

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

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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

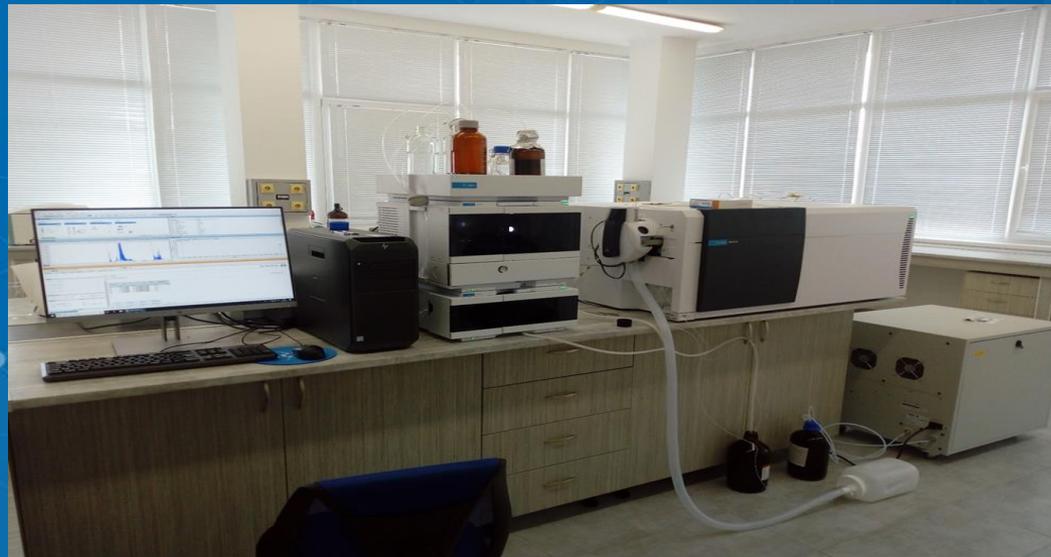
In the late elimination phase plasma concentrations are low and difficult to be quantified. Sensitive methods for analysis are needed.

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 through 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. Additionally, 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.

The screenshot displays the Agilent MassHunter Workstation Data Acquisition software interface. The main window is titled "Agilent MassHunter Workstation Data Acquisition" and shows the "Instrument Status" section with the following components:

- Sampler:** Idle
- Quat. Pump:** Not Ready
- Column Oven:** Not Ready
- QQQ:** Standby

The "Actuals" table shows the following parameters and values:

Parameter	Value
QQQ: Capillary	19 V
QQQ: Capillary Current	34 nA
QQQ: Gas Flow	3.0 l/min
QQQ: Gas Temp	300 °C
QQQ: Instrument State	standby
QQQ: Collision Gas	on
QQQ: Rough Vac	1.78E+0 Torr
QQQ: High Vac	2.04E-5 Torr
QQQ: Sheath Gas Flow	3.0 l/min
QQQ: Sheath Gas Temp	125 °C
QQQ: Error State	no_error

The "Method Editor" section shows the "Quat. Pump (G7111B)" configuration. The "Flow" section is set to 0.300 mL/min. The "Solvents" section shows the following configuration:

Solvent	Percentage
A	90.0 %
B	10.0 %
C	0.0 %
D	0.0 %

The "Pressure Limits" section shows a minimum of 0.00 bar and a maximum of 600.00 bar. The "Stop/Posttime" section shows a stop time of 12.00 min and a posttime of 4.50 min. The "Advanced" section shows a timetable with 4100 events:

Time [min]	A [%]	B [%]	C [%]	D [%]	Flow [mL/min]	Max. Pressure Limit [bar]
0.00	90.0	10.0	0.0	0.0	0.300	600.00
0.50	90.0	10.0	0.0	0.0	0.300	---
8.00	2.0	98.0	0.0	0.0	0.300	---

The "Chromatogram Plot" section shows a blank plot with a y-axis labeled "AU" and an x-axis labeled "min". The "Spectrum Pane" section shows a blank spectrum plot.

Material and methods

AJS ESI ion source was used with N₂ gas

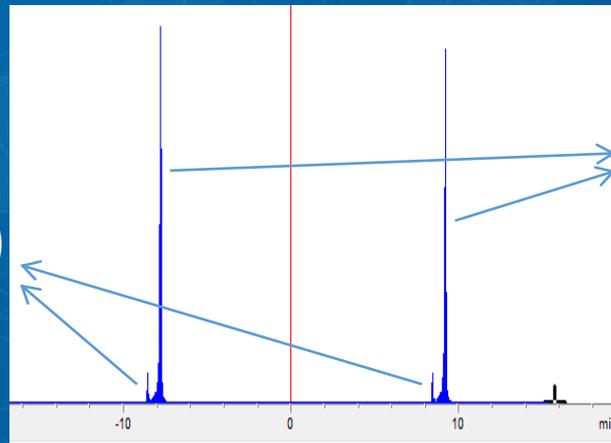
The screenshot displays the Agilent MassHunter Acquisition Method Editor interface. The 'Acquisition' tab is active, showing a table of scan segments. The table includes columns for Compound Group, Compound Name, ISTD?, Precursor Ion, MS1 Res, Product Ion, MS2 Res, Dwell, Fragmentor, Collision Energy, Cell Accelerator Voltage, and Polarity. The scan segments are as follows:

Compound Group	Compound Name	ISTD?	Precursor Ion	MS1 Res	Product Ion	MS2 Res	Dwell	Fragmentor	Collision Energy	Cell Accelerator Voltage	Polarity
0xYD0xY	Oxytetracycline	<input type="checkbox"/>	461.1	Unit	444	Unit	200	146	16	4	Positive
0xYD0xY	Oxytetracycline	<input type="checkbox"/>	461.1	Unit	443.1	Unit	200	146	6	4	Positive
0xYD0xY	Doxycycline	<input type="checkbox"/>	445.1	Unit	428.1	Unit	200	113	16	4	Positive
0xYD0xY	Doxycycline	<input type="checkbox"/>	445.1	Unit	410	Unit	200	113	24	4	Positive

Additional parameters shown in the interface include: Tune file: jatlunes_20191209_022338.TUNE>ML; Ion source: AJS ESI; Time segments: 1 (Start Time: 0.01, Scan Type: MRM, Div Valve: To Waste, Delta EMV (+): 200, Delta EMV (-): 0, Stored: checked); and instrument settings: 1.23 cycles/s, 814.0 ms/cycle.

Retention time:

8.4 min (Oxytetracycline)



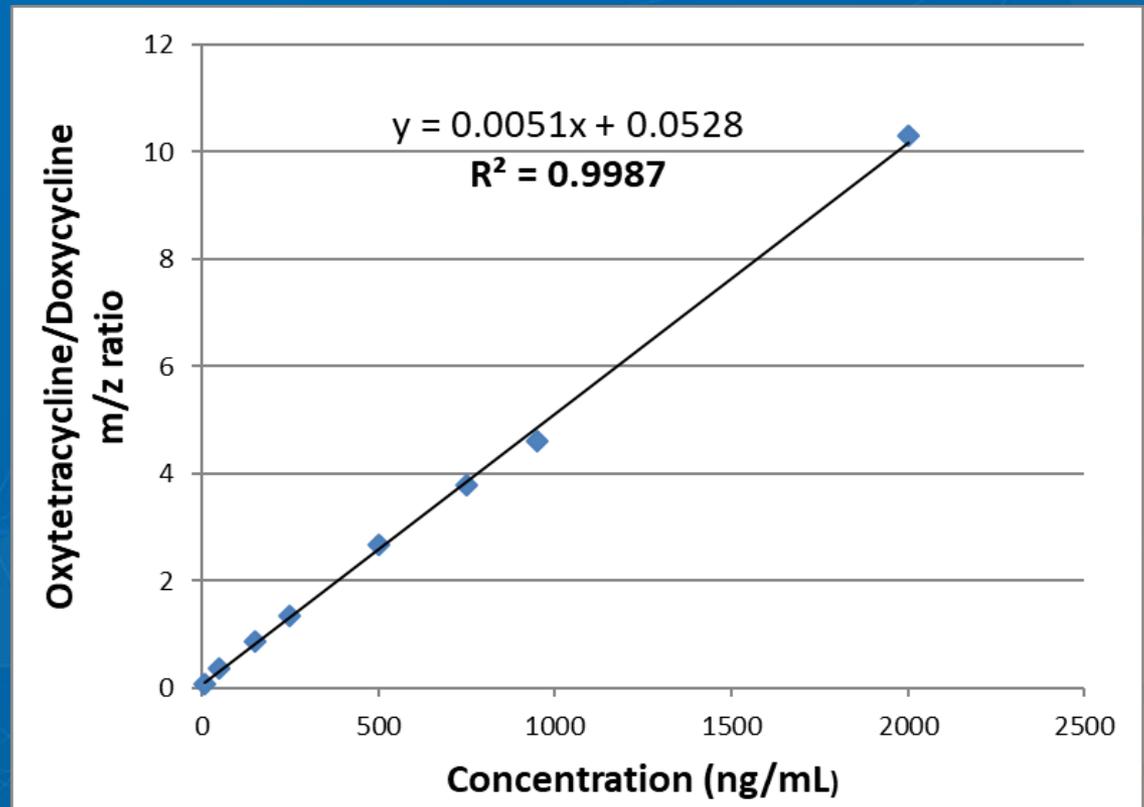
9.2 min (Doxycycline)

Results

Linearity: 10 - 2000 ng/mL

LOD: 6, 92 ng/mL

LOQ: 20.98 ng/mL



Results

Biological matrix	Oxytetracycline concentration ($\mu\text{g}/\text{mL}\pm\text{SD}$)	Accuracy (%)	Extraction recovery ($\%\pm\text{SD}$)	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

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Thank you for your attention!