An investigation into the occurrence in food of chemicals used in pharmaceuticals, veterinary medicines, and personal care products

Report to the Food Standards Agency

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Glossary of Main Terms

Term or Acronym	General Meaning Of Term
ADBI	Celestolide
AHTN	Tonalide (7 acetyl -1,1,3,4,4,6 hexamethyl, 1,2,3,4 tetra-hydro naphthalene)
AMGs	Aminoglycoside compounds
C_{18}	Reversed phase solid phase sorbent material
Cefas	Centre for Environment, Fisheries & Aquaculture Science
DHS	Dihydrostreptomycin
DNC	Dinitrocarbanilide
dSPE	Solid Phase Extraction in the dispersive mode
EDTA	Ethylendiaminetetraacetic Acid
EMEA	European Medicine Agency
FSA	Food Standards Agency
GC-MS	Gas Chromatography – Mass Spectrometry
HFBA	Heptafluorobutyric Acid
HHCB	Galaxolide
HPs	Human Pharmaceuticals
IS	Internal Standard
LC-MS/MS	Liquid Chromatography- Tandem Quadrupole Mass Spectrometry
LIMS	Laboratory Information Management System
Log Dow	pH dependent octanol-water distribution coefficient
Log *Kow*	Octanol-water partition coefficient defined as the ratio of the concentration of a chemical in *n*-octanol and water at equilibrium.
MeP	Methyl Paraben
MS	Mass Spectrometry
NH_2	Amine Solid Phase material
PCPs	Personal Care Products
PEC	Predicted Environmental Concentration
PrP	Propyl Paraben
PSA	Primary Secondary Amine solid phase material
PTFE	Polytetrafluoroethylene
PTV	Programmed Temperature Vaporising Injector
SIM	Selected Ion Monitoring
SPE	Solid Phase Extraction
VMD	Veterinary Medicines Directorate
VMs	Veterinary Medicines

Executive Summary

Human exposure to emerging contaminants by indirect routes is of increasing interest. It is now understood that some groups of compounds, previously not considered as a risk, may enter the environment and subsequently the food chain by various pathways during their production, usage or disposal. This pilot research project has been undertaken to make an initial assessment of the potential for the contamination of food by selected human pharmaceuticals (HPs), veterinary medicines (VMs) and personal care products (PCPs). The **first phase** of the project was a desk study to prioritise those HPs, VMs and PCPs on the basis of their potential to contaminate food. The prioritisation considered many factors including usage, persistence, uptake and bioaccumulation potential, results from previous prioritisation exercises, and reported environmental occurrence. The **second phase** involved the development and validation of suitable analytical methods for the analysis of the prioritised contaminants at low ng/g concentrations. In the **third and final phase** the validated methods were employed for the analysis of samples of mushrooms, vegetables, fodder crops, aquaculture products and animal tissues collected from scenarios considered to present a potential risk of contamination. Some samples of aquaculture products imported from South East Asia were also included because of frequent detection of residues and reports in the EU Rapid Alert System for Food and Feed.

Approximately 400 individual food/crop samples were collected. Sample preparation (including compositing, and separation of component plant parts) produced around 200 samples for analysis. The analytical methods developed included a multi-analyte LC-MS/MS procedure for the determination of 36 different VMs and HPs compounds, and separate chemical-class specific LC-MS/MS methods for the determination of aminoglycosides, coccidiostats and parabens, respectively. A method based on GC-high resolution mass spectrometry (HRMS) was developed and validated for the analysis of 6 musk compounds. Not all of the methods were applied to all samples and not all analytes in the prioritisation list were included in the methods because of cost limitations. The analysis of all sample-method combinations produced around 9000 results. Taking into account the quality control procedures (recovery spikes, etc.) a total of approximately 18000 determinations were performed.

Around 325 individual residues were detected in 118 individual samples, but mostly at low ng/g concentrations.

Residues of methyl and propyl paraben occurred at higher frequency (at concentrations up to 2.84 and 1.3 ng/g respectively) in trout collected in the UK compared to fish imported from South East Asia. Likewise, residues of all 6 musk compounds tested occurred at higher concentrations (up to

50 ng/g) and higher frequency (100%) in trout collected in the UK compared to imported fish from South East Asia. Residues of musks up to 0.3 ng/g were found in 70% of the fish samples from South East Asia. Interestingly, the highest concentrations of musks occurred in UK trout from fish farms located downstream of large urban sewage treatment works effluent release points. By contrast, all fish samples from Vietnam contained residues of enrofloxacin, a contaminant which was not detected in any of the UK trout samples. Shrimp imported from South East Asia were comparatively 'clean' and found to contain low concentrations of musk xylene in two of the samples and musk ketone in one sample.

Residues of trimethoprim, a potentiator for sulphonamide antibiotics were detected at very low concentration (below 1 ng/g), in the majority (73%) of mushroom samples tested. Trimethoprim was not detected in any of the associated compost samples, but this was possibly because of difficulties with the extraction of trimethoprim. To provide evidence of uptake would require detection of trimethoprim in the mushroom compost. This in turn requires further method development to improve the extraction efficiency of the analytical method. Interestingly, residues of coccidiostats, detected at estimated concentrations up to 50 ng/g in mushroom composts, were not detected in the mushroom samples.

Residues of decoquinate, diclofenac and tilmicosin in bovine liver were not unexpected. The occurrence of musk compounds, in bovine liver, bovine kidney, wheat and sugar beet leaves indicates that musk compounds are ubiquitous environmental contaminants.

No formal risk assessment has yet been undertaken, but on the basis of reported toxicological effects in combination with the low concentrations detected, it is unlikely that the findings will pose concerns to human health.

1. Study Background

Human exposure to 'emerging contaminants' by indirect routes is becoming an ever increasingly important issue. It is now understood that some groups of compounds previously not considered as a risk may enter the environment and subsequently the food chain by various pathways during their production, usage or disposal as illustrated in Figure 1. Compounds considered in this study were Human Pharmaceuticals (HPs), Veterinary Medicines (VMs) and Personal Care Products (PCPs).

After use, HPs and PCPs are eventually discharged into the sewage system and following treatment may be released into surface waters through the effluent from sewage treatment works. These classes of compounds can then enter agricultural soils through irrigation with contaminated surface water or through the application of biosolids containing HPs and PCPs. Similarly the principle route for VMs to enter agricultural soils is after the application of manure from farm animals. Theoretically, there is the potential for growing crops to uptake compounds that are present in the contaminated soil. Another possibility for consideration is the direct uptake of VMs by mushrooms cultivated using compost based on poultry litter. If agricultural crops grown for food or animal fodder can uptake chemicals from the environment, then it follows that there is the potential for human exposure via consumption of these food crops or meat from animals exposed to contaminated feed and/or water.

The uptake of the different chemicals by crops and aquaculture products (e.g. fish, crustaceans) is dependent on many factors including: the physico-chemical properties of the compounds, the actual usage of the chemicals, the fate of the chemicals, and environmental factors (e.g. rainfall etc.). By way of example, the factors taken into account in determining indirect exposure of crops to HPs and PCPs through irrigation of contaminated surface waters include: estimation of predicted environmental concentration in surface waters (considering compound usage, wastewater production per inhabitant), partitioning to sewage sludge, dilution of wastewater effluent (and the subsequent average irrigation rate), soil incorporation depth, uptake into roots and transport to above ground parts of the plant. The details of the relevant factors and associated calculations for all of the scenarios evaluated are described in detail in an interim report which was dated May 2011 and is appended to this final report.

The objective of this project was to assess the potential for the contamination of food by human pharmaceuticals (HPs), veterinary medicines (VMs) and personal care products (PCPs). The process to achieve this objective was to analyse samples of mushrooms, vegetables, fodder crops, aquaculture products and animal tissues collected from scenarios identified as presenting a

particular risk of contamination. For example, crops cultivated on land after application of biosolids and fish reared in water downstream of outflows from sewage treatment works.

Because of the complexity of factors, the project was divided into 3 separate phases. The **first phase** was to identify and prioritise those HPs, VMs and PCPs that might be of greatest concern regarding human health if they are present in food. The prioritisation considered many factors including usage (except for PCPs), persistence, uptake and bioaccumulation potential, results from previous prioritisation exercises and reported environmental occurrence. The **second phase** involved the development and validation of analytical methods to determine the presence of those chemicals, categorised as 'high priority, in different representative food and fodder commodities. The analysis of all of compounds in the high priority category would, because of their diverse chemical and physical properties, require a large number of different analytical methods. Since the associated high costs were outside of the budget of the project, it was agreed to employ a small number of multi-analyte methods in an attempt to screen for as many high priority chemicals as possible at a realistic cost. The **third and final phase** of the project was a pilot survey that involved the analysis of samples collected from aquaculture and agriculture scenarios that present a possible risk of contamination and uptake. Around 400 individual samples of liver, kidney, fish, fodder crops and mushrooms, were collected from different scenarios in the UK. Samples of fish and shrimp imported from South East Asia and retail samples of mushrooms were also obtained. The scenarios evaluated in the UK included the uptake of VMs by mushrooms grown in compost derived from chicken manure, the uptake of chemicals by fish from farms downstream of sewage treatment outfalls, the general uptake of chemicals by cattle, and the uptake of all chemical classes by fodder crops grown on land amended with biosolids or manure.

2. Experimental

2.1 Overview of Phase 1; Prioritisation Exercise

The main objective of **Phase 1** was the identification and prioritisation of HPs, VMs, and PCPs that have the greatest potential to enter the food chain from the natural environment. The methods employed and the results (**study objective 1**) have been previously described in detail in an interim report, which was dated May 2011 and is appended to this current report.

Prior to starting this current project it was evident that a number of studies had already been performed by researchers attempting to identify/prioritise emerging contaminants, and in particular HPs, VMs and PCPs relevant to this project. These existing studies were reviewed to enable existing data to be utilised as much as possible and to avoid duplication of previous efforts. Also, it was anticipated that, methodologies described in the literature could be considered for use during this current project.

The main conclusions from consideration of pre-existing studies were:

- The focus of the previous prioritisation studies were in the order VMs>HPs>>PCPs;
- Most studies followed a risk based approach considering both exposure and effects to certain environmental compartments, predominantly surface waters;
- There was very limited consideration of human exposure through food;
- Regulatory methodologies for estimating concentrations in environmental matrices were available for HPs and VMs from the EMEA and some of the existing approaches have utilised these accepted methodologies;
- No prioritisation exercises considered the transfer of these contaminants from environmental matrices such as soil and surface waters to food;
- 90 VMs had been identified as high and/or medium priority in at least one of the previous studies, with 10 identified in at least five of the six studies considering the prioritisation of this class;
- With reference to HPs 78 compounds had been identified as high and/or medium priority in at least one previous study, with 6 identified in at least three other studies; and
- The prioritisation of PCPs has only been considered in one study, which identified 24 compounds as high and/or medium priority

This information was used to prioritise the compounds most likely to be taken up into food in scenarios relevant to the UK. HPs, VMs, and PCPs were evaluated and prioritised within their own

class because of difficulties in obtaining equivalent and appropriate usage data across the different chemical classes. In fact a suitable source of usage data was not identified for PCPs and therefore a priority list for PCPs was determined on the basis of information from the scientific and grey literature combined with expert opinion. Polycyclic and nitro musk compounds, which have been used extensively in PCPs are recognised as common contaminants in environmental compartments and, in particular, in the aquatic environment. Relatively little is known about their occurrence in foods or agricultural crops. Previous concern arising from the use and persistence of nitro musks has led to restrictions on their use. In recent times they have been replaced by other products such as the polycyclic musks, in particular galaxolide (HHCB) and tonalide (AHTN) which reportedly account for around 90% of polycyclic musk usage in Europe during the last decade (HERA, 2004).

2.2 Sample Collection and Preparation

Approximately 400 individual food/crop samples were collected for analysis. Sample preparation (including compositing, and separation of component plant parts) gave around 200 samples for analysis. In addition to plant and animal samples, soil and compost material were also collected and stored frozen in case they would be required for further investigation.

Upon collection, samples were double bagged in pre-labelled polyethylene bags and then sealed. Where the duration between sample collection and sample receipt was greater than 12 hours then samples were typically cooled during transit. The transportation of offal (kidney, liver) and fish was undertaken in compliance with official regulations.

On receipt at the laboratory each prepared sample was given a unique laboratory reference number and the sample details were logged into a LIMS database. After initial preparation (separation and/or compositing) the prepared laboratory samples were homogenised under cryogenic conditions using dry ice. Cryogenic homogenisation is known to provide good stability of certain chemical contaminants and also sufficient homogeneity to permit the use of small amounts of sample and to facilitate the implementation of small-scale methods. After cryogenic homogenisation the sub-samples requiring screening for musk compounds were also freeze-dried. The freeze-dried sample powders were mixed thoroughly and aliquots of these were used for analyses.

Further information on the sources of samples, number of samples and any specific sample pre-treatments are given below.

2.2.1 Samples of Mushrooms and Mushroom Compost

A total of 33 mushroom samples were collected from both retail sources and growers in England between January 2011 and March 2012. The retail samples (each approx. 1 kg) comprised different varieties of mushrooms, originating from the UK, Ireland and China.

Mushroom samples (*Agaricus bisporus*) cultivated using compost from the two main commercial suppliers in the UK, were obtained. The samples were harvested from the first, second and third 'flushes' (typically 3 crops are harvested before the compost is replaced). The mushrooms were all close to marketable size and each sample consisted of a minimum of 1 kg of mushrooms. Samples of compost were also collected with each sample of cultivated mushrooms.

The mushroom samples collected in the early stages of the project were brushed and rinsed (tap water) to remove compost adhering to the surface. Whole mushrooms were frozen and then homogenised in the presence of dry ice. A number of mushroom samples collected during the later stages in the project were peeled and destalked prior to extraction. The homogenised caps, and peel/stalk (not homogenised), were analysed as two separate samples.

Selected samples of compost were also cryogenically processed (comminuted) in the presence of dry ice.

2.2.2 Samples of Fish and Shrimp

Areas of intensive livestock production (particularly pigs) will have requirements to dispose of large volumes of animal manures and/or slurries to land. Fish from fish farms that source waters from such catchments have the potential to uptake any VMs present in those waters.

Large conurbations will have the requirement to release large volumes of treated effluent to environmental surface waters. Any fish from fish farms that source waters downstream of large urban sewage treatment effluent release points will have the potential to uptake any HPs and PCPs present in those waters.

A total of 30 samples of table ready fish were collected (three samples from ten fish farms), from areas with high density of cattle and/or pigs, and from farms located downstream from sewage treatment works serving large human populations. Samples were collected by sampling officers from the Centre for Environment, Fisheries and Aquaculture Science (Cefas).

On receipt, the heads and tails were removed and the fish gutted and filleted to remove bones. The flesh including the subcutaneous fat was homogenised in the presence of dry ice.

In addition, a total of 21 samples of fish and 32 samples of shrimp imported from countries in South East Asia were also analysed. These samples were originally collected and analysed for residues of veterinary medicines as part of the Veterinary Medicines Directorate (VMD) surveillance programme. Since the samples were scheduled to be discarded VMD gave permission for the samples to be analysed for a broader scope of compounds within this current project. Peeled shrimp and filleted fish samples were cryogenically milled.

2.2.3 Samples of Root and Foliage Crops

HPs and PCPs have the potential to contaminate root or foliage crops if grown at sites that have previously received applications of biosolids. Similarly the uptake of VMs into root or foliage crops could occur after the application of animal manures to agricultural land. Based on assessment of the data received, pig slurry appeared to be the most relevant scenario since a considerable number of the VMs could be present in pig slurry.

Sludge managers from some of the 29 water companies in Great Britain provided information on sites of biosolid application. It became evident that very few sites treated with biosolids are used for growing crops for human consumption. The majority of soils amended with biosolids are used to grow fodder, turnips and kale for animal feed. In view of the difficulty in obtaining samples intended for human consumption, it was agreed by the FSA that samples of any crops available would be acceptable to assess possible uptake by plants. Thus, a total of 22 samples of sugar beet, oil seed rape and wheat were collected. The leaves of the sugar beet were removed from the roots and each analysed separately. Similarly the stalks and heads of wheat were separated and treated as individual samples. For oil seed rape the oil seeds were separated from the foliage. The foliage was analysed, but there were insufficient oil seeds for analysis. All samples were cryogenically milled in the presence of dry ice.

2.2.4 Samples of Bovine Offal (Kidney and Liver)

Samples were collected from ten selected slaughter houses, of which 5 five received animals from areas where uptake of PCPs was considered a possibility whilst the other five received animals from areas where uptake of VMs was a possibility. Samples were collected from each individual slaughter house on three separate sampling days to ensure that the samples collected covered a large number of animals from different farms.

Samples of bovine liver and bovine kidney were to be collected from the selected abattoirs by an Animal Health Officer from the Food Standards Agency (FSA). In compliance with official regulations samples were frozen and triple sealed in plastic bags for transportation to the

laboratory. Slices of kidney (typically 100g) from 105 individual animals, and slices of liver (typically 100g) from another 95 individual animals were collected. On receipt at the laboratory the tissues were combined (5 samples representing 5 different animals, collected on the same day from the same abattoir) to produce a total of 40 composited samples (21 kidney and 19 liver) for analysis. This approach was employed to increase the chance of detecting residues since a greater number of animals would ultimately be tested.

All composited samples were cryogenically milled in the presence of dry ice.

2.3 Assessment of Analytical Possibilities for the Analysis of Prioritised Chemicals

The list of compounds resulting from the prioritisation exercise was carefully considered in order to determine an appropriate analytical strategy that would provide as many analytical results for as many different compounds as possible, within the financial budget of the project. It was concluded that a multi-analyte, multi-class method based on LC-MS/MS, a multi-analyte GC-MS based method for the detection of musk compounds, and a class specific multi-analyte LC-MS/MS method for aminoglycosides (required for mushrooms only) was the most appropriate option. It was accepted that the multi-analyte approach would compromise the analytical performance for specific compounds but would enable screening for a greater number of compounds overall. If required as a consequence of emerging results, and dependent on cost, then specific methods could be implemented as was the case for the analysis of coccidiostats in mushrooms.

It was recognised that implementation of this approach inevitably meant that some compounds included in the finalised prioritisation list would be excluded from the project. Of the total of 79 different chemicals included in the prioritisation list it was predicted that 47 could be included in the multi-analyte approach. Of the remaining compounds parabens were considered by the FSA to be of particular interest. During the course of the project a specific method for 7 parabens was therefore developed and validated for the analysis of fish and shrimp. There was insufficient time and resources to apply the method to the analysis of the other commodities. For many of the other compounds on the priority list, particularly the stearates, flame retardants (hexabromocyclododecane, tetrabromobisphenol A), butylated hydroxyl anisole (antioxidant and food preservative), methylene bis-benzatrazoyl (sunscreen) and tocopherol acetate (vitamin supplement) there is the potential for direct uptake from other non-food sources. Since their contribution from food is likely to be relatively low and the cost of analysis relatively high, these compounds were not included in the project. Also some recent occurrence data for some compounds such as brominated fame retardants is available for food consumed in the UK. The quaternary ammonium compounds (e.g. benzalkonium chloride) and ionic surfactants (e.g. sodium dodecylbenzene- sulphonate, sodium lauryl ethersulphate) require specialist costly methods whilst

in the case of propoxyphene and dextropropoxyphene, analytical standards were not readily available.

2.4 Analytical Methodology

A summary of the commodities analysed by the different methods is given in Table1.

2.4.1 Multi-analyte, Multi-class Method based on LC-MS/MS

The objective was to screen for residues at a concentration as low as possible, realistically around a target concentration of 1 ng/g in the sample. The method was based on a small-scale method capable of extracting analytes across a wide range of polarities (Tarbin & Fussell, 2013). However this method only includes a crude clean-up (SPE in the dispersive mode) and consequently the final extracts contain relatively high concentrations of co-extractives. This could result in low analyte response (due to matrix induced suppression of ionisation in the MS) and hence higher detection limits depending on the specific composition of the sample. Since individual samples, even of the same sample type, can exhibit different suppression effects a sample 'over-spiking' approach (also referred to as a single point standard addition) was employed in an attempt to ensure the validity of each individual commodity-analyte result. In this approach each sample was analysed twice, once without addition of analyte and once following addition of a known amount of analyte (typically equivalent to 1-5 ng/g in the sample). This enabled the detection of the analyte at the spiked concentration to be verified for each individual sample and to be compared with the sample with no addition of analyte. Furthermore, the addition of deuterated/^{13}C-labelled analogues, where available, provided some evidence to the validity of every individual extraction (including the non-spiked sample) and provided more accurate and precise quantification of the recovery of the equivalent non labelled analytes. For information the recovery of analytes for each of the spiked extracts was calculated against matrix-matched calibration standards (typically prepared using a single sample known to be blank)

Briefly, cryogenically milled analytical portions (5 g) of the samples were analysed with and without spiking. Deuterated/^{13}C-labelled analogues where available were added to all samples and non-labelled analytes were added to the 'over-spike' samples. Water (2 to 5 mL depending on the sample) was added and the extraction performed using 1% oxalic acid in acetonitrile (15 mL). Anhydrous sodium sulphate (5 g) was added to reduce the water content prior to a clean-up step using dispersive solid-phase extraction (dSPE) with C18 and primary secondary amine (PSA) to reduce the matrix co-extractives. Finally, an aliquot (3 mL) of the extract was evaporated and reconstituted with acetonitrile:water (1:1, 1 mL). The final solvent composition was selected as a compromise between sufficient analyte solubility and acceptable chromatographic peak shapes for

early eluting polar compounds. All extracts were filtered through 0.22 µm polytetrafluoroethylene (PTFE) filters.

In the case of kidney and liver, highly complex chromatograms were obtained using the above extraction procedure. In order to improve the selectivity of the extraction, the influence of slight modifications (e.g. different amount of sorbents in the clean-up step, extraction with 1% acetic acid in acetonitrile) to the method were assessed. The use of 1% acetic acid (instead of oxalic acid) in acetonitrile (Stubbings & Bigwood, 2009) resulted in lower amounts of co-extractives in the final extract and was thus implemented for the analyses of kidney and liver.

Analytical measurements were carried out using ultra high-performance liquid chromatography-electrospray tandem mass spectrometry (UPLC-ES-MS/MS). All analytes were infused into the system (Acquity UPLC-Xevo TQ-S, Waters, Manchester, UK) to optimise the parameters to obtain best MS/MS response. The MS detector in multiple reaction monitoring (MRM) mode was used for quantitative analysis using the two most intense transitions for target analytes and one transition for internal standards. All compounds were measured in the positive mode only.

Different columns and mobile phase compositions were evaluated for the best peak shape, selectivity and sensitivity. Due to the different chemical nature/diversity of analytes included in the method, final conditions were a compromise to achieve the best results for the greater number of compounds. The separation was carried out using a Phenomenex Kinetex XB-C18 column (100 x 2.1 mm, 2.6 µm) maintained at 40 $^{\circ}$C and gradient elution using (A) 0.1% formic acid in water and (B) 0.1% formic acid in methanol:acetonitrile (1:1). The gradient programme was: 95% A (0 - 0.2 min), 5% A (3.0 - 6.6 min), 95% A (6.7 - 8.0 min). The flow rate during the separation was set at 0.4 mL/min, but increased to 0.5 mL/min between 3.6 and 7.5 min to minimise the time required to clean the column after elution of the analytes. The injection volume was 3 µL.

2.4.2 Determination of Parabens using LC-MS/MS

A method was developed and validated for the screening of parabens in samples of fish and shrimp. Due to the widespread use of these compounds the control of the background contamination during handling and extraction of samples was crucial. Cleanliness of the laboratory environment, and careful control and monitoring of materials and reagents was essential in order to achieve acceptably low detection limits.

In brief, cryogenically comminuted samples (5 g) spiked with ^{13}C-labelled methyl paraben (and with target compounds in the case of ‘over-spiked samples’) were extracted with acetonitrile (10 mL). Magnesium sulphate (2 g) was added to reduce the volume of water in the final extract. Resultant

extracts were purified by means of dSPE using C_{18} and PSA. Finally, an aliquot (4 mL) of the extract was concentrated to give a final volume of 1 mL in a mixture acetonitrile:water (1:1). All extracts were filtered through 0.22 µm PTFE filters.

Methyl paraben was internally standardised with a ^{13}C labelled analogue while all other compounds were quantified using external calibration with matrix-matched standards. These standards were prepared using fish/shrimp material free of residues (if not available, the matrix with the lowest residue concentrations was used instead).

Analytical measurements were carried out using UPLC-ES-MS/MS. All analytes were infused into the system (Acquity UPLC-Xevo TQ-S, Waters, Manchester, UK) to optimise the parameters to obtain best MS/MS response. The MS detector in MRM mode was used for quantitative analysis using the two most intense transitions for target analytes and one transition for the internal standard. All compounds were analysed in the negative mode.

Target compounds were separated using a Phenomenex Kinetex XB-C18 column (100 x 2.1 mm, 2.6 µm) maintained at 40 °C using gradient elution with (A) 1 mM ammonium acetate in water and (B) 1 mM ammonium acetate in methanol. The gradient programme was: 60% A (0-0.2 min), 45% A (2.5 - 4.9 min), 5% A (5.1 - 6.1 min), 60% A (6.3 - 7.8 min). The mobile phase flow used was 0.4 mL/min. The injection volume (1 µL) was a critical parameter to achieve repeatable retention times.

2.4.3 Determination of Aminoglycosides using LC-MS/MS

Aminoglycosides (AMGs) residues were extracted from the mushroom samples using a phosphate (20 mM KH_2PO4) and EDTA (0.4 mM Na_2EDTA) buffer containing trichloroacetic acid (2%) to precipitate protein. The extract was then neutralised and cleaned-up using a weak cation exchange solid-phase extraction cartridge which was eluted with 10% acetic acid in methanol (3 mL). The methanolic eluate was evaporated and reconstituted in an aqueous solution of an ion-pair reagent (400 µL of 20 mM heptafluorobutyric acid, HFBA). Extracts were then analysed by ion-pair reversed-phase HPLC-ES-MS/MS. All analytes were quantified using external calibration with matrix-matched standards.

AMGs analyses were performed using a HPLC-ES-MS/MS (Alliance 2695- Quattro Ultima, Waters, Manchester, UK). All analytes were infused to optimise the parameters to achieve best MS/MS response for each individual analyte. The two most intense transitions were monitored in the positive mode. AMGs were separated using a Phenomenex Gemini C_{18} column (100 x 2 mm, 3 µm particle size, maintained at 30 °C) and a ternary gradient with water (A), acetonitrile (B), and 100 mM HFBA aqueous solution (C). The gradient was the following: 75% A, 5% B, 20% C (0-0.5

min); 20% A, 60% B, 20% C (1 min); 10% A, 70% B, 20% C (12 min); 0% A, 80% B, 20% C (12.1-15.0 min); 75% A, 5% B, 20% C (15.1 min). The injection volume was 30 µL and the flow rate set at 0.25 mL/min.

2.4.4 Determination of Coccidiostats using LC-MS/MS

A number of coccidiostats (not included in the priority list) were added to the analytical suite after residues of monensin, narasin and dinitrocarbanilide (DNC) were detected in selected samples of compost. At this point, it was decided to peel mushroom samples and analyse the stalks/peels, separately from the peeled cups, in an attempt to assess any contamination on the surface of the mushrooms.

Cryogenically milled mushroom samples (5 g), spiked with a deuterated analogue of DNC (and with target compounds in the case of 'over-spiked' samples) were extracted with 1% acetic acid acetonitrile (15 mL). Anhydrous sodium sulphate (5 g) was added to reduce the volume of water, prior to clean-up with dSPE using C_{18} and NH_2. Finally an aliquot (3 mL) of the cleaned-up extract was evaporated and reconstituted with acetonitrile:water (3:1). All extracts were filtered through 0.22 µm PTFE filters.

Coccidiostat analytes were determined by UPLC-ES-MS/MS. All analytes were infused into the system (Acquity UPLC-Xevo TQ-S, Waters, Manchester, UK) to optimise the settings to obtain best MS/MS response. The MS detector in MRM mode was used for quantitative analysis using the two most intense transitions for target analytes and one transition for the internal standard. DNC and its deuterated analogue were ionised in the negative mode while the other ionophores were ionised in the positive mode. DNC and diclazuril were internally standardised while the other coccidiostats were externally standardised.

Chromatographic separation was achieved using a Waters Acquity CSH phenyl-hexyl column (100 x 2.1 mm, 1.7 µm) maintained at 40 °C and gradient elution using: (A) 0.1% formic acid in water and (B) 0.1% formic acid in methanol:acetonitrile. The gradient programme was: 95% A (0 - 0.2 min), 60% A (1 min), 5% A (3.0 - 5.6 min), 95% A (5.7 - 7 min). The mobile phase flow was set at 0.4 mL/min (0.5 - 0.6 mL/min from 3.5 - 5.7 min) and the injection volume was 1 µL.

2.4.5 Analytical Quality Control for Methods based on LC-MS/MS

As described above, an over-spike approach was employed for all analyses conducted using LC-MS/MS. This approach takes into account the matrix specificity and was used to validate the method for each sample in all batches. Additionally, measurement of the recovery of concentration of analytes in the over-spiked samples was obtained by comparing the response with matrix-matched calibration standards (typically comprised of 5 concentration levels prepared using a selected sample of the type relevant to the sample batch). This provided information on the performance of the method for each individual sample.

Furthermore, a procedural/method blank was included in all batches to check/monitor the possible contamination of the samples from reagents, solvents and materials employed during the analysis. The method blank (reagents and solvents, with no addition of sample) was subject to the same procedures as the sample.

The results of this project were assessed against the analytical quality control (AQC) criteria specified Commission Decision 2002/657/EC.

For a specific analyte to be considered present in a sample extract then specific criteria for identification of the analyte must be met:

i) the relative retention time of the analyte must be comparable (tolerance set at ± 2.5%) to those of an internal standard, if available, and to authentic analytical standards of each analyte
ii) the peak must have the correct mass transition
iii) the signal to noise ratio of any peak must be greater than 3:1, and
iv) the relative ions intensities of the two transitions must be within the expected range

In order to demonstrate that a compound is not present at or above a specified reporting concentration then: the internal standard, if added, must be present in all extracts and the 'over-spiked' extract must show a peak for the target compound at the required retention time. Also, the blank (non-spiked) extract must not show a signal at or above the reporting limit at the retention time of the target analyte.

2.4.6 Determination of Musk Compounds using GC-MS

An aliquot of the freeze-dried material equivalent to 10 g of sample was ultra-turraxed in 30 mL of dichloromethane:hexane (40:60). The extract was extracted using an ultrasonic bath set at 40 ˚C

for 30 min. The extract was cooled, filtered through sodium sulphate (supported by glass-fibre frits/silanised glass wool), and then concentrated to ~ 0.5 mL.

The concentrated extract was purified on 6 g of activated alumina, previously washed with ethyl acetate and topped with 1.5 g of sodium sulphate. The alumina column was washed with hexane (100 mL) followed by ethyl ethyl acetate:hexane (1:10), before elution with ethyl acetate:hexane (1:2) and ethyl acetate. The extracts were concentrated to ~ 250 µL with the addition of ^{13}C-labelled PCB77 internal standard. For some matrices, in particular kidneys, the final extracts were allowed to stand refrigerated, overnight and were then centrifuged to remove any solid material (e.g. salts) that may have precipitated, prior to analysis.

No ^{13}C musk surrogates are available for the musk compounds, and deuterated musk xylene and deuterated tonalide which were investigated for use as internal standards were found to be unstable during analysis.

The measurements were performed using a Micromass Autospec high resolution mass spectrometer coupled to a Agilent 6890N gas chromatograph fitted with a J&W DB-5 MS fused silica capillary column (60 m x 0.25 mm i.d., 0.25 µm film thickness) and a programmable temperature vaporisation (PTV) injector operated in constant flow (~1 mL/min helium) mode. The mass spectrometer was operated in electron ionisation (EI) mode at a mass resolution of ~10000 (at 10% peak height). Two ions were monitored for each analyte in two discrete groups based on the molecular mass range and chromatographic retention. These were monitored in the selected ion monitoring (SIM) mode. An acceleration voltage of 7 kV was used in conjunction with an electron energy of 32-37 eV and a trap current of 450 µA. The GC-MS interface was set to 280 °C.

Standard solutions and sample extracts were introduced by 5 µL injections into the PTV injector at 60 °C using a CTC Analytics GC PAL auto-sampler. Analyte transfer to the GC column was achieved using a PTV injector programme which consisted of a 3 minute isothermal period at 60°C followed by heating at 12 °C/sec to 320 °C, for 10 min, then at 12 °C/sec to 340 °C to the end of the run.

Chromatographic separation was achieved using a GC oven temperature programme consisting of a 5 minute isothermal period at 60 °C followed by heating at 24 °C/min to 160 °C for 2 min, and then at 3 °C/min to 250 °C followed by 5 °C/min to 300 °C for 3 min (see Figure 2).

3. Results and Discussion

Results for the validation of the methods are summarised in Tables 2-6, an overview of the reporting limits is presented in Tables 7-15, and the residues detected collated in Tables 16-21.

3.1. Validation of the Multi-analyte Method based on LC-MS/MS

The method developed initially included 36 different VM and HP compounds. The MS/MS response for the individual analytes varied from matrix to matrix, most likely due to the presence of different co-extractives. Depending on the response obtained during preliminary experiments, the over-spiking concentration for an individual compound was set at 1 or 5 ng/g. Typically, the lowest calibrated concentration was set at one fifth of the spiking concentrations. Labelled internal standards, available for 12 of the compounds, were also spiked onto the sample prior to extraction. During analysis the recovery of analytes spiked onto each sample were calculated against calibration standards prepared in a single matrix. Although variability is inevitable, due to differences in response between different sample matrices, these results (see Tables 2-6) are considered to represent a more robust and more accurate assessment of method performance.

The assessment of the results from the samples of fish and shrimp over-spiked at 1 ng/g (24 analytes) and 5 ng/g (12 analytes) demonstrate that the method was fit for purpose. The majority of recoveries were in the range 60-120% and associated % RSDs were generally less than 20 (see Table 2). For the analysis of both imported fish and shrimp, enrofloxacin, chlortetracycline and flucloxacillin suffered from irreproducible calibration. The method did not produce reliable results and/or the response was insufficient for cefalexin, chlorhexidine, florfenicol amine, sulphasalazine and tramadol.

The analyte responses in mushroom (see Table 8) were generally not as good as in fish (see Table 7). The total number of analytes that could be detected in mushroom at 1 and 5 ng/g were 24 and 9 respectively. Seventeen out of 35 analytes tested could be detected and identified (sufficient response for two MS/MS transitions) at 0.2 ng/g. Florfenicol amine and chlorhexidine gave no detectable response at any of the concentrations (up to 10 ng/g) tested, possibly because of suppression of the response by matrix co-extractives.

Since each sample was over-spiked and each batch included a calibration sequence then it was possible to calculate the recovery of each analyte from each sample.

The extraction method for liver and kidney using 1% acetic acid in acetonitrile was validated by performing seven replicate extractions of blank samples of each matrix spiked at 1 or 5 ng/g depending on the analyte (data not shown). After initial validation a more extensive on-going validation was performed by spiking each individual composite sample with analytes at 1-5 ng/g. The majority of recoveries for analytes spiked into each composite sample of liver were in the range of 57-104% with associated % RSD below 29. The exception was lasalocid (recovery 36%). For kidney composite samples recoveries were generally in the range 50-127% with associated % RSDs below 23. The exceptions were ciprofloxacin (32 % RSD) and lasolocid (recovery 42%).

Oil seed rape proved to be the most challenging plant crop matrix. Ten of the 33 compounds that were detected in other matrices (e.g. sugar beet) could not be reported in oil seed rape due to lack of sufficient selectivity (due to co-eluting interferences) or sensitivity (matrix suppression). For sugar beet samples (roots and leaves) recoveries ranged from 52 to 135%, excluding salinomycin and quinine. Recoveries for wheat heads and stalks were quite similar and above 50% with the exception of salinomycin and with % RSD less than 20% excluding chlortetracycline and robenidine (see Table 3).

3.1.2 Validation of the Method for the Determination of Parabens

The method was initially validated using two different trout samples (7 replicates of each) spiked at 1 ng/g. The recoveries were calculated against calibration standards prepared in one of the matrices and ranged between 95 and 107% with % RSD between 2 and 8% (data not shown). The collated recovery results (from the over spiking of each of the 83 individual samples of fish and shrimp with 1 ng/g of each of the 7 parabens) were in the 79-125% with associated % RSDs in the range 4-23 (see Table 5).

3.1.3 Validation of the Method for the Determination of Aminoglycosides

Using matrix-extracted calibration in combination with a specialist ion pairing LC-ES-MS/MS method, ten aminoglycosides were detected at 2 to 5 ng/g in mushroom during the initial validation. For eight of the aminoglycoside compounds spiked at 2 ng/g, recoveries were in the range 98-127% with associated RSDs in the range 7-20% (data not shown). The method only included one MS/MS transition for some compounds in order to optimise the response of the primary transition for the purpose of screening. Any samples found to contain potential residues of amino glycosides would require further analysis to verify the identity of the analyte. Unfortunately the response of the LC-MS/MS could not be maintained during the analysis of samples batches, presumably because of differences in matrix effects, and thus the reporting limits had to be raised.

The collated recovery results from the over spiking of each 25 individual samples of mushrooms at 10 ng/g for 8 aminoglycosides are summarised in Table 4. Recoveries were in the 79-122% with associated RSDs in the range 4-16%.

Two aminoglycosides (streptomycin and spectinomycin) were not detected at the highest spiking concentration (10 ng/g), and hence no results were obtained for these two compounds.

3.1.4 Validation of the Method for the Determination of Coccidiostats

The method is validated and in routine use for the analyses of animal tissues, but not mushrooms. In this case the over-spike data for mushroom matrix demonstrated that the method is 'fit for purpose' (see Table 4). Recoveries were in the 41-106% with associated % RSDs below 13 except for diclazuril which was more variable.

3.1.5 Validation of the Method for the Determination of Musk Compounds

The method limits of detection varied depending on the particular compound and the matrix and were influenced by the presence of incurred residues of the targeted analytes in the samples. For celestolide and musk xylene LODs were as low as 0.01 ng/g, whereas the LOD for galaxolide was around 0.8 ng/g because of the frequent occurrence of residues at low concentrations and hence the difficulty of obtaining 'blank' samples. In general the LODs achieved are considerably lower than literature values quoted for environmental matrices and biota samples, particularly fish (Fromme *et al*., 2001; Kallenborn *et al*., 2001; Kannan *et al*., 2005; Nakata, 2005; Osemwengie & Steinberg, 2003).

The linearity of measurement with target musk compounds normalised to ^{13}C-PCB77 was confirmed over a range corresponding to 0.2 ng/g to 50 ng/g. Preliminary measurement precision was in the range of 11-22%.

Analytical recoveries were typically in the range of 50-100% but musk xylene which is more labile generally showed lower recoveries (typically 30-40%).

The method proved to be suitable for the analysis of fish, shrimp, root vegetables, wheat, foliage crops, liver and kidney.

3.2 Study findings

3.2.1 Fish and shrimp

The number of studies devoted to the analysis of parabens in food samples is scarce. In this current study, concentrations of residues of parabens in fish samples from South East Asia were in general lower than those found in trout samples from the UK. Individual trout samples from the UK contained up to 8 different residues whilst fish and shrimp from South East Asia were found to contain up to 5 of the targeted compounds.

Methyl paraben (MeP) was found in the concentration range 0.6-2.8 ng/g in 23 samples (77%) of UK trout and propyl paraben (PrP) in 19 samples (63%) at concentrations in the range 0.4-1.3 ng/g. Figure 3 shows a chromatogram of a fish sample containing incurred residues. By contrast these compounds were detected at lower concentrations in fish and shrimp imported from South East Asia. However, Ramaswamy *et al*. (2011) analysed 58 samples belonging to 20 different fish species from the Manila Bay in the Philippines and detected methyl, propyl and butyl paraben in more than 90% of the samples and ethyl paraben in about 70%. Methyl paraben was found to be the predominant compound with concentrations ranging from <0.05 to 3600 ng/g. The concentrations of the other 3 paraben species varied from <0.011 to 1100 ng/g. Since parabens are approved for use as food preservatives (European Parliament and Council Directive 95/2/EC, 1995), the low concentrations detected are not likely to give cause for concern to human health.

There is more information on the occurrence of paraben compounds in environmental samples compared to food. According to the literature, methyl and propyl paraben (as well as ethyl paraben) are the most commonly found paraben species in the environment (Nieto *et al*., 2009; Canosa *et al*., 2007; Gonzalez-Mariño *et al*., 2011). Methyl and propyl paraben have been reported to occur in raw wastewater at concentrations up to 10 and 2.8 µg/L, respectively (Gonzalez-Mariño *et al*., 2011). However, their elimination rates in wastewater treatment plants have been reported to be high and thus very low levels are found in effluent samples (Lee *et al*., 2005, Gonzalez-Mariño *et al*., 2011). Therefore, the fact that we have not detected higher concentrations of parabens in fish samples obtained from fish farms located downstream wastewater treatment plants compared to other scenarios is consistent with published data.

Enrofloxacin proved difficult to quantify in fish and shrimp from South East Asia due to an irreproducible MS response possibly caused by the effects of matrix co-extractives. Thus calculated concentrations are indicative for most of the samples. Identification of the residues was based on the ratio of two transitions compared to matrix-matched standard. Additional experiments were carried out in an attempt to provide a more accurate quantification. Different

clean-up strategies (e.g. SPE) and dilution of extracts were evaluated but did not result in any improvement in the data. More satisfactory quantification was achieved using standard addition compared to external standard calibration. In some cases it was necessary to allow the spiked sample to stand (>20 hours) in order to provide sufficient interaction between the spiked compound and the matrix. Due to time constraints this approach was only used for a limited number of samples in order to verify its applicability to different fish matrices.

Residues of enrofloxacin (0.3 - 5.1 ng/g) were detected in 21 samples (100%) of fish and in 2 samples (6%) of shrimp (1.3 and 4.7 ng/g) from South East Asia. Figure 4 shows the extracted ion chromatograms for enrofloxacin in incurred fish samples. In addition 1 sample of shrimp from Bangladesh contained a residue of lincomycin at 1.0 ng/g. By contrast no residues of enrofloxacin were detected in UK trout. Enrofloxacin is a fluoroquinolone antibiotic which is not authorised for use on fish in the UK.

The results obtained for enrofloxacin are in general agreement with reports in the literature. Enrofloxacin has been detected in monitoring studies on surface waters and sediments downstream of wastewater treatment discharge sites (Gibs *et al.,* 2013). In our study, this compound was not prioritised as a potential contaminant of fish due to the predicted low concentration in surface waters using standard exposure algorithms and this decision is consistent with the fact that no residues of enrofloxacin were detected in UK trout.

He et al. (2012) reported the occurrence of selected fluoroquinolones (norfloxacin, ciprofloxacin and enrofloxacin) in nine species of fish collected from six sites in two marine aquaculture regions of the Pearl River Delta, China. All 3 fluoroquinolones were detected in all fish samples, and the concentrations were higher in liver tissues than those in muscle tissues. Interestingly the results showed that concentrations of enrofloxacin were below the limits of quantification (LOQ) in water and sediment samples. In our study all of the fish originating from Vietnam and 2 shrimp samples also contained residues of enrofloxacin at low concentration. The presence of this fluoroquinolone in farmed shrimp from Vietnam has also been reported in the recent years (Seafish, 2011).

The fish and shrimp samples from South East Asia were also screened using a LC-time of flight (TOF) instrument against a database containing more than 3000 compounds (mainly veterinary drugs). The sensitivity of this instrument is much less than LC-MS/MS and did not permit screening as low as the 1 ng/g concentration. Residues of enrofloxacin in fish and some shrimp samples detected with the triple quadrupole instrument could not be confirmed by accurate mass data due to insufficient sensitivity, but tentative residues of phyto-oestrogens were detected.

Residues of all 6 musk compounds tested occurred at higher concentrations (up to 50 ng/g) and higher frequency (100%) in trout collected in the UK compared to imported fish from South East Asia, where 70% of the samples contained residues up to 0.3 ng/g. Shrimp imported from South East Asia were comparatively 'clean' with residues of musk xylene at 0.04 and 0.06 ng/g in two samples and musk ketone at 0.02 ng/g in one sample.

The highest concentrations of galaxolide and tonalide occurred in fish collected from fish farms located downstream of large urban sewage treatment effluent release points. As only one species of freshwater fish (trout) was investigated, species selective uptake cannot be ruled out. However, other studies on contaminants in freshwater fish indicate a greater dependence of contaminant occurrence on local pollution levels.

The occurrence of the individual musk compounds in fish and shrimp confirms the potential for human dietary exposure, and may also indicate the chronology and magnitude of usage. "Older" compounds such as musk-xylene and musk-ketone which are no longer used, were either not detected or occurred at a much lower frequency than macrolytic musk compounds such as galaxolide and tonalide. The use of these is more recent, accounting for >90% of polycyclic musk usage. Similarly, the lower occurrence of cashmeran and celestolide may reflect their lower general usage.

3.2.2 Mushrooms

Residues of trimethoprim, which is used as a potentiator for sulphonamide antibiotics, were detected at approximately 0.07-0.73 ng/g in 18 (72%) mushroom samples. Residues were found in peeled mushroom caps and peelings/stalks at similar concentrations, indicating that occurrence was not due to surface contamination. Figure 5 shows the chromatograms of mushroom samples containing incurred residues. Although trimethoprim was not detected in the selected compost samples analysed (possibly due to deficiencies of the method for composts) the relatively consistent concentration of trimethoprim throughout the mushroom does suggest possible uptake. Further analysis of the compost using improved methodology is required to attempt to detect residues in the compost, and thus provide further evidence for possible uptake.

Identification of trimethoprim was based on the ratio of two transitions in the sample compared to the ratios in a matrix matched standard. Selected samples were re-analysed using a different column and including an additional third transition in the method to provide more confidence on the identification of these residues at low concentration. For selected samples, additional evidence of identification of trimethoprim was provided by MS/MS (Thermo Scientific orbitrap technology) with measurement of the fragment ions at 35,000 resolution. The ratios of 3 transitions in the samples

were in good agreement with the ratios in solvent standards, and hence compliant with the EU guideline criteria for identification.

Trimethoprim was considered in both the HP and VMP prioritisations. The estimated concentration of the compound in chicken litter that could subsequently be used for mushroom compost production was 6.05 mg/kg. Compared to other poultry VMP's this was low with at least eleven compounds with estimated concentrations at least an order or magnitude higher. However these estimates used a 'total residue' approach and did not consider how metabolism, manure degradation and/or compost production would change the concentrations in the final litter. Trimethorpin has been detected in waters from urban and agricultural areas (Gibs *et al.*, 2013; Kasprzyk-Hordern *et al*., 2008).

No residues of trimethoprim were detected in 4 retail samples (Shiitake, Oyster, closed cup and baby button) mushrooms.

No residues of aminoglycosides were detected in mushrooms at or above 10 ng/g. The method of analysis was problematic for mushrooms and particularly compost. Without reliable detection of aminoglycosides in mushroom litter it is difficult to conclude that no uptake occurred. Mushroom samples were not analysed for musk compounds or parabens.

Selected compost samples associated to some mushroom samples were screened for veterinary drugs. The mushroom compost matrix proved to be very complex, giving rise to chemical interferences and hence the results obtained must be interpreted with caution. No residues of the sulphonamides sought were detected in the compost. This could have been as a result of poor extraction using the methods employed. Residues of coccidiostats were detected in selected composts at estimated concentrations up to 60 ng/g of DNC, 54 ng/g of monensin and 1 ng/g of narasin. Although a specific method for the analysis of coccidiostats was employed, the recovery of coccidiostats spiked into the compost matrix were variable and less than 40% indicating that the extraction efficiency for incurred residues is likely to be poor. Further investigation using a more appropriate method is required to be able to provide a more definitive conclusion. No residues of coccidiostats were detected in any of the mushroom samples analysed.

3.2.3 Bovine offal (Kidney and Liver)

Composite liver and kidney samples contained up to 5 different residues. Residues of decoquinate (0.38 and 0.49 ng/g), diclofenac (0.2 ng/g) and tilmicosin (0.35 and around 7.89 ng/g) were found in composite samples of liver. Decoquinate at 0.5 ng/g was also found in 1 composite sample of

kidney. As these residues were not unexpected the individual samples that made up the composites were not analysed.

Diclofenac was high up on the priority list for HP's because of the potential to indirectly contaminate root crops, meat and fish due to its high usage, potential for the transport to the soil compartment and high potential for the subsequent uptake by plants. Diclofenac residues have previously been detected around the world in waste waters, sewage sludge, freshwater and marine surface waters, ground waters and drinking waters (Tweari *et al.*, 2013; Felix-Cañedo *et al.,* 2013; Boxall *et al*., 2011; Thomas & Hilton, 2004; Ternes, 1998) and has recently been added to the 'watch list' as part of the Water Framework Directive.

Decoquinate also placed close to the top of the prioritisation for root crops due to its moderate potential for transport to the soil compartment and high potential for subsequent uptake into crops.

Tilmicosin, a macrolide antibiotic used for the treatment of bovine respiratory diseases, was towards the top of the prioritisations for VMP's because of the potential to transfer to the soil compartment and indirectly contaminate root crops and the high potential for gastrointestinal tract transfer. Decoquinate and tilmicosin residues have been detected in environmental waters and soils, however monitoring for these compounds has been considerably less comprehensive as for other more high profile pharmaceuticals such as diclofenac (Iglesias, 2012). Residues of tilmicosin have been reported in bovine tissues, with the highest concentrations occurring in liver and kidney of treated cattle (Donoho *et al*., 1992). Thus the residue found in one composite sample of liver is not unexpected.

Diclofenac, decoquinate and tilmicosin are all approved for use in cattle in the UK, therefore it is not possible to differentiate whether the residues detected in the bovine liver and kidney samples where a result of normal medication or indirect exposure through the contamination of food and/or drinking water.

Musk residues found in offal samples were at much lower concentrations than those detected in fish samples. In liver samples, galaxolide showed the highest concentrations (0.83 - 2.30 ng/g in 7 composite samples) followed by tonalide (0.36 - 1.19 ng/g in 3 composite samples). Residues of cashmeran, celestolide and musk-xylene were below 0.3 ng/g and not present in all samples. Musk-ketone was not detected in any of the composite samples. In the case of kidney, neither musk-xylene, musk-ketone nor cashmeran were detected in composite samples. As for liver samples the highest residue concentrations corresponded to galaxolide (0.94 ng/g - ca. 2.55 ng/g) followed by tonalide (0.22 – 0.40 ng/g) and celestolide (3 samples contained 0.02 ng/g).

3.2.4 Root and Foliage Crops

Musk compounds were the only residues detected in crops. Residues of all 6 musks were detected at or above respective reporting limits in wheat samples. Two out of 3 samples of sugar beet leaves contained cashmeran at 0.2 ng/g. None of the three samples of sugar beet roots contained musk compounds at detectable concentrations. This suggests that contamination of foliage, either by aerial deposition or from surface contact with contaminated soil is a possibility, but the reasoning can only be tentative given the low number of samples analysed.

Hu *et al*. (2010) systematically evaluated the occurrence and potential uptake of ten veterinary antibiotics by crops (radish, rape, celery and coriander) in fields amended with manure in Northern China. Uptake in different crops was variable. Radish leaves were found to contain 9 antibiotics at concentrations between 0.1-57 ng/g, whilst in roots 3 compounds were detected: oxytetracycline at 8.3 ng/g, sulfadoxine at 0.1-0.4 ng/g and lincomycin at 1.5-3.9 ng/g. Across all of the crops in this study the highest concentrations were found in leaves followed by stems and roots. In our study only a limited number of plant crop samples were analysed. Musk residues were detected in sugar beet leaves but not in the roots (data based only on 3 samples). Since sugar beet roots, compared to radish are relative large, any low concentration residues may have been further diluted by growth of the root. Migliore *et al*. (1998) found that the concentration of antibiotics was higher in roots of cereals than in leaves (wheat). In our study we did not separate the roots, stems and leaves. It is likely that the morphology and growth stages of different vegetables will have an effect on their distribution.

Peteghem *et al*. (2012) assessed the uptake of selected coccidiostats in vegetable samples (carrot, lettuce, potato, tomato and courgette) grown in spiked and manure-amended soils. One or more coccidiostats were detected in all vegetables, except tomato, cultivated on soils spiked with coccidiostats. The uptake from manure-amended soils, a more realistic scenario, was limited. Nicarbazin in carrots and monensin in lettuce were found at concentrations around 1 ng/g. Some of these coccidiostats (salinomycin and lasalocid) were included in the project multi-residue method but no residues were found in the samples analysed in this current project.

Recent studies have demonstrated the natural occurrence and subsequent uptake of chloramphenicol (CAP) into crops as a result of production by microbial populations in the soil (Berendsen *et al.*, 2010; Berendsen *et al.*, 2013). Although this chemical has not been included in this study, the analysis of plant materials to evaluate the occurrence of this compound in the UK could provide information to explain noncompliant findings of CAP in animal-derived food products and support future legislative measures to ensure the safety of the food supply.

4. Conclusions

This project has generated more than 9000 results for a list of selected human pharmaceuticals (HPs), veterinary medicines (VMs) and personal care products (PCPs) which were prioritised considering the potential to contaminate food through indirect exposure.

Multi-class and/or multi-residue methods for the analysis of the priority contaminants at low ng/g concentrations were developed and validated prior to the analysis of samples of mushrooms, vegetables, fodder crops, aquaculture products and animal tissues produced in scenarios identified as a potential risk of contamination.

Results indicate that the uptake of target chemicals was limited and found residues were mostly at low ng/g concentrations.

Some differences were observed between samples depending on the country of origin or sampling location. For instance, musk compounds tested occurred at higher concentrations and frequency in trout collected in the UK compared to imported fish and the highest concentrations were detected in fish from fish farms located downstream of large urban sewage treatment works effluent release points. On the other hand, all fish samples from Vietnam contained residues of enrofloxacin (a fluoroquinolone antibiotic) whereas the trout samples from the UK were free of fluoroquinolones. Additionally, the concentrations of parabens in trout samples were higher than those in fish from South East Asia.

Most of the mushroom samples tested contained residues of trimethoprim at very low concentration, below 1 ng/g, but the analysis of the associated compost is necessary to provide further evidence for possible uptake.

Residues of coccidiostats were detected at estimated concentrations up to ca. 50 ng/g in selected composts associated with some mushroom samples. However, residues of coccidiostats were not detected in the mushroom samples analysed. The analysis of more compost samples is necessary to draw any conclusion about the uptake.

No formal risk assessment has yet been undertaken, but on the basis of reported toxicological effects in combination with the low concentrations detected, it is unlikely that the findings will pose concerns to human health.

5. Proposals for future work

Analysis of compost associated to mushroom samples

The analysis of compost associated with the mushroom samples is essential to draw any conclusion about the possible uptake of trimethoprim, fluoroquinolines and coccidiostats. This will require the development of more suitable methods for the analysis of this highly complex matrix in an attempt to achieve more acceptable and consistent recoveries

Analysis of sulphonamide compounds in mushroom samples

Sulphasalazine and sulphadiazine were included in the multi-class, multi-residue method but no residues were detected. However, trimethoprim, frequently used as a potentiator of sulphonamide antibiotics, was detected in most of the mushroom samples analysed. Thus there is a possibility that sulphonamide compounds that have not been included in the prioritisation study could be present in the samples collected. A suitable method for the analysis of sulphonamide compounds not included in this study should be developed and applied to a selected number of mushroom samples containing trimethoprim residues.

Evaluation of the uptake of endocrine active compounds

Although estrogenic compounds were not included in the prioritised list, some residues were detected in samples from South East Asia when screened by LC-Q-ToF-MS against a database of more than 300 compounds (mainly veterinary drugs). The instrument is not particularly sensitive so it is possible that these compounds respond exceptionally well or more likely are present at relatively high concentrations. The identification and prioritisation of estrogenic compounds and the development of a suitable method for their analysis in different food matrices would provide data about their fate and uptake. Endocrine active substances that should be considered are those prioritised as Category 1 (evidence of endocrine disrupting activity in at least one species using intact animals) by the European Union. Examples include steroids, anti-oestrogens, anti-androgens and aromatase inhibitors (European Chemicals Agency, 2013).

Further investigation of difficulties experienced for the quantification of enrofloxacin incurred residues at low concentrations in aquaculture samples

Enrofloxacin was difficult to quantify by external calibration using matrix-matched standards due to an irreproducible MS response possibly caused by the effects of matrix co-extractives. Satisfactory results were obtained for some samples using the standard addition approach, but in some cases only after allowing spiked samples to stand several hours before extraction. A better understanding of the chemistry is required to assist in the development of a more robust method and to provide a more detailed explanation of the results achieved to date. Additional experiments using high resolution-mass spectrometry with ion mobility to study intra-molecular protonation may help to provide a feasible explanation to the issues with external calibration.

Further investigation of difficulties experienced for the quantification of aminoglycosides in samples of mushrooms

The analysis of aminoglycosides is extremely difficult. In this study results streptomycin, dihyrostreptomycin and spectinomycin could not be reported due to difficulties with the method. Some initial experiments with Flow injection-mass spectrometry showed promise, but this technique could not be fully evaluated because of limited access to the instrumentation within the timescale of the project. A manufacturer has offered to provide access to the instrumentation but further development is not possible without further funding.

Analysis of further samples for musk compounds

The relatively high cost of musk analysis meant that only a few samples of imported fish and plant crops could be analysed within the budget of the project. Since residues of musk compounds were detected in most samples analysed, then consideration should be given to provision of funding to allow development of a more cost effective method and hence the analysis of additional samples (including analysis of individual samples of liver), subject to the success of any developments.

Analysis of samples from other sources

Since completion of the project we have been informed that samples of vegetables cultivated on waste from on-farm anaerobic digesters or digested biosolid waste may possibly be available from a UK university. Should this prove to be the case then consideration should be given to the analysis of available samples in order to assess possible uptake.

Development of screening methods to assess occurrence and uptake of chemicals

From the literature there are a few compounds that have been reported to occur as residues in plants, but were not included in the priority list in this current study. Given the large number of possible chemicals this is inevitable. Since completion of the project new technologies have been developed for the multi-analyte screening of chemical contaminants. It would be extremely beneficial to future studies if these technologies could be implemented. Therefore consideration should be given to a proof of concept project to assess the detection, identification capabilities of emerging mass spectrometry technologies (e.g. Orbitrap, and GC-Time of Flight) and associated software. The assessment could include re-analysis of samples found to contain incurred residues in this project, to assess the detection limits, and increase the scope (i.e. to detect compounds not detected in this current project). If successful then this approach could be applied to other projects and food issues. As always, successful implementation is dependent on the new technologies providing sufficient sensitivity and selectivity.

6. References

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7. Appendices

7.1. Appendix 1: Figures

Figure 1: Potential Routes of Exposure of VMs, HPs and PCPs into Food

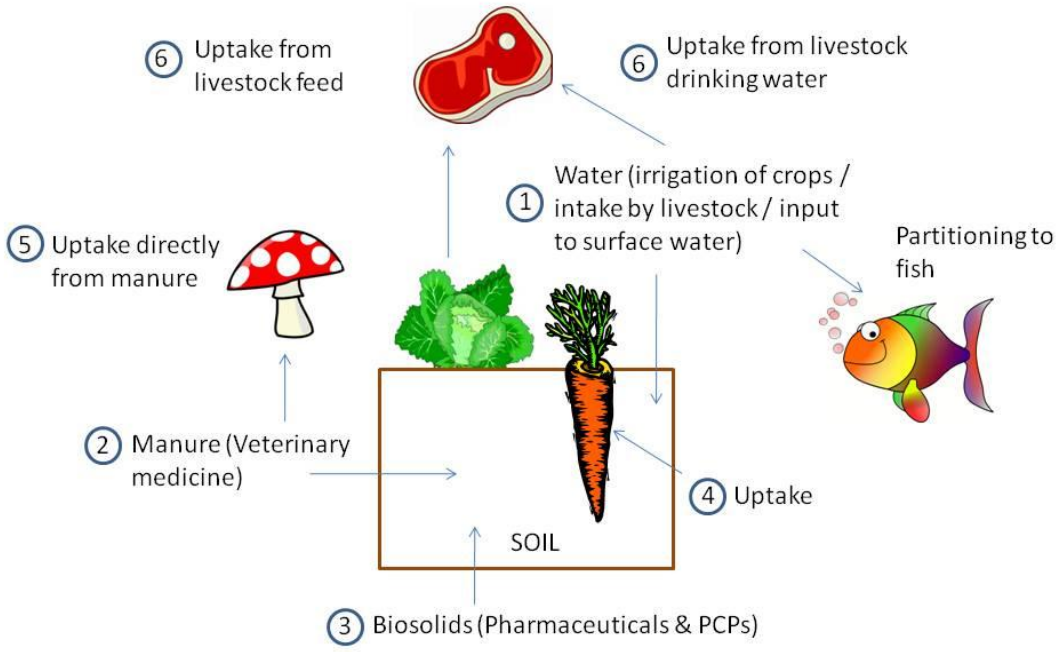


Figure 2: HRGC- HRMS Traces for Musk Compounds in a Fortified Trout Matrix

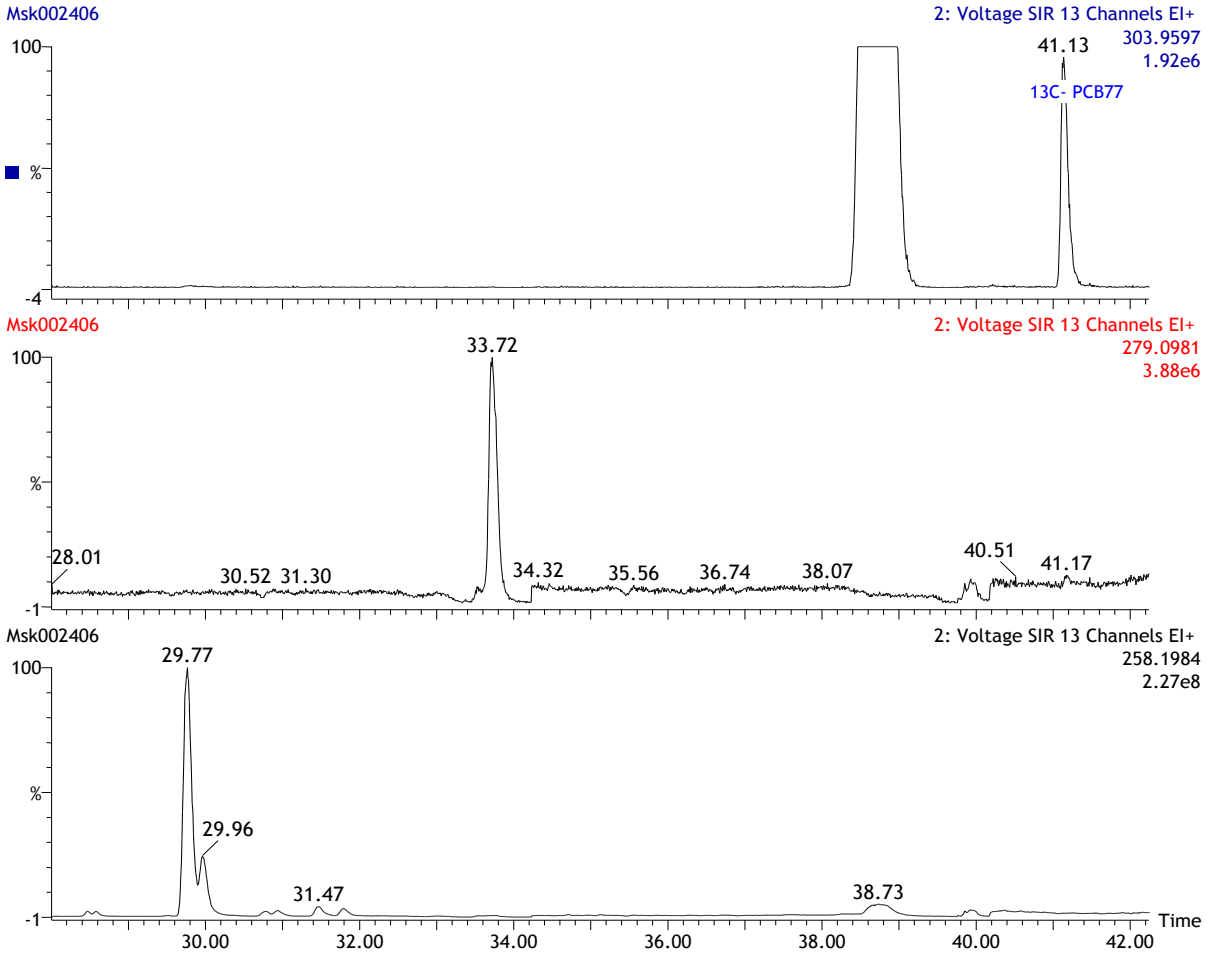
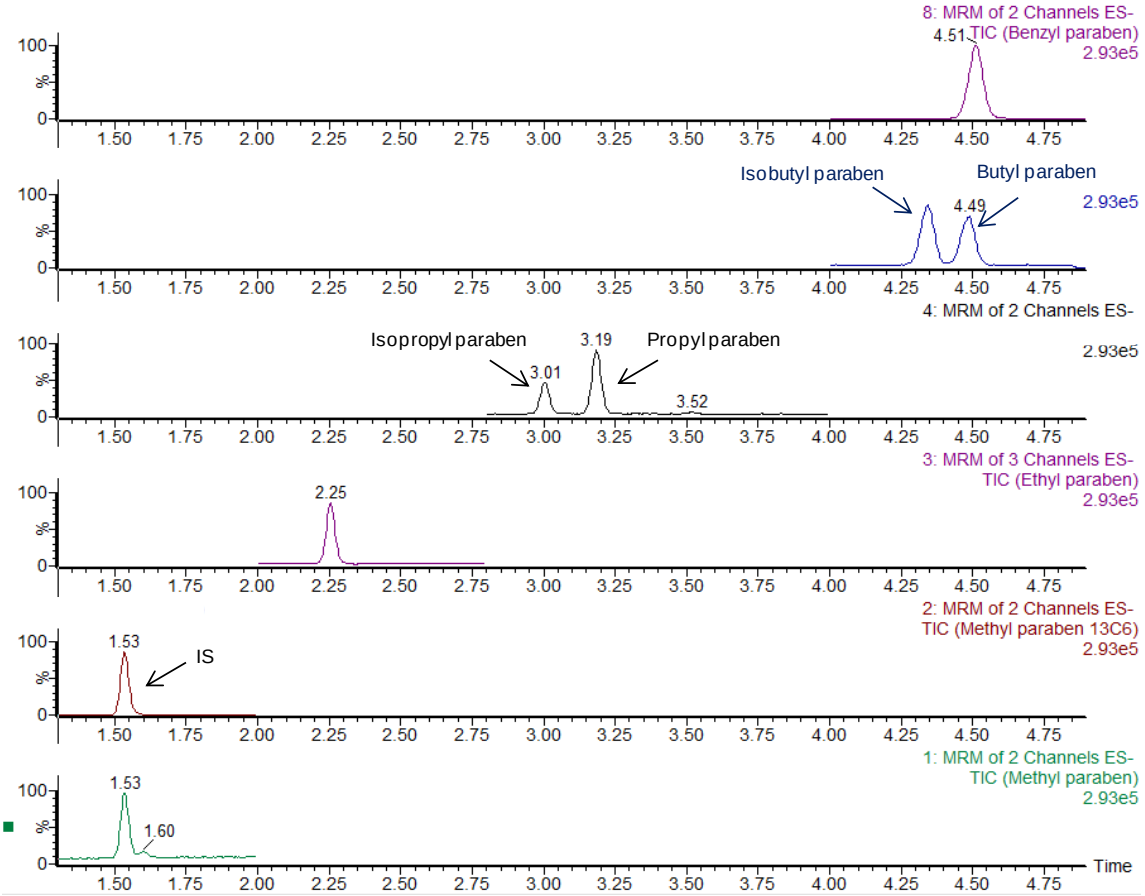


Figure 3: LC-MS/MS Chromatograms for Parabens in Fish

Total ion chromatogram (TIC) for (A) a fish sample spiked with 7 paraben compounds and the internal standard at 1 ng/g and (B) an incurred fish sample (S12-004027).

(A)



(B)

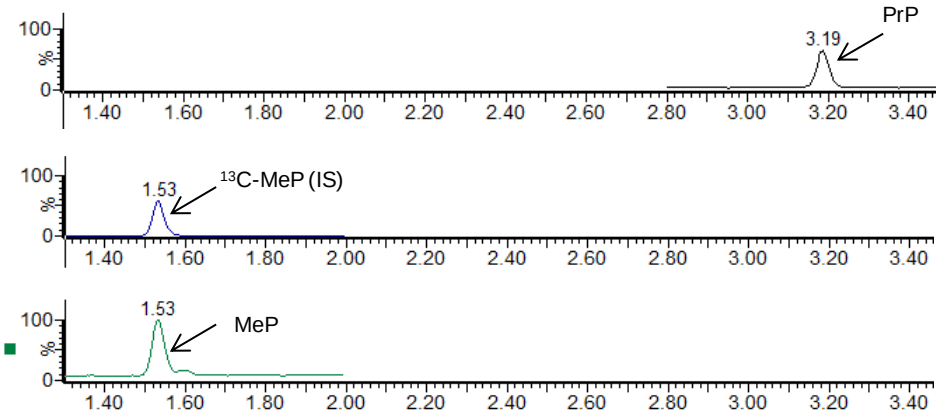


Figure 4: LC-MS/MS Chromatograms for Enrofloxacin in Fish

(Left) Overlaid LC-MS/MS extracted ion chromatograms of enrofloxacin (360 > 316) for a trout blank sample (purple trace), a matrix-matched standard at 1 ng/g (green trace) and an incurred fish sample (blue trace).

(Right) LC-MS/MS extracted ion chromatogram traces showing 2 transitions (2 top traces) corresponding to incurred residues of enrofloxacin in a fish sample from South East Asia (S12-030764) and the deuterated standard (bottom trace, 365 > 347) used for internal standardisation.

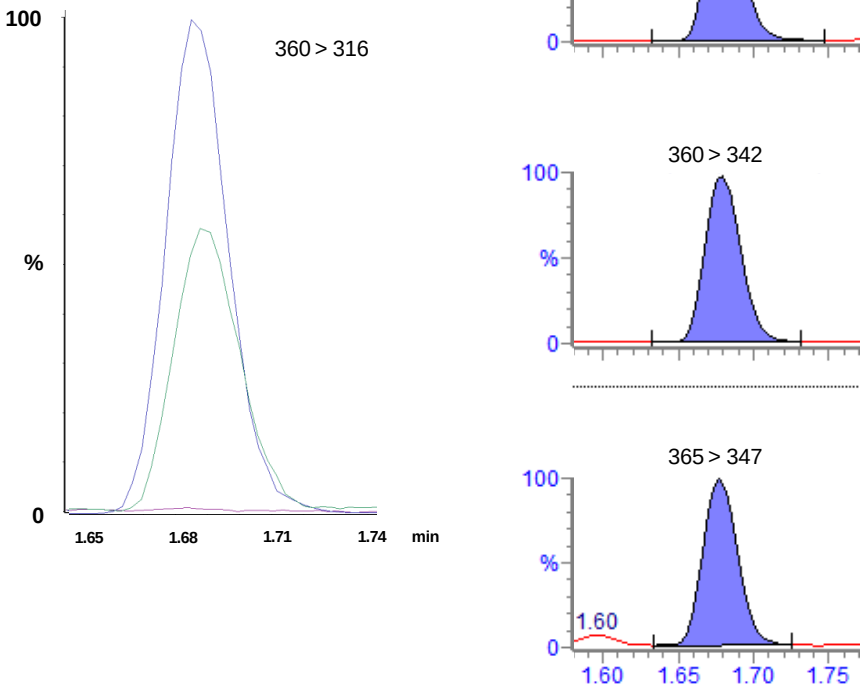
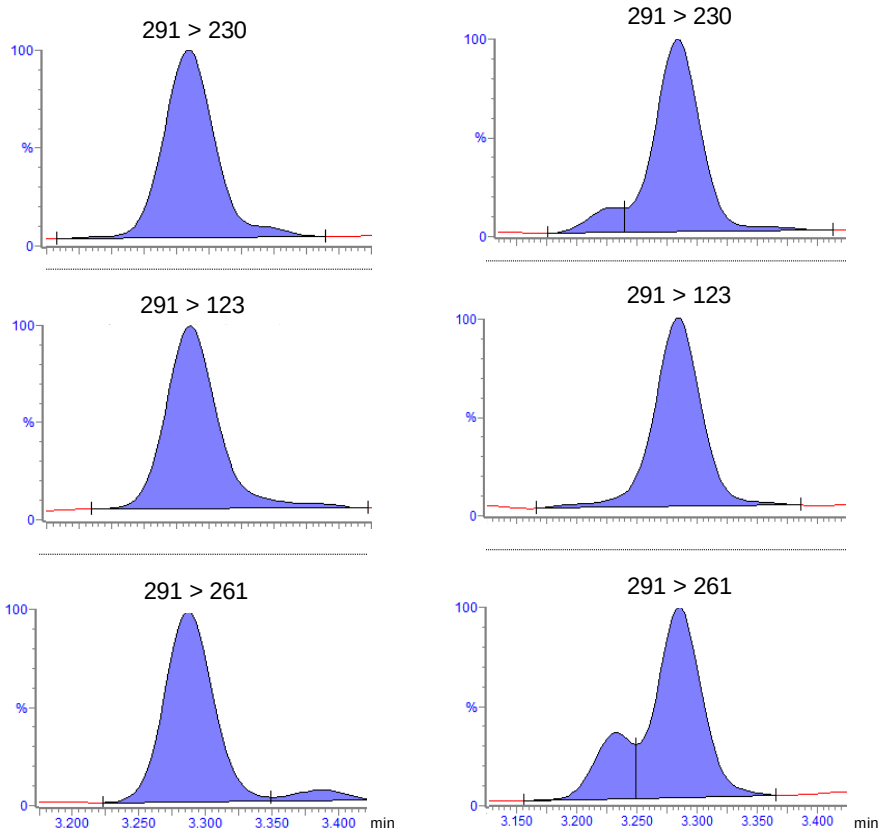


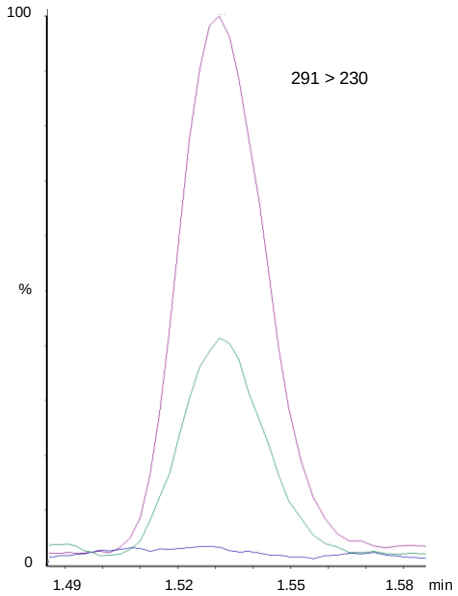
Figure 5: LC-MS/MS Chromatograms for Trimethoprim in Mushrooms

(A) LC-MS/MS chromatogram showing 3 different transitions of trimethoprim incurred residues in the cap (left) and stalk/peelings (right) of a mushroom sample (S12-042231); (B) Overlaid LC-MS/MS traces (291 > 230) for a mushroom blank sample (blue, S12-059026), a sample containing trimethoprim incurred residues (green, S12-042234) and the same sample over-spiked with 1 ng/g of trimethoprim (purple).

(A)



(B)



7.2. Appendix 2: Tables

Table 1. Overview of Analysed Commodities and Compounds Classes Tested

	Multi-residue	Aminoglycosides	Parabens	Coccidiostats	Musks
Mushrooms	✓	✓		✓	
Trout	✓		✓		✓
Imported Fish	✓		✓		✓
Imported Shrimp	✓		✓		✓
Plant crops	✓				✓
Offal	✓				✓

Table 2. Inter- batch Validation of the LC-MS/MS Multi-Analyte Method for Samples of Fish and Shrimp

		Average Recoveries % and (RSD) at 1 or 5 ng/g		
ANALYTES	**IS**	**Imported shrimp [32]**	**Imported fish [21]**	**Trout [30]**
2-Aminoflubendazole		69 (10)	66 (11)	53 (8)
Cimetidine		60 (34)	81 (21)	35 (25)
Ciprofloxacin	✓	114 (18)	92 (9)	106 (23)
Codeine		65 (19)	55 (17)	59 (11)
Danofloxacin	✓	130 (18)	78 (7)	101 (13)
Decoquinate		73 (13)	107 (17)	65 (15)
Diclofenac	✓	101 (18)	126 (21)	101 (15)
Difloxacin	✓	95 (17)	63 (15)	100 (12)
Dipyridamole		83 (10)	73 (8)	75 (16)
Doxyclycline	✓	93 (15)	83 (5)	101 (19)
Enrofloxacin	✓	*	*	93 (12)
Erythromycin	✓	96 (7)	88 (11)	102 (10)
Flubendazole	✓	96 (7)	94 (12)	101 (10)
Gliclazide		89 (6)	98 (16)	77 (14)
Irbestan		85 (7)	96 (9)	79 (9)
Lasalocid		61 (20)	75 (11)	57 (36)
Lincomycin	✓	67 (32)	57 (10)	98 (10)
Maduramycin		61 (16)	57 (11)	60 (10)
Mebeverine		74 (11)	55 (9)	74 (12)
Mefenamic acid		84 (13)	110 (9)	72 (12)
Sulphasalazine		80 (14)	ISN	ISN
Sulphadiazine	✓	96 (11)	92 (14)	104 (13)
Tilmicosin		65 (18)	43 (16)	64 (55)
Trimethoprim		69 (15)	69 (12)	59 (13)
Cefalexin		ISN	ISN	59 (49)
Chlortetracycline	✓	*	*	60 (25)
Dicyclanil		64 (19)	65 (13)	70 (11)
Florfenicol amine		ISN	ISN	47(66)
Flucloxacillin		*	*	79 (23)
Quinine		50 (23)	50 (22)	54 (6)
Robenidine hydrochloride		74 (30)	105 (17)	81 (26)
Salinomycin sodium		49 (18)	53 (9)	55 (10)
Tetracycline	✓	99 (16)	67 (26)	100 (9)
Toltrazuril sulphoxide		84 (21)	124 (16)	81 (9)
Tramadol		70 (14)	ISN	84 (27)

Notes: R% for samples spiked at 1 or 5 ng/g depending on analyte (see table 7)
IS, internal standard
Number in square brackets = number of samples analysed
* Calibration issues
ISN = insufficient S/N

Table 3. Inter-batch Validation of the LC-MS/MS Multi-Analyte Method for Mushrooms and Various Plant Commodities

		Average Recoveries % and (RSD) at 1 or 5 ng/g					
ANALYTES	**IS**	**Mushrooms [25]**	**Wheat heads [4]**	**Wheat stalks [4]**	**Oil seed rape [2]**	**Sugar beet roots [16]**	**Sugar beet leaves [16]**
2-AminoFlubendazole		78 (9)	67 (11)	68 (2)	70 (9)	89 (8)	90 (22)
Cimetidine		38 (32)	60 (16)	50 (14)	67 (17)	60 (18)	60 (18)
Ciprofloxacin	✓	83 (26)*	#	#	ISN	90 (21)	99 (14)
Codeine		59 (19)	66 (9)	75 (11)	61 (8)	75 (25)	77 (9)
Danofloxacin	✓	79 (33)*	92 (13)	95 (3)	ISN	87 (13)	125 (15)
Decoquinate		68 (14)	66 (10)	71 (7)	56 (2)	94 (14)	77 (6)
Diclofenac	✓	101 (10)	90 (7)	105 (6)	110 (0.6)	86 (6)	92 (4)
Difloxacin	✓	84 (26)*	112 (9)	106 (4)	92 (3)	65 (32)	135 (19)
Dipyridamole		84 (11)	74 (10)	78 (2)	70 (8)	98 (7)	91 (8)
Doxyclycline	✓	96 (12)	128 (3)	120 (2)	ISN	84 (7)	98 (5)
Enrofloxacin	✓	90 (16)*	98 (8)	96 (4)	66 (21)	53 (45)	74 (15)
Erythromycin	✓	105 (10)	87 (9)	95 (3)	111 (2)	91 (13)	97 (7)
Flubendazole	✓	105 (9)	89 (14)	94 (9)	78 (1)	94 (7)	104 (11)
Gliclazide		86 (10)	84 (7)	94 (7)	80 (1)	97 (7)	92 (9)
Irbestan		107 (18)	63 (12)	90 (4)	82 (0.1)	98 (7)	94 (20)
Lasalocid		69 (8)	66 (10)	71 (6)	46 (0.1)	89 (9)	72 (7)
Lincomycin	✓	70 (32)	75 (10)	76 (2)	68 (4)	55 (44)	57 (29)
Maduramycin		68 (13)	48 (10)	53 (5)	38 (1)	72 (10)	62 (7)
Mebeverine		77 (9)	74 (10)	71 (6)	49 (2)	78 (12)	82 (9)
Mefenamic acid		89 (11)	79 (9)	89 (8)	78 (0.5)	98 (7)	90 (7)
Sulphasalazine		90 (12)	86 (11)	107 (5)	ISN	87 (16)	92 (7)
Sulphadiazine	✓	103 (12)	90 (10)	98 (6)	#	92 (12)	98 (7)
Tilmicosin		73 (11)	58 (8)	55 (6)	ISN	70 (18)	83 (31)
Trimethoprim		81 (16)	70 (12)	67 (4)	ISN	84 (8)	89 (14)
Cefalexin		77 (9) *	ISN	ISN	ISN	¥	¥
Chlortetracycline	✓	83 (25)	132 (12)	100 (25)	ISN	62 (7)	81 (27)
Dicyclanil		53 (27)	55 (9)	46 (9)	49 (33)	52 (14)	73 (10)
Flucloxacillin		99 (13)	93 (15)	81 (17)	83 (37)	98 (29)	106 (24)
Quinine		35 (17)	40 (14)	23 (12)	143 (1)**	20 (33)	53 (19)
Robenidine hydrochloride		91 (30)	41 (36)	71 (4)	59 (7)	104 (15)	86 (17)
Salinomycin sodium		41 (25)	21 (16)	28 (13)	20 (8)	16 (21)	30 (31)
Tetracycline	✓	97 (13)	128 (2)	122 (3)	ISN	84 (8)	98 (5)
Tramadol		60 (32)*	ISN	ISN	ISN	88 (18)	78 (13)
Toltrazuril sulphoxide		91 (11)	86 (16)	83 (5)	87 (4)	99 (8)	112 (18)

Notes: R% for samples spiked at 1 or 5 ng/g depending on analyte (see Table 8) relative standard deviation in brackets
IS, internal standard
Number in square brackets = number of samples analysed
25 different mushroom samples: caps and stalks/peelings analysed separately for 16 samples
*The number of spiked recovery measurements used (including caps, peels, stalks but taking into account isobaric intereferences) to calculate Recovery % was variable:

- 30 for ciprofloxacin, danofloxacin, difloxacin, enrofloxacin and tilmicosin in mushrooms
- 9 for cefalexin and tramadol in mushrooms
- 4 for tramadol in sugar beet leaves

ISN = insufficient signal/noise
**R% calculated on samples over-spiked at 10 ng/g
Insufficient selectivity: interference overlapped
¥ insufficient calibration points to provide quantitative data

Table 4. Inter-batch Validation of the LC-MS/MS Method for the Analysis of Aminoglycosides and Coccidiostats in Mushrooms

		Average Recoveries % and (RSD) at 10 ng/g
AMINOGLYCOSIDES	**IS**	**Mushrooms [25]**
Apramycin		58 (20)
Dihydrostreptomycin		86 (14)
Gentamicin C1		61 (13)
Gentamicin C1a		60 (18)
Gentamicin C2+C2a		60 (13)
Kanamycin A		85 (13)
Neomycin B		27 (33)
Paromomycin		61 (9)
COCCIDIOSTATS	**IS**	**Mushrooms [29]**
Diclazuril	✓	87 (40)
Dinitrocarbanilide	✓	106 (13)
Lasalocid		61 (13)
Maduramycin		47 (16)
Monensin		74 (10)
Narasin		41 (14)
Salinomycin sodium		51 (12)

Notes: number in square brackets = number of samples analysed
IS, internal standard
25 different mushroom samples: caps and stalks/peelings analysed separately for 16 mushroom samples
R% for DHS and neomycin B based on 18 and 19 samples respectively due to insufficient S/N and/or calibration failure

Table 5. Inter-batch Validation of the LC-MS/MS Method for the Analysis of Parabens in Fish and Shrimp

		Average Recovery % and (RSD) at 1 ng/g		
PARABENS	**IS**	**Imported shrimp [32]**	**Imported fish [21]**	**Trout [30]**
Methyl paraben	✓	125 (15)	117 (13)	99 (23)
Ethyl paraben		92 (10)	80 (4)	90 (10)
Isopropyl paraben		88 (8)	81 (4)	87 (8)
Propyl paraben		89 (9)	79 (5)	86 (16)
Isobutyl paraben		87 (8)	81 (5)	85 (7)
Butyl paraben		87 (9)	79 (12)	85 (9)
Benzyl paraben		88 (8)	80 (6)	87 (8)

Notes: number in square brackets = number of samples analysed
IS, internal standard

Table 6. Inter-batch Validation of the LC-MS/MS Multi-Analyte Method for Samples of Liver and Kidney

		Average Recoveries % and (RSD) at 1 or 5 ng/g	
ANALYTES	**IS**	**Liver composite samples [19]**	**Kidney composite samples [21]**
2-AminoFlubendazole		57 (23)	65 (8)
Cimetidine		62 (22)	53 (11)
Ciprofloxacin	✓	74 (29)	60 (32)
Codeine		58 (14)	63 (9)
Danofloxacin	✓	75 (12)	75 (15)
Decoquinate		78 (11)	67 (18)
Diclofenac	✓	89 (27)	97 (15)
Difloxacin	✓	93 (18)	95 (12)*
Dipyridamole		85 (19)	127 (23)*
Doxyclycline	✓	100 (15)	93 (12)
Enrofloxacin	✓	88 (10)	90 (11)
Erythromycin	✓	89 (10)	97 (11)
Flubendazole	✓	94 (7)	95 (9)
Gliclazide		82 (9)	85 (6)
Irbestan		98 (25)	75 (7)
Lasalocid		36 (13)*	42 (12)
Lincomycin	✓	87 (16)	94 (16)
Maduramycin		61 (18)	53 (11)
Mebeverine		83 (11)	77 (14)
Mefenamic acid		64 (14)	77 (7)
Sulphasalazine		78 (14)	83 (9)
Sulphadiazine	✓	84 (13)	94 (11)
Tilmicosin		95 (19)	81 (15)
Trimethoprim		66 (9)	72 (4)
Chlortetracycline	✓	104 (27)	83 (10)
Dicyclanil		88 (22)	79 (19)
Flucloxacillin		95 (25)	74 (13)
Quinine		75 (25)	71 (12)
Robenidine hydrochloride		76 (29)	56 (21)
Salinomycin sodium		60 (17)	50 (7)
Tetracycline	✓	101 (15)	94 (10)
Tramadol		66 (20)	88 (16)
Toltrazuril sulphoxide		90 (22)	78 (12)

Notes: number in square brackets = number of samples analysed
IS, internal standard
*R% for difloxacin, dipyridamole and lasalocid is based on 18, 11 and 7 samples, respectively

Table 7. Overview of Reporting Limits obtained from the LC-MS/MS Multi-Analyte /Multi-Class Analysis of Samples of Fish and Shrimp

			Reporting limit (RL), ng/g		
			Fish		Shrimp
ANALYTES	IS	Spike, ng/g	UK trout (30)	Imported fish (21)	Imported shrimp (32)
2-Aminoflubendazole		1	< 0.2	< 0.2	< 0.2
Cimetidine		1	< 0.2	< 0.2	< 0.2
Ciprofloxacin	✓	1	< 0.2	< 0.75	< 0.2
Codeine		1	< 0.2	< 0.2	< 0.2
Danofloxacin	✓	1	< 0.2	< 0.5	< 0.2
Decoquinate		1	< 0.2	< 0.2	< 0.2
Diclofenac	✓	1	< 0.2	< 0.2	< 0.3*
Difloxacin	✓	1	< 0.2	< 0.5	< 0.2
Dipyridamole		1	< 0.2	< 0.2	< 0.2
Doxycycline	✓	1	< 0.2	< 0.2	< 0.2
Enrofloxacin	✓	1	< 0.2	< 0.2*	< 0.2*
Erythromycin	✓	1	< 0.2	< 0.2	< 0.2
Flubendazole	✓	1	< 0.2	< 0.2	< 0.2
Gliclazide		1	< 0.2	< 0.2	< 0.2
Irbestan		1	< 0.2	< 0.2	< 0.2
Lasalocid		1	< 0.2	< 0.2	< 0.2
Lincomycin	✓	1	< 0.2	< 0.2	< 0.2*
Maduramycin		1	< 0.2	< 0.2	< 0.2
Mebeverine		1	< 0.2	< 0.2	< 0.2
Mefenamic acid		1	< 0.2	< 0.2	< 0.2
Sulphasalazine		1	ISN	ISN	< 0.5
Sulphadiazine	✓	1	< 0.2	< 0.2	< 0.2
Tilmicosin		1	< 0.2	< 0.2	< 0.2
Trimethoprim		1	< 0.2	< 0.2	< 0.2
Cefalexin		5	< 1	ISN	< 5
Chlorhexidine		5	ISN	ISN	ISN
Chlortetracycline	✓	5	< 1	< 2.5	< 1
Dicyclanil		5	< 1	<1	< 1
Florfenicol amine		5	< 1	ISN	ISN
Flucloxacillin		5	< 2.5	<1	< 1
Quinine		5	< 2.5	<1	< 1
Robenidine hydrochloride		5	< 1	<1	< 1
Salinomycin sodium		5	< 1	<1	< 1
Tetracycline (TC)	✓	5	< 1	<1	< 1
Toltrazuril sulphoxide		5	< 1	<1	< 1
Tramadol		5	< 1	ISN	< 5

Notes: number in brackets = number of samples analysed
* Some samples contain potential residues at a concentration above respective reporting limit (see tables 16 and 19)
ISN = insufficient sensitivity (S/N); IS, internal standard

Origin of imported fish and shrimp was South East Asia

Table 8. Overview of Reporting Limits obtained from the LC-MS/MS Multi-Analyte /Multi-Class Analysis of Samples of Mushrooms and Various Plant Commodities

			Reporting limit (RL), ng/g			
				Plant commodities		
ANALYTES	**IS**	**Spike, ng/g**	**Mushrooms (25)**	**Wheat (4)**	**Oil seed rape (2)**	**Sugar beet (16)**
2-Aminoflubendazole		1	< 0.2	< 0.5	< 0.5	< 0.5
Cimetidine		1	< 0.2	< 0.5	< 1	< 0.5
Ciprofloxacin	✓	1	< 0.5 #1	< 5 #3	< 2.5	<1
Codeine		1	< 0.5	< 0.5	< 1	< 0.5
Danofloxacin	✓	1	< 0.2 #1	< 0.5	< 2.5	< 1
Decoquinate		1	< 0.2	< 0.5	< 0.5	< 0.5
Diclofenac	✓	1	< 0.2	< 0.9	< 0.7	< 0.5
Difloxacin	✓	1	< 0.5*	< 0.5	< 1	< 0.5
Dipyridamole		1	< 0.2	< 0.5	< 0.5	< 0.5
Doxycycline	✓	1	< 0.2	< 0.5	< 1	< 0.5
Enrofloxacin	✓	1	< 0.5	< 0.5	<1	< 0.5
Erythromycin	✓	1	< 0.2	< 0.5	< 0.5	< 0.5
Flubendazole	✓	1	< 0.2	< 0.5	< 0.5	< 0.5
Gliclazide		1	< 0.2	< 0.5	< 0.5	< 0.5
Irbestan		1	< 0.2	< 0.5	< 0.5	< 0.5
Lasalocid		1	< 0.2	< 0.5	< 0.5	< 0.5
Lincomycin	✓	1	< 0.2	< 0.5	< 0.5	< 0.5
Maduramycin		1	< 0.2	< 0.5	< 0.5	< 0.5
Mebeverine		1	< 0.2	< 0.5	< 0.5	< 0.5
Mefenamic acid		1	< 0.2	< 0.5	< 0.5	< 0.5
Sulphasalazine		1	< 0.5	< 0.5	< 5	< 0.5
Sulphadiazine	✓	1	< 0.2	< 0.5	NR	< 0.5
Tilmicosin		1	< 0.2	< 0.5	< 5	< 0.5
Trimethoprim		1	< 0.7*	< 0.5	< 5	< 0.5
Cefalexin		5	< 5 #2	< 5	< 5	< 2.5
Chlorhexidine		5	ISN	ISN	ISN	ISN
Chlortetracycline	✓	5	< 1	< 2.5	< 2.5	< 2.5
Dicyclanil		5	< 1	< 2.5	< 2.5	< 2.5
Florfenicol amine		5	ISN	ISN	ISN	ISN
Flucloxacillin		5	< 1	< 2.5	< 2.5	< 2.5
Quinine		5	< 1	< 2.5	< 5	< 2.5
Robenidine hydrochloride		5	< 1	< 2.5	< 2.5	< 2.5
Salinomycin sodium		5	< 1	< 2.5	< 2.5	< 2.5
Tetracycline	✓	5	< 1	< 2.5	< 2.5	< 2.5
Toltrazuril sulphoxide		5	< 1	< 2.5	< 2.5	< 2.5
Tramadol		5	< 1	< 5	ISN	< 2.5

Notes: number in brackets = number of samples analysed
* Some samples contain potential residues at a concentration above respective reporting limit (see tables 17 and 20); ISN = insufficient sensitivity (S/N); NR = not reportable due to interference
#1 RL < 2.5 ng/g for one of the batches; #2 ISN for one batch; #3 not reportable for stalks; IS, internal standard
Mushroom samples: caps and peelings/stalks were analysed separately for 16 samples; Sugar beet samples: roots and foliage were analysed separately; Wheat samples: heads and stalks were analysed separately

Table 9. Overview of Reporting Limits obtained from the LC-MS/MS Multi-Analyte/Multi-Class Analysis of Bovine Offal

			Reporting limit (RL), ng/g	
ANALYTES	**IS**	**Spike, ng/g**	**Liver Composite samples (19)**	**Kidney Composite samples (21)**
2-Aminoflubendazole		1	< 0.5	< 0.2
Cimetidine		1	< 0.5	< 0.2
Ciprofloxacin	✓	1	< 1	< 1
Codeine		1	< 0.2	< 0.2
Danofloxacin	✓	1	< 0.2	< 1
Decoquinate		1	< 0.3 *	< 0.2*
Diclofenac	✓	1	< 0.2	< 0.2
Difloxacin	✓	1	< 0.2	< 1
Dipyridamole		1	< 0.2	< 0.2
Doxycycline	✓	1	< 0.2	< 1
Enrofloxacin	✓	1	< 0.2	< 0.5
Erythromycin	✓	1	< 0.2	< 0.2
Flubendazole	✓	1	< 0.2	< 0.2
Gliclazide		1	< 0.2	< 0.2
Irbestan		1	< 0.2	< 0.2
Lasalocid		1	< 1.3	< 0.2
Lincomycin	✓	1	< 0.2	< 0.2
Maduramycin		1	< 0.2	< 0.2
Mebeverine		1	< 0.2	< 0.2
Mefenamic acid		1	< 0.2	< 0.2
Sulphasalazine		1	< 0.2	< 1
Sulphadiazine	✓	1	< 0.2	< 0.2
Tilmicosin		1	< 0.2*	< 0.2
Trimethoprim		1	< 0.2*	< 0.2
Cefalexin		5	ISN	ISN
Chlorhexidine		5	ISN	ISN
Chlortetracycline	✓	5	< 2.5	< 1
Dicyclanil		5	< 1	< 2.5
Florfenicol amine		5	ISN	ISN
Flucloxacillin		5	< 1	< 1
Quinine		5	< 1	< 1
Robenidine hydrochloride		5	< 1	< 1
Salinomycin sodium		5	< 1	< 1
Tetracycline (TC)	✓	5	< 1	< 5
Toltrazuril sulphoxide		5	< 1	< 1
Tramadol		5	< 1	< 1

Notes: number in brackets = number of samples analysed
ISN, insufficient sensitivity (S/N); IS, internal standard
*Some samples contain potential residues at a concentration above respective reporting limit (see tables 18 and 21)
Reporting limits provided in the table correspond to composite sample. Individual samples reporting limits are 5 times higher.

Table 10. Overview of Reporting Limits obtained for Parabens from the LC-MS/MS Analysis of Fish and Shrimp

			Reporting limit (RL), ng/g		
PARABENS	**IS**	**Spike, ng/g**	**Imported shrimp (32)**	**Imported fish (21)**	**UK trout (30)**
Methyl paraben	✓	1	< 0.4 or 1*	< 0.5	< 0.6*
Ethyl paraben		1	< 1.2	< 0.2	< 0.3
Isopropyl paraben		1	< 0.2	< 0.2	< 0.2
Propyl paraben		1	< 0.2*	< 0.5	< 0.4*
Isobutyl paraben		1	< 0.2	< 0.2	< 0.2
Butyl paraben		1	< 0.2	< 0.2	< 0.2
Benzyl paraben		1	< 0.2	< 0.2	< 0.2

Notes: number in brackets = number of samples analysed
IS, internal standard
* Some samples contain potential residues at a concentration above respective reporting limit (see tables16 and 19)
Origin of imported fish and shrimp was South East Asia

Table 11. Overview of Reporting Limits obtained for Aminoglycosides from the LC-MS/MS Analysis of Mushrooms

			Reporting limit (RL), ng/g
AMINOGLYCOSIDES	**IS**	**Spike, ng/g**	**Mushrooms (25)**
Apramycin		10	< 5
Dihydrostreptomycin		10	ISN
Gentamicin C1		10	< 5
Gentamicin C1a		10	< 5
Gentamicin C2+C2a		10	< 5
Kanamycin A		10	< 5
Neomycin B		10	< 5
Paromomycin		10	< 5
Spectinomycin		10	ISN
Streptomycin		10	ISN

Notes: number in brackets = number of samples analysed
IS, internal standard
ISN, insufficient sensitivity (S/N)

Table 12. Overview of Reporting Limits obtained for Coccidiostats from the LC-MS/MS Analysis of Mushrooms

			Reporting limit (RL), ng/g
COCCIDIOSTATS	**IS**	**Spike, ng/g**	**Mushrooms (25)**
Diclazuril	✓	1	< 0.2
Dinitrocarbanilide	✓	1	< 0.2
Lasalocid		1	< 0.2
Maduramycin		1	< 0.2
Monensin		1	< 0.2
Narasin		1	< 0.2
Salinomycin sodium		1	< 0.2

Notes: number in brackets = number of samples analysed
IS, internal standard

Table 13. Overview of Reporting Limits obtained for Musk Compounds from the GC-MS Analysis of Samples of Fish and Shrimp

	Reporting limit (RL), ng/g		
MUSKS	**Imported Shrimp (10)**	**Imported Fish (10)**	**Trout (29)**
Cashmeran	<0.01	<0.07*	< 0.09
Celestolide (ADBI)	<0.01	<0.01*	< 0.08
Galaxolide (HHCB)	<0.68	<0.91*	< 1.12
Tonalide (AGTN)	<0.26	<0.15*	< 1.01
Musk-xylene	<0.03*	<0.02*	<0.03
Musk-ketone	<0.02*	<0.01*	<0.02

Notes: number in brackets = number of samples analysed
* Some samples contain potential residues at a concentration above respective reporting limit (see table 19)

Table 14. Overview of Reporting Limits obtained for Musk Compounds from the GC-MS Analysis of Samples of Liver and Kidney

	Reporting limit (RL), ng/g	
ANALYTES	**Composite liver samples (17)**	**Composite kidney samples (18)**
Cashmeran	< 0.11	< 0.02 - < 0.04
Celestolide	< 0.01 or < 0.03	< 0.01
Galaxolide	<0.82 or < 1.17	< 0.89 or < 0.93
Tonalide	< 0.32 or < 0.45	< 0.19 - < 0.24
Musk-xylene	< 0.02 - < 0.06	< 0.01 - < 0.03
Musk-ketone	< 0.02 or < 0.03	< 0.01

Notes: number in brackets = number of samples analysed
* Some samples contain potential residues at a concentration above respective reporting limit (see tables 18 and 21)

Table 15. Overview of Reporting Limits obtained for Musk Compounds from the GC-MS Analysis of Samples of Plant Crops

	Reporting limit (RL), ng/g		
MUSKS	**Wheat (2)**	**Sugar Beet Roots (3)**	**Sugar Beet Leaves (3)**
Cashmeran	<0.24*	<0.13	<0.08*
Celestolide (ADBI)	<0.02*	<0.01	<0.01
Galaxolide (HHCB)	<2.84*	<1.5	<0.97
Tonalide (AGTN)	<0.41*	<0.22	<0.14
Musk-xylene	<0.06*	<0.03	<0.02
Musk-ketone	<0.02	<0.01	<0.01

Notes: number in brackets = number of samples analysed
Sugar beet samples: roots and foliage were analysed separately
Wheat samples: heads and stalks were analysed separately
* Some samples contain potential residues at a concentration above respective reporting limit (see table 17)

Table 16. Residues found in Samples of Fish and Shrimp

	Residues, ng/g		
ANALYTES	**UK trout**	**Imported Fish**	**Imported Shrimp**
Enrofloxacin		0.35i-5.05* (21)	1.34i, 4.72i
Lincomycin			1.10
Methyl paraben	0.60-2.84 (23)		0.36i-4.98 (8)
Propyl paraben	0.40-1.30 (19)		7.35i
Cashmeran	0.10 - 2.8 (14)	0.09, 0.22	
Celestolide (ADBI)	0.08 - 0.94 (29)	0.01 (2)	
Galaxolide (HHCB)	1.68 - 50.3 (24)	1.10	
Tonalide (AGTN)	1.01 - 34.3 (29)	0.15-0.26 (6)	
Musk-xylene	0.03 - 0.73 (24)	0.02-0.06 (4)	0.04, 0.06
Musk-ketone	0.02 - 1.96 (23)	0.01-0.03 (4)	0.02i

Notes: number in brackets = number of samples containing residues above RL
i: indicative value (residues concentration outside calibration range or calibration issues)

Table 17. Residues found in Samples of Mushrooms and Plant Commodities

	Residues, ng/g		
ANALYTES	**Mushrooms**	**Wheat**	**Sugar Beet Leaves**
Trimethoprim	0.07i-0.73 (18)		
Cashmeran		0.26i	0.16, 0.22
Celestolide (ADBI)		0.02-0.05 (2)	
Galaxolide (HHCB)		3.65, 4.46	
Tonalide (AGTN)		0.44, 0.62	
Musk-xylene		0.09	

Notes: number in brackets = number of samples containing residues above RL
i: indicative value (residues concentration outside calibration range)
Sugar beet samples: roots and foliage were analysed separately
Wheat samples: heads and stalks were analysed separately

Table 18. Residues found in Composite Samples of Liver and Kidney

	Residues, ng/g	
ANALYTES	**Composite liver samples**	**Composite kidney samples**
Decoquinate	0.38, 0.49	0.51
Diclofenac	0.20, 0.23	
Tilmicosin	0.35, 7.89i	
Cashmeran	0.11 - 0.27 (11)	
Celestolide	0.01 - 0.13i (4)	0.02 (3)
Galaxolide	0.83 - 2.30 (7)	0.94 - 2.55i (6)
Tonalide	0.36 - 1.19 (3)	0.22 - 0.40 (9)
Musk-xylene	0.05 - 0.15 (10)	

Notes: number in brackets = number of samples containing residues above RL
i: indicative value (residues concentration outside calibration range)
Individual samples have not been analysed

Table 19. Overview of all Residues detected at or above the Reporting Limits in Samples of Fish and Shrimp

		Residues, ng/g									
		UK trout									
Sample ID	**Scenario**	**MeP**	**PrP**	**Cashmeran**	**ADBI**	**HHCB**	**AHTN**	**Musk-xylene**	**Musk-ketone**	**Enrofloxacin**	**Lincomycin**
S12-003995	VMs, cattle and pig culture, UK	0.97			0.17		2.55	0.03	0.04i		
S12-003996	VMs, cattle and pig culture, UK	0.93									
S12-003997	VMs, cattle and pig culture, UK	1.98	0.45		0.13		1.53	0.03	0.04		
S12-003998	VMs, cattle and pig culture, UK	1.00		0.35i	0.20	13.63	5.65	0.06	0.18		
S12-003999	VMs, cattle and pig culture, UK	1.05		0.37i	0.35	13.70	5.42	0.07	0.17		
S12-004000	VMs, cattle and pig culture, UK	2.06	0.73		0.35	13.70	5.42	0.07	0.17		
S12-004024	Downstream STP, UK	1.06	0.47	1.50i	0.37	3.06	19.86	0.69	1.28		
S12-004025	Downstream STP, UK	2.84	0.98	2.79i	0.40	3.77	22.49	0.73	1.31		
S12-004026	Downstream STP, UK	0.85		0.83i	0.94	27.75	34.3	0.65	1.05i		
S12-004027	Downstream STP, UK	1.97	0.66	0.41	0.59	50.27	29.68	0.15	0.25i		
S12-004028	Downstream STP, UK	0.67	0.42	0.28	0.48	44.34	14.47	0.15i	0.55		
S12-004029	Downstream STP, UK	0.98	0.46		0.34	32.37	8.57	0.11i	0.83		
S12-004030	Downstream STP, UK	0.69		0.22	0.38	37.26	5.02	0.15i	0.65		
S12-004031	Downstream STP, UK	0.70		0.49	0.57	48.81	8.14	0.13i	0.32		
S12-004032	Downstream STP, UK	0.81	0.48	0.28	0.48	34.26	9.63	0.11i	0.21i		
S12-004089	VMs, pig culture, UK			0.10	0.14	4.75	1.27				
S12-004090	VMs, pig culture, UK		0.46		0.14	6.25	1.5				
S12-004091	VMs, pig culture, UK		0.44	0.18	0.12	5.42	1.4				
S12-004092	VMs, pig culture, UK	0.60	0.88	0.12	0.22	14.75	3.73				
S12-004093	VMs, pig culture, UK	0.60	0.44		0.09	2.03i	1.80	0.11	0.09		
S12-004094	VMs, pig culture, UK				0.09		1.25	0.08	0.08		
S12-004095	Downstream STP, UK	0.70	0.51		0.23	9.25	3.75	0.14	0.18		
S12-004096	Downstream STP, UK	1.12	0.73		0.3	12.74	3.86	0.15	0.36i		

Table 19. Overview of all Residues detected at or above the Reporting Limits in Samples of Fish and Shrimp (*continued*)

Sample ID	Scenario	MeP	PrP	Cashmeran	ADBI	HHCB	AHTN	Musk-xylene	Musk-ketone	Enrofloxacin	Lincomycin
S12-004097	Downstream STP, UK		0.50		0.15	1.86	2.89	0.20i	0.18i		
S12-004098	VMs, pig culture, UK		1.30		0.11	1.60	1.01	0.04			
S12-004099	VMs, pig culture, UK	0.82	0.40		0.16		1.81	0.07			
S12-004100	VMs, pig culture, UK	0.63			0.08		0.74				
S12-004101	Downstream STP, UK	2.10	0.61		0.21	2.98	8.88	0.31	1.70		
S12-004102	Downstream STP, UK		0.89		0.29	9.05	14.97	0.35	1.96		
S12-004103	Downstream STP, UK	0.63			0.34	24.85	10.34	0.13i	0.29		
							Imported farmed fish				
Sample ID	**Scenario**	**MeP**	**PrP**	**Cashmeran**	**ADBI**	**HHCB**	**AHTN**	**Musk-xylene**	**Musk-ketone**	**Enrofloxacin**	**Lincomycin**
S12-028338	Vietnam									0.73i	
S12-028339	Vietnam									*1.813 by SA*	
S12-030746	Vietnam									0.35i	
S12-030763	Vietnam				0.01		0.19			*5.05 by SA*	
S12-030764	Vietnam									*1.61 by SA*	
S12-030771	Vietnam						0.19	0.02	0.01	*1.10 by SA*	
S12-030774	Vietnam			0.22i	0.01		0.26		0.01	1.52i	
S12-030789	Vietnam									*3.24 by SA*	
S12-030791	Vietnam									*3.42 by SA*	
S12-030792	Vietnam				0.01		0.22	0.06	0.02	*2.31 by SA*	
S12-030807	Vietnam									0.97i	
S12-030811	Vietnam			0.09				0.03	0.02	0.43i	
S12-032600	Vietnam									0.35i	
S12-032610	Vietnam									0.63i	
S12-032627	Vietnam						0.22		0.03	1.50i	
S12-032640	Vietnam									2.45i	
S12-032641	Vietnam					1.10	0.15	0.03		1.04i	

Table 19. Overview of all Residues detected at or above the Reporting Limits in Samples of Fish and Shrimp (*continued*)

Sample ID	Scenario	MeP	PrP	Cashmeran	ADBI	HHCB	AHTN	Musk-xylene	Musk-ketone	Enrofloxacin	Lincomycin
S12-032643	Vietnam									2.20i	
S12-032657	Vietnam									1.29	
S12-032679	Vietnam									1.83i	
S12-032695	Vietnam									0.41i	
							Imported farmed shrimp				
Sample ID	**Scenario**	**MeP**	**PrP**	**Cashmeran**	**ADBI**	**HHCB**	**AHTN**	**Musk-xylene**	**Musk-ketone**	**Enrofloxacin**	**Lincomycin**
S12-028321	Vietnam	0.55									
S12-028322	Vietnam	4.98	7.35i					0.06			
S12-028346	India	0.36									
S12-028347	Bangladesh										1.10
S12-030768	India	0.64									
S12-30785	Vietnam									1.34i	
S12-032595	Vietnam	1.08i									
S12-032596	Vietnam									4.72i	
S12-032615	Thailand	0.84i									
S12-032620	Vietnam							0.04	0.02i		
S12-032673	India	0.72i									
S12-032674	Thailand	0.87i									

Notes: i: indicative value (residues concentration outside calibration range or calibration issues)
MeP: methyl paraben; PrP: propyl paraben; ADBI: cestolide; HHCB: galaxolide; AHTN: tonalide;
SA, standard addition
Residues in italics have been confirmed by a 2nd analysis

Table 20. Overview of all Residues detected at or above the Reporting Limits in Mushroom Samples

	Residues, ng/g
Sample ID	**Mushrooms**
S12-003589	Trimethoprim (0.09i)
S12-003591	Trimethoprim (0.14i)
S12-003593	Trimethoprim (0.15i)
S12-003595	Trimethoprim (0.19i)
S12-003597	Trimethoprim (0.15i)
S12-003599	Trimethoprim (0.07i)
S12-004068	Trimethoprim (0.17i)
S12-004070	Trimethoprim (0.36i)
S12-004072	Trimethoprim (0.20i)
S12-042229	Trimethoprim (*0.24 caps*/*0.23i s+p*)
S12-042230	Trimethoprim (*0.36 caps*/*0.27i s+p*)
S12-042231	Trimethoprim (*0.18 caps*/*0.24i s+p*)
S12-042232	Trimethoprim (0.24i caps/ 0.21i s+p)
S12-042234	Trimethoprim (*0.40 caps*/0.22i s+p)
S12-042235	Trimethoprim (*0.73 caps*/0.35i s+p)
S12-042236	Trimethoprim (*0.44 caps*/0.33i s+p)
S12-042237	Trimethoprim (*0.54 caps*/0.33i s+p)
S12-059024	Trimethoprim (0.46)

Notes: number in brackets = concentration in ng/g
i: indicative value (residues concentration outside calibration range)
S+P, stalks/peelings
Residues in italics have been confirmed by a 2nd analysis

Table 21. Overview of all Residues detected at or above the Reporting Limits in Bovine Offal Samples

	Residues, ng/g								
	Composite liver samples								
Sample ID	Diclofenac	Tilmicosin	Decoquinate	Trimethoprim	Cashmeran	ADBI	HHCB	AHTN	Musk-xylene
S12-017624	0.20								
S12-017624						0.01	0.83		0.08
S12-017626	0.23								
S12-017626									0.05
S12-017772		7.89i			0.27	0.05	2.30	1.19	0.11
S12-017774		0.35			0.11				0.10
S12-017972					0.14	0.03			
S12-017974					0.13	0.13i	0.95		0.08
S12-017976					0.17		1.28		0.15
S12-018639					0.12				
S12-018640					0.29				0.09
S12-018642					0.12				
S12-018644							0.87	0.38	0.05
S12-018646			0.49	0.63					
S12-018646					0.14				0.07
S12-018648					0.16				
S12-018676							0.89		
S12-019489					0.13		1.08	0.36	0.06
S12-045062			0.38						

Table 21. Overview of all Residues detected at or above the Reporting Limits in Bovine Offal Samples (*continued*)

	Composite kidney samples								
Sample ID	**Diclofenac**	**Tilmicosin**	**Decoquinate**	**Trimethoprim**	**Cashmeran**	**ADBI**	**HHCB**	**AHTN**	**Musk-xylene**
S12-045063			0.51						
S12-017625							2.55i		
S12-017627								0.23	
S12-017773							1.42i	0.40	
S12-017775								0.29	
S12-017975							0.94	0.29	
S12-017977						0.02		0.30	
S12-018641							1.45	0.23	
S12-018643							1.31i	0.22	
S12-018645								0.22	
S12-018647							1.22	0.25	
S12-019349						0.02			
S12-019490						0.02			

Notes: number in brackets = concentration in ng/g
i: indicative value (residues concentration outside calibration range)
ADBI: celestolide; HHCB: galaxolide; AHTN: tonalide;
i: indicative value

Table 22. Overview of all Residues detected at or above the Reporting Limits in Plant Samples

	Residues, ng/g				
	Sugar Beet Leaves				
Sample ID	Cashmeran	ADBI	HHCB	AHTN	Musk-xylene
S12-042868	0.22				
S12-042876	0.16				
	Wheat				
Sample ID	Cashmeran	ADBI	HHCB	AHTN	Musk-xylene
S12-030452		0.03			0.09
S12-030460 (heads)		0.02	3.65	0.44	
S12-030460 (stalks)	0.26i	0.05	4.46	0.62	

Notes: number in brackets = concentration in ng/g
i: indicative value (residues concentration outside calibration range)
ADBI: cestolide; HHCB: galaxolide; AHTN: tonalide

7.3. Appendix 3: Prioritisation report

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This report has been prepared by FERA after exercise of all reasonable care and skill, but is provided without liability in its application and use.

Opinions and interpretation are outside the scope of UKAS accreditation.