through a more extensive TOF-MS data set involving more matrices analysed over a longer period of time, in a similar way as was conducted in the GC-MS. The UPLC-TOF-MS results are encouraging in that the sample extracts were not subjected to any clean-up before injection. By contrast, the GC-MS sample extracts were cleaned by tandem cartridge SPE to improve the detection rates. Clearly, neither technique will be able to cover all pesticides, so both approaches will be required in practice in order to provide a comprehensive scope at a realistic cost. For those pesticides that are amenable to both GC and LC, reliability of detection will be further increased. Both examples also show the limitations, of instruments in terms of sensitivity and specificity, and the capabilities of the software packages and libraries. These limitations serve to emphasize the need for effective method validation of

qualitative MRMs prior to their implementation for monitoring and enforcement purposes.

As for quantitative MRMs, initial method validation of qualitative MRMs cannot be totally comprehensive. The use of appropriate ongoing AQC procedures is critical in order to broaden the scope of validation as well as ensuring that the method remains under control. This paper does not cover all the aspects of validation of qualitative MRMs, but the two examples serve to highlight the most important factors that need to be considered. The examples also demonstrate that qualitative MRMs that rely on MS detectors are suitable for detecting the presence of a large number of target pesticides in a broad range of food commodities. Within Europe this has been recognized and the latest version of the EU SANCO document,[9] were implemented on 1st January 2012, and include an expansion of the existing section on method validation of qualitative methods.

Conflicts of interest

The author has no conflicts of interest.

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