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Fenominal method for determination of betalactums in milk using mass spectrometry

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Abstract
Veterinary drugs are antibacterial antibiotics in a broad-spectrum, and these are used to treat animal diseases. Many countries already have abolished the usage of veterinary drugs due to creating residues like nitrofurans, chloramphenicol etc. People already were administered veterinary drugs to increase milk production in animals for more profit and economic lure. This is the reason for increasing usage of illegal drugs for animals' milk production volume increase and that is practically not useful for consumers. Consumers who deliberately consume the drug affected animal-derived food products, as many antimicrobial residues are left. It causes serious health diseases, even cancer and allergic diseases among milk Consumers. This calls for a restriction in illegal drug usage for veterinary purposes, Government in India also needs to restrict this drug usage in animals. The identification and confirmation of various veterinary drug residues in milks are available; animal milk products can be scrutinized through various analytical techniques like mass spectrometry and liquid chromatography. All these are sophisticated as well as equipment are widely available these days. The European Commission has described the analytical method validation should be under 2021/808/EC. The veterinary drug application continuously poses a big challenge in terms of its efficiency, long-term usage and authorisation. There are numerous analytical processes that can be used effectively for protecting consumers' health in a very less time; more groups of compounds are considered such as sulphonamides, tetracycline and macrolides etc. in a single multi-residual method. All these methods are used under distinct validation parameters and include calibration curve, accuracy, precision, detection limit and quantification. The current study has aimed to obtain multi-residual rapid test in less time from the milk samples.

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I. Introduction

There are many antimicrobial agents that are widely considered in the dairy cattle management sector. Disease therapy is the main concerning area of such cattle management and utilizing those antimicrobial agents [1]. Farmers' improper administrations and actions of veterinarians when they fail to observe the conditions of treated animals and its necessary withdrawal time then some consequences take place. These include the existence of antimicrobial residues in milk and mild food products which contribute to microbial effects in drug resistance, also it spreads the bacterial resistance including serious health diseases [2]. Veterinary drugs mainly are used to inhibit any bacterial growth in the animal body and it is used widely to cure animal health ailments [23].The studies previously have been conducted on drug residues/antimicrobials in various food products that are likely to derive from animals; it began late in 1960 and early 1970s mostly in European regions, for instance: Netherlands, Belgium and Luxembourg [24]. The reasons are use of drugs/antimicrobials' capacity to leave residues in the dairy-animal made foodstuffs; due to unlawful usage of unlicensed drugs/antibiotics, extra label doses, failed observations during the withdrawal period, severe contaminations of the animal feed and antimicrobials-treated animals etc. Even banned antibiotic residuals when leaving residues in the food-producing animals it also affects the animal byproducts (meat and milk); which is an illegal venture [3]. In the recent scenario, mostly dairy products pose its significant and unique properties (especially in goat milk, the amino acid and milk protein ratios and its easy digestive property). This is how, veterinarian harmful medicinal effects can cause consequences on human health. It is important to understand the role of surveillance in the antimicrobial residue control [4]. In the EU, a monitoring has been already imposed on veterinarian drugs usage and how the residues can be controlled in the border line. This is done through a set down requirements in a ***"council Directive*** 96/23/EC"**.** A commission decision "97/747/EC" was also aided in that requirement. In line with all those requirements, a Croatian Program of residue monitoring has been implemented since 2000, especially on animal products, live animals etc. The sample distribution over the all-monitoring groups taken under surveillance of the

subjected substances; these are examined [5]. A stipulation by the Council directive "96/23/EC" has been made for sample testing and analysis of the antimicrobial substances frequently with milk samples, collected from animal species. The Commission's decisions were 97/747/EC and 98/179/EC of the simple examination stipulation. Now, as per this entire surveillance and annual monitoring plans, programs the paramount role is of State Veterinary Inspection groups and they need to conduct annual administrative controls on this whole matter of scrutiny on animal products, veterinary drug applications process etc [6].

Sometimes, the HPLC method is used to estimate the drug residue but this is not that much efficient [15]. This quantification methodology has some drawbacks [8]. It consumes a lot of time gathering instruments, different compound groups at a time; and also it is not that much easy to go through the process right. It is important to conduct a different type of quantification method for different groups and a more sophisticated method Lc-MS/MS conditions are currently considered [7]. This is a less time-consuming process and quantification on the certain numbers of compounds is very easy as compared to the HPLC. The analysis of such veterinary drug residues with various groups of antibiotics in just a single multi-residue process [9]. This is through LC-MS/MS conditions and the major advantage that can be achieved through this process is; not only quantitative but also confirmative analysis at a very low level [10]. Here, a Mass-Spectrometry Chromatography has been considered.

Aim and objectives

The aim of this research is to conduct a rapid test, optimization and validation for determining the veterinary drug residues in Milk products by using a liquid chromatography Mass-Spectrometry. The objectives are the following:

- Optimization of extractions procedure of veterinary drug residues in milk
- Optimization of Compound and source dependent parameters
- Method development and validation of veterinary drugs residues in milk using liquid chromatography mass-spectrometry as per regulatory 2021/808/EC

II. Experimental phases

Chemicals and experimental reagents

The commercial standards have been maintained for the antibiotics (Acetonitrile at Gradient/HPLC, water-Milli Q at Gradient/HPLC, Methanol at Gradient/HPLC/MS, Formic Acid at MS Grade, Magnesium Sulphate at GR/Ar, Acetic Acid at GR/AR, Sodium Chloride at Gr/AR, Ammonium formate at MS grade, and C_{18} at Gr/AR and Magnesium sulfate at Gr/AR) [11]. Other ANTHELMINTICS group analyte names which have been considered for the veterinary drug groups and range of testing include Albendazole (100µg/kg, range of testing 10-200), Febantel (100µg/kg, range of testing 10-200), Fenbendazole (100µg/kg, range of testing 10-200), Oxyfendazole (100µg/kg, range of testing 10-200), Parbendazole (10µg/kg, 1.0-20), Praziquantel (10 µg/kg, 1.0-20), Thiabendazole (100 µg/kg, 10-200), and Doramectin (15 µg/kg, 1.5-30). These are all certified reference materials based on HPC standards, only Parbendazole, Praziquantel are of Pherma A2S, and Doramectin is from Sigma-Aldrich [12].

Stock standard solutions also have been used for individual compounds (with a concentration level between 200 and 300 mg/L). These were developed by following an exact weighing of the 100ml methanol and powder. The powder was dissolved in 100ml methanol (HPLC grade, mainly Sigma) [12]. Each compound has been used by maintaining appropriate diluted stock solutions with stored methanol; it was in screw-capped glass tubes at -20 degree Celsius. The ultrapure water has been obtained from Milli-Q gradient water method [16].

Preparation of mobile phase and buffer mode

First a DSPE cleanup process has been implemented at the weight~50mg; and 150mg magnesium sulfate into the safe lock reaction vessels. Then 1% acetic acid has been used at acetonitrile (v/v) phase by filling a 100ml volumetric flask with 1 ml glacial acetic acid (Concentrated acetic acid). There was a fill up mark with acetonitrile mix properly. In terms of mobile phase A (5mM ammonium format and 0.1% formic acid in water) the ammonium format has been prepared of 0.3153g weight with 1 ml dissolved formic acid in 100% Q milli water. This has been mixed well and sonicated. Next was mobile phase B (0.1% formic acid in Acetonitrile) [14]. Here, 1 ml formic acid has been dissolved in 1000ml of acetonitrile mix and sonicated well.

Standard preparation

First, a standard stock solution process was followed, it was through a standard purchasing from the reputed certified sources and stored at the defined temperatures. It Weighs the ~10mg of standard and transfers into a 10ml of volumetric flask. Standard dissolved in 10ml of suitable solvent. And calculate the concentration as per below calculation.

Stock solution conc. (mg/L)

= Standard weight in (mg) x Purity x 1000

Make up volume (ml) x 100

Next, the working solution mix process was prepared through transferring the 100µL of each 1000µg/ml stock standard (volumes may vary with respect to the concentrations of the stock standards) into a 10mL volumetric flask and make up with acetonitrile and label as working standard solution of 10µg/ml. Then, it was Transferred with 1mL of 10µg/ml standard into a 10mL volumetric flask and made up with the same label as 1000 ng/ml [13]. After this, it was the calibration curve standard formation phase, here tables are followed by dilutions (see table 1) with solvent by using the working solution mix of 1000 ng/ml and 100 ng/ml solution.

Table 1: Calibration curve

S. No.	*Linearity*	*Linearity (ppb)*	*Sample Weight (g)*	*Available (ppb)*	*Volume Taken (ul)*
1	Calibration Curve-1	0.5	5	100	25
2	Calibration Curve-2	1	5	100	50
3	Calibration Curve-3	2	5	100	100
4	Calibration Curve-4	5	5	1000	25
5	Calibration Curve-5	10	5	1000	50
6	Calibration Curve-6	15	5	1000	75
7	Calibration Curve-7	20	5	1000	100

Equipment

The equipment used for this chromatographic analysis was the UPLC system. That system includes MA, USA, waters and Milford. This is an aqcuity UPLC BEH C18 column ((“100mm*2.1mm, 1.7ɯ particular size”). Therefore, main equipment are LC/MS (AB SCIEX-LC MS MS-4500), Analytical balance (AUX 220D, 0.0001 to 220g), Centrifuge (NEYA 16 R (2000 to 10000 RPM), and Micro pipettes (MICROLIT, 10-100 µL, 10-200µL,100-1000 µL). The following table 2 has been followed in terms of Chromatography:

LC-MS/MS conditions

The optimization of parameters in LC-MS/MS conditions have been considered in terms of positive and negative Ion mode at ESI for Ion Sources, Exion LC standard for HPLC, autosampler temperature at 15 ºC; Column over temperature at 40 ºC, Mass was AB SCIEX TRIPLE QUAD-4500; in mobile phase the Eluent A – water 0.1 % formic acid+5mM ammonium format, Eluent B – Acetonitrile+ 0.1 % formic acid has been followed. In terms of flow rate 0.600 mL/min, the diluent was made at Water: Acetonitrile (90:10) + 0.1 % Formic Acid, injection volume was 4 µL, MRM scan type and 15 minutes run time were used. In all cases, Ions are always abundant except ivermectin and abamectin; and also these ions have acted as great precursors for understanding the most sensitive transition for the confirmation and quantification purpose. Some published papers also consider APCI usage probe in negative mode for determining the anthelmintics levels; good sensitivity also can be obtained when positive ESI mode is used in MS/MS conditions.

Chromatographic Separation

Chromatographic separation in this paper plays a paramount role to achieve best retention and separation of the analytes used. Firstly, several mobile preparations testing with methanol has been performed; it was at different concentrations at acetic acid. The following HPLC programming has been used to develop chromatographic separation:

Step	*Total Time (min)*	*Flow Rate (µl/min)*	*A (%)*	*B (%)*
1	0.00	600	80	20
2	1.00	600	80	20
3	6.00	600	2	98
4	10.00	600	2	98
5	11.00	600	80	20
6	15.00	600	80	20

Table 2: HPLC Gradient Programming

Several gradient profiles and column temperature, injection volume, flow rates, total time etc. have been studied to obtain a fast and reliable chromatographic separation.

Extraction procedure optimization

The test sample is homogenized thoroughly, weighing 5.0 ±0.1g into the 50ml PP centrifuge tube. Then, 10 ml of 1% acetic acid in acetonitrile was added. The tube was closed and vortexed for 5 min. It was by a high-

speed vortex (by multi tube vortex). Then 0.5g $MgSO_4$ and 0.5g NaCl were added and the tube was then closed. Then, immediately it was shaken vigorously for 1min and centrifuged for 5min at 4200rpm.

Note: In the presence of water, magnesium sulfate tends to form lumps, which can harden rapidly. This can be avoided, if shaken vigorously immediately after the addition of the salt mixture.

Next, an aliquot of 4ml transferred into a ria vial and dSPE cleanup salt mixture, the tube was closed with cap, shaken vigorously for 30 sec and centrifuged for 5 min at 10,000rpm. Finally, it was transferred 1mL of supernatant in a ria vial. An Evaporated process was necessary for it to dry under N2 at 40ºC. Then, reconstituted with 0.5 mL of 9:1 (Water: ACN) with 0.1% formic acid and sonicate the contents, vortex, centrifuge and filter through 0.22µm syringe filter into autosampler vial and injected in LC-MS/MS.

Samples

In this stage, the applicability checking of the methods have been done, different milk samples (full cream, skimmed milk and semi-skimmed milk) were collected from local market milk suppliers. Milk samples then were scrutinized using all the procedures (described in next section). A total of 200 samples have been collected from the local market. Special attention has been given on differentiated milk brands from different locations. Then, the samples were kept in the refrigerator at 4 degrees Celsius until it was analysed within two days. Calibration standards also have been maintained, and blank milk samples have been recognized based on absence of antibiotics. This has been done for the study of validation.

III. Result and Discussion

The aim of this study was to conduct a rapid test, optimization and validation for determining the veterinary drug residues in Milk products by using a liquid chromatography Mass-Spectrometry. The main objectives are arrayed in Optimization of extractions procedure of veterinary drug residues in milk. The source dependent parameters, method development, validation in milk liquid chromatography samples all are imparted with the validation study, the mass-spectrometry under 2021/808/EC regulation has been conducted.

Optimization of instrumental parameters and extraction of parameters

Sample preparation has remained a critical step in this study as usual and the reason is, this is a multiresidue antibiotic methodological rapid test. Here, various properties of the selected substances are subjected to extraction simultaneously. Furthermore, the antibiotic extraction from milk used to be conducted through a traditional protein precipitation process with an organic solvent or a combination of strong acid such as trichloroacetic acid followed by cleaning up with SPE and sample enrichment. This process has been simplified with the buffered QuEChERS process.

S. No.	*Q1*	*Q3*	*Analyte*	*DP*	*EP*	*CE*	*CXP*
1	266.3	234	Albendazole-01	40	10	40	10
2	266.3	190.9	Albendazole-02	40	10	44	10
3	447.1	383.1	Febantel-01	130	10	25	10
4	447.1	415.2	Febantel-02	130	10	18	10
5	300.1	268.1	Fenbendazole-01	50	10	25	8
6	300.1	159	Fenbendazole-02	50	10	33	8
7	316.2	191	Oxyfendazole-01	110	10	31	6
8	316.2	159.1	Oxyfendazole-02	110	10	31	6
9	316.2	284.1	Oxyfendazole-03	110	10	26	9
10	248	173.1	Parbendazole-01	65	10	47	10
11	248	160.1	Parbendazole-02	65	10	45	10
12	313.3	203.1	Praziquantel-01	90	10	25	10
13	313.3	83.1	Praziquantel-02	90	10	30	10
14	202.6	175.9	Thiabendazole-01	62	10	44	9
15	202.6	132	Thiabendazole-02	62	10	44	9
16	916.6	145	Doramectin-01	100	10	45	9
17	916.6	331	Doramectin-02	100	10	45	8

Table 3: Optimization of the parameters_instrumental and extraction of those in the study

Evolution of Matrix

ESI was used in the ionization process in the mass spectrometry, the main issue having arrived with the analytes enhancements, signal suppression due to the other elements present in the matrix effects. In order to evaluate these matric effects, three differentiated milk samples (skimmed, semi-skimmed and full-cream milk) were considered to be used for this validation study. Different concentration standards were analysed in pure solvents in the matrices.

Validation

The entire process was developed and validated through many steps. These steps include certain points of intraday, sensitivity as well as linearity factor. Method linearity factor was assayed by calibration curve performance and using all the matrix-matched calibration spiked milk samples (selected ranges of the antibiotics are 5-200 µg/kg); A linear response has been seen in the assayed range in terms of coefficient determination. The coefficient determination was higher than 0.99 in all the used cases. Also, recovery study was conducted at two distinct levels (10 and 50 µg/kg), six blank milk samples fortified with each fortified antibiotics level. The obtained results have been presented below in the following tables. Therefore, the main validation steps in the work include system precision, specificity and selectivity, recovery study, Precision: Repeatability Study (RSDr), reproducibility, limit of detection, limit of quantifications, ruggedness and Uncertainty of Measurements.

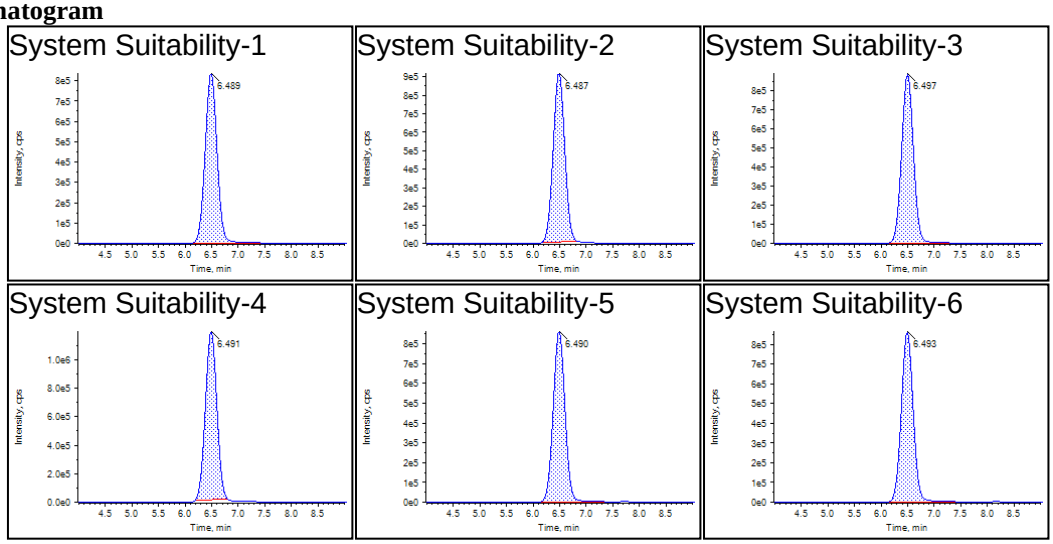
Analyte

Analyte: Albendazole-01 (266.3 / 234.0)

Data File	Data Antibiotics Validation in Milk_02022023.wiff	*Result Table*	Antibiotics validation in Milk_System Suitability
Acquisition Date	02/02/2023 1:01:11 AM	Algorithm Used	MQ4
Acquisition Method	Antibiotics MRM.dam	Instrument Name	Triple Quad 4500
Project	Default		

Sample Name	Sample Type	Acquistion Date	Area (cps)	RT (min)	Target [Conc] (ppb)	Calculated Conc (ppb)	ACCURACY %	[ION RATIO]
System Suitability-1	Standard	02/02/2023 1:01:11 AM	13079629	6.49	N/A	<2 points	N/A	1.480
System Suitability-2	Standard	02/02/2023 1:17:04 AM	13813529	6.49	N/A	<2 points	N/A	1.381
System Suitability-3	Standard	02/02/2023 1:32:58 AM	13727128	6.50	N/A	<2 points	N/A	1.367
System Suitability-4	Standard	02/02/2023 1:48:53 AM	17581788	6.49	N/A	<2 points	N/A	0.954
System Suitability-5	Standard	02/02/2023 2:04:48 AM	13329029	6.49	N/A	<2 points	N/A	1.402
System Suitability-6	Standard	02/02/2023 2:20:42 AM	13381946	6.49	N/A	<2 points	N/A	1.464

Chromatogram

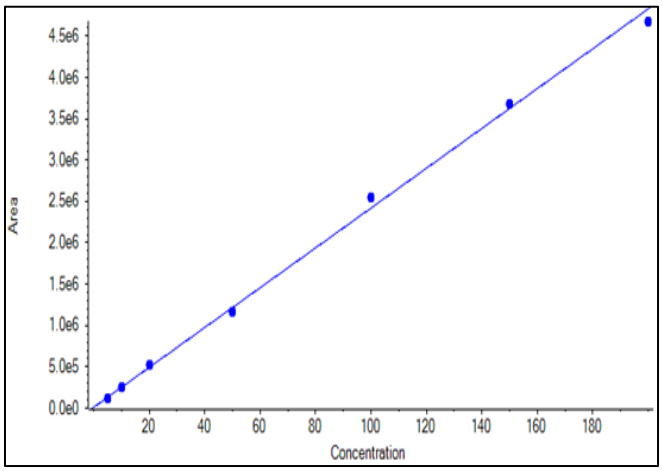


Analyte Febantel-01 (447.1/415.2)

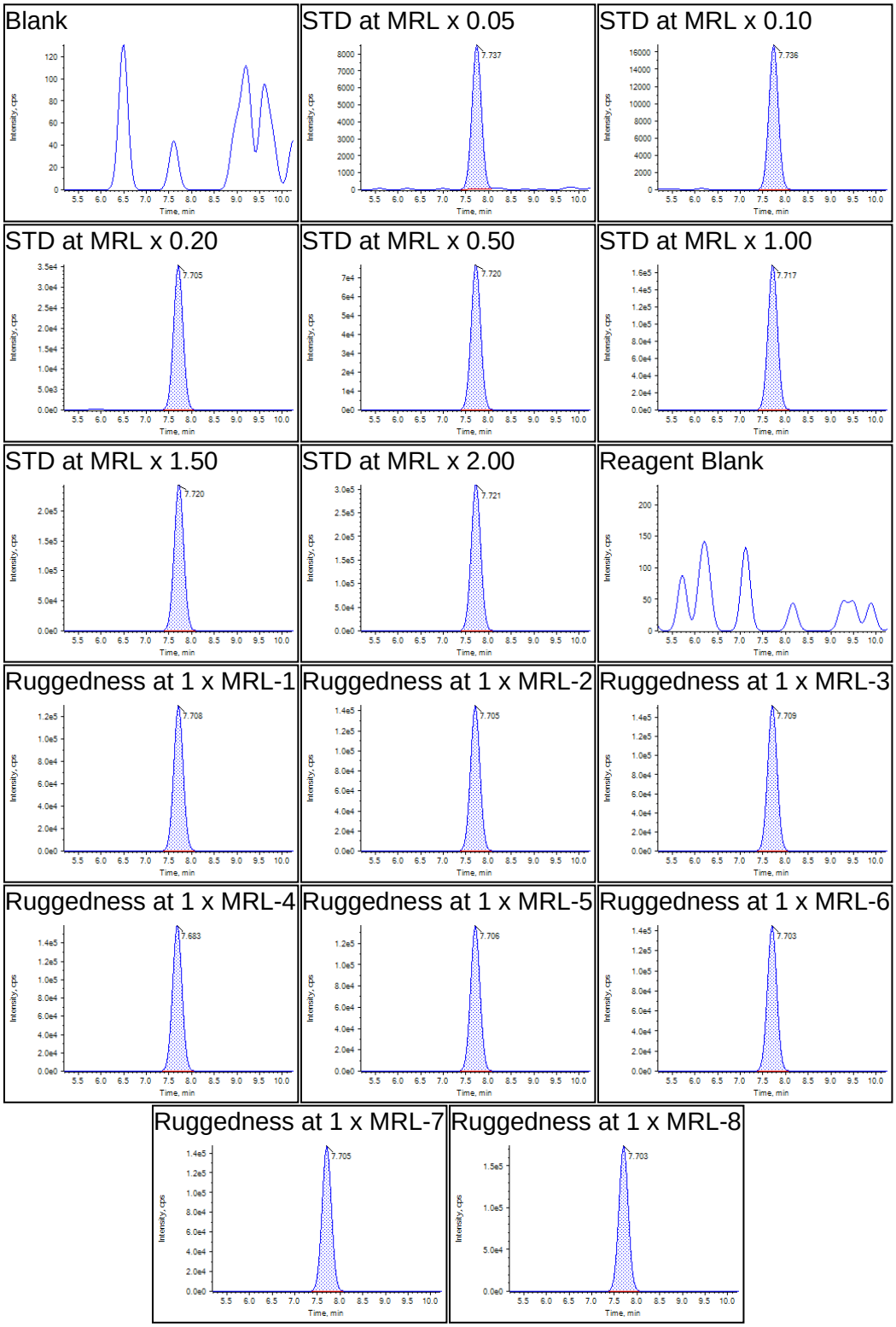
Data File	Data Antibiotics Validation in Milk_30122022.wiff	*Result Table*	Antibiotics validation in Milk_Ruggedness
Acquisition Date	12/30/2022 10:55:11 AM	Algorithm Used	MQ4
Acquisition Method	Antibiotics MRM.dam	Instrument Name	Triple Quad 4500
Project	Default		

Sample Name	Sample Type	Acquistion Date	Area (cps)	RT (min)	Target [Conc] (ppb)	Calculated Conc (ppb)	ACCURACY %	[ION RATIO]
Blank	Unknown	12/30/2022 10:55:11 AM	N/A	N/A	N/A	N/A	N/A	N/A
STD at MRL x 0.05	Standard	12/30/2022 11:11:13 AM	125271	7.74	5.00	4.750	95.08	0.587
STD at MRL x 0.10	Standard	12/30/2022 11:27:07 AM	249655	7.74	10.00	9.920	99.19	0.671
STD at MRL x 0.20	Standard	12/30/2022 11:43:02 AM	524663	7.71	20.00	21.340	106.69	0.553
STD at MRL x 0.50	Standard	12/30/2022 11:58:57 AM	1159294	7.72	50.00	47.690	95.38	0.501
STD at MRL x 1.00	Standard	12/30/2022 12:14:50 PM	2542889	7.72	100.00	105.150	105.15	0.532
STD at MRL x 1.50	Standard	12/30/2022 12:30:43 PM	3684839	7.72	150.00	152.560	101.71	0.475
STD at MRL x 2.00	Standard	12/30/2022 12:46:36 PM	4672722	7.72	200.00	193.590	96.79	0.537
Reagent Blank	Unknown	12/30/2022 1:02:30 PM	N/A	N/A	N/A	N/A	N/A	N/A
Ruggedness at 1 x MRL-1	Quality Control	12/30/2022 1:18:24 PM	1934678	7.71	100.00	79.890	79.89	0.390
Ruggedness at 1 x MRL-2	Quality Control	12/30/2022 1:34:20 PM	2162106	7.70	100.00	89.330	89.33	0.597
Ruggedness at 1 x MRL-3	Quality Control	12/30/2022 1:50:16 PM	2268552	7.71	100.00	93.750	93.75	0.490
Ruggedness at 1 x MRL-4	Quality Control	12/30/2022 2:06:11 PM	2386960	7.68	100.00	98.670	98.67	0.502
Redness at 1 x MRL-5	Quality Control	12/30/2022 2:22:09 PM	2025393	7.71	100.00	83.660	83.66	0.523
Ruggedness at 1 x MRL-6	Quality Control	12/30/2022 2:38:04 PM	2159905	7.70	100.00	89.240	89.24	0.560
Ruggedness at 1 x MRL-7	Quality Control	12/30/2022 2:53:58 PM	2190080	7.71	100.00	90.490	90.49	0.502
Ruggedness at 1 x MRL-8	Quality Control	12/30/2022 3:09:53 PM	2578849	7.70	100.00	106.640	106.64	0.527

Regression Equation: y = 24081.97625 x + 10782.26647 (r = 0.99912, r^2 = 0.99824) (weighting: 1 / x)



Chromatogram



Sample analysis

The developed method has been applied for the antibiotic residue determination in terms of all the 20 milk samples. The sample analysis table has given below:

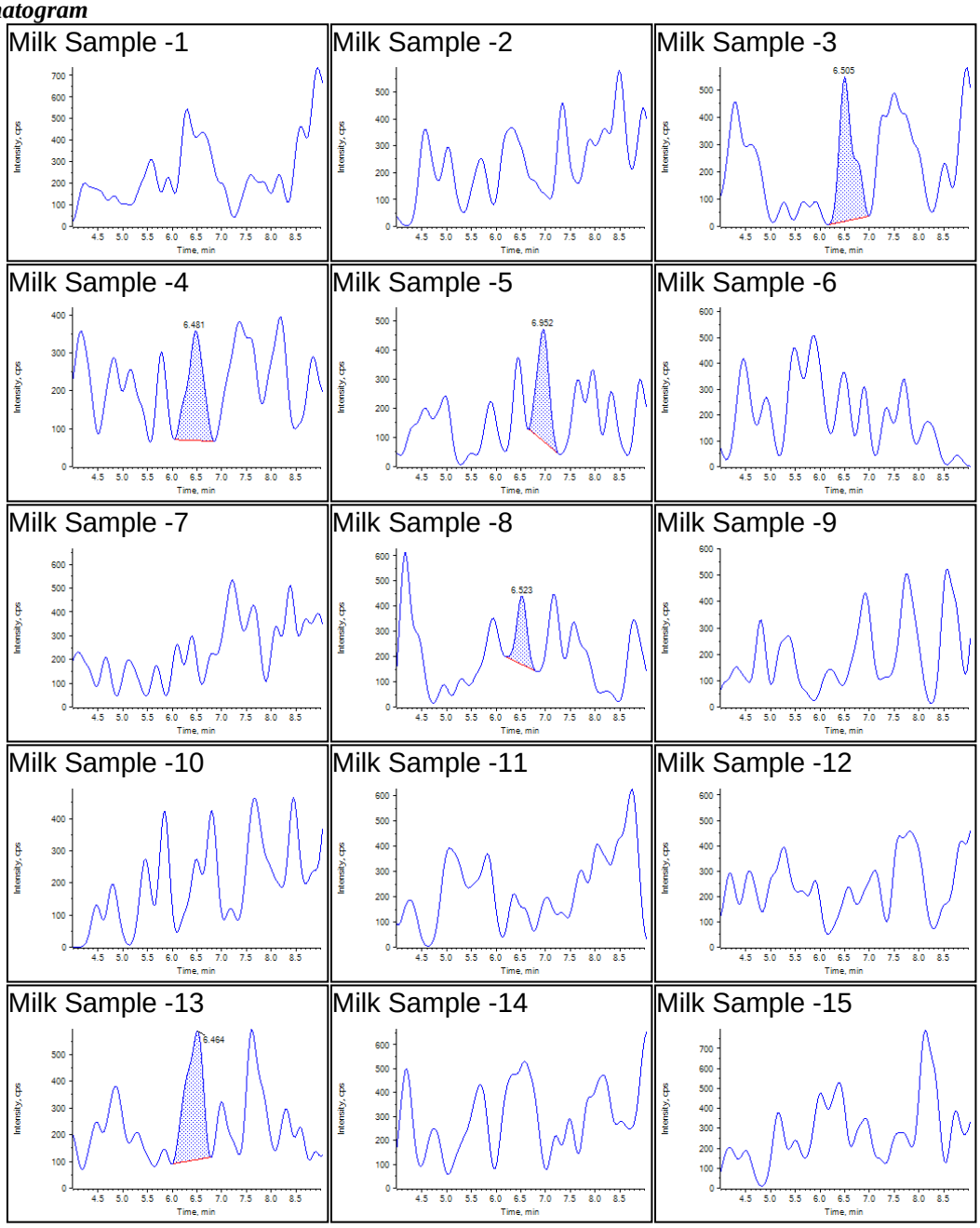
Milk Samples

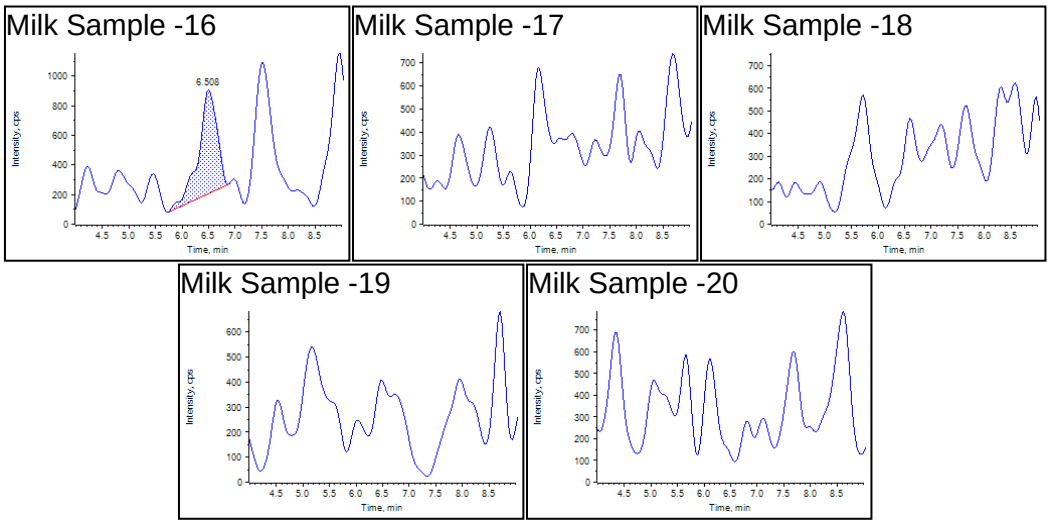
Analyte Albendazole-02 (266.3/190.9)

Data File	Data Veterinary Drugs in Milk Samples _25122022.wiff	*Result Table*	Veterinary Drugs in Milk Samples
Acquisition Date	12/25/2022 7:42:46 PM	Algorithm Used	MQ4
Acquisition Method	Antibiotics MRM.dam	Instrument Name	Triple Quad 4500
Project	Default		

Sample Name	Sample Type	Acquistion Date	Area (cps)	RT (min)	Target [Conc] (ppb)	Calculated Conc (ppb)	ACCURACY %	[ION RATIO]
Milk Sample-1	Unknown	12/25/2022 27:42:46 PM	N/A	N/A	N/A	N/A	N/A	N/A
Milk Sample-2	Unknown	12/25/2022 7:58:39 PM	N/A	N/A	N/A	N/A	N/A	N/A
Milk Sample-3	Unknown	12/25/2022 8:14:35 PM	106	6.51	N/A	<2 points	N/A	N/A
Milk Sample-4	Unknown	12/25/2022 8:30:27 PM	65	6.48	N/A	<2 points	N/A	N/A
Milk Sample-5	Unknown	12/25/2022 8:46:23 PM	64	6.95	N/A	<2 points	N/A	N/A
Milk Sample-6	Unknown	12/25/2022 9:02:17 PM	N/A	N/A	N/A	N/A	N/A	N/A
Milk Sample-7	Unknown	12/25/2022 9:18:12 PM	N/A	N/A	N/A	N/A	N/A	N/A
Milk Sample-8	Unknown	12/25/2022 9:34:16 PM	39	6.52	N/A	<2 points	N/A	N/A
Milk Sample-9	Unknown	12/25/2022 9:50:12 PM	N/A	N/A	N/A	N/A	N/A	N/A
Milk Sample-10	Unknown	12/25/2022 10:06:07 PM	N/A	N/A	N/A	N/A	N/A	N/A
Milk Sample-11	Unknown	12/25/2022 10:22:01 PM	N/A	N/A	N/A	N/A	N/A	N/A
Milk Sample-12	Unknown	12/25/2022 10:37:56 PM	N/A	N/A	N/A	N/A	N/A	N/A
Milk Sample-13	Unknown	12/25/2022 10:53:51 PM	11582	6.46	N/A	<2 points	N/A	N/A
Milk Sample-14	Unknown	12/25/2022 11:09:46 PM	N/A	N/A	N/A	N/A	N/A	N/A
Milk Sample-15	Unknown	12/25/2022 11:25:41 PM	N/A	N/A	N/A	N/A	N/A	N/A
Milk Sample-16	Unknown	12/25/2022 11:41:38 PM	16	6.51	N/A	<2 points	N/A	N/A
Milk Sample-17	Unknown	12/25/2022 11:57:34 PM	N/A	N/A	N/A	N/A	N/A	N/A
Milk Sample-18	Unknown	12/26/2022 12:13:28 AM	N/A	N/A	N/A	N/A	N/A	N/A
Milk Sample-19	Unknown	12/26/2022 12:29:22 AM	N/A	N/A	N/A	N/A	N/A	N/A
Milk Sample-20	Unknown	12/26/2022 12:45:16 AM	N/A	N/A	N/A	N/A	N/A	N/A

Chromatogram





IV. Conclusion

All the techniques that have been used in this study have distinct importance based on their application process. Here, a systematic procedure has been followed step by step to analyse antibiotic residues in the milk samples. The other methods such as LCMS, HPLC and ELISA are certain useful techniques for this purpose. ELISA is considered when antigen-antibody reaction is to be examined; this ELISA is highly a knit-based method and semi-quantitative in natur. HPLC has become specifically a useful technique to assess the microbial antibiotic existence quantitatively. This technique has become more useful due to its usefulness in more unknown sample variety. The sample and interpretation both have been matched well; also same wavelength standard, phermaceutical conditions with same RT have been followed. These all are subjected to be applied in food industries, various research areas as well. Good validation parameters have been used in in terms of precision, recovery, linearity etc. The number of examined validation samples are 15, in just less than 2 hours the total process of extractin has taken place. The ESI technique also is very well-known and suitable for mid-polar and polar compounds; these include any pestiside residues or antibiotique residues. The APPI is usefulfor weekly polar and non-polar compunds in terms of soft ionization process. The proposed method here along with MS/MS has conducted to improve sensitivity and resolution. It can widely detect unknown residues of veterinary drugs. Also, analyte procedure has been performed in skimmed, semi-skimmed and full-cream milk three types of samples. The compounds from this study significantly present a vast range of physico-chemical properties indicating QuEChERS potentiality for the veterinary antibiotic multi residual extraction in milk

References

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