Development and validation of a HPLC Assay for the Determination of Temocillin in Serum of Haemodialysis Patients

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INTRODUCTION

Temocillin (6-α-methoxy ticarcillin) is well known for its resistance to degradation by β-lactamases including most ESBL.[1,2] It is therefore considered as a sparing drug for carbapenems.[2]

Its pharmacokinetic is unknown in haemodialysis patients, which often present a wide pharmacokinetic variability that can lead to subtherapeutic concentrations.

Determining temocillin in the serum of these patients is challenged by the fact they receive several other medications and accumulate metabolites that can interfere in the assay.

AIM

To develop and validate a new method to analyse temocillin in serum from haemodialysis patients, and to quantify it by an HPLC system.

METHODS

A

Solid phase extraction of temocillin from human serum with Waters Oasis® MAX cartridges (sorbent mass 1cc/30mg)

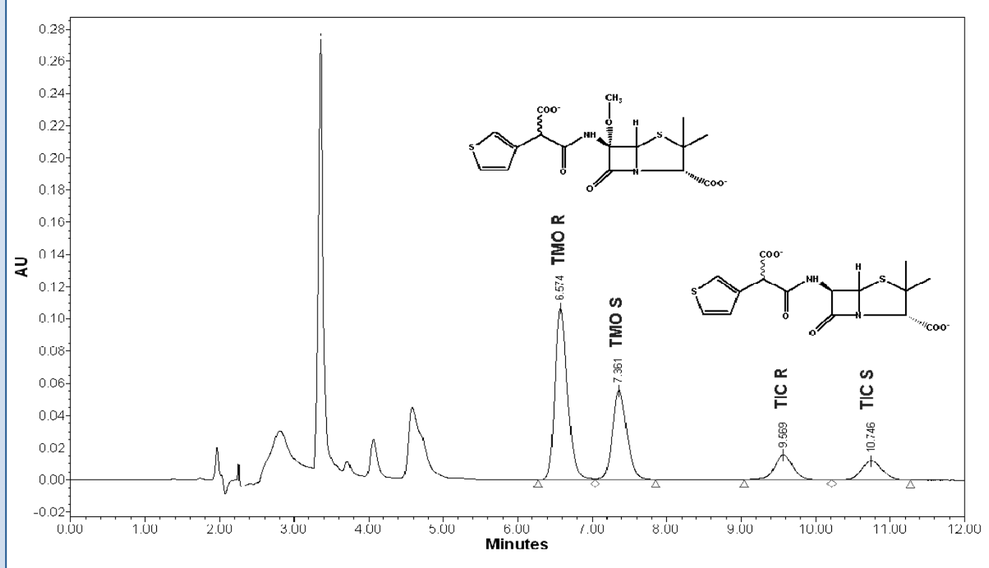
HPLC analysis coupled to a photodiode array detector (HPLC-UV)
RP-18 LiChrospher® column (250 x 4 mm, 100Å, 5µm) with isocratic elution of 100mM sodium acetate buffer pH 7 / acetonitrile (95:5, v/v); flow rate 1 mL/min; ticarcillin was internal standard.

Method validation
The method was validated according to the accuracy profile methodology, using total error to verify the trueness, precision and overall accuracy.[3] The results were evaluated according to the FDA acceptance criteria.

B

Clinical evaluation in 4 haemodialysis patients - degree of dialyzability of temocillin (extraction ratio – ER, haemodialysis clearance – CL**HD**)

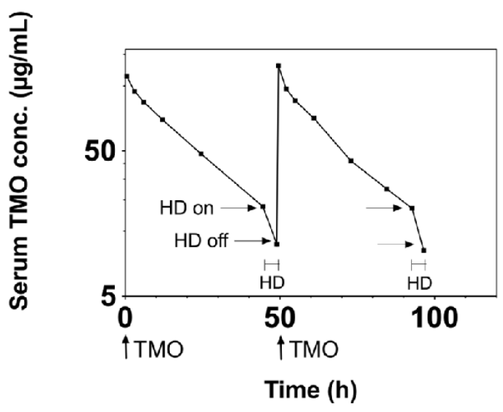
RESULTS



Chromatogram of a representative human serum sample from haemodialysis patient. The elution profiles of temocillin (R [TMO R] and S [TMO S]) isomers are well separated from that of other substances absorbing at the detection wavelength (235 nm) and from ticarcillin (internal standard, with also the corresponding R and S isomers).

Clinical Evaluation in Haemodialysis Patients

(a)



Concentration-time profile for TMO in serum in a haemodialysis patient. Initial (t=0) and post dialysis (t=48) dose was 2g. The beginning (HD on) and end (HD off) of the haemodialysis are marked with horizontal arrows, and haemodialysis periods (4hours) are shown by horizontal bars (HD).

(b)

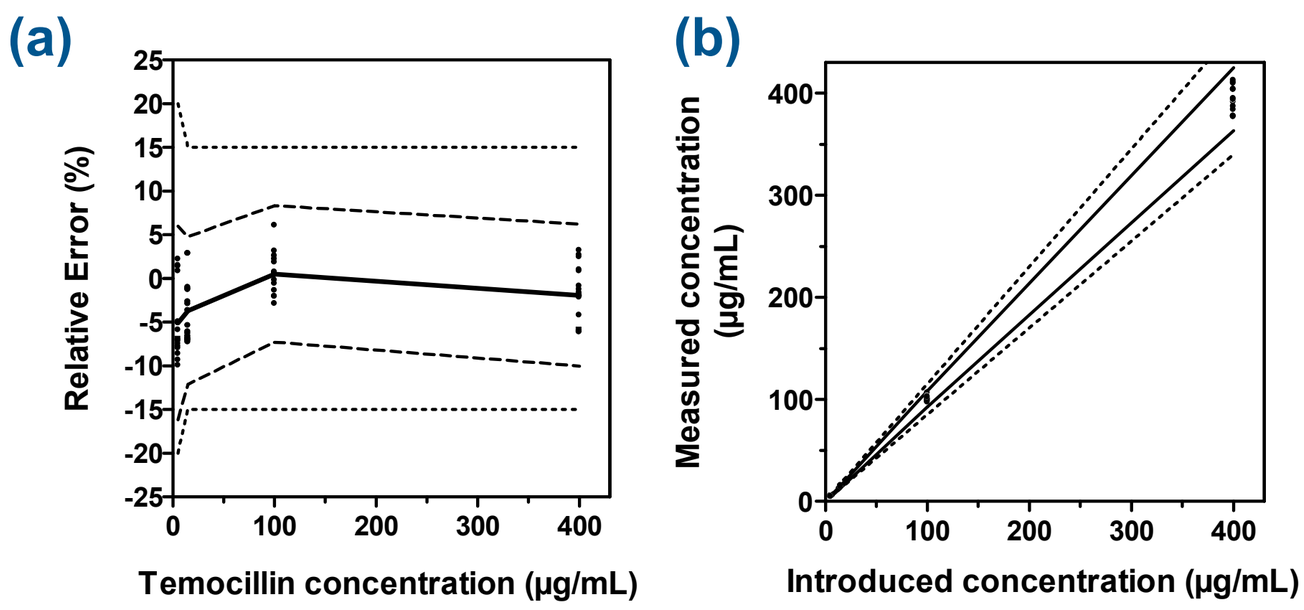
$$ER = \frac{C_{s,IN} - C_{s,OUT}}{C_{s,IN}} \qquad CL_{HD} = Q_{dial} \cdot ER$$

Temocillin dialysability was investigated in samples from 4 patients, showing a mean extraction ratio of 56.9% (range 44.8 - 71.1%), and a mean CL_{HD} of 10.87 L/h (range 8.03 – 13.0 L/h). ER – extraction ratio, $C_{s,IN}$, $C_{s,OUT}$ – serum concentrations of TMO flowing into and out of the dialyser (µg/mL), CL_{HD} – haemodialysis clearance (L/h), Q_{dial} – blood flow into and out of the dialyser (L/h)

Assay Validation Results for Quantification of Total Temocillin in Human Serum.

Sample	Nominal concentration (µg/mL)	Trueness		Precision		Accuracy	
		Absolute bias (µg/mL)	Relative bias (%)	Repeatability (RSD %)	Intermediate precision (RSD %)	β-expectation tolerance intervals (µg/mL)	Relative β-expectation tolerance limits (%)
LOQ	5	-0.256	-5.129	2.062	5.120	[4.223 ; 5.264]	[-16.095 ; 5.836]
QC1	15	-0.553	-3.687	2.336	4.006	[13.230 ; 15.664]	[-12.101 ; 4.734]
QC2	100	-0.497	0.497	2.205	2.410	[92.642 ; 108.353]	[-7.319 ; 8.314]
QC3	400	-5.889	-1.472	0.957	3.611	[363.344 ; 424.877]	[-9.279 ; 6.334]

RSD: relative standard deviation; **QC**: quality control



(a) Accuracy profile of the concentration (µg/mL) of temocillin using a linear regression model. The continuous line is the relative bias, the dashed line is the upper and lower 80% β-expectation tolerance limits, and the dotted lines are the upper and lower FDA acceptance limits. The dots represent the relative error of the measured concentrations for each QC sample and are plotted with respect to their target concentration. (b) Linear profile of temocillin in human serum. The dotted lines are the upper and lower FDA acceptance limits, and the dashed lines are the upper and lower 80% β-expectation tolerance limits, both expressed in concentration units. The dots represent the data points for the different quality control samples across the concentration range 5 – 400 µg/mL.

Stability Results for Temocillin in Human Serum

Nominal concentration (µg/mL)	Freeze-Thaw Stability* (%)	Benchtop Stability* (%)	Autosampler Stability* (%)	6months Stability* (%)
5	89.81 ± 3.20	95.67 ± 2.34	93.50 ± 2.46	93.73 ± 3.07
100	94.14 ± 1.05	94.67 ± 1.40	96.91 ± 7.26	90.97 ± 6.64
400	95.78 ± 0.12	95.40 ± 2.5	98.10 ± 1.98	88.96 ± 2.26
* Mean ± SD				

CONCLUSIONS

This fully validated method enables detection and quantification of temocillin in serum of haemodialysis patients in a simple, robust and reproducible manner. It can prove beneficial for optimal management of haemodialysis patients.

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