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Determination of Spectinomycin Sulfate Tetrahydrate and Lincomycin Hydrochloride by HPLC in Veterinary Products (Mycospectin oral powder)

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Abstract
developing a high-performance liquid chromatography separation technique that is quick and precise and quantification of both spectinomycin sulphate tetrahydrate and lincomycin hydrochloride in the veterinary formulation drug. This method is applied on an HPLC Agilent 1260 with a UV detector using 150X 4.6 mm BDS hypersil cyano column, Particle size 5 µm. Mobile phase: 95% buffer (dissolve 3.5 g potassium dihydrogen phosphate in 1000 ml distilled water and adjust pH to 6.5 with 1N sodium hydroxide):5 % acetonitrile, Flow rate 1.0 ml/min, Wavelength 220 nm, Injection volume 10.0 µl and ambient column temperature.. The calibration curve of spectinomycin sulphate tetrahydrate and lincomycin hydrochloride was linear with a correlation coefficient that should be more than 0.99 between 50% and 150% of the desired concentrations. It is determined by preparing five concentrations of spectinomycin sulfate tetrahydrate (6.72, 10.08, 13.44, 16.8 and 20.16 mg/ml) and lincomycin hydrochloride (2.513, 3.7695, 5.026, 6.2825 and 7.539 mg/ml). Linearity results of spectinomycin sulfate r=0.9999 NLT, Linearity results for Lincomycin hydrochloride = 0.999 so the method is found to be linear as r is more than 0.99, The result of relative standard deviation was ≤2. The suggested technique was verified and successfully used for the separation and quantification of both spectinomycin sulphate and lincomycin hydrochloride in the veterinary drug.

Keywords: RP-HPLC; Spectinomycin sulphate tetrahydrate; Lincomycin hydrochloride; Forced degradation; Method Validation.

1. Introduction

The antibiotics spectinomycin sulphate and lincomycin hydrochloride, which are respectively aminoglycoside and lincosamide, have synergistic and complimentary effects on one another's antibacterial spectra and processes. The antibacterial mechanism of spectinomycin sulphate, which inhibits bacterial protein synthesis by acting on the 30S subunit of ribosomes, primarily includes inhibiting the binding of messenger ribonucleic acid and ribosomes. This prevents protein synthesis and has bactericidal effects [1, 24]. Lincomycin HCL works by attaching to the bacterial ribosomal 50S subunit and inhibiting peptide acyltransferase, which prevents the production of bacterial proteins and has bactericidal effects. [2,25]. Spectinomycin sulphate is more active against Gram-negative bacteria than Gram-positive bacteria, Lincomycin HCL, however, has a potent antibacterial impact on Gram-positive bacteria, so can effect on Mycoplasma hyopneumoniae which is a species of gram-positive bacteria, is highly contagious and causes a chronic respiratory disease [3]. When disease caused by strains of Neisseria gonorrhoeae that are susceptible to spectinomycin which is used to treat acute gonorrheal urethritis and proctitis in males as well as acute gonorrheal cervicitis and proctitis in females. [4]. As a result, Mycoplasma hyopneumoniae and Mycoplasma pneumonia infections, which cause chronic respiratory disorders in chickens& piglet diarrhea are commonly treated with combination of spectinomycin sulphate and lincomycin HCL [5-7,27]. In broiler-type hens, therapy with a combination of lincomycin HCl and spectinomycin sulphate during the first week of life can prevent the enterococcus cecorum EC-associated illness and has a significant effect on the formation of the cecal microbiota. [8,26]. There are numerous published techniques for determining spectinomycin sulphate and lincomycin hydrochloride. To determine the presence of spectinomycin, The HPLC approach employed post-column oxidation and derivatization for fluorometric detection after ion-pair solid phase extraction for clean-up. [9] and with tandem mass spectrometry [10] Method with A pre-column

derivatized HPLC technique and UV detection [11] in addition to HPLC with ELSD for analysis [12] Lincomycin detection by UV detector and micellar electrokinetic capillary chromatography [13] also LC combined with tandem mass spectrometry [14] gas chromatography for the detection of nitrogen and phosphorus [15] as well as a method of pre-anodized screen-printed carbon electrode [16] RP-HPLC with TMS [17]and with RP-HPLC only [28] An immunochromatographic assay and an enzyme-linked immunosorbent assay (ELISA) were created. [18] Qualitative evaluation of lincomycin and spectinomycin by ASE followed by GC-MS [19,20] And combined with GC-NPD [21] the only reported method used charged aerosol detection techniques with liquid chromatography. (LC–CAD) [22] Also HPLC with gradient mobile phase, UV detector but in a gradient way not preferable where it takes time and, so not recommended being routine QC/QA chromatographic assay [23]. Our procedure yields a straightforward, quick, and precise reverse phase HPLC method for measuring both lincomycin hydrochloride [251.3 mg/g] and spectinomycin sulphate [672 mg/gm] in the formulation of veterinary products in an isocratic way where the method uses column 150*4.6 mm DBS hypersil cyano column particle size 5 µ, flow rate 1.0 m/min, injection volume 10.0 µl wavelength 220 nm, mobile phase ratio 95%buffer (dissolve 3.5g potassium dihydrogen phosphate in 1000ml distilled water and use 1 N sodium hydroxide to set the pH to 6.5) : 5% acetonitrile and by using a UV detector . For the analysis and quantification of both substances in bulk and pharmaceutical dosage form, the presented procedures are a straightforward, conventional QC/QA chromatographic assay.

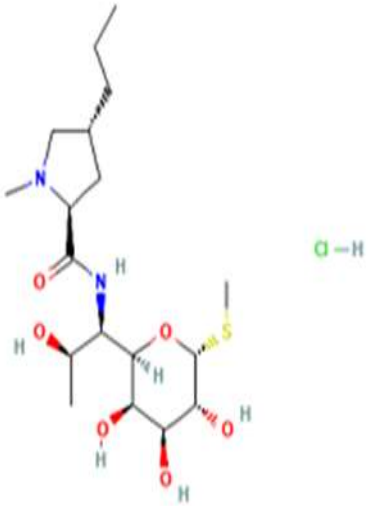

Fig. (1) Chemical structure of lincomycin HCl

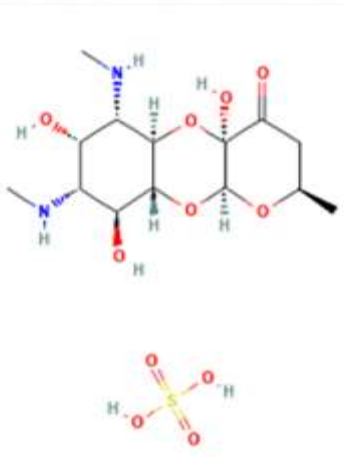

Fig. (2) Chemical structure spectinomycin sulphate

2. Material and Methods

Reagents and chemicals

Sodium hydroxide, Potassium dihydrogen ortho-phosphate, Acetonitrile HPLC grade, ortho-phosphoric acid 85% HPLC grade and water all purchased Through Merck (Germany). A 0.45-Mm nylon membrane filter, manufactured by Chrom Tech(UK)., was used to filter the mobile phase

Standards and Samples

Lincomycin hydrochloride gets from Ningxia taiyicin biotech CO.LTD (China) & spectinomycin sulfate tetrahydrate get from Hebei shengxue Dacheng pharmaceutical (Tangshan) CO.LTD (China), Pharmaceutical products containing spectinomycin sulphate and lincomycin hydrochloride (Mycospectin oral powder) were purchased from the Egyptian pharmaceuticals market

Instrumentation and Chromatographic conditions

HPLC Agilent 1260 is used to analyze drugs by using (150X 4.6 mm) BDS hypersil cyano column.Particle size 5µ, withn UV detector. Chemstation, Peak areas are integrated using an Agilent LC solution software programme, and data was recorded using LC solution software.

Schimadzu AUY 220 balance, 780 pH meter, and ultrasonic cleaner from skymen (JP-060S, china) is used to degas mobile phase.

The separation and quantification of both spectinomycin sulphate and lincomycin hydrochloride carried using HPLC with a 150X 4.6 mm BDS hypersil cyano column, Particle size 5 µ, with a UV detector, when the detector's wavelength was set at 220 nm. 95% buffer (3.5 g potassium dihydrogen phosphate in 1000 ml distilled water, pH adjusted to 6.5 with 1N sodium hydroxide): 5% acetonitrile makes up the mobile phase with flow rate of 1.0 ml/min, injection volume of sample was 10.0 µl, and the column temperature is ambient.

Preparing a solution

Standard solution preparation:

Transfer 672 mg Spectinomycin sulfate working standard and 251.3 mg lincomycin hydrochloride working standard add 30 ml of

mobile phase to a 50 ml volumetric flask, sonicate for 15 minutes, and then cool to room temperature and reach to volume while using the mobile phase. To obtain Final concentration of 13.440 and 5.026 mg/ml respectively.

Preparation of a sample solution

Transfer 1.0 g of the mixture (corresponding to 672 mg of spectinomycin sulphate and 251.3 mg of lincomycin hydrochloride) to a 50 ml volumetric flask. Add 30 ml of the mobile phase, sonicate for 15 minutes, allow the mixture to cool to room temperature, and then add the mobile phase to fill the flask to capacity.

Test method validation

Specificity

If the main peak is clearly separated from any other peak by a resolution of at least 2, the method is considered selective.

Forced degradation study

The stress conditions that were put to use include oxidative, acidic, and basic stressors, according to ICH [24].

Acid hydrolysis:

In order to force degradation in acidic media, transferring 1g sample to 50ml volumetric flask, adding 5 ml 1 N HCL, leaving at 75℃ temperature for 1 hr then neutralize using 5 ml 1 N NaOH, Add 30 ml of mobile phase, sonicate for 15 minutes, let the mixture cool to room temperature, and then add the mobile phase to finish the volume.

Base hydrolysis:

In order to force degradation in basic media was performed by transferring 1g sample to 50ml volumetric flask, adding 5 ml 1 N NaOH, leaving at 75 ℃ temperature for 1 hr. then neutralize using 5 ml 1N HCL, Add 30 ml of mobile phase, sonicate for 15 minutes, let the mixture cool to room temperature, and then add the mobile phase to finish the volume.

Oxidative hydrolysis:

In order to force degradation in oxidative media was performed by transferring 1g sample to 50ml volumetric flask, add 5 ml 3% H_2O_2, leave at 75℃ for 1 hr. Add 30 ml of mobile phase, sonicate for 15 minutes, let the mixture cool to room temperature, and then add the mobile phase to finish the volume.

Linearity:

It is determined by calculating the correlation coefficient which should be more than 0.99 between 50% and 150% of the desired concentrations. It is determined by preparing five concentrations of spectinomycin sulfate (6.72. 10.08, 13.44, 16.8 and 20.16 mg/ml) and lincomycin hydrochloride (2.513, 3.7695, 5.026, 6.2825 and 7.539 mg/ml). Each solution was injected twice.

Accuracy

It is achieved by determining the active recovery from placebo at three levels (50%, 100%, and 150%), with three preparations at each level.

System suitability

Six replicates of the standard solution at 100% concentration and six measurements of the homogeneous sample were used to test the system's repeatability.

Ruggedness

The ruggedness is determined by performing the analysis of a homogenous sample under different conditions like different days and different analysts.

Day to day: It is done by analyzing 6 different test preparations at 100 % concentration; each preparation was injected twice and repeated on the second day by the same analyst.

Analyst to analyst: It is done by analyzing 6 different test preparations at 100 % concentration; each preparation was injected twice and repeated by second analyst on the same day.

Robustness

It is determined by observing how the method stands up to slight variations in the chromatographic condition such as flow rate or the make-up of the mobile phase,mobile phase was changed from 5% to 6% acetonitrile, and the flow rate was increased from 1.0 to 1.1 ml/min.

Limit of Quantitation and Limit of Detection:

Limit of detection is the minimum concentration that can be detected by the analytical procedure, where concentration levels provide a 3:1 signal-to-noise ratio. The minimum concentration is the limit of quantitation. That can be quantified by the analytical procedure with an accepted precision, where the concentration level provides a 10:1 signal-to-noise ratio.

3. Results and discussion

Specificity and forced degradation study

Spectinomycin and lincomycin demonstrated reduction in the peak areas under acidic and basic conditions while the formulation shows good stability under oxidative conditions.

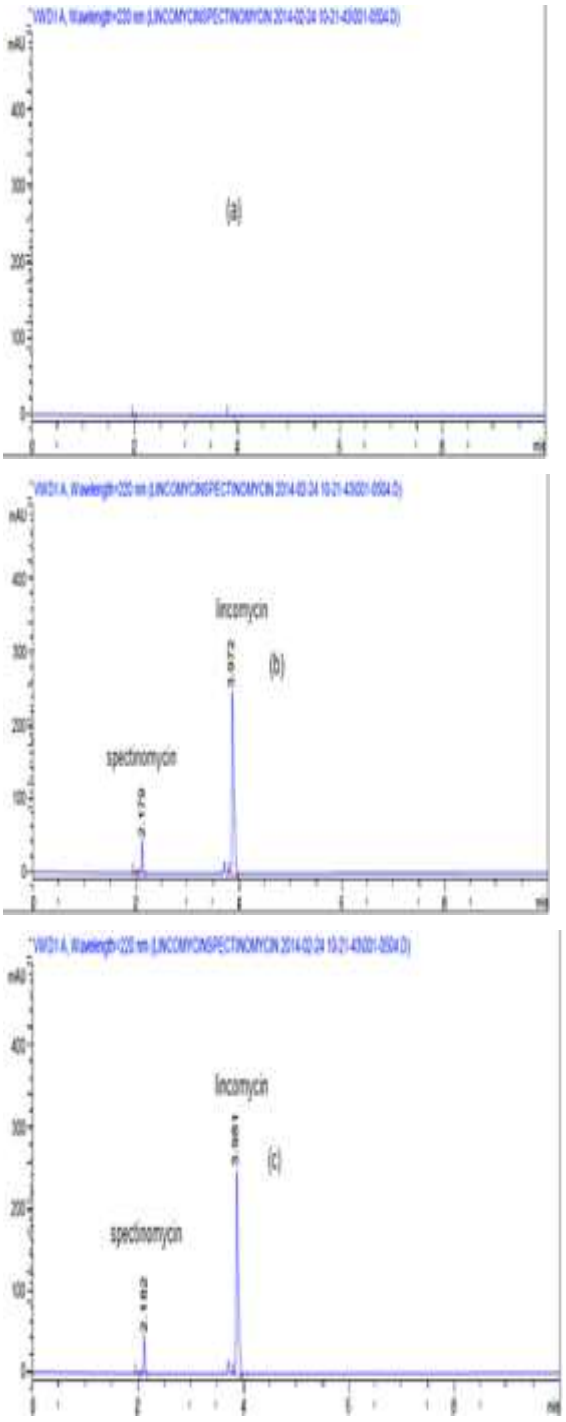

Figure (3) HPLC chromatogram of (a) placebo, (b) reference substance, and (c) test substance

Linearity:

Linearity method shows its suitability for analysis in over the concentration range figures 4 and 5, the results showed that the goodness of fit (R2) $R^2 = 0.99$ for Spectinomycin sulphate and $R^2 = 0.99$ for Lincomycin HCl revealing a linear correlation between the analyte's concentration and the observed peak area.Spectinomycin sulphate: $y = 26.504x + 4.8099$. $R^2 = 0.99$

Lincomycin HCl: $y = 738.8x + 80.564$. $R^2 = 0.99$

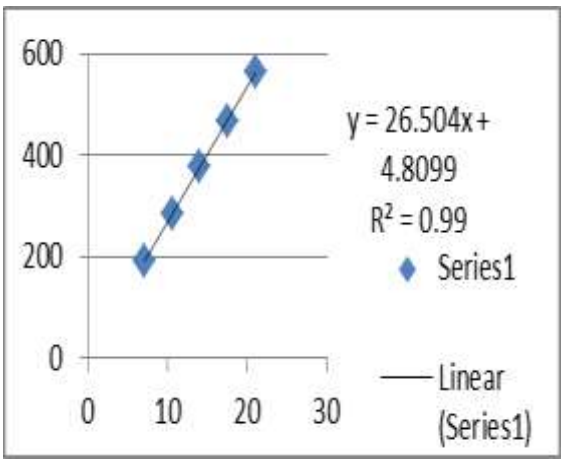

Figure (4) Linearity curve of spectinomycin sulphate

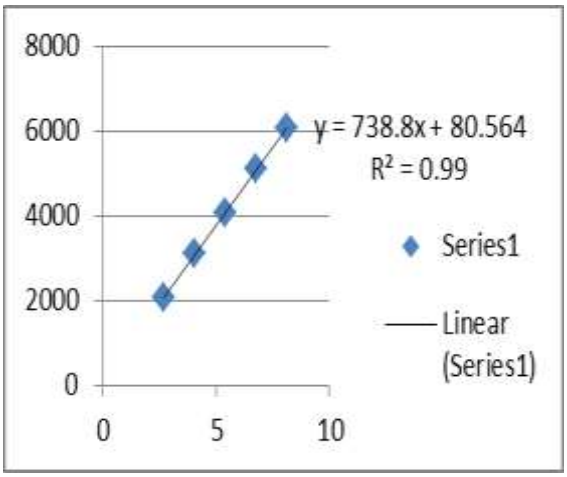

Figure (5) Linearity curve of lincomycin HCl

Within the target concentration range and the % RSD, the method displayed remarkable accuracy. For both active ingredients are within acceptable limit ≤2. Percentage recovery for each test solution within limits (100±2). The data summarized in tables 1 and 2.

System suitability: An analytical technique demonstrates that all of the required system suitability parameters are within allowable bounds. For spectinomycin sulphate and lincomycin hydrochloride, it was determined that the respective column efficiency (N) values were 3025 and 3015 theoretical plates. The symmetry and tailing factors at the peak are 1.01 and 0.97, respectively. The two active ingredients' peaks are separated by 7.16 between them.

Table (1) Recovery results of Spectinomycin sulfate:

S.NO	theoretical conc. (mg/ml)	Found conc.(mg/ml)	Recovery %
1-	6.72	6.74	100.30
2-	6.72	6.73	100.15
3-	6.72	6.71	99.85
1-	13.44	13.43	99.93
2-	13.44	13.41	99.78
3-	13.44	13.43	99.93
1-	20.16	20.18	100.10
2-	20.16	20.17	100.05
3-	20.16	20.17	100.05
Mean			100.01
SD			0.161
RSD			0.161

Table (2) Recovery results of lincomycin hydrochloride**:**

S.NO	theoretical conc. (mg/ml)	Found conc.(mg/ml)	Recovery %
1-	2.513	2.511	99.92
2-	2.513	2.512	99.96
3-	2.513	2.51	99.88
1-	5.026	5.024	99.96
2-	5.026	5.022	99.92
3-	5.026	5.02	99.88
1-	7.539	7.53	99.88
2-	7.539	7.52	99.75
3-	7.539	7.51	99.62
Mean			99.86
SD			0.112
RSD			0.113

Table (3) System suitability results

Parameters	Limits	Value for spectinomycin sulphate	Value for lincomycin HCl
Tailing factor (T)	NMT 2	1.01	0.97
Retention time (min)	--	2.2	4.0
Number of theoretical plates(N)	NLT 2000	3025	3015
Resolution	NLT 2	7.16	

Ruggedness& Robustness:

The methods indicate that small adjustments to the experimental conditions have no impact on the assay or the procedures' abilities to identify and quantify active substances. RSD ≤ 2.0 % & Pooled RSD ≤ 3.0% for every change which ensures the robust and the rugged of the method.

Limit of detection &Limit of Quantitation:

The limit of quantification (LOQ) is the lowest amount of analyte in a sample that can be measured with acceptable precision and accuracy, as contrast to the detection limit (LOD), which is the lowest amount of analyte that can be recognized in a sample.. LOD and LOQ were determined statistically from the calibration curve. LOD for spectinomycin sulphate is 0.70115 mg/ml while LOQ is 2.10345 mg/ml. LOD for Lincomycin hydrochloride is 0.03 mg/ml while LOQ is 0.08mg/ml.

Table (4) Assay validation report for the suggested technique

Parameters	Spectinomycin Sulphate	Lincomycin HCl
System suitability (%RSD)	0.23	0.34
Calibration range(mg/ml)	6.72 – 20.16	2.51-7.53
Slope	26.50	738.8
Intercept	4.81	80.56
Correlation coefficient	0.99	0.99
Intermediate precision (POOLED RSD%)	0.49	0.70
LOD (mg/ml)	0.70	0.03
LOQ (mg/ml)	2.10	0.08

5. Conclusion

An innovative, reliable, and appropriate HPLC test utilizing UV detection for analysis of an injectable lincomycin hydrochloride, Spectinomycin sulphate combination. This method has many advantages compared to other accepted techniques for analyzing these active ingredients. It is affordable and quickly assays the two ingredients. The developed method was validated per both FDA and ICH guidelines and shows excellent linearity, accuracy, selectivity, and system suitability within the standards for acceptance. The technique has been used in stability-indicating studies, and this suggests that it is appropriate for applications involving both purity and degradation evaluation..

Conflict of Interest

There are no apparent conflicts.

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References

[1] Butler MM, Waidyarachchi SL, Connolly KL, Jerse AE, Chai W, Lee RE, Kohlhoff SA, Shinabarger DL, Bowlin TL. Aminomethyl spectinomycins as therapeutics for drug-resistant gonorrhea and chlamydia coinfections. Antimicrobial agents and chemotherapy. 2018 May 1;62(5):e00325-18.

[2] Ishizuka M, Imai Y, Mukai K, Shimono K, Hamauzu R, Ochi K, Hosaka T. A possible mechanism for lincomycin induction of secondary metabolism in Streptomyces coelicolor A3 (2). Antonie Van Leeuwenhoek. 2018 May;111(5):705-16.

[3] Mateusen B, Maes D, Verdonck M, de Kruif A, Van Goubergen M. Effectiveness of treatment with lincomycin hydrochloride and/or vaccination against Mycoplasma hyopneumoniae for controlling chronic respiratory disease in a herd of pigs. Veterinary record. 2002 Aug;151(5):135-40. https://go.drugbank.com/drugs/DB00919https://go.drugbank.com/drugs/DB00919

[5] Verrette L, Fairbrother JM, Boulianne M. Effect of cessation of ceftiofur and substitution with lincomycin-spectinomycin on extended-spectrum-β-lactamase/AmpC genes and multidrug resistance in escherichia coli from a canadian broiler production pyramid. Applied and environmental microbiology. 2019 Jun 17;85(13):e00037-19.

[6] Pokrant E, Maddaleno A, Lobos R, Trincado L, Lapierre L, San Martín B, Cornejo J. Assessing the depletion of lincomycin in feathers from treated broiler chickens: a comparison with the concentration of its residues in edible tissues. Food Additives & Contaminants: Part A. 2019 Nov 2;36(11):1647-53.

[7] Yu Y, Fang JT, Zheng M, Zhang Q, Walsh TR, Liao XP, Sun J, Liu YH. Combination therapy strategies against multiple-resistant Streptococcus suis. Frontiers in pharmacology. 2018 May 15;9:489.

[8] Schreier J, Karasova D, Crhanova M, Rychlik I, Rautenschlein S, Jung A. Influence of lincomycin-spectinomycin treatment on the outcome of Enterococcus cecorum infection and on the cecal microbiota in broilers. Gut Pathogens. 2022 Dec;14(1):1-3. https://www.sciencedirect.com/science/article/abs/pii/037843479500211Z?via%3Dihub

[10] Hornish RE, Wiest JR. Quantitation of spectinomycin residues in bovine tissues by ion-exchange high-performance liquid chromatography with post-column derivatization and confirmation by reversed-phase high-performance liquid chromatography–atmospheric pressure chemical ionization tandem mass spectrometry. Journal of Chromatography A. 1998 Jul 3;812(1-2):123-33.

[11] Yan H, Xu P, Huang H, Qiu J. Analysis of spectinomycin in fermentation broth by

reversed-phase chromatography. Chemical Papers. 2009 Dec 1;63(6):635-40.

[12] Wang MJ, Hu CQ. Analysis of spectinomycin by HPLC with evaporative light-scattering detection. Chromatographia. 2006 Mar;63(5):255-60.

[13] Kowalski P, Konieczna L, Olędzka I, Plenis A, Bączek T. Development and validation of electromigration technique for the determination of lincomycin and clindamycin residues in poultry tissues. Food Analytical Methods. 2014 Feb;7(2):276-82.

[14] Juan C, Moltó JC, Mañes J, Font G. Determination of macrolide and lincosamide antibiotics by pressurised liquid extraction and liquid chromatography-tandem mass spectrometry in meat and milk. Food Control. 2010 Dec 1;21(12):1703-9.

[15] Luo W, Yin B, Ang CY, Rushing L, Thompson Jr HC. Determination of lincomycin residues in salmon tissues by gas chromatography with nitrogen-phosphorus detection. Journal of Chromatography B: Biomedical Sciences and Applications. 1996 Dec 13;687(2):405-11.

[16] Chiu MH, Yang HH, Liu CH, Zen JM. Determination of lincomycin in urine and some foodstuffs by flow injection analysis coupled with liquid chromatography and electrochemical detection with a preanodized screen-printed carbon electrode. Journal of Chromatography B. 2009 Apr 1;877(10):991-4.

[17] Thompson TS, Noot DK, Calvert J, Pernal SF. Determination of lincomycin and tylosin residues in honey using solid-phase extraction and liquid chromatography-atmospheric pressure chemical ionization mass spectrometry. Journal of chromatography A. 2003 Dec 12;1020(2):241-50.

[18] Cao S, Song S, Liu L, Kong N, Kuang H, Xu C. Comparison of an enzyme-linked immunosorbent assay with an immunochromatographic assay for detection of lincomycin in milk and honey. Immunological Investigations. 2015 Jul 4;44(5):438-50.

[19] Wang B, Wang Y, Xie X, Diao Z, Xie K, Zhang G, Zhang T, Dai G. Quantitative analysis of spectinomycin and lincomycin in poultry eggs by accelerated solvent extraction coupled with gas chromatography tandem mass spectrometry. Foods. 2020 May;9(5):651.

[20] Guo Y, Xie X, Diao Z, Wang Y, Wang B, Xie K, Wang X, Zhang P. Detection and determination of spectinomycin and lincomycin in poultry muscles and pork by ASE-SPE-GC–MS/MS. Journal of Food Composition and Analysis. 2021 Aug 1;101:103979.

[21] Tao Y, Chen D, Yu G, Yu H, Pan Y, Wang Y, Huang L, Yuan Z. Simultaneous determination of lincomycin and spectinomycin residues in animal tissues by gas chromatography-nitrogen phosphorus detection and gas chromatography-mass spectrometry with accelerated solvent extraction. Food Additives and Contaminants. 2011 Feb 1;28(2):145-54.

[22] Stypulkowska K, Blazewicz A, Brudzikowska A, Warowna–Grzeskiewicz M, Sarna K, Fijalek Z. Development of high performance liquid chromatography methods with charged aerosol detection for the determination of lincomycin, spectinomycin and its impurities in pharmaceutical products. Journal of Pharmaceutical and Biomedical Analysis. 2015 Aug 10;112:8-14.

[23] Abualhasan MN, Batrawi N, Sutcliffe OB, Zaid AN. A validated stability-indicating HPLC method for routine analysis of an injectable lincomycin and spectinomycin formulation. Scientia Pharmaceutica. 2012 Dec;80(4):977-86.

[24] Washington JA, Yu PK. In vitro antibacterial activity of spectinomycin. Antimicrobial Agents and Chemotherapy. 1972 Dec;2(6):427-30.

[25] Bals M, Bratu M. Action of lincomycin on staphylococci. Applied Microbiology. 1966 Jul;14(4):582-3.

[26] Schreier J, Karasova D, Crhanova M, Rychlik I, Rautenschlein S, Jung A. Influence of lincomycin-spectinomycin treatment on the outcome of Enterococcus cecorum infection and on the cecal microbiota in broilers. Gut pathogens. 2022 Dec;14(1):1-3.

[27] Holloway WJ. Spectinomycin. The Medical Clinics of North America. 1982 Jan 1;66(1):169-73.

[28] Kumar PR, Rajeevkumar RE. A validated analytical HPLC method for the quantification of lincomycin hydrochloride in bulk and solid dosage form. Int J Appl Pharm. 2017;9:42-.