Analysis of tranquilizers in pork tissue using LC-MS/MS

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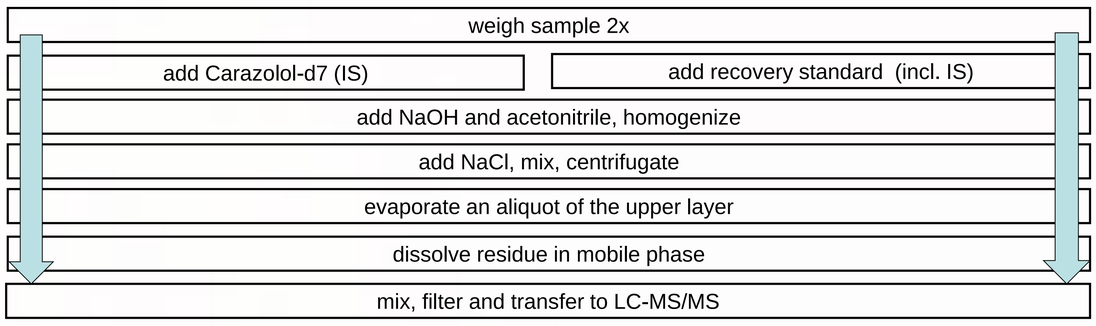
Introduction

Tranquilizers are often used in animal production, especially in pigs, because during transport from the farm to the slaughterhouse and prior to slaughter they are under considerable stress. Poor-quality meat, called PSE (Pale Soft Exudative) and high mortality rates can be the consequence, resulting in financial losses for the farmers. Furthermore aggressive vigorous animals, such as pigs and cattle can become a danger for people who deal with them at the transport. Therefore veterinary drugs with sedative and muscle relaxant effects were administered to these animals. Even if the degradation of the substances takes place within hours, there may be residues in meat due to an administration shortly before slaughter.

In the EU the use of veterinary drugs is regulated by Commission Regulation (EU) 37/2010 establishing Maximum Residue Limits (MRL) and listing prohibited substances. While residues of Azaperone and his metabolite Azaperol, which was mainly used in pig farming, are regulated in pig muscle with 100 µg/kg as sum, the MRL of Carazolol in pig and cattle muscle is set to 5 µg/kg and no MRL is required for Ketamine and Xylazine. Due to the potential risk to consumers health Chlorpromazine is listed in the Commission Regulation (EU) 37/2010 in table 2 of prohibited substances and the Community Reference Laboratories (CRLs) recommend a ccbeta of 10 µg/kg for the analysis of Chlorpromazine and 50 µg/kg of Acepromazine, Propionylpromazine and Haloperidol in kidney. Because these substances can be harmful to consumers and tranquilizers and their metabolites have been found in slaughtered animals a quick and reliable method for the analysis of tranquilizers in meat is necessary.

Until now several methods for the extraction and detection of residues of tranquilizers in pork tissue are published, mainly based on liquid extraction followed by clean-up with solid phase extraction (SPE) and measured by LC-MS/MS. [1][2][3][4][5] In this poster we present a quick and easy method for the analysis of residues of tranquilizers in pork tissue based on liquid/liquid extraction and detection by LC-MS/MS, which is capable of confirming and quantifying the tranquilizers, according to the EU requirements of Maximum Residue Limits (MRL), laid down in the Commission Regulation (EU) No 37/2010, and which fulfills the recommended requirements of the CRL Guidance Paper (2007).

Method



Each sample is weighed twice. One is spiked with the internal standard (IS) only, the other with a standard solution containing all targeted compounds and the internal standard. Experience has revealed that each matrix has a different effect on the sensitivity of the MS/MS signals. Therefore, the recovery rate of each sample is determined individually.

HPLC	Agilent 1100 (pump, degasser, autosampler, column oven)
Column	Phenomenex Luna PFP, 100 x 4.6 mm, 3 µm, 40°C
Eluent	Water/ACN + 0.1 % Formic acid
Gradient	linear, 0.700 ml/min
MS/MS	AB Sciex API 4000, TurbolonSpray®Source
Mode	ESI positive, MRM mode

Conclusion

The proposed method has been successfully validated and is suitable for the simultaneous determination of tranquilizers in pork tissue without time consuming SPE clean-up. The recovery (105%-115%), the within-lab reproducibility (3.00%-11.70%) and the repeatability (2.81-19.57%) comply with the Guidelines for the Implementation of Decision 2002/657/EC, concerning the performance of analytical methods and the interpretation of results. A summary of the validation data is shown in the table below.

Tranquilizers	MRL (µg/kg)	LPL (µg/kg)	LOD (µg/kg)	LOQ (µg/kg)	cca (µg/kg)	ccb (µg/kg)	Repeat-ability CV (%)	within-lab reproducibility CV (%)	recovery (%)
Azaperol	Σ100		0.40	1.20	5.69	6.39	19.57	8.10	110
Azaperone			0.56	1.69	5.92	6.84	12.40	11.70	106
Acepromazine		5	0.50	1.50	5.31	5.63	8.70	3.80	111
Carazolol	5		0.52	1.56	5.26	5.53	2.81	3.00	115
Chlorpromazine		5	0.73	2.19	5.34	5.82	6.43	4.30	105
Haloperidol		5	0.82	2.46	5.63	6.26	5.64	7.40	112
Ketamine		5	0.79	2.37	5.54	6.09	11.78	7.20	108
Propionylpromazine		5	0.54	1.62	5.54	6.09	6.61	6.50	107
Xylazine		5	0.70	2.10	5.52	6.04	9.43	6.80	109

Results

Figure 1 to 3 show chromatograms obtained by applying the proposed method to routine analysis of meat samples.

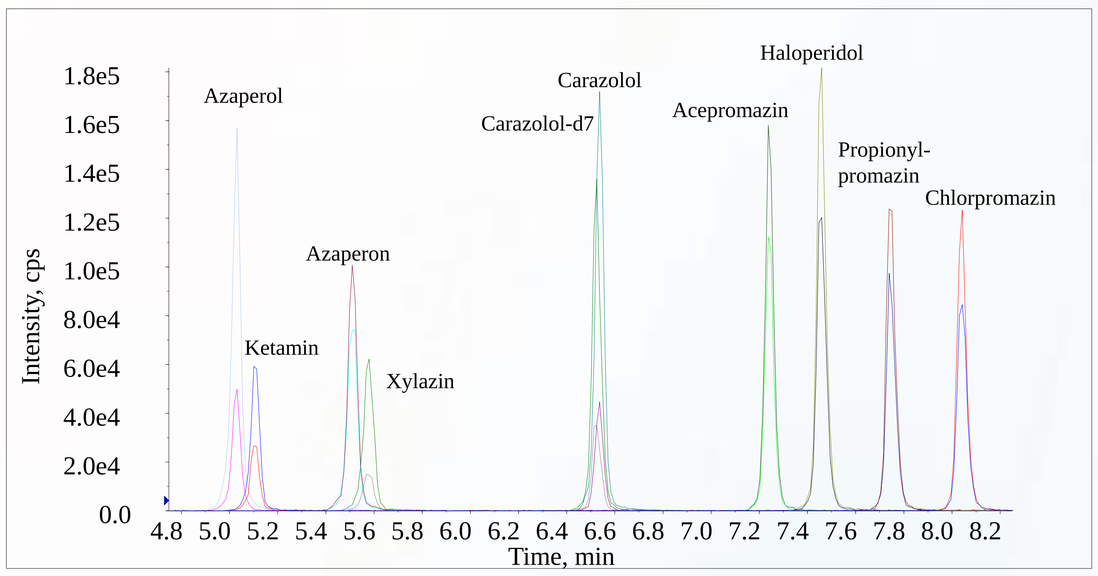


Fig. 1: MRM-chromatogram of a standard mix containing 5 µg/L of each compound

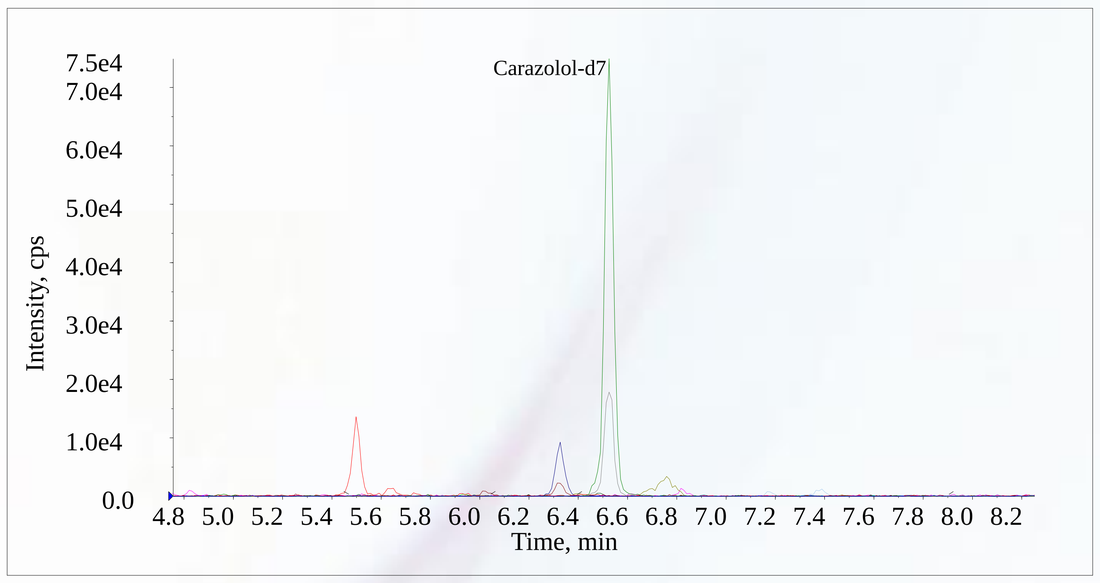


Fig. 2: MRM-chromatogram of a pork sample spiked with 5 µg/kg Carazolol-d7 (Internal Standard)

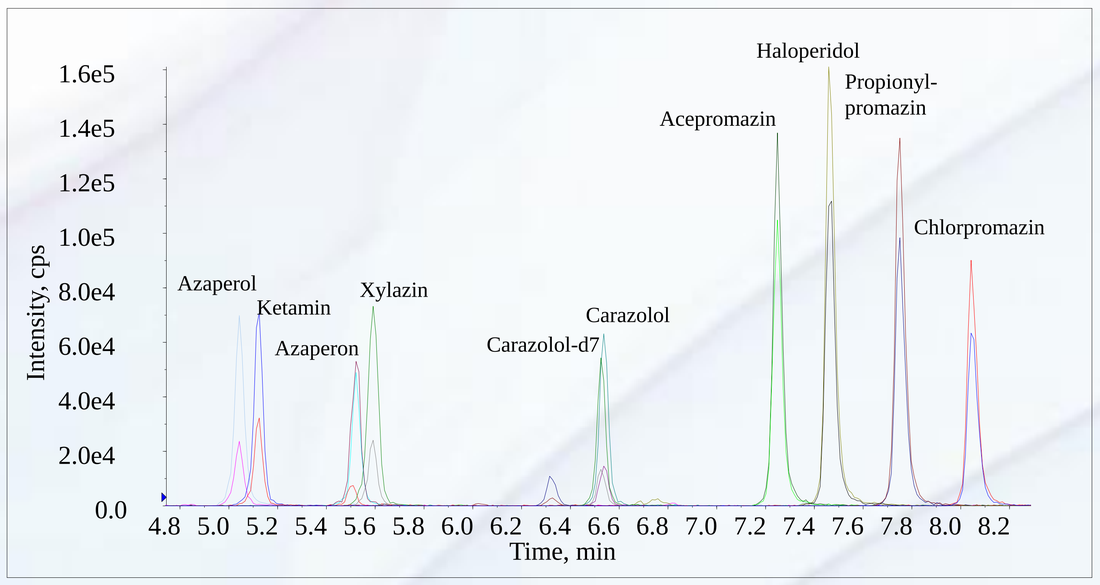


Fig. 3: MRM-chromatogram of a pork sample spiked with 5 µg/kg of each target compound (incl. IS)

References

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