Determination of quaternary ammonium salts and veterinary drugs in food

Helmut Riering[1], Norbert Helle[2], Franziska Chmelka[2], Mariia Matkovskaia[2], Judith Bauer[2]
[1]MACHEREY-NAGEL GmbH & Co. KG, Neumann-Neander-Str., 52355 Düren, Germany · [2]Tela GmbH, Handelspark 4-6, 27624 Geestland, Germany

There is widespread interest in the reliable and sensitive determination of residues in food and beverages. Although the analytical instrumentation is getting more sensitive the need of a good chromatographic separation is nevertheless necessary and important to produce relieable results.

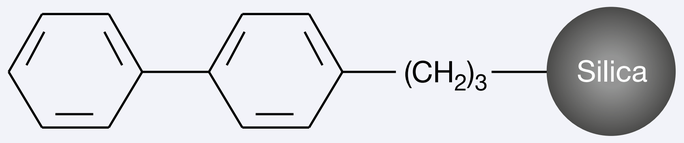
In this work a new chromatographic sorbent NUCLEODUR® π² is tested for the determination of veterinary drug residues and quaternary ammonium compounds.

NUCLEODUR® π² is a silica based chromatographic support with biphenylpropyl groups. Stationary phases with alternative separation mechanisms are still of interest for HPLC. Aromatic ligands on the sorbent are particularly suitable for this [1], because they are chemically inert and provide as hydrocarbons sufficient hydrophobicity. However, due to the π,π-interactions their selectivity is different compared with alkyl modified silicas [2,3].

Due to the large aromatic system in combination with the flexible propyl spacer π,π- and hydrophobic interactions of NUCLEODUR® π² are both enhanced [4].

NUCLEODUR® π²

Pore size:	110 Å	Surface area:	340 m²/g
Pore volume:	0,9 mL/g	pH stability:	1,5–10
Ligand:	Biphenylpropyl (USP L11)	Carbon content:	17 %



Suitable for aqueous eluents

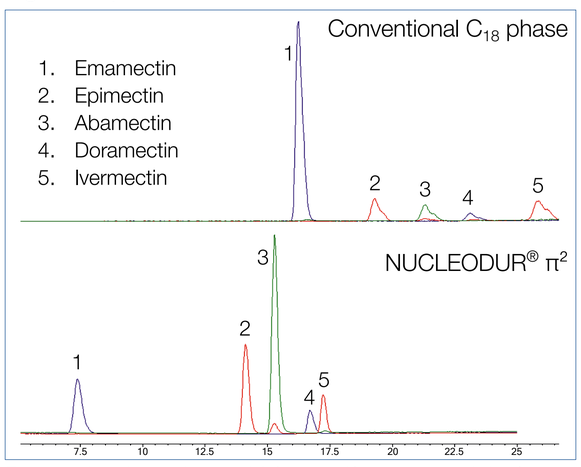
Results

Multi veterinary drug residues

Several different veterinary drugs are used for prevention and treatment of disease in animal production. This use often leads to drug residues in food. Usually for each group of veterinary drugs an individual method for analysis is applied.

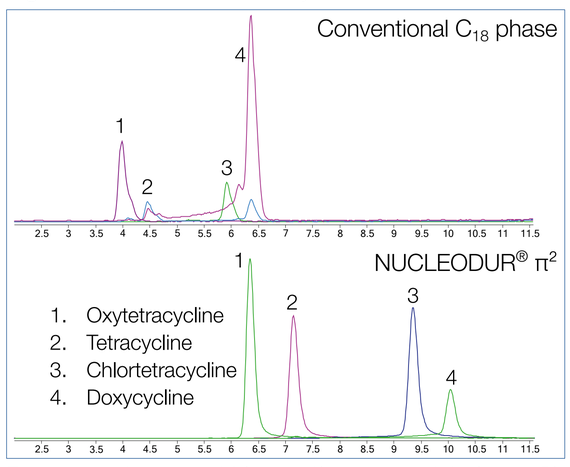
First NUCLEODUR® π² is tested for the analysis of mectins. Figure 1 shows the separation of mectins on a conventional C_{18} phase and on NUCLEODUR® π². Except for Emamectin the peaks of NUCLEODUR® π² are quite higher and show a better peak shape.

Fig. 1: Separation of mectins



Eluents: formic acid 100mM (eluent A) and acetonitrile (eluent B), gradient: 0 min - 20 % B, 12 min - 50 % B, 15 min - 95 % B, 18 min - 95 % B, 18.1 min - 20 % B, 0.25 mL/min

Fig. 2: Separation of tetracyclines



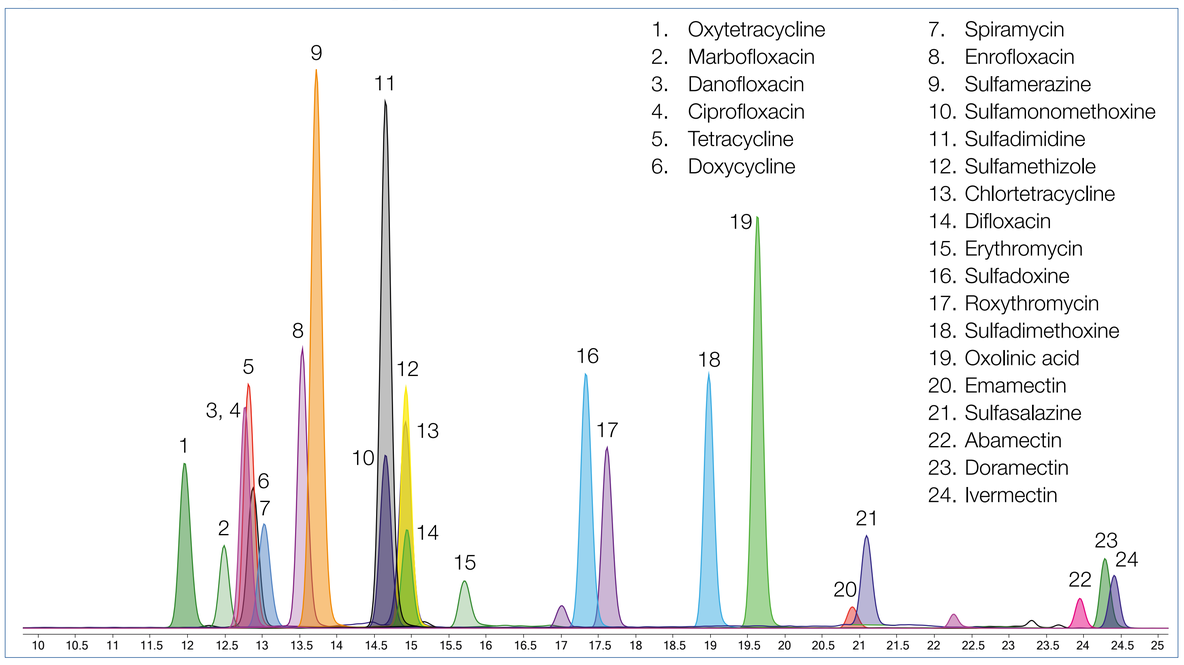
Eluents: formic acid 5mM (eluent A) and acetonitrile (eluent B), gradient: 0 min - 50 % B, 10 min - 85 % B, 25 min - 90 % B, 30 min - 100 % B, 31 min - 50 % B, 0.25 mL/min

Satisfied by these results we decided to test the NUCLEODUR® π² for the separation of tetracyclines. Using conventional C_{18} columns tetracyclines usually shows broad signals and peak fronting and tailing effects under these conditions (see figure 2). The NUCLEODUR® π² column enables a peak-tailing-free elution of the tetracyclines (see figure 2).

Because of the good separation of these two groups of veterinary drugs we make up our mind to develop a multi veterinary drug method that allows to measure mectins, tetracyclines, chinolones, sulfonamides and mycins in one run. To reach a good separation for all groups of veterinary drugs we had to optimize the chromatographic conditions.

Figure 3 shows a chromatogram of a standard mixture containing different drugs. The NUCLEODUR® π² allows a measurement of a wide range of veterinary drug residues in one run. This will save a lot of analysis time and costs in the analysis of drug residues.

Fig. 3: Separation of veterinary drugs



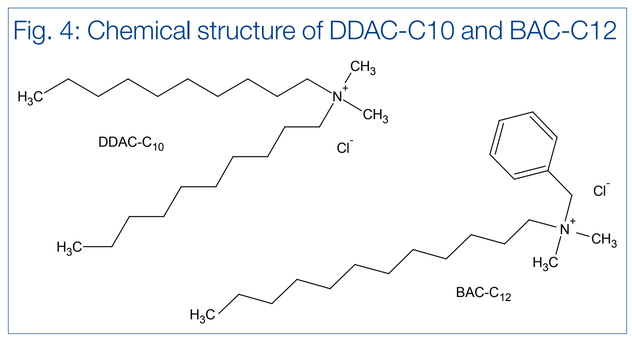
Column: MACHEREY-NAGEL NUCLEODUR® π², 250 x 2 mm, 5 µm, eluent: formic acid 100 mM (eluent A) und acetonitrile (eluent B), 0 min - 10 % B, 4 min - 10 % B, 13 min - 35 % B, 19 min - 60 % B, 20 min - 98 % B, 27 min - 98 % B, 27.1 min - 10 % B, flow: 0.25 mL/min, column temperature: 50 °C, injection volume: 5 µL, detection: Agilent 6495 Triple Quadrupole ESI positive Jet Stream (MRM)

Quaternary ammonium salts

Quaternary ammonium salts are classified as biocides because of their disinfecting effects. They are found in disinfectants, fabric softeners and surfactants among other things. The most common substances are didecyldimethylammonium chloride (DDAC) and benzalkonium chloride (BAC). Both DDAC as well as BAC are mixtures of quarternary ammonium salts with different alkyl chain lengths. Figure 4 shows the chemical structure of DDAC-C_{10} and BAC-C_{12}.

Quaternary ammonium compounds are used in the food production for clean-up and disinfection. This way residues of quaternary ammonium salts can enter food during the production processes. Until 2012 a MRL (maximum residue level) of 0.01 mg/kg for DDAC and BAC was applied based on Regulation (EC) No 396 / 2005 for maximum residue levels of pesticides in food.

Fig. 4: Chemical structure of DDAC-C10 and BAC-C12

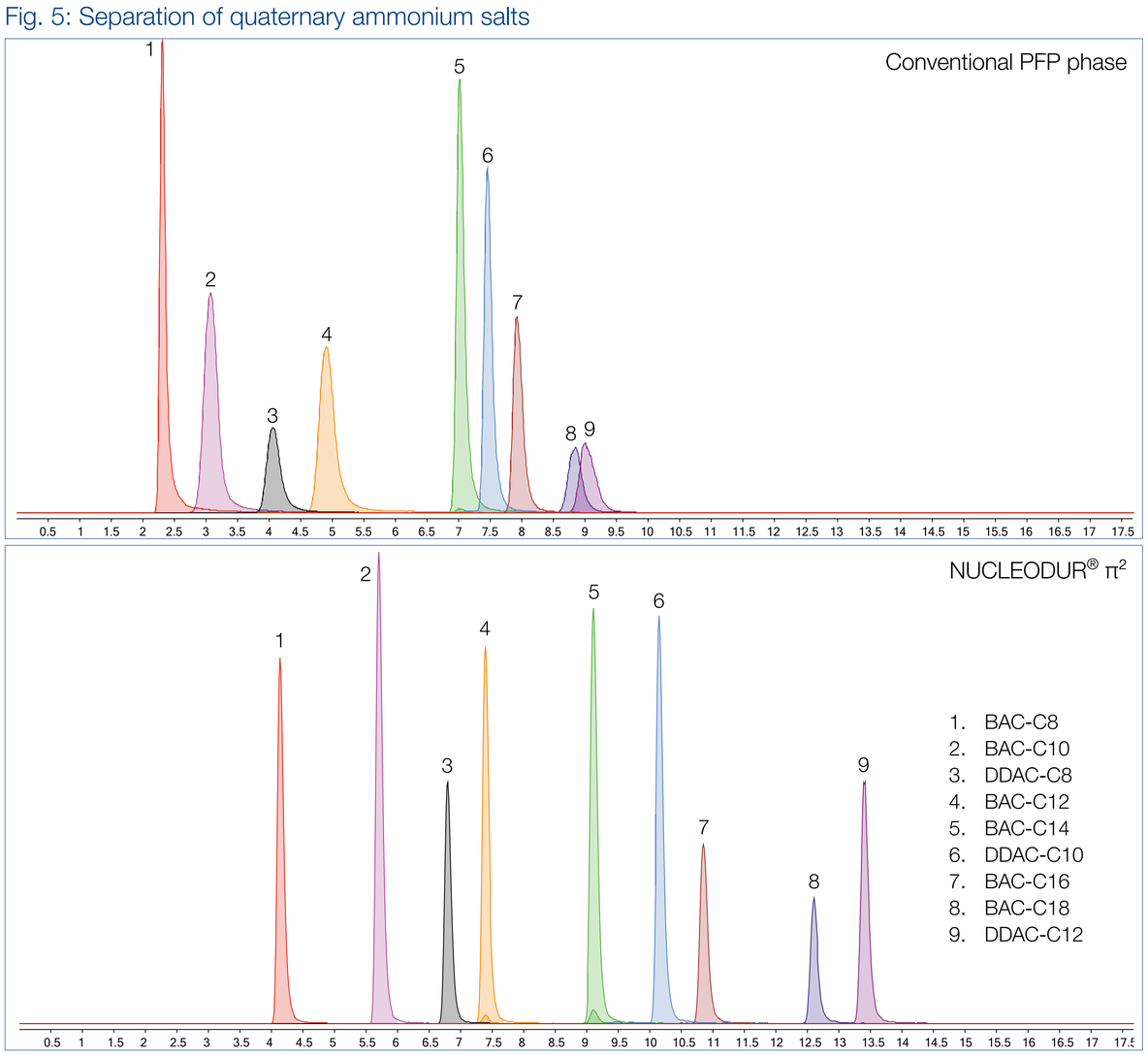


Because of a lot of positive results above the MRL of 0.01 mg/kg the EU agreed on an enforcement level of 0.5 mg/kg. Therefore a sensitive determination of DDAC and BAC is necessary.

We chromatographically separated and detected nine different quaternary ammonium compounds using an Agilent 1260 HPLC system coupled with an Agilent 6460 Triple Quadrupole.

Figure 5 shows the chromatograms of a standard mixture containing BAC-C8, -C10, -C12, -C14, -C16, -C18, DDAC-C8, -C10 and -C12 separated on a conventional PFP and a NUCLEODUR® π² column. The biphenyl sorbent elutes the compounds with better selectivity and peak shape. According to the higher carbon content of NUCLEODUR® π², the analytes elute with longer retention times.

Fig. 5: Separation of quaternary ammonium salts



Column: MACHEREY-NAGEL NUCLEODUR® π², 250 x 2 mm, 5 µm, eluent: formic acid 50 mM (eluent A) und methanol (eluent B), 0 min - 45 % B, 14 min - 90 % B, 18 min - 100 % B, 22 min - 100 % B, 22.1 min - 45 % B, flow: 0.3 mL/min, column temperature: 50 °C, injection volume: 5 µL, detection: Agilent 6460 Triple Quadrupole ESI positive Jet Stream (MRM)

Conclusion

NUCLEODUR® π² is a good choice for the analysis of different compound classes in foodstuff. A multi-veterinary drug method which includes mectins, tetracyclines, chinolones, sulfonamides and mycins and a separation of quaternary ammonium salts could be successfully developed. The peak shape and selectivity of NUCLEODUR® π² are equal or better in comparison to conventional RP columns.

References

[1] F. Chan, L.S. Yeung, R. LoBrutto, Y.V. Kazakevich J. Chromatogr. A 1069 (2005) 217 – 224

[2] M.R. Euerby, P. Petersson, W. Campbell, W. Roe J. Chromatogr. A 1154 (2007) 138 – 151

[3] K. Croes, A. Steffens, D.H. Marchand, L.R. Snyder J. Chromatogr. A 1098 (2005) 123 –130

[4] Helmut Riering, Natalie Bilmann, Giovanna Cozzupoli, 2016 Comparison of various aryl and alkyl modified Sorbents in RP chromatography, Poster ISC 2016, Cork (Online available: *www.mn-net.com/NUCLEODUR* (Poster: Comparison of RP Sorbents)).