Temocillin quantification in human serum using a high performance liquid chromatography-tandem mass spectrometry

P. Ngougni Pokem[1,3], A.C. Miranda Bastos[1,2,3], P.M. Tulkens[1,3], F. Van Bambeke[1,3], A. Capron[4]

Louvain Drug Research Institute: (1) Pharmacologie cellulaire et moléculaire, (2) Clinical Pharmacy Research Group, (3) Center for Clinical Pharmacy, Cliniques Universitaires Saint-Luc: (4) Department of Clinical Chemistry, Université Catholique de Louvain, Brussels, Belgium.

Contact: francoise.vanbambeke@uclouvain.be

Introduction

Temocillin (TMO) is a beta-lactam antibiotic that has recently seen both its usage and associated research increased, due to its remarkable resistance to beta-lactamases. [1]

Measuring TMO serum concentrations can be clinically useful for optimal patient management. A method using HPLC coupled to a UV detector has recently been developed and validated [2]. Yet, UV detection being not specific, interferences could occur when assaying TMO in the serum of patients taking multiple medications.

Aim of the study

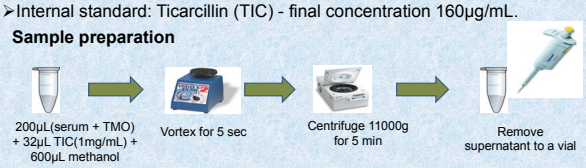
To develop and validate a new HPLC method coupled to MS-MS detection for the analysis of temocillin in human serum.

Methods

Methanol protein precipitation was used as the extraction method.

- TMO calibration standards: 1 to 500 µg/mL.
- TMO quality standards: 5 to 450µg/mL.
- Internal standard: Ticarcillin (TIC) - final concentration 160µg/mL.

Sample preparation



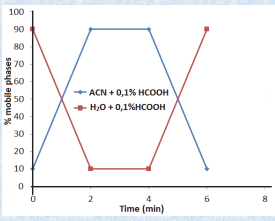
200µL(serum + TMO) + 32µL TIC(1mg/mL) + 600µL methanol → Vortex for 5 sec → Centrifuge 11000g for 5 min → Remove supernatant to a vial

Equipment

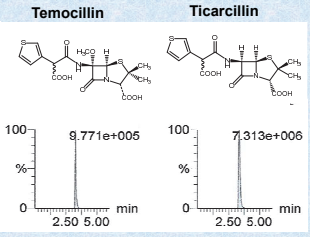
HPLC (Alliance 2796 Waters)		MS-MS	
Column	Xbrigde phenyl (50 x 2,1mm; 3,5µm)	Ionisation mode	ESi+
Mobile phases	A: H2O+ 0,1%HCOOH B: ACN + 0,1% HCOOH	Acquisition mode	MRM
Temperature	40° C	Transition (Da)	415.34 > 339.10 (TMO) 385.31 > 160.30 (TIC)
Flow rate	0,3 mL/min	Cone voltage (V)	20 (TMO) 25 (TIC)
Injection Volume	10µL	Collision Energies (eV)	12 (TMO) 14 (TIC)
Run time	6min		

Method validation according FDA acceptance criteria.

Gradient elution



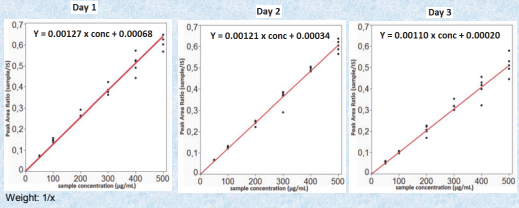
Chromatogram of a representative human serum sample at the LOQ



Results

Linearity

Linearity of HPLC MS-MS detection of temocillin: Peak area ratio (TMO/IS) over the assay concentration range 1- 500 µg/ml



5 replicates of calibration standards over 3 days

	R^2	slope
Day 1	0.993	0.00127 ; P<0.001
Day 2	0.996	0.00121 ; P<0.001
Day 3	0.991	0.00101 ; P<0.001
Mean	0.993	0.00116; P<0.001
Standard deviation	0.0025	0.000136
Coefficient of variation (CV%)	0.25	11.7

✓ **Method is linear**

Precision and Accuracy

TMO theoretical concentration µg/mL	Mean estimated concentration µg/mL	intra-day (n=5) Precision (CV%)	intra-day (n=5) Accuracy (MRPE %)	inter-day (n=15) Precision (CV%)	inter-day (n=15) Accuracy (MRPE %)
LLOQ 1	1.07	17.52	15.73	13.09	6.80
LQC 5	5.48	9.17	13.02	7.05	13.06
MQC 250	259.19	7.21	6.85	6.48	3.67
HQC 450	460.18	7.14	11.68	8.88	2.26

- **LLOQ: CV< 20%**
- **Intra-day: CV < 15%**
- **Inter- day: CV < 15%**

✓ **Method is precise**

- **LLOQ: MRPE<20%**
- **Intra-day : |MRPE%| < 15%**
- **Inter- day: |MRPE%| < 15%**

✓ **Method is accurate**

LLOQ, lower limit of quantification
LQC, low quality control; MQC, medium quality control; HQC, high quality control
CV, coefficient of variation; MRPE, Mean relative prediction error

Recovery and Precision

TMO theoretical concentration µg/mL	Mean % recovery (n=6)	Standard deviation	Coefficient of variation (CV%)
LQC 5	85.80	6.90	8.04
LQC 25	89.15	10.63	11.92
MQC 250	90.96	6.86	7.54
HQC 450	99.40	7.50	7.55

Recovery is high, precise, reproducible, and is minimally affected by concentration

. comparison of analytical results for extracted samples at 3 concentrations (low, medium, and high) with unextracted standards
. LQC, low quality control; MQC, medium quality control ; HQC, high quality control

Carry-over

Peak areas of TMO 500 µg/mL	Peak areas of blank sample (methanol)	Peak areas of LLOQ (1µg/mL)	Carry-over (%) (n=5)
359680.60	60.69	907.25	0.017
270024.06	37.79	327.17	0.014
260330.53	31.86	296.91	0.012
268586.41	34.09	233.98	0.012
263777.00	32.12	251.28	0.012

Carry-over < 0.1 % of LLOQ

✓**Carry-over is in the acceptable range**

. 5 successive injections of blank samples after the highest calibrator concentration.

Conclusion

This is the first report describing the quantification of temocillin by HPLC-MS/MS. This method proved fast, specific, and sensitive enough for determining temocillin levels in serum. It could be used for both pharmacokinetic studies and therapeutic monitoring purposes and should avoid any interference with other medications taken by the patients.

References

[1] Livermore DM, Tulkens PM. J Antimicrob Chemother (2009) 63: 243-5.

[2] AC Miranda Bastos et al. J Pharm Biomed Anal. (2014) 90:192-197.

[3] Guidance for Industry: Bioanalytical Method Validation, U.S. Department ofHealth and Human Services, Food and Drug Administration, Center for DrugEvaluation and Research, 2001.

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