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A Comprehensive Evaluation of The Clean-Up Step in The QuEChERS Procedure for The Determination of Six Groups of Veterinary Drugs in Poultry Using Ultra Performance Liquid Chromatography Tandem Mass Spectrometry (UPLC-MS/MS)

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ABSTRACT

The primary aim of this study is to establish a reliable, quick, and cost-effective QuEChERS method for extracting and cleaning six groups of veterinary drugs from poultry feed for subsequent analysis. Several factors were explored, including the type of solvent used for extraction, clean-up process optimization using graphitized carbon black (GCB), ethyl acetate, n-heptane, and temperature suitable for the concentration process. Prior research was considered to choose the best extraction solvent, and a mix of 0.1 M EDTA-McIlvaine buffer, acetonitrile (ACN), and methanol (MeOH) was used. After thorough analysis, a solvent composition called OSC-B, containing 10 mL ACN and 4 mL MeOH, was preferred due to its superior extraction properties for specific drugs. GCB was added during the clean-up process, and 15 mg was found to be the most effective amount. Ethyl acetate and n-heptane were added to reduce matrix interference from the feed, resulting in three distinct layers during extraction. The optimal sample volume to withdraw from the second layer was 7 mL, consistently producing clear samples suitable for analysis. The solution concentration process was optimized at different temperatures, with 45°C selected as the ideal temperature due to its shorter evaporation time and acceptable analyte peak shapes. The recovery of all six groups is close to 100%, indicating trustworthy results. This study comprehensively optimizes extraction and clean-up processes, offering a robust methodology for analyzing five groups in the positive mode and one group in the negative mode of veterinary drug residue in poultry feed. This study is vital in ensuring the safety and quality of poultry products for consumers.

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INTRODUCTION

As humans, we require food to survive. With the advancements in technology and economics, the demand for high-quality food has increased. Food's nutritional value and safety are the primary concerns when searching for it [1]. A significant problem in ensuring food safety is the presence of contaminants. Therefore, these substances are considered undesirable and serve no purpose in food or its production. Unfortunately, they cannot be avoided and can be found in various types of food due to advancements in analytical detection techniques that are now more sensitive to detecting them [2]. Researchers must devise many approaches to effectively identify and validate food quality to ensure consumers access high-quality food products. These methods must be reliable, accurate, and efficient to maintain consumers' safety and satisfaction [3].

Food safety is a significant concern due to contaminants like veterinary drugs found in food. These medications are utilized to manage animal diseases. When used appropriately following good veterinary practices (GVP), they contribute to producing high-quality animal-based food such as meat, milk, eggs, and honey. However, if GVP standards are not observed, harmful effects may occur, including drug residues in food exceeding safe levels for humans [4]. Misusing drugs, for example, administering them excessively or without supervision, can result in deposition in the eggs and edible poultry tissue. This residue can cause health problems. Drug remnants in animal-derived food products can lead to different harmful impacts such as cancer-causing, allergic, poisonous, neurological disorders, and microbial effects. [5]. Consumers may develop antimicrobial resistance from animal food contaminated with drug overdose [6].

When analyzing complex samples, several necessary steps must be followed. These include sampling, sample preparation, separation, quantification, statistical evaluation, and decision-making. Each step is crucial to ensure accurate and informative results that guide optimal decision-making. One of the most critical steps is selecting the appropriate sample preparation method. This step aims to isolate the target analytes from the matrix because most analytical instruments are designed to handle only analytes, not the matrix. Sample preparation typically involves extraction and "clean-up" procedures for very complex and "dirty" samples. Concentrating the analytes to a level suitable for detection is essential, which is why enrichment is typically included in sample preparation methods. There are several types of extraction techniques available, including gas-phase extraction, liquid-liquid extraction, extraction of solids by a fluid, digestion methods, solid-phase extraction, solid-phase microextraction techniques, membrane extraction, and automated and high-throughput extraction methods using advanced flow technology [7].

Anastassiades proposed dispersive solid phase extraction (DSPE) for determining mycotoxins in wheat semolina [8,9]. QuEChERS, which stands for Quick, Easy, Cheap, Effective, Rugged, and Safe [10], is another name for DSPE, which was developed originally for the determination of pesticide residues in plants [8,11]. This method has been used to analyze mycotoxins in different food types, including dried fruits [12], eggs [13], and cereals [14, 15]. In these approaches, mycotoxins were extracted using acetonitrile or a mixture of water and ethyl acetate. The extract was then purified using a combination of MgSO4, NaCl, primary secondary amine (PSA), graphitized carbon black (GCB), or an SPE cartridge.

An initial salting-out liquid-liquid extraction and dispersive-SPE clean-up had proven to be highly successful in detecting multiple residues of pesticides, veterinary drugs, and mycotoxins in a diverse range of food and agricultural products. Recently, this approach has earned more attention due to its ability to eliminate harmful impurities from consumable items [16]. The QuEChERS method is preferred as an eco-friendly analytical approach for various reasons, including its ability to reduce solvent usage and waste generation, making it an ideal choice for those seeking to minimize their environmental impact. This method is highly efficient, user-friendly, and affordable, making it a popular choice in many laboratory

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applications. Its effectiveness in analyzing different samples has made it a go-to option for many. In summary, the QuEChERS method is a trustworthy and practical solution for analytical analysis, which has proven effective in numerous laboratory settings [17].

It is imperative to acknowledge that each QuEChERS technique may exhibit variations concerning its individual steps and subtleties. Thus, it is of utmost significance to meticulously scrutinize the particulars of every QuEChERS method utilized [18]. Several essential elements must be considered when making changes to the QuEChERS method. These include the characteristics of the substances being examined, the composition of the samples, and the equipment utilized. There are various ways to customize the process, such as adjusting the sample quantity employed, the solvent used during extraction, and the specific sorbents and salts required.

Tailoring the approach to the specific variables at play can optimize the effectiveness and accuracy of the QuEChERS method for each unique situation. Modifications are carried out to maximize the recovery and selectivity of analytes while diminishing matrix effects through effective extract clean-up in complex matrices to achieve this. In particular, solid-phase extraction (SPE) clean-up is crucial in removing matrix and fatty compounds, which can extend the lifespan of a liquid chromatography machine and maintain its efficiency in identification and quantification. Such optimization of the QuEChERS method can enhance its utility in analytical and research contexts [19]. Using C18 sorbent in d-SPE has effectively eliminated non-polar interferences, such as lipids, from extracts with high-fat content ranging from 2% to 20%. This purification method does not impair the recovery of analytes, making it a valuable technique in analytical chemistry [20].

Analyzing veterinary drugs is a recurring task requiring frequent performance throughout the year. To curtail the analysis cost, the QuEChERS method proves to be the most appropriate substitute for SPE. Besides being a prompt and economical technique, the QuEChERS method has demonstrated its efficacy in extracting substantial quantities of veterinary drugs in egg samples [21] and detecting mycotoxins in a wide range of feeds and foods [22]. The employment of the QuEChERS methodology has been observed to enhance the sample yield compared to solid-phase extraction (SPE) and matrix solid-phase dispersion techniques [21].

The present study aims to evaluate the efficacy of the QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) method for extracting samples, specifically in UPLC analysis of poultry matrices. The study seeks to determine the ability of the method to effectively clean up complex matrices and provide reliable and accurate results for the analysis of veterinary drugs in poultry feed. The findings of this study are expected to contribute to further developing and refining analytical methods for detecting and quantifying these analytes in food products.

EXPERIMENTAL

Reagents and Materials

Poultry Feed Sample

The Department of Veterinary Services, Malaysia (DVS) provided the poultry feed samples utilized in this study.

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Standards and Reagents

LC-MS grade reagents were purchased from Merck (Darmstadt, Germany), which included Acetonitrile (ACN), Methanol (MeOH), mobile phase buffer Ammonium Acetate (NH4OAc), and Formic Acid (FA). Optima™ LC/MS Grade water was used for the mobile phase. Sample extraction involved purchasing Disodium Hydrogen Phosphate, Citric Acid Monohydrate, Magnesium Sulphate Anhydrous (MgSO4), and Sodium Chloride (NaCl) from Merck. Classic Chemicals (Selangor, Malaysia) supplied Ethylenediaminetetraacetic Acid Disodium (EDTA) salt. Graphitized Carbon Black (GCB), Primary Secondary Amine (PSA), and C18 End-Capped Chemicals were provided by Agilent Technologies (Santa Clara, CA, USA). Nitrogen (99.999%) was generated using a nitrogen generator from Peak Scientific, NM32LA, to produce desolvation and nebulizer gas. The MS-MS collision gas Argon (99.999%) was supplied by Malaysian Oxygen (Kuala Lumpur, Malaysia).

Standards

All standards, including 18 analytes of interest, were of high purity grade (>99.0%). The following veterinary drugs were purchased: erythromycin (ERY) and sulfaquinoxaline (SQX) from Sigma-Aldrich (St. Louis, MO, USA), sulfamethazine (SMZ), sulfadiazine (SDZ), tylosin (TYL), tilmicosin (TIL), chloramphenicol (CAP), florfenicol (FF) and thiamphenicol (TAF), from GmbG (Augsburg, Germany), ciprofloxacin (CIP) and dimetridazole (DMZ) from Fluka (St. Gallen, Switzerland), enrofloxacin (ENR), ipronidazole (IPZ), clenbuterol (CBR), salbutamol (SBM), ractopamine (RPM), metronidazole (MNZ) and norfloxacin (NOR) from Witega (Berlin, Germany).

Poultry Feed Sample Preparation

Each finely ground sample of poultry feed (weighing 0.50 ± 0.05 g) was meticulously transferred to a 50 mL Falcon™ polypropylene tube. Subsequently, homogenization was carried out over 15 min. Following this, 20 mL of a composition comprising methanol, acetonitrile, and 0.1 M EDTA-Mcllvaine buffer in a volumetric ratio of 20:50:30 was added to the tube. The mixture was then vortexed for 60 seconds and placed in an ultrasonic bath for 10 minutes. Notably, the buffer was prepared with a pH range of 4 to 4.5. Finally, centrifugation was performed for 10 minutes at 4000 rpm and 5±1 °C.

Poultry Feed Extraction and Clean-up

Following centrifugation, the supernatant was subjected to liquid-liquid partitioning by transferring it into a 50 ml Falcon™ polypropylene tube containing 1 g of sodium chloride and

4 g of magnesium sulphate. To prevent the formation of bulk magnesium sulfate crystals during the hydration process, the tube was sealed expeditiously and agitated vigorously for 1 minute. After that, 10 ml of ethyl acetate and n-heptane (50:50) was infused into the tube, which was then centrifuged for 10 min at 4000 rpm for 5±1 °C. Subsequently, the organic phase of the supernatant on the second layer (7 ml) was meticulously transferred into a 15 ml boiling tube. Under a stream of nitrogen, the sample was evaporated to dryness on a sample concentrator at 45 °C. The residue was subsequently reconstituted in 1.5 ml 14% ACN in 0.1% formic acid in water and agitated for 30 s before the extract was transferred into 2 ml micro test tubes containing 15 mg of GCB, 40 mg of PSA, and 10 mg C18 for the d-SPE clean-up method. The micro test tube was subsequently agitated for 30 s before being centrifuged at 15,000 rpm and 5 °C in a microcentrifuge. The aqueous phase was filtered through a 0.45 µm PTFE filter directly into the HPLC vial. The vial was capped and arranged on the LC autosampler. Finally, a 10 µl of the supernatant was injected into the LC system.

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UPLC–MS/MS Analysis for Veterinary Drugs

The present study employed ultra-high-performance liquid chromatography (UPLC) using an Acquity UPLC™ I-Class FTN system (Waters, Manchester, UK) equipped with an electrospray ionization interface (ESI) for chromatographic separation. An Acquity UPLC BEH C18 1.7 µm particle size analytical column 100 mm × 2.1 mm was utilized with a mobile phase containing two mM NH4Ac in water and methanol. The gradient program consisted of A (90%) (0.25 min) and A (10%) (7.25 min), with a subsequent re-equilibration time of 2.50 min, resulting in a total run time of 10 min. The flow rate employed for complete analysis was 0.35 mL/min under a column pressure of less than 18,000 psi. In contrast, the column temperature was maintained at 45 °C, and the sample manager temperature was kept at 15 °C. The sample injection volume per analysis was 10 µL. For MS/MS detection, an Acquity TQS tandem quadrupole mass spectrometer (Waters, Manchester, UK) was employed. The ESI interface was utilized in both positive ion (ESI+) and negative ion (ESI-) mode, with the following settings: capillary voltage of 1.0 kV; source temperature of 150 °C; desolvation temperature of 600 °C; cone gas flow of 150 L/h; and desolvation gas flow of 1000 L/h. The transitions ion for quantification and qualifier ion of each analyte were determined by directly infusing the respective solutions (at 0.005 mg/L in water and acetonitrile (50:50)) into the mass spectrometer ion source at a flow rate of 0.8 ml/min. Two multiple reaction monitoring (MRM) transitions were recorded for the 18 compounds, with dwell times being automatically selected to obtain enough points per peak. Data analysis and quantification were performed using the Waters MassLynx and TargetLynx software.

RESULTS AND DISCUSSION

This study aimed to develop a robust and efficient extraction method. Firstly, it compared different clean-up processes to ensure optimal removal of impurities. Afterward, the focus shifted to the extraction solvent, investigating the effectiveness of methanol, acetonitrile, and a buffer solution. Following solvent selection, the amount of graphitized carbon black was optimized by testing various concentrations (5, 15, 25, and 35 mg). The influence of adding ethyl acetate and n-heptane was evaluated to refine the process further. Finally, the concentration temperature was optimized at three different settings (35 ºC, 45 ºC, and 55 °C) to achieve the most efficient extraction.

The optimization process was carried out considering the method that would be low-cost, rapid, and simple to execute. By implementing these optimization techniques, the study established a sound methodology for extraction that can be deemed academically reliable. Fig. 1 shows the summarised flowchart for extraction method optimization.

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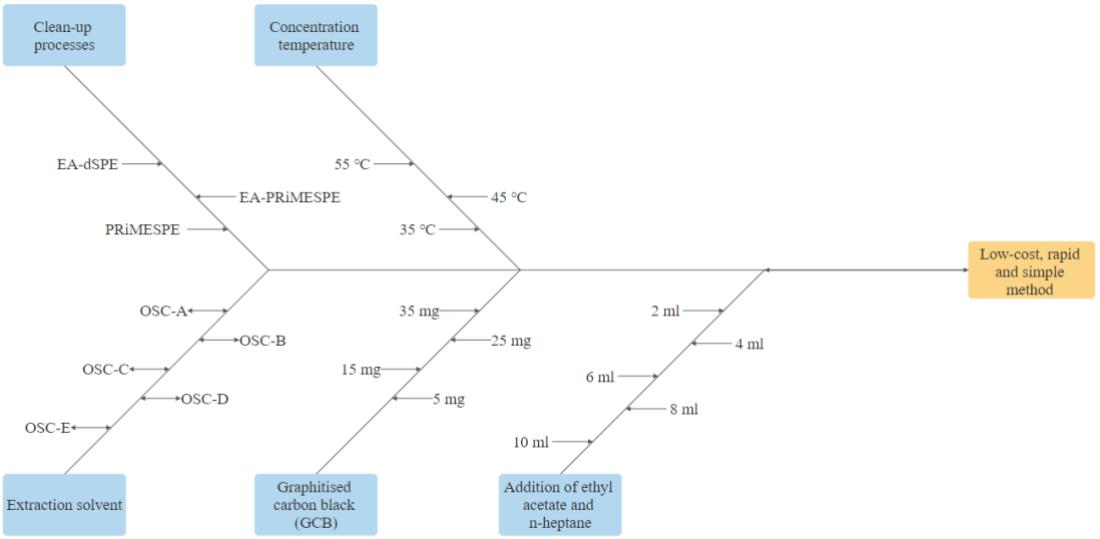

Fig. 1. Summarised details on extraction method optimization

Clean-up process

Three methods were compared to compare the clean-up process. One method used dispersive solid phase extraction (d-SPE), while the other two used Oasis PRiME HLB (PRiME-SPE), a product Waters developed that offers a single and faster extraction process. This product eliminates the need for SPE conditioning, resulting in a quicker clean-up. Two simple processes can be done using PRiME-SPE: catch and release, which involves load, wash, and elute, and pass through, which only includes wash and rinse. Three duplicate samples were used to study the differences between the clean-up processes of d-SPE and PRiME-SPE. Three clean-up systems were compared to determine their effectiveness for multidrug extraction on poultry feed. The first system, EA-dSPE, utilized the extraction method developed in the study and underwent clean-up using d-SPE. The second system, PRiMESPE, followed the method used by Wang et al. on chicken and pig manure and underwent clean-up using SPE. The third system, EA-PRiMESPE, combined the extraction method developed in the study and clean-up using SPE, which was the same method used by Wang et al. The findings revealed that the extraction method developed in the study (EA-dSPE) outperformed the other two methods by effectively extracting all drugs. Fig. 2 summarises the average recovery for poultry feed with three different clean-ups.

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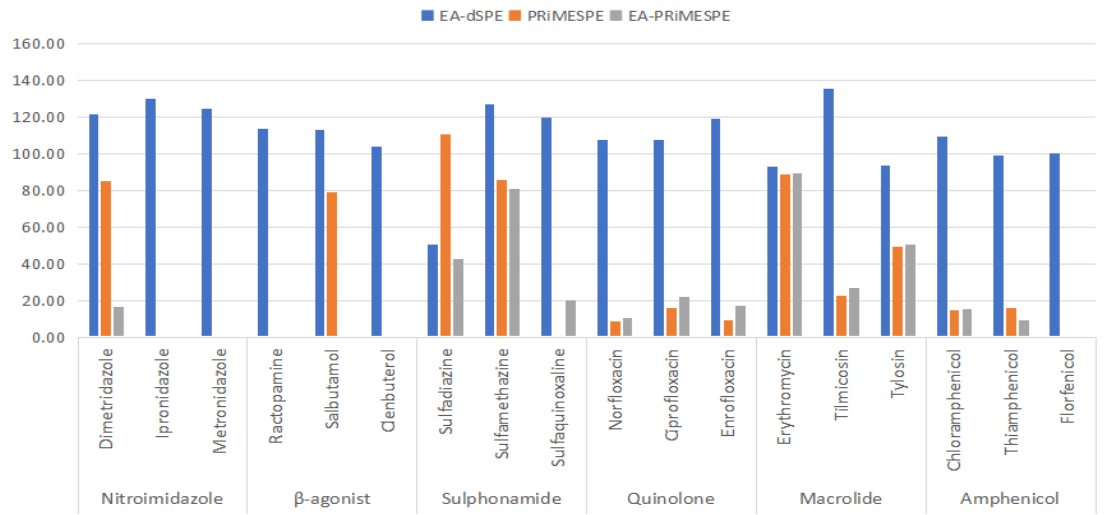

Fig. 2. Percentage recoveries for six groups of veterinary drugs in poultry feed using different clean-up methods

Extraction solvent

The present analysis employs a solvent extraction method comprising a 0.1 M EDTA-Mcllvaine buffer composite, ACN, and MeOH. This solvent was selected based on prior research that used a modified QuEChERS technique to quantify veterinary antibiotics in swine manure [24]. EDTA-Mcllvaine buffer was added as a chelating agent in sample preparation [25,26]. The combination of methanol, a polar-protic solvent, and acetonitrile, a polar-aprotic solvent, with water as a ternary mixture, has exhibited intermediate selectivity for numerous compounds [26]. Table 1 shows the set of extraction solvent compositions that were used to optimize.

Table 1. Composition of extraction solvent for optimization

Label	Acetonitrile	Methanol	Water
OSC-A	12	2	6
OSC-B	10	4	6
OSC-C	7	7	6
OSC-D	4	10	6
OSC-E	2	12	6

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After a thorough analysis, it was revealed that both organic solvent compositions, namely OSC-A and OSC-B, were equally competent in extracting all 18 drugs. However, after careful consideration and evaluation, OSC-B was preferred over OSC-A as the extraction solvent. OSC-B comprises 10 mL ACN and 4 mL MeOH, exhibiting superior extraction properties, particularly for ESI- drugs such as CAP, TAF, and FF. The extraction of ESI- drugs was observed to produce inconsistent results using other organic compositions, making it crucial to select the best organic composition for their extraction. Table 2 shows the average recoveries of the veterinary drugs tested with different organic compositions.

Table 2. Average recovery (%) for 18 drugs with five different organic compositions

Compound	OSCA	OSCB	OSCC	OSCD	OSCE
Dimetridazole	74.66	70.25	42.68	32.81	0.00
Ipronidazole	77.30	70.84	15.56	18.08	0.00
Metronidazole	76.97	70.90	15.35	17.55	0.00
Ractopamine	66.15	69.18	55.48	91.48	37.10
Salbutamol	78.99	72.84	75.62	98.58	0.00
Clenbuterol	78.49	78.14	87.88	73.09	0.00
Sulfadiazine	42.69	57.83	67.11	86.36	0.00
Sulfamethazine	88.21	87.50	83.99	96.10	0.00
Sulfaquinoxaline	49.15	54.36	69.78	72.53	54.30
Norfloxacin	54.82	41.85	73.28	25.51	0.00
Ciprofloxacin	57.11	48.76	85.75	39.03	0.00
Enrofloxacin	75.77	82.51	92.76	94.44	0.00
Erythromycin	37.30	84.58	37.05	7.35	0.00
Tilmicosin	83.28	66.61	72.80	53.81	0.00
Tylosin	40.26	78.69	0.00	12.62	0.00
Chloramphenicol	57.76	79.52	65.83	32.00	61.44
Thiamphenicol	60.13	97.75	55.99	20.27	45.67
Florfenicol	78.69	71.91	41.76	10.43	22.37

Addition of Ethyl Acetate and n-Heptane

After storing solid fat in the refrigerator overnight, a transparent-yellow color was observed in the final sample formation. However, excessive matrix interference from the feed posed a problem even after a clean-up process was done. To solve this issue, ethyl acetate was added to the extraction solvent as a dilutor, while n-heptane was added as a fat remover after the salting-out process. This process formed three layers, with n-heptane as the top layer, followed by acetonitrile/methanol/ethyl acetate and a salt layer. Diluting the mixture with ethyl acetate helped reduce matrix interference's effect and induce negative ion formation. The amount of sample to be withdrawn from the second layer was decided by comparing the color and concentration of the final sample. The optimization was done by changing the volume removed from the second layer, starting with 2 mL followed by 4 mL, 6 mL, 8 mL, and 10 mL. After optimizing the volume withdrawn from the second layer, it was found that 7 mL produced a consistent and intended colorless sample needed for better instrumental care. Fig. 3 shows the final samples for different volumes withdrawSolution Concentration Process

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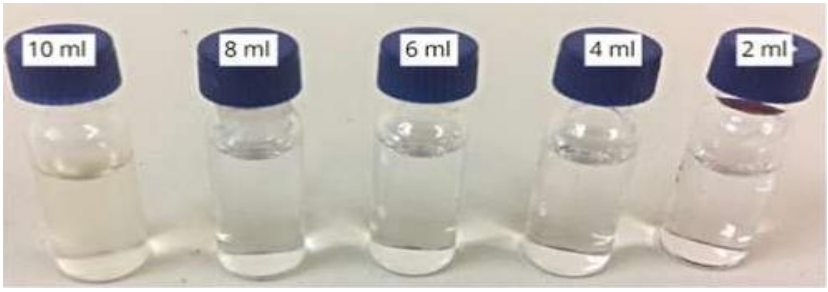

Fig. 3. Observation of the final samples for different volumes withdrawn.

Solution Concentration Process

The solution from the second layer was transferred into test tubes to be concentrated to increase the compound of interest in a solution. The optimization was done at three different temperatures: 35°C, 45°C, and 55°C. An optimization was conducted to find the best temperature for evaporation, ensuring that the solution evaporates in a shorter time while preserving the analytes. At 35°C, the solution took over 90 minutes to dry completely. Around 60 to 90 minutes were needed for the evaporation temperature of 45°C and less than 60 min for the temperature of 55°C. After the evaporation and reconstitution step in an appropriate diluent, samples were subjected to MS analysis. The results showed that samples dried using evaporation temperatures of 35°C and 45°C produced good analyte peaks, while samples dried at 55°C had broad and unsymmetrical analyte peaks. Therefore, the optimized temperature for evaporation was chosen to be 45°C, giving a faster sample evaporation time with an acceptable analyte peak.

Addition of Graphitized Carbon Black (GCB)

The function of a GCB sorbent is to eliminate the presence of plant pigment [28]. Accordingly, it is employed in the clean-up process to remove the yellowish hue commonly encountered in the final sample. Nevertheless, excessive use of GCB may prove disadvantageous as the sorbent can absorb the analyte of interest, reducing concentration. To optimize the amount of GCB needed to achieve optimal analyte recovery during the clean-up process, four different amounts of sorbent were selected: 5 mg, 15 mg, 25 mg, and 35 mg. Fig. 4 illustrates the four distinct extracts obtained using different quantities of GCB during the clean-up process. Of the four extracts, only the 5 mg GCB extract yielded a negative result, displaying the presence of a yellowish hue. Upon comparing the results obtained using 15 mg, 25 mg, and 35 mg of GCB, the 15 mg variable was deemed optimal as it yielded a more concentrated sample with positive observations in the final sample.

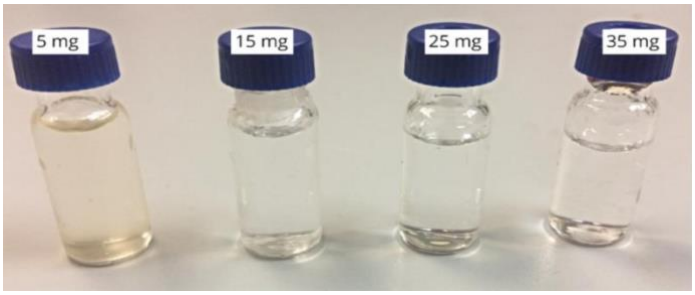

Fig. 4. Extracts after the clean-up process using different GCB amounts.

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Recovery Rates of Veterinary Drug Groups Using Optimised Method

Ensuring a high success rate in the recovery process is crucial in building confidence in the effectiveness and consistency of the approach used. The accuracy and consistency of the analytical method employed are essential in achieving a positive outcome [29]. Summarise recoveries of the six groups of veterinary drugs, which are tabulated in Table 3. An analysis of different drug groups revealed that the Nitroimidazole (recovery rates ranging from 99.13% to 102.50%, with an average recovery of 101.08%) and β-agonist groups (recovery rates for these drugs vary from 100.07% to 103.17%, with an average recovery of 101.37%) exhibit slightly lower average recovery rate. The Sulphonamide group (recovery rates ranging from 98.53% to 100.33%, with an average recovery of 99.70%) had close to 100% recovery rates, indicating high accuracy. In comparison, the accuracy of the Quinolone group (recovery rates ranging from 98.13% to 101.27%, with an average recovery of 99.30%) was somewhat lower. The Macrolide group (recovery rates ranging from 95.83% to 101.47%, with an average recovery of 98.12%) had the lowest average recovery rate, indicating a lower level of accuracy. The Amphenicol group (recovery rates ranging from 100.90% to 103.03%, with an average recovery of 101.97%) demonstrated a high level of accuracy, with an average recovery rate close to 102%. Overall, the recovery rates for veterinary drug analytes were excellent and reliable, with all groups displaying close to 100% recovery rates.

Table 3. Average recovery (%) for six groups of veterinary drugs

Veterinary drug groups	Analyte	Recovery (%)	Average recovery by group (%)
Nitroimidazole	Dimetridazole	101.60	101.08
(ESI+)	Ipronidazole	99.13	
	Metronidazole	102.50	
β-agonist	Ractopamine	100.87	101.37
(ESI+)	Salbutamol	100.07	
	Clenbuterol	103.17	
Sulphonamide	Sulfadiazine	98.53	99.70
(ESI+)	Sulfamethazine	100.23	
	Sulfaquinoxaline	100.33	
Quinolone	Norfloxacin	98.50	99.30
(ESI+)	Ciprofloxacin	98.13	
	Enrofloxacin	101.27	
Macrolide	Erythromycin	97.07	98.12
(ESI+)	Tilmicosin	95.83	
	Tylosin	101.47	
Amphenicol	Chloramphenicol	101.97	101.97
(ESI-)	Thiamphenicol	100.90	
	Florfenicol	103.03	

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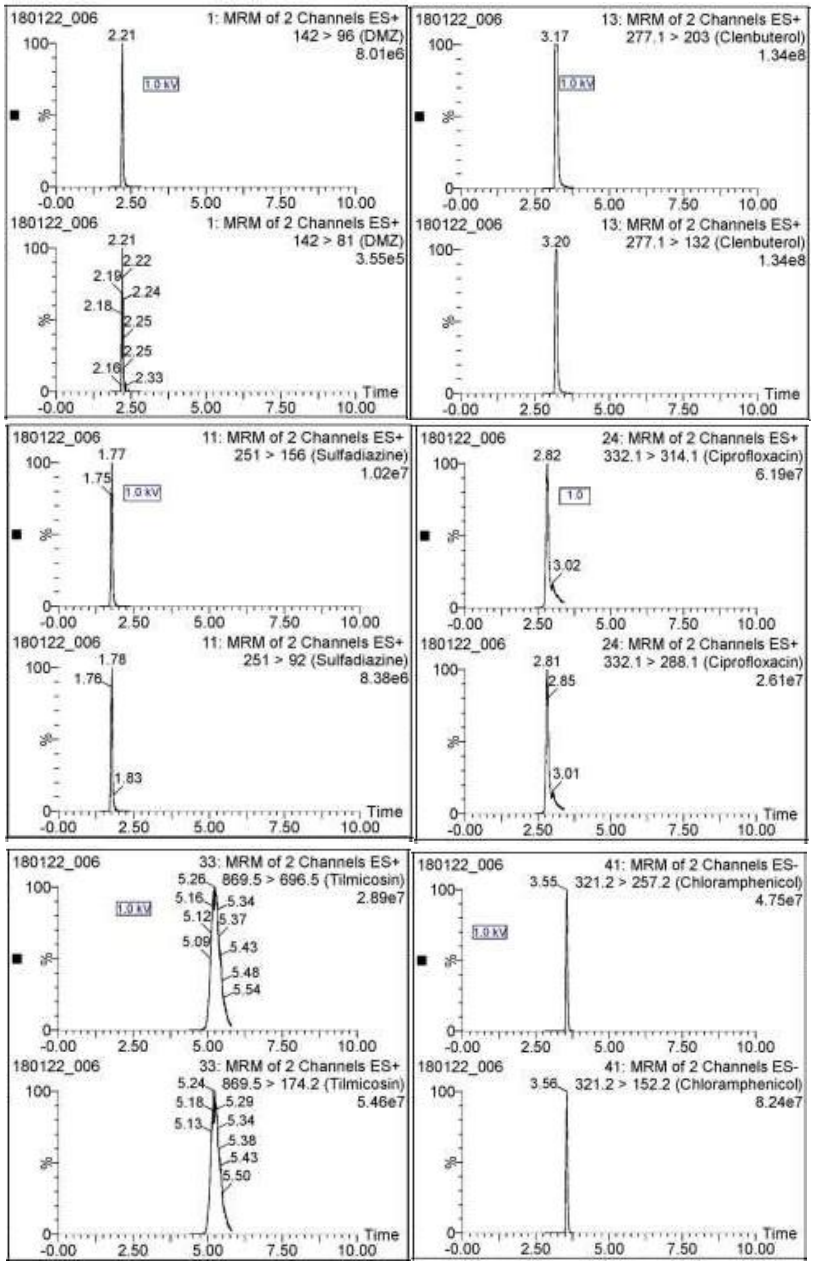

Fig. 5. Chromatograms of Nitroimidazole (Dimetridazole), β-agonist (Clenbuterol), Sulphonamide (Sulfadiazine), Quinolone (Ciprofloxacin), Macrolide (Tilmicosin) and Amphenicol (Chloramphenicol) standards.

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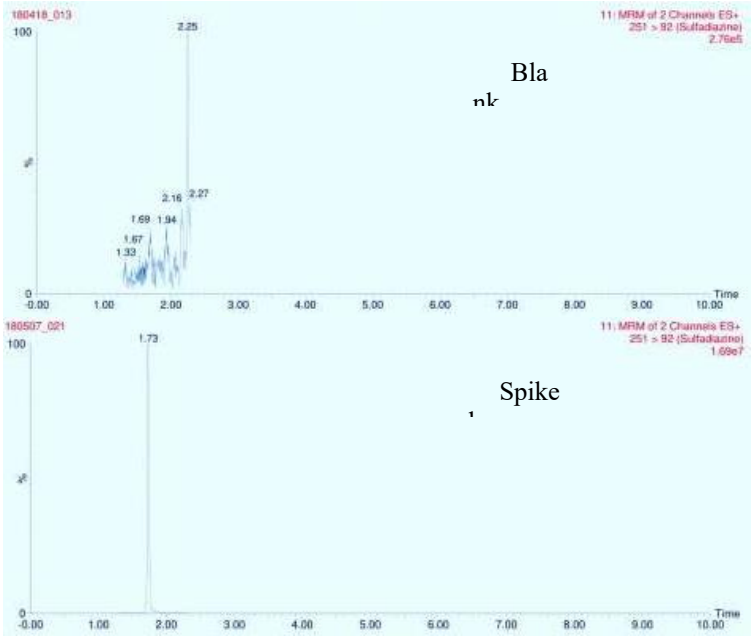

Fig. 6. Chromatogram of blank sample and spiked sample (Sulfadiazine).

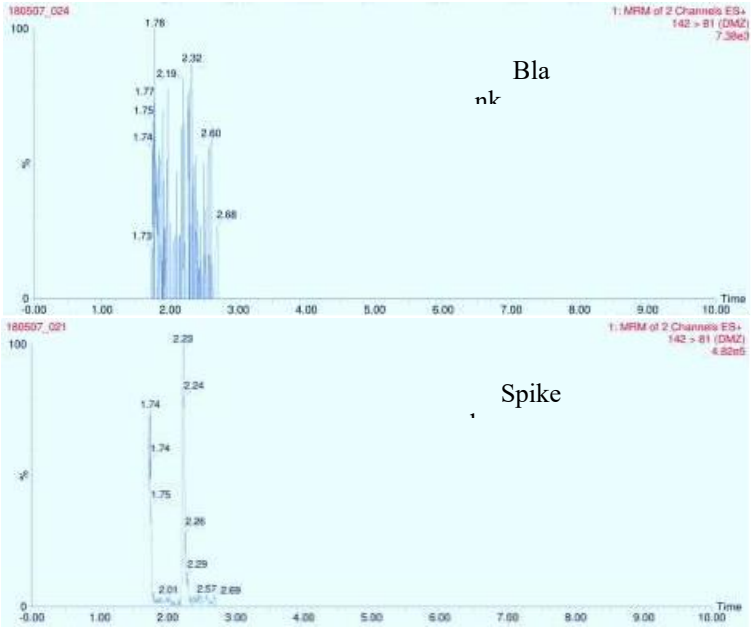

Fig. 7. Chromatogram of blank sample and spiked sample (Dimetridazole).

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CONCLUSION

In conclusion, this research has effectively optimized the extraction and clean-up procedures to analyze six diverse groups of veterinary drugs in poultry feed. The main objective was to establish a dependable, efficient, cost-effective methodology. A systematic investigation was conducted to identify the optimal approach, considering several factors such as the extraction solvent, graphitized carbon black (GCB) quantity for clean-up, ethyl acetate, and n-heptane, and the concentration temperature. After careful consideration and experimentation, a solvent composition named OSC-B was chosen, which consists of 10 mL of acetonitrile and 4 mL of methanol. Further, adding 15 mg of GCB during the clean-up process effectively eliminated unwanted matrix interference from the poultry feed. Incorporating ethyl acetate and n-heptane led to a three-layer separation during extraction, with the second layer consistently producing clear samples ideal for analysis. In addition, the sample volume withdrawal from the second layer was optimized, and it was determined that 7 mL was the ideal volume for producing high-quality samples. Moreover, it was found that a concentration temperature of 45°C offered the best results, with shorter evaporation times and acceptable analyte peak shapes. The most significant finding of this study was the nearly 100% recovery rate for all six groups of veterinary drugs, indicating the reliability of the methodology. These findings provide a comprehensive and robust approach for analyzing five groups in the positive mode and one in the negative mode of veterinary drug residues in poultry feed. The importance of this research lies in its contribution to ensuring the safety and quality of poultry products for consumers, making it a valuable resource for industry.

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AUTHOR'S CONTRIBUTION

The article was written and revised by Muhammad Isfaiz Iskandar. Ahmad Talhah Suhaimi did the research. Marni Sapar conceptualized the main idea for the research, provided the theoretical foundation, designed the study, and oversaw its progress. Zaidah Zainal Ariffin and Muhd Fauzi Safian anchored the review, approved corrections, and approved the publication submission.

CONFLICT OF INTEREST STATEMENT

The authors affirm that there are no competing interests regarding the publication of this paper.

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