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Determination of Veterinary Drug Residues in Foods of Animal Origin using QuEChERS methodology by LC-MS/MS

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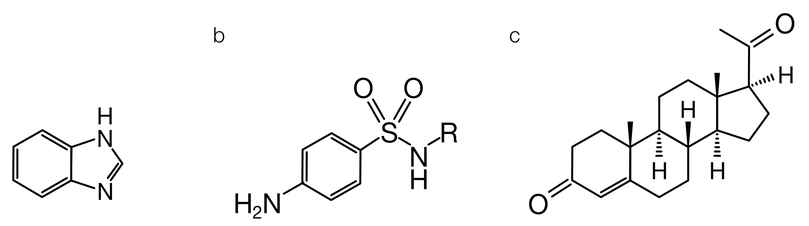
Introduction

The control of veterinary drug residues in food is of paramount importance for ensuring the quality and safety of food products in the European marketplace. Therefore, the European Parliament and of the Council the European Union have placed with the Directive 2019/06 a regulatory framework. This regulation lays down rules for the placing on the market, manufacturing, import, export, supply, distribution, pharmacovigilance, control and use of veterinary medicinal products [1]. The availability of safe and effective veterinary medicines is essential - to protect animals themselves, but also to protect humans from the transmission of diseases by animals, the so-called zoonoses [2, 3]. For industry and national regulatory laboratories, the challenges of controlling veterinary drug residues in food include the high number of drugs (antibiotics, antiparasitics, anti-inflammatory agents, etc.) and the diversity of foods of animal origin. It is critical to use an efficient sample pretreatment method for analyte extraction, concentration and matrix clean-up.

QuEChERS became the method of choice in sample preparation for the analysis of residues and other processing contaminants in fruit, vegetables and other food products. In this essay, a sensitive QuEChERS method with an efficient cleanup for animal origin sample matrices like milk, eggs and beef was developed. The sample raw extract was purified with a cleanup-mix with customized composition. Sodium sulfate was used instead of traditionally used magnesium sulfate to allow establishing multi-residue methods because of certain veterinary drug groups tend to chelate with magnesium ions. High recovery rates of veterinary drugs like benzimidazoles, glucocorticoids and sulfonamides and the matrix-reduction for different sample materials are presented and discussed. The identification and the quantification of the focused analyts was finally carried out by ESI mass spectrometry on NUCLEOSHELL® RP18 column.

Figure 1:



Examples for the respective group of veterinary drugs: a Benzimidazole, b Sulfonamide, c Progesterone.

Sample pretreatment

Extraction, clean-up, and concentration methodology

Test portion preparation:

1. Weight two 1.00 g ± 0.05 g test portions of the laboratory sample into two separate 50 mL polypropylene tubes.
2. The test portion fortfilled with 30 µL of the standard solution (β = 1 µg/mL in methanol). Homogenize the test portion immediately by hand-shake or by vortexing. Make sure that the spiked volume is totally absorbed by the matrix.

Extraction:

1. Add 8 mL of water to the fortified test portion.
2. Hand-shake for 3 min. Check that the whole sample is completely dissolved/solubilized or dispersed in solution. No lumps should be visible.
3. Add 30 mL of acetonitrile and 30 µL of concentrated formic acid.
4. Shake for 3 min.
5. Add 0.1 mL of internal standard solution (0.1 µg/mL each compound in methanol) and 0.1 mL of native standard solution (0.1 µg/mL each compound in methanol) for determining recovery rate.
6. Add CHROMABOND® QuEChERS Mix LV (4000 mg 900 mg Na2SO4, 1000 mg NaCl, 500 mg Na2H citrate x 1.5 H2O, 1000 mg Na3 citrate x 2 H2O, REF 7300022).
7. Shake the mixture for 1 min.
8. Centrifuge the mixture for 10 min at 4500 rpm at 5 °C.

Clean-up:

1. Transfer 6 mL supernatant to a 15 mL centrifuge tube, which is prefilled with CHROMABOND® QuEChERS Mix LII (900 mg Na_2SO_4, 50 mg CHROMABOND® Diamino, 150 mg CHROMABOND® C18 ec, REF 7300019).
2. Shake for 3 min.
3. Centrifuge again for 10 min at 4500 rpm at 5 °C.
4. Transfer 3 mL of the supernatant into a sample tube.

Eluent exchange and concentration:

1. Evaporate the supernatant to dryness under with a gentle stream of nitrogen gas at 40 ± 5 °C. Reconstitute the residue with 250 µL of a methanol–water mixture (15/85, v/v).
2. Vortex and sonicate for about 1 min to re-suspend the residue.
3. Transfer the extract into a 1.5 mL polypropylene tube and centrifuge at 13500rpm at room temperature for 10 min.
4. Transfer the clean supernatant into a HPLC vial for further LC–MS/MS analysis.

Analysis by HPLC-MS / MS

Chromatographic conditions

Column	EC 100/2 NUCLEOSHELL® RP18, 2.7 µm (REF 763134.20)
Eluent A	0.1 % formic acid in water
Eluent B	0.1 % formic acid in acetonitrile
Gradient	In 10 min from 5 % B to 100 % B, hold 100 % B for 2 min, in 0.1 min from 100 % B to 5 % B, hold % % B for 3.9 min
Flow rate	0.4 mL/min
Temperature	40 °C
Injection volume	2 µL

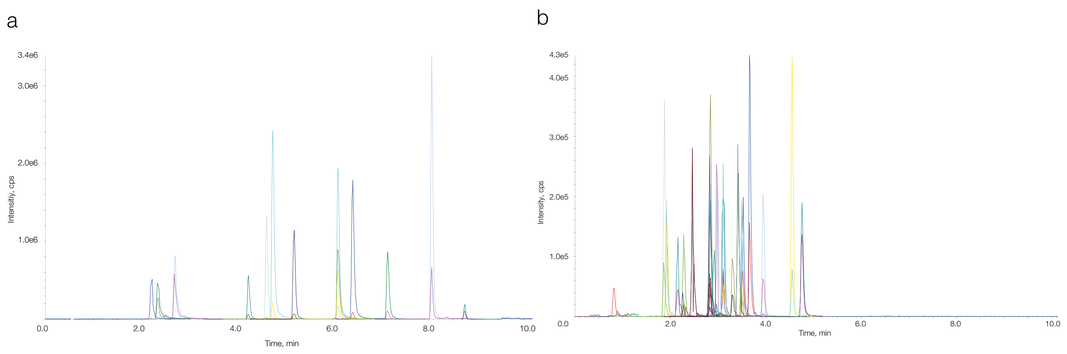
MS conditions for AB Sciex API 5500

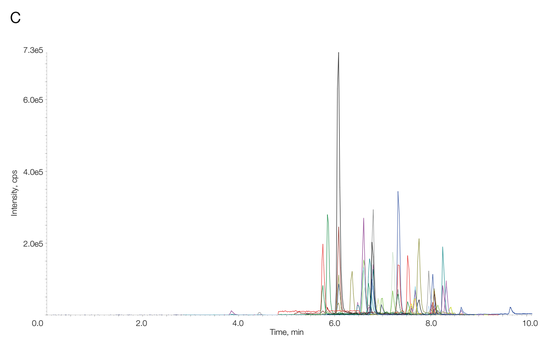
Aquisition mode	MRM
Interface	ESI
Polarity	positive
Curain Gas	30 psig
Ionspry voltage	5000 V
Temperature	550 °C
ion Source Gas 1	40 psig
Ion Source Gas 2	40 psig
Detection Windows	90 sec

MRM transitions see MN Appl. No.129590

Chromatogramms

Figure 2 a–c:

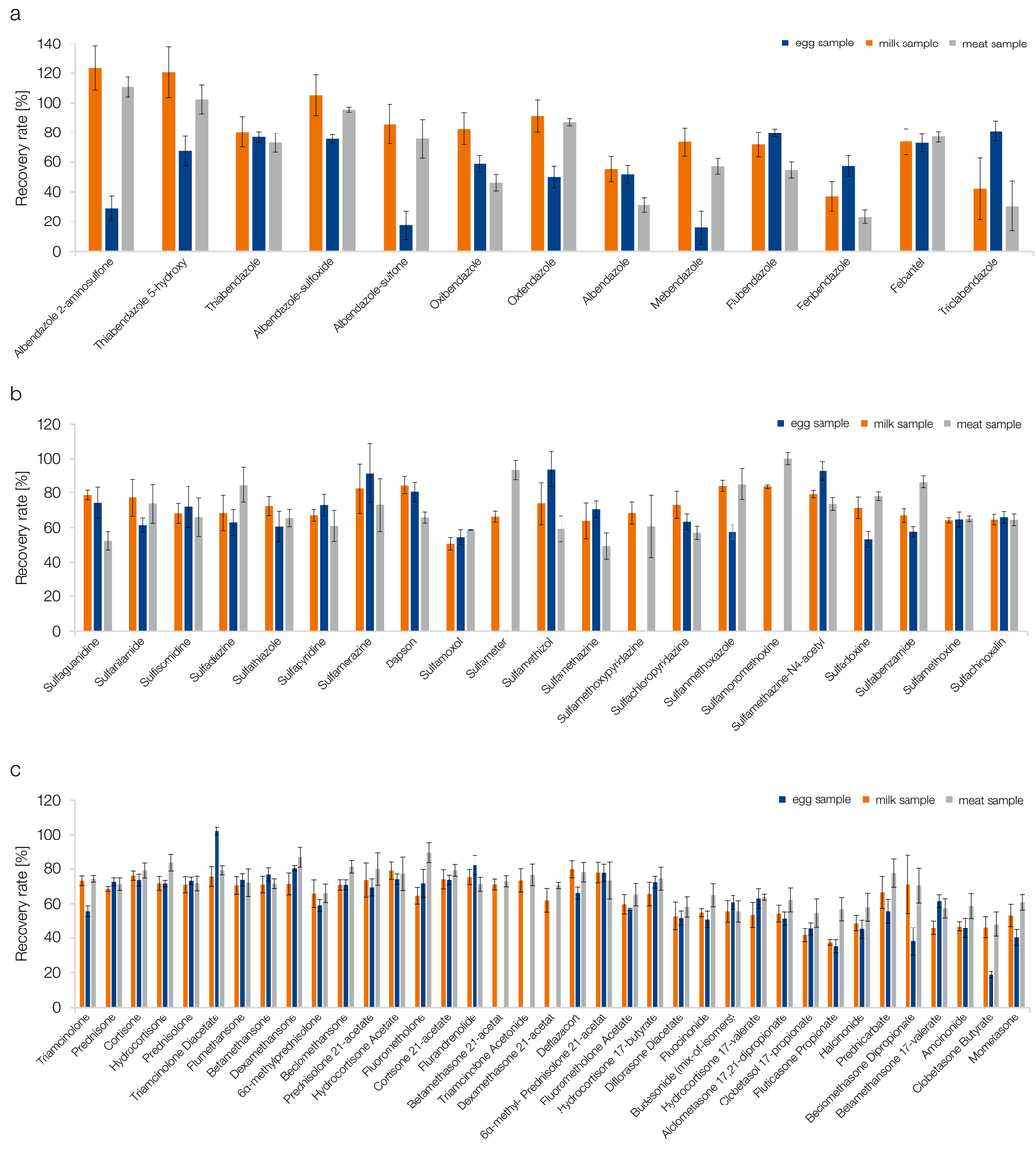




Chromatogram of sample eluate (β = 12 ng/mL for each component in methanol-water mixture (15/85, v/v))
a: Benzimidazoles from meat sample, b: Sulfonamides from meat sample, c: Glucocorticoids from milk sample.

Recovery rates

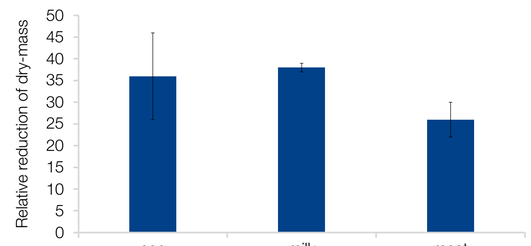
Figure 3 a–c:



Recovery rates of benzimidazoles, sulfonamides and glucocorticoids from different matrices.
a: Benzimidazoles, b: Sulfonamides, c: Glucocorticoids.

Matrix reduction

Figure 4:



Relative matrix reduction of dry mass.

Conclusion

The reliable and successful determination of more than 70 veterinary drug compounds from several matrices of animal origin could be presented. By using CHROMABOND® QuEChERS Mix LV and CHROMABOND® QuEChERS Mix LII, it was possible to achieve high recovery rates for the veterinary drug classes of benzimidazoles, sulfonamides and glucocorticoids from three food matrices (egg, milk meat) with good reproducibility. The used QuEChERS clean-up mix shows effective matrix reduction. Figure 4 shows that the amounts of matrix components in QuEChERS extracts are effectively reduced round up to 30 %. Replacing $MgSO_4$ to Na_2SO_4 as water-binding salt component allows establishing multi-residue methods because of certain veterinary drug groups tend to chelate with magnesium ions. The identification and the quantification of benzimidazoles, sulfonamides and glucocorticoids in food were finally carried out by ESI mass spectrometry on a NUCLEOSHELL® RP18 column. Using the same chromatographic conditions allows combining veterinary drug compounds in one HPLC method.

References

[1] REGULATION (EU) 2019/6 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 11 December 2018 on veterinary medicinal products and repealing Directive 2001/82/EC.

[2] Lekshmi, M., Ammini, P., Kumar, S., & Varela, M. F. (2017) Microorganisms 5, 11 – 36. doi:10.3390/microorganisms5010011.

[3] WHO (2011) Critically Important Antimicrobials for Human Medicine, 3rd ed.