Analytica Chimica Acta 856 (2015) 54–67

Contents lists available at ScienceDirect

Analytica Chimica Acta

journal homepage: www.elsevier.com/locate/aca

Reliability of veterinary drug residue confirmation: High resolution mass spectrometry versus tandem mass spectrometry

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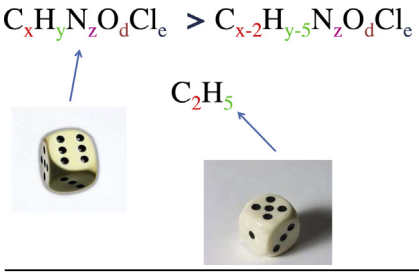
HIGHLIGHTS

- A HRMS based confirmation criteria for residue analysis is proposed.
- The criteria was comprehensively compared to existing criteria.
- Less false-positives and false-negatives was observed.
- Limitations of existing confirmation criteria are critically discussed.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 19 August 2014
Received in revised form 10 October 2014
Accepted 25 November 2014
Available online 29 November 2014

Keywords:
High Resolution mass spectrometry
Residue
Confirmation
Commission Decision 93/256/EC
Food safety

ABSTRACT

Confirmation of suspected residues has been a long time domain of tandem triple quadrupole mass spectrometry (QqQ). The currently most widely used confirmation strategy relies on the use of two selected reaction monitoring signals (SRM). The details of this confirmation procedure are described in detail in the Commission Decision 93/256/EC (CD). On the other hand, high resolution mass spectrometry (HRMS) is nowadays increasingly used for trace analysis. Yet its utility for confirmatory purposes has not been well explored and utilized, since established confirmation strategies like the CD do not yet include rules for modern HRMS technologies.

It is the focus of this paper to evaluate the likelihood of false positive and false negative confirmation results, when using a variety of HRMS based measurement modes as compared to conventional QqQ mass spectrometry. The experimental strategy relies on the chromatographic separation of a complex blank sample (bovine liver extract) and the subsequent monitoring of a number of dummy transitions respectively dummy accurate masses. The term "dummy" refers to precursor and derived product ions (based on a realistic neutral loss) whose elemental compositions ($C_xH_yN_zO_dCl_e$) were produced by a random number generator. Monitoring a large number of such hypothetical SRM's, or accurate masses inevitably produces a number of mass traces containing chromatographic peaks (false detects) which are caused by eluting matrix compounds. The number and intensity of these peaks were recorded and standardized to permit a comparison among the two employed MS technologies. QqQ performance (compounds which happen to produce a response in two SRM traces at identical retention time) was compared with a number of different $HRMS^1$ and $HRMS^2$ detection based modes. A HRMS confirmation criterion based on two full scans (an unfragmented and an all ion fragmented) was proposed. Compared to the CD criteria, a significantly lower probability of false positive and false negative findings is obtained by utilizing this criterion.

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http://dx.doi.org/10.1016/j.aca.2014.11.034
0003-2670/

1. Introduction

Confirmation is the important final step before a found and quantified compound (residue) is being reported. The process of confirmation is intended to ensure that the compound responsible for the observed detector signal is truly identical with the finally reported analyte. Reporting, false positive findings of hazardous residues in food or the environment can have huge financial and juristic implications. Hence, norms were developed to ensure that the likelihood of false positive findings is extremely small.

Within this paper the terminology "false detect" is used when an analyte selective transition or accurate mass traces shows a chromatographic peak which is not related to the searched analyte but caused by a non-related matrix compound. The terminology "false positive" is used when such a false detect signal survives all further investigations (e.g., second SRM trace retention time and ion ratio) and finally leads to the wrong conclusion that the investigated analyte is considered to be confirmed.

For selectivity and sensitivity reasons, mass spectrometry has become the preferred tool for residue confirmatory work. Currently, most often used is unit mass resolving tandem quadrupole technology (QqQ). The observation of two transitions, both showing a chromatographic peak at the expected analyte retention time, producing the analyte typical peak area ratio (ion ratio) has been considered to be a solid confirmatory criteria. The details (maximum deviations of retention time and peak area ratio of the two SRM traces) have been clearly described in the Commission Decision 93/256/EC (CD). The use of this norm [1] is required in all EU member countries, if a veterinary drug found in animal derived food (e.g., meat) is going to be reported. A slightly different concept consisting of three levels (detection, identification and confirmation) has been proposed as well [2]. That concept requires the use of a second independent analytical measurement method for a successful confirmation step. According to the CD, a banned compound (e.g., veterinary drug in meat intended for human consumption) is considered to be sufficiently confirmed when 4 identification points (IP) are obtained. One point can result from the low resolution (LR) mass resolved precursor ion and 2×1.5 points by monitoring precursor selected and LR resolved product ions. The CD also defines identification points for high resolution based measurements. However, they are based on a mass resolving power of 10,000 based on a 10% valley. This definition was commonly employed for sector field mass spectrometers. Currently used time of flight (TOF) or Orbitrap instruments specify resolution at full width at half maximum (FWHM). Hence, is not entirely clear, how the CD shall be used for HRMS data. This has been one reason why residue analysts have been reluctant in employing HRMS technology. In spite of this, HRMS is increasingly recognized to be a versatile and promising emerging technology [3–5]. It is therefore important to have well defined criteria of how HRMS confirmatory data are to be weighted and interpreted. Hence, there is a need for HRMS based confirmatory rules which ensure an equal reliable confirmation as currently available for LR QqQ based MS.

It is important to recognize that the CD is a framework based on combined expert opinion [6–8]. The document (CD) neither provides scientific data nor cites scientific literature regarding the establishment of the proposed identification point system. There is no data available, how the likelihood of false positive is affected when, for example, relying on three LR non-precursor selected analyte ions (3 identification points) or relying on a LR precursor selected analyte and two derived product ions (4 identification points). Yet monitoring of two product ions (after LR based precursor selection) has become the commonly used benchmark for any confirmation. Hence, any alternative HRMS based confirmation strategy has to produce an at least equally small likelihood of false positive and false negative findings.

Although the CD is to be considered the benchmark, limitations of this norm have been criticized. False negative findings were observed when analyzing some qinolones in meat matrix [9]. Some members of this drug family (e.g., ciprofloxacin) produce two singly charged isobaric ions (protomers) in the electrospray interface. These two ions produce two completely different sets of product ions. Unfortunately, matrix dependent signal suppression affects the generation of these two isobaric precursor ions in a different extent. As a result, signal abundance of the two utilized confirmation SRM's will be differently affected as long as the involved product ions do not originate from the same precursor (protomer). Hence, SRM ratio derived from the analyte present in a blank standard versus spiked in matrix can differ that much that a false negative finding will result. Such false negative findings are not analyte concentration dependent. Observed false negative findings (benzophenone in water) were reported [10] to be caused by a co-eluting endogenous compound (called Harman). There were reports of false positive findings where pharmaceutical metabolites share common fragment ions [11] and where some matrix compounds mimicked the expected, analyte specific SRM ratio. Reported examples were the suspected finding of a pesticide in tarragon [12] and the finding of a nitroimidazol antibiotic in honey [13], smoked salmon [14] as well as muscle tissue [15]. Described were circumstances where signal suppression affected the SRM ratio of polypeptide antibiotics [16]. Polypeptides are analyzed as multiple charged ions. SRM ratio based on product ions originating from double and triple charged precursors were found to be differently affected by signal suppression. The observed deviations were that extensive, that a confirmation became impossible. Hence SRM reference ratios based on a spiked matrix extracts were suggested [5,9,16] to circumvent this problem.

False positive findings certainly have serious financial and juristic implications. On the other and false negative findings will go mostly undetected. However, the enforcement of food safety relies on the capability to avoid false negative findings. Therefore, the CD introduced the parameter of detection capability (CCβ). This parameter indicates that a residue present at that concentration will be detected with a likelihood of 95%. In other words, a given method will not miss a compound present at or above this concentration [1,7,8]. Unfortunately, this parameter which is intended to ensure the absence of false negative findings can still be overturned if the following, compulsory confirmatory step fails. Such a false negative finding is mostly linked to an incorrect SRM ratio. Examples have been given above. A mismatch of QqQ performance is directly linked to such findings. The sensitivity of QqQ based MS instruments has dramatically increased over the last decade. On the other hand, no relevant improvements of QqQ selectivity has been achieved. This has created a situation where even an analyzed, blank samples contain matrix compounds which are capable in producing a chromatographically resolved peak. Worse, one SRM trace may show a chromatographic peak, exactly corresponding the analyte retention time. Such a peak will most likely be recognized as a false detect, since the SRM ratio will be unlikely to correspond the ratio typical for the analyte. However, there will be situations, where the analyte as well as an interfering matrix compound will be present in a given sample. The presence of such an interfering compound will therefore shift the resulting SRM ratio. A sufficiently intensive matrix compound, or a relatively low analyte concentration will lead to the situation that the truly present analyte cannot anymore be confirmed. This scenario was a very unlikely issue during the time the CD was written. However, currently employed MS instruments are now one to two orders of magnitude more sensitive. The combined effects of higher sensitivity and unchanged selectivity of LR MS/MS has therefore