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Review

Liquid chromatographic analysis of antibacterial drug residues in food products of animal origin

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ABSTRACT

This paper reviews recent developments in the liquid chromatographic (LC) methods of analysis for the residues of antibiotics (aminoglycosides, chloramphenicol, sulfonamides, tetracyclines, macrolides, β-lactams, etc.) in food products of animal origin. The review also covers clean-up procedures, such as, ultrafiltration, liquid–liquid partition, solid-phase extraction, immunoaffinity, and matrix solid-phase dispersion, for use as extraction, deproteination, and concentration steps. The LC methods offer considerable potential for rapid automated analysis, and some may be used as direct screening for residues in meat and milk.

CONTENTS

1. INTRODUCTION

Antibiotics are used in food-producing animals not only for treatment of disease, but also subtherapeutically to maintain health and promote growth. The use of unauthorized antibiotics or the failure to follow label directions for approved antibiotics could result in unsafe antibiotic residues in food products. Therefore, monitoring antibiotic residues in food forms part of a general policy to prevent unapproved uses of antibiotics.

Traditionally, most antibiotics have been determined by microbiological assay. However, it is very difficult to distinguish one antibiotic from another

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using microbiological methods. The United States Department of Agricultures's Food Safety Inspection Service frequently finds microbial inhibitors in animal tissues which can not be identified by standard multiresidue procedures. There is increasing recognition of the need for improved procedures for identification and quantitation of suspect residues detected by screening methods. Liquid chromatography (LC) has emerged as the method of choice for determination of antibiotics which are rather polar, non-volatile, and sometimes heat sensitive. Other chromatographic modes such as gas–liquid and supercritical fluid chromatography have had very limited application to the determination of antibiotics. Thin-layer chromatography could be an inexpensive alternative, although it lacks sensitivity and reproducibility and quantitation is more difficult.

Many methods have been described for determination of antibiotics in formulations and in biological fluids for clinical applications. For residue analysis, isolation from more complex substrates and greater sensitivity to meet the established tolerance is required. To be useful, chromatographic methods should equal or exceed the sensitivity of screening tests. Otherwise, doubt will remain as to the identity of residues detected by screening tests if they are not detectable by chromatographic methods.

Development of methods with adequate sensitivity has proven elusive for many compounds. However, significant progress has been made in recent years. This paper discusses recent progress in the applications of LC methods for determination of antibiotic residues in food products of animal origin.

2. AMINOGLYCOSIDE ANTIBIOTICS

In recent years, LC has been increasingly used as a method of choice for the determination of aminoglycoside antibiotic residues in tissues and milk of food-producing animals. A detailed review of physical-chemical methods, including LC methods for aminoglycoside antibiotics in tissues and fluids of food-producing animals was reported in 1985 [1]. The overwhelming majority of procedures for aminoglycoside antibiotics use paired-ion chromatography on reversed-phase columns. Generally, post-column derivatization has been used with the paired-ion technique. In pre-column derivatization, the chromophore is linked to the primary amine group of aminoglycosides, yielding a less polar solute which then is readily separated by reversed-phase partition. Before LC analysis, sample pretreatment is needed to remove endogenous substances so that they do not interfere with the compounds of interest during LC analysis. Shaikh and co-workers [2–4] reported determination of neomycin in tissues and milk by LC using ion-pairing mobile phase, post-column derivatization with *o*-pthalaldehyde (OPA), and fluorometric detection. The limit of determination of neomycin in kidney tissue and milk was 1 mg/kg and 0.15 μg/ml, respectively. Buffer extraction and heat deproteination was used to extract neomycin from the tissues. Direct centrifugation of whole milk at 4°C was used to separate lipid material from the aqueous part of the milk. This was followed by deproteination with trichloroacetic acid before LC analysis. This LC procedure has also been applied to the determination of gentamicin in milk [5]. The above defattening and deproteination procedures were also used for the determination of gentamicin in milk. An additional solid-phase extraction (SPE) step, as reported by D'Souza and Ogilvie [6], was included to concentrate gentamicin on an SPE column to lower the limit of determination to 30 ng/ml. The injection volume was 500 ul and LC condition used were same as reported in ref. 4 above.

Schenck [7] used a matrix solid-phase disperson (MSPD) technique to extract neomycin from bovine kidney tissue and quantitated by using post-column LC, as reported previously [2]. The limit of determination was 2.5 mg/kg.

McLaughlin *et al.* [8] used Schenck's MSPD technique [7] to extract a number of aminoglycosides from bovine kidney tissue. Formic acid instead of sulfuric acid was used to elute the analytes from the MSPD column to obtain improved peak shapes. The LC separation was performed on a minibore YMCbasic (C_8) column using a gradient mobile phase containing acetonitrile and pentafluoropropionic acid as ion-pairing agent. The detection was by mass spectrometry (MS) using ion spray interface. This LC–MS system was used to detect aminoglycosides such as, neomycin, gentamicin, streptomycin and dihydrostreptomycin in fortified bovine kidney tissue below 1 mg/kg. However, no recoveries from fortified kidney were reported for any of the aminoglycosides.

Agarwal used pre-column derivatization for the determination of gentamicin in bovine muscle tissue and milk [9,10] and neomycin in milk [11]. For gentamicin, CM-Sephadex was used to remove endogenous interfering compounds from both tissue and milk, followed by further purification and on-column derivatization with OPA on silica Sep-Pak cartridges. The detection limit for gentamicin in both tissue and milk was 0.2 μg/ml. However, only two of the three major components of gentamicin, C_{1a} and C_2, were resolved from interfering background compounds in tissue. A different weak cation-exchange resin, Amberlite CG-50, was used for isolation of neomycin from milk. This was followed by on-column derivatization with OPA. A HISEP reversed-phase LC column, ion-pairing mobile phase, and fluorometric detection was used for LC analysis. The detection limit was 50 ng/ml. However, the OPA derivative of neomycin formed two peaks and had to be stored in the freezer for 15 min to achieve complete derivatization, before LC analysis.

Okayama *et al.* [12] reported LC determination of streptomycin in meat using ninhydrin as a post-column derivatization reagent. The method included extraction with perchloric acid solution and clean-up using a C_8 pretreatment SPE column. The LC conditions employed were as follows: reversed-phase C_{18} column; mobile phase of water–acetonitrile containing disodium 1,2-ethanesulfonate, sodium 1-octanesulfonate, and ninhydrin; post-column reaction solution of 0.3 *M* sodium hydroxide; and fluorescence detection. The method was used to determine streptomycin in chicken meat, and the recovery of added streptomycin at the 2 μg/g level was about 67%.

Shaikh *et al.* [13] developed LC conditions for the separation and determination of streptomycin and dihydrostreptomycin reference standards. The conditions used were as follows: reversed-phase ODS column (Spherisorb 5 ODS 2, 15 cm × 4.5 mm I.D.) at 50°C; mobile phase, 20 m*M* sodium hexane sulfonate, 25 m*M* tribasic sodium phosphate, 5 m*M* ninhydrin in acetonitrile–water (8:92), pH adjusted to 3.0 with phosphoric acid; post-column reagent, 0.5 *M* sodium hydroxide; and fluorescence detection at 400 and 495 nm excitation and emission wavelengths, respectively. A number of clean-up systems were also evaluated to isolate streptomycin and dihydrostreptomycin from bovine kidney tissue. The most promising was the extraction procedure of Okayama *et al.* [12] followed by additional clean-up using polymeric materials, Polysorb MP-1 solid-phase extraction cartridges (Interaction Chromatography, San Jose, CA, USA). However, the overall determinative procedure is not completed.

Recently, Gerhardt *et al.* [14] reported determination of streptomycin in porcine and bovine tissue by reversed-phase LC. Streptomycin is extracted with 3.6% perchloric acid as reported by Okayama *et al.* [12]. The extract is further purified on cation-exchange SPE column and analyzed using an inline column enrichment–post-column derivatization LC system with fluorescent detection. The limit of detection was 20 μg/kg, and the mean recovery from fortified tissue was 61.5%. However, the standard curve was prepared in tissue extracts for quantitative analysis.

3. CHLORAMPHENICOL

Allen [15] reviewed chromatographic methods, including LC, for the determination of chloramphenicol (CAP) in food products of animal origin. All methods used ethylacetate extraction followed by liquid–liquid partition and in some cases purification on SPE cartridges before HPLC analysis. Allen provided a detailed review of LC methods; therefore, only additional developments since then will be reviewed.

Sanders *et al.* [16] reported LC determination of CAP in calf tissue. The method employs ethylacetate for extraction followed by liquid–liquid partition with hexane–chloroform–water. The LC analysis consists of a reversed-phase column, acetonitrile–phosphate buffer mobile phase, and UV detection at 275 nm. The detection limit in muscle was 1 μg/kg. CAP was also found to be stable in muscle at −20°C for 180 days.

Van de Water and Haagsma [17] reported analysis of CAP residues in swine tissues and milk. The authors used silica gel SPE cartridges and antibody-mediated clean-up (AMC) as sample pretreatment procedures. Originally, the SPE procedure was developed for isolation of CAP from swine tissue [18] and later modified for use in milk. The milk method employs ethylacetate extraction, SPE clean-up, and LC analysis. The AMC procedure is based on a very specific clean-up and concentration of CAP from

aqueous meat extracts and defatted milk using immobilized monoclonal antibodies directed against CAP [16,18]. The monoclonal antibodies are covalently bound to immunoaffinity gel (carbonyldiimidazole-activated trisacryl GF-2000). The sample solutions are passed through these immunoaffinity columns by means of a peristaltic pump. The columns are washed with phosphate-buffered saline (PBS). The antibody-bound CAP was eluted with 20 ml of a solution containing 0.2 *M* glycine and 0.5 *M* NaCl (pH 2.8). The HPLC consisted of reversed-phase column, acetonitrile–0.01 *M* sodium acetate buffer (1:3, v/v), and UV detection at 280 nm. The limit of determination was 1 μg/kg in milk and 10 μg/kg in swine tissue. The results of LC procedures compared well with two enzyme-linked immunosorbent assay (ELISA) screening procedures.

4. SULFONAMIDES

Recently Agarwal [19] provided an exhaustive and updated review of LC methods for the determination of sulfonamides in tissues, milk, and eggs. Therefore, only general approaches to the LC determination of sulfonamides will be discussed here. Traditionally, the extraction of sulfonamides from various matrices has involved use of organic solvents such as chloroform, acetonitrile, or acetone followed by extraction with hexane to remove lipids. For example, Weber and Smedley [20] quantitated 10 ng/ml and above sulfamethazine in milk using a simple chloroform extraction followed by partitioning between potassium phosphate buffer and hexane to remove lipids. They further extended this work to the determination of ten sulfonamides in milk [21] by using a chloroform–acetone extraction. In many cases, an additional [19] liquid–liquid extraction is also carried out to further purify the sample extracts before LC analysis. However, in the recent past, use of SPE columns has been introduced to replace liquid–liquid extraction steps. The SPE columns used include Cyclobond-1, where β-cyclodextrin is bonded to silica [22], C_{18} [23–25] and cation-exchange resins [26]. The use of SPE columns has not only provided cleaner extracts but significantly reduced use of organic solvents and hence contributed to the reduction of amounts of hazardous waste generated. Long *et al.* [27] extracted sulfonamides from tissue or milk using MSPD techniques, where the sample is directly blended with C_{18} material. This was followed by LC analysis with a limit of detection of 31 μg/kg. The LC analysis in most cases was carried out on C_{18} columns. However, in some cases, C_8 and C_2 columns were also used. Detection in most cases was UV with a limit of determination of 5–10 μg/kg. However, fluorescence derivatization with *p*-dimethylaminobenzyldehyde (DMAB) [23] and electrochemical detection [28] were also employed. Fluorescence detection provided reduced background levels and was more discriminatory for sulfonamides resulting in increased sensitivity. Electrochemical detection was comparable to UV detection.

5. TETRACYCLINES

Significant progress has been reported in recent years on development of HPLC methods for determination of the tetracycline group of antibiotics in food substrates including honey, milk, tissues and eggs. A variety of approaches to extraction, clean-up, and HPLC analysis have been used.

Honey has been analyzed directly for oxytetracycline with no sample preparation other than dilution and filtration [29,30]. In this case, the LC method is comparable in speed and simplicity to screening methods. Others used a preliminary extraction and clean-up for determination of oxytetracycline in honey [31,32].

Other food substrates require some type of extraction procedure. For determination of residues in milk, Thomas [33] used ultrafiltration followed by direct injection of the filtrate. Recoveries were near 100% but separation from interferences was less satisfactory, limiting sensitivity. Kijak [34] used Thomas' ultrafiltration [33] in combination with a modification of Oka's *et al.* [35] C_{18} SPE procedure to obtain cleaner extracts for use in LC–MS. Fletouris *et al.* [36] and White *et al.* [37] used extraction with HCl–acetonitrile. Fletouris *et al.* [36] used a partitioning clean-up. White *et al.* [37] injected the water layer formed by adding methylene chloride and hexane to the filtrate. Farrington *et al.* [38] described a method using extraction with pH 4.0 buffer followed by clean-up on chelating sepharose and XAD-2 resin. This procedure was further modified by Carson and co-workers [39,40] for determination of seven tetracyclines in milk. For analysis, Thomas

[33] used a bonded ODS column with oxalate buffer at pH 2.0. Fletouris *et al.* [36] used an ODS column with phosphoric acid. They found it necessary to saturate the column with chlortetracycline for satisfactory results. White *et al.* [37] used a polymeric PLRP-S column (Polymer Labs, Amherst, MA, USA) with pH 2.0 oxalate buffer containing sodium decanesulfonate as an ion-pair to improve separation from interferences. Carson [40] also found that use of a polymeric column for analysis was advantageous. The method of Thomas [33] will detect residues at 10–20 ng/ml and the other procedures can determine tetracyclines at levels of less than 10 ng/ml in milk. This is well under official levels of concern which are 80, 30 and 30 ng/ml for tetracycline, oxytetracycline, and chlortetracycline, respectively, in the USA [41] and also below the maximum levels of 100 ng/ml in milk recommended by the World Health Organization (WHO) [42].

Long *et al.* [43,44] used MSPD to extract oxytetracycline, tetracycline, and chlorotetracycline from milk and oxytetracycline from catfish. For analysis, a reversed-phase ODS column was used with a mobile phase of 0.01 *M* oxalic acid–acetonitrile (70:30) for milk and 0.02 *M* oxalic acid–acetonitrile–methanol (70:27.5:2.5) for catfish. The limit of determination was 100 ng/ml for milk and 50 μg/kg for catfish.

Sharma and Bevill [45] described a procedure for extraction of tetracyclines from tissues into methylene chloride using the complexing agents phenylbutazone, calcium chloride, and sodium barbital. The tetracyclines were then extracted with 0.33 *M* phosphoric acid prior to analysis. The recoveries were good with a sensitivity limit of 0.5 mg/kg in tissues. The authors noted the need for extensive conditioning of the bonded ODS column prior to use.

Bocker and Estler [46] found that 0.03 *M* H_3PO_4–acetonitrile was superior to extraction with acid alone for recovery of residues from tissues. Aliquots of the extract were filtered and analyzed using a C_8 bonded column with NaH_2PO_4 buffer adjusted to pH 2.4 with 0.1 *M* HNO_3 as described by Sharma *et al.* [47].

Onji *et al.* [48] used extraction with 1 *M* HCl to recover tetracyclines from meat and tissue. Residues were concentrated by clean-up on an XAD-2 column. They noted losses during evaporation, especially of chlortetracycline. Analysis was on either dimethyl silica or polystyrene columns.

Ashworth [49] used acid and heat to convert tetracyclines in tissues to the anhydro forms which could then be extracted with chloroform. He described problems encountered with the use of silica-based reversed-phase columns.

Nelis and De Leenheer [50] extracted doxycycline from human tissue with 0.1 *M* HCl followed by partitioning into ethyl acetate from phosphate–sulfite buffer. For analysis, a LiChrosorb-RP-8 column was used with 0.1 *M* citric acid–acetonitrile (75:25) as the mobile phase.

Oka and co-workers [35,51] extracted tetracycline from tissues using pH 4.0 Na_2EDTA–McIlvaine buffer. For clean-up a Baker C_{18} SPE cartridge was used. They found that retention of tetracyclines on commercial C_{18} SPE cartridges differed considerably. For analysis, a LiChrosorb RP-8 column was used with a mobile phase of methanol–acetonitrile–0.01 *M* pH 2.0 oxalic acid (2:3:5). This procedure was applied to a number of substrates including milk and eggs. Ikai *et al.* [52] reported further studies with this procedure which has reported detection limits of 0.01 mg/kg in tissues.

Moats [53] found that optimum extractions from tissues were obtained using 1 *M* HCl–acetonitrile. The tetracycline could be recovered in the water layer formed when hexane and methylene chloride were added to the filtrates. Multiple injections were used to concentrate the tetracycline on the HPLC column. They were then eluted with an acetonitrile gradient. A polymeric PLRP-S column was used for analysis which avoided the problems reported with silica-based columns. For analysis, a mobile phase of 0.01 *M* H_3PO_4–methanol–acetonitrile was used with a gradient of 80:20:0 (0.2 min)–30:20:50 (25 min).

Mulders and Van de Lagemaat [54] used the extraction system described by Oka *et al.* [35] for animal tissues. A Sep-Pak C_{18} cartridge was used for clean-up after silylation of the cartridge. For analysis a NovaPak Phenyl Radial-Pak column was used with a Resolve CN guard cartridge. The mobile phase was acetonitrile–0.02 *M* oxalic acid–methanol 15:80:5 (0 min)–27:60:13 (23 min).

Nordlander *et al.* [55], extracted fish tissue with 1 *M* HCl–trichloracetic acid. For clean-up, a Sep-Pak

C_{18} cartridge was used. For analysis, a Shandon ODS Hypersil column was used. The mobile phase was pH 2.5 phosphate buffer with diethanol amine–acetonitrile–dimethylformamide (81:19:6).

Kondo *et al.* [56] extracted tetracycline from bovine tissue into ethyl acetate using the procedure described above [45]. The compounds were then extracted into phosphoric acid. For analysis, a μBondapak C_{18} column was used with a mobile phase similar to that described by Nordlander *et al.* [55].

Reimer and Young [57] used an extraction and clean-up procedure for fish similar to that described by Oka *et al.* [35]. For analysis, a Merck Hibar LiChro CART RP-18 column was used with a mobile phase of 0.01 *M* oxalic acid–acetonitrile–methanol (73:17:10).

Rogstad *et al.* [58] extracted fish tissue with 0.1 *M* Na_2EDTA in pH 4.2 phosphate buffer. Clean-up was on C_8 SPE column. For analysis, a Supelcosil LC-18-DB column was used with pH 2 phosphate buffer–acetonitrile–tetrahydrofuran (81:10:9).

Murray *et al.* [59] extracted fish tissue with HCl and $HClO_4$ mixture followed by clean-up on an XAD-2 column. For analysis, a Hypersil SAS column was used with a mobile phase consisting of citrate buffer and acetonitrile (70:30) with added Na_2EDTA.

Farrington *et al.* [38] used pH 4.0 succinate buffer containing EDTA for extraction of residues from tissues. As with milk, a chelating Sepharose column was used. A second clean-up step on an XAD-2 column was included. For analysis, a LiChrosorb RP-8 column was used with 0.01 *M* oxalic acid–acetonitrile (1:1) as the mobile phase.

Botsoglou *et al.* [60] described a procedure for determination of tetracyclines in eggs using extraction into methylene chloride with the aid of complexing agents. The tetracyclines were then recovered in acid. Analysis was on an ODS reversed-phase column using pH 2.6 phosphate buffer–acetonitrile.

Blanchflower *et al.* [61] described a procedure for determination of chlortetracycline in tissues in which tissues were extracted with 1 *M* HCl–glycine. For clean-up, the extract was passed through Bond-Elut cyclohexyl cartridges. The chlortetracycline was converted to a fluorescent derivative in pH 12 glycine buffer. For analysis, a polymer PLRP-S column was used with pH 12 glycine buffer.

Walsh *et al.* [62] determined tetracyclines in beef and pork muscle by HPLC. The tissues were homogenized in EDTA–McIlvaine buffer, centrifuged, and precipitated with trichloroacetic acid. For clean-up, Sep-Pak cartridges were used. For analysis, a NovaPak C_{18} column was used with phosphate–citrate–acetonitrile buffer.

Riond *et al.* [63] described a procedure for determining doxycycline in bovine tissues and body fluids. Ultrafiltration was used for clean-up of extracts.

Tolerances for tetracycline in edible tissues vary from 0.1–4 mg/kg in the USA [49]; and in Canada, tolerances have been set at 1, 2 and 0.25 mg/kg in edible tissue for chlorotetracycline, oxytetracycline, and tetracycline, respectively [64]. Thus, extremely high sensitivity may not be required for regulatory purposes.

6. MACROLIDE ANTIBIOTICS

There are relatively few reports of the application of LC methods to the determination of macrolide antibiotic residues in milk and tissues. However, methods have been described for determination of tylosin [65–67], spiramycin [68,69], and sedecamycin and its metabolites [70].

Moats *et al.* [65,66] described a method for determination of tylosin (tylosin A) in blood serum and tissues. Tissues were blended with 3 volumes (v/w) of water or 0.2 *M* pH 2.2 buffer for liver and kidney. Then 4 volumes of acetonitrile (v/v) were added to the homogenate, and the supernatant was filtered. Tylosin was extracted with methylene chloride, evaporated to dryness and taken up in acetonitrile. Analysis was on reversed-phase LC column using 0.005 *M* $NH_4H_2PO_4$–acetonitrile–methanol (10:60:30). The proportions were varied to improve separations depending on the matrix. As with many basic compounds on silica-based reversed-phase columns, tylosin interacts with the silica support as well as the bonded phase. As the organic solvent concentration was increased to more than 50%, the effect of the silica support became dominant as was observed with other basic compounds [71,72]. Tylosin was loaded in acetonitrile as for normal-phase chromatography on silica rather than in water as would be normal practice for reversed-phase chromatography. The interaction with the silica support

aided separation from interferences [72]. Detection limits were about 0.05 mg/kg in serum and tissues.

Horie *et al.* [67], described a procedure for tylosin A, B, C, and D in tissues using an extraction and clean-up procedure similar to that of Moats *et al.* [65,66]. Analysis was on a bonded ODS column using NaH_2PO_4–acetonitrile (65:35). Detection limits were about 0.05 mg/kg.

Horie *et al.* [68], described a procedure for determination of spiramycin in tissues using extraction with 0.5% H_3PO_4–methanol and clean-up by partitioning into methylene chloride. For analysis, a Nucleosil 5 C_{18} column was used with a mobile phase of 0.05 *M* NaH_2PO_4–acetonitrile (72:38) with UV detection at 232 nm.

Nagata and Saeki [69] extracted spiramycin residues from chicken muscle with acetonitrile. Cleanup was by partitioning into $CHCl_3$. For analysis, a Zorbax DB-C8 column was used with methanol–0.4% H_3PO_4, 0.2% sodium heptanesulfonate (7:3) with UV detection at 231 nm. The detection limit was about 0.05 mg/kg.

Okada and Kondo [70] described a procedure for determination of sedecamycin and metabolites in swine plasma and tissues. Residues were extracted with ethyl acetate. Florisil and silica columns were used for clean-up. For analysis, two types of columns, silica (μPorasil) and bonded reversed-phase (μBondapak C_{18}) and three mobile phases were used: (a) *n*-hexane–isopropanol (80:20), (b) *n*-hexane–isopropanol–acetic acid (75:25:0.2) for normal-phase chromatography, and (c) 0.01 *M* pH 8.2 phosphate buffer–acetonitrile (60:40) for reversed-phase chromatography. Detection limits were about 0.05 mg/kg.

7. β-LACTAM ANTIBIOTICS

LC methods for determination of β-lactam antibiotics were recently reviewed by Moats [73] and by Petz [74]. As with other antibiotics, LC methods are generally the procedures of choice although other chromatographic methods have been used [73,74]. For β-lactam antibiotics, several screening tests will detect residues at levels of 2–5 μg/kg [75]. Regulatory requirements are also quite stringent, especially for milk. In the USA, the levels of concern in milk are 5 ng/ml for penicillin G; 10 ng/ml for amoxicillin, ampicillin, and cloxacillin; and 20 ng/ml for cephapirin [76]. The Food and Agriculture Organization (FAO)/WHO Expert Committee has recommended a limit of 4 ng/ml for penicillin G in milk [74]. A level of 50 μg/kg of penicillin G in tissues is widely accepted as the international standard [74]. Recently, several sensitive LC methods have been described for determination of penicillins with neutral side-chains at levels of < 10 ng/ml in milk [77–81] and tissues [82,83]. Development of methods of comparable sensitivity for determination of amphoteric β-lactams has proven elusive. Except for a method recently described by Moats for cephapirin [84] using LC fractionation, none will detect < 10 μg/kg of amphoteric β-lactams [85–88]. The only procedure reported for determination of amphoteric compounds in tissues is one described for ampicillin in fish [89].

All the procedures used require some type of extraction from the matrix. A variety of approaches have been used including extraction/deproteinization with acetonitrile [77–80,82,84,90], tungstic acid [83,91], methanol [89], ultrafiltration (milk only [85,87]), direct solid-phase extraction (of milk [84,88]), and partitioning into dichlormethane at acid pH [81]. Generally, further clean-up was required. Methods used for clean-up included solid-phase extraction [83,88,89,91,92], partitioning between buffers and organic solvents [77,78,81,82,90], and HPLC fractionation [79,80,84]. Detection methods included direct UV absorbance [79–82, 84–89,91,92], and derivatization with either UV [78–83] or fluorometric detection [77,90].

8. OTHER ANTIBIOTICS

Virginiamycin is added to feed as a growth promotor. It is actually a mixture; the principle components have been designated M_1 and S. The antimicrobial activity is dependent on synergism between the two components and is affected by the ratio of the two. The M_1 component is the predominant component of the mixture.

Nogase and Fukamachi [93] described a procedure for determination of both the S and M_1 components in muscle. Virginiamycin was extracted from tissues with acetonitrile. Clean-up was by partitioning into $CHCl_3$ and then into the HPLC mobile phase. Analysis was on an ODS column with fluorescence detection. Moats and Leskinen [94]

blended tissue with 0.2 *M* $NH_4H_2PO_4$, added methanol at a volume equal to that of the blend, and homogenized again. The filtrate was extracted into methylene chloride–petroleum ether and then into HPLC mobile phase. For analysis, a Supelco LC-18 column was used with 0.01 *M* $NH_4H_2PO_4$–acetonitrile gradient. Detection was based on the M_1 component. Saito *et al.* [95] extracted virginiamycin from tissues using methanol-phosphotungstic acid. Clean-up was based on partitioning followed by solid-phase extraction. For analysis, an ODS column was used with acetonitrile–water. Detection was based on the M_1 component. Further studies by Moats and Leskinen [94] demonstrated that only traces of virginiamycin were found in tissue of swine fed very high levels. Since this is the only mode of administration to farm animals, it is therefore unlikely that violative residue will be found in animal tissues.

Moats and Leskinen [96] described a rapid procedure for determination of novobiocin in milk and tissues. Milk or tissue were homogenized with 0.2 *M* $(NH_4)H_2PO_4$, methanol added at a volume equal to that of the blend, and homogenized again (twice the volume with liver and kidney). The filtrates (diluted with water if necessary) were concentrated on-line on an ODS column and eluted with an acetonitrile gradient for analysis with UV detection at 320 nm.

Moats [97] described a procedure for lincomycin in milk and tissues. Milk and tissue was blended in 0.1 *M* $(NH_4)H_2PO_4$, methanol added at a volume equal to that of the blend, and homogenized again. The resulting filtrate was mixed with 1.5 volumes of acetonitrile and refiltered. The filtrate was evaporated to remove acetonitrile. For clean-up, solid-phase extraction and fractionation using the HPLC system was used. Detection was UV at 210 nm.

9. CONCLUSIONS

In the last decade many LC methods have been developed for the determination of antibiotic residues in meat and milk. Many of these methods are relatively simple, specific, and able to analyze at tolerance levels. However, in order for them to be practical and rugged for residue monitoring, they must be subjected to collaborative studies or validated in various other laboratories.

Some LC methods require minimal sample preparation and can be completed in one h or less (*e.g.* refs. 29, 37 and 96). They are therefore comparable in speed and cost with some screening tests. The LC methods can provide a more specific result in a short time while most conventional screening tests can only establish the need for further testing.

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