Determination of veterinary drugs in European reference material

SpeedExtractor E-916:
Determination of Nitroimidazoles in Pork Muscle using the SpeedExtractor E-916

The determination of veterinary drugs in food is important concerning to legal regulation. A fast determination of different nitroimidazoles in lyophilized pork muscle (ERM) is introduced below. The sample is extracted using the SpeedExtractor E-916.

1. Introduction

The residual amount of veterinary drugs in food is regulated by legislation, for example in the COMMISSION REGULATION (EU) No 37/2010. Nitroimidazoles derived from antibiotics, like metronidazole or ronidazole and their metabolites are prohibited [1]. Hence to protect consumers, fast and reliable extraction techniques are important for the detection of such prohibited substances.

2. Experimental

Equipment: SpeedExtractor E-916

Sample: Pork muscle ERM-BB124, lyophilized

Determination: The frozen sample is warmed to ambient temperature before opening. An aliquot of 1.25 g is accurately weighed out and 3.75 g of distilled water added and mixed. The sample is mixed with 5.0 g diatomaceous earth until homogeneous.

The extraction was performed using the E-916, with the parameters specified in Table 1.

Table 1: Parameters for the extraction with E-916

Pressure	100 bar
Temperature	70 °C
Cell	40 mL
Solvent	Acetonitrile:Acetic acid (98:2)
Vial	240 mL
No. of cycles	2
Heat-up	1/1 min
Hold	5/5 min
Discharge	2/2 min
Flush with solvent	3 min
Flush with gas	2 min
Total extraction time	35 min

After extraction 10 mL of the extract was concentrated under nitrogen at 50 °C. The Syncore® could also have been used for this purpose.The residue was redissolved in 300 µL in 2 % formic acid/methanol (90:10) and filtered with a fine mesh filter (Spartan, 13/0.45 RC). Another 10 mL of the extract was spiked with a standard mix of nitroimidazoles and reconstituted as above.

The samples were analysed using UPLC-MS/MS (Acquity UPLC, Waters) with electrospray ionization (ESI). The conditions were as following:

- Column: HSS T3 1.8 µm, 2.1x100 mm, Waters
- Injection volume: 2 µL
- Flow rate: 0.24 mL/min
- Eluent A: 100 µg malonic acid + 100 µL formic acid / 1 L water
- Eluent B: methanol

The gradient is specified as follows:

0 min	95 % A	5 % B
1 min	95 % A	5 % B
6 min	5 % A	95 % B
10 min	5 % A	95 % B
10.1 min	95 % A	5 % B

3. Results

The recoveries of the different nitroimidazoles were between 70 % and 85 %. As an example Figure 1 shows the determined amount of ronidazole (RNZ) compared to the certified value.

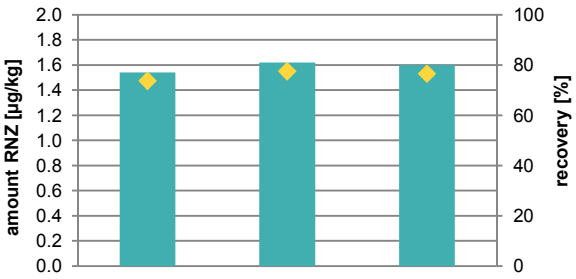

Figure 1: Determined amount of ronidazole compared to the certified value of ronidazole (shown as recovery)

The extraction parameters were not fully optimized, however, two cycles gave reasonable recoveries. Similar recoveries (up to 80 %) were achieved for the determination of nitroimidazole in pork muscle by solid phase extraction [2].

Recoveries for nitroimidalzole spikes done directly before the UPLC-MS/MS determination were between 70 and 80 % showing the difficulty faced in this type of analysis.

4. Conclusion

The determination of nitroimidazoles in pork muscle using the SpeedExtractor E-916 provides reliable and reproducible results. Using pressurized solvent extraction as the chosen extraction technique provides the results in quick timeframe.

5. Acknowledgement

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6. References

[1] COMMISSION REGULATION (EU) No 37/2010
[2] Shen et al.: Journal of AOAC International Vol. 86, No. 3, 2003

- Operation Manual of SpeedExtractor E-914/E-916