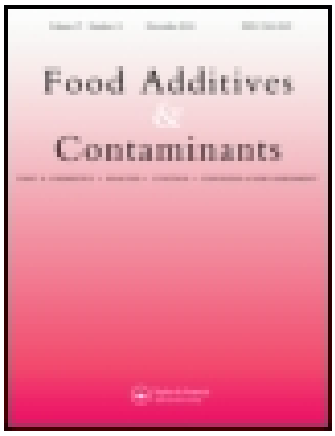

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Development and validation of a multi-residue screening method for veterinary drugs, their metabolites and pesticide in meat using liquid chromatography - tandem mass spectrometry

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Development and validation of a multi-residue screening method for veterinary drugs, their metabolites and pesticide in meat using liquid chromatography - tandem mass spectrometry

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ABSTRACT

A rapid multi-residue screening method which includes 128 veterinary anti-parasitic drugs and metabolites in meat of chicken, porcine and bovine has been developed. The scope of the method focuses on screening the following main families of veterinary anti-parasitic drugs: avermectines, benzimidazoles, the polyether ionophore, anti-tapeworm, anti-trematode, anti-piroplasmosis and chemical classes of coccodiostats. The method described QuEChERS sample preparation procedure prior to LC-MS/MS analysis. The modified QuEChERS technology minimizes the sample complexity and ion suppression effects. The method was validated according to the

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EU guidelines (2002/657/EC) for a quantitative screening method. The validation results demonstrate that the described LC-MS/MS method provides sensitive, repeatable and meeting the residue screening monitoring requirements.

Key words: Veterinary antiparasite drugs; Screening; QuEChERS; HPLC-MS/MS; Multi-residues

1 Introduction

Veterinary anti-parasites are a kind of common medicine which can kill or/and remove parasite in animal vivo and/or vitro, or many other effects such as improving feed conversion rates. According to the pharmacological effects, they can also be divided into anthelmintic, coccidiostats and pesticide, and so on. Coccidiostats are widely used for prevention and treatment of coccidiosis, a disease, which causes serious economic consequences in animal production. Veterinary anti-parasites play an important role on animal husbandry, but incorrect use of drugs in veterinary anti-parasites practice may leave residues in edible tissues (McKellar, & Scott, 1990; Mohammad, Faris, Rhayma, & Ahmed, 2006; Lankas, & Peter, 1992). These residues may have direct toxic effects on consumer e.g. allergic reaction in hypersensitive individuals, or for example antibiotics may cause problems indirectly through induction of resistant strains of bacteria. The occurrence of unavoidable carry-over of feed additives in non-target feed may result in the presence of residues of veterinary anti-parasites in animal products like meat. The European Commission has set maximum levels (MLs) for coccidiostats in various foods from non-target animals [EMEA, 2377/90 (EEC), 1996, 1998, 2000, 2003, 2004, 2007].

In order to detect such residues in food and tissues, microbiological or bioassay techniques are widely used as screening methods. These methods generally do not distinguish between members of a class of veterinary anti-parasitic, but provide a semi-quantitative estimate of 'total' residues detected. A way to improve cost-effectiveness is maximize the number of analytes that may be determined by a single procedure or from a single portion of test material. Such an approach is extremely effective when multi-compound techniques, such as liquid chromatography in combination with triple-quadrupole mass-spectrometric detection are used (Romero-González et al., 2011). There were many reports for multi-compound screening of veterinary drugs in animal tissue or product. A very interesting example for multi-compound screening of veterinary drugs in urine is described by Kaufmann (Kaufmann, et al., 2007). These methods covered more than 100 analytes belonging to different classes of veterinary drugs. But most of the interested coumpounds were antibiotic drugs, only including few of veterinary anti-parasite drugs. Whelan (Whelan et al., 2010) developed a quantitative and confirmatory method for 38 kinds of anti-parasites drugs including benzimidazoles, abamectin, and other anthelmintic drugs residues in milk using acetonitrile as extracting solvent and purified by dispersive solid phase extraction (D-SPE) as it is quicker and easier than traditional solid phase. It enables clean extracts to be obtained for the multiresidue analysis in an easy and quick procedure. The D-SPE involves, in general, the use of one or more sorbents together with magnesium sulfate anhydrous in a centrifuge tube to remove the residual water and the co-extractives from the organic extract. D-SPE reduces extraction time, troublesome, costs, solvent, waste, glassware and minimal training is required compared to SPE. Kinsella (Kinsella et al., 2009; Kinsella et al., 2010) developed another quantitative and confirmatory method for 38 kinds of antiparasites

drugs including benzimidazoles, macrolides, and other anthelmintic drugs residue in bovine liver and milk.

Sample preparation is the major bottleneck in analytical procedure for the screening determination of veterinary residues in animal-derived food. The complex biological matrix and various compounds with defferent chemical properties make sample preparation a challenge. The QuEChERS (quick, easy, cheap, effective, rugged and safe) method was initially applied to pesticide residue analysis. The QuEChERS approach has many advantages over extraction and clean-up strategies, because it could simplifies analytical and reduces the time (Anastassiades et al., 2003). And it is a flexible method that permits modifications depending on analytes, matrices or analyst preferences (León et al., 2012; Jeong et al., 2012). The QuEChERS approach consist of two steps: quick, easy extractions procedures which sample components such as water, proteins and fats are removed and analytes are extracted into extraction solvent and maintaining high recoveries. Recently, different QuEChERS procedures have been applied in the multi-determination of veterinary drugs residues in animal tissue (Zhao, et al., 2011; Stubbings et al., 2009; Pérez-Burgos et al., 2012; Park et al., 2012; Kang J et al., 2010), milk (Jeong et al., 2012; Martínez et al., 2010; Aguilera-Luiz et al., 2008), eggs (Wang et al., 2012; Olejnik et al., 2010) and other products (Romero-González et al., 2011; Tomasini et al., 2012)

The aim of the present study was, thus, to develop a multi-residue sreeening method for 128 kinds of veterinary antiparasite drugs (Avermectins, Benzimidazoles, Polyether antibiotics, Chemical synthesis coccidiostats, Sulfonamides, Organophosphorus, Pyrethroids, Organochlorine, Carbamates, Organic arsenic, other Pesticides, other antinematode drug, other Anthelmintic, Antiprotozoal) residues in

meat using QuEChERS sample preparation and LC-MS/MS. The optimization of QuEChERS sample pretreatment and MS parameters was discussed in detail. The method has adequate specificity, sensitivity and selectivity without exhaustive clean-up step. Compounds were successfully identified in spiked samples from their accurate mass and LC retention times from the acquired full-scan chromatogram. Further analysis of the data for real samples allowed the identification of the targeted antiparasite residues but also non-targeted compounds, such as some of their metabolites.

2 Materials and method

2.1 Chemicals and sample

Veterinary anti-parasitic drug reference standards used in this study were above 95% or available highest purity. Ivermectin, avermectin, doramectin, eprinomectin, moxidectin, emamectin, dinitolmide, 3-ANOT, methylbenzoquate, decoquinate, toltrazuril, diclazuril, cyromazine, clopidol, nicarbazin, amprolium, ethopabate, robenidine, niclosamide, fenbendazole, levamisol, pyrantel pamoate, morantel, salinomycin, maduramicin, halofuginone lactate, naphthalophos, acetofenate, cypermethrin, diethylcarbamazine citrate, praziquantel, closantel, rafoxanide, nitroxinil, oxyclozanide, imidocarb, arecoline hydrobromide were purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany). Narasin, lasalocid, dithiazanine iodide, pyrantel, diminazene, monensin, carbofuran, bendiocarb were purchased from Sigma–Aldrich Chemie b.v (Zwijndrecht, the Netherlands). Methiocarb, propoxur, metacrate, carbaryl, etrofolan, pirimicarb, carbosulfan, furathiocarb, benfuracarb, amitraz, diazinon, phoxim, propetamphos, rogor, lindane, permethrin, fenvalerate, ronnel, fenthion, dibrom, crufomate, azamethiphos, tetramethrin were purchased from Accu.

Standard Inc. (New Haven, USA). Artesunate, bithionol, metyridine, albendazole, sulfadimidine were purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). Nitroscanate were purchased from J&K Chemical Inc. (Beijing, China). Halofuginone, toltrazuril sulfone were purchased from Toronto Research Chemicals Inc. (Toronto, Canada). Amino-flubendazole, amino-Mebendazole, hydroxy-Mebendazole, fenbendazole sulphone, triclabendazole sulphone, triclabendazole sulfoxide were purchased from Witega Laboratories Berlin-Aldershof GmbH (Berlin, Germany). Milbemycin oxime, parbendazole were purchased from U.S. Pharmacopeia (MD, USA). Trichlorfon, dichlorvos, coumaphos, malathion, deltamethrin were purchased from Agro-Environmental Protection Institute of Ministry of Agriculture (Tianjin, China). Nanchangmycin were purchased from Selleck Chemicals LLC (Houston, TX, USA). Stock solutions with concentrations at 1000 mg/L for each veterinary antiparasitic drugs: Avermectin, doramectin, mamectin, moxidectin, milbemycin oxime, lasalocid, propetamphos, decoquinate, diclazuril, cyromazin, nicarbazin, closantel, febantel which were dissolved in Acetonitrile; Nitroscanate, imidocarb, albendazole, fubendazole, mebendazole, amino-Mebendazole, hydroxy-Mebendazole, fenbendazole, fenbendazole sulphone, oxfendazole, oxibendazole, thiabendazole which were dissolved in dimethyl sulfoxide; other drugs were dissolved in methanol and stored at -20^{o}C. The working mixed standard solution was prepared by diluting the multi-analyte intermediate standard solutions or stock solutions.

All samples tested were collected from MAO Key Laboratory of Food Safety Evaluation/National Reference Laboratory of Veterinary Drug Residues (Wuhan, China). Meat samples were muscle, liver, from swine, chicken and bovine. The samples were stored in frozen conditions and were kept frozen (-20 ^{o}C) until analysis.

2.2 Instrumentation

2.2.1 Chromatographic separation

Samples were analysed using high performance liquid chromatograph (Finnigan Surveyor LC consisting of: degasser, dual pump system, autosampler and column oven) coupled with ESI-TSQ Quantum Access MS/MS detector (Thermofisher, USA). Separations were performed using a Thermo® Hypersil C_{18} column (150*2.1mm, 5μm) at a temperature of 40 ^{o}C. The mobile phase consisted of 12.5mM aqueous ammonium formate at pH4 (A) and 12.5mM ammonium formate in acetonitrile/methanol (50/50, v/v) (B). The chromatographic separation was performed in a gradient mode (0 min: A/B 98/2, v/v; 10 min: A/B 0/100; 30min: A/B 0/100; 30.5 min: A/B 98/2; 40 min: A/B 98/2) at a flow of 0.2 mL/min for a total run time of 40min, and 10 μL of the extract were injected.

2.2.2 ESI-TSQ-MS/MS condition

Electrospray ionisation (ESI) was used in the MS with both positive and negative ionisation with polarity switching in a single chromatographic analysis with a spray voltage of 4500V, a capillary temperature of 350 ^{o}C, a sheath gas pressure of 50psi and an aux gas pressure of 15arbitrary units. The Sheath Gas and Aux Gas Pressure were carried out by using nitrogen and the Collision Gas was argon. The resolution of Q1 was 0.7 amu and Q3 was 0.7 amu. This method was based on the identification of the veterinary antiparasitic drugs using parent-ion scan, selecting one product ion or one common fragment for each kinds of veterinary anti-parasitic drugs. Table 1 shows the MS/MS conditions (selected product ion or common fragment, as well as the corresponding cone voltage and collision energies) for each kind of veterinary anti-parasitic drugs.

2.3 Sample preparation

Veterinary anti-parasitic drugs were extracted from meat using an extraction procedure based on QuEChERS methodology. The procedure was as follows: 2g meat tissue homogenate was weighed into polypropylene centrifuge tubes (50mL), then 10mL acetonitrile/ethyl acetate (1/1, v/v) was added to the sample, vortexed for 30s and ultrasonic for 1min, and then 1.0g of anhydrous $MgSO_4$ was added. The samples were mixed with the vortex for a few seconds, then centrifuged at 5000g for 10min at room temperature. Transfer the upper layer into a polypropylene tube, then 1mL of 5% $NH_3 \cdot H_2O$ aqueous was added to the tube and vortexed for 30s. The supernatant was transferred into another tube containing 200mg ODS and 1.5g anhydrous $MgSO_4$, then vortexed for 30s and centrifuged again at 5000g for 5min. Transfer the upper layer into a polypropylene tube and evaporated under nitrogen at 40 ^{o}C. Dissolve the residue with 1.0 mL mobile phase and ready for the LC-MS/MS system.

2.4 Validation

The method was validated by EU regulation 2002/657/EC for a quantitative screening method. It was more suitable than the classical approach of validation usually applied to quantitative physicochemical methods (trueness, precision, linearity, etc.). It was more relevant to monitor many samples at the level of interest in order to assess statistically the capacity of detection of the method. The requirement for screening methods as described in Decision 2002/657/EC is that these methods have the capability of a high sample throughput and allow the detection of all targeted substances with a false compliant rate below 5% at the level of interest. It means that the CCβ of the method should be found below this level of interest. The analytical response (relative chromatographic peak area) was determined for each analyte at the

relative retention time (2.5%) of the corresponding MRM transition.

The method was considered validated for a target compound if none of the responses of the spiked samples fell within the range of responses of the blanks. VLs (validation levels, VLs) for meat of swine, chicken and bovine were chosen according to the MRL and MRPL values of the target drugs in the corresponding laws and regulations and were set at half the MRL or the MRPL (minimum required performance limit, MRPL) value (table 2). The blank samples of swine meat, chicken meat and bovine meat 20 pieces each were spiked at the level of interest (MRL or ½MRL) with the different analytes and tested within the same day. Generally, the samples were spiked at the MRL level. The half-MRL level was chosen for some compounds especially when the MRL is established for the parent drug plus its metabolite or for the sum of different compounds (e.g. Albendazole, Albendazole oxide, Fenbendazole, Flubendazole, Mebendazole, Morantel, sulfonamides, Zoalene). The validation was then carried out per family in order to obtain information about the potential interferences. The concentrations evaluated during the validation are given in table 2. This step was repeated three times in order to obtain independent data for each analyte at the level of interest. The repetitions were carried out on three different days distributed over three different weeks.

The selectivity/specificity of the method was established by checking for the absence of interfering peaks at the expected relative retention time in negative samples. The specificity of a method is its ability to distinguish one specific analyte from the other analytes. It can be determined as being the number of negative agreements divided by the number of true negative samples expressed as a percentage. The sensitivity of a method can be determined as being the number of positive agreements divided by the number of true positive samples expressed as a percentage.

The consequence is the CCβ is below the MRL level.

3 Results and discussion

3.1 The optimization of QuEChERS sample pretreatment

The challenge for the analysts in the development of a multi-residue method was to find suitable extraction conditions for a large range of targeted analytes displaying different chemical properties (lipophilicity, hydrophilicity, alcaline and acidic characteristics, etc.). Avermectins were a class of macrocyclic lactone with the basic structure of macrolide antibiotics, which were easily soluble in methanol, ethyl acetate, DMF, DMSO. Benzimidazoles contained a benzimidazole structure and were moderate polarity easily soluble in DMSO, DMF, water insoluble in dilute acetic acid. Polyether antibiotics have a chain of four or five chain structure and an ether group, organic acid group and were fat-soluble hydrophobic. These drugs are generally soluble or soluble in organic solvents, such as acetonitrile, methanol, ethyl acetate, slightly soluble or insoluble in water. As it is almost impossible to optimize the clean-up process for all analytes, the purification step has generally to be as short and simple as possible. For sample preparation the QuEChERS approach was chosen due to its advantages in terms of speed, high throughput and low cost.

From the literatures in the field of veterinary antiparasitic drugs residues, the most extraction solvents such as acetonitrile, ethyl acetate, methanol and water were considered in muscle. For optimization, 2 g of sample was spiked with 128 kinds of veterinary antiparasitic drugs at the level of 10 μg/kg were added to a glass mortar, then extracted with 10mL acetonitrile, ethyl acetate, methanol, water and acetonitrile/ethyl acetate (1/1, v/v) separately. Fig. 1 a showed the results of extraction effects. The first was acetonitrile extraction, which is a common solvent

used for many veterinary antiparasitic drugs. This solvent was found to be sufficiently effective for the extraction of avermectins, imidazo thiazole, synthetic coccidiostats, flooding nematode drugs, taeniafuge drugs, flooding fluke drugs and insecticides. The second was an extraction using ethyl acetate under basic conditions which was found to be suitable for benzimidazoles, polyether antibiotics and carbamates. cetonitrile/ethyl acetate (v/v, 1/1) have complementary advantages, so acetonitrile/ethyl acetate (v/v, 1/1) was considered to be extraction solvent. Considering some alkaline drugs such as benzimidazoles and niclosamide can reach higher recovery under alkaline conditions using ethyl acetate extraction. Before the ethyl acetate extraction, 5% of ammonia solution is adopted to build alkaline environment. For liquid samples, such as eggs and milk, by contrast, found that after the first step extracted by acetonitrile, the second step extract is not necessary. So in order to achieve simple and time saving, for several liquid samples using acetonitrile and ethyl acetate mixed solvent and one-time extraction.

Then the selection test for the ratio between matrix and sorbent material was optimized. Four kinds of sorbent materials such as anhydrous $MgSO_4$-separate water from the organic solvent; PSA, NH_2-organic acids, pigments sugars and fatty acids; ODS-non-polar interfering substances like lipids; GCB-sterols and pigments were for the purification experiments. The 2 g samples were spiked with 128 kinds of veterinary anti-parasitic drugs at the level of 10 μg/kg after been extracted by acetonitrile/ethyl acetate (v/v, 1/1), the extracting solvents were added to an equal amount 500mg of the adsorbent for purification of four impurities, after vortex, centrifuged, and the supernatant was dried by nitrogen gas stream at a low speed. The results were shown in fig 1 b, the adsorbents purification effect were: PSA, NH2, ODS, GCB. With the above optimization, the ODS can ensure high recovery,

purification capacity. Thus, ODS was selected for the purification. Then we also optimized the amount of ODS (200mg, 500mg, 800mg, 1000mg). Fig. 1 c showed that ODS (200mg) was the best selection.

The last procedure after optimization was as follows: 2g tissue was extracted by 10mL acetonitrile/ethyl acetate (1/1, v/v) with 5% $NH_3 \cdot H2O$ aqueous, then 200mg ODS and 1.5g anhydrous $MgSO_4$ for purification.

3.2 The optimization of LC-MS/MS method

Reverse phase chromatography is a kind of liquid chromatographic separation mode, its surface nonpolar carrier as stationary phase, with relatively strong polarity solvent as mobile phase. This study involved more than drugs for small molecule medium polarity or weak polar compounds, so choose to adopt the most appropriate C_{18} reversed-phase chromatography column. The liquid chromatographic analysis of the veterinary anti-parasitic drugs was studied in order to achieve fast detection and acceptable separation of analytes by this screening method developed in this manuscript. The polarity of the 128 drugs are not identical, although unlike affinity chromatography column, but in order to better split drugs, the gradient elution mode: in view of the reversed phase chromatographic column, first with a low ratio of water phase and the high proportion of organic phase, the polarity of the target was cleared out; Gradually reduce the proportion of water phase and improve the proportion of organic phase to 100%, using pure organic phase elution, the polarity weak target elution, finally return to the initial proportion of mobile phase. In different time Settings of different proportion of mobile phase, and then make the retention time of drug in the post, making drugs in different time the peak, improve the detection sensitivity. For that purpose,

The LC programmed method was optimized in order to achieve the good separation of analytes with widely differing polarities and the optimum conditions were found to be that the initial percentage of organic phase (12.5mM ammonium formate in MeCN:MeOH (50:50, v/v)) was modified from 10% to 2%, reaching 100% of organic phase.

MS/MS conditions for this screening method was designed by employing parent-ion scan mode of work in MS/MS for the identification of several families of veterinary anti-parasitic drugs namely avermectins, benzimidazoles, polyether antibiotics, chemical synthesis coccidiostats, organophosphorus, carbamates pyrethroids, organic arsenic and other pesticides. The selection of product ions was carried out injecting a standard solution of each veterinary anti-parasitic drug (1 mg /L) in a mixture of methanol–aqueous solution of 0.05% formic acid (50:50v/v). For screening purposes and because of structural variations within the same group, benzimidazoles were separated in two groups. On the other hand, the loss of CH_3OH was selected for fenbendazole, mebendazole, and albendazole. Afterwards, the cone voltages and collision energies were optimized for each one. Furthermore, MS/MS parameters, such as resolution of Q1 and Q3 and scan time were evaluated. It can be indicated that when the resolution of Q1 was modified, no significant differences on the intensity of the signals were observed. However, resolution of Q3 has a great impact on the intensity of the signals. When Q1 and Q3 were both 0.7 amu, the result was observed the higher intensity peak. Table 1 shows the MS/MS parameters to monitor each family of compounds, including the scan time and the mass range selected in Q1. Some analytes do not form protonated molecules, but Na or K or NH_3 adducts were used as precursor ions. The molecular formula of Ivermectin is $C_{48}H_{74}O_{14}$ (M = 875). The precursor ion is $[M+Na]^+$ as $C_{48}H_{74}O_{14}Na$ (M = 897).

Similarly, the precursors of Avermectin, Doramectin, Eprinomectin, Milbemycin oxime, Monensin, Salinomycin, Narasin, Maduramicin, Lasalocid, Nanchangmycin were $[M+Na]^+$. The precursors of Aldicarb, Metacrate, Deltamethrin, Fenvalerate were $[M+NH_4]^+$. The precursors of Permethrin and Lindane were $[M+K]^+$. The precursors of amprolium M = 278 and Robenidine M=370 were $[M-Cl]^-$

3.3 Method validation results

The validation process previously described had already been tested for biological screening methods but was totally new for an LC/MS-MS method. The suitability of this validation scheme applied to a physicochemical screening method was demonstrated and gave more useful information than the classical approach of validation usually applied to quantitative physicochemical methods. The limit of detection, specificity, sensitivity and CCβ were assessed during the validation and the results are described below for each anti-parasitic family. The specificity of the method was checked by analysing 20 meat blank samples of different origins and the specificity was 100% for all the analytes as no peak was detected in these samples at the retention time corresponding to each analyte. This fact underlines the very high specificity of the tandem mass spectrometer. The chromatography is avermectins and benzimidazoles (Fig 2a), polyether antibiotics and chemical synthesis coccidiostats (Fig 2b), organophosphorus and carbamates(Fig 2c), pyrethroids, organic arsenic and other pesticides (Fig 2d) in spiked swine muscle.

For Avermectins, meat samples were spiked at 10 µg/kg for ivermectin, 20 µg/kg for avermectin, 40 µg/kg for doramectin, 5 µg/kg for eprinomectin, moxidectin, milbemycin oxime and emamectin. Results are presented in table 2. These results mean that the CCβ of the seven avermectins are below the level of concentrations tested during the validation, i.e. below MRL for ivermectin, avermectin, doramectin.

The limits of detection were calculated and ranged between 2 and 5 μg/kg. Retention times ranged from 16.3 to 22.5 min. For Benzimidazoles, the level of validation for each analyte is given in table 2. The estimated CCβ was below the level of validation. The limits of detection were below 0.5 μg/kg for most of the analytes and up to 2 μg/kg for fenbendazole, oxibendazole. Retention times ranged 12.5 to 17.1 min for benzimidazoles. For Polyether antibiotics, meat samples were spiked at 2 μg/kg for monensin, 600 μg/kg for salinomycin and narasin, 240 μg/kg for maduramicin, 20 μg/kg for lasalocid and nanchangmycin. These results mean that the CCβ of the six polyether antibiotics are below the level of concentrations tested during the validation. The limits of detection were calculated and ranged between 2 and 10 μg/kg. Retention times ranged from 20.7 to 25.4 min. For Synthetic coccidiostats**,** the levels of validation for each nitrobenzene amides, morpholino, triazines, pyridine, benzene urea, plant bases, antithiamine class, synergist class, guanidines, sulfonamides, imidazo and thiazole are given in table 2. Results were very satisfactory as the CCβ was assessed to be below the level of validation for all analytes. The limits of detection were calculated and ranged between 0.5 and 5 μg/kg. Retention times ranged from 3.4 to 24.9 min. For Insecticides (Organophosphate insecticides, Pyrethroids, Organochlorine insecticides, Carbamates, Organic arsenic pesticides), the levels of validation for each insecticides were ranged from 1 to 10 μg/kg. Results were very satisfactory as the CCβ was assessed to be below the level of validation for all analytes (table 2). The limits of detection were calculated and ranged between 0.5 and 5 μg/kg. Retention times ranged from 12.7 to 29.7 min. For Flooding nematode drugs, Taeniafuge drugs, Flooding fluke drugs, Anti-trypanosome drug, Piroplasma anti-drug, meat samples were spiked at 1-10 μg/kg for the left 20 kinds drugs. Twenty spiked samples were analysed per day and

during 3 days. In total, 60 samples were analysed for each analyte. These results mean that the CCβ of the 20 kinds of drugs are below the level of concentrations tested during the validation. The limits of detection were calculated and ranged between 1 and 10 μg/kg. Retention times ranged from 7.3 to 19.2 min.

4 Conclusion

A simple method was developed for the screening of 128 veterinary anti-parasitic in animal meat. The QuEChERS sample preparation procedure made available the detection of the majority of the analytes with high specificity, sensitivity and selectivity. This technique requires no exhaustive clean-up steps and uses small amounts of solvent, being therefore simpler, cheaper and faster than the traditional procedure. This possibility, in conjunction with the opportunity of expanding the present screening methodology to other animal matrices, is under investigation in our laboratory.

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Fig.1. a. The optimization of different extracting solvents;

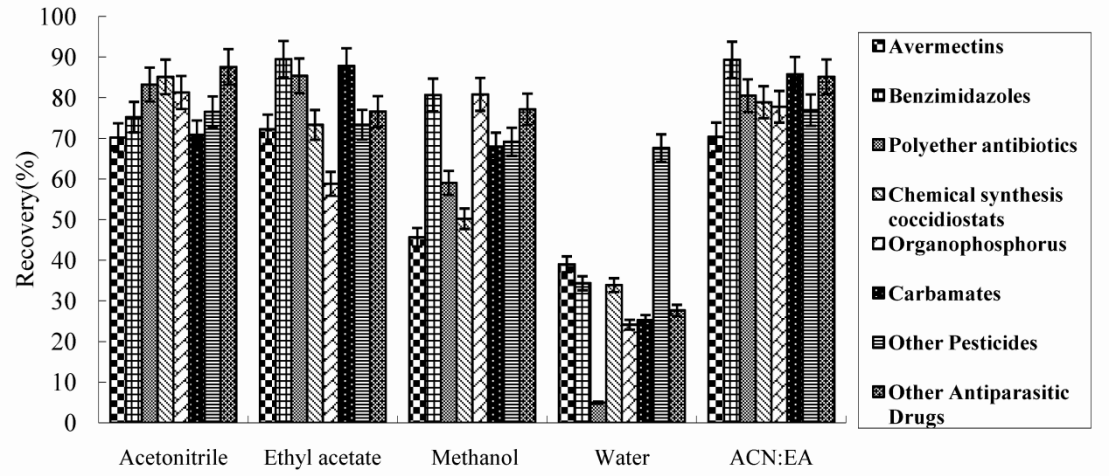


Fig.1. b. The optimization of adsorbent materials;

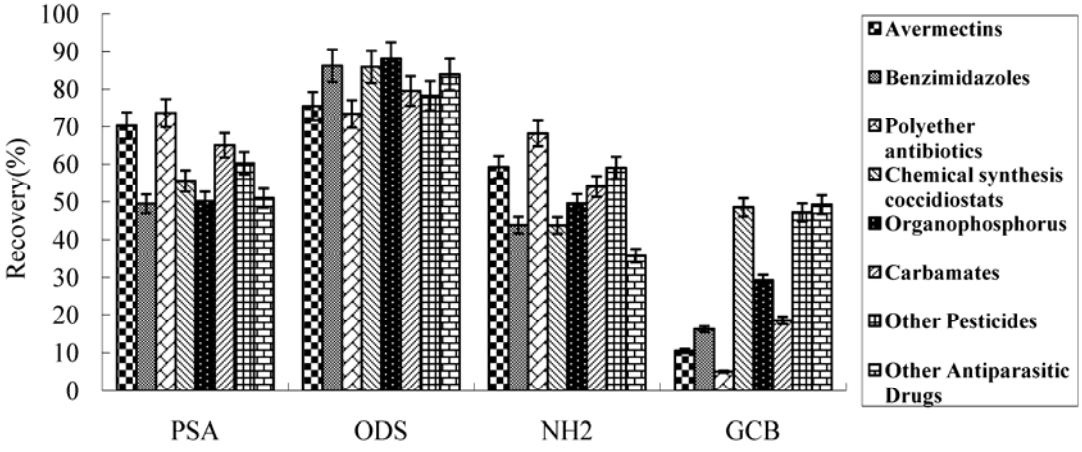


Fig.1. c. The optimization of different addition amount.

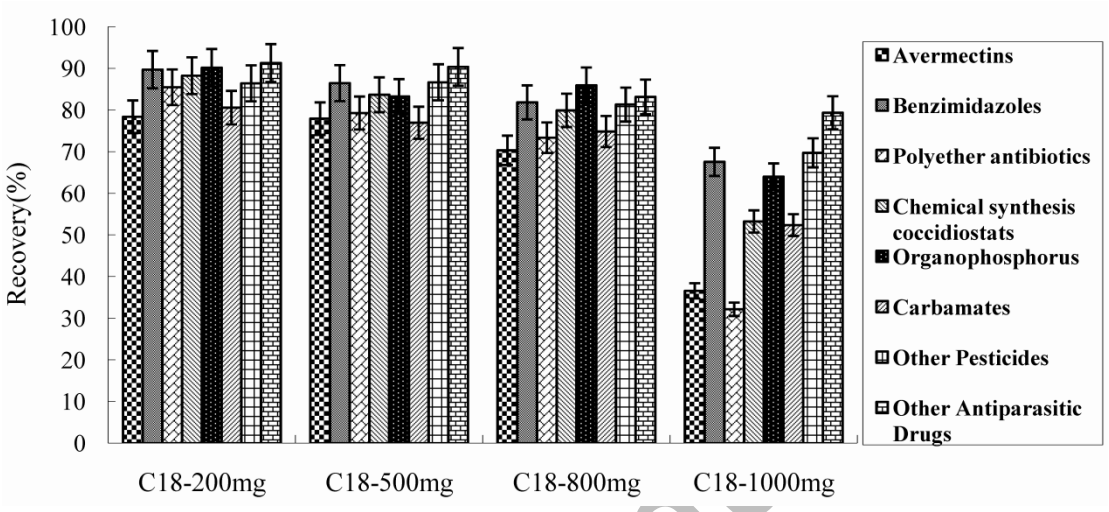


Fig.2.a. The chromatography of quantitative ions of Avermectins (10 μg/kg for ivermectin, 20 μg/kg for avermectin, 40 μg/kg for doramectin, 5 μg/kg for eprinomectin, moxidectin, milbemycin oxime and emamectin)

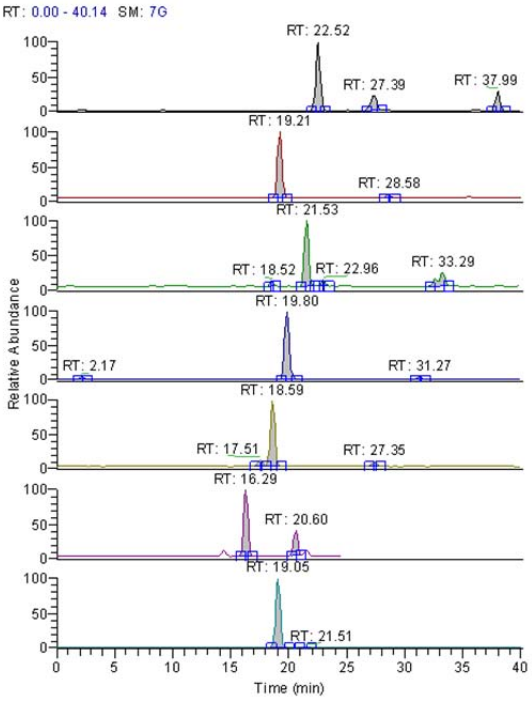


Fig.2.b. The chromatography of quantitative ions of Benzimidazoles (2μg/kg-25μg/kg) in spiked swine muscle;

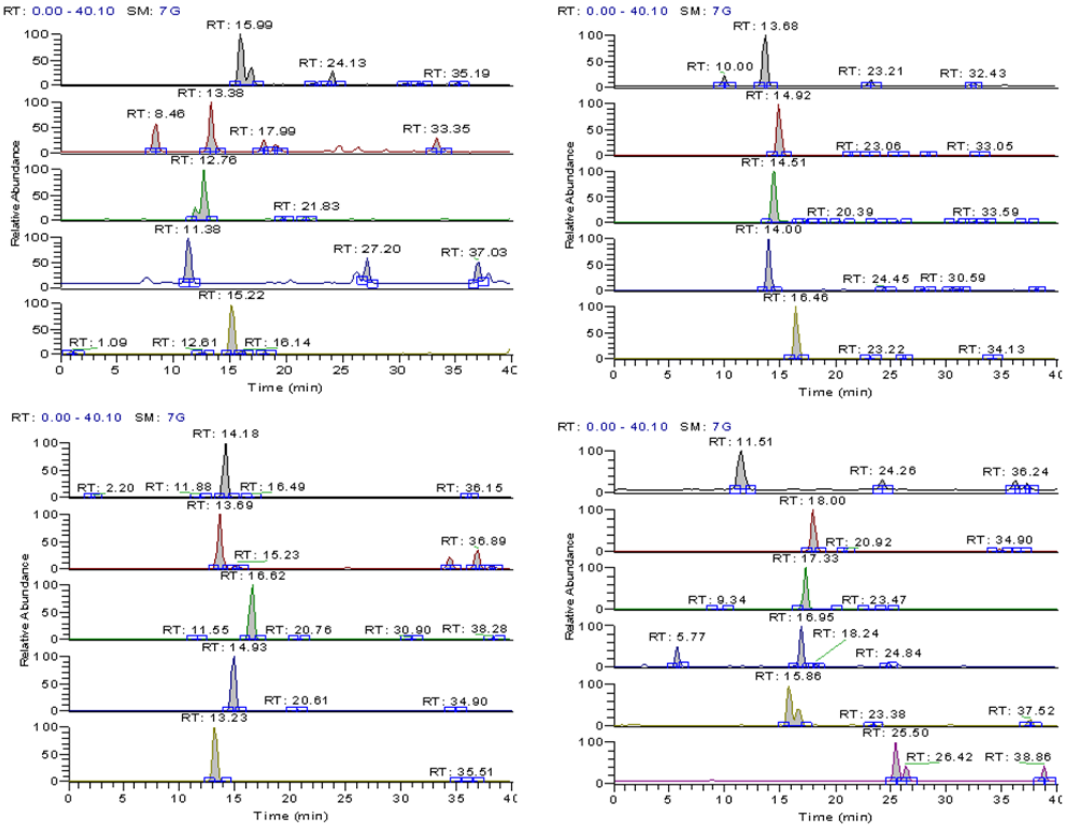


Fig.2.c. The chromatography of quantitative ions of Polyether antibiotics (2μg/kg-20μg/kg) in spiked swine muscle;

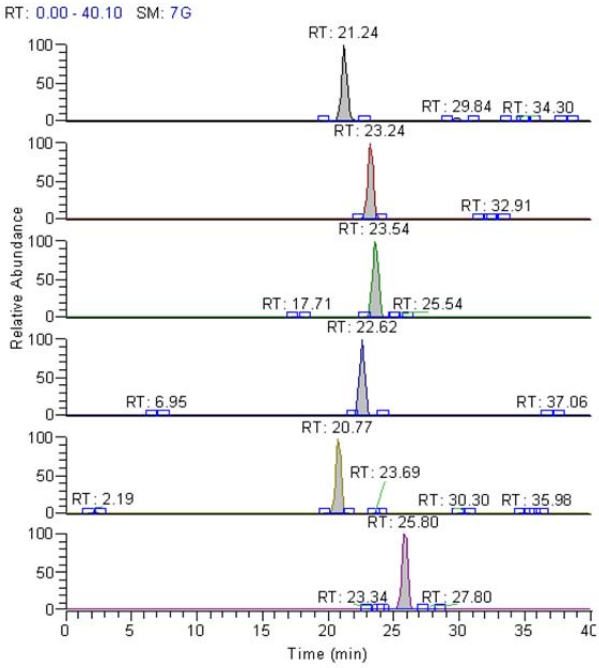


Fig.2.d. The chromatography of quantitative ions of chemical synthesis coccidiostats (2μg/kg-4μg/kg) in spiked swine muscle;

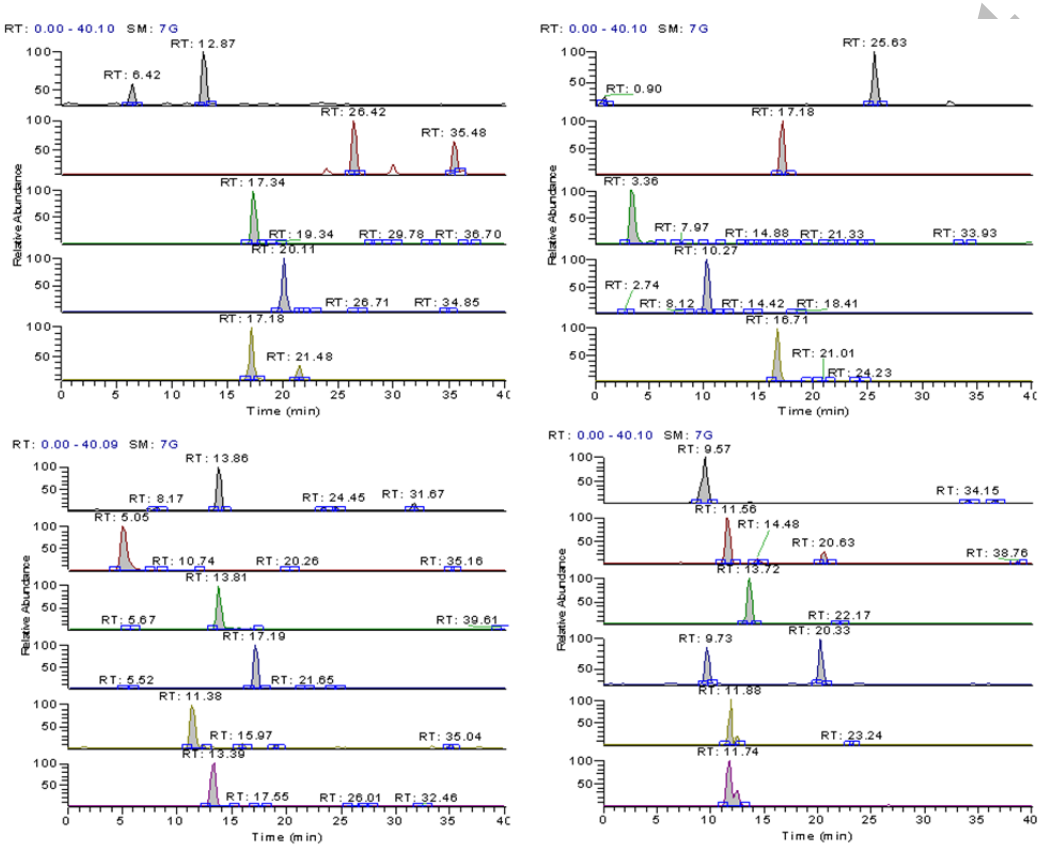


Fig.2.e. The chromatography of quantitative ions of Organophosphorus (2μg/kg-10μg/kg) in spiked swine muscle;

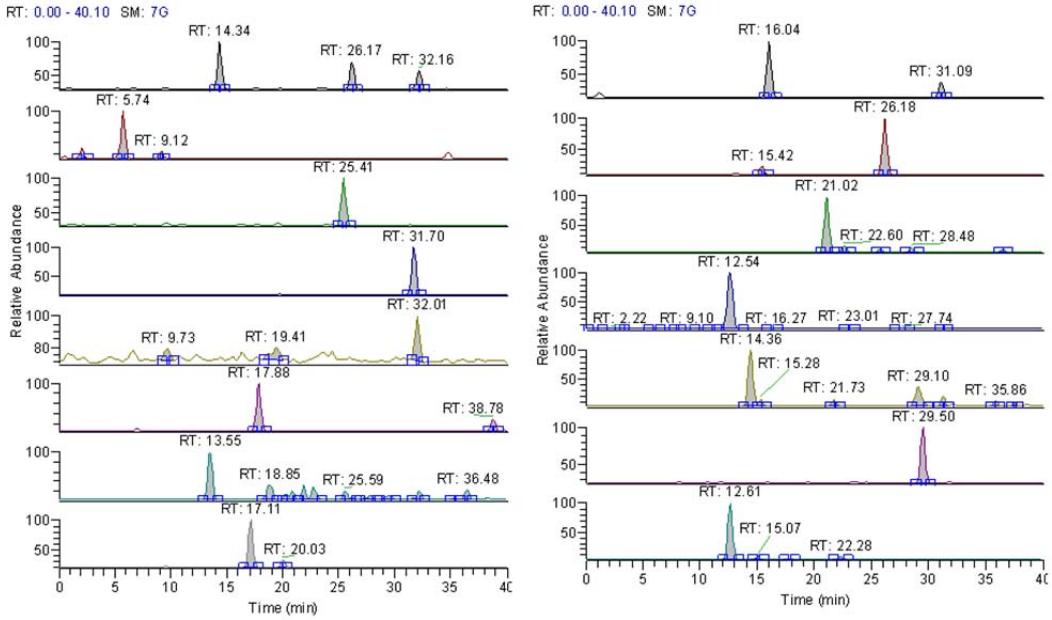


Fig.2.f. The chromatography of quantitative ions of Carbamates (2μg/kg-25μg/kg)in spiked swine muscle;

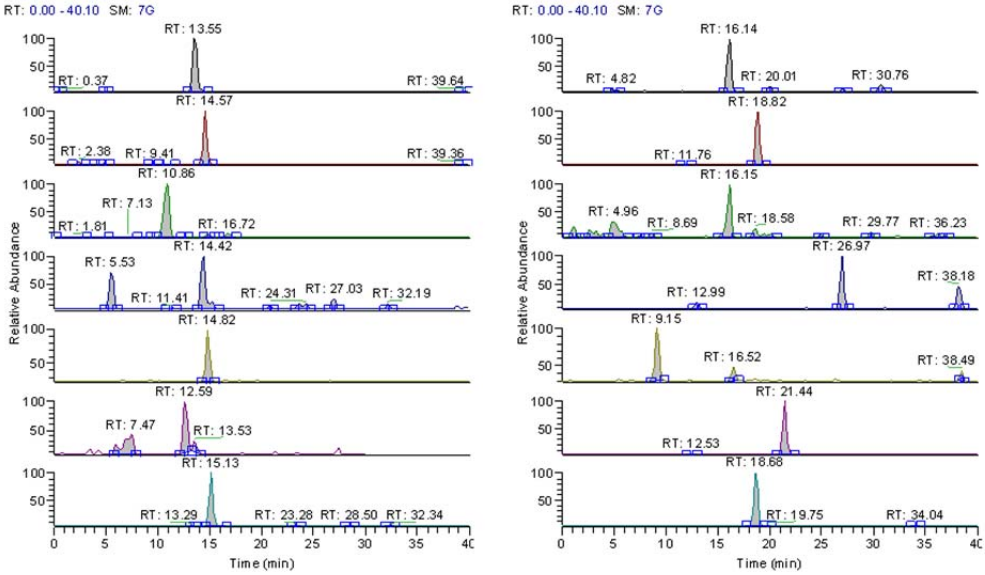


Fig.2.g. The chromatography of Pyrethroids (2μg/kg-20μg/kg) in spiked swine muscle

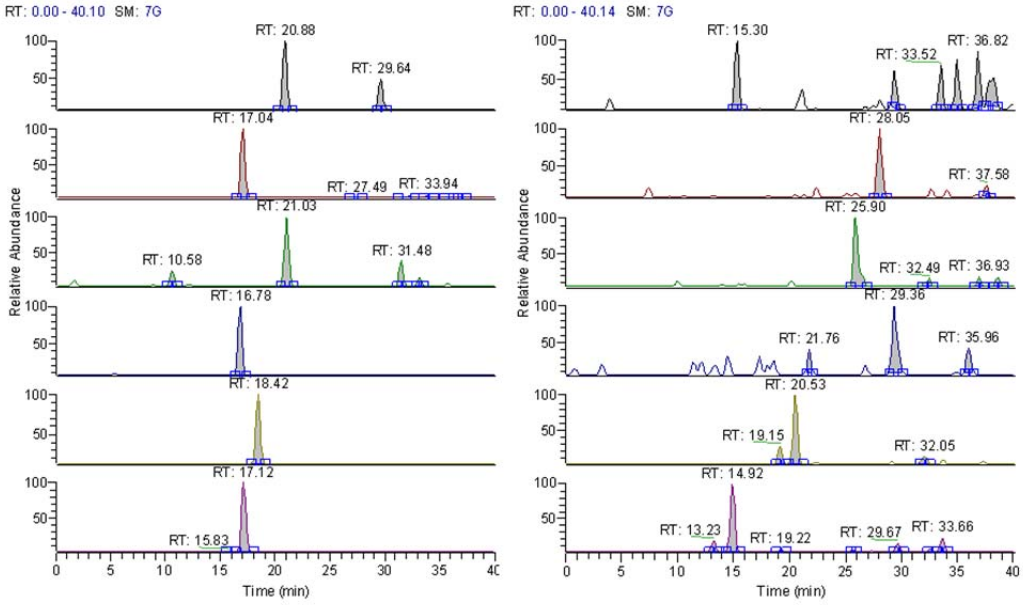


Fig.2.h. The chromatography of Organic arsenic (2μg/kg-25μg/kg) in spiked swine muscle

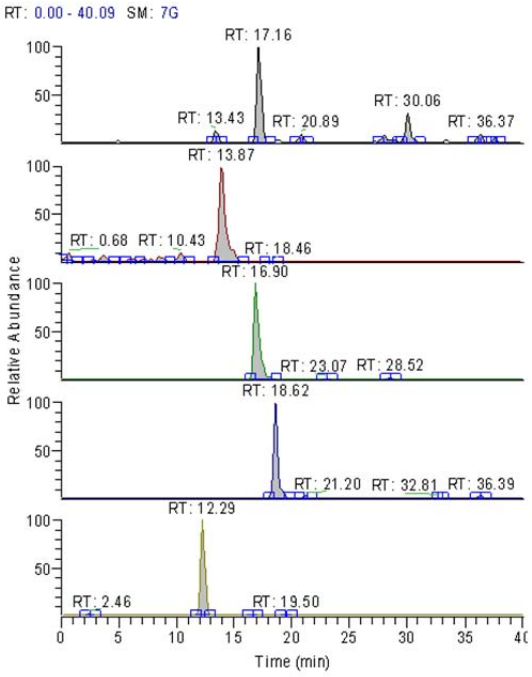
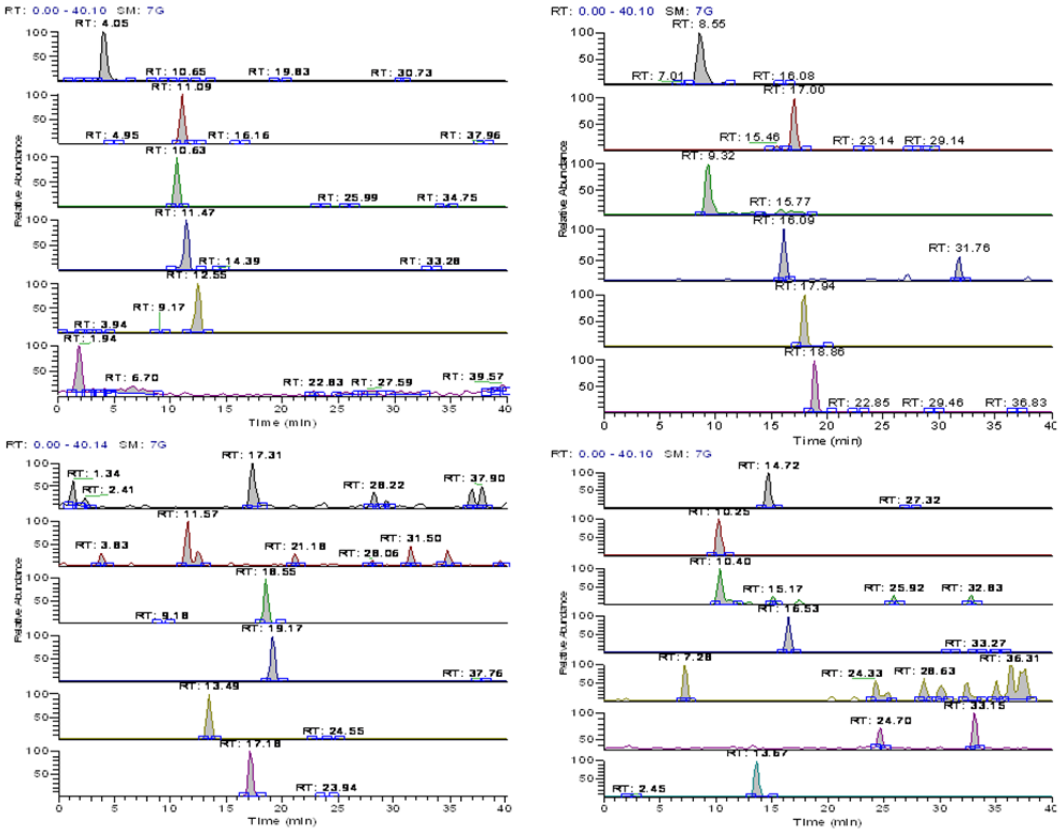


Fig.2.i. The chromatography of other pesticides (2μg/kg-50μg/kg) in spiked swine muscle



A Graphical Abstract

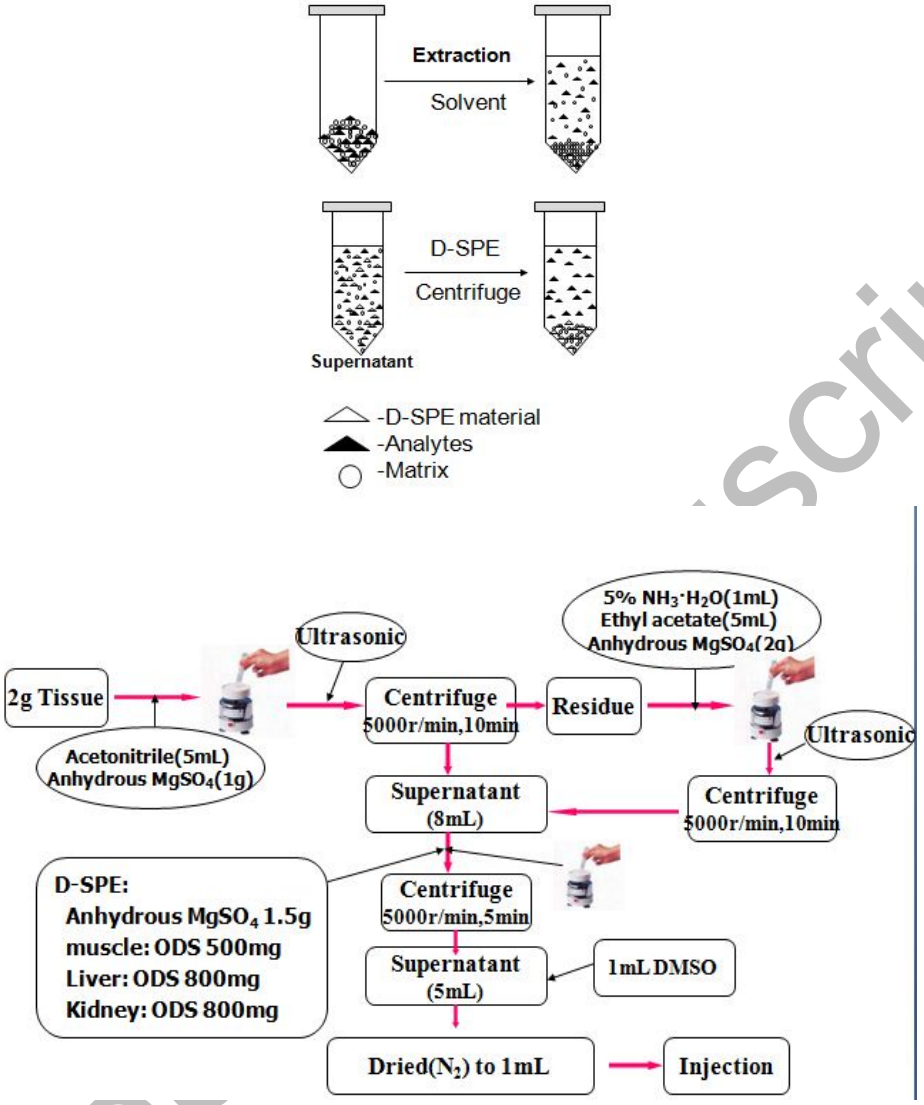


Table 1 Molecular weights, retention times, t_R (acceptable ranges, ±3 SD), ion transitions, optimized MS/MS parameters, and ion ratios (acceptable ranges, ±3 SD) vs. concentrations of theantiparasitic drugs and pesticides by LC–MS/MS

Number serial	Analyte	Elemental composition	M.W	t_R(min)	Quantification transition(m/z)	CE (eV)	Confirmation transition(m/z)	CE(eV)	Ion ratio (average of ion ratio% ±RSD% for 0.5MRL-1.5MRL)
1	Ivermectin $[M+Na]^+$	C48H74O14	875.1	22.5±0.03	897.3＞311.1	48	897.3＞240.2	49	25%±8%
2	Avermectin $[M+Na]^+$	C48H72O14	873.1	19.2±0.02	895.5＞328.6	19	895.5＞327.1	42	34%±14%
3	Doramectin $[M+Na]^+$	C50H74O14	899.1	21.5±0.01	921.4＞217.2	46	921.4＞183.0	51	63%±16%
4	Eprinomectin $[M+Na]^+$	C50H75NO14	914.1	19.8±0.02	936.3＞352.1	53	936.3＞223.9	52	39%±15%
5	Moxidectin	C37H53NO8	639.8	18.6±0.02	640.3＞498.2	8	640.3＞199.3	27	34%±9%
6	Milbemycin oxime $[M+Na]^+$	C63H88N2O14	1097.4	16.3±0.02	1119.4＞577.9	31	1119.4＞563.8	28	53%±10%
7	Emamectin	C49H75NO13	886.1	19.1±0.03	886.6＞126.3	30	886.6＞82.4	48	25%±7%
8	Albendazole	C12H15N3O2S	265.3	15.7±0.06	266.2＞191.4	32	266.2＞159.3	39	78±8%
9	Albendazole sulphone	C12H15N3O4S	297.3	13.1±0.04	298.2＞224.3	25	298.2＞159.4	35	61%±12%
10	Albendazole sulfoxide	C12H15N3O3S	281.3	12.5±0.04	282.2＞208.3	24	282.2＞159.4	33	63%±3%
11	Albendazole-2-amino-sulfone	C10H13N3O2S	239.3	10.9±0.03	240.2＞133.4	28	240.2＞106.5	41	60%±16%
12	Fubendazole	C16H12FN3O3	313.3	14.9±0.03	314.2＞123.4	32	314.2＞95.4	45	74%±16%
13	Amino-flubendazole	C14H10FN3O	255.3	13.4±0.05	256.2＞113.5	33	256.2＞95.5	36	68%±3%
14	Mebendazole	C16H13N3O3	295.3	14.7±0.05	296.2＞105.4	31	296.2＞77.5	42	89%±13%
15	Amino-Mebendazole	C14H11N3O	238.3	12.9±0.01	238.3＞95.6	27	238.3＞77.5	33	78%±20%
16	Hydroxy-Mebendazole	C16H14N3O3	297.3	13.7±0.06	298.2＞160.4	32	298.2＞79.6	34	74%±9%
17	Fenbendazole	C15H13N3	29	16.2	300.2＞	29	300.2＞	34	64%±2%

Number serial	Analyte	Elemental composition	M.W	t_R(min)	Quantification transition(m/z)	CE (eV)	Confirmation transition(m/z)	CE(eV)	Ion ratio (average of ion ratio% ±RSD% for 0.5MRL-1.5MRL)
		O2S	9.4	±0.03	190.3		159.3		
18	Fenbendazole sulphone	C15H13N3O4S	331.4	13.4 ±0.03	332.2＞175.1	20	332.2＞159.4	34	68%±18%
19	Oxfendazole	C15H13N3O3S	315.4	16.4 ±0.03	316.2＞191.4	20	316.2＞159.3	34	56%±3%
20	Parbendazole	C13H17N3O2	247.3	14.7 ±0.01	248.3＞173.3	30	248.3＞160.4	28	47%±19%
21	Oxibendazole	C12H15N3O3	249.3	12.9 ±0.01	250.3＞176.3	27	250.3＞148.3	35	78%±10%
22	Thiabendazole	C10H7N3S	201.3	11.1 ±0.05	202.2＞131.4	31	202.2＞92.5	33	67%±7%
23	5-Hydroxy Thiabendazole	C10H7N3OS	217.3	14±0.04	218.0＞147.1	31	218.0＞108.2	30	66%±16%
24	Triclabendazole	C14H9Cl3N2OS	359.7	17.7 ±0.04	359.0＞198.0	20	359.0＞165.4	41	56%±12%
25	Triclabendazole sulfoxide $[M-H]^-$	C14H9Cl3N2O2S	375.7	17.1 ±0.05	374.5＞213.5	33	374.5＞181.3	48	54%±10%
26	Triclabendazole sulphone	C14H9Cl3N2O3S	391.7	17.1 ±0.06	391.1＞242.4	34	391.1＞167.5	38	46%±8%
27	Benomyl	C14H18N4O3	290.3	15.7 ±0.06	290.4＞228.6	16	290.4＞146.3	15	35%±3%
28	Febantel	C20H22N4O6S	446.5	16.9 ±0.02	447.0＞382.9	18	447.0＞311.9	23	87%±6%
29	Monensin$[M+Na]^+$	C36H62O11	670.9	21±0.02	693.5＞461.4	46	693.5＞321.2	49	91%±15%
30	Salinomycin $[M+Na]^+$	C42H70O11	751	22.9 ±0.02	773.5＞413.2	42	773.5＞265.4	44	58%±5%
31	Narasin$[M+Na]^+$	C43H72O11	765	23.3 ±0.01	787.6＞413.4	45	787.6＞279.2	50	62%±10%
32	Maduramicin $[M+Na]^+$	C47H80O17	917.1	22.3 ±0.01	939.6＞877.3	23	939.6＞859.7	54	45%±12%
33	Lasalocid $[M+Na]^+$	C34H54O8	590.8	20.7 ±0.05	613.4＞377.5	32	613.4＞359.4	37	61%±19%
34	Nanchangmycin $[M+Na]^+$	C48H80O13	865.1	25.4 ±0.04	889.4＞447.1	49	889.4＞292.8	64	42%±14%
35	Dinitolmide (-)*	C8H7N3O5	225.2	12.6 ±0.03	224.0＞181.0	14	224.0＞151.0	20	14%±6%
36	3-ANOT	C8H9N3O3	195.2	7.2±0.04	195.8＞125.5	23	195.8＞106.4	10	24%±14%

Number serial	Analyte	Elemental composition	M.W	t_R(min)	Quantification transition(m/z)	CE (eV)	Confirmation transition(m/z)	CE(eV)	Ion ratio (average of ion ratio% ±RSD% for 0.5MRL-1.5MRL)
37	Methylbenzoquate	C22H23NO4	365.4	17.2±0.02	366.2>201.0	31	366.2>145.0	43	34%±7%
38	Decoquinate	C24H35NO5	417.5	19.8±0.02	418.4>206.2	33	418.4>204.2	40	24%±16%
39	Toltrazuril (-)	C18H3N3O4S	425.4	12.8±0.03	423.7	0	423.7	0	33%±6%
40	Toltrazuril sulfone (-)	C18H3N3O6S	457.4	16.6±0.01	455.9	0	455.9	0	37%±15%
41	Diclazuril (-)	C17H9Cl3N4O2	407.6	17.1±0.03	404.8>334.9	18	404.8>333.9	20	47%±7%
42	Cyromazine	C6H10N6	166.2	2.3±0.03	167.0>108.3	21	167.0>85.4	17	58%±13%
43	Clopidol	C7H7Cl2NO	192	9.6±0.02	193.9>103.2	32	193.9>100.8	25	25%±10%
44	Nicarbazin	C13H10N4O5.C6H8	426.4	16.3±0.02	301.0>137.1	20	301.0>107.1	40	18%±12%
45	Amprolium	C14H19ClN4	278.8	1.3±0.02	243.2>135.4	24	243.2>122.3	24	54%±17%
46	Ethopabate	C12H15NO4	237.3	13.6±0.06	238.0>164.3	20	238.0>136.4	21	60%±5%
47	Robenidine	C15H13Cl2N5.HCl	370.7	16.5±0.03	334.0>138.2	26	334.0>111.2	52	23%±14%
48	Halofuginone	C16H17BrClN3O3	414.7	11.4±0.02	415.7>120.0	17	415.7>99.9	18	35%±22%
49	Sulfaclozine	C10H9ClN4O2S	284.7	11.6±0.03	285.0>108.1	24	285.0>92.2	27	51%±10%
50	Sulfaquinoxaline	C14H12N4O2S	300.3	13.4±0.04	301.0>108.1	25	301.0>92.1	29	52%±17%
51	Sulfadiazine	C10H10N4O2S	250.3	11.7±0.04	251.0>108.1	21	251.0>92.2	26	65%±26%
52	Sulfadimidine	C12H14N4O2S	278.3	1.5±0.05	279.0>186.0	16	279.0>124.1	27	39%±7%
53	Sulfadimethoxine	C12H14N4O4S	310.3	11.4±0.03	311.0>156.0	20	311.0>108.1	26	32%±3%
54	Sulfamethoxazole,	C10H11N3O3S	253.3	11.7±0.05	254.0>108.1	23	254.0>92.2	26	45%±12%
55	Sulfamonomethoxine	C11H12N4O3S	280.3	13.4±0.04	281.0>215.0	15	281.0>108.1	26	50%±6%
56	Sulfamethoxydiazine	C11H12N4O3S	280.3	18.8±0.06	281.0>215.0	17	281.0>108.1	27	34%±14%

Number serial	Analyte	Elemental composition	M.W	t_R(min)	Quantification transition(m/z)	CE (eV)	Confirmation transition(m/z)	CE(eV)	Ion ratio (average of ion ratio% ±RSD% for 0.5MRL-1.5MRL)
57	Sulfamethoxypyridazine	C11H12N4O3S	280.3	3.4±0.05	281.0>108.1	25	281.0>92.1	27	43%±9%
58	Trichlorfon	C4H8Cl3O4P	257.4	1.1±0.05	259.1>221.3	12	259.1>127.4	15	57%±20%
59	Dichlorvos	C4H7Cl2O4P	221	17.4±0.06	221.1>109.4	17	221.1>79.4	26	39%±6%
60	Coumaphos	C14H16ClO5PS	362.8	15.8±0.04	363.1>227.3	24	363.1>211.3	30	56%±7%
61	Coumaphos-oxon	C14H16ClO6P	346.7	16.6±0.04	347.0>291.0	19	347.0>211.2	30	43%±4%
62	Malathion	C10H19O6PS2	330.4	17.6±0.01	331.2>125.3	27	331.2>99.4	25	72%±14%
63	Diazinon	C12H21N2O3PS	304.3	18.3±0.02	305.2>153.4	20	305.2>97.4	33	28%±10%
64	Fenthion	C10H15O3PS2	278.3	17±0.01	279.2>169.3	18	279.2>105.5	28	46%±20%
65	Crufomate	C12H19ClNO3P	291.7	16.6±0.03	292.2>126.4	25	292.2>108.5	26	22%±2%
66	Phoxim	C12H15N2O3PS	298.3	24.9±0.03	299.2>97.2	29	299.2>77.5	30	86%±2%
67	Propetamphos	C10H20NO4PS	281.3	17±0.04	282.2>124.4	23	282.2>110.3	29	31%±4%
68	Dibrom	C4H7Br2Cl2O4P	380.8	12.7±0.04	380.9>127.4	12	380.9>109.5	38	64%±13%
69	Rogor	C5H12NO3PS2	229.3	15.8±0.05	230.2>171.3	14	230.2>125.3	23	24%±6%
70	Azamethiphos	C9H10ClN2O5PS	324.7	14.3±0.06	325.1>139.3	24	325.1>112.4	32	43%±18%
71	Temephos	C16H20O6P2S3	466.5	22.3±0.05	466.7>404.8	15	466.7>124.7	32	52%±18%
72	Ronnel	C8H8Cl3O3PS	321.6	12.9±0.06	323.0>264.9	11	323.0>223.2	20	36%±19%
73	Aldicarb $[M+NH_4]^+$	C7H14N2O2S	190.3	13.7±0.03	208.1>89.3	15	208.1>61.3	22	54%±12%
74	Propoxur	C11H15NO3	209.2	14.4±0.04	210.0>93.2	26	210.0>65.3	33	37%±12%
75	Carbofuran	C12H15NO3	221.3	14.6±0.02	222.0>123.1	22	222.0>77.2	38	62%±18%
76	Metacrate $[M+NH_4]^+$	C9H11NO	165.	14±0.01	183.1>109.2	14	183.1>94.2	32	53%±17%

Number serial	Analyte	Elemental composition	M.W	t_R(min)	Quantification transition(m/z)	CE (eV)	Confirmation transition(m/z)	CE(eV)	Ion ratio (average of ion ratio% ±RSD% for 0.5MRL-1.5MRL)
77	Carbaryl	C12H11NO2	201.2	14.7±0.01	202.0>127.1	27	202.0>117.1	22	53%±5%
78	Etrofolan	C11H15NO2	193.2	15.3±0.01	194.1>95.2	14	194.1>77.3	33	60%±7%
79	Pirimicarb	C11H18N4O2	238.3	14.9±0.03	239.1>182.2	15	239.1>72.3	20	16%±5%
80	Diethofencarb	C14H21NO4	267.3	15.9±0.03	268.0>152.1	22	268.0>124.1	31	64%±2%
81	Furathiocarb	C18H26N2O5S	382.5	18.6±0.05	383.0>167.0	25	383.0>162.0	31	66%±15%
82	Methiocarb	C11H15NO2S	225.3	16±0.03	226.0>121.2	18	226.0>91.2	32	29%±17%
83	Bendiocarb	C11H13NO4	223.3	14.4±0.01	224.0>109.1	18	224.0>81.2	33	34%±18%
84	Fenobucarb	C12H17NO2	207.3	16±0.02	208.3>95.5	15	208.3>77.5	34	62%±26%
85	Carbosulfan	C20H30N2O3S	380.5	17±0.02	381.3>118.4	18	381.3>76.4	30	36%±13%
86	Benfuracarb	C20H30N2O5S	410.5	18.7±0.02	411.3>162.4	31	411.3>102.4	29	51%±14%
87	Deltamethrin $[M+NH_4]^+$	C22H19Br2NO3	505.2	20.9±0.06	523.1>506.3	6	523.1>280.6	5	81%±21%
88	Permethrin $[M+K]^+$	C21H20Cl2O3	391.3	17±0.06	432.3>135.5	20	432.3>119.5	18	50%±3%
89	Tetramethrin	C19H25NO4	331.4	21±0.05	332.3>286.4	10	332.3>164.4	24	76%±5%
90	Fenvalerate $[M+NH_4]^+$	C25H22ClNO3	419.9	16.8±0.04	437.3>317.6	18	437.3>303.5	27	57%±12%
91	Cypermethrin $[M-Cl]^-$	C22H19Cl2NO3	416.3	18.4±0.05	381.3>285.0	18	381.3>253.3	31	61%±13%
92	Cyfluthrin	C22H18Cl2FNO3	434.3	17.1±0.03	437.1>303.1	23	437.1>273.1	21	45%±13%
93	Arsanilic acid	C6H8AsNO3	217.1	15.3±0.02	246.1>108.4	22	246.1>80.5	37	58%±25%
94	Roxarsone	C6H6AsNO6	263	28.1±0.04	292.1>198.1	18	292.1>91.2	28	51%±2%
95	4-nitrophenylarsonic acid	C6H6AsNO5	247	25.9±0.04	276.1>168.3	17	276.1>140.3	27	42%±2%

Number serial	Analyte	Elemental composition	M.W	t_R(min)	Quantification transition(m/z)	CE (eV)	Confirmation transition(m/z)	CE(eV)	Ion ratio (average of ion ratio% ±RSD% for 0.5MRL-1.5MRL)
96	Carbarsone	C7H9AsN2O4	260.1	29.4±0.02	289.1>182.3	23	289.1>108.4	28	57%±21%
97	Lindane [M+K]+	C6H6Cl6	290.8	20.5±0.03	330.4>85.6	26	330.4>70.5	28	63%±16%
98	Acetofenate	C10H7Cl5O2	336.4	14.9±0.02	337.0>280.8	20	337.0>204.3	17	56%±2%
99	Amitraz	C19H23N3	293.4	17.2±0.01	294.3>122.4	29	294.3>107.5	37	47%±12%
100	2,4-DMA	C10H14N2	121.2	13.7±0.01	122.3>105.5	15	122.3>77.5	30	54%±16%
101	Diflubenzuron (-)	C14H9ClF2N2O2	310.7	16.9±0.04	309.0>113.1	32	309.0>93.1	55	48%±17%
102	Fluazuron (-)	C20H10Cl5N3O3	506.2	18.6±0.05	503.8>304.9	17	503.8>261.9	29	75%±19%
103	Chlordimeform	C10H13ClN2	196.7	12.3±0.05	196.7>116.9	29	196.7>90.0	43	60%±7%
104	Metronidazole	C6H9N3O3	171.2	4.1±0.06	171.9>111.1	19	171.9>82.2	22	53%±16%
105	Dimetridazole	C5H7N3O2	141.1	11.1±0.06	141.9>95.2	20	141.9>81.2	25	47%±6%
106	Levamisol	C11H28N2S	204.3	10.6±0.03	205.2>123.4	27	205.2>91.5	34	23%±4%
107	Pyrantel	C11H14N2S	206.3	11.5±0.03	207.1>109.2	45	207.1>97.4	21	53%±3%
108	Morantel	C12H16N2S	190.3	12.6±0.04	221.0>123.2	35	221.0>111.1	21	63%±9%
109	N-methyl-1, 3-propane diamine	C4H12N2	88.2	1.9±0.04	89.0>58.4	11	89.0>57.4	20	39%±16%
110	Diethylcarbamazine citrate	C16H29N3O8	391.4	8.6±0.01	200.4>127.3	12	200.4>100.3	14	82%±13%
111	Dithiazanine Iodide	C23H23N2S2I	518.5	17±0.02	391.3>214.3	30	391.3>188.2	26	48%±8%
112	Metyridine	C8H11NO	137.2	9.3±0.03	138.1>106.3	13	138.1>78.3	28	53%±15%
113	Praziquantel	C19H24N2O2	312.4	16.1±0.02	313.2>202.3	4	313.2>173.8	40	72%±13%
114	Niclosamide	C13H8Cl2N2O4	327.1	17.9±0.01	324.8>171.0	28	324.8>135.0	42	40%±13%
115	Bithionol	C12H6Cl4O2S	356.	18.8±0.0	354.7>191.9	27	354.7>161.0	26	63%±1%

Number serial	Analyte	Elemental composition	M.W	t_R(min)	Quantification transition(m/z)	CE (eV)	Confirmation transition(m/z)	CE(eV)	Ion ratio (average of ion ratio% ±RSD% for 0.5MRL-1.5MRL)
116	Nitroscanate	C13H8N2O3S	272.3	17.3±0.01	274.2>106.4	21	274.2>88.3	22	53%±14%
117	Arecoline hydrobromide	C8H14BrNO2	236.1	11.7±0.02	156.1>124.2	11	156.1>113.2	14	47%±13%
118	Closantel (-)	C22H14Cl2I2N2O2	663.1	18.6±0.04	660.7>278.9	42	660.7>126.9	46	76%±17%
119	Rafoxanide (-)	C19H11Cl2I2NO3	626	19.2±0.05	623.6>344.6	33	623.6>126.9	43	55%±2%
120	Nitroxinil (-)	C7H2O3	290	13.5±0.05	288.8>162.0	22	288.8>126.9	28	59%±14%
121	Oxyclozanide (-)	C13H6Cl5NO3	401.5	17.2±0.06	400.0>201.9	24	400.0>175.9	29	21%±6%
122	Disophenol (-)	C6H3I2NO3	390.9	14.7±0.04	389.7>234.8	33	389.7>126.9	43	66%±9%
123	Diminazene	C14H15N7	281.3	10.3±0.04	282.0>236.8	3	282.0>220.0	6	53%±7%
124	Imidocarb	C19H20N6O	348.4	10.4±0.06	349.3>188.2	26	349.3>162.4	21	75%±14%
125	Artesunate	C19H28O8	384.4	16.5±0.03	267.3>117.0	28	267.3>105.4	22	52%±14%
126	Piperazine	C4H10N2	86.1	7.3±0.02	87.0>68.4	17	87.0>44.5	17	23%±6%
127	Clorsulon (-)	C8H8Cl3N3O4S2	380.6	13.5±0.01	377.8>276.8	22	377.8>241.9	23	39%±8%
128	Pyrimethamine	$C_{12}H_{13}ClN_4$	248.7	13.7±0.02	248.8>128.0	46	248.8>110.9	43	48%±7%

*(-), the Ionization mode is ESI-; others are ESI+.

Table 2. Regulatory limits (RLs), Validation concentration (VL) and CCß for meat tissues.

Analyte	Swine meat			chicken meat			bovine meat		
	RL in meat (µg/kg)	Validation concentration (VL) (µg/kg)	CCß (µg/kg)	RL in meat (µg/kg)	Validation concentration (VL) (µg/kg)	CCß (µg/kg)	RL in meat (µg/kg)	Validation concentration (VL) (µg/kg)	CCß (µg/kg)
Ivermectin	/	20	20.14	/	20	20.21	10	10	10.15
Avermectin	/	20	20.16	/	20	20.2	/	10	10.1
Doramectin	40	40	40.11	/	40	40.17	40	40	40.11
Eprinomectin	/	10	10.22	/	10	10.24	50	50	50.1
Moxidectin	/	10	10.12	/	10	10.23	50	50	50.13
Milbemycin oxime	/	10	10.27	/	10	10.23	/	10	10.07
Emamectin	/	20	20.17	/	20	20.31	/	10	10.07
Albendazole	/	10	10.17	/	10	10.34	100	50	50.38
Albendazole sulphone	/	4	4.25	/	4	4.29	100	50	50.3
Albendazole sulfoxide	/	2	2.31	/	2	2.3	100	50	50.33
Albendazole-2-amino-sulfone	/	10	10.31	/	10	10.28	100	50	50.4
Fubendazole	50	25	25.28	50	25	25.27	/	10	10.36
Amino-flubendazole	50	25	25.29	50	25	25.33	/	10	10.25
Mebendazole	/	20	20.36	/	20	20.41	/	10	10.3
Amino-Mebendazole	/	2	2.38	/	2	2.22	/	10	10.2
Hydroxy-Mebendazole	/	4	4.36	/	4	4.21	/	10	10.17
Fenbendazole	50	25	25.13	/	4	4.4	50	25	25.37
Fenbendazole sulphone	50	25	25.35	/	50	50.25	50	25	25.36
Oxfendazole	50	25	25.34	/	50	50.35	50	25	25.32
Parbendazole	/	2	2.37	/	2	2.25	/	10	10.4
Oxibendazole	100	50	50.37	/	100	100.28	/	10	10.18
Thiabendazole	100	50	50.34	/	100	100.37	100	50	50.33
5-Hydroxy Thiabendazole	100	50	50.36	/	100	100.34	100	50	50.16

Triclabendazole	/	2	2.35	1500	750	750.37	200	100	100.27
Triclabendazole sulfoxide	/	2	2.36	1500	750	750.23	200	100	100.41
Triclabendazole sulphone	/	10	10.32	1500	750	750.24	200	100	100.38
Benomyl	/	20	20.34	/	20	20.21	/	10	10.27
Febantel	/	2	2.19	/	2	2.36	/	10	10.25
Monensin	/	4	4.19	1500	1500	1500.08	2	2	2.09
Salinomycin	/	10	10.19	600	600	600.14	/	10	10.19
Narasin	/	4	4.24	600	600	600.16	/	10	10.1
Maduramicin	/	20	20.19	240	240	240.09	/	10	10.09
Lasalocid	/	10	10.22	20	20	20.16	/	10	10.14
Nanchangmycin	/	4	4.3	1500	1500	1500.14	/	10	10.11
Dinitolmide (-)	/	20	20.27	3000	1500	1500.16	/	10	10.19
3-ANOT	/	10	10.06	3000	1500	1500.16	/	10	10.17
Methylbenzoquate	/	10	10.31	/	10	10.05	/	10	10.19
Decoquinate	/	4	4.26	2000	2000	2000.07	/	10	10.15
Toltrazuril (-)	100	50	50.24	100	50	50.1	100	50	50.22
Toltrazuril sulfone (-)	100	50	50.2	100	50	50.13	100	50	50.14
Diclazuril (-)	/	100	100.24	500	500	500.15	/	10	10.3
Cyromazine	/	2	2.27	50	50	50.17	300	300	300.27
Clopidol	200	10	10.24	5000	5000	5000.15	200	200	200.16
Nicarbazin	/	200	200.12	200	200	200.2	/	10	10.14
Amprolium	/	10	10.2	/	10	10.14	10	10	10.17
Ethopabate	/	10	10.26	/	10	10.18	500	500	500.26
Robenidine	/	20	20.19	500	500	500.16	/	10	10.24
Halofuginone	/	4	4.2	100	100	100.18	/	10	10.15
Sulfaclozine	100	50	50.15	100	50	50.17	100	50	50.2
Sulfaquinoxaline	100	50	50.13	100	50	50.09	100	50	50.13
Sulfadiazine	100	50	50.25	100	50	50.24	100	50	50.12
Sulfadimidine	100	50	50.26	100	50	50.21	100	50	50.18

Sulfadimethoxine	100	50	50.22	100	50	50.23	100	50	50.12
Sulfamethoxazole,	100	50	50.24	100	50	50.12	100	50	50.2
Sulfamonomethoxine	100	50	50.14	100	50	50.19	100	50	50.25
Sulfamethoxydiazine	100	50	50.27	100	50	50.1	100	50	50.14
Sulfamethoxypyridazine	100	50	50.26	100	50	50.23	100	50	50.15
Trichlorfon	/	100	100.3	/	20	20.21	50	50	50.16
Dichlorvos	100	20	20.22	50	50	50.18	/	10	10.16
Coumaphos	/	100	100.24	/	10	10.2	/	10	10.12
Coumaphos-oxon	/	2	2.26	/	2	2.23	/	10	10.21
Malathion	4000	20	20.15	4000	4000	4000.07	4000	4000	4000.12
Diazinon	20	4000	4000.26	/	10	10.22	20	20	20.1
Fenthion	/	20	20.14	/	10	10.17	/	10	10.17
Crufomate	100	10	10.27	100	100	100.2	100	100	100.17
Phoxim	/	100	100.17	/	10	10.21	/	10	10.13
Propetamphos	20	20	20.24	25	25	25.22	50	50	50.17
Dibrom	/	20	20.22	/	20	20.29	/	20	20.1
Rogor	/	10	10.28	/	10	10.17	/	10	10.16
Azamethiphos	/	20	20.13	/	20	20.28	/	20	20.24
Temephos	/	4	4.24	/	4	4.17	/	4	4.14
Ronnel	/	2	2.17	/	2	2.31	/	2	2.1
Aldicarb	/	4	4.3	/	4	4.25	/	4	4.2
Propoxur	/	4	4.16	/	4	4.18	/	4	4.16
Carbofuran	/	2	2.19	/	2	2.29	/	2	2.24
Metacrate	/	4	4.16	/	4	4.23	/	4	4.15
Carbaryl	/	4	4.29	/	4	4.15	/	4	4.18
Etrofolan	/	4	4.16	/	4	4.13	/	4	4.12
Pirimicarb	/	4	4.26	/	4	4.2	/	4	4.28
Diethofencarb	/	2	2.27	/	2	2.18	/	2	2.21
Furathiocarb	/	10	10.29	/	10	10.09	/	10	10.19
Methiocarb	/	10	10.28	/	10	10.22	/	10	10.14
Bendiocarb	/	10	10.28	/	10	10.25	/	10	10.16
Fenobucarb	/	4	4.29	/	4	4.18	/	4	4.15
Carbosulfan	/	4	4.27	/	4	4.07	/	4	4.09

Benfuracarb	/	20	20.28	/	20	20.21	/	20	20.24
Deltamethrin	/	10	10.21	30	30	30.15	10	10	10.16
Permethrin	/	4	4.24	/	4	4.16	50	50	50.22
Tetramethrin	/	4	4.26	/	4	4.19	/	10	10.25
Fenvalerate	1000	10	10.24	/	10	10.16	25	25	25.17
Cypermethrin	/	10	10.14	/	10	10.09	20	20	20.18
Cyfluthrin	/	2	2.13	/	2	2.16	10	10	10.19
Arsanilic acid	500	500	500.22	500	500	500.16	/	10	10.2
Roxarsone	500	500	500.2	500	500	500.12	/	10	10.24
4-nitrophenylarsonic acid	500	500	500.21	500	500	500.19	/	10	10.19
Carbarsone	500	500	500.17	500	500	500.21	/	10	10.13
Lindane	/	2	2.17	/	2	2.23	/	10	10.25
Acetofenate	/	10	10.15	/	10	10.17	/	10	10.19
Amitraz	/	2	2.19	10	10	10.24	/	10	10.16
2,4-DMA	/	2	2.3	10	10	10.21	/	10	10.19
Diflubenzuron (-)	/	2	2.24	/	2	2.15	/	10	10.28
Fluazuron (-)	/	2	2.24	/	2	2.27	/	10	10.17
Chlordimeform	/	10	10.23	/	10	10.25	200	200	200.14
Metronidazole	banned	1	1.16	banned	1	1.17	banned	1	1.12
Dimetridazole	banned	1	1.26	banned	1	1.28	banned	1	1.16
Levamisol	10	2	2.27	10	10	10.23	10	10	10.11
Pyrantel	/	2	2.21	/	2	2.26	/	2	2.2
Morantel	/	10	10.17	/	10	10.25	100	100	100.22
N-methyl-1, 3-propane diamine	/	2	2.17	/	2	2.27	100	100	100.23
Diethylcarbamazine citrate	/	4	4.2	/	4	4.17	/	10	10.19
Dithiazanine Iodide	/	4	4.22	/	4	4.22	/	10	10.14
Metyridine	/	10	10.18	/	10	10.22	/	10	10.15
Praziquantel	/	4	4.2	/	4	4.15	/	10	10.21
Niclosamide	/	10	10.13	/	10	10.29	/	10	10.16

Bithionol	/	10	10.31	/	10	10.24	/	10	10.25
Nitroscanate	/	2	2.25	/	2	2.11	/	10	10.15
Arecoline hydrobromide	/	2	2.26	/	2	2.23	/	10	10.16
Closantel	/	20	20.26	/	20	20.21	1000	1000	1000.22
Rafoxanide	/	20	20.25	/	20	20.31	30	30	30.13
Nitroxinil	/	20	20.2	/	20	20.2	400	400	400.13
Oxyclozanide	/	10	10.27	/	10	10.18	20	20	20.08
Disophenol	/	10	10.23	/	10	10.28	/	10	10.15
Diminazene	/	2	2.2	/	2	2.29	500	500	500.12
Imidocarb	/	20	20.18	/	20	20.29	300	300	300.13
Artesunate	/	40	40.23	/	40	40.2	/	10	10.19
Piperazine	400	400	400.19	/	400	400.16	/	10	10.17
Clorsulon	/	20	20.29	/	20	20.25	35	35	35.11
Pyrimethamine	/	4	4.29	/	4	4.14	/	10	10.17

RL: Regulatory limits and others with MRL according to EU regulation No 2010/37.

/: No MRL in meat.