Food Control 145 (2023) 109488

Contents lists available at ScienceDirect

Food Control

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

Use of high-resolution mass spectrometry for veterinary drug multi-residue analysis

Esmer Jongedijk [a,*], Markus Fifeik [b], Ane Arrizabalaga-Larrañaga [a], Joachim Polzer [b], Marco Blokland [a], Saskia Sterk [a]

[a] Wageningen Food Safety Research (WFSR), Part of Wageningen University & Research, European Union Reference Laboratory for Residues, P.O. Box 230, 6700, AE Wageningen, the Netherlands

[b] German Federal Office of Consumer Protection and Food Safety (BVL), Unit 502 "European Union Reference Laboratory (EURL)", P.O. Box 11 02 60, 10832, Berlin, Germany

ARTICLE INFO

Keywords:
HRMS
Risk-based monitoring
Growth promotors
Antibiotics
Residues
Food safety

ABSTRACT

National and international food and feed safety authorities are shifting from routine-to risk-based monitoring. Risk-based monitoring requires flexibility in the scope of analytes, matrices, and sampling. Also, risk-based monitoring implies a desire for retrospective analysis using different scope(s) to follow trends, identify new food safety threats, and monitor the effectiveness of policy interventions. The current availability of sensitive and accurate high-resolution mass spectrometry (HRMS) fits within this approach. This writing reviews the applicability of HRMS techniques for food control laboratories in the analysis of veterinary medicinal products and hormones in food, using HRMS and legislative background. Different HRMS measurement and data evaluation strategies are identified and discussed. Among them, routine screening and confirmation, suspect screening, semi-untargeted analysis (common mass pattern search), metabolite and degradation product identification, profiling for deviating samples, physiological markers or treatments, and identification of unknowns can be found. The food safety competent authorities could shift from methods with predefined scope to real risk-based monitoring by implementing HRMS for routine food and feed analysis.

1. Introduction

Nowadays, food safety laboratories perform most of the routine analysis of veterinary drugs and hormones in food products by triple quadrupole mass analysers. Triple quadrupole mass analysers working in multiple reaction monitoring (MRM) mode are usually very sensitive and specific and fits current legislation ("general food law" EC No. 178/2002) (European Commission, 2002b). However, due to the specific MRM measurement, only a pre-defined number of compounds can be detected. Also, low-resolution mass spectrometry cannot differentiate or avoid isobaric interferences (substances with the same nominal *m/z* ratio but different elemental compositions) or separate coeluting isobaric compounds, which are especially frequent when analysing food samples with complex matrices (van der Heeft et al., 2009). Under these circumstances, mass analysers such as time of flight (TOF) and Orbitrap allow the acquisition of high-resolution full scan mass spectra providing high accurate mass measurements (<1–3 ppm) in combination with resolutions greater than 20 000 and, in most cases, with sensitivity and selectivity comparable to triple quadrupoles in MRM mode (Geib, Sleno, Hall, Stokes, & Volmer, 2016; Kaufmann & Butcher, 2006; Malato, Lozano, Mezcua, Agüera, & Fernandez-Alba, 2011). Thereby, the mass accuracy and the separation of overlapped isotope cluster ions allow the tentative assignment of the molecular formula to each ion signal, making the separation of most isobaric compounds possible.

The high sensitivity and selectivity provided by current high-resolution mass analysers in full-scan mode enable an untargeted and retrospective analysis to identify multiple families of suspected contaminants in the food safety field (Capriotti et al., 2021; Domínguez, Garrido Frenich, & Romero-González, 2020; Gavage, Delahaut, & Gillard, 2021; Rocchetti, Ghilardelli, Masoero, & Gallo, 2021; Yan, Zhang, Zhou, Li, & Feng, 2022). Additionally, high-resolution hybrid mass analysers such as quadrupole-(Q-)Orbitrap, Ion trap-Orbitrap, and Q-TOF can perform tandem mass spectrometry (MS/MS) whereby the product ions are measured by the HRMS. These HRMS product ion spectra can be used to identify and narrow down the list of potential candidates which were already defined by the obtained elemental

* Corresponding author.
E-mail address: esmer.jongedijk@wur.nl (E. Jongedijk).

https://doi.org/10.1016/j.foodcont.2022.109488
Received 18 August 2022; Received in revised form 25 October 2022; Accepted 31 October 2022
Available online 8 November 2022

composition of the precursor ion. Due to its capability to identify and perform untargeted measurements, HRMS would fit in a future risk-based measuring approach with a flexible scope of matrices and analytes (Fig. 1).

An overview of identified new risks in the past years showed that new risks are most often first discovered by the reporting of adverse health effects (intoxications) in humans or animals (Gerssen, Bovee, van Ginkel, van Iersel, & Hoogenboom, 2019). The authors of this overview conclude that the untargeted collection of HRMS data plays a crucial role in identifying risk compounds, thus preventing intoxications in the future. The first HRMS methods published for veterinary drugs and hormones showed the potential of this technique for residue analysis (Kaufmann, 2012; Peters et al., 2010; Stolker et al., 2008). Nowadays, HRMS is already used by some routine laboratories in Europe, mainly for multi-residue screening methods. These methods demonstrate that HRMS can be applied for the screening of these compounds at relevant levels.

Despite the high potential of HRMS to detect these compounds, the possibilities of HRMS are not fully explored for food safety analysis. This is partly due to the lack of advanced data-processing software at the time these methods were developed (Sturm, Jones, Mulvana, & Lowes, 2016) and also because current HRMS instruments can combine more scan events in one run due to increased scan speed. Also, the dynamic range has increased over the years (Steiner, Malachova, Sulyok, & Krska, 2021). Nowadays, HRMS equipment can cover 3–5 orders of magnitude and are therefore capable of quantitative analysis as well.

However, the underexploiting of HRMS in the area of veterinary drug residue analysis might also be explained significantly by a lack of daily routine and/or awareness of its added value in the relevant control laboratories. Therefore, this position paper serves to researchers and control laboratories that might extend the application of HRMS in their monitoring activities in the near future. This paper presents the main applications of HRMS in the veterinary drug residue field. It describes the different HRMS approaches available in food safety laboratories, focusing on the application of HRMS for small molecules in the field of veterinary drugs and growth promoters (hormones). Besides, the results are presented of an inquiry among the European Union Reference Laboratories (EURL), National Reference Laboratories (NRL), and routine laboratories about the current situation and future perspective of HRMS use for multi-residue screening. Additionally, the possibilities to fit HRMS in current and future legislation for veterinary drug residues are discussed. An overview of available methods and workflows that were published in the last 5 years is presented (2017–2022). From these insights, we identified scenarios for control laboratories for which it could be relevant to apply HRMS using different strategies.

2. Survey HRMS in EURL and NRL network

A survey was carried out among European Union and National Reference Laboratories (EURL and NRL) for certain substances of veterinary drugs and growth promoters. A total of 35 laboratories from 30 countries participated in this survey. In the context of official laboratories, this can be considered representative. The survey determined the general use of the analytical systems and the type of application, and the use of HRMS for different substance groups.

From the survey respondents 58% of the laboratories use HRMS systems for various purposes and reasons. The users of HRMS systems see the greatest benefits in the possibility of retrospective analysis (25%), the high selectivity or mass resolution (25%), and the possibility of examining many substances simultaneously (35%). Regarding the selection of systems, TOF/QTOF and Orbitrap are preferred compared to magnetic sector instruments. They are used in 65% and 50% of the laboratories, respectively, while magnetic sector instruments are only used in 20% of the laboratories. The experience level varies greatly: 25% of the users have been working with high resolution for 1–2 years, 30% for 2–5 years, 25% for 5–10 years, and 20% have been working with HRMS systems for 10–20 years.

The use in routine laboratory work covers a wide range of screening approaches (Fig. 2A). 75% of the laboratories use HRMS for confirmatory analysis, although all laboratories also use their systems for target screening. The application of the non-target screening is divided into “unknown screening” and “known unknown screening”, which are applied in 65% and 50% of the laboratories, respectively. Whereas in an “unknown screening”, no data nor information are previously available, in a “known unknown screening”, information on the substance is available in existing libraries or databases, even if the substance itself is unknown in the laboratory’s field of application.

Among EURL and NRLs, HRMS has been applied for the analysis of a wide range of substance classes (Fig. 2B). Most of the covered substances belong to the group of veterinary drugs, e.g. coccidiostats, non-steroidal anti-inflammatory drugs (NSAIDs) or anthelmintics. This is not surprising since, as mentioned before, the participants of the survey mainly work in this field. However, further compounds such as polycyclic aromatic hydrocarbons (PAH), polychlorinated biphenyls (PCB) and pesticides are also included in the analysis of known and unknown substances.

Additionally, the answer to the question why almost half of the participants currently do not use HRMS techniques, can be partly deduced from the replies in Fig. 2C. The majority of the respondents who do not use HRMS, state that the main challenge of HRMS compared to triple quadrupole MS is the lack of expertise required to exploit the full potential of this technology. They also mention the diminished

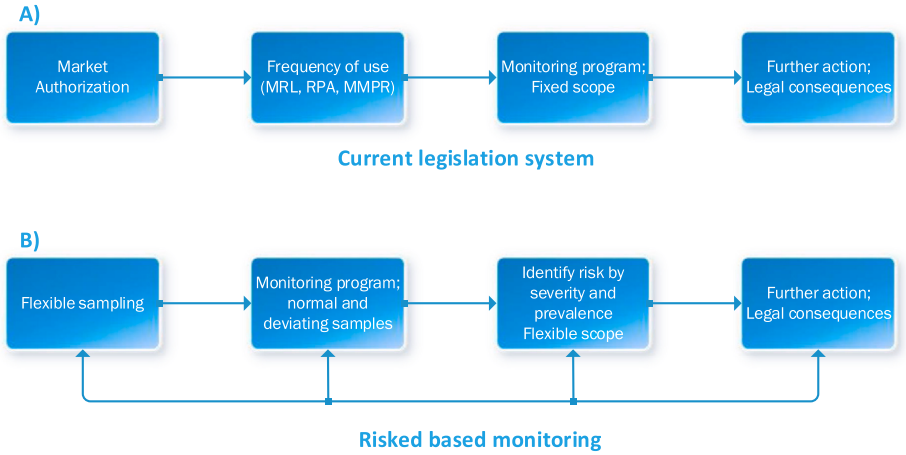


Fig. 1. Paradigm shift towards risk-based monitoring. A) current legislation system, B) risk-based monitoring.

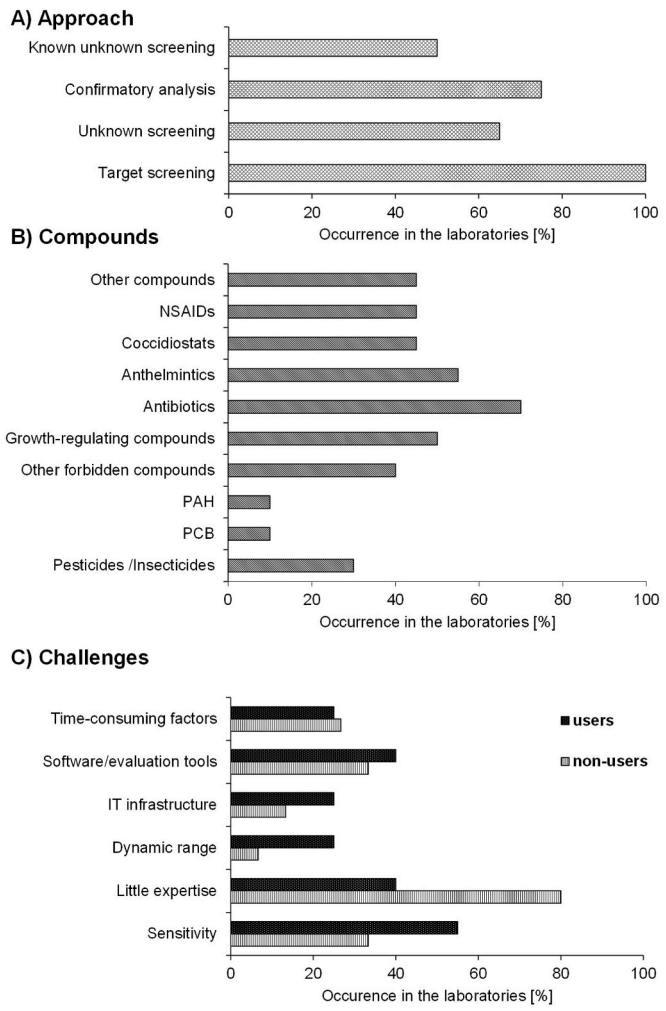


Fig. 2. Results from the survey about HRMS in EURL and NRL network. A) Overview of the approach areas, B) Overview of the compound groups analyzed with HRMS; "other compounds" includes individual substances such as dioxins and mycotoxins, but also proteomics, metabolomics and others, C) Challenges of HMRS from the laboratories' perspective; the results reflect the number of laboratories in each case.

sensitivity, the complex evaluation tools and the very time-consuming data processing as major challenges. For experienced users, however, the challenges shift. With the increasing experience of the users, the lack of expertise as a challenge decreases significantly. Nevertheless, although reduced, the experience seems to remain a consistent problem even among current users. In addition, performance problems such as sensitivity and dynamic range come to the fore for current users. Moreover, the IT infrastructure is increasingly cited as a challenge. This refers to the size of the resulting data, which quickly takes up the size of several gigabytes of data storage. Therefore, the storage capacities must be taken into account from the beginning in order to be able to handle this data, especially if data are acquired on a regular basis.

Irrespective of these challenges, the fact that 75% of the laboratories already have validated methods on HRMS systems, shows that the HRMS technology is applied for analyzing a wide variety of substances. Although some of these methods are established on both triple quadrupole MS and HRMS, the majority (63%) are purely based on HRMS. This underlines that there is already a high level of confidence in the technology, which is also supported by the fact that 60% of the respondents already use the systems in their routine analysis. Together with the generally high acceptance and the broad areas of application, it gives rise to hopes that this selective technology will find even wider acceptance among users and laboratories in the years to come. Moreover, the fact that powerful tools in the form of methods can be generated with HRMS, even with little previous knowledge, should help to motivate current non-users.

3. Fitting HRMS in legislation

Current EU legislation on laying down procedures for food safety is documented in regulation EC No. 178/2002, the "general food law" (European Commission, 2002b). It uses a fixed scope of allowed and prohibited substances, defined in Annex I of Council Directive 96/23/EC (European Commission, 1996b), specified for individual substances by Commission Regulation (EU) No 37/2010 (European Commission, 2009). This regulation contains two tables, "Table 1" with allowed substances (group B), for which a maximum residue limit (MRL) is established based on toxicology. These are, for example, several registered veterinary medicines, antibiotics and anthelminthics (worming agents). The other table in this regulation, "Table 2" (group A) lists selected prohibited substances for which there are no MRL, e.g. chloramphenicol, single nitroimidazoles and nitrofurans. In addition a general ban of substances having a hormonal or thyrostatic action and of beta-agonists in livestock farming is fixed in Council Directive 96/22/EC (European Commission, 1996a). Next to this the Commission regulation (EU) 2019/1871 describes the prohibited substances with an Reference Point for Action (RPA) (European Commission, 2019) and a Guidance document on Minimum Method Performance Requirements (MMPR) describes the recommended concentration at which official laboratories need to be able to analyze prohibited substances (Verdon, Polzer, & Sterk, 2020). Based on the substances listed in these tables, EU countries develop annually their monitoring program with a fixed analyte scope. The basis of this legislation originates still from the nineties and is not any more effectual for current wishes of risk-based monitoring. This legislation is presently being revised and shall take into account a more risk based approach (Table 1).

Besides a scope for substances, food control legislation gives directives for the performance of analytical methods used to analyze the substances. Until June 2021 the specifications for analytical methods and their validation were given by Commission Decision (CD) 2002/657/EC (European Commission, 2002a). This decision has since been superseded by Commission Implementing Regulation (EU) 2021/808 (European Commission, 2021). This regulation specifies the requirements for the performance of analytical methods and the interpretation of results. In the previous decision for HRMS there was only a definition for resolution given. It was defined that the resolution shall typically be greater than 10 000 for the entire mass range at 10% valley and was referring mainly to magnetic sector field instruments. With Commission Implementing Regulation (EU) 2021/808, the definition was amended by the full width at half peak maximum (FWHM) approach, which is meanwhile the preferred calculation approach (also adopted in the pesticide field). In addition, a requirement for mass accuracy was specified. Here it is required that for successful confirmation, the mass deviation of all diagnostic ions shall be below 5 ppm (or in case of $m/z < 200$ below 1 mDa).

Furthermore, different scan modes for mass spectrometric detection are listed, as well as detailed/general requirements/considerations. Linked with the respective operation mode of the MS, as full scan recording, precursor ion selection, fragment und product ions, mass resolution are given. These specifications are the basis for successful identification of a substance by the identification point system as described in Commission Implementing Regulation (EU) 2021/8081.2.4.2 in Annex I. Five identification points are needed for a successful confirmation of an unauthorised substance, 4 identification points for a MRL substance. Usually, 1 point origins from the application of a chromatographic separation technique before the detection. Depending on the kind of the ion (LR-MS/HR-MS ion and precursor/product ion) a different number of identification points (1.0–2.5) can be

Table 1
Current legislation Commission Implementing Regulation (CIR) 2021/808/EC (implementing Council Directive 96/23/EC) and suggestions for future legislation related to application of HRMS for veterinary drug analysis.

	Current legislation (CIR 2021/808)	Suggestions for future legislation
Scope	Fixed scope of allowed substances and prohibited substances	(Un)targeted residue identification from flexible risk-based sampling
Definition HRMS	Resolution >10 000 by the 10% valley measurement or 20 000 by the FWHM measurement for the entire mass range. Mass deviation all diagnostic ions <5 ppm (if m/z < 200 below 1 mDa)	–
Identification point system	Points per ion, more for HRMS than for LRMS. Ion ratio criteria (described in CIR 2021/808; in table 3 of 1.2.4.2 annex I of that CIR) A general criterium for ratio deviation, 40%	Full scan mode: isotopic pattern should match molecular formula Complete spectrum evaluation
Definition scan modes	Full scan in case the precursor selection has a mass selection window of more than one Dalton	Description of other scan modes
(Internal) standards	Relation to standards defined, ion ratios and co-chromatography Absence of internal standard	Strategy how to deal if no (internal) standards are available
Yet to be exploited	–	Putative molecular formula Putative molecular structure Co-elution and deconvolution: connect fragment ions to precursor ion Metabolite analysis/data clustering Different fragmentation options: ddMS2, AIF, vDIA, SWATH

gained, with 1.0 points for a LR-MS ion to a maximum of 2.5 points for a HRMS product ion. Together with additionally listed specifications (relative intensities for ion ratios, signal-to-noise ratios, retention time deviations in chromatographic systems), this concept can be applied in general to different kinds of systems and combinations of systems while maintaining uniform criteria for successful substance identification (Table 1). Even though Commission Implementing Regulation (EU) 2021/808 gives examples of how a successful identification can be achieved, especially in the area of modern HRMS instruments not all possible operation modes (e.g. HRMS scan modes, such as ddMS2, vDIA, AIF, data independent acquisition and SWATH) are explicitly mentioned and leaves some space for interpretation. Also, criteria for the application of computer-aided library search of spectra (which were present in CD 2002/657/EC) have not been adopted to the new regulation. In case of recording full scan spectra, only the selection of diagnostic ions is addressed: diagnostic ions with a relative intensity of more than 10% in the reference spectrum (which can derive from the calibration standard, matrix-matched standard or matrix-fortified standards) are suitable. Diagnostic ions shall include the molecular ion (if present at $\geq$ 10% intensity of the base peak) and characteristic fragment or product ions. I.e. this listing refers more to a single ion evaluation rather than a complete spectrum evaluation, including the determination of a critical match factor for library search during validation (Table 1).

Also, additional techniques which might contribute to a unique identification of substances (e.g. ion mobility, NMR) are not mentioned. Currently, also the co-measuring of a calibration standard (standard solution/matrix standard) is always required.

The regulation only states that GC-MS using electron impact ionization is regarded as being a different technique to GC-MS using chemical ionization. Additionally, it does not state anything about the nature or orthogonality of the techniques combined.

In the field of official control of pesticides in food and feed, a guidance document, SANTE 11312/2021 (EURL Pesticides, 2021), also lays down provisions for the identification of analytes. These identification provisions, although not identical, are quite similar to the ones laid down in (EU) 2021/808.

A trend is to use HRMS to identify new emerging risks (see 'Strategies of using HRMS in the field of veterinary drugs and hormones residue analysis' section). Especially for food fraud analysis untargeted analysis is sometimes the only way to go, as the distinguishing compound(s) are not always known. Cavanna et al. proposed a harmonized validation protocol for untargeted analysis of food fraud, including marker validation, that can be used for specific predetermined types of fraud (Cavanna, Righetti, Elliott, & Suman, 2018).

Monitoring according to (EU) 2017/625 states "Competent authorities shall perform official controls on all operators regularly, on a risk basis and with appropriate frequency" (European Commission, 2017). A risk could be decomposed in a certain severity with a certain prevalence. For new ways of analysis, such as retrospective analysis of datasets, or profiling analysis, HRMS can be of large contribution in determining prevalence of known and new compounds.

4. Strategies of using HRMS in the field of veterinary drugs and hormones residue analysis

Risk-based monitoring asks to determine the prevalence of known and new compounds (EU) 2017/625) (European Commission, 2017). Key steps of a risk-based workflow (Fig. 1) are to analyze food matrices in a targeted and untargeted way and identify deviating materials in every sense of the word. HRMS could play a key role in realizing a risk-based workflow. Indeed, the application of HRMS in the field of veterinary drugs and growth promoters is exponentially increased. This is mainly for screening purposes, but also for other strategies of application. An overview of available methods and workflows that were published in the past five years is presented in Table 2. Table 2 is structured by highlighting the applied HRMS strategy in those writings,

Table 2
Available HRMS methods and workflows in the field of food safety for the analysis of small molecules veterinary drugs and growth promoters, published 2017–2022.

Compounds (number of analytes[a])	Matrix	Sample preprocessing method	Chromatographic separation	Mass Spectrometry	Validation	Reference
Screening						
Steroids (46)	Urine	Deconjugation, LLE, derivatization	GC	Comparing Q-Orbitrap and Q-TOF[b], EI[c]	–	Abushareeda et al. (2018)
Veterinary drugs (87)	Milk, veal muscle, egg, honey	(SA)LLE[d], QuEChERS[e]	nano-LC	Q-Orbitrap, ESI[f]+, Full Scan, AIF[g]	–	Alcantara-Duran et al. (2018)
Pharmaceuticals/personal care products (6)	Fresh water invertebrates	QuEChERS	LC	Q-Orbitrap, ESI+/−, Full Scan	–	Althakafy et al. (2018)
Veterinary drugs (182), pesticides (524), pollutants (32), marine toxins (18)	Aquaculture products	LE, (d)SPE[h]	LC	Q-TOF, ESI+, Full Scan, target mode	SANTE/12682/2019	Bai et al. (2022)
Quinolones (7)	Honey	Diluting	LC	Q-TOF, ESI+, Full Scan	CD 2002/657/EC	Bandini and Spisso (2021)
Veterinary drugs (100)	Liver, muscle, urine	Variable	LC	Variable	Inter-laboratory study	Berendsen et al. (2017)
Steroids (40)	Urine	SPE, deconjugation, derivatization	GCxGC	TOF, EI	–	Bileck et al. (2018)
Sulphonamides, β-agonists and (steroid) hormones (53)	Urine	Deconjugation, SPE	LCxLC	TOF, ESI+, Full Scan	–	Blokland et al. (2018)
Veterinary drugs, pesticides, natural toxins (15)	–	–	–	MAI[i], DAPSI[j], TM-DART[k], CBS[l], Orbitrap, Full Scan	–	Blokland et al. (2019)
Progestagens (22)	Milk	(SA)LLE, SPE	LC	Q-Orbitrap, ESI+, PRM[m]	NY/T 1896, CD 2002/657/EC	Decheng et al. (2021)
Veterinary drugs (29), pesticides (25), mycotoxins (23)	Eadible insects	SLE, SPE	LC	Q-Orbitrap, ESI+/−, Full Scan	CD 2002/657/EC, SANCO	De Paepe et al. (2019)
Sulfonamides (8)	Milk	LLE, Magnetic SPE	LC	Q-Orbitrap, ESI+, Full Scan	Partly	Di et al. (2020)
Prohibited substances (111)	Urine	Deconjugation, SPE	LC	Q-Orbitrap, ESI+, Full Scan and vDIA[n]	Partly	Han et al. (2019)
Veterinary drugs (63), pesticides (13)	Egg	QuEChERS	LC	Q-TOF, ESI+	Partly	Hou et al. (2020)
Antibiotics (36)	Milk, fresh cheese, whey	QuEChERS, SPE	LC	Q-Orbitrap, ESI+/−, Full Scan	CD 2022/657/EC	Igualada et al. (2022)
Veterinary drugs, mycotoxins, pesticides (382)	Infant formula	(SA)LLE, QuEChERS	LC	Q-Orbitrap, ESI+/−, Full Scan, DIA	CD 2002/657/EC (45 substances)	Izzo et al. (2022)
Veterinary drugs (137)	Tilapia	(SA)LLE, pipette tip SPE	LC	Q-Orbitrap, ESI+/−, Full Scan, vDIA	CD 2002/657/EC, SANCO	Jia et al. (2017)
Veterinary drugs (114)	Feed, feather meal	LLE	LC	Q-Orbitrap, ESI+, comparing vDIA with AIF/ddMS2[o]	–	Jansen et al. (2022)
Corticosteroids (44)	Plasma	LLE	LC	Q-Orbitrap, ESI+, Full Scan, DIA	Partly	Karakka Kal et al. (2021)
Steroids, stilbenes, resorcylic acid lactones (42)	Urine, muscle, liver, serum, blood	Thermal denaturation, deconjugation, LLE, defatting	LC	Q-Orbitrap and Q-TOF, ESI+/−, Full Scan, MS/MS	CD 2002/657/EC, with minor deviations	Kaufmann et al. (2019)
β-agonists (20)	Muscle, liver, plasma, milk, urine	Deconjugation, LLE	LC	Q-Orbitrap and Q-TOF, ESI+, Full Scan, MS/MS, AIF, PRM (depending on analyte)	CD 2002/657/EC	Kaufmann et al. (2021)
Antibiotics (18)	Water	Online SPE	LC	Q-Orbitrap, ESI+, Full Scan	Partly	Kim et al. (2018)
Pharmaceuticals (>200)	Carp, shrimp, crab, eel, mussel	LLE	LC	Q-Orbitrap, Full Scan, ddMS2	Partly	Kong et al. (2018)
Veterinary drugs (141)	Pork meat	LE, SPE	LC	Q-TOF, ESI+/−, Full Scan, MS/MS	CD 2002/657/EC, SANTE/11813/2017, GB/T 27404	Li et al. (2020)
Antibiotics (66)	Meat, milk, eggs	HTpSPE[p]	LC	Q-Orbitrap, ESI+, Full Scan, vDIA	Partly, CD 2022/657/EC	Mehl et al. (2021)
Veterinary drugs (81)	Meat, milk, eggs, honey	HTpSPE	LC	Q-Orbitrap, ESI+, Full Scan, vDIA	(EU) 2021/808	Mehl et al. (2022)
Veterinary drugs (173), mycotoxins (9), pesticides (122)	Meat, wheat flower	LE	LC	Q-Orbitrap, ESI+/−, Full Scan, ddMS2	CD 2002/657/EC	Moretti et al. (2020)
Anticoccidials (17)	Poultry, egg	LE	LC	Q-Orbitrap, ESI+/−, Full Scan, ddMS2	CD 2002/657/EC	Rusko et al. (2019)
Veterinary drugs (155)	Milk	Protein precipitation, SPE	LC	Q-Orbitrap, ESI+, Full Scan, ddMS2	–	Tan et al. (2022)
Veterinary drugs (>300)	Fish	LE, SPE	LC	Q-Orbitrap, ESI+/−, Full Scan, AIF, ddMS2	FDA	Turnipseed et al. (2017)
Antibiotics (91)	Meat, fish	LE, QuEChERS	LC	Q-TOF, ESI+/−, DIA (All ions MS/MS)	CD 2002/657/EC	Varenina et al. (2022)
Antibiotics, steroids (156)	Feces	(SA)LLE, QuEChERS	LC	Q-TOF, Full Scan	AOAC	(K. Wang, Li, et al., 2020)

(*continued on next page*)

Table 2 (*continued*)

Compounds (number of analytes[a])	Matrix	Sample preprocessing method	Chromatographic separation	Mass Spectrometry	Validation	Reference
Veterinary drugs (52)	Fish blood microsample	micro-LLE	DART	Q-Orbitrap, Full Scan, ddMS2	Partly	Wang et al. (2021)
Veterinary drugs (124)	Urine	SLE[q]	LC	Q-Orbitrap, ESI+/−, Full Scan, DIA	Partly	Wong et al. (2020)
Glucocorticoids (39)	Meat, milk, egg	(SA)LLE, online SPE	LC	Q-Orbitrap, ESI+, Full Scan, ddMS2	Partly	Yan et al. (2021)
Veterinary drugs, pesticides (49)	Infant formula	LE, (d)SPE	LC	Q-Orbitrap, ESI+, PRM	Partly	Zhang et al. (2020)
Confirmation & follow-up						
Pharmaceuticals/personal care products (6)	Fresh water invertebrates	QuEChERS	LC	Q-Orbitrap, ESI+/−, PRM	–	Althakafy et al. (2018)
Steroids, stilbenes, resorcylic acid lactones (42)	Urine, muscle, liver, serum, blood	Thermal denaturation, deconjugation, LLE, defatting	LC	Q-Orbitrap and Q-TOF, ESI+/−, Full Scan, MS/MS	CD 2002/657/EC, with minor deviations	Kaufmann et al. (2019)
Veterinary drugs (112)	Liver	LE, SPE	LC, IMS	Q-TOF, ESI+	–	Kaufmann, Butcher, Maden, Walker, and Widmer (2020)
Veterinary drugs and growth promoters (14)	Liver	Thermal denaturation, deconjugation, LLE, defatting or LE, SPE	LC	Q-TOF, ESI+, comparing AIF, PRM, SWATH[r]	–	Kaufmann, Maden, and Walker (2020)
Antibiotics (18)	Water	Online SPE	LC	Q-Orbitrap, ESI+, Full Scan, MS/MS	Partly	Kim et al. (2018)
Anticoccidials (17)	Poultry, egg	LE	LC	Q-Orbitrap, ESI+/−, Full Scan, ddMS2	CD 2002/657/EC	Rusko et al. (2019)
Antibiotics (91)	Meat, fish	LE, QuEChERS	LC	Q-TOF, ESI+/−, DIA (All ions MS/MS)	CD 2002/657/EC	Varenina et al. (2022)
Common patterns						
Sulfonamides	Salmon	(SA)LLE, pipette tip SPE	LC	Q-Orbitrap, ESI+, Full Scan, vDIA	CD 2002/657/EC (27 confirmed analytes)	Jia et al. (2018)
Sulfonamides	Goat milk	LLE, Magnetic SPE	LC	Q-Orbitrap, ESI+/−, Full Scan, vDIA	CD 2002/657/EC (35 confirmed analytes)	Jia et al. (2021)
Sulfonamides	Meat, plasma, liver, urine	QuEChERS	LC	Q-Orbitrap, different scan modes compared	–	Jia et al. (2022)
Sulfonamides	Dietary supplements	LLE	LC	Q-TOF, ESI-, Full Scan, MS/MS	IHC (35 confirmed analytes)	Ki et al. (2019)
Steroids (12 newly identified)	Urine	SPE, deconjugation, derivatization	GCxGC, learning algorithm	TOF	63 steroids	Randazzo et al. (2020)
Metabolism, degradation, transformation studies						
Eprinomectin shelf life degradation	–	–	LC	Q-TOF, ESI+, Full Scan, MS/MS	–	Adhikari and Rustum (2022)
Sulfonamides	Meat, plasma, liver, urine	QuEChERS	LC	Q-Orbitrap, different scan modes compared	–	Jia et al. (2022)
Salinomycin electrochemical and liver microsome transformation	–	–	LC	Q-TOF, ESI+, Full Scan, MS2	–	Knoche et al. (2022)
Moxidectin electro- and photochemical transformation	–	–	LC	Q-TOF, ESI+, Full Scan, IDA[s]	–	Kotthoff et al. (2020)
Altrenogest metabolism	Urine	Hydrolysis (with/without), SPE	LC	Q-Orbitrap, ESI+/−, Full Scan, ddMS2	Partly	Liesenfeld et al. (2022)
7-keto-DHEA metabolism	Urine	Deconjugation, LLE, derivatization	GC	Q-TOF, EI	–	Martinez-Brito et al. (2019)
Gamithromycin metabolism	Sheep edible tissues	LE, defatting, SPE	LC	Q-Orbitrap, ESI+, Full Scan, ddMS2	–	Tong et al. (2022)
Physiological marker profiles						
–	Muscle, kidney, non-complient for veterinary drugs	LE, SPE	LC	Q-Orbitrap, Q-TOF, ESI+, Full Scan, ddMS2	–	Liesenfeld et al. (2020)
Altrenogest treatment	Urine	Deconjugation (with/without), SPE	LC	Q-Orbitrap, ESI+/−, Full Scan, ddMS2	Partly	Liesenfeld et al. (2022)

(*continued on next page*)

Table 2 (*continued*)

Compounds (number of analytes[a])	Matrix	Sample preprocessing method	Chromatographic separation	Mass Spectrometry	Validation	Reference
Deviating sample						
–	Serum	LLE	LC	Q-TOF, ESI-, Full Scan, dd mode, targeted mode	–	Schiffman et al. (2019)
Unknowns analysis						
(2 unexpected identified)	Feed	LE	LC fractionation, LC	Q-Orbitrap	–	Wegh et al. (2017)
(1 unexpected identified)	Tilapia	(SA)LLE, pipette tip SPE	LC	Q-Orbitrap, ESI+/−, Full Scan, vDIA	CD 2002/657/EC, SANCO (137 veterinary drugs)	Jia et al. (2017)
Pharmaceutically active substances (1068 suspect screened)	Pak choi	LE, SPE	LC	Q-TOF, ESI+/−, DIA (All ions MS/MS)	–	Chen et al. (2021)
Veterinary drugs (3 unexpected identified)	Feed, feather meal	LLE	LC	Q-Orbitrap, ESI+, Full Scan, AIF/ddMS2	False positive/false negative rate veterinary drugs evaluated (114)	Jansen et al. (2022)
Veterinary drugs	Milk	Protein precipitation, SPE	LC	Q-Orbitrap, ESI+, Full Scan, ddMS2, DIA	Test identification of 180 veterinary drugs	Sun et al. (2021)
Veterinary drugs	Eel	LE, SPE	LC	Q-Orbitrap, ESI+, Full Scan, AIF/DIA	Test identification of 68 veterinary drugs	Wu et al. (2020)
Veterinary drugs	Pork meat	LE, SPE	LC	Q-Orbitrap, ESI+, Full Scan, BE-DDA[t]	Test identification of 48 veterinary drugs	Zhu et al. (2022)

[a] Where applicable, number of analytes is given between brackets.
[b] Time of flight.
[c] Electron ionization.
[d] (Salting-out supported) liquid extraction.
[e] Quick, easy, cheap, effective, rugged, and safe.
[f] Electron spray ionization.
[g] All ion fragmentation.
[h] (Dispersive) solid-phase extraction.
[i] Matrix assisted inlet ionization.
[j] Desorption atmospheric pressure chemical ionization.
[k] Transmission-mode direct analysis in real time.
[l] Coated blade spray.
[m] Parallel reaction monitoring.
[n] Variable data-independent acquisition.
[o] Data-dependent MS2.
[p] High-throughput planar solid phase extraction.
[q] Solid-supported liquid extraction.
[r] Sequential window acquisition of all theoretical mass spectra.
[s] Information dependent acquisition.
[t] Background exclusion data-dependent acquisition.

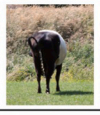
Scenario for control laboratories	Strategy of HRMS application						
	(A) Screening	(B) Confirmation and follow-up	(C) Common patterns	(D) Metabolites identification	(E) Physiological marker profiles	(F) Deviating sample	(G) Unknowns analysis
							
(1) Reliable enforcement of misuse	✓	✓	–	–	–	–	–
(2) Easy expansion of methods with new compounds	✓	–	✓	✓	–	–	–
(3) Fill-in risk-based analysis by means of a broad survey, including finding unexpected or neglected risks	✓	–	–	–	✓	✓	✓
(4) Explaining an observed worrying activity, effect or signal that could not yet be explained by the confirmatory methods in place	–	–	–	–	–	✓	✓
(5) Retrospective analysis, a.o. supporting research to the origin of residues and trend analysis for evaluating interventions	✓	✓	✓	✓	✓	✓	✓
Implementing 808/2021 Identification points; flexible scope validation	✓	✓	–	–	–	–	–

Fig. 3. Applications of HRMS technique for veterinary drug residue analysis in food. A) Screening, B) Confirmation and follow-up, C) Common patterns, D) Metabolites identification, E) Physiological marker profiles, F) Deviating sample, G) Unknown analysis. (Photos by Rob Kregting).

which distinguishes screening, confirmation and follow-up, common patterns, metabolites identification, physiological marker profiles, deviating sample and unknowns analysis. The literature research methods (used databases, search terms, and filters) are described in Supplemental Information A.

Based on the different scenarios needed by relevant control laboratories, one or more of these described strategies for using HRMS might be applied. Following the information from the survey, the legislative requirements and the available literature that has been presented in Table 2, Fig. 3 shows how those necessities and strategies are interrelated.

As listed in Table 2, the applied HRMS strategies are categorized, ranging from completely targeted to full untargeted. Each scenario listed in Table 2 is discussed in the following paragraphs: targeted screening (4.1) followed by confirmation and follow-up of samples that were screened in other ways (4.2) and semi-untargeted application by searching common patterns of compounds (e.g. fragmentation patterns or isotopic patterns) (4.3). Then, the identification of new metabolites (4.4), untargeted physiological marker profiling (4.5), the identification of a deviating sample (4.6), and unknowns analysis, that describes both suspect screening and so-called 'truly unknown' workflows (4.7). All described strategies can be applied straight-away directly after analysis, or, if the method was designed appropriately, also in retrospective, which is discussed in section 4.8.

4.1. Screening

The most common way of HRMS use is multi-residue or as a multi-class, targeted screening method (Table 2, Fig. 3A). The current trend is to measure more and more analytes per run, resulting in so-called mega methods (Mol et al., 2008; Monteiro, Lehotay, Sapozhnikova, Ninga, & Lightfield, 2021). Screening methods with a large scope of analytes using HRMS have been published for several food and animal matrices for veterinary drugs (Alcantara-Duran, Moreno-Gonzalez, Gilbert-Lopez, Molina-Diaz, & Garcia-Reyes, 2018; Althakafy, Kulsing, Grace, & Marriott, 2018; Bandini & Spisso, 2021; Berendsen, Meijer, Mol, van Ginkel, & Nielen, 2017; Chitescu, Kaklamanos, Nicolau, & Stolker, 2015; Di, Yu, Chen, Zhu, & Zhu, 2020; Igualada, Giraldo, Font, & Yusà, 2022; Jansen et al., 2022; Jia et al., 2017; Kaufmann, Butcher, Maden, Walker, & Widmer, 2015a; Kim, Ryu, Chung, & Kim, 2018; Kong, Wang, Huang, & Yu, 2018; Li et al., 2020; Mehl, Schmidt, Schmidt, & Morlock, 2021; Mehl, Hudel, Bücker, & Morlock, 2022; Romero-Gonzalez, Aguilera-Luiz, Plaza-Bolanos, Frenich, & Vidal, 2011; Rusko, Jansons, Pugajeva, Zacs, & Bartkevics, 2019; Solliec, Roy-Lachapelle, & Sauve, 2015; Tan et al., 2022; Turnipseed et al., 2017; Turnipseed et al., 2018; Varenina, Bilandžić, Luburić, Kolanović, & Varga, 2022; Wang et al., 2021; C. Wang, Li, et al., 2020; Wong et al., 2020; Zhang et al., 2016), hormones and growth promoters (Abusharbeeda et al., 2018; Bileck, Verouti, Escher, Vogt, & Groessl, 2018; Decheng et al., 2021; Han, Min, Jeon, Kang, & Son, 2019; Karakka Kal et al., 2021; Kaufmann et al., 2019, 2021; Yan et al., 2021), the combination thereof (Blokland et al., 2018; K. Wang, Wang, et al., 2020) and also in combination with other residue analysis fields, such as pesticides (Bai et al., 2022; Cotton et al., 2016; Gomez-Perez et al., 2014; Hou, Xu, Xu, Han, & Qiu, 2020; Jia, Chu, Ling, Huang, & Chang, 2014; Zhang et al., 2020), mycotoxins (Kellmann, Muenster, Zomer, & Mol, 2009; Moretti et al., 2020), up to three or more multi-class combinations (Blokland, Gerssen, Zoontjes, Pawliszyn, & Nielen, 2019; De Paepe et al., 2019; Izzo et al., 2022; Moretti et al., 2020). For this kind of multi-class screening methods, simple generic sample pre-treatment is applied (Mol et al., 2008), aiming for an as broad as possible screening scope that can analyze hundreds of analytes at the same time. Multiclass methods and the used mass spectrometry techniques have been reviewed (Domínguez et al., 2020; Turnipseed & Jayasuriya, 2020). Possibly the broad scope

has a smaller or larger trade off in recovery and/or sensitivity. In the case of molecules that exhibit poor fragmentation, such as some avermectins (anti-worming agents) and steroids (growth promoters), HRMS screening showed enhanced precision and/or sensitivity (Kaufmann, 2020) compared to low resolution MS/MS methods. Generic screening is performed using full scan mode, which is the most distinctive and gives lowest numbers of false positives and false negatives (Kaufmann, Butcher, Maden, Walker, & Widmer, 2015b), possibly in combination with data dependent MS2 or data independent MS2 for further confirmation of identity. The advantages of performing routine screening by HRMS is that the scope of the methods can easily be expanded with new molecules, due to the possibility of applying universal pre-processing methods and the untargeted nature of the data acquisition. This scope expansion could even be performed retrospectively. Of course the method needs to be set-up in a way that facilitates these advantages as much as possible (e.g. by independent data acquisition settings, good balance between mass resolution and number of datapoints per peak, good balance between sample clean-up to lower matrix effect but still universal enough to allow detection of a broad scope, etc.).

4.2. Confirmation and follow-up

Despite the growing number of HRMS screening methods that are available in literature, only a limited number of multi-residue methods have fully been validated according to CD 2002/657/EC as a quantitative confirmation method (European Commission, 2002a; Jia et al., 2014; Kaufmann et al., 2015a, 2019; Kim et al., 2018; Romero-Gonzalez et al., 2011; Rusko et al., 2019; Varenina et al., 2022) (Table 2, Fig. 3B). This might be because of the elaborate amount of work necessary to fully validate such a method according to current criteria (see section 3. Fitting HRMS in legislation). These validated confirmatory methods make use of, mostly data-dependent, MS/MS scan, in order to have enough identification points and sensitivity (Table 2). Generally, this is done in addition to, or alternating with, a full scan acquisition. Other options are to make use of PRM (Althakafy et al., 2018) or SWATH (Kaufmann, Maden, & Walker, 2020) modes, or combine LC separation with ion mobility (Kaufmann, Butcher, Maden, Walker, & Widmer, 2020) to increase sensitivity or separation power respectively. Next to screening and confirmation in a targeted multi-residue, routine way, HRMS is also suitable as a way to confirm or reject findings as a follow-up from other screening methods, such as anti-microbial activity screening on plates or LC-MS/MS, in case the nature of the method limits conclusive judgment. A common case is when two analytes in LC-MS/MS, or an analyte/background signal combination, share the same retention time (RT) and nominal mass, but have a different exact mass in HRMS. An example is the growth promoter 17β-trenbolone, a compound that has difficulties in confirmatory analysis by LC-MS/MS, due to co-eluting interferences with similar, relatively non-selective, neutral losses (Berendsen, Stolker, & Nielen, 2013). However, an alternative strategy was proposed for trenbolone using accurate mass measurement with LC-TOF, where it could be readily distinguished as the only match from bovine urine matrix, using a mass accuracy better than 3 ppm of the quasi-molecular ion and a fragment (Berendsen et al., 2013; Blokland et al., 2008).

4.3. Common patterns

As the data acquisition in HRMS is generally untargeted, e.g. by full scan acquisition mode alternated with data independent MS2, the results could be mined in an intelligently designed 'semi-untargeted' way by common pattern analysis at isotope patterns or fragmentation patterns that are related with known illegal contaminants (Table 2, Fig. 3C). Sulfonamides, which are antibiotics that may be administered in livestock are the most well-known example of this type of analysis. Sulfonamides are compounds that share very similar fragmentation patterns in MS2 within their compound class (Xia et al., 2013). Therefore, new sulfonamide structures could be tracked down and putatively identified by searching HRMS data using the expected m/z patterns (Borras, Kaufmann, & Companyo, 2013; Jia et al., 2021, 2022; Ki et al., 2019; Majewsky, Glauner, & Horn, 2015). With this strategy, suspect samples have been identified in real samples of salmon (Jia, Shi, & Chu, 2018). Besides sulfonamides, Borras et al. describe an application of common patterns for the molecular group of penicillins (Borras et al., 2013). Besides, steroids share similar structures and fragmentation patterns. Randazzo et al. newly identified 12 steroids based on HRMS data, by making use of a learning algorithm exploiting data of known steroids that were acquired on GCxGC-TOF (Randazzo, Bileck, Danani, Vogt, & Groessl, 2020). The idea of common patterns could be exploited as well using isotopic patterns. E.g. chlorine is an element that has a characteristic isotope pattern and might occur mainly in ectopically administered compounds and not in animal matrices.

4.4. Metabolites identification

The idea of linking masses to each other through data processing after untargeted data acquisition can be applied as well for metabolism and degradation studies (Table 2, Fig. 3D). Finding metabolites of administered compounds is important for health risk assessment and the design of proper abuse detection methods. Plenty of examples of such studies are available from human drugs studies. For example, by using HRMS, metabolites (e.g. dehydration, methylation, acetylation, reduction, conjugation) were identified after treatments with human medicinal prescriptions in mice and rats (Jiang et al., 2020; Karkoula et al., 2020). Additionally, resolved isotope patterns of certain ectopic elements (e.g. chlorine, bromine) can track down metabolites in an untargeted way (Sanchez-Ponce & Guengerich, 2007). In the field of food safety research, the strategy is relatively new, and analytical methods for monitoring are still mainly focused on the administered form of molecules, so metabolites are still under-attended. Nevertheless, HRMS has been exploited for metabolite studies, of for example, sulfonamide (Jia et al., 2022; Pfeifer, Tuerk, & Fuchs, 2005), altrenogest (Liesenfeld, Steliopoulos, Wenig, Gottstein, & Hamscher, 2022), 7-keto-DHEA (Martinez-Brito, de la Torre, Colamonici, Curcio, & Botre, 2019) and gamithromycin (Tong et al., 2022) administration, measuring urine, manure and/or eadible tissues. Also, shelf life and physiochemical transformation products have been studied, e.g. of the anti-worming agents eprinomycin (Adhikari & Rustum, 2022), moxidectin (Kotthoff, O'Callaghan, Lisec, Schwerdtle, & Koch, 2020) and the antibiotic/coccidiostat salinomycin (Knoche, Lisec, Schwerdtle, & Koch, 2022). By finding all relevant metabolites related to a certain treatment, HRMS could improve risk based food monitoring strategies.

4.5. Physiological marker profiles

HRMS data consisting of mass spectra and RT of food matrices can also be used for an untargeted physiological profiling analysis (Table 2, Fig. 3E). This is nowadays a quite common practice in the area of food fraud, for example to determine adulteration of olive oil or juices (Cavanna et al., 2018; Esteki, Regueiro, & Simal-Gándara, 2019). In this approach, groups are made of 'conform' and 'non-conform' sample data, which allows statistical determination of distinguishing markers by for example Principal Component Analysis (PCA) (Fu, Zhao, Lu, & Xu, 2017). The approach has been shown to be useful in the area of veterinary treatment and food safety, when residues are also naturally occurring (e.g. steroids) (Dervilly-Pinel et al., 2012). A targeted way of profiling has been done previously by steroid profiling using LC-MS/MS analysis of known hormones (Blokland, van Tricht, van Ginkel, & Sterk, 2017; Narduzzi et al., 2021; Thevis, Kuuranne, Geyer, & Schanzer, 2017). The advantage of profiling by HRMS might be that the distinguishing markers or marker combinations do not have to be previously known, or not even have to be identified, to be able to classify samples to a group reliably. This becomes clear for profiling of

treatments with growth promoters. Dervilly et al. could discriminate between β-agonist treated and untreated cows by directly analyzing urine samples without sample cleanup, using an untargeted metabolomics approach (Dervilly-Pinel, Chereau, Cesbron, Monteau, & Le Bizec, 2014). Such treatment could not be directly tracked down by analyzing β-agonists themselves, as these concentrations would be below the detection limit. Similarly, discrimination could be made for altrenogest, clenbuterol, DHEA, prednisolone and pregnenolone treatment by HRMS profiles and metabolomics approaches (Courant et al., 2009; De Clercq et al., 2015; Liesenfeld et al., 2022; Rijk et al., 2009). The strategy of profiling can also be useful for pointing out treated *vs*. untreated samples, independent of the specific veterinary drug that has been applied (Liesenfeld, Steliopoulos, & Hamscher, 2020). It has to be stated that for a statistically sound profiling analysis, sufficient amounts of samples need to be available for all 'profiles' to be investigated (e.g. treated *vs.* untreated, pure *vs.* adulterated, etc) and only that specific same type of treatment or fraud can be identified statistically (Cavanna et al., 2018).

4.6. Deviating sample

HRMS profiling could also give rise to deviating samples from the 'normal' *m/z* and RT profiles, untargeted, without a prior dataset available from a certain type of abuse (Table 2, Fig. 3F). These samples could still be identified, provided that a large enough group of proper blank samples are available as a control group. They could be identified from HRMS data differential analysis, or on any property in the sample taking, processing and analyzing work, such as physical appearance of the animal, color of the extract, cleanness of the chromatogram, etc (Schiffman et al., 2019). Documenting spectral relative standard deviations (RSDs) could assist as a practical benchmark (Parsons, Ekman, Collette, & Viant, 2009). To the best of our knowledge, such a 'metabolomics-like' quality check approach has not yet been applied for food safety analysis. The samples containing such a 'deviating' profile could be of interest to track down possible distinctive markers, putative identification of their HRMS mass spectra and software tools, and further analysis with other principles, like anti-microbial activity fractionation, NMR, or for unknown analysis (see below).

4.7. Unknowns analysis

HRMS is an emerging tool as a basis for the so-called 'unknowns analysis' (Table 2, Fig. 3G). The full dataset of untargeted full scan acquisition mass spectra, and maybe in combination with MS2 data, can be mined for unknowns. By matching the MS-spectra of broad on-line databases, such as PubChem, and, when working with electron ionization, NIST, unknown peaks can be tentatively identified. Systematic workflows e.g. (Fu et al., 2017), acquisition modes e.g. (Fenaille, Barbier Saint-Hilaire, Rousseau, & Junot, 2017) and data processing tools e.g. (Kind et al., 2018) for untargeted analysis have been reviewed. The workflows usually consist of data pretreatment such as peak picking, deconvolution, alignment, data reduction and filtering of masses based on differential analysis and/or chemical principles. Afterwards, hits will be putatively identified based on database search (Kind et al., 2018), possibly assisted by in-silico simulation of retention time and fragmentation (Hu et al., 2018; Kaufmann, Butcher, Maden, Walker, & Widmer, 2017), or truly identified by further analysis. Unexpected knowns could be confirmed by means of an analytical standard, or completely unknown masses could be further identified by NMR analysis. Some examples of successful identifications of unexpected veterinary drugs residues present in food matrices have been published using untargeted approaches. Jia et al. found an unexpected residue (robenidine) in tilapia. Jansen et al. found three unexpected residues in feed an feather meals (gatifloxacin, levofloxacin and azithromycin). Solliec et al. were able to identify one hormone (medroxyprogesterone), one analgesic (acetaminophen), seven antibiotics and some of their isomeric metabolites in swine manure samples using an untargeted workflow (Solliec et al., 2015). Fu et al. found an unexpected residue in untargeted acquired fish data, and could identify it as difloxacin (Fu et al., 2016). A good practice is, before application on real samples, to validate unknown workflows and accessory LC and MS settings on a test set of model veterinary drugs, evaluating the identification of structures and false positive/negative findings (Table 2) (Jansen et al., 2022; Jia et al., 2017; Sun et al., 2021; Wu, Turnipseed, Storey, Andersen, & Madson, 2020; Zhu et al., 2022). Such so-called 'suspect screening' workflows can deal with a large number of compounds at the same time without elaborate one-by-one validation procedures, as hits are based on library-matching. Chen et al. evaluated 1068 pharmaceutically active substances in the vegetable pak choi by suspect screening using library matching (Chen, Lin, Huang, Peng, & Ling, 2021). In the upcoming field of non-targeted and suspect screening approaches, the harmonization, reproducibility, and quality control of used methods are under development (Pourchet et al., 2020). Standardization and documentation of methods have also been reported (Knolhof, Premo, & Fisher, 2021; Peter et al., 2021; Pourchet et al., 2020). Proposals for risk assessment based on unknown analysis, with an essential role for HRMS, have been published (Gerssen et al., 2019; Wegh et al., 2017). Important for estimating relevance of hits is the coupling between HRMS spectra and other properties of the molecules, such as anti-microbial or hormonal activity, by e.g. screening and fractionation approaches. Wegh et al. used such workflow consisting of untargeted HRMS in combination with fractionation and anti-microbial activity test, and were able to identify the new anti-microbial didecyldimethylammonium chloride in animal feed, as well as finding back spiked components (roxithromycin and cryptotanshinone) in two similarly designed test cases (Wegh et al., 2017). The high-resolution *m/z* data play an essential role in putative identification after separation in order to prioritize hits and decide on the subsequent steps.

4.8. Retrospective analysis

All strategies described here to apply HRMS for risk-based food monitoring (Table 2, Fig. 3), contribute in their specific way to improve food safety. Due to the untargeted nature of the HRMS data acquisition, the strategies as shown in Fig. 3 could be applied retrospectively on data sets that were acquired for another purpose. Such was done by Li et al., who could include in their 'traditional' screening of veterinary drugs in pork meat, immediately a metabolite study of a positive finding (a sulfonamide), due to the untargeted nature of the data acquisition on LC-Q-TOF (Li et al., 2020). Good practice would be to have in mind application for multiple purposes when developing HRMS methods, and design sample preprocessing (e.g. generic simple cleanup) and instrument settings (e.g. including full scan and possibly data-independent MS2 in the acquisition) in a way that the data will be suitable for retrospective analysis (Jansen et al., 2022). In practice, this could typically mean that when applying screening by HRMS in a routine way for national monitoring programs, the so built-up datasets will be analyzed annually retrospectively by one or more of the other strategies (profiling, metabolism studies, unknown analysis) and trend analysis will be performed, to set actual risk-based monitoring strategies for the coming year(s). For this purpose HRMS as a versatile technique is an essential link to progress in real risk-based analysis.

5. Conclusions

In the field of veterinary drug and hormone residue analysis the use of HRMS technique has been increased significantly in the last few years, mainly for multi-residue screening purposes. Multi-residue screening of veterinary drugs and hormones by HRMS is the way to go to increase the number of analytes per run with satisfactory distinguishing power. Besides screening and confirmation of currently legislated compounds, HRMS can be deployed for (semi)-untargeted food residue analysis,

which is mainly of interest to identify unexpected or unknown molecules or metabolites. HRMS can be applied retrospectively for risk-based monitoring and a combination of analysis strategies. Recent advances in scan speed, data-processing software and dynamic range enable intelligent data acquisition and quantitative analysis. However, HRMS still needs to become more sensitive to detect residues at the lowest relevant concentrations, mainly for forbidden compounds and complex matrices. Future legislation could consider to include suggested updates, thus facilitating the use of the broad potential of strategies for HRMS use. Currently 58% of the European official control laboratories in the residue field are using HRMS. Next to exploiting instrument advances, in order to have this technique become the dominant one in residue analysis, guidance on workflows and validation are needed. When these improvements are made and implemented, HRMS will be the technique of choice in the near future. The authors believe that HRMS is an essential link to progress in real risk-based monitoring programs.

Funding

This project was financially supported by the EC DG SANTE and the Dutch Ministry of Agriculture, Nature and Food Quality.

CRediT authorship contribution statement

Esmer Jongedijk: Conceptualization, Methodology, Visualization, Writing – original draft, Writing – review & editing. **Markus Fifeik:** Methodology, Visualization, Writing – original draft. **Ane Arrizabalaga-Larrañaga:** Visualization, Writing – original draft, Writing – review & editing. **Joachim Polzer:** Writing – original draft, Writing – review & editing. **Marco Blokland:** Conceptualization, Visualization, Writing – review & editing. **Saskia Sterk:** Writing – review & editing, Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this paper.

Data availability

Data will be made available on request.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodcont.2022.109488.

References

Abushareeda, W., Tienstra, M., Lommen, A., Blokland, M., Sterk, S., Kraiem, S., et al. (2018). Comparison of gas chromatography/quadrupole time-of-flight and quadrupole orbitrap mass spectrometry in anti-doping analysis: I. Detection of anabolic-androgenic steroids. *Rapid Communications in Mass Spectrometry, 32*(23), 2055–2064. https://doi.org/10.1002/rcm.8281

Adhikari, S., & Rustum, A. M. (2022). Identification and characterization of major degradation products of Eprinomectin drug substance including degradation pathways using LC-HRMS and NMR. *Journal of Pharmacy Biomedicine Analytical, 215*, Article 114767. https://doi.org/10.1016/j.jpba.2022.114767

Alcantara-Duran, J., Moreno-Gonzalez, D., Gilbert-Lopez, B., Molina-Diaz, A., & Garcia-Reyes, J. F. (2018). Matrix-effect free multi-residue analysis of veterinary drugs in food samples of animal origin by nanoflow liquid chromatography high resolution mass spectrometry. *Food Chemistry, 245*, 29–38. https://doi.org/10.1016/j.foodchem.2017.10.083

Althakafy, J. T., Kulsing, C., Grace, M. R., & Marriott, P. J. (2018). Determination of selected emerging contaminants in freshwater invertebrates using a universal extraction technique and liquid chromatography accurate mass spectrometry. *Journal of Separation Science, 41*(19), 3706–3715. https://doi.org/10.1002/jssc.201800507

Bai, M., Tang, R., Li, G., She, W., Chen, G., Shen, H., et al. (2022). High-throughput screening of 756 chemical contaminants in aquaculture products using liquid chromatography/quadrupole time-of-flight mass spectrometry. *Food Chemistry, X*, 15. https://doi.org/10.1016/j.fochx.2022.100380

Bandini, T. B., & Spisso, B. F. (2021). Development and validation of an LC-HRMS method for the determination of pyrrolizidine alkaloids and quinolones in honey employing a simple alkaline sample dilution. *Journal of Food Measurement and Characterization, 15*(5), 4758–4770. https://doi.org/10.1007/s11694-021-01048-9

Berendsen, B. J. A., Meijer, T., Mol, H. G. J., van Ginkel, L., & Nielen, M. W. F. (2017). A global inter-laboratory study to assess acquisition modes for multi-compound confirmatory analysis of veterinary drugs using liquid chromatography coupled to triple quadrupole, time of flight and orbitrap mass spectrometry. *Analytica Chimica Acta, 962*, 60–72. https://doi.org/10.1016/j.aca.2017.01.046

Berendsen, B. J., Stolker, L. A., & Nielen, M. W. (2013). The (un)certainty of selectivity in liquid chromatography tandem mass spectrometry. *Journal of the American Society for Mass Spectrometry, 24*(1), 154–163. https://doi.org/10.1007/s13361-012-0501-0

Bileck, A., Verouti, S. N., Escher, G., Vogt, B., & Groessl, M. (2018). A comprehensive urinary steroid analysis strategy using two-dimensional gas chromatography - time of flight mass spectrometry. *Analyst, 143*(18), 4484–4494. https://doi.org/10.1039/c7an01990d

Blokland, M. H., Gerssen, A., Zoontjes, P. W., Pawliszyn, J., & Nielen, M. W. F. (2019). Potential of recent ambient ionization techniques for future food contaminant analysis using (Trans)Portable mass spectrometry. *Food Analytical Methods, 13*(3), 706–717. https://doi.org/10.1007/s12161-019-01666-6

Blokland, M. H., van Tricht, E. F., van Ginkel, L. A., & Sterk, S. S. (2017). Applicability of an innovative steroid-profiling method to determine synthetic growth promoter abuse in cattle. *The Journal of Steroid Biochemistry and Molecular Biology, 174*, 265–275. https://doi.org/10.1016/j.jsbmb.2017.10.007

Blokland, M. H., Zoontjes, P. W., Sterk, S. S., Stephany, R. W., Zweigenbaum, J., & van Ginkel, L. A. (2008). Confirmatory analysis of Trenbolone using accurate mass measurement with LC/TOF-MS. *Analytica Chimica Acta, 618*(1), 86–93. https://doi.org/10.1016/j.aca.2008.04.040

Blokland, M. H., Zoontjes, P. W., Van Ginkel, L. A., Van De Schans, M. G. M., Sterk, S. S., & Bovee, T. F. H. (2018). Multiclass screening in urine by comprehensive two-dimensional liquid chromatography time of flight mass spectrometry for residues of sulphonamides, beta-agonists and steroids. *Food Additives & Contaminants Part A Chem Anal Control Expo Risk Assess, 35*(9), 1703–1715. https://doi.org/10.1080/19440049.2018.1506160

Borras, S., Kaufmann, A., & Companyo, R. (2013). Correlation of precursor and product ions in single-stage high resolution mass spectrometry. A tool for detecting diagnostic ions and improving the precursor elemental composition elucidation. *Analytica Chimica Acta, 772*, 47–58. https://doi.org/10.1016/j.aca.2013.02.012

Capriotti, A. L., Cannazza, G., Catani, M., Cavaliere, C., Cavazzini, A., Cerrato, A., et al. (2021). Recent applications of mass spectrometry for the characterization of cannabis and hemp phytocannabinoids: From targeted to untargeted analysis. *Journal of Chromatography A, 1655*, Article 462492. https://doi.org/10.1016/j.chroma.2021.462492

Cavanna, D., Righetti, L., Elliott, C., & Suman, M. (2018). The scientific challenges in moving from targeted to non-targeted mass spectrometric methods for food fraud analysis: A proposed validation workflow to bring about a harmonized approach. *Trends in Food Science & Technology, 80*, 223–241. https://doi.org/10.1016/j.tifs.2018.08.007

Chen, W. L., Lin, S. C., Huang, C. H., Peng, S. Y., & Ling, Y. S. (2021). Wide-scope screening for pharmaceutically active substances in a leafy vegetable cultivated under biogas slurry irrigation. *Science of the Total Environment, 750*, Article 141519. https://doi.org/10.1016/j.scitotenv.2020.141519

Chitescu, C. L., Kaklamanos, G., Nicolau, A. I., & Stolker, A. A. (2015). High sensitive multiresidue analysis of pharmaceuticals and antifungals in surface water using U-HPLC-Q-Exactive Orbitrap HRMS. Application to the Danube river basin on the Romanian territory. *Science of the Total Environment, 532*, 501–511. https://doi.org/10.1016/j.scitotenv.2015.06.010

Cotton, J., Leroux, F., Broudin, S., Poirel, M., Corman, B., Junot, C., et al. (2016). Development and validation of a multiresidue method for the analysis of more than 500 pesticides and drugs in water based on on-line and liquid chromatography coupled to high resolution mass spectrometry. *Water Research, 104*, 20–27. https://doi.org/10.1016/j.watres.2016.07.075

Courant, F., Pinel, G., Bichon, E., Monteau, F., Antignac, J. P., & Le Bizec, B. (2009). Development of a metabolomic approach based on liquid chromatography-high resolution mass spectrometry to screen for clenbuterol abuse in calves. *Analyst, 134*(8), 1637–1646. https://doi.org/10.1039/b901813a

De Clercq, N., Bussche, J. V., Van Meulebroek, L., Croubels, S., Delahaut, P., Buyst, D., et al. (2015). Metabolic fingerprinting reveals a novel candidate biomarker for prednisolone treatment in cattle. *Metabolomics, 12*(1). https://doi.org/10.1007/s11306-015-0887-3

De Paepe, E., Wauters, J., Van Der Borght, M., Claes, J., Huysman, S., Croubels, S., et al. (2019). Ultra-high-performance liquid chromatography coupled to quadrupole orbitrap high-resolution mass spectrometry for multi-residue screening of pesticides, (veterinary) drugs and mycotoxins in edible insects. *Food Chemistry, 293*, 187–196. https://doi.org/10.1016/j.foodchem.2019.04.082

Decheng, S., Xia, F., Zhiming, X., Shulin, W., Shi, W., & Peilong, W. (2021). Trace analysis of progesterone and 21 progestins in milk by ultra-performance liquid chromatography coupled with high-field quadrupole-orbitrap high-resolution mass spectrometry. *Food Chemistry, 361*, Article 130115. https://doi.org/10.1016/j.foodchem.2021.130115

Dervilly-Pinel, G., Chereau, S., Cesbron, N., Monteau, F., & Le Bizec, B. (2014). LC-HRMS based metabolomics screening model to detect various β-agonists treatments in bovines. *Metabolomics, 11*(2), 403–411. https://doi.org/10.1007/s11306-014-0705-3

Dervilly-Pinel, G., Courant, F., Chereau, S., Royer, A. L., Boyard-Kieken, F., Antignac, J. P., et al. (2012). Metabolomics in food analysis: Application to the control of forbidden substances. *Drug Testing and Analysis, 4*(Suppl 1), 59–69. https://doi.org/10.1002/dta.1349

Di, S., Yu, J., Chen, P., Zhu, G., & Zhu, S. (2020). Net-like mesoporous carbon nanocomposites for magnetic solid-phase extraction of sulfonamides prior to their quantitation by UPLC-HRMS. *Mikrochimica Acta, 187*(2), 112. https://doi.org/10.1007/s00604-019-4072-7

Domínguez, I., Garrido Frenich, A., & Romero-González, R. (2020). Mass spectrometry approaches to ensure food safety. *Analytical Methods, 12*(9), 1148–1162. https://doi.org/10.1039/c9ay02681a

Esteki, M., Regueiro, J., & Simal-Gándara, J. (2019). Tackling fraudsters with global strategies to expose fraud in the food chain. *Comprehensive Reviews in Food Science and Food Safety, 18*(2), 425–440. https://doi.org/10.1111/1541-4337.12419

European Commission. (1996a). *Council directive 96/22/EC*. Official Journal of the European Union.

European Commission. (1996b). *Council directive 96/23/EC*. Official Journal of the European Union.

European Commission. (2002a). *Commission decision 2002/657/EC*. Official Journal of the European Union.

European Commission. (2002b). *Regulation (EU) 178/2002*. Official Journal of the European Union.

European Commission. (2009). *Regulation (EU) 37/2010*. Official Journal of the European Union.

European Commission. (2017). *Regulation (EU) 2017/625*. Official Journal of the European Union.

European Commission. (2019). *Commission regulation 2019/1871*. Official Journal of the European Union.

European Commission. (2021). *Commission implementing regulation 2021/808*. Official Journal of the European Union.

Fenaille, F., Barbier Saint-Hilaire, P., Rousseau, K., & Junot, C. (2017). Data acquisition workflows in liquid chromatography coupled to high resolution mass spectrometry-based metabolomics: Where do we stand? *Journal of Chromatography A, 1526*, 1–12. https://doi.org/10.1016/j.chroma.2017.10.043

Fu, Y., Zhao, C., Lu, X., & Xu, G. (2017). Nontargeted screening of chemical contaminants and illegal additives in food based on liquid chromatography–high resolution mass spectrometry. *TrAC, Trends in Analytical Chemistry, 96*, 89–98. https://doi.org/10.1016/j.trac.2017.07.014

Fu, Y., Zhou, Z., Kong, H., Lu, X., Zhao, X., Chen, Y., et al. (2016). Nontargeted screening method for illegal additives based on ultrahigh-performance liquid chromatography-high-resolution mass spectrometry. *Analytical Chemistry, 88*(17), 8870–8877. https://doi.org/10.1021/acs.analchem.6b02482

Gavage, M., Delahaut, P., & Gillard, N. (2021). Suitability of high-resolution mass spectrometry for routine analysis of small molecules in food, feed and water for safety and authenticity purposes: A review. *Foods, 10*(3), 601. https://www.mdpi.com/2304-8158/10/3/601.

Geib, T., Sleno, L., Hall, R. A., Stokes, C. S., & Volmer, D. A. (2016). Triple quadrupole versus high resolution quadrupole-time-of-flight mass spectrometry for quantitative LC-MS/MS analysis of 25-hydroxyvitamin D in human serum. *Journal of the American Society for Mass Spectrometry, 27*(8), 1404–1410. https://doi.org/10.1007/s13361-016-1412-2

Gerssen, A., Bovee, T. H. F., van Ginkel, L. A., van Iersel, M. L. P. S., & Hoogenboom, R. L. A. P. (2019). Food and feed safety: Cases and approaches to identify the responsible toxins and toxicants. *Food Control, 98*, 9–18. https://doi.org/10.1016/j.foodcont.2018.10.028

Gomez-Perez, M. L., Romero-Gonzalez, R., Plaza-Bolanos, P., Genin, E., Martinez Vidal, J. L., & Garrido Frenich, A. (2014). Wide-scope analysis of pesticide and veterinary drug residues in meat matrices by high resolution MS: Detection and identification using exactive-orbitrap. *Journal of Mass Spectrometry, 49*(1), 27–36. https://doi.org/10.1002/jms.3309

Han, B., Min, H., Jeon, M., Kang, B., & Son, J. (2019). A rapid non-target screening method for determining prohibited substances in human urine using liquid chromatography/high-resolution tandem mass spectrometry. *Drug Testing and Analysis, 11*(3), 382–391. https://doi.org/10.1002/dta.2495

van der Heeft, E., Bolck, Y. J. C., Beumer, B., Nijrolder, A. W. J. M., Stolker, A. A. M., & Nielen, M. W. F. (2009). Full-scan accurate mass selectivity of ultra-performance liquid chromatography combined with time-of-flight and orbitrap mass spectrometry in hormone and veterinary drug residue analysis. *Journal of the American Society for Mass Spectrometry, 20*(3), 451–463. https://doi.org/10.1016/j.jasms.2008.11.002

Hou, X., Xu, X., Xu, X., Han, M., & Qiu, S. (2020). Application of a multiclass screening method for veterinary drugs and pesticides using HPLC-QTOF-MS in egg samples. *Food Chemistry, 309*, Article 125746. https://doi.org/10.1016/j.foodchem.2019.125746

Hu, M., Muller, E., Schymanski, E. L., Ruttkies, C., Schulze, T., Brack, W., et al. (2018). Performance of combined fragmentation and retention prediction for the identification of organic micropollutants by LC-HRMS. *Analytical and Bioanalytical Chemistry, 410*(7), 1931–1941. https://doi.org/10.1007/s00216-018-0857-5

Igualada, C., Giraldo, J., Font, G., & Yusà, V. (2022). Validation of a multi-residue UHPLC-HRMS method for antibiotics screening in milk, fresh cheese, and whey. *Journal of Food Composition and Analysis, 106*. https://doi.org/10.1016/j.jfca.2021.104265

Izzo, L., Narvaez, A., Castaldo, L., Gaspari, A., Rodriguez-Carrasco, Y., Grosso, M., et al. (2022). Multiclass and multi-residue screening of mycotoxins, pharmacologically active substances, and pesticides in infant milk formulas through ultra-high-performance liquid chromatography coupled with high-resolution mass spectrometry analysis. *Journal of Dairy Science, 105*(4), 2948–2962. https://doi.org/10.3168/jds.2021-21123

Jansen, L. J. M., Nijssen, R., Bolck, Y. J. C., Wegh, R. S., van de Schans, M. G. M., & Berendsen, B. J. A. (2022). Systematic assessment of acquisition and data-processing parameters in the suspect screening of veterinary drugs in archive matrices using LC-HRMS. *Food Additives & Contaminants Part A Chem Anal Control Expo Risk Assess, 39*(2), 272–284. https://doi.org/10.1080/19440049.2021.1999507

Jia, W., Chu, X., Chang, J., Wang, P. G., Chen, Y., & Zhang, F. (2017). High-throughput untargeted screening of veterinary drug residues and metabolites in tilapia using high resolution orbitrap mass spectrometry. *Analytica Chimica Acta, 957*, 29–39. https://doi.org/10.1016/j.aca.2016.12.038

Jia, W., Chu, X., Ling, Y., Huang, J., & Chang, J. (2014). High-throughput screening of pesticide and veterinary drug residues in baby food by liquid chromatography coupled to quadrupole Orbitrap mass spectrometry. *Journal of Chromatography A, 1347*, 122–128. https://doi.org/10.1016/j.chroma.2014.04.081

Jia, W., Du, A., Fan, Z., & Shi, L. (2021). High-coverage screening of sulfonamide metabolites in goat milk by magnetic doped S graphene combined with ultrahigh-performance liquid chromatography-high-resolution mass spectrometry. *Journal of Agricultural and Food Chemistry, 69*(16), 4755–4765. https://doi.org/10.1021/acs.jafc.1c01431

Jiang, X., Zhou, L., Zuo, L., Wang, X., Shi, Y., Du, X., et al. (2020). Pharmacokinetics and metabolism research of shenkang injection in rats based on UHPLC-MS/MS and UHPLC-Q-orbitrap HRMS. *Drug Design, Development and Therapy, 14*, 1837–1850. https://doi.org/10.2147/DDDT.S235646

Jia, W., Shi, L., & Chu, X. (2018). Untargeted screening of sulfonamides and their metabolites in salmon using liquid chromatography coupled to quadrupole Orbitrap mass spectrometry. *Food Chemistry, 239*, 427–433. https://doi.org/10.1016/j.foodchem.2017.06.143

Jia, W., Zhang, M., Zhu, J., & Shi, L. (2022). Strategies for studying in vivo biochemical formation pathways and multilevel distributions of sulfanilamide metabolites in food (2012-2022). *Food Chemistry, 388*, Article 133039. https://doi.org/10.1016/j.foodchem.2022.133039

Karakka Kal, A. K., Karatt, T. K., Nalakath, J., Subhahar, M. B., Koshy, S. A., Perwad, Z., et al. (2021). Simultaneous analysis of 44 frequently abused corticosteroid drugs using polysaccharide-based chiral column-HRMS approach. *Analytical Science Advances, 2*(9–10), 427–439. https://doi.org/10.1002/ansa.202000166

Karkoula, E., Dagla, I. V., Baira, E., Kokras, N., Dalla, C., Skaltsounis, A. L., et al. (2020). A novel UHPLC-HRMS-based metabolomics strategy enables the discovery of potential neuroactive metabolites in mice plasma, following i.p. administration of the main Crocus sativus L. bioactive component. *Journal of Pharmacy Biomedicine Analytical, 177*, Article 112878. https://doi.org/10.1016/j.jpba.2019.112878

Kaufmann, A. (2012). The current role of high-resolution mass spectrometry in food analysis. *Analytical and Bioanalytical Chemistry, 403*(5), 1233–1249. https://doi.org/10.1007/s00216-011-5629-4

Kaufmann, A. (2020). High-resolution mass spectrometry for bioanalytical applications: Is this the new gold standard? *Journal of Mass Spectrometry, 55*(9), e4533. https://doi.org/10.1002/jms.4533

Kaufmann, A., & Butcher, P. (2006). Strategies to avoid false negative findings in residue analysis using liquid chromatography coupled to time-of-flight mass spectrometry. *Rapid Communications in Mass Spectrometry, 20*(23), 3566–3572. https://doi.org/10.1002/rcm.2762

Kaufmann, A., Butcher, P., Maden, K., Walker, S., & Widmer, M. (2015a). Determination of nitrofuran and chloramphenicol residues by high resolution mass spectrometry versus tandem quadrupole mass spectrometry. *Analytica Chimica Acta, 862*, 41–52. https://doi.org/10.1016/j.aca.2014.12.036

Kaufmann, A., Butcher, P., Maden, K., Walker, S., & Widmer, M. (2015b). Reliability of veterinary drug residue confirmation: High resolution mass spectrometry versus tandem mass spectrometry. *Analytica Chimica Acta, 856*, 54–67. https://doi.org/10.1016/j.aca.2014.11.034

Kaufmann, A., Butcher, P., Maden, K., Walker, S., & Widmer, M. (2017). Using in silico fragmentation to improve routine residue screening in complex matrices. *Journal of the American Society for Mass Spectrometry, 28*(12), 2705–2715. https://doi.org/10.1007/s13361-017-1800-2

Kaufmann, A., Butcher, P., Maden, K., Walker, S., & Widmer, M. (2019). High-resolution mass spectrometry-based multi-residue method covering relevant steroids, stilbenes and resorcylic acid lactones in a variety of animal-based matrices. *Analytica Chimica Acta, 1054*, 59–73. https://doi.org/10.1016/j.aca.2018.12.012

Kaufmann, A., Butcher, P., Maden, K., Walker, S., & Widmer, M. (2020). Does the ion mobility resolving power as provided by commercially available ion mobility quadrupole time-of-flight mass spectrometry instruments permit the unambiguous identification of small molecules in complex matrices? *Analytica Chimica Acta, 1107*, 113–126. https://doi.org/10.1016/j.aca.2020.02.032

Kaufmann, A., Maden, K., & Walker, S. (2020). Partially overlapping sequential window acquisition of all theoretical mass spectra: A methodology to improve the spectra quality of veterinary drugs present at low concentrations in highly complex biological matrices. *Rapid Communications in Mass Spectrometry, 34*(7), Article e8638. https://doi.org/10.1002/rcm.8638

Kaufmann, A., Widmer, M., Maden, K., Butcher, P., & Walker, S. (2021). High resolution mass spectrometry-based detection and quantification of beta-agonists at relevant trace levels in a variety of animal-based food matrices. *Food Additives & Contaminants Part A Chem Anal Control Expo Risk Assess, 38*(8), 1350–1363. https://doi.org/10.1080/19440049.2021.1922759

Kellmann, M., Muenster, H., Zomer, P., & Mol, H. (2009). Full scan MS in comprehensive qualitative and quantitative residue analysis in food and feed matrices: How much resolving power is required? *Journal of the American Society for Mass Spectrometry, 20*(8), 1464–1476. https://doi.org/10.1016/j.jasms.2009.05.010

Ki, N. Y., Hur, J., Kim, B. H., Kim, K. H., Moon, B. J., Oh, H. B., et al. (2019). Rapid screening of sulfonamides in dietary supplements based on extracted common ion chromatogram and neutral loss scan by LC-Q/TOF-mass spectrometry. *Journal of Food and Drug Analysis, 27*(1), 164–174. https://doi.org/10.1016/j.jfda.2018.08.006

Kim, C., Ryu, H. D., Chung, E. G., & Kim, Y. (2018). Determination of 18 veterinary antibiotics in environmental water using high-performance liquid chromatography-q-orbitrap combined with on-line solid-phase extraction. *Journal of Chromatography, B: Analytical Technologies in the Biomedical and Life Sciences, 1084*, 158–165. https://doi.org/10.1016/j.jchromb.2018.03.038

Kind, T., Tsugawa, H., Cajka, T., Ma, Y., Lai, Z., Mehta, S. S., et al. (2018). Identification of small molecules using accurate mass MS/MS search. *Mass Spectrometry Reviews, 37*(4), 513–532. https://doi.org/10.1002/mas.21535

Knoche, L., Lisec, J., Schwerdtle, T., & Koch, M. (2022). LC-HRMS-Based identification of transformation products of the drug salinomycin generated by electrochemistry and liver microsome. *Antibiotics, 11*(2). https://doi.org/10.3390/antibiotics11020155

Knolhof, A. M., Premo, J. H., & Fisher, C. M. (2021). A proposed quality control standard mixture and its uses for evaluating nontargeted and suspect screening LC/HR-MS method performance. *Analytical Chemistry, 93*, 1596–1603. https://doi.org/10.1021/acs.analchem.0c04036

Kong, C., Wang, Y., Huang, Y., & Yu, H. (2018). Multiclass screening of >200 pharmaceutical and other residues in aquatic foods by ultrahigh-performance liquid chromatography-quadrupole-Orbitrap mass spectrometry. *Analytical and Bioanalytical Chemistry, 410*(22), 5545–5553. https://doi.org/10.1007/s00216-018-1124-5

Kotthoff, L., O'Callaghan, S. L., Lisec, J., Schwerdtle, T., & Koch, M. (2020). Structural annotation of electro- and photochemically generated transformation products of moxidectin using high-resolution mass spectrometry. *Analytical and Bioanalytical Chemistry, 412*(13), 3141–3152. https://doi.org/10.1007/s00216-020-02572-1

Li, X., Chi, Q., Xia, S., Pan, Y., Chen, Y., & Wang, K. (2020). Untargeted multi-residue method for the simultaneous determination of 141 veterinary drugs and their metabolites in pork by high-performance liquid chromatography time-of-flight mass spectrometry. *Journal of Chromatography A, 1634*, Article 461671. https://doi.org/10.1016/j.chroma.2020.461671

Liesenfeld, S., Steliopoulos, P., & Hamscher, G. (2020). Comprehensive metabolomics analysis of nontargeted LC-HRMS data provides valuable insights regarding the origin of veterinary drug residues. *Journal of Agricultural and Food Chemistry, 68*(44), 12493–12502. https://doi.org/10.1021/acs.jafc.0c05163

Liesenfeld, S., Steliopoulos, P., Wenig, S., Gottstein, V., & Hamscher, G. (2022). Comprehensive LC-HRMS metabolomics analyses for the estimation of environmental inputs of altrenogest in pig breeding. *Chemosphere, 287*(Pt 3), Article 132353. https://doi.org/10.1016/j.chemosphere.2021.132353

Majewsky, M., Glauner, T., & Horn, H. (2015). Systematic suspect screening and identification of sulfonamide antibiotic transformation products in the aquatic environment. *Analytical and Bioanalytical Chemistry, 407*(19), 5707–5717. https://doi.org/10.1007/s00216-015-8748-5

Malato, O., Lozano, A., Mezcua, M., Agüera, A., & Fernandez-Alba, A. R. (2011). Benefits and pitfalls of the application of screening methods for the analysis of pesticide residues in fruits and vegetables. *Journal of Chromatography A, 1218*(42), 7615–7626. https://doi.org/10.1016/j.chroma.2011.06.110

Martinez-Brito, D., de la Torre, X., Colamonici, C., Curcio, D., & Botre, F. (2019). 7-keto-DHEAmetabolism in humans. Pitfalls in interpreting the analytical results in the antidoping field. *Drug Testing and Analysis, 11*(11–12), 1629–1643. https://doi.org/10.1002/dta.2734

Mehl, A., Hudel, L., Bücker, M., & Morlock, G. E. (2022). Validated screening method for 81 multiclass veterinary drug residues in food via online-coupling high-throughput planar solid-phase extraction to high-performance liquid chromatography–orbitrap tandem mass spectrometry. *Journal of Agricultural and Food Chemistry, 70*(35), 10886–10898. https://doi.org/10.1021/acs.jafc.2c03925

Mehl, A., Schmidt, L. J., Schmidt, L., & Morlock, G. E. (2021). High-throughput planar solid-phase extraction coupled to orbitrap high-resolution mass spectrometry via the autoTLC-MS interface for screening of 66 multi-class antibiotic residues in food of animal origin. *Food Chemistry, 351*, Article 129211. https://doi.org/10.1016/j.foodchem.2021.129211

Mol, H. G., Plaza-Bolanos, P., Zomer, P., de Rijk, T. C., Stolker, A. A., & Mulder, P. P. (2008). Toward a generic extraction method for simultaneous determination of pesticides, mycotoxins, plant toxins, and veterinary drugs in feed and food matrixes. *Analytical Chemistry, 80*(24), 9450–9459. https://doi.org/10.1021/ac801557f

Monteiro, S. H., Lehotay, S. J., Sapozhnikova, Y., Ninga, E., & Lightfield, A. R. (2021). High-throughput mega-method for the analysis of pesticides, veterinary drugs, and environmental contaminants by ultra-high-performance liquid chromatography-tandem mass spectrometry and robotic mini-solid-phase extraction cleanup + low-pressure gas chromatography-tandem mass spectrometry, Part 1: Beef. *Journal of Agricultural and Food Chemistry*. https://doi.org/10.1021/acs.jafc.0c00710

Moretti, S., Cavanna, D., Lambertini, F., Catellani, D., Sammarco, G., Barola, C., et al. (2020). Practical approach to develop a multi-group screening method for detection of mycotoxins, pesticides and veterinary drugs in food. *Journal of Mass Spectrometry*. https://doi.org/10.1002/jms.4618

Narduzzi, L., Buisson, C., Morvan, M. L., Marchand, A., Audran, M., Le Bouc, Y., et al. (2021). Coupling complete blood count and steroidomics to track low doses administration of recombinant growth hormone: An anti-doping perspective. *Frontiers in Molecular Biosciences, 8*, Article 683675. https://doi.org/10.3389/fmolb.2021.683675

Parsons, H. M., Ekman, D. R., Collette, T. W., & Viant, M. R. (2009). Spectral relative standard deviation: A practical benchmark in metabolomics. *Analyst, 134*(3), 478–485. https://doi.org/10.1039/b808986h

EURL Pesticides. (2021). *SANTE/11312/2021 analytical quality control and method validation procedures for pesticide residues analysis in food and feed*.

Peter, K. T., Phillips, A. L., Knolhoff, A. M., Gardinali, P. R., Manzano, C. A., Miller, K. E., et al. (2021). Nontargeted analysis study reporting tool: A framework to improve research transparency and reproducibility. *Analytical Chemistry, 93*, 13870–13879. https://doi.org/10.1021/acs.analchem.1c02621

Peters, R. J. B., Stolker, A. A. M., Mol, J. G. J., Lommen, A., Lyris, E., Angelis, Y., et al. (2010). Screening in veterinary drug analysis and sports doping control based on full-scan, accurate-mass spectrometry. *TrAC, Trends in Analytical Chemistry, 29*(11), 1250–1268. https://doi.org/10.1016/j.trac.2010.07.012

Pfeifer, T., Tuerk, J., & Fuchs, R. (2005). Structural characterization of sulfadiazine metabolites using H/D exchange combined with various MS/MS experiments. *Journal of the American Society for Mass Spectrometry, 16*(10), 1687–1694. https://doi.org/10.1016/j.jasms.2005.06.008

Pourchet, M., Debrauwer, L., Klanova, J., Price, E. J., Covaci, A., Caballero-Casero, N., et al. (2020). Suspect and non-targeted screening of chemicals of emerging concern for human biomonitoring, environmental health studies and support to risk assessment: From promises to challenges and harmonisation issue. *Environment International, 139*, Article 105545. https://doi.org/10.1016/j.envint.2020.105545

Randazzo, G. M., Bileck, A., Danani, A., Vogt, B., & Groessl, M. (2020). Steroid identification via deep learning retention time predictions and two-dimensional gas chromatography-high resolution mass spectrometry. *Journal of Chromatography A, 1612*, Article 460661. https://doi.org/10.1016/j.chroma.2019.460661

Rijk, J. C., Lommen, A., Essers, M. L., Groot, M. J., Van Hende, J. M., Doeswijk, T. G., et al. (2009). Metabolomics approach to anabolic steroid urine profiling of bovines treated with prohormones. *Analytical Chemistry, 81*(16), 6879–6888. https://doi.org/10.1021/ac900874m

Rocchetti, G., Ghilardelli, F., Masoero, F., & Gallo, A. (2021). Screening of regulated and emerging mycotoxins in bulk milk samples by high-resolution mass spectrometry. *Foods, 10*(9), 2025. https://doi.org/10.3390/foods10092025

Romero-Gonzalez, R., Aguilera-Luiz, M. M., Plaza-Bolanos, P., Frenich, A. G., & Vidal, J. L. (2011). Food contaminant analysis at high resolution mass spectrometry: Application for the determination of veterinary drugs in milk. *Journal of Chromatography A, 1218*(52), 9353–9365. https://doi.org/10.1016/j.chroma.2011.10.074

Rusko, J., Jansons, M., Pugajeva, I., Zacs, D., & Bartkevics, V. (2019). Development and optimization of confirmatory liquid chromatography-Orbitrap mass spectrometry method for the determination of 17 anticoccidials in poultry and eggs. *Journal of Pharmacy Biomedicine Analytical, 164*, 402–412. https://doi.org/10.1016/j.jpba.2018.10.056

Sanchez-Ponce, R., & Guengerich, F. P. (2007). Untargeted analysis of mass spectrometry data for elucidation of metabolites and function of enzymes. *Analytical Chemistry, 79*(9), 3355–3362. https://doi.org/10.1021/ac0622781

Schiffman, C., Petrick, L., Perttula, K., Yano, Y., Carlsson, H., Whitehead, T., et al. (2019). Filtering procedures for untargeted LC-MS metabolomics data. *BMC Bioinformatics, 20*(1), 334. https://doi.org/10.1186/s12859-019-2871-9

Solliec, M., Roy-Lachapelle, A., & Sauve, S. (2015). Development of a suspect and non-target screening approach to detect veterinary antibiotic residues in a complex biological matrix using liquid chromatography/high-resolution mass spectrometry. *Rapid Communications in Mass Spectrometry, 29*(24), 2361–2373. https://doi.org/10.1002/rcm.7405

Steiner, D., Malachova, A., Sulyok, M., & Krska, R. (2021). Challenges and future directions in LC-MS-based multiclass method development for the quantification of food contaminants. *Analytical and Bioanalytical Chemistry, 413*(1), 25–34. https://doi.org/10.1007/s00216-020-03015-7

Stolker, A. A., Rutgers, P., Oosterink, E., Lasaroms, J. J., Peters, R. J., van Rhijn, J. A., et al. (2008). Comprehensive screening and quantification of veterinary drugs in milk using UPLC-ToF-MS. *Analytical and Bioanalytical Chemistry, 391*(6), 2309–2322. https://doi.org/10.1007/s00216-008-2168-8

Sturm, R. M., Jones, B. R., Mulvana, D. E., & Lowes, S. (2016). HRMS using a Q-exactive series mass spectrometer for regulated quantitative bioanalysis: How, when, and why to implement. *Bioanalysis, 8*(16), 1709–1721. https://doi.org/10.4155/bio-2016-0079

Sun, F., Tan, H., Li, Y., De Boevre, M., Zhang, H., Zhou, J., et al. (2021). An integrated data-dependent and data-independent acquisition method for hazardous compounds screening in foods using a single UHPLC-Q-Orbitrap run. *Journal of Hazardous Materials, 401*, Article 123266. https://doi.org/10.1016/j.jhazmat.2020.123266

Tan, H., Sun, F., Abdallah, M. F., Li, J., Zhou, J., Li, Y., et al. (2022). Background ions into exclusion list: A new strategy to enhance the efficiency of DDA data collection for high-throughput screening of chemical contaminations in food. *Food Chemistry, 385*, Article 132669. https://doi.org/10.1016/j.foodchem.2022.132669

Thevis, M., Kuuranne, T., Geyer, H., & Schanzer, W. (2017). Annual banned-substance review: Analytical approaches in human sports drug testing. *Drug Testing and Analysis, 9*(1), 6–29. https://doi.org/10.1002/dta.2139

Tong, W., Huang, R., Zuo, H., Zarabadipour, C., Moore, A., Hamel, D., et al. (2022). Feasibility of establishing a veterinary marker to total residue in edible tissues with non-radiolabeled study using high-resolution mass spectrometry. *Research in Veterinary Science, 149*, 60–70. https://doi.org/10.1016/j.rvsc.2022.06.006

Turnipseed, S. B., & Jayasuriya, H. (2020). Analytical methods for mixed organic chemical residues and contaminants in food. *Analytical and Bioanalytical Chemistry, 412*(24), 5969–5980. https://doi.org/10.1007/s00216-020-02668-8

Turnipseed, S. B., Storey, J. M., Lohne, J. J., Andersen, W. C., Burger, R., Johnson, A. S., et al. (2017). Wide-scope screening method for multiclass veterinary drug residues in fish, shrimp, and eel using liquid chromatography-quadrupole high-resolution mass spectrometry. *Journal of Agricultural and Food Chemistry, 65*(34), 7252–7267. https://doi.org/10.1021/acs.jafc.6b04717

Turnipseed, S. B., Storey, J. M., Wu, I. L., Gieseker, C. M., Hasbrouck, N. R., Crosby, T. C., et al. (2018). Application and evaluation of a high-resolution mass spectrometry screening method for veterinary drug residues in incurred fish and imported aquaculture samples. *Analytical and Bioanalytical Chemistry, 410*(22), 5529–5544. https://doi.org/10.1007/s00216-018-0917-x

Varenina, I., Bilandžić, N., Luburić, Đ. B., Kolanović, B. S., & Varga, I. (2022). High resolution mass spectrometry method for the determination of 13 antibiotic groups in bovine, swine, poultry and fish meat: An effective screening and confirmation analysis approach for routine laboratories. *Food Control, 133*. https://doi.org/10.1016/j.foodcont.2021.108576

Verdon, E., Polzer, J., & Sterk, S. (2020). *EURL guidance on Minimum method performance requirements (MMPRs) for specific pharmacologically active substances in specific animal matrices*.

Wang, C., Chen, M., Hu, Q., Bai, H., Wang, C., & Ma, Q. (2021). Non-lethal microsampling and rapid identification of multi-residue veterinary drugs in aquacultured fish by direct analysis in real time coupled with quadrupole-Orbitrap high-resolution mass spectrometry. *Microchemical Journal, 160*. https://doi.org/10.1016/j.microc.2020.105673

Wang, C., Li, X., Yu, F., Wang, Y., Ye, D., Hu, X., et al. (2020). Multi-class analysis of veterinary drugs in eggs using dispersive-solid phase extraction and ultra-high performance liquid chromatography-tandem mass spectrometry. *Food Chemistry, 334*, Article 127598. https://doi.org/10.1016/j.foodchem.2020.127598

Wang, K., Wang, X., Xu, Z., & Yang, S. (2020). Simultaneous determination of multi-class antibiotics and steroid hormones drugs in livestock and poultry faeces using liquid chromatography-quadrupole time-of-flight mass spectrometry. *Food Additives & Contaminants Part A Chem Anal Control Expo Risk Assess*, 1–14. https://doi.org/10.1080/19440049.2020.1776900

Wegh, R. S., Berendsen, B. J. A., Driessen-Van Lankveld, W. D. M., Pikkemaat, M. G., Zuidema, T., & Van Ginkel, L. A. (2017). Non-targeted workflow for identification of antimicrobial compounds in animal feed using bioassay-directed screening in combination with liquid chromatography-high resolution mass spectrometry. *Food Additives & Contaminants Part A Chem Anal Control Expo Risk Assess, 34*(11), 1935–1947. https://doi.org/10.1080/19440049.2017.1364431

Wong, J. K. Y., Chan, G. H. M., Choi, T. L. S., Kwok, K. Y., Lau, M. Y., Leung, G. N. W., et al. (2020). A high-throughput and broad-spectrum screening method for analysing over 120 drugs in horse urine using liquid chromatography-high-resolution mass spectrometry. *Drug Testing and Analysis, 12*(7), 900–917. https://doi.org/10.1002/dta.2799

Wu, I. L., Turnipseed, S. B., Storey, J. M., Andersen, W. C., & Madson, M. R. (2020). Comparison of data acquisition modes with Orbitrap high-resolution mass spectrometry for targeted and non-targeted residue screening in aquacultured eel. *Rapid Communications in Mass Spectrometry, 34*(7), Article e8642. https://doi.org/10.1002/rcm.8642

Xia, B., Liu, X., Gu, Y. C., Zhang, Z. H., Wang, H. Y., Ding, L. S., et al. (2013). Non-target screening of veterinary drugs using tandem mass spectrometry on SmartMass. *Journal of the American Society for Mass Spectrometry, 24*(5), 789–793. https://doi.org/10.1007/s13361-013-0578-0

Yan, Y., Ai, L., Zhang, H., Kang, W., Zhang, Y., & Lian, K. (2021). Development an automated and high-throughput analytical platform for screening 39 glucocorticoids in animal-derived food for doping control. *Microchemical Journal, 165*. https://doi.org/10.1016/j.microc.2021.106142

Yan, X.-t., Zhang, Y., Zhou, Y., Li, G.-h., & Feng, X.-s. (2022). Technical overview of orbitrap high resolution mass spectrometry and its application to the detection of small molecules in food (update since 2012). *Critical Reviews in Analytical Chemistry, 52*(3), 593–626. https://doi.org/10.1080/10408347.2020.1815168

Zhang, Y., Liu, X., Li, X., Zhang, J., Cao, Y., Su, M., et al. (2016). Rapid screening and quantification of multi-class multi-residue veterinary drugs in royal jelly by ultra performance liquid chromatography coupled to quadrupole time-of-flight mass spectrometry. *Food Control, 60*, 667–676. https://doi.org/10.1016/j.foodcont.2015.09.010

Zhang, L. Q., Zhang, X. M., Zhang, H. W., Wang, H., Xu, H., Wang, F. M., et al. (2020). Multiclass and multiresidue screening of veterinary drugs and pesticides in infant formula using Quadrupole-Orbitrap MS with PRM scan mode. *Journal of Mass Spectrometry, 55*(3), e4497. https://doi.org/10.1002/jms.4497

Zhu, C., Lai, G., Jin, Y., Xu, D., Chen, J., Jiang, X., et al. (2022). Suspect screening and untargeted analysis of veterinary drugs in food by LC-HRMS: Application of background exclusion-dependent acquisition for retrospective analysis of unknown xenobiotics. *Journal of Pharmacy Biomedicine Analytical, 210*, Article 114583. https://doi.org/10.1016/j.jpba.2022.114583