

CHROMATOGRAPHY

Think about **food**
Care about **safety**

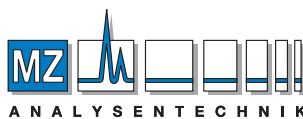


Vol. 3
Antibiotics



MACHEREY-NAGEL

www.mn-net.com



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Since 1911

Focus on antibiotics

The global production and trading of food and feed products makes monitoring and control difficult.

Only analysis of food and raw materials for the production of food can ensure safe and edible food. Food safety is an essential part of our quality of life.

MN shows examples for food analysis that will help you with your work and make our food safer!

Vol. 3: Antibiotics in food of animal origin

Healthy animals provide healthy food.

Antibiotics or antimicrobials are primarily used to treat infections caused by bacteria and other microorganisms but also they show growth promoting effects.

In order to have the possibility to prevent diseases a judicious use of antimicrobials is necessary since bacteria can be changed and then antibiotics are no longer effective in people who need them to treat infections. Antibiotic resistance is now a major threat to public health. [WHO – World Health Organisation – <http://www.who.int/mediacentre/news/releases/2014/amr-report/en/>]

There are different classes of antimicrobials, e.g.,

- Penicillins
- Aminoglycosides
- Arsenicals
- Polypeptides
- Glycolipids
- Tetracyclines
- Macrolides
- Lincosamides
- β -Lactams
- Quinolones / fluoroquinolone
- Streptogramins
- Sulfonamides
- and others

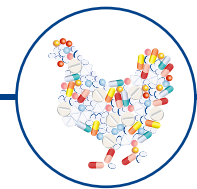
[Wikipedia – http://en.wikipedia.org/wiki/List_of_antibiotics].

Most of these examples are antibiotics that were first isolated from a natural source. Nowadays they are mostly produced synthetically and new antibiotics are usually semisynthetic modifications.

Most of the antibiotics used in food animals are the same as those used in humans.

Due to the importance of antibiotic resistance as a public health problem, many governments and professional societies have reviewed existing scientific evidence and developed recommendations to limit all types of antibiotic use, including use in food animals [National Antimicrobial Resistance Monitoring System (NARMS) – FDA: <http://www.fda.gov/AnimalVeterinary/SafetyHealth/AntimicrobialResistance/NationalAntimicrobialResistanceMonitoringSystem/default.htm> – CDC: <http://www.cdc.gov/narms> – USDA: <http://www.ars.usda.gov/saa/bear/narms>].



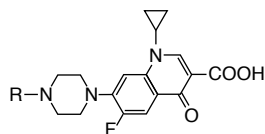


Chemical structures of important antibiotics

Quinolones

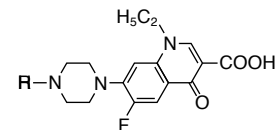
Ciprofloxacin (R = H)

Enrofloxacin (R = C₂H₅)



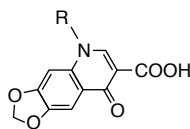
Norfloxacin (R = H)

Pefloxacin (R = CH₃)

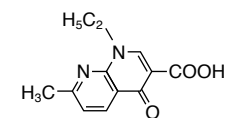


Miloxacin (R = OCH₃)

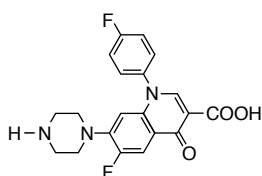
Oxolinic acid (R = C₂H₅)



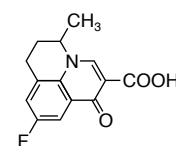
Nalidixic acid



Sarafloxacin

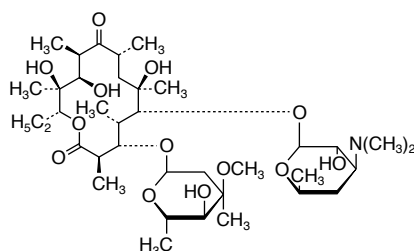


Flumequine

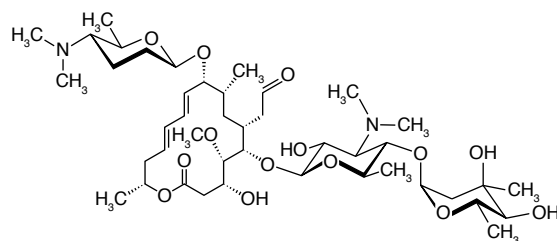


Macrolides

Erythromycin

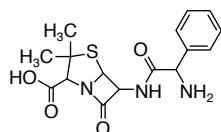


Spiramycin

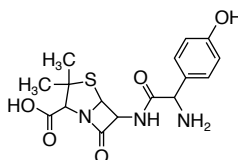


β-Lactams

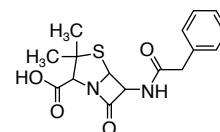
Ampicillin



Amoxicillin

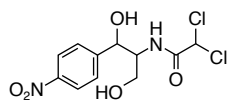


Penicillin G

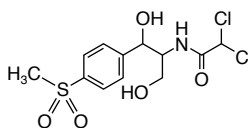


Miscellaneous

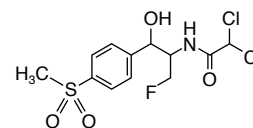
Chloramphenicol



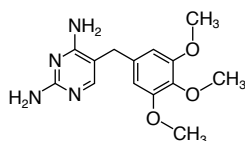
Thiamphenicol



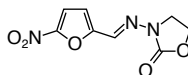
Florfenicol



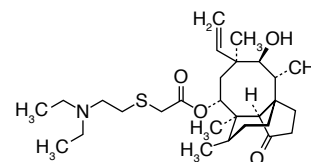
Trimethoprim



Furazolidone



Tiamulin



Chemical structures of sulfonamides and tetracyclines see page 5.

Typical samples, preparation

SPE of veterinary drugs from fish

MN Appl. No. 305080

J.M. Stevens et al., GIT Spezial Separation 1/2009, 16–17

Sample pretreatment:

see www.mn-net.com/apps

Column type:

CHROMABOND® SA, 3 mL, 500 mg
REF 730077

Column conditioning:

2 x 3 mL *n*-hexane, drying by vacuum, then 3 mL 0.5 % acetic acid in ethyl acetate; do not let run the column dry!

Sample aspiration:

5–8 mL/min, then 10–12 min drying

Column washing:

10 mL methanol

Elution:

5 mL 10 % triethanolamine in methanol

Compounds investigated:

sulfamerazine, sulfadimidine, sulfadimethoxine, trimethoprim, sulfadiazine, chlortetracycline, oxytetracycline, ampicillin, amoxicillin, benzylpenicillin, ciprofloxacin, enrofloxacin, norfloxacin, oxolinic acid, pefloxacin, flumequine, sarafloxacin, furazolidone, erythromycin, spiramycin, gentamicin, chloramphenicol, florfenicol, thiamphenicol, tiamulin, nalidixic acid, miloxacin

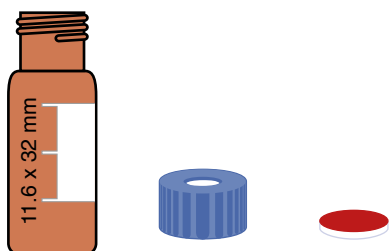
Recovery rates: 85 to 100 % for spiked samples with concentrations from 0.001 to 0.100 mg/mL

Sample filtration and storage

It is recommended to filter the prepared sample through disposable filters **CHROMAFIL® Xtra PVDF-45/25**
REF 729219



Transfer into a sample vial, e.g., 1.5 mL screw neck vials N 9, amber, flat bottom, label and scale, wide opening, REF 702284 and ready assembled screw closures N 9, blue, center hole with septum silicone / PTFE, REF 702287.1



SPE of tetracyclines from musculature

MN Appl. No. 302030

Courtesy of Mr. Lippold, Chemisches Landesuntersuchungsamt (Chem. Research Agency) Freiburg, Germany

Sample pretreatment:

see www.mn-net.com/apps

Column type:

CHROMABOND® Tetracycline, 6 mL, 500 mg
REF 730315

Column conditioning:

1 column volume methanol, 1 column volume dist. water, then 1 column volume EDTA-succinate buffer (see description at www.mn-net.com/apps); do not let run the column dry!

Sample application:

Slowly force or aspirate the eluate of the sample pretreatment through the column.

Column washing:

2 mL dist. water (removal of Cu ions), 1 mL *n*-hexane

Elution:

Elute with 7.5 mL methanol in a 25 mL tapered flask. Add 1 mL of an ethylene glycol – methanol mixture (22 g ethylene glycol are filled up with methanol to 100 mL) and evaporate with a rotation evaporator (max. 40 °C) to dryness. Fill up the residue with 0.1 mol/L Mcllvain-EDTA buffer (dissolve 52.5 g citric acid x H₂O, 44.5 g Na₂HPO₄ x H₂O and 93 g Titriplex III in 2.5 L dist. water, adjust with NaOH to pH 4) to 400 mL.

Compounds investigated:

tetracycline, oxytetracycline, chlortetracycline, 100–500 mg/kg

Recovery rates:

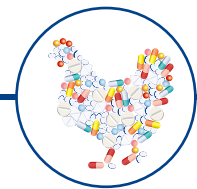
tetracycline, chlortetracycline: ca. 50–70 %

oxytetracycline: ca. 60–80 %

CHROMABOND® vacuum manifold for 12 columns
REF 730150



For other applications see www.mn-net.com/apps

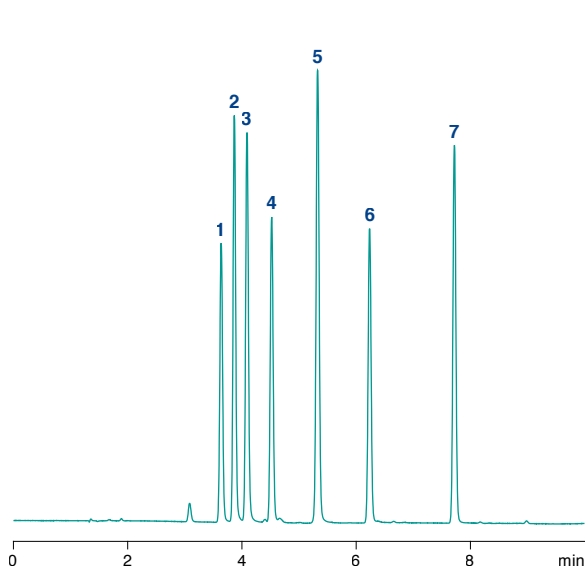


Subsequent analysis: HPLC

Sulfonamides

MN Appl. No. 125860

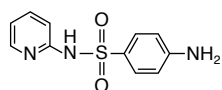
Column: EC 150/3 NUCLEODUR® Phenyl-Hexyl, 3 µm
REF 760578.30



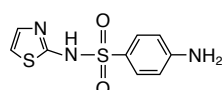
Peaks

1. Sulfadiazine
2. Sulfachloropyridazine
3. Sulfapyridine
4. Sulfamerazine
5. Sulfadimidine
6. Sulfathiazole
7. Sulfadimethoxine

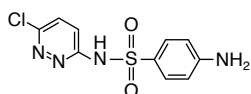
Sulfapyridine



Sulfathiazole



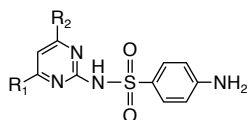
Sulfachloropyridazine



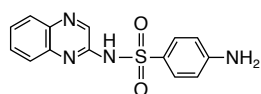
Sulfadiazine $R_1=R_2=H$

Sulfamerazine $R_1 = H, R_2 = CH_3$

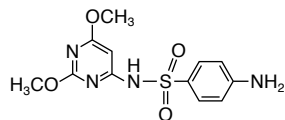
Sulfadimidine $R_1 = R_2 = CH_3$



Sulfaquinoxaline



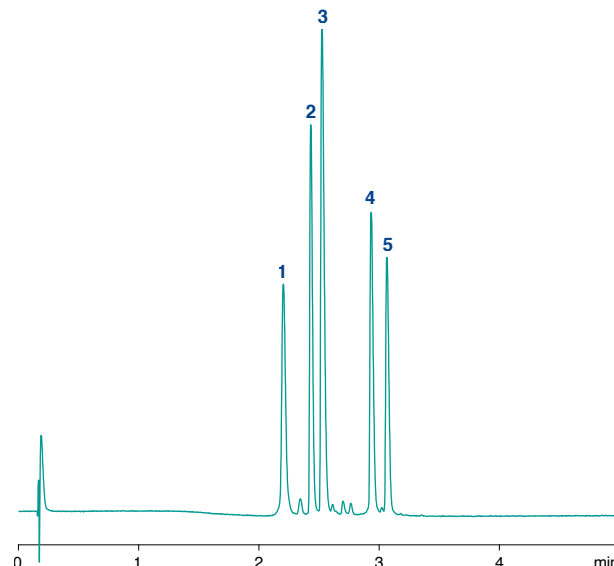
Sulfadimethoxine



Tetracyclines

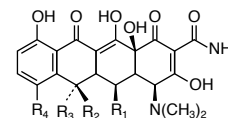
MN Appl. No. 127050

Column: EC 50/2 NUCLEOSHELL® RP 18plus, 2.7 µm
REF 763232.20



Peaks

1. Minocycline
2. Oxytetracycline
3. Tetracycline
4. Chlortetracycline
5. Doxycycline



	R ₁	R ₂	R ₃	R ₄
1. Minocycline	H	H	H	NH ₂
2. Oxytetracycline	OH	OH	CH ₃	H
3. Tetracycline	H	OH	CH ₃	H
4. Chlortetracycline	H	OH	CH ₃	Cl
5. Doxycycline	OH	H	CH ₃	H



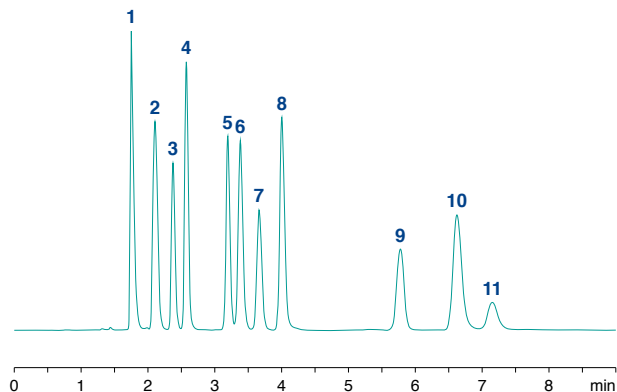
Detailed conditions are available online at www.mn-net.com/apps

Analysis

Antibacterial agents

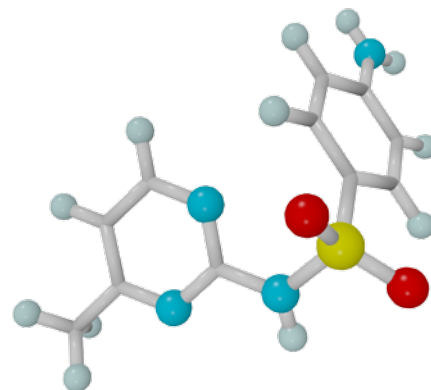
MN Appl. No. 124190

Column: EC 150/4.6 NUCLEODUR® C₁₈ HTec, 3 µm
REF 760325.46



Peaks:

1. Sulfadiazine
2. Sulfachloropyridazine
3. Sulfathiazole
4. Sulfamerazine
5. Sulfapyridine
6. Sulfaquinoxaline
7. Sulfadimethoxine
8. Sulfadimidine
9. Furazolidone
10. Nalidixic acid
11. Oxolinic acid

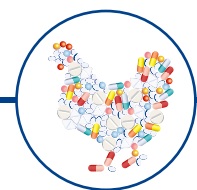


Detailed conditions are available online at www.mn-net.com/apps

MN suggests using guard columns to protect your main column and significantly increase column life.

The **Column Protection System** (REF 718966) is suitable for all analytical HPLC columns with 1/16" fittings!





Ordering information

Step	Product	Pack of	REF
SPE	CHROMABOND® SA, 3 mL, 500 mg	50	730077
	Especially recommended for tetracyclines: CHROMABOND® Tetracycline, 6 mL, 500 mg	30	730315
Filtration	Disposable filters CHROMAFIL® Xtra PVDF-45/25	100	729219
Vials and caps	1.5 mL screw neck vials N 9, amber, label and scale	100	702284
	Ready assembled screw closures N 9, blue, center hole with septum Silicone white / PTFE red	100	702287.1
HPLC 1			
Column	EC 150/3 NUCLEODUR® Phenyl-Hexyl, 3 µm	1	760578.30
Guard column	EC 4/3 NUCLEODUR® Phenyl-Hexyl, 3 µm	3	761986.30
Guard column holder	Column Protection System	1	718966
HPLC 2			
Column	EC 50/3 NUCLEOSHELL® RP 18plus, 2.7 µm	1	763232.30
Guard column	EC 4/3 NUCLEOSHELL® RP 18plus, 2.7 µm	3	763238.30
Guard column holder	Column Protection System	1	718966
HPLC 3			
Column	EC 150/4.6 NUCLEODUR® C ₁₈ HTec, 3 µm	1	760325.46
Guard column	EC 4/3 NUCLEODUR® C ₁₈ HTec, 3 µm	3	761926.30
Guard column holder	Column Protection System	1	718966

Visit us at www.mn-net.com/chroma to get more helpful information

Selection tools for

Vials and caps

Syringe filters

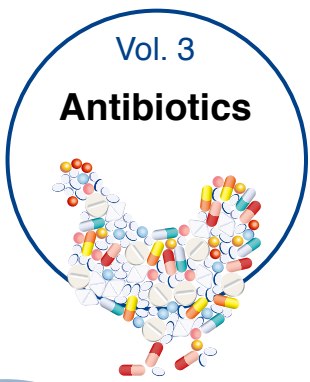
Troubleshooting guides

GC and HPLC

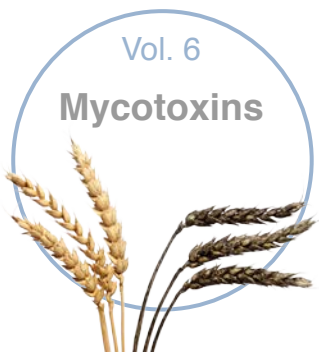
Detailed product information

...and much more

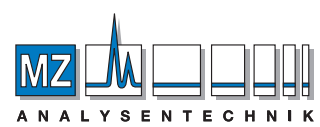




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