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Original Research Article

Determination of albendazole and ivermectin residues in cattle and poultry-derived samples from India by micellar liquid chromatography

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ABSTRACT

We have developed a method, based on micellar liquid chromatography, to determine albendazole and ivermectin in dairy products and biological waste from bovine, as well as edible tissues from poultry. Anthelmitics were resolved in less than 10 min using a C18 column and a mobile phase of 0.15 mol/L sodium dodecyl sulfate – 6% 1-pentanol at buffered at pH 7 with a 0.01 M phosphate salt, running under isocratic mode at 1 mL/min. Detection was by absorbance at 292 nm. Method was successfully validated following official validation guidelines, in terms of: selectivity, sensitivity, calibration range (0.0125−0.5 mg/kg to 25−50 mg/kg), linearity ($r^2 > 9990$), trueness (86.3–105.6%), precision (<12.2 %), carry-over effect, robustness and stability. Procedure was easy-to-conduct, safe, ecofriendly, economical and with a high sample throughput. It can be used as screening method prior to a confirmatory one, like liquid chromatography-mass spectrometry. Finally, it was applied to incurred samples. In nearly half of the food samples, the analytes were found at concentrations (albendazole 0.20–4.1 mg/kg and ivermectin, 0.31–8.73 mg/kg) over the maximum residue limits stated by international regulations. This exposes consumer to the risk of suffering from severe diseases and points out an inappropriate administration of anthelmitic-based treatments.

1. Introduction

Anthelmintic is a term commonly referred to a wide range of chemical substances which either kill or inhibit the growth of helminths (worms) (Bowman, 2014). They can be classified into many types among which benzimidazoles and macrocyclic lactones are the most important groups of anthelmintics because of their broad-spectrum activity against helminths and other infective agents. Among these classes, albendazole (ALB), a benzimidazole, and ivermectin (IVE), a macrocyclic lactone, which structures are depicted in Figure SM-1 (Kim et al., 2016) are largely used to prevent and cure helminth infections in human, animal, and poultry (Bowman, 2014; Jedziniak et al., 2009; Puspitari et al., 2016). Although IVE was initially used to treat parasitic infections in animals, soon its potential was identified and recommended to cure human parasitic infections (Dubroca et al., 2003; González Canga et al., 2008; Danaher et al., 2006). Albendazole (molecular mass = 265.33 g/mol: log Po/w = 3.2; acidic pKa = 9.5; basic pKa = 4.3; charge at pH 7 = +0; charge at pH 5 = +0.16; charge at pH 3 = +0.95) and IVE (molecular mass = 875.1 g/mol; log Po/w = 5.8; without neither acidic nor basic pKa; and charge at pH 3−7 = 0) (Kim et al., 2016; Wishart et al., 2017) are frequently prescribed either as single dose or in combined dosage form as per the requirement. These compounds are lipophilic in nature and have long withdrawal periods. Thus, their rampant and extensive use in animal increases the possibility of ocurrence of residues in food stuffs of animal and poultry origin what leads to several adverse effects on chronic exposure. The adverse effects associated with benzimidazole compounds are teratogenicity, congenic malformations, polyploidy, diarrhea, anemia, pulmonary ademas *etc.* (Hu et al., 2010). Therefore, use of albendazole has been approved in cattle but it is not recommended in dairy cattle of breeding age (Bowman, 2014). Similarly, drug resistance to dung beetles, intestinal microbes and other beneficial plants are some of the noted adverse effects of IVE (Jacobs and Scholtz, 2015). ALB and IVE are also reported as the environmental and agricultural contaminant. Thus, there is a significant risk that wild

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worms can be affected by high concentrations of these drugs found in the environment. Exposure to low concentrations of these compounds in the environment and agriculture samples may also encourage the development of resistant strains of parasites (Horvat et al., 2012).

Therefore, to protect the consumers from risk associated with these compounds, maximum residue limits (MRLs) have been set by several national and international regulatory bodies such as U.S. Food and Drug Administration (FDA) (FDA, 2019), Codex Alimentarius (FAO, 2018), EU Commission (European Commission, 2010), Food Safety and Standard Authority of India (FSSAI) (Media et al., 2019), etc. for the parent drugs and their metabolites in various food products of animal origin, like meat, meat products, poultry, fish, milk etc. The value of MRLs varies depending on the compounds and sample of interest. By and large, MRL for ALB are: for bovine milk and milk products (European Commission, 2010; Media et al., 2019) and chicken fat and muscle (FAO, 2018; Media et al., 2019), 0.1 mg/kg, and for chicken liver and kidney, 5 mg/kg (FAO, 2018; Media et al., 2019). MRL for IVE in milk and milk products has been set to 0.01 mg/kg (FAO, 2018; Media et al., 2019). However, these two anthelmintic drugs are largely used by cattle and poultry farmers in the area of Sagar (India), and some of them may be inadequately administrating ALB and IVE to the animals (Pawar et al., 2019).

Bovine dairy products and meat poultry are among the animal-derived foodstuff most produced and consumed in India. Therefore, the quality of finished products has a significant impact on Indian diet and economy. The unwanted presence of these substances in food items ought to be checked before launching the product to the market, as it poses a potential threat to human health and could also lead to the development of resistance among disease-causing agents (Puspitari et al., 2016; Pawar et al., 2020). The analytes should also be checked in biological samples and nitrogenous waste from the animals, as their presence in these matrices can be used as a proof of inadequate administration of the drugs (Bowman, 2014; Wishart et al., 2017) and it is a common way these contaminants reach and damage the environment (Beynon, 2012). Besides, food products manufactured in India should comply with the regulations of the different national and international agencies to allow their exportation and generate and keep an image of Indian producers as reliable and Indian food products as healthy and safe (Pawar et al., 2020).

Several analytical methods such as thin layer chromatography (TLC) (Floate et al., 1997; Long et al., 1989), high performance liquid chromatography (HPLC) (Legrae et al., 2020; Dos Santos Magalhaes, 2014), liquid chromatography-tandem mass spectrometry (LC–MS/MS) (Garrido Frenich et al., 2010; García-Gómez et al., 2012; Pugajeva et al., 2019) and ultra-high performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) (Pérez et al., 2013; Wang et al., 2021) have been developed and validated for the determination of the ALB and IVE and their metabolites in food and biological samples such as dung, milk, cheese, meat, urine and eggs, among others.. However, the above-mentioned methods require tedious and long sample extraction steps.. Although, LC–MS is a selective and sensitive technique, it is excessively expensive and sophisticated. Therefore, it needs a prior sample screening technique as most of the times, to discard moderately-highly contaminated samples and restrain the use of LC–MS for samples which concentration ranges from 0 to slightly above the MRL, or where the identification is doubtful, as a confirmatory method to determine the accurate concentration. However, as far as we know, there is no bibliography about the determination of these two anthelmintic drugs in dairy products, biological waste and edible tissues by HPLC-Absorbance detection for screening purposes.

Micellar liquid chromatography (MLC), a branch of HPLC, has been demonstrated as an interesting alternative to determine drugs in food and biological waste (Pawar et al., 2020), either solid or liquid. It uses mobile phases made up of a surfactant, usually the anionic sodium dodecyl sulphate (SDS), and a low proportion of an organic solvent (hybrid micellar solutions). These solutions have shown high performances as extractant (for solid matrices) and dilution (for liquid samples), to-be-used in the sample treatment. Indeed, micellar media have a strong solubilization capacity, as they are able to solve substances in a large range of hydrophobicity, including biological macromolecules (*e.g.* proteins); since micelles have electrostatic, hydrophilic and hydrophobic sites. Besides, the obtained solution can be directly injected in the column, thus avoiding clean-up and purification steps, what shortens the analysis time. As they strongly bind the micelles, the macromolecules are harmless eluted at the front of the chromatogram, instead of precipitating in the chromatographic system. Besides, hybrid micellar solutions contain less than <15 % of toxic and volatile solvent (far less than required in hydro-organic RP-HPLC), and the SDS is innocuous and biodegradable, thus being eco-friendlier and safer (Esteve-Romero et al., 2005; Peris-Vicente et al., 2014; Peris-Vicente et al., 2016). As far as we know, green HPLC techniques have not previously used to determine albendazole and ivermectin in food samples.

The aim of the research was the development of a reliable and practical screening method based on micellar liquid chromatography for the determination of ivermectin and albendazole in bovine (milk, curd, butter milk, butter, cheese, dung, urine and saliva) and poultry (chicken gizzard, muscles, liver and kidney) samples. It can be applied to rule largely contaminated samples out LC—MS analysis, what would give rise to a significant cost saving for the laboratory.. The effect of the main chromatographic conditions on the retention were carefully studied to select the optimal values (maximizing resolution and minimizing analysis time). The method was validated by official guidelines, to check its suitability (Peris-Vicente et al., 2015), and finally applied to incurred samples obtained from several supermarkets, shops and producers in Sagar (Madhya Pradesh, India) area.

2. Materials and methods

2.1. Sample collection and treatment

All samples were randomly taken from Sagar and Chhindwara city in the state of Madhya Pradesh, India.

Bovine (milk, curd, butter milk, butter and cheese) and chicken (gizzard, muscles, liver and kidney) food samples were collected from several local dairy shops and markets. Milk samples were also collected from the milkman. Bovine biological waste, like dung, urine and saliva were sampled from several farms were animals are raised as an intensive way. Incurred samples were those from animals treated with ivermectin and albendazole, while blank samples were from animals having no previous history of any kind of medical treatment. Solid samples (curd, butter, cheese and poultry tissues) were finely ground using a mincer (Model MZ10, Petra Electric, Burgau, Germany) at 5000 rpm for 5 min and stored in vacuum bags at -20 °C, while liquid samples (milk, butter milk, urine and saliva) were directly kept under the same conditions. Thawing process was as follows: samples were kept at +4 °C the night before the analysis and left on the workbench the same day of the analysis until the room temperature was reached.

Incurred and blank samples were treated as the same way. Solid samples were processed by a batch stirring-assisted solid-to-liquid extraction (BSASLE) followed by a batch ultrasound-assisted solid-to-liquid extraction (BUASLE) so that the analyte end up in a solution (Pawar et al., 2020). The same procedure was applied to liquid samples, despite the analytes were already in a liquid matrix, to favour the separation of ALB and IVE from the original matrices. Five grams of liquid (except for milk, which was 2.5 g as described by Peris-Vicente et al., 2016) or minced solid were mixed with 20 mL of mobile phase in an Erlenmeyer flask, shaken using a magnetic stirrer for 1 h and ultrasonicated for 15 min. The mixture was transferred to a volumetric flask, which was filled up to 25 mL. The supernatant was filtered and directly injected. Samples were treated the same day of the analysis and none of the obtained solutions were stored.

In the case of fortified samples, a definite volume of drug solution

was injected into the weighed sample or liquid, which were left one day to attain the complete integration of the drugs with the matrix. Those samples imitate the "naturally" contaminated ones (Peris-Vicente et al., 2019). For samples over the upper limit of quantification, the appropriate extra-dilution was carried out, using mobile phase as solvent (Mishra et al., 2018).

2.2. Chemical and reagents

Solid standards of Albendazole (>98 %), Ivermectin (>99 %) and anhydrous sodium acetate ((CH_3COONa, purity >99.0 %) were purchased from Sigma-Aldrich (St. Louis, MO, USA). SDS ($C_{12}H_{25}SO_4Na$, >98 %), sodium dihydrogen phosphate monohydrate (NaH_2PO_4, >99.0 %) and HPLC grade methanol (CH_3OH), 1-butanol (C_4H_9OH) and 1-pentanol ($C_5H_{11}OH$) were bought from Scharlab (Barcelona, Spain). HPLC grade 1-propanol (C_3H_7OH) was supplied by Merck KGaA (Darmstadt, Germany). Hydrochloric acid (HCl, 37.0 %) came from J.T. Baker (Deventer, The Netherlands). Sodium hydroxide (NaOH; >98 %) was purchased from Riedel-deHaën (Hannover, Germany). Ultrapure water was in-lab produced from deionized water (provided by the University as tap water) using an ultrapure generator device Simplicity UV (Millipore S.A.S., Molsheim, France). All the aqueous solutions were prepared using freshly made ultrapure water. Prepared ultrapure water was not stored.

2.3. Preparation of solutions

Stock solutions of standard ALB and IVE were prepared at a concentration of 1 mg/mL for each compound by dissolving appropriate amount of the drug separately in methanol. These solutions were sonicated for 5 min using an ultrasonic bath for the proper dissolution and finally stored in amber volumetric flask at 4 °C. Standard solutions for validation studies were freshly prepared by appropriate dilution of the stock solutions with the mobile phase, and again stored at 4 °C.

Micellar solutions were prepared as follows: the proper amount of SDS and buffer salt (sodium dihydrogen phosphate monohydrate for pH 3 and 7; and sodium acetate for pH 5) were solved in ultrapure water, and the pH was adjusted to the desired value by adding drops of HCl or NaOH solutions and introduced in a volumetric flask. Afterwards, we added the adequate volume of the organic solvent, and then we fill up the flask with ultrapure water. The solution was ultrasonicated for 15 min to achieve solubilization and filtered through a 0.45-μm-Nylon filter (Micron Separations, Westboro, MA, USA) placed in a Büchner flask, with the aid of a vacuum pump. These solutions were stored in amber bottles.

2.4. Chromatographic conditions

Chromatographic analyses were carried out using an HP1100 (Agilent Technologies, Palo Alto, CA, USA) system equipped with an isocratic pump, a degasser, an autosampler, a 20-μL loop, a C18 Kromasil (Scharlab, Barcelona, Spain) column (length, 150 mm; internal diameter, 4.6 mm; particle size, 5 μm; pore size, 10 nm) and an UV–vis absorbance diode array detector (DAD); connected to a PC. No module worked at a regulated temperature. Mobile phase was an aqueous solution of 0.15 M SDS, 6% pentanol (v/v) buffered at pH 3 with 0.01 M phosphate salt, running at 1 mL/min under isocratic mode. The detection was performed by absorbance at 292 nm. All the samples to be injected were filtered by manual pushing through a 0.45-μm-Nylon membrane filter, with the aid of a 3 mL syringe, and placed in amber vials.

The software Chemstation Rev.A.10.01 (Agilent Technologies) was used to control the instrumentation as well as to register and process the signal. The dead time ($t_0 \approx 1.0$ min) and the retention time (t_R) were directly taken from the chromatogram. We calculated the retention factor (k) (Harris, 2007), efficiency (N, number of theoretical plates), asymmetry (B/A), and resolution (Rs) (Erner and Miller, 2005). The working and cleaning specifications regarding the utilization of the chromatographic instrumentation when using micellar mobile phases were described by Mishra et al., 2019.

2.5. Method validation

Validation of the procedure was carried out by the guidelines of the EU Commission Decision 2002/657/EC (European Commission, 2002) for food samples and Standard Practices for Method Validation in Forensic Toxicology for biological waste (Scientific Working Group for Forensic Toxicology (SWGTOX, 2013); and assisted by other documents about validation and statistical calculations (Peris-Vicente et al., 2015; Erner and Miller, 2005). The general parameters were determined, i.e. linearity, instrumental calibration range and sensitivity, robustness and carry-over effect in standard solution, while, precision, trueness, method sensitivity and calibration range in matrix using spiked blanks (For each kind of food, the "blank samples" was a sample food free of both albendazole and ivermectin).

2.5.1. Calibration range, linearity and sensitivity

In order to study linearity of the ALB and IVE, nine dilutions (n = 9) were prepared from the stock solutions. Analysis was performed in the range of 0.025–5.0 mg/L by n = 5 injections of each compound at different concentrations. The calibration curve was constructed using the average peak area vs. concentration. The slope, y-intercept, coefficient of determination (r2) and residual standard deviation were calculated by the least square linear regression method.

Limit of detection (LOD) is the smallest concentration providing a signal distinguishable from the baseline noise, while the limit of quantification (LOQ) was the minimal concentration reliably quantifiable. They were calculated as 3.3 and 10 times, respectively, the standard deviation of the y-intercept divided by the slope of the calibration curve. Upper limit of quantification (ULOQ) was the maximum point of the calibration curve.

2.5.2. Robustness

Robustness of the developed method was determined by the analysis of a standard solution containing 0.5 mg/L of both analytes, slightly changing the chromatographic factor like concentration of SDS, percentage of pentanol (v/v), pH and the flow rate of the mobile phase from the optimal values, while the other conditions remain at their optimal value (one-factor-a-time strategy). The considered intervals for each factor are in Table SM-1. The studied responses were retention time, peak area and efficiency.

2.5.3. Carry-over effect

Cross contamination between samples successively injected was evaluated. Indeed, this would result in a false positive, what could provoke the unfair refusal of the batch and legal consequences to the producer or distributor. We analyse a standard solution containing 5 mg/L of both drugs, and a blank immediately after.

2.5.4. Selectivity

We checked the possible coelution or overlapping of the drugs with other compounds of the matrix. Firstly, we analysed a blank sample of all the studied matrices: bovine (milk, curd, butter milk, butter; cheese; dung, urine and saliva) and chicken (gizzard, muscles, liver and kidney). Furthermore, those samples were fortified at the appropriate concentration to get an injected solution of 0.5 mg/L, and analyzed (that means 5 mg/kg for milk and 2.5 mg/kg for the other samples).

2.5.5. Method calibration range and sensitivity

The method detection limit (MDL), method quantification limit (MQL), and upper method quantification limit (UMQL) were calculated from instrumental LOD, LOQ and ULOQ, respectively, taking into

account the sample processing.

2.5.6. Trueness and precision

These parameters were determined at three levels (values in Table 1) for each one of the studied matrices: bovine milk, curd, butter milk, butter, cheese, dung, urine and saliva, as well as poultry chicken gizzard, muscles, liver and kidney. For each sort of sample, six blanks were spiked at the corresponding concentration and analyzed in the same run. The same assay was applied five times in five days (using renewed samples) over a three-month period.

Trueness was calculated over the five runs as the percentage of the amount that was spiked into the food matrix (the quotient of the grand mean of found concentrations and the true value, relative recovery). Repeatability was the relative standard deviation (RSD) of the six found concentrations from the run with the higher dispersion. Intermediate precision calculated over the five runs, as the RSD of the grand mean. Acceptance criteria were taken from the following documents: European Commission, 2002 and Scientific Working Group for Forensic Toxicology (SWGTOX, 2013 for food and biological samples, respectively.

2.5.7. Stability

The resistance of the drugs to chemical change through time was evaluated in standard solution and matrix (food and biological waste) at their usual long-term storage conditions, respectively (+4 °C and -20 °C, both in darkness), as well as bench-top in mobile phase (like processed samples) under at room temperature for two days (maximum time a processed sample is expected to spend between preparation and injection). Decomposition was appraised by monitoring the peak area and the appearance of other peaks (considered as decay products). Maximum storage time was the time at which the peak area fell (compared to the fresh one): 5% for standard solution, and the corresponding trueness acceptance criteria for each matrix, respectively. The procedure to evaluate this parameter was adapted to each sort of stability, as described below:

a) Standard solution (0.5 mg/L) was analyzed (fresh sample) and thereafter placed in the fridge at -4 °C in darkness. Each day for two months, an aliquot was taken and analyzed.

b) Thirty-one blank samples were fortified at 2.5 mg/kg (5 mg/kg for milk). The first one was analyzed (fresh sample) and the other ones were frozen at -20 °C. Each two days, for two months, one sample was thawed and analyzed. This procedure was separately performed for all the studied samples (see section 2.5.6).

c) A standard solution (0.5 mg/L) was placed in the autosampler tray and analyzed each 15 min for two days.

3. Results and discussion

Bovine dairy products and poultry edible tissues were selected for the study. as they are highly elaborated and consumed in India (the most among animal-derived food), and then the related industry has a relevant weight on Indian economy. Therefore, the elaboration and distribution of food with anthelmitic residues by unethical farmers could be dramatic in terms of impact on health, (many consumers may be affected) and economy (the image of the entire industry can be harmed). As a consequence, it is of the utmost importance to detect contaminated samples before being launched to the market. The analysis of biological waste can be used an evidence of misadministration of these two anthelmintic drugs. Otherwise, biological waste is the primary source of IVE and ALB pollution to the environment, and then it should be controlled for environmental reasons.

3.1. Optimization of the chromatographic conditions

The tandem C18/SDS as column and surfactant has been demonstrated as a suitable choice to determine drugs in edible tissues of animals (Peris-Vicente et al., 2019), dairy products, and biological waste

Table 1
Trueness and precision of the proposed method (trueness[a], %/repeatability[a], %/intermediate precision[b], %).

Matrix	Concentration	Albendazole	Ivermectin	Acceptance criteria
Milk	0.5 mg/kg	93.6/4.4/5.8	105.6/4.9/6.0	80–110/<11.9
	5 mg/kg	97.2/3.2/3.8	103.8/3.6/4.7	80–110/<8,4
	15 mg/kg	100.5/2.0/2.8	100.8/1.5/2.9	80–110/<7.1
Curd	0.25 mg/kg	90.2/7.3/9.9	89.4/8.4/10.5	80–110/<13.2
	2.5 mg/kg	92.6/5.4/7.5	93.2/6.2/8.7	80–110/<9.3
	10 mg/kg	98.2/3.8/4.3	98.1/4.0/4.9	80–110/<7.6
Cheese	0.25 mg/kg	87.9/8.6/11.2	89.2/7.3/10.9	80–110/<13.2
	2.5 mg/kg	93.2/6.3/7.4	94.6/6.7/7.5	80–110/<9.3
	10 mg/kg	97.9/3.5/4.1	98.5/3.9/4.9	80–110/<7.6
Butter milk	0.25 mg/kg	95.7/3.2/4.4	96.3/3.8/4.7	80–110/<13.2
	2.5 mg/kg	97.8/2.0/2.8	98.5/3.1/3.5	80–110/<9.3
	10 mg/kg	100.3/1.9/3.1	99.8/1.3/2.0	80–110/<7.6
Butter	0.25 mg/kg	93.8/6.5/8.7	90.5/6.7/7.4	80–110/<13.2
	2.5 mg/kg	95.7/4.6/5.9	103.6/5.8/6.4	80–110/<8.3
	10 mg/kg	102.4/2.9/3.7	97.5/2.4/3.5	80–110/<7.6
Urine	0.25 mg/kg	94.6/4.8/5.9	97.6/4.2/5.2	80–120/<20
	2.5 mg/kg	97.5/3.8/3.9	98.1/3.4/4.0	
	10 mg/kg	100.8/2.6/2.6	99.9/2.0/3.5	
Dung	0.25 mg/kg	86.3/9.5/11.9	88.9/7.6/10.4	80–120/<20
	2.5 mg/kg	91.8/7.3/8.5	90.1/5.2/7.3	
	10.0 mg/kg	97.8/4.4/5.9	96.4/3.8/4.7	
Saliva	0.25 mg/kg	103.4/3.5/4.4	97.7/3.3/4.0	80–120/<20
	2.5 mg/kg	100.8/2.8/3.2	99.9/4.6/5.1	
	10 mg/kg	99.78/1.6/2.2	101.5/1.8/2.3	
Chicken muscle	0.25 mg/kg	93.5/7.5/9.7	89.7/8.9/9.4	80–110/<13.2
	2.5 mg/kg	101.6/4.9/6.8	95.9/5.8/7.5	80–110/<9.3
	10.0 mg/kg	97.6/2.4/3.5	98.6/2.6/4.2	80–110/<7.6
Chicken kidney	0.25 mg/kg	94.3/8.3/11.5	92.3/9.4/12.2	80–110/<13.2
	2.5 mg/kg	99.8/6.2/8.5	98.6/7.8/8.9	80–110/<9.3
	10.0 mg/kg	96.5/3.7/4.8	96.2/4.3/5.4	80–110/<7.6
Chicken liver	0.25 mg/kg	95.8/9.4/11.5	91.5/7.5/9.7	80–110/<13.2
	2.5 mg/kg	92.5/7.1/8.4	96.9/7.2/8.7	80–110/<9.3
	10 mg/kg	98.5/4.0/5.6	97.4/3.4/5.2	80–110/<7.6
Chicken gizzard	0.25 mg/kg	98.7/8.4/10.8	101.6/8.8/9.4	80–110/<13.2
	2.5 mg/kg	94.3/5.4/6.7	93.5/6.9/8.2	80–110/<9.3
	10.0 mg/kg	90.6/4.5/6.6	91.6/3.9/4.8	80–110/<7.6

[a] n = 6.

[b] n = 5.

(Pawar et al., 2020). As pure micellar mobile phases exhibit weak elution power, the addition of an organic solvent is usually required for the main of the drugs to achieve untroublesome retention times and relatively Gaussian peak shape, because of the decrease in polarity and viscosity of the bulk mobile phase. Injection volume, flow, and temperature were set at their usual values (Peris-Vicente et al., 2014).

The experimental chromatographic conditions optimized in the frame of this study were: composition of the mobile phase (nature of organic solvent, concentration of SDS and organic solvent and pH) and detection conditions.

3.1.1. Selection of the pH

The pH of the mobile phase has strong implications in the retention mechanism and has to be properly selected to achieve a refined resolution and selectivity. Firstly, it acts on the stationary phase, as the pKa of the silanol groups is 4–5; and the neutralization of the silanol groups usually lead to an enhance of the asymmetry (Harris, 2007). Secondarily, it influences the charge of the solutes, what also depends on their acidic/basic characteristics. The optimum pH used for separation is generally related to its effect on the resolution, by the examination of its effect on the retention of each analyte. The working pH is restricted by the stability window of the alkyl-silica bonds (2.5–7.5).

When working with SDS, the stationary phase exhibits a negatively charged layer, because SDS-monomers tend to adsorb on the C18-alkyl chains, with the anionic head oriented to the mobile phase. Negatively charged compounds are repelled by electrostatics, thus strongly reducing the retention. Neutral and cationic compounds interact with the stationary phase by hydrophobicity and/or electrostatics and display a higher retention. However, these compounds interact as the same way with the micelles, and then the higher concentration of SDS, the earlier elution they show. In general, MLC conditions can be optimized to allow the resolution in the same run of charged and neutral analytes (Esteve-Romero et al., 2005).

The pH study was performed by keeping the concentration of organic modifier and surfactant constant at the optimal value, whereas the pH was varied to 3, 5 and 7. Retention of ALB decreased at increasing values of pH, as it gradually changes from monocationic to neutral. Ivermectin does not show very pronounced effects of the pH since it remains as neutral in the studied interval. Finally, pH 3 was selected as the optimum pH to carry out the analysis of ALB and IVE. The reason for selecting pH 3 lies in the fact that at pH 5 and 7, ALB was co-eluting with IVE. Therefore, in order to avoid overlapping peaks and achieve good resolution, pH 3 was selected as the optimum value for further separation.

3.1.2. Selection of the SDS concentration

SDS concentration was optimized in the interval 0.05−0.15 M (0.05 – 0.075 – 0.10 – 0.125 – 0.15 M) at pH 3. As a strong retention is expected using pure micellar mobile phases (because of the moderate hydrophobicity and positive charge of ALB, and extreme hydrophobicity of IVE), 6% 1-pentanol was added. The tested concentrations were clearly above the critical micellar concentration (CMC) of SDS (8 mM), in order to ensure the formation of micelles. We know the number of aggregation is constant, and then, the increase of the concentration of SDS results in an increase in the number of micelles, while the concentration of free monomer in the bulk mobile phase (equal to the CMC) and the amount of SDS-monomers adsorbed on the mobile phase remains invariant (Peris-Vicente et al., 2016).

We observed that higher the concentration of SDS, the shorter was the retention time. That proves micelles get to link with the analytes (by electrostatics combined with hydrophobic attraction for ALB, while pure hydrophobic for IVE), thus reducing the interaction of the solute with the stationary phase, what indicates a binding behaviour of the analytes with the micelles. Finally, the SDS concentration was set to 0.15 M, as the other values provide longer retention capacities, without a significant improvement of the resolution.

3.1.3. Selection of the alcohol

The effect of the addition of the organic solvents 1-propanol, 1-butanol and 1-pentanol was examined. The anthelminthic drugs were determined using four mobile phases containing 0.15 M SDS, buffered at pH 3, and: the maximal concentration recommended for each alcohol, i.e. 12.5 % 1-propanol (v/v), 7 % 1-butanol (v/v), 6 % 1-pentanol (v/v) and without alcohol.

The obtained results indicated that 1-pentanol reduces the retention time to less than 10 min. with a respectable resolution between two peaks, whereas 1-butanol; 1-propanol and the pure mobile phase took 25, 40 and 50 min, respectively. We notice that the effect of the alcohol in the elution strength was directly proportional to the length of carbon chain, as usual in MLC (Peris-Vicente et al., 2014). Thus, 1-pentanol at 6% was selected as the optimum modifier for rapid analysis.

3.1.4. Selection of the detection conditions

Both ALB and IVE are UV active compounds due to the presence of conjugated saturated and unsaturated bonds as well as the presence of heteroatoms like nitrogen, sulphur and oxygen (Figure SM-1). However, the spectroscopic properties considerably depend on the chemical environment, and then the bibliographic data could not be used (Peris-Vicente et al., 2014). The absorbance spectra of both drugs were in-line taken during the analysis using the optimal separation conditions. Maximum absorbance was 305 nm for ALB and 245 nm for IVE, respectively. As both compounds exhibit adequate absorbance at 292 nm, this value was considered as optimal.

3.1.5. System suitability testing

Finally, the optimum mobile phase was an aqueous solution containing 0.15 M SDS, 6% 1-pentanol (v/v), pH 3; and the registered signal was the absorbance at 292 nm. A system suitability test (SST) was performed, by the consecutive injection (n = 6) of a standard solution containing 0.5 mg/L of both analytes. Chromatograms obtained by the analysis of ALB, IVE and their mixture can be seen in Figure SM-1. Entire absorption spectra were taken at the t_R, and leading and tailing edge at 5- and 50 % height for further peak purity studies. Results of the system suitability test (t_0 = 1.5 min) were (albendazole; ivermectin; acceptance criteria, taken from (Harris, 2007; Erner and Miller, 2005): retention time, min (9.21 ± 0.09; 8.12 ± 0.08; —); relative standard deviation (RSD) of t_R, % (1.0; 1.0; <2.0); RSD of peak area, % (0.8; 0.9; 2.0); retention factor (5.14; 4.41; 0.5–20); number of theoretical plates (2106; 2036; >2000); asymmetry (1.2; 1.4; 0.8–1.6) and resolution (1.2 between both compounds; >2.0).

Retention times were appropriate to avoid overlapping with endogenous compounds or excipients, which are expected to elute at the dead time. Results were inside the acceptance criteria, indicating the experimental conditions are suitable for the analysis, and the instrumentation provides stable responses. The only exception was resolution. Nevertheless, the overlapping is minimal and does not obstruct the quantification.

Satisfactory resolution was obtained using an eco-friendly and safe aqueous mobile phase (only 6% of toxic and volatile solvent), whereas hydroorganic mobile phases are highly pollutant (up to 100 %). In addition, the separation was performed using mobile phase running under isocratic mode, despite the difference of nature between the two drugs, thus avoiding the typical problems of gradient (lack of baseline stability, appearance of spurious peaks and a higher variability in response, need of a stabilization time between two injections, as stated by Peris-Vicente et al., 2019. This was acquired by the remarkable properties of micellar mobile phases.

It was surprising the close retention time of both compounds, regardless of the difference of hydrophobicity. Indeed, ALB is

monocationic, what increases retention, while IVE molecules is bulky and due to stearic hindrance, it is not properly retained on the chromatographic column (Ruiz-Ángel et al., 2009).

3.2. Method validation

3.2.1. Calibration range, linearity and sensitivity

The highest and the lowest variance were found equal by a

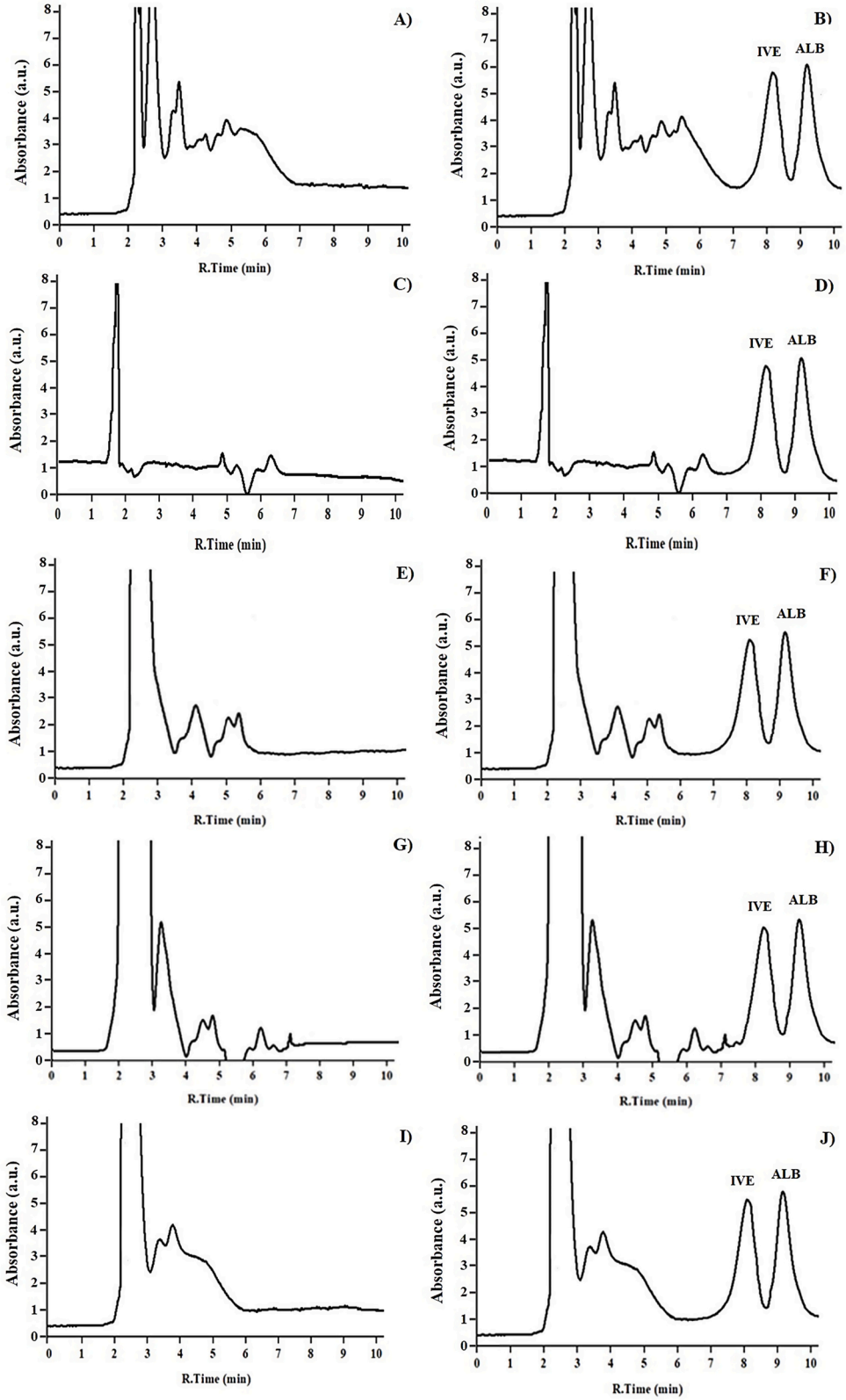

Fig. 1. Chromatograms obtained by the analysis of blank (A, C, E, G and I) and spiked (B, D, F, H and J) (injected concentration of 0.5 mg/L for both analytes) bovine dairy products: A,B) milk; C,D) curd; E,F) butter; G,H) butter milk and I,J) cheese.

Snedecor's F test (α = 5%), and then the residuals were homoscedastic. The goodness-of-fit of the model was verified by the following tests:

- Normality of the residuals: no trend was observed in the plot residual *v.s.* concentration, either by visual observation or by applying a Wald-Wolfowitz run test (significance level 5%).

- r^2 was 0.9994 for both compounds, and the relative residual standard deviation were 1.0 % (ALB) and 1.3 % (IVE) (Acceptance criteria of 0.995 and 1.5 %, respectively). A significant correlation was found by a *t*-test on r (p = 0.000 %, acceptance criteria<5%)

- No systematic error was detected, as the y-intercept represents <1.5 % of the target concentration (0.5 mg/L).

- No outliers were detected, as the squared Cook distance was <1 and the relative residuals were <3.

. LOD, LOQ and ULOQ, for both drugs, were 0.01; 0.025 and 5 mg/L, respectively.

3.2.2. Robustness

Results of robustness study are shown in Table SM-1. Even though no acceptance criteria are usually considered (Little, 2016), we state a variation over 5% is significant. Variation in the flow rate had a stronger influence on the retention time of both compounds as expected, while 1-pentanol affects the retention time of ALB. We consider the method provides consistent and stable responses, even though the employed operating conditions somewhat differs from the optimal ones.

3.2.3. Carry-over effect

No peak was observed in the second chromatogram (obtained from the blank sample) at the window time of the analytes, thus pointing out the absence of carry-over effect.

3.2.4. Selectivity

In the blank samples, peaks corresponding to the endogenous compounds were eluted before 7.0 min; and no peak was detected at the window time of the analytes. In the fortified samples, no overlapping of the analytes with the front of the chromatogram or other matrix compounds was observed. The chromatograms corresponding to the analysis

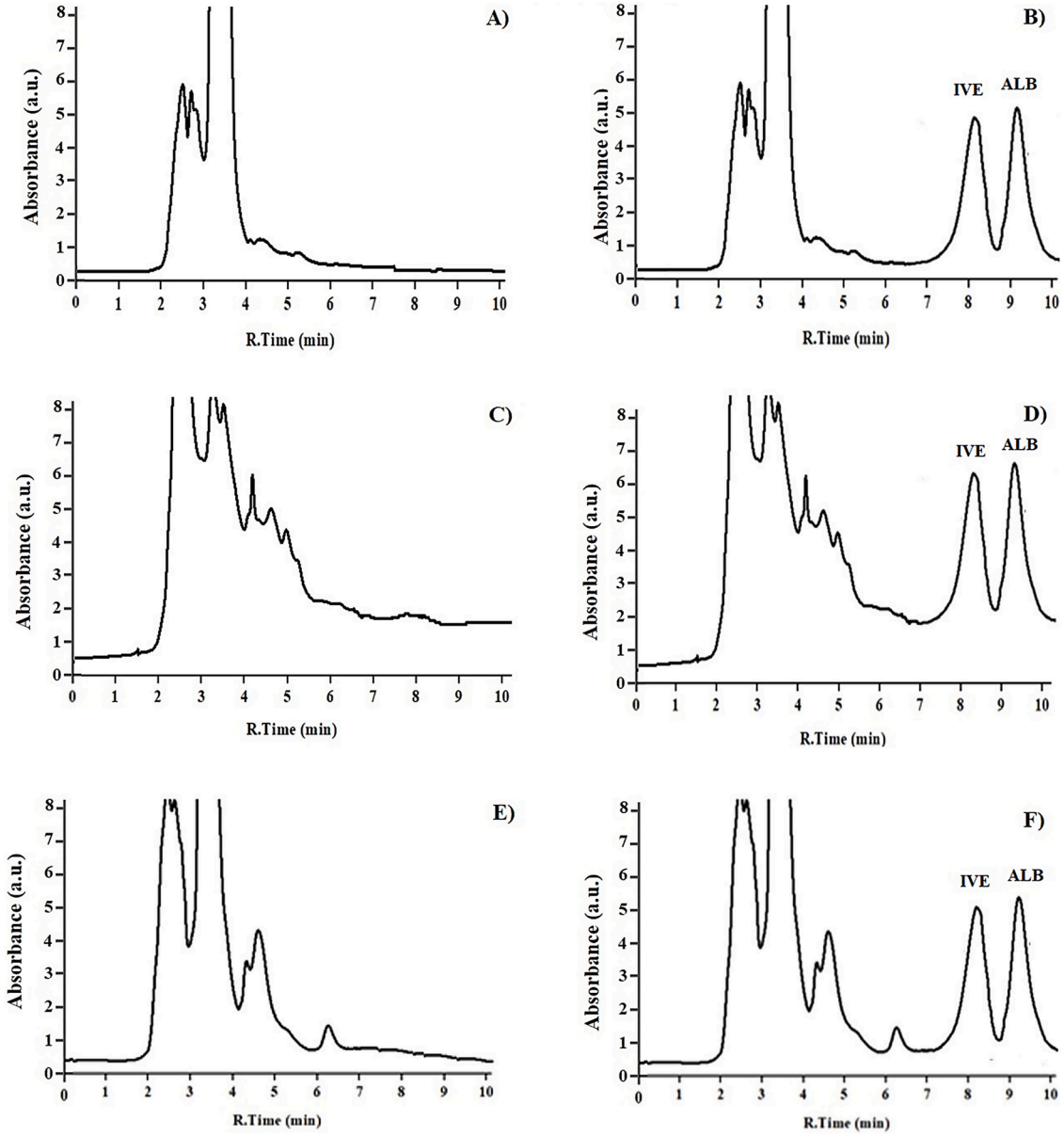

Fig. 2. Chromatograms obtained by the analysis of blank (A, C and E) and spiked (B, D and F) (injected concentration of 0.5 mg/L for both analytes) bovine biological waste samples: A,B) urine; C,D) dung; and E,F) saliva.

of blank and spiked samples of bovine dairy products, bovine biological waste and chicken edible tissues can be seen in Figs. 1, 2 and 3 .

The chromatograms were compared by overlaying with those obtained from the standard solution. For ALB and IVE, differences in retention time was <2.5 % (proving the obtained peaks are effectively the anthelmintic drugs), and peak shape and area were similar. Finally, we carried out a peak-purity study, hence the entire absorbance spectra were taken at the same points as in 3.1.5. For each matrix and analyte, we compare by overlaying and visual observation the absorbance spectra obtained at each point of the peak to those from the standard

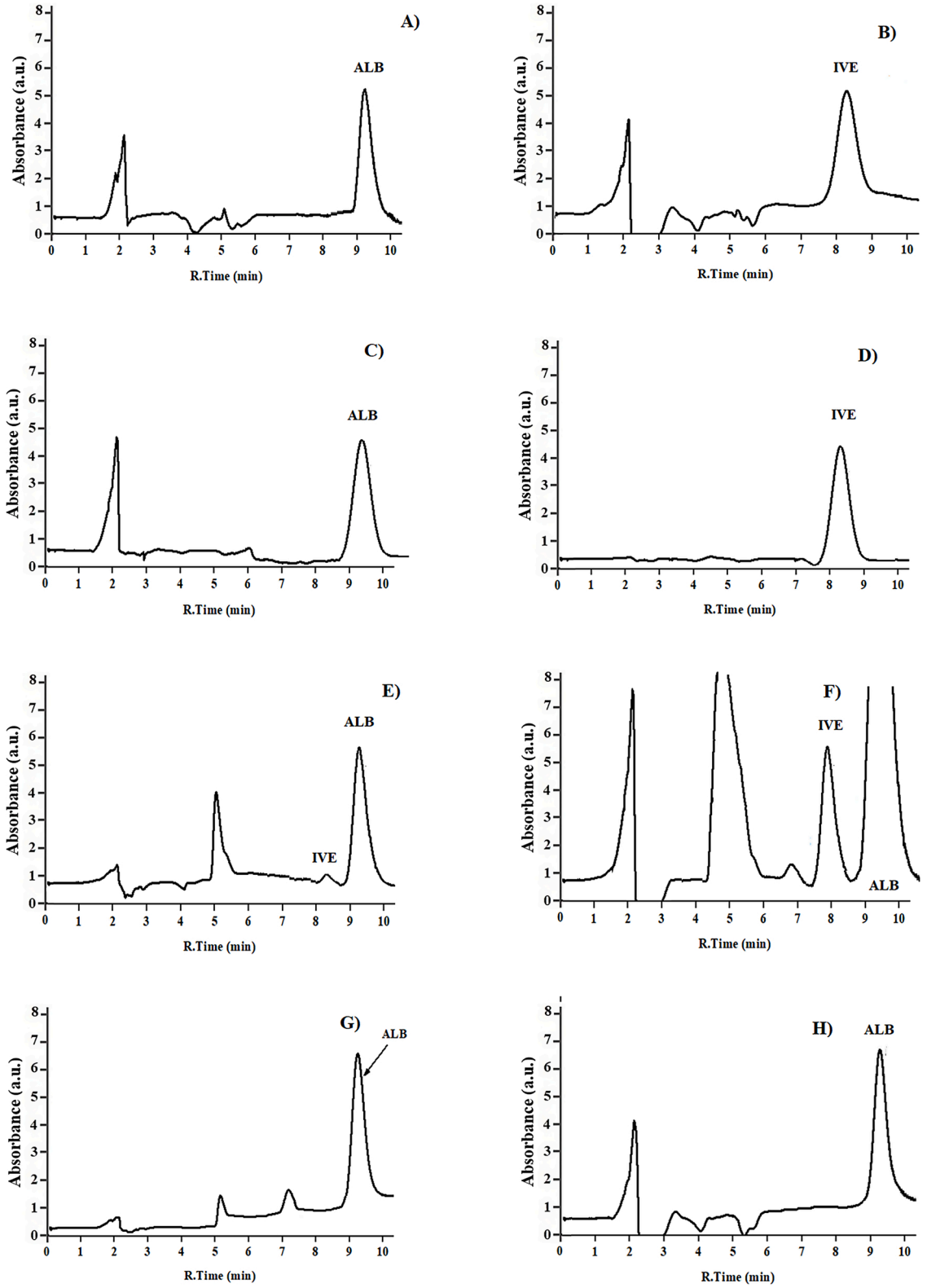

Fig. 3. Chromatrograms obtained by the analysis of blank (A, C, E and G) and spiked (B, D, F, and H) (injected concentration of 0.5 mg/L for both analytes) chicken edible tissues: A,B) muscle; C,D) kidney; E,F) liver; and G,H) gizzard.

solution; and no significant difference was obtained. Therefore, we deduce there is not any coeluting compound; and then the entire peak area corresponds to the supposed analyte. From these results, we infer the absence of interfering compounds in the matrix; and the ALB and IVE can be reliably identified and quantified.

3.2.5. Method calibration range and sensitivity

Sample was 1/5 dilution prior injection, and then the concentration in sample is five times the injected concentration. Therefore, for both veterinary drugs, MDL, MQL and UMQL were 0.1; 0.25 and 50 mg/ for milk, 0.05; 0.125 and 25 mg/kg for the other matrices. We can deduce the method exhibit enough sensitivity to detect distinguish between compliant and uncompliant samples in all the studied matrix regarding ALB residues (as the MRL is in the detection range), but it is unable to reach the MRL for IVE in detect milk samples. This can be considered a serious limitation of the procedure.

3.2.6. Trueness and precision

The results of this study can be seen in Table 1. Relative recovery (86.3–105.6%) and dispersion of the results (RSD < 12.2 %) were acceptable and inside the acceptance criteria of the guidelines. Therefore, the method provides reliable quantitative information for each kind of sample, despite their complexity. Besides, due to the low variability, the concentration obtained for a single measurement is valid; thus, avoiding the need of replicate analysis in incurred samples, what results in a higher sample throughput and saving of resources, reagents, and time. These performances were attained because of the simplification of the treatment and the quantitative injection of the sample, the absence of purification steps on the supernatant, thus limiting the loss of analyte and sources of variance, as well as the strong solubilization power of micellar solutions.

3.2.7. Stability

In the three cases, the drugs were considered stable for the duration of the study. Therefore, the standard/solutions can be stored at least two months without producing a significant deviation in the quantitative results; and a processed sample can be analyzed until two days after preparation, if stored at the autosampler tray. In addition, the food samples can be analyzed until two months after being collected, if kept in the freezer.

3.3. Analysis of incurred samples

The method was applied to the determination of incurred samples collected as indicated in section 2.1, to check it suitability for routine analysis; evaluate food safety and the compliance of producers with the regulation about administration of these veterinary anthelmintic drugs.

A hundred samples were collected and analyzed: ten and five samples for each bovine and chicken matrix, respectively. Analytes were not detected (<MDL) in any of cheese and butter samples. Results for the other samples, including the interval of concentrations of ALB and IVE found, can be seen in Table 2. Chromatograms obtained from the analysis of some samples can be seen in Fig. 4.

The proportion of food samples compliant with the regulation were: bovine milk and curd, 40 %; butter and cheese, 100 %; butter milk, 50 %, chicken muscle, 40 %, liver and kidney, 100 % and gizzard, 60 %. The proportion of bovine waste free of antihelmitics were: urine and dung, 60 % and saliva, 70 %. These samples were considered uncontaminated.

Among all samples wherein at least one of the drug was detected, the concentration of ALB found in milk and milk products ranged from 0.46 to 3.61 mg/kg whereas in biological fluids and waste its concentration ranged from 0.14 to 6.10 mg/kg. ALB was also found in various poultry products in the range of 0.13 – 0.68 mg/kg. The level of IVE in milk and milk products was in the range 0.31–8.73 mg/kg, while in biological fluids, 0.4–7.24 mg/kg. Ivermectin was not found in poultry products. In milk and other dairy products, the highest concentration of ALB was found in milk while the highest concentration of the IVE was found in the curd samples. In the biological fluid and waste obtained from cattle, the highest concentration of ALB and IVE was found in dung it is because a large percentage of the unchanged IVE is excreted through urine and dung (Wishart et al., 2017).

Table 2
Concentrations found in different samples analysed for albendazole (ALB) and ivermectin (IVE). (*indicate the level of antihelmintic exceeds the corresponding maximum residue limit stated in the Introduction, then the sample is uncompliant with the regulation).

Origin of samples	Sample type	ALB (mg/kg)	IVE (mg/kg)	Sample type	ALB (mg/kg)	IVE (mg/kg)
Bovine animals (Cow & Buffalo)	Milk (1)	4.10*	n.d.	Milk (2)	2.33*	n.d.
	Milk (3)	1.57*	0.45*	Milk (4)	0.46*	n.d.
	Milk (5)	5.30*	3.80*	Milk (6)	0.87*	n.d.
	Curd (1)	0.51*	n.d.	Curd (2)	0.74*	8.73*
	Curd (3)	n.d.	0.31*	Curd (4)	0.63*	4.65*
	Curd (5)	0.71*	2.42*	Curd (6)	0.55*	n.d.
	Butter milk (1)	0.84*	1.90*	Butter milk (2)	2.10*	0.24*
	Butter milk (3)	0.52*	n.d.	Butter milk (4)	3.61*	n.d.
	Butter milk (5)	1.57*	n.d.			
	Urine (1)	n.d.	1.15	Urine (2)	0.14	0.73
	Urine (3)	0.95	0.86	Urine (4)	3.96	n.d.
	Dung (1)	6.10	n.d.	Dung (2)	n.d.	7.24
	Dung (3)	0.32	0.40	Dung (4)	0.24	n.d.
	Saliva (1)	2.75	n.d.	Saliva (2)	1.23	n.d.
	Saliva (3)	0.87	n.d.			
	Muscle (1)	0.68*	n.d.	Muscle (2)	0.43*	n.d.
	Muscle (3)	0.23*	n.d.			
Poultry (Chicken)	Kidney (1)	0.16	n.d.			
	Liver (1)	0.19	n.d.	Liver (2)	0.13	n.d.
	Gizzard (1)	0.20*	n.d.	Gizzard (2)	0.24*	n.d.
	Gizzard (3)	0.39*	n.d.			

The number of contaminated samples was higher as expected. In these samples, the concentration of ALB and IVE is quite high. The reason behind the presence of both drugs might be the unawareness in the cattle rearer as they do not go to the veterinary doctors and give high or repeated dose of these drugs to the cattle and poultry. Apart from this, the set withdrawal period does not keep in mind during the milking and meat distribution of the treated cattle and poultry. This fact of extensive appearance of ALB and IVE in such samples cannot be ignored. Studies have revealed the presence of ALB and IVE in milk, milk products and meat products as a common phenomenon. The presence of anthelmintic drugs in dairy products is particularly serious as these are largely consumed, especially by infants and children.

As per the reported facts, the concentrations detected for ALB and IVE in the analysed milk and milk products obtained from the cattle as well as poultry (chicken) samples could indicate a risk to public health, as it may cause unwanted chronic exposure to the end users depending on their dietary habits. This kind of exposure can produce severe health effects like resistance to the wild parasites, intestinal nematodes and beneficial microorganism present inside human and animal. Apart from this, the concentration of unchanged ALB and IVE present in urine and dung may also adversely affect to the flora and fauna of agricultural land as well as small beneficial living creatures of the ecosystem (Dubroca et al., 2003). Hence, to minimize the risk of unwanted exposure of these compounds, proper care must be taken during the treatment of cattle and poultry.

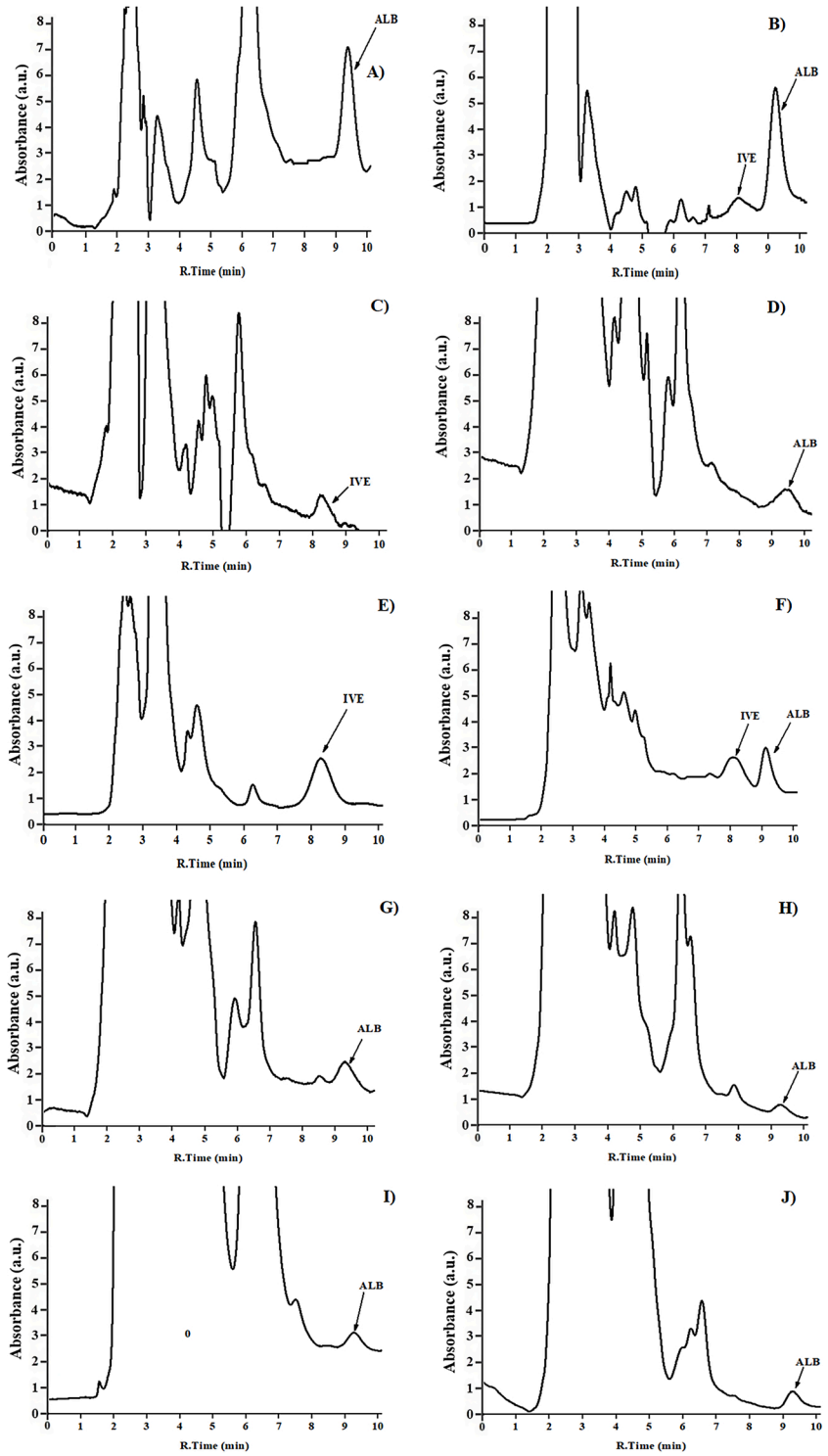

Fig. 4. Chromatograms obtained by the analysis of a contaminated sample of: A) bovine milk (4,1 mg/kg); B) bovine butter milk (ALB: 2.1 mg/kg and IVE: 0.24 mg/kg); C) bovine curd (IVE: 0.31 mg/kg); D) bovine saliva (ALB: 2.75 mg/kg); E) bovine urine (IVE: 1.15 mg/kg); F) bovine dung (IVE: 0.4 mg/kg and ALB: 0.32 mg/kg); G) chicken muscle (ALB: 0.23 mg/kg); H) Chicken kidney (ALB: 0.16 mg/kg); I) Chicken liver (ALB: 0.19 mg/kg) and J) Chicken gizzard (ALB: 0.2 mg/kg).

3.4. Advantages of the procedure

Even though the large number of samples, the entire set (Table 2), was analysed in four days with a minimal participation of the operator during the sample pretreatment (only mixing aliquots and extracting solutions, as the longer phase, the stirring, was semi-automated as indicated by Pawar et al., 2020). Therefore, the work time was fully optimized, thus resulting in a high sample throughput. Only one solution was prepared (mobile phase, used in all the steps) and the volume of hazardous and volatile organic modifier handled and wasted was minimal. Furthermore, only available, and economical reagents, laboratory material and instrumentation were required. Consequently, the method exhibited interesting practical features, as highly sample-throughput, economic, safe and barely pollutant, and then useful for routine analysis. Furthermore, the developed MLC method is found to be good enough to determine ALB and IVE concentrations in all the considered

matrices. No overlapping was noticed, thus proving the selectivity results obtained during the validation process are applicable to all kind of samples.

4. Conclusions

A suitable micellar liquid chromatographic method has been developed and validated for the simultaneous determination of ALB and IVE in a large number of diverse samples, like bovine milk and milk products, bovine biological waste, and poultry (chicken) edible tissues. Main advantages of the method are the simplification of sample treatment (based on direct injection), thanks to the strong solubilization power of micellar solutions, the reproducibility and consistency of the retention mechanism, and the low environmental impact and workplace hazard. The procedure has been separately validated for each kind of matrix, using in each case the appropriate guideline, with satisfactory results. The developed method was proven to be reliable, easy-to-handle, sensitive enough, inexpensive, eco-friendly and with a highly sample-throughput. It can be used as a screening method to find largely contaminated samples and then limit LC–MS analysis to samples where the concentration of ALB and IVE is low or their identification is doubtful (which would result in a significant reduction of costs), in several areas associated to public health assessment: food safety (to recognize contaminated food and identify producers misusing IVE and ALB-based treatments), environmental (to control a potential way of pollutant release)-

From the obtained results, we found a too large number of samples with unacceptable level of anthelmintics, which poses in danger the consumer, and then awareness must be created among rearers to follow norms and time set for the withdrawal period and care must be taken while obtaining food products from the cattle and poultry under treatment.

Author contributions

Conceptualization, J.A.C., J.P.V. and D.B.; methodology, R.P.P., J.E. R. and A.B.; software, J.A.C. and D.B.; validation, S.C.B., J.P.V. and R.P. P.; formal analysis, R.P.P. and D.B.; investigation, R.P.P. and D.B.; resources, S.C.B. and D.B.; data curation, J.A.C., J.P.V. and S.C.B.; writing—original draft preparation, A.B. and D.B.; writing—review and editing, J.P.V. and J.E.R.; visualization, A.B. and J.E.R.; supervision, D. B. and J.E.R.; project administration and funding acquisition, D.B. and S. C.B. All authors have read and agreed to the published version of the manuscript.

Ethical statement for experiments involving animals

The farmers, who had participated in the study, have taken dung, urine and saliva of the animals, and given to the laboratory researchers. The sampling has been performed without harming the animals and respecting their welfare.

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The founding sources were not involved in any aspect of the development of the work.

Declaration of Competing Interest

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

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jfca.2021.104111.

References

Beynon, S.A., 2012. Potential environmental consequences of administration of anthelmiths to sheep. Vet. Parasitol. 189 (1), 113–124. https://doi.org/10.1016/j.vetpar.2012.03.040.

Bowman, D.D., 2014. Georgis' Parasitology for Veterinarians, tenth ed. Saunders, Philadelphia, PA, USA.

Danaher, M., Howells, L., Crooks, S., Cerkvenik-Flajs, V., O'Keeffe, M., 2006. Review of methodology for the determination of macrocyclic lactone residues in biological matrices. J. Chromatogr. B 844 (2), 175–203. https://doi.org/10.1016/j.jchromb.2006.07.035.

Dos Santos Magalhaes, I.R., 2014. Current bioanalytical methods for pharmacokinetic studies of drugs used in neglected tropical diseases. Bioanalysis 6 (12), 1711–1723. https://doi.org/10.4155/bio.14.122.

Dubroca, J., Ducrot, P., Alvinerie, M., Galtier, P., Mougin, C., Kollmann, A., 2003. Fate of the veterinary medicine ivermectin in soil. Environ. Chem. Lett. 1 (2), 131–134. https://doi.org/10.1007/s10311-003-0032-9.

Erner, J., Miller, J.H. (Eds.), 2005. Method Validation in Pharmaceutical Analysis. A Guide of Best Practice, third ed. Wiley-VCH GmbH & Co, Weinheim, Germany.

Esteve-Romero, J., Carda-Broch, S., Gil-Agustí, M., Capella-Peiró, M., Bose, D., 2005. Micellar liquid chromatography for the determination of drug materials in pharmaceutical preparations and biological samples. Trends Analyt. Chem. 24 (2), 75–91.

European Commission, 2002. Commission decision of 12 August 2002 implementing council directive 96/23/EC concerning the performance of analytical methods and the interpretation of results (2002/657/EC. Off. J. Eur. Communities L221, 8–36. Retrieved from: https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32002D0657&from=ES Accessed on June 15 2020).

European Commission, 2010. Commission regulation (EU) No 37/2010 of 22 December 2009 on pharmacologically active substances and their classification regarding maximum residue limits in foodstuffs of animal origin. Official Journal of the European Communities (L15), 1–72. https://ec.europa.eu/health/sites/default/files/files/eudralex/vol-5/reg_2010_37/reg_2010_37_en.pdf.

FAO, 2018. Maximum Residue Limits (MRLs) and Risk Management Recommendations (RMRs), CX/MRL 2-2018. Retrieved from: http://www.fao.org/fao-whocodexalimentarius/shproxy/en/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FStandards%252FCAC%2BMRL%2B2%252FMRL2e.pdf Accessed on June 15 2020.

FDA, 2019. CFR - Code of Federal Regulations Title 21. Retrieved from:https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfCFR/CFRSearch.cfm?CFRPart=556&showFR=1 Accessed on June 15 2020.

Floate, K.D., Taylor, W.G., Spooner, R.W., 1997. Thin-layer chromatographic detection of ivermectin in cattle dung. J. Chromatogr. B, Biomed. Appl. 694 (1), 246–251. https://doi.org/10.1016/S0378-4347(97)00153-9.

García-Gómez, D., García-Hernández, M., Rodríguez-Gonzalo, E., Carabias-Martínez, R., 2012. A fast and reliable method for the quantitative determination of benzimidazoles and metabolites in milk by LC-MS/MS with on-line sample treatment. Anal. Bioanal. Chem. 404 (10), 2909–2914. https://doi.org/10.1007/s00216-012-6415-7.

Garrido Frenich, A., Aguilera-Luiz, M., Martínez Vidal, J., Romero-González, R., 2010. Comparison of several extraction techniques for multiclass analysis of veterinary drugs in eggs using ultra-high pressure liquid chromatography- tandem mass spectrometry. Anal. Chim. Acta 661 (2), 150–160. https://doi.org/10.1016/j.aca.2009.12.016.

González Canga, A., Sahagún Prieto, A., Diez Liébana, M., Fernández Martínez, N., Sierra Vega, M., García Vieitez, J., 2008. The pharmacokinetics and interactions of ivermectin in humans—a mini-review. AAPS J. 10 (1), 42–46. https://doi.org/10.1208/s12248-007-9000-9.

Harris, D.C., 2007. Quantitative Chemical Analysis, seventh ed. W.H. Freeman and Company, New York, NY, USA.

Horvat, A.J., Babić, S., Pavlović, D.M., Ašperger, D., Pelko, S., Kaštelan-Macan, Petrovic, M., Mance, A.D., 2012. Analysis, occurrence and fate of anthelmintics and their transformation products in the environment. Trends Analyt. Chem. 31, 61–84. https://doi.org/10.1016/j.trac.2011.06.023.

Hu, X., Wang, J., Feng, Y., 2010. Determination of benzimidazole residues in edible animal food by polymer monolith microextraction combined with liquid chromatography−mass spectrometry. J. Agric. Food Chem. 58 (1), 112–119. https://doi.org/10.1021/jf902888a.

Jacobs, C., Scholtz, C., 2015. A review on the effect of macrocyclic lactones on dung-dwelling insects: toxicity of macrocyclic lactones to dung beetles. Onderstepoort J. Vet. Res. 82 (1), 858. https://doi.org/10.4102/ojvr.v82i1.858.

Jedziniak, P., Szprengier-Juszkiewicz, T., Olejnik, M., 2009. Determination of benzimidazoles and levamisole residues in milk by liquid chromatography–mass spectrometry: screening method development and validation. J. Chromatogr. A 1216 (46), 8165–8172. https://doi.org/10.1016/j.chroma.2009.07.036.

Kim, S., Thiessen, P.A., Bolton, E.E., Chen, J., Fu, G., Gindulyte, A., Han, L., He, J., He, S., Shoemaker, B.A., Wang, J., Yu, B., Zhang, J., Bryant, S.H., 2016. PubChem substance and compound databases. Nucleic Acids Res. 44, D1202–213. Retrieved from: https://pubchem.ncbi.nlm.nih.gov/ Accessed June 15, 2020).

Legrae, L.H., Deida, M.F., Abdellahi, B.M.L., Elkory, M.B., Ndiaye, I., Bouajila, J., 2020. An easy efficient method of veterinary drug residue analysis in raw milk by RP-HPLC-UV with application to raw milk. Curr. Pharm. Anal. 16 (7), 942–949. https://doi.org/10.2174/1573412915666190416115517.

Little, T., 2016. Establishing acceptance criteria for analytical methods. BioPharm Int. 29 (10), 44–48.

Long, A.R., Hsieh, L.C., Malbrough, M.S., Short, C.R., Barker, S.A., 1989. Multiresidue method for isolation and liquid chromatographic determination of seven benzimidazole anthelmintics in milk. J. Assoc. Off. Anal. Chem. 72 (5), 739–741. https://doi.org/10.1093/jaoac/72.5.739.

Media, F., Us, A., Media, F., 2019. FSSAI Amends Tolerance Limit of Antibiotics and Pharmacology Active Substances - Food Safety Helpline. Retrieved from:https://foodsafetyhelpline.com/antibiotics-pharmacology/ Accessed on June 5 2020.

Mishra, P., Albiol-Chiva, J., Bose, D., Durgbanshi, A., Peris-Vicente, J., Carda-Broch, S., Esteve-Romero, J., 2018. Optimization and validation of a chromatographic method for the quantification of isoniazid in urine of tuberculosis patients according to the European medicines agency guideline. Antibiotics 7, 107. https://doi.org/10.3390/antibiotics7040107.

Mishra, P., Pawar, R.P., Bose, D., Durgbanshi, A., Albiol-Chiva, J., Peris-Vicente, J., Esteve-Romero, J., Jain, A., 2019. Stability studies of rifampicin in plasma and urine of tuberculosis patients according to the European Medicines Agency Guidelines. Bioanalysis 11 (08), 713–726. https://doi.org/10.4155/bio-2018-0174.

Pawar, R.P., Mishra, P., Durgbanshi, A., Bose, D., 2019. A rapid thin layer chromatographic method for simultaneous screening of albendazole and ivermectin in formulations prescribed for human or veterinary use. Asian J. Chem. 31 (4), 896–900. Pages.

Pawar, R.P., Mishra, P., Durgbanshi, A., Bose, D., Albiol-Chiva, J., Peris-Vicente, J., García-Ferrer, D., Esteve-Romero, J., 2020. Use of micellar liquid chromatography to determine mebendazole in diary products and breeding waste from bovine animals. Antibiotics 9 (2), 86. https://doi.org/10.3390/antibiotics9020086.

Pérez, M., Romero-González, R., Vidal, J., Frenich, A., 2013. Analysis of veterinary drug residues in cheese by ultra-high-performance LC coupled to triple quadrupole MS/MS. J. Sep. Sci. 36 (7), 1223–1230. https://doi.org/10.1002/jssc.201201003.

Peris-Vicente, J., Casas-Breva, I., Roca-Genovés, P., Esteve-Romero, J., 2014. Application of micellar liquid chromatography for the determination of antitumoral and antiretroviral drugs in plasma. Bioanalysis 6 (14), 1975–1988. https://doi.org/10.4155/BIO.14.154.

Peris-Vicente, J., Esteve-Romero, J., Carda-Broch, S., 2015. Validation of analytical methods based on chromatographic techniques: an overview. Anal. Sep. Sci. 5, 1757–1808. https://doi.org/10.1002/9783527678129.assep064.

Peris-Vicente, J., Albiol-Chiva, J., Roca-Genovés, P., Esteve-Romero, J., 2016. Advances on melamine determination by micellar liquid chromatography: a review. J. Liq. Chromatogr. Relat. Technol. 39 (7), 325–338. https://doi.org/10.1080/10826076.2016.1152482.

Peris-Vicente, J., Iborra-Millet, J.J., Albiol-Chiva, J., Carda-Broch, S., Esteve-Romero, J., 2019. A rapid and reliable assay to determine flumequine, marbofloxacin, difloxacin, and sarafloxacin in commonly consumed meat by micellar liquid chromatography. J. Sci. Food Agric. 99, 1375–1383. https://doi.org/10.1002/jsfa.9314.

Pugajeva, I., Ikkere, L.E., Judjallo, E., Bartkevics, V., 2019. Determination of residues and metabolites of more than 140 pharmacologically active substances in meat by liquid chromatography coupled to high resolution Orbitrap mass spectrometry. J. Pharm. Biomed. Anal. 166, 252–263. https://doi.org/10.1016/j.jpba.2019.01.024.

Puspitari, S., Farajallah, A., Sulistiawati, E., Muladno, 2016. Efectiveness of Ivermectin and Albendazole against Haemonchus contortus in Sheep in West Java, Indonesia. Trop. Life Sci. Res. 44 (1), 135–144.

Ruiz-Ángel, M.J., Carda-Broch, S., Torres-Lapasió, J.R., García-Álvarez-Coque, M.C., 2009. Retention mechanisms in micellar liquid chromatography. J. Chromatogr. A 1216, 1798–1814. https://doi.org/10.1016/j.chroma.2008.09.053.

Scientific Working Group for Forensic Toxicology (SWGTOX), 2013. Standard practices for method validation in forensic toxicology. J. Anal. Toxicol. 37, 452–474. https://doi.org/10.1093/jat/bkt054.

Wang, X., Wang, K.W., Xu, Z.Z., Yang, X.M., Zhao, Y., 2021. Development and validation of a multi-residue analytical method for veterinarian and human pharmaceuticals in livestock urine and blood using UHPLC-QTOF. J. Chromatogr. B 1167, 122564. https://doi.org/10.1016/j.jchromb.2021.122564.

Wishart, D.S., Feunang, Y.D., Guo, A.C., Lo, E.J., Marcu, A., Grant, J.R., Sajed, T., Johnson, D., Li, C., Sayeeda, Z., Assempour, N., Iynkkaran, I., Liu, Y., Maciejewski, A., Gale, N., Wilson, A., Chin, L., Cummings, R., Le, D., Pon, A., Knox, C., Wilson, M., 2017. DrugBank 5.0: a major update to the DrugBank database for 2018. Nucleic Acids Res. https://doi.org/10.1093/nar/gkx1037. Retrieved from: Available online: https://www.drugbank.ca/ Accessed June 15 2020.