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ARTICLES

Identification and Determination of Antibiotics from Various Classes in Food- and Feedstuffs by Matrix- or Surface-Assisted Laser Desorption/Ionization Mass Spectrometry

V. G. Amelin[a, b] and T. A. Krasnova[a, b]

[a] *Federal Center for Animal Health, Yur'evets, Vladimir, 600901, Russia*
[b] *Vladimir State University, ul. Gor'kogo 87, Vladimir 600000, Russia*
e-mail: amelinvg@mail.ru

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Abstract—Methods are proposed for the identification and determination of residual quantities of antibiotics from various classes in food- and feedstuffs for animals by matrix- or surface-assisted laser desorption/ionization mass spectrometry (MALDI/SALDI MS). It is shown that acetonitrile is efficient for the extraction of antibiotics without additional extract purification. The limits of detection for antibiotics in the indicated products are 0.01–0.3 μg/kg in MALDI MS and 0.001–0.03 μg/kg in SALDI MS at the signal-to-noise ratio 4. The relative standard deviation of the results of determination of antibiotics does not exceed 10% in feedstuffs and premixes at the analytical range 20–400 mg/kg for monensin and narasin, 0.2–200 mg/kg for tilmicosin, and 20–1000 mg/kg for avilamycin and a weighed portion of feedstuff 1.0 g. The time of analysis is 40–50 min.

Keywords: MALDI/SALDI MS, antibiotics, identification, determination, foodstuff, feedstuff
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The use of various antibiotics in veterinary for the prevention of diseases of poultry and cattle sometimes results in their presence in food of animal origin (meat, eggs, milk, etc.). The use of food containing residual quantities of antibiotics substantially harms human health. According to the current regulatory documents, antibiotics in the food of animal origin are determined by microbiological methods based on the use of bacteria sensitive to antibiotics and on their capability to multiply in foodstuffs [1]. Moreover, solid-phase enzyme immunoassay is used for the determination of antibiotics [2]. Microbiological studies and enzyme immunoassay require complicated sample preparation; the time of analysis reached several hours.

In the last decade, new methods have been proposed for the determination of some antibiotics by tandem liquid chromatography–electrospray ionization mass spectrometry. For example, a method for the determination of chloramphenicol in meat and seafood was proposed in [3]. Method [4] for the simultaneous determination of 120 antibiotics from various classes in kidneys of animals was proposed. Such methods require thorough purification of extract of antibiotics from coextracted impurities (proteins, fats, lipids, etc.) in the solid-phase extraction.

Taking into account the complexity of the sample preparation stage in the above methods, the search for a simpler method for the identification and determination of antibiotics is continued. One of the rapidly developing methods for the study of organic compounds is matrix- or surface-assisted laser desorption/ionization mass spectrometry with a time-off-flight mass analyzer [5, 6]. The determination of antibiotic monensin in soil, water, and urine using colloidal silver as a matrix [7], and tylosin, tilmicosin, spiramycin, and erythromycin in urine using α-cyano-4-hydroxycinnamic acid as a matrix was described [8].

The aim of the present work was to demonstrate the capabilities of MALDI/SALDI MS for the identification of antibiotics in food of animal origin and the determination of antibiotics in feedstuffs and premixes.

EXPERIMENTAL

Equipment. A MALDI mass spectrometer with an Autoflex III smart beam time-off-flight mass analyzer (Bruker Daltonik, Germany) was used; the reflectron operated in the positive and negative ion modes in the mass range 200–7000 Da (PepMix standard mode); the main parameters used in the study were as follows: an ultraviolet nitrogen laser with the wavelength 337 nm, pulse length 3 ns, and power of laser radiation 10^6–10^7 W/cm^2. Mass spectra were corded using the FlexControl ver. 3.3 software. Spectra were analyzed using the FlexAnalysis ver. 3.3 software (Bruker Daltonik, Germany). An MTP 384 ground-steel TF plate and a Nanosys MSP 96 disposable substrate with inorganic

nanothreads (Bruker Daltonik, Germany) were used for sample application.

Reagents. 4-Hydroxy-3,5-dimethoxycinnamic acid, α-cyano-4-hydroxycinnamic acid (**CHCA**), 2,5-dihydroxybenzoic acid, as well as Nanosys MSP 96 nanosurface (Bruker Daltonik, Germany) were used as matrices.

To graduate the mass spectrometer for work in the PepMix positive and negative ion mode, we used a standard peptide mixture (Peptide Calibration Standard, Bruker Daltonik, Germany) consisting of 7 compounds: angiotensin I (1047.19 Da), angiotensin II (1297.49 Da), substance P (1348.64 Da), bombesin (1620.86 Da), ACTH 1–17 (2094.43 Da), ACTH 18–39 (2466.69 Da), and somatostatin 28 (3149.57 Da). Standard samples of individual antibiotics, monensin, narasin, and avilamycin (Elly Lilly & Company, United States); tilmicosin, kanamycin, neomycin, streptomycin, dihydrostreptomycin, lasalocid, laidlomycin, erythromycin, salinomycin, josamycin, spiramycin, rifabutin, semduramicin, maduramicin, and tylosin (Sigma–Aldrich, Switzerland); spectinomycin, ivermectin, abamectin, rifampicin, amikacin, polymyxin sulfate, valinomycin, bacitracin, and ceftiofur (Dr. Ehrenstorfer, Germany) were used. Solutions of antibiotics with the concentration 1 mg/mL were prepared by dissolving corresponding weighed portions of substances in acetonitrile. Working solutions were prepared in the day of use by the dilution of stock solutions with acetonitrile. Matrices of the concentration 20 mg/mL were prepared using acetone (Ekokhimtech, Russia) and trifluoroacetic acid (Sigma-Aldrich, Switzerland). Acetonitrile (Prolabo, Austria), $MgSO_4$, NaCl (Panreac, Spain), trisodium citrate dihydrate $Na_3C_6H_5O_7 \cdot 2H_2O$, disodium citrate sesquihydrate $Na_2C_6H_5O_7 \cdot 1.5H_2O$ (Sigma-Aldrich, Switzerland), and adsorbents Bondesil PSA and Discovery DSC-18 (Supelco, United States) were used. Deionized water with the resistivity not less than 18 MOhm was obtained on a Water Pro PS system (Labconco, United States).

Sample preparation. *Dairy products*. Milk or a dairy product (5.0 g) were placed in a 50-mL plastic tube; 10.0 mL of acetonitrile and 5.0 mL of deionized water were added; the mixture was shaken for 5 min, 4 g of NaCl was added, and the mixture was vigorously shaken for 1–2 min and centrifuged for 5 min at 4500 rpm.

Food of animal origin. Homogenated food (5.0 g) was placed in a 50-mL plastic tube, 10.0 mL of acetonitrile was added, and the mixture was shaken for 15 min and centrifuged for 5 min at 4500 rpm.

Application of QuEChERS. A weighed portion (5.0 g) of a test sample was placed in a 50-mL centrifuge tube, 10.0 mL of acetonitrile and 0.1 mL of conc. formic acid were added, and the tube was capped and vigorously shaken for 1 min. Then a mixture of 4.0 g of $MgSO_4$, 1.0 g of NaCl, 1.0 g of $Na_3C_6H_5O_7 \cdot 2H_2O$, and 0.5 g of $Na_2C_6H_6O_7 \cdot 1.5\ H_2O$ was added. After the addition of salts, the mixture was shaken for 1 min (to prevent lump formation), centrifuged for 5 min at 4500 rpm, and 5 mL of the upper portion of the extract was taken and transferred into a 15-mL centrifuge tube containing a mixture of adsorbents Bondesil PSA (0.15 g), C_{18} (0.15 g), and $MgSO_4$ (0.9 g). The tube was vigorously shaken for 1 min and centrifuged for 5 min at 2700 rpm.

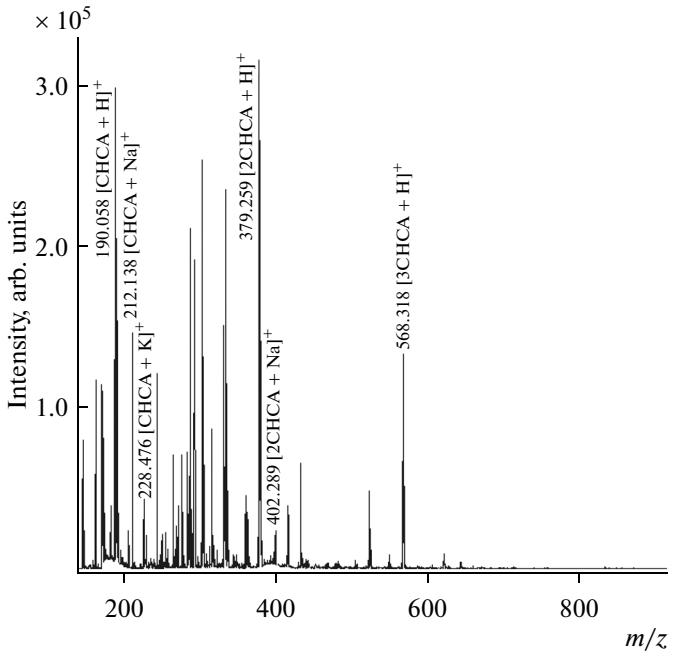

Fig. 1. Mass spectrum of α-cyano-4-hydroxycinnamic acid in the positive ion mode.

The extracts obtained were mixed with a matrix (α-cyano-4-hydroxycinnamic acid, 20 mg/mL) in the ratio 1 : 1 (v/v) and 1 μL of the mixture was applied onto a steel substrate (MTP 384 ground-steel TF plate) for MALDI MS, or extracts were mixed with twice-distilled water in the ratio 1 : 1 (v/v) and 1 μL of the mixture was applied on a Nanosys MSP 96 substrate for SALDI MS.

The identification of antibiotics was performed by comparing the mass lists of the recorded spectra with masses of the major ions of antibiotics obtained in the study of individual standards (taking into account the natural isotope ratio).

Determination of antibiotics in feedstuffs and premixes. A homogenized feedstuff (1.0 g) or 0.05 g of a premix were placed into a 50-mL plastic tube, 9.0 mL of acetonitrile and 1.0 mL of deionized water were added, and the mixture was shaken for 15 min and centrifuged for 5 min at 4500 rpm. The extract obtained was mixed with an internal standard (10 μg/mL josamycin solution) in the ratio 1 : 1 (v/v). The mixture of the analyzed solution with the internal standard was mixed with the matrix (α-cyano-4-hydroxycinnamic acid) in the ratio 1 : 1 (v/v) and 1 μL of the mixture was applied onto a steel substrate.

The efficiency of sample preparation was characterized by recovery (R):

Table 1. Characteristic masses of ions of antibiotics of various classes obtained by MALDI obtained by MALDI MS and SALDI MS in the registration of positive ions

Antibiotic, molecular mass, class	MALDI MS		SALDI MS	
	ion	m/z	ion	m/z
Kanamycin, 484.50, aminoglycosides	$[KNM + H]^+$	485.420	$[KNM + H]^+$	485.478
Ceftiofur, 523.57, cephalosporins	$[CFT + H]^+$ $[CFT + Na]^+$ $[CFT + K]^+$	524.198 546.200 562.181	$[CFT]^+$ $[CFT + H]^+$	523.010 524.198
Streptomycin, 581.58, aminoglycosides	$[STM + H]^+$	582.764	$[STM + H]^+$	582.751
Dihydrostreptomycin, 584.62, aminoglycosides	$[DHS]^+$	584.721	$[DHS]^+$	584.635
Amikacin, 585.61, aminoglycosides	$[AMK + H]^+$ $[AMK + Na]^+$	586.484 608.487	$[AMK + H]^+$	586.483
Lasalocid, 588.78, ionophores	$[LSC + H]^+$ $[LSC + Na]^+$	589.756 611.770	$[LSC + H]^+$ $[LSC + Na]^+$	589.643 611.634
Neomycin, 614.65, aminoglycosides	$[NMC + H]^+$	615.523	$[NMC + H]^+$	615.465
Monensin, 670.88, ionophores	$[MNS + Na]^+$ $[MNS + K]^+$ $[MNS + Na - H_2O]^+$	693.636 709.611 675.616	$[MNS + Na]^+$ $[MNS + Na - H_2O]^+$	693.636 675.510
Laidlomycin, 698.89, ionophores	$[LDM + Na]^+$ $[LDM + K]^+$	720.458 736.354	$[LDM + Na]^+$ $[LDM + K]^+$	720.678 736.657
Erythromycin, 733.94, macrolides	$[ERM + Na]^+$ $[ERM + K]^+$ $[ERM + H]^+$	756.689 772.669 734.684	$[ERM + H]^+$ $[ERM + Na]^+$ $[ERM + K]^+$	734.786 756.853 771.900
Salinomycin, 751.01, ionophores	$[SLM + H]^+$ $[SLM + Na]^+$ $[SLM + K]^+$	752.453 774.245 790.383	$[SLM + H]^+$ $[SLM + Na]^+$ $[SLM + K]^+$	752.235 774.458 790.355
Narasin, 764.04, ionophores	$[NRS + Na]^+$ $[NRS + K]^+$	787.721 803.705	$[NRS + Na]^+$ $[NRS + K]^+$	787.775 803.760
Rifampicin, 823.95, ansamycins	$[RFP + H]^+$ $[RFP + Na]^+$ $[RFP + K]^+$	824.323 846.315 862.400	$[RFP + H]^+$ $[RFP + Na]^+$ $[RFP + K]^+$	824.345 846.675 862.445
Josamycin, 828.01, macrolides	$[JM + H]^+$ $[JM + Na]^+$ $[JM + K]^+$	828.630 850.644 866.620	$[JM + H]^+$ $[JM + K]^+$ $[JM + Na]^+$	828.767 866.620 850.644
Spiramycin, 843.07, macrolides	$[SPM + H]^+$ $[SPM + Na]^+$ $[SPM + K]^+$	843.683 865.695 881.673	$[SPM + H]^+$	843.862
Rifabutin, 846.02, ansamycins	$[RFB + H]^+$ $[RFB + Na]^+$ $[RFB + K]^+$	847.435 870.478 885.639	$[RFB + H]^+$ $[RFB + Na]^+$ $[RFB + K]^+$	847.560 870.564 885.625
Tilmicosin, 868.15, macrolides	$[TMC + H]^+$ $[TMC + Na]^+$	869.835 891.816	$[TMC + H]^+$	869.794

Table 1. (Contd.)

Antibiotic, molecular mass, class	MALDI MS		SALDI MS	
	ion	m/z	ion	m/z
Abamectin, 874.10, avermectins	$[AMT + K]^+$	912.229	$[AMT + K]^+$	912.301
Semduramicin, 873.09, ionophores	$[SDM + H]^+$ $[SDM + Na]^+$ $[SDM + K]^+$	874.383 896.195 912.373	$[SDM + H]^+$ $[SDM + Na]^+$ $[SDM + K]^+$	874.437 896.789 912.569
Ivermectin, 875.11, avermectins	$[IMT + K]^+$	914.230	$[IMT + K]^+$	913.347
Tylosin, 916.11, macrolides	$[TLS + H]^+$ $[TLS + Na]^+$	916.702 938.713	$[TLS + H]^+$ $[TLS + Na]^+$	916.829 938.864
Maduramicin, 917.14, ionophores	$[MDM + H]^+$ $[MDM + Na]^+$ $[MDM + K]^+$	918.234 940.476 956.532	$[MDM + H]^+$ $[MDM + Na]^+$ $[MDM + K]^+$	918.349 940.568 956.543
Valinomycin, 1111.34, ionophores	$[VLM + H]^+$ $[VLM + K]^+$	1112.092 1150.078	$[VLM + H]^+$ $[VLM + K]^+$	1112.125 1150.085
Polymyxin sulfate, 1203.50, polypeptides	$[PLM + H]^+$ $[PLM + K - 3NH_3]^+$	1204.097 1190.071	$[PLM + H]^+$ $[PLM + K - 3NH_3]^+$	1204.085 1190.063
Avilamycin, 1404.25, ortozomocins	$[AVM + K]^+$ $[AVM + 2K - H_2O - H]^+$	1443.137 1461.715	$[AVM + K]^+$	1444.022
Bacitracin, 1422.72, polypeptides	$[BCC + H]^+$ $[BCC + K - 3NH_3]^+$	1423.183 1409.129	$[BCC + H]^+$ $[BCC + K - 3NH_3]^+$	1423.167 1409.115

$$R = \frac{c_f V_f}{c_0 V_0} \times 100,$$

where c_f and c_0 are the concentrations of analyte in the final solution and in the initial sample, respectively, and V_f and V_0 are the volumes of the final analyzed solution concentrate and of the sample, respectively.

RESULTS AND DISCUSSION

Several classes of antibiotics widely used in veterinary were used, i.e., macrolides, polypeptides, cephalosporins, and aminoglycosides (Table 1). It was found that, for the work with the indicated substances, the standard RP PepMix mode with the reflectron is optimal for the registration of positive ions. In the work with negative ions (RN PepMix), the peaks of antibiotic ions were not detected in the mass spectra. The optimum intensity level of analyte peaks was attained at 55% of the maximum of laser power and reflectron voltage of 1569 V. The summary mass spectra of 2000 laser impacts was stored, while the results of the first 20 impacts were rejected.

Selection of a matrix in the study of antibiotics from various classes. The most widely used organic matrices for drugs are CHCA, 2,5-dihydroxybenzoic acid, and 4-hydroxy-3,5-dimethoxycinnamic acid. It was found in recording mass spectra with these matrices that only with CHCA analysts could detect peaks of ions of all the studied antibiotics. It should be noted that the itself is responsible for the appearance of ions in the mass range from 100 to 950 Da, which can result in the superposition of the masses of the matrix and analyte (Fig. 1). As it can be seen in Fig. 1, the main spectrum of the matrix is in the mass range 150–800 Da when a MTR 384 ground steel TF substrate is used. The peaks of ions in the range 180–400 Da are most intense. Intense peaks of the matrix were also observed in the vicinity of 530 and 580 Da. Nevertheless, taking into account the molecular masses of the studied antibiotics (Table 1), the use of CHCA as a matrix was justified. In the case of macrolides and polypeptides, no

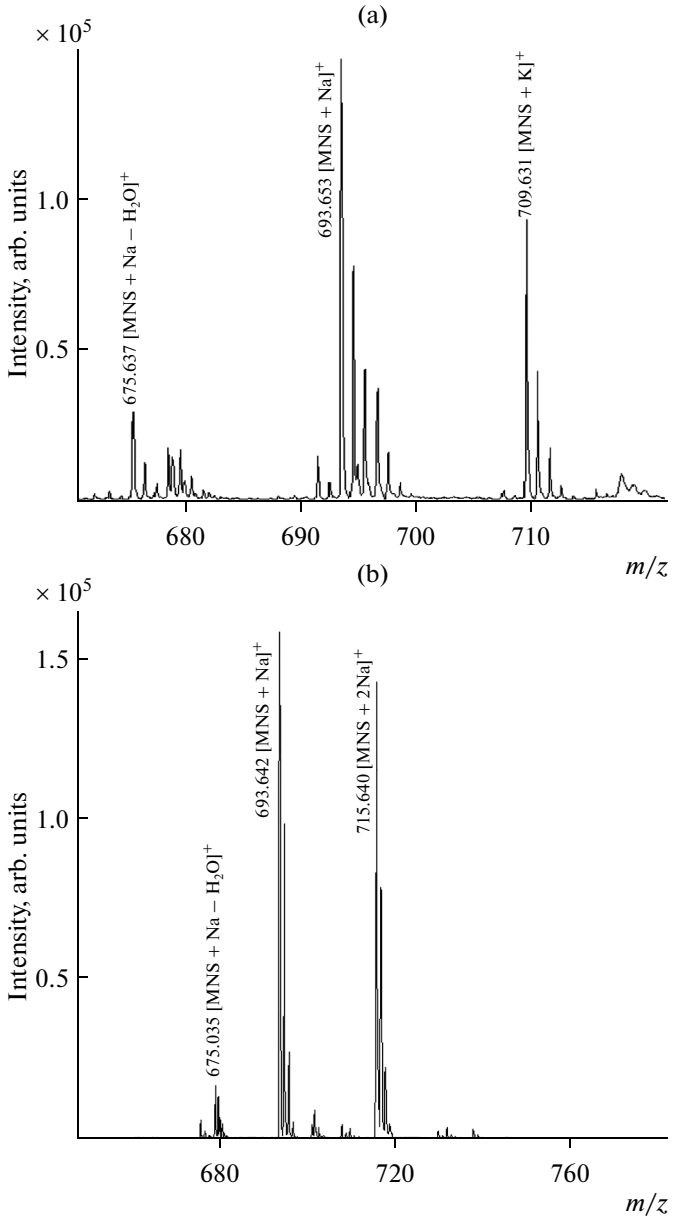

Fig. 2. Mass spectra (a) MALDI MS and (b) SALDI MS of monensin.

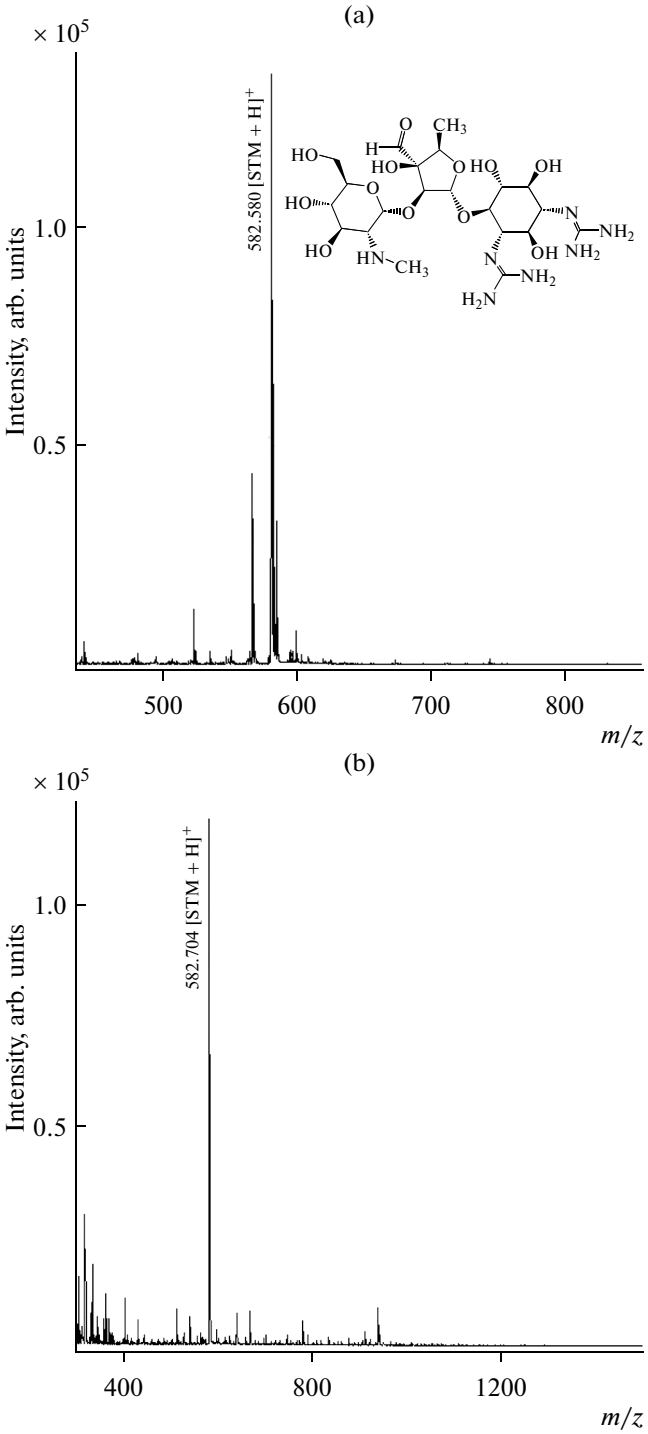

Fig. 3. Mass spectra (a) MALDI MS and (b) SALDI MS of streptomycin.

matrix effect on mass spectra was observed, because their molecular masses were greater than the maximum masses of matrix ions. It was found in the study of antibiotics from all classes that CHCA was the optimum organic matrix at the component ratio in the system matrix : analyte 1 : 1 (v/v) and matrix concentration of 20 mg/mL.

It should be emphasized that, when a Nanosys MSP 96 inorganic matrix was used, peaks of different ions were found in the range 300–700 Da; however, the intensity of lines in the spectrum was very low. On the addition of an analyte, the indicated peaks disappeared, which can be explained by the prevalence of the ionization/desorption of analyte ions and, consequently, insufficient energy for the ionization/desorption of matrix ions.

The mass spectra of antibiotics in the registration of positive ions are characterized by a small number of well resolved peaks of ions characteristic for a particular compound, mainly protonated ions $[M + H]^+$ and adducts with sodium or potassium $[M + Na]^+$ or

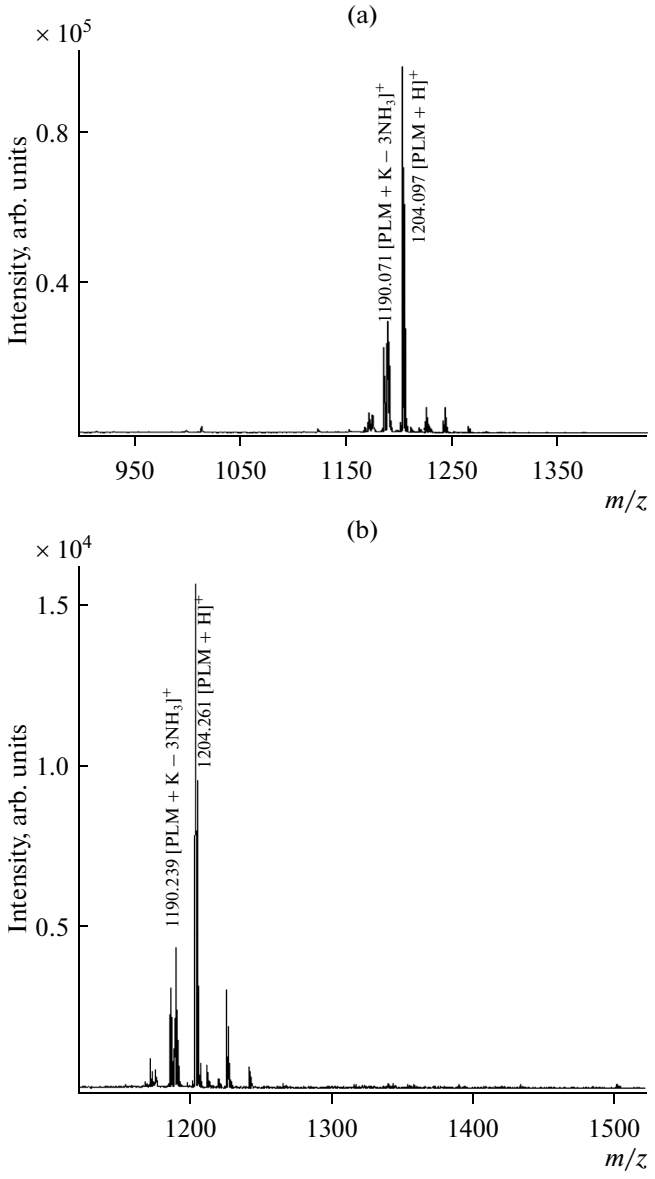

Fig. 4. Mass spectra (a) MALDI MS and (b) SALDI MS of polymyxin.

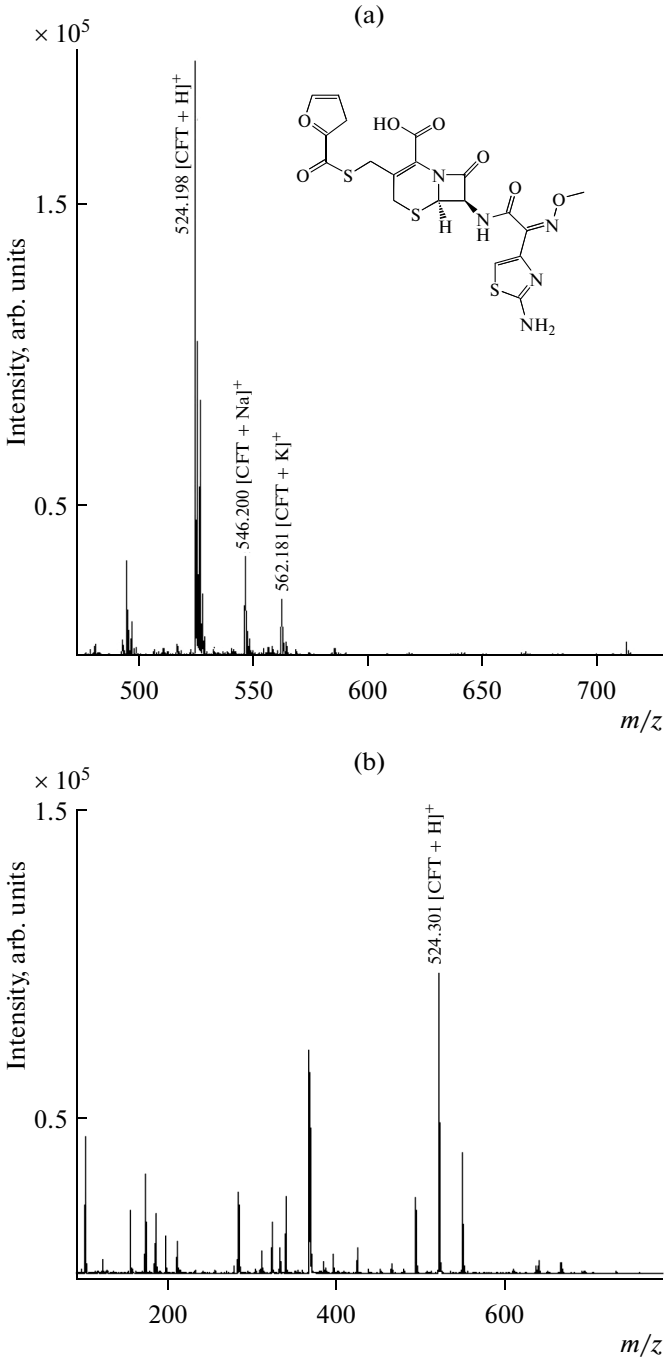

Fig. 5. Mass spectra (a) MALDI MS and (b) SALDI MS of ceftiofur.

$[M + K]^+$. In the study of avilamycin, ionofores and polypeptides, peaks of ions associated with the defragmentation of molecules were observed in the mass spectra. Thus, the elimination of water and ammonia molecules was characteristic especially for avilamycin, monensin and polypeptides: $[M + Na - H_2O]^+$, $[M + K - H_2O]^+$, and $[M + K - 3NH_3]^+$ (Table 1). The indicated ions were observed in the spectra obtained both by MALDI MS and SALDI MS.

Figures 2–5 present mass spectra of antibiotics of certain studied classes. For monensin, one of the most widely used ionophore, peaks of ions containing adducts with Na^+ and K^+, as well as ions with sodium were observed on water elimination. Essentially all representatives of this class are characterized by the presence of peaks of adducts with sodium and potassium in the mass spectra (Table 1). As protonated forms of ions are characteristic for aminoglycosides, peaks of ions formed with the addition of sodium or potassium were not found (Fig. 3) in their mass spectra. In the case of polypeptides, the predominant formation of ions of the protonated form was observed, while for antibiotics from this class fragment ions were found as well, primarily the elimination of ammonia with the simultaneous addition of sodium or potas-

Table 2. Limits of detection for antibiotics in the study of extracts from various samples ($S/N = 4$, $n = 3$)

Antibiotic	MALDI MS, μg/mL	SALDI MS, ng/mL
Monensin	0.8	0.01
Narasin	0.5	0.03
Tilmicosin	0.003	0.0005
Avilamycin	0.9	0.01

sium was observed (Fig. 4). For cephalosporins, the presence of adduct ions with sodium or potassium was detected, as well as of protonated ions (Fig. 5).

Sample preparation for the detection of antibiotics in foodstuffs of animal origin and feedstuffs. For the selection of the conditions for the sample preparation of food of animal origin and feedstuffs, various methods of extraction of antibiotics were studied. The efficiencies of extraction by QuEChERS both with and without purification of the extract were compared. The extraction of organic compounds by QuEChERS was performed with acetonitrile (more rarely with ethyl acetate) in the presence of salting out agents and buffer mixtures [9]. The purification of extracts from organic acids, lipids, fats, and proteins was performed by bulk adsorbents Bondesil PSA (mixture of primary and secondary amines), C_{18}, graphitized carbon black, ion exchange resins, and their combinations. The identification and determination of antibiotics in foodstuffs of animal origin is a complicated task because of the presence of lipids, carbohydrates, and proteins in the food. The use of sample preparation according to QuEChERS for the extraction, preconcentration, and purification of food extracts has recently become a common practice in such cases.

It was found in this work that unpurified extracts give a more *clean* spectrum in the mass region below 1000 Da. In the extracts obtained by QuEChERS, the number of peaks from various ions was significantly higher. This could be associated with the additional introduction of various cations during sample preparation, capable of forming adducts with the matrix and with the substances present in the extract and resulting in the formation of new ions in the spectrum in the indicated mass region. Therefore, we selected extraction with acetonitrile using sodium chloride as a salting-out agent as the main method of the extraction of antibiotics. This method of extraction makes sample preparation significantly easier even compared to the simple QuEChERS method. It was found that the recovery of antibiotics from feedstuffs in extraction with acetonitrile was 80–100%.

Identification and determination of antibiotics in feedstuffs and premixes. The possibility of the detection and determination of antibiotics was studied on an example of analysis of mixtures of macrolides most often used in veterinary, i.e., monensin, narasin, tilmicosin, and avilamycin. A comparative study of the mass spectra of extracts from real samples with additives of the above antibiotics showed that the attained

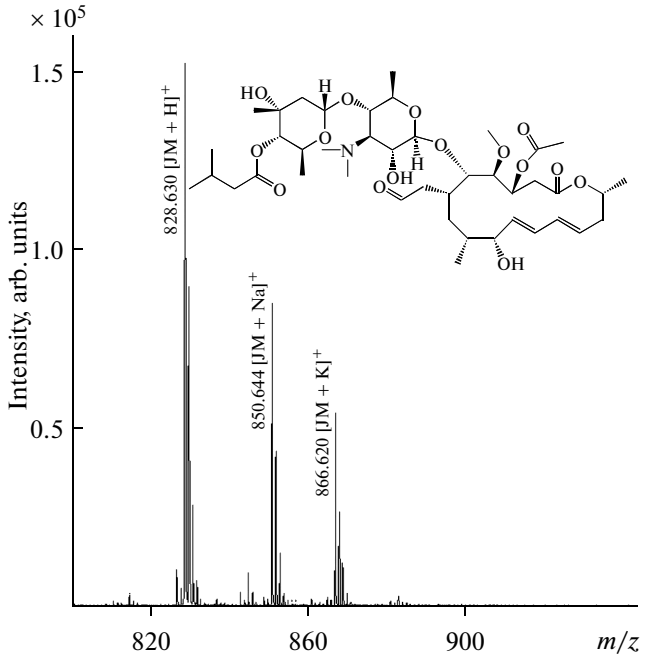

Fig. 6. Mass spectrum of josamycin ($c = 10$ μg/mL).

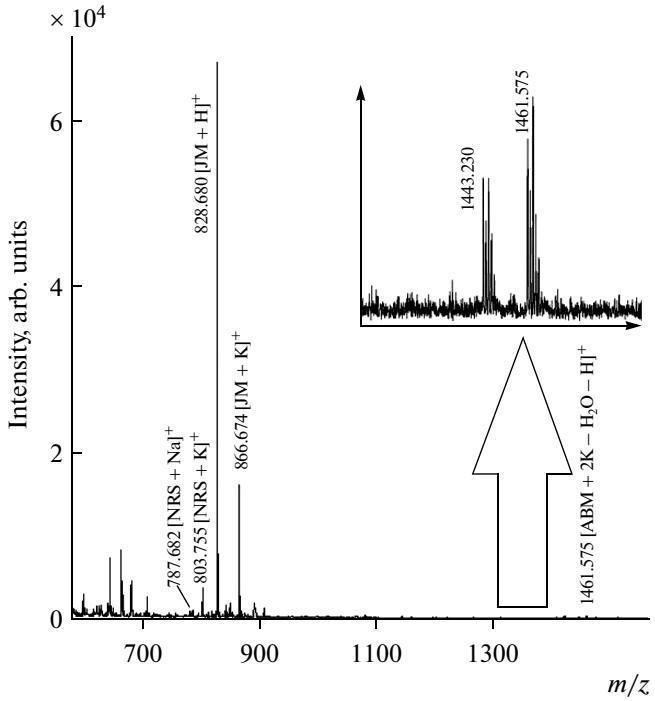

Fig. 7. Mass spectrum of an extract from a premix with an addition of an internal standard josamycin.

Table 3. Limits of detection and linearity ranges of calibration dependencies in determination of antibiotics

Antibiotic	LOD	LR	Equation of calibration curve	R^2
Monensin	0.3	1–20	$y = 4.858x$	0.991
Narasin	0.3	1–20	$y = 13.04x$	0.999
Josamycin	0.05	0.1–50	Internal standard	–
Tilmicosin	0.003	0.01–10	$y = 136.4x$	0.992
Avilamycin	0.3	1–50	$y = 0.7350x$	0.995

LOD is limit of detection and LR is linearity range (g/mL)

limits of detection found by the S/N ratio was 4 using SALDI MS and 3–4 orders of magnitude higher using MALDI MS (Table 2).

In the study of the MALDI MS and SALDI MS mass spectra of mixed poultry feed, it was found that narasin, monensin, and avilamycin were present in the samples. The possibility of the determination of antibiotics in the feed and premixes was studied using the analysis of a mixture of antibiotics monensin, narasin, tilmicosin, and avilamycin as an example. For quantitative determination by MALDI MS, an internal standard was used. As was found in the result of study of various antibiotics from the macrolide group, the best internal standard for the indicated mixture of antibiotics was josamycin (Fig. 6).

As was shown in the study of mass spectra of mixtures of extracts with the internal standard in the ratios 1 : 1, 1 : 2, 1 : 3, 2 :1, 3 :1 (v/v) with the josamycin concentration 10, 20, 30, and 50 μg/mL, volume ratio of 1 : 1 and josamycin concentration of 10 μg/mL were the best. In the determination of calibration dependences, the sum of peak areas in mass spectra for narasin $[NRS + Na]^+$ $m/z = 787$ and $[NRS + K]^+$ $m/z = 803$; monensin $[MNS + Na]^+$ $m/z = 693$, $[MNS + K]^+$ $m/z = 709$, and $[MNS + Na - H_2O]^+$ $m/z = 675$; tilmicosin $[TMC + Na]^+$ $m/z = 891$ and $[TMC + H]^+$ $m/z = 869$; avilamycin $[AVM + K]^+$ $m/z = 1443$, and $[AVM + 2K - H_2O - H]^+$ $m/z = 1461$ were related to the sum of peak areas of josamycin ions $[JM + H]^+$ $m/z = 828$, $[JM + K]^+$ $m/z = 866$, and $[JM + Na]^+$ $m/z = 850$.

The calibration dependences were linear in the range 1–20 μg/mL for monensin and narasin, 0.01–10 μg/mL for tilmicosin, and 1–50 μg/mL for avilamycin at RSD ≤ 0.09% (Table 3). The limits of detection were determined at $S/N = 4$. The analytical ranges at the weighed portions of feed 1.0 g were (mg/kg) 20–400 for monensin and narasin, 0.2–200 for tilmicosin, and 20–1000 for avilamycin. The accuracy of the results of determinations was tested using the added–found method on an example of feed containing no antibiotics (Table 4).

Table 4. Results (mg/kg) of determination of antibiotics in feedstuffs using the added–found method ($n = 3$, $P = 0.95$)

Antibiotic	Added	Found	RSD, %
Narasin	200	219 ± 35	9
Monensin	100	107 ± 22	7
	200	230 ± 37	10
Tilmicosin	50	40 ± 5	1
Avilamycin	200	174 ± 30	9
	500	419 ± 51	10

Table 5. Determination of narasin and avilamycin in premixes using MALDI MS ($n = 3$, $P = 0.95$)

Antibiotic	Sample	Found, mg/kg	RSD, %
Narasin	Feed no.1	23 ± 5	10
	Feed no. 2	35 ± 2	2
	Feed no. 3	31 ± 6	8
	Premix	230 ± 21	4
Avilamycin	Premix no. 1	46 ± 8	7
	Premix no. 2	150 ± 32	8
	Premix no. 3	214 ± 55	10

Table 6. Identification of residual amounts of antibiotics in foodstuffs of animal origin by MALDI MS (SALDI MS in milk)

Sample	Characteristic masses, m/z, Da	S/N ratio	Peak height, arb. units	Peak area, arb. units	Identified antibiotic
Egg	869.743	4.5	51	7	Tilmicosin
Pork	586.461	5.4	455	56	Amikacin
	584.437	5.1	428	59	Dihydrostreptomycin
	709.567	8.2	465	61	Monensin
	756.733	4.4	232	22	Erythromycin
Lard	615.390	9.0	1364	180	Neomycin
	333.254	54.0	13635	1056	Spectinomycin
	787.808	41.4	3471	651	Narasin
Beef	586.430	6.3	255	27	Amikacin
	615.332	9.3	350	35	Neomycin
	333.230	21.6	550	36	Spectinomycin
	584.423	4.1	170	24	Dihydrostreptomycin
	803.383	4.0	102	14	Narasin
Pork liver	608.464	9.5	410	51	Amikacin
	615.348	10.6	453	59	Neomycin
	333.231	13.1	715	47	Spectinomycin
	584.440	18.7	824	98	Dihydrostreptomycin
Chicken meet	333.230	13.4	1100	78	Spectinomycin
Cow milk 3.2%	523.483	39.3	2462	268	Ceftiofur
	734.856	71.0	18802	2997	Erythromycin
	787.930	11.7	3330	565	Narasin
	582.754	56.8	18680	2285	Streptomycin
	611.820	21.4	6938	1263	Lasalocid
	692.836	15.5	4846	819	Laidlomycin
	693.845	6.5	2354	383	Monensin
	774.458	11.5	3442	503	Salinomycin
Gout milk 1.5%	824.842	10.9	724	75	Rifampicin

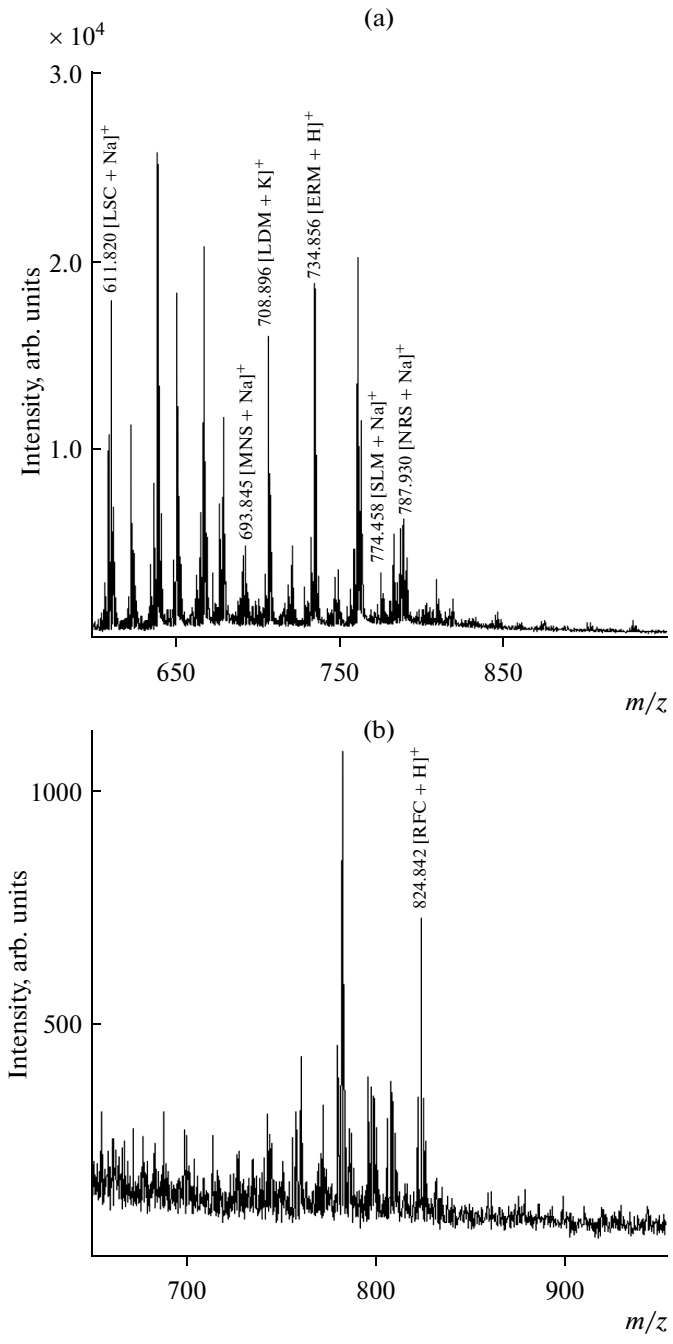

Fig. 8. SALDI mass spectra of extracts from (a) cow milk and (b) goat milk. LSC is lasalocid, MNS is monensin, LDM is laidlomycin, ERM is erythromycin, SLM is salinomycin, NRS is narasin, and RFC is rifampicin.

Table 5 presents the results of determination of antibiotics in feed and premixes; it can be seen that RSD ≤ 10% (n= 3). A MALDI MS mass spectrum of an extract of premix with an addition of josamycin in shown in Fig. 7.

Identification of antibiotics in foodstuffs of animal origin. Peaks of some antibiotics were found in the mass spectra of acetonitrile extracts from foodstuffs of animal origin. The antibiotics were identified by the comparison of mass spectra of extracts and mass spectra of standard solutions of antibiotics. In addition to the correspondence of the m/z values for the peaks, we used the correspondence of their isotope distributions as one of identification parameters. Residual amounts of tilmicosin, amikacin, monensin, erythromycin, narasin, and other antibiotics were found in samples of pork, beef, pork liver, lard, chicken meat, and chicken eggs (Table 6). Macrolides practically in all mass spectra of these samples were presented by the ions $[M + Na]^+$ and $[M + K]^+$. The limits of detection for antibiotics in the indicated products at the ratio $S/N = 4$ were 0.01–0.3 μg/kg for MALDI MS and 0.001–0.03 μg/kg for SALDI MS.

Antibiotics of the studied groups were found in samples of cow and goat milk using SALDI MS (Table 6, Fig. 8). As was found from the obtained mass spectra of extracts from milk taking into account peak areas and peak intensities for antibiotics ions, the amounts of avilamycin, narasin, erythromycin, lasalocid, ceftiofur, streptomycin, and rifampicin exceeded the permissible levels.

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