LC-MS / MS method for determination of oxytetracycline in bovine plasma

Rositsa Mileva, Aneliya Milanova, Nasko Vasilev,
Ivan Fasulkov, Manol Karadaev

Department of Pharmacology, Animal Physiology
and Physiological Chemistry
Department of Obstetrics, Reproduction and Reproductive Disorders

Faculty of Veterinary Medicine, Trakia University, 2020

Background

- ✓ Precise knowledge on antibiotic pharmacokinetics requires reliable methods for analysis of the free plasma concentrations in the samples from the treated animals.

- ✓ Oxytetracycline as a tetracycline antibiotic is widely used in the treatment of various pathological conditions in cattle.

Aim

In the late elimination phase plasma concentrations are low and difficult to be quantified. Sensitive methods for analysis are needed.

Therefore an LC – MS / MS method for determination of oxytetracycline in bovine plasma was validated.

Material and methods

The study was conducted in the Department of Pharmacology, Animal Physiology and Physiological Chemistry, Faculty of Veterinary Medicine, Trakia University

Agilent 6460C Triple Quadrupole LC–MS/MS system was used for the analysis.

Material and methods

Drugs and reagents:

- Oxytetracycline hydrochloride(Sigma-Aldrich)
- Doxycycline hyclate as internal standard (Sigma-Aldrich)
- All the other reagents were LS-MS grade (Sigma-Aldrich)

Drug free blood for calibration curves was obtained by venepuncture of subcutaneous abdominal vein from healthy untreated cows.
Blood samples were collected in heparin tubes (2.5 ml Lithium heparin, FL Medical, Italy) . Plasma was obtained by centrifugation.

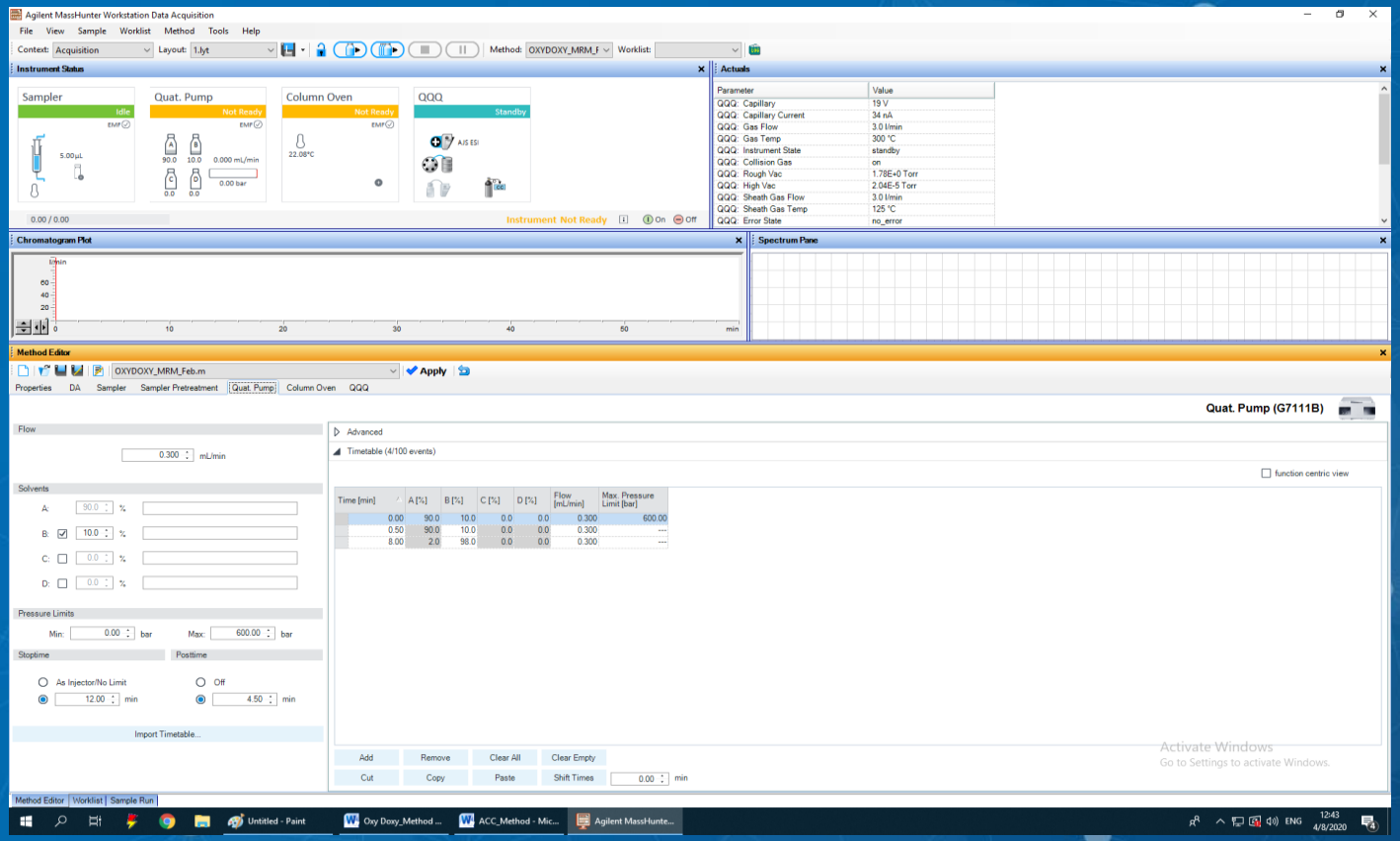
Preparation of the standard solutions

- ✓ Standard solutions of oxytetracycline in bovine plasma: 10, 50, 150, 250, 500, 750, 950 and 2000 ng/mL were prepared.
- ✓ Each standard was spiked with internal standard (final doxycycline concentration 200 ng/mL).
- ✓ TFA was used as a precipitation agent.
- ✓ The mixture was vortexed and centrifuged at 10 800g for 10 min at 25˚C. The supernatant was filtered trough syringe filter (0,20µm, SFCA membrane, Corning) into MS vials.
- ✓ 5µL of each standard was injected into the system in triplicate in 3 different days. Additionaly, standard solutions of oxytetracycline in water were prepared for determination of the recovery.

Material and methods

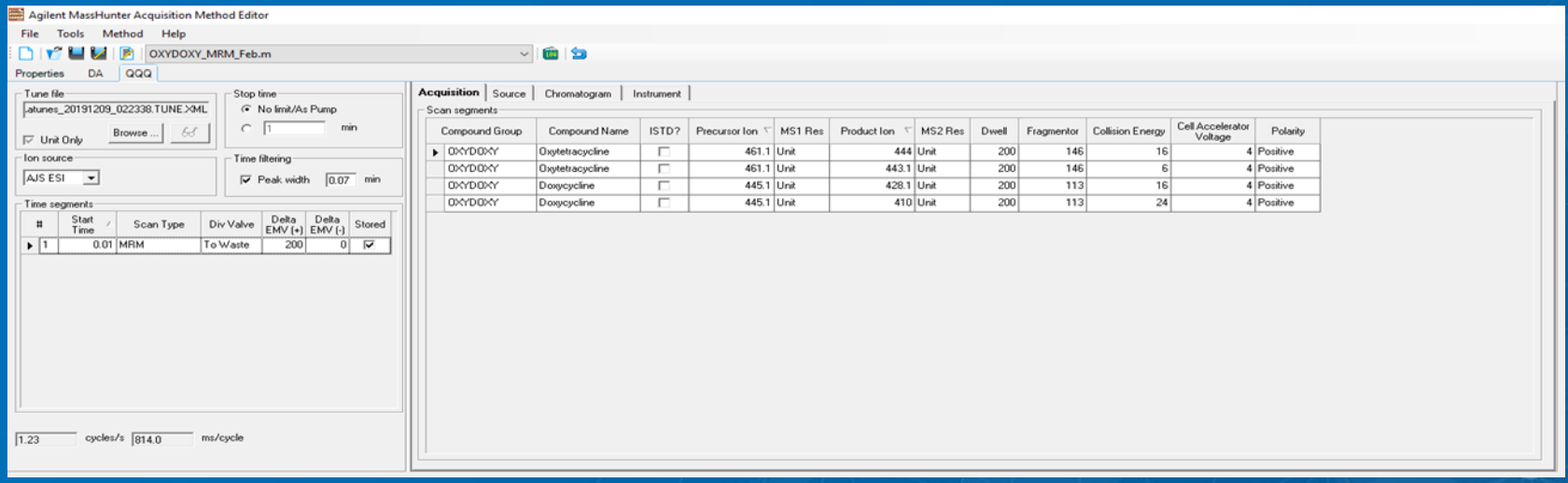
Mobile phase consisted of 0.1% Formic acid and Acetonitrile.

Gradient mode was applied.



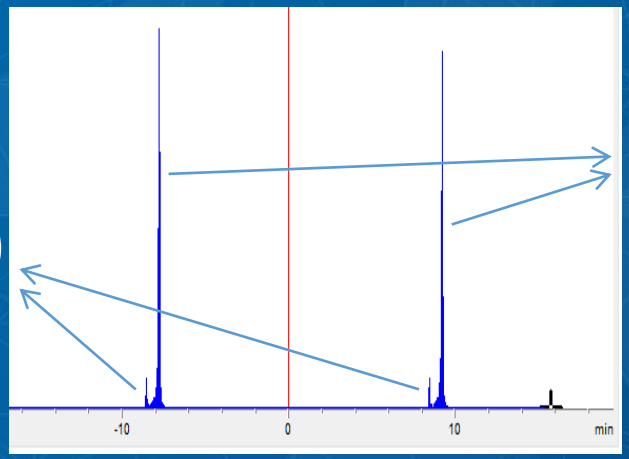
Material and methods

AJS ESI ion source was used with N_2 gas



Retention time:

8.4 min (Oxytetracycline)



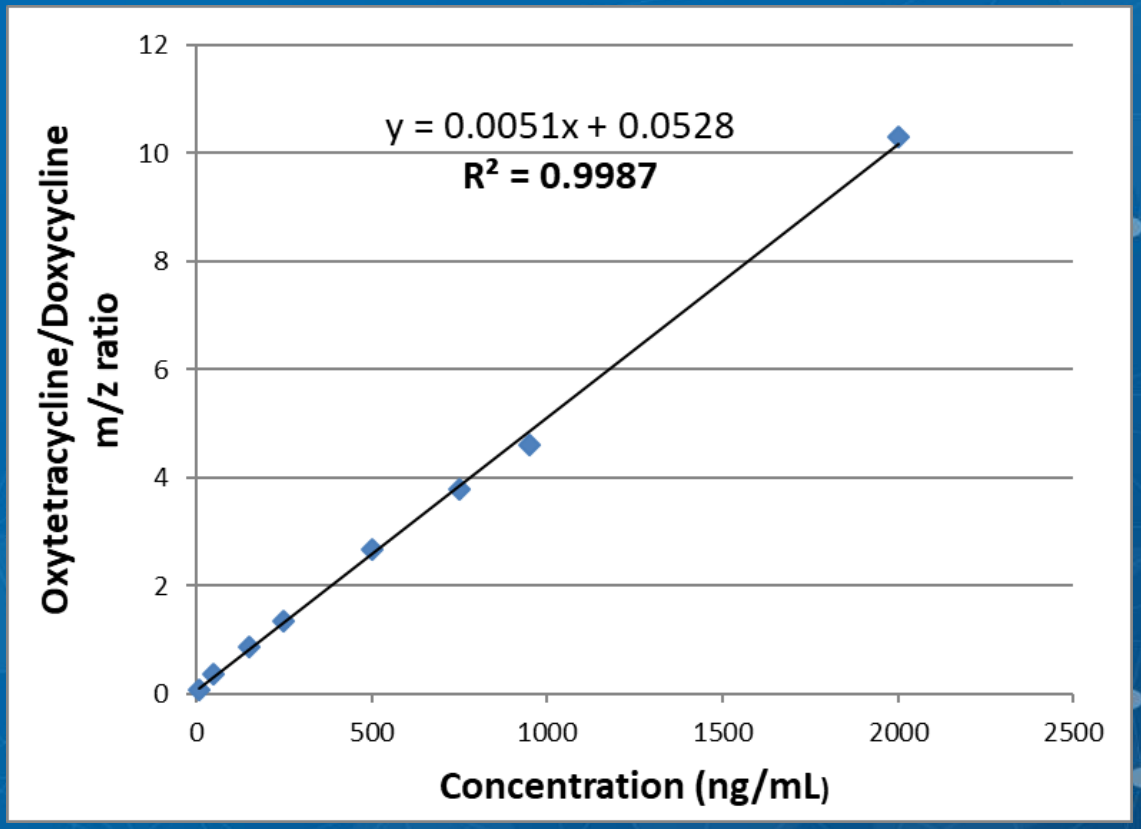
9.2 min (Doxycycline)

Results

Linearity: 10 - 2000 ng/mL

LOD: 6, 92 ng/mL

LOQ: 20.98 ng/mL



Results

Biological matrix	Oxytetracycline concentration (µg/mL±SD)	Accuracy (%)	Extraction recovery (%±SD)	Precision (RSD%)	
				Intra-Assay	Inter-Assay
Bovine plasma	50	109.62	100.49	5.03	14.31
	250	86.59	76.83	1.55	5.95
	750	90.17	92.87	2.92	8.02

Conclusion

The developed LC-MS method is more sensitive than the HPLC methods, allowing detection of lower concentrations than 100 ng/ml (MRL oxytetracycline).

The developed method fulfills the validation criteria and can be used for routine determination of oxytetracycline concentrations in bovine plasma for pharmacokinetic studies.

Acknowledgments

The study was supported by National scientific program “Reproductive biotechnologies in breeding in Bulgaria” - REPROBIOTECH funded by Ministry of Education and Science.

Thank you for your attention!