Quantification of four immunosuppressant drugs by Liquid Chomatography-Tandem Mass Spectrometry using Direct Injection.

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Introduction

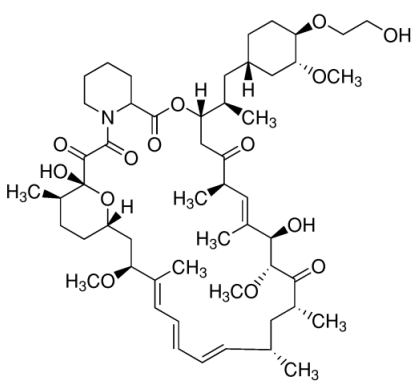
- Immunosuppressors: drugs used in transplanted patients to avoid graft rejection.
- Therapeutic drug monitoring (TDM) of immunosuppressors is important due to its narrow therapeutic window.
- Methods used for TDM should have adequate analytical performance and turn-around times.

Introduction

- Analytical methods available for quantification include immunoassay and chromatographic methods (HPLC-UV; LC-MS/MS).
- Liquid chromatography tandem-mass spectrometry (LC-MS/MS) provide accuracy, speed and sensitivity compared to immunoassay based techniques.
- LC-MS/MS methods have the ability of measure the active compound and not drug metabolites.

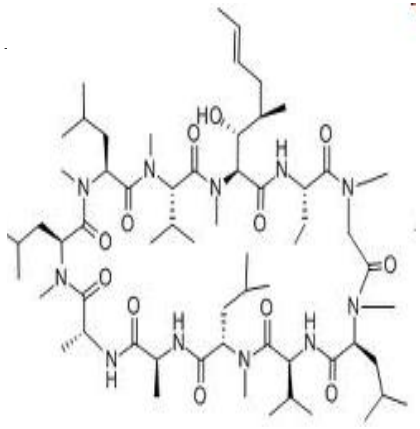
Purpose

- Implementation of a rapid and simple LC-MS/MS method for simultaneous analysis of 4 immunosuppressors in whole blood:



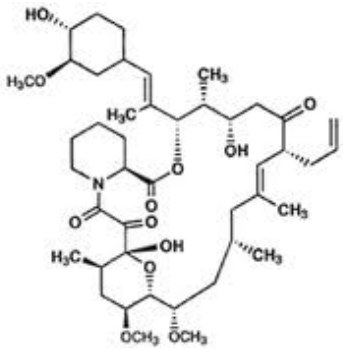
Cyclosporine A (CsA)

Mw 1202.8



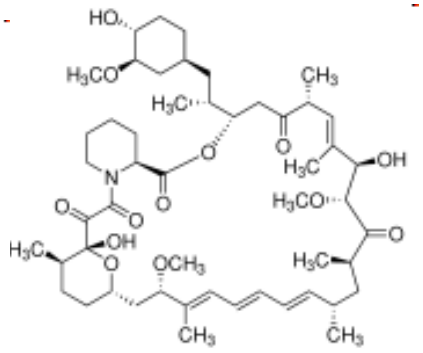
Everolimus (Ever)

Mw 958.2



Tacrolimus (Tac)

Mw:804.0



Sirolimus (Sir)

Mw:914.2

Material and Methods

- Equipment: LC-MS/MS API 3200, AB SCIEX, coupled to HPLC Agilent 1200.
- Controls and calibrators: available from RECIPE or Chromsystems.
- Method: iMethod from AB SCIEX for simultaneous analysis of cyclosporine A, everolimus, tacrolimus and sirolimus.

Material and Methods

MS conditions: ESI positive; MRM mode

MRM transitions for analytes and internal standards

Analyte	Q1 (m/z)	Q3 (m/z)	RT (min.)
Cyclosporine A	1202.8	425.4	1.17
Everolimus	975.7	908.7	1.09
Tacrolimus	821.5	768.4	1.09
Sirolimus	931.7	864.6	1.09
Internal standards			
Cyclosporine D	1216.8	425.4	1.17
Ascomicin	809.5	756.4	1.09

Material and Methods

HPLC analytical conditions	
Mode	Gradient
Mobile phase A	water: ammonium acetate: formic acid (997:2:1)
Mobile phase B	metanol: ammonium acetate: formic acid (997:2:1)
Flow rate	0.750 mL/min
Column oven temperature	60°C
Autosampler temperature	10°C
Pre-column	C_{18}; 4x3 mm
Injection volume	50 µL
Run time	2 min.

Material and Methods

Sample preparation

200 µL of protein precipitation protein

↓

Add 100 µL of whole blood

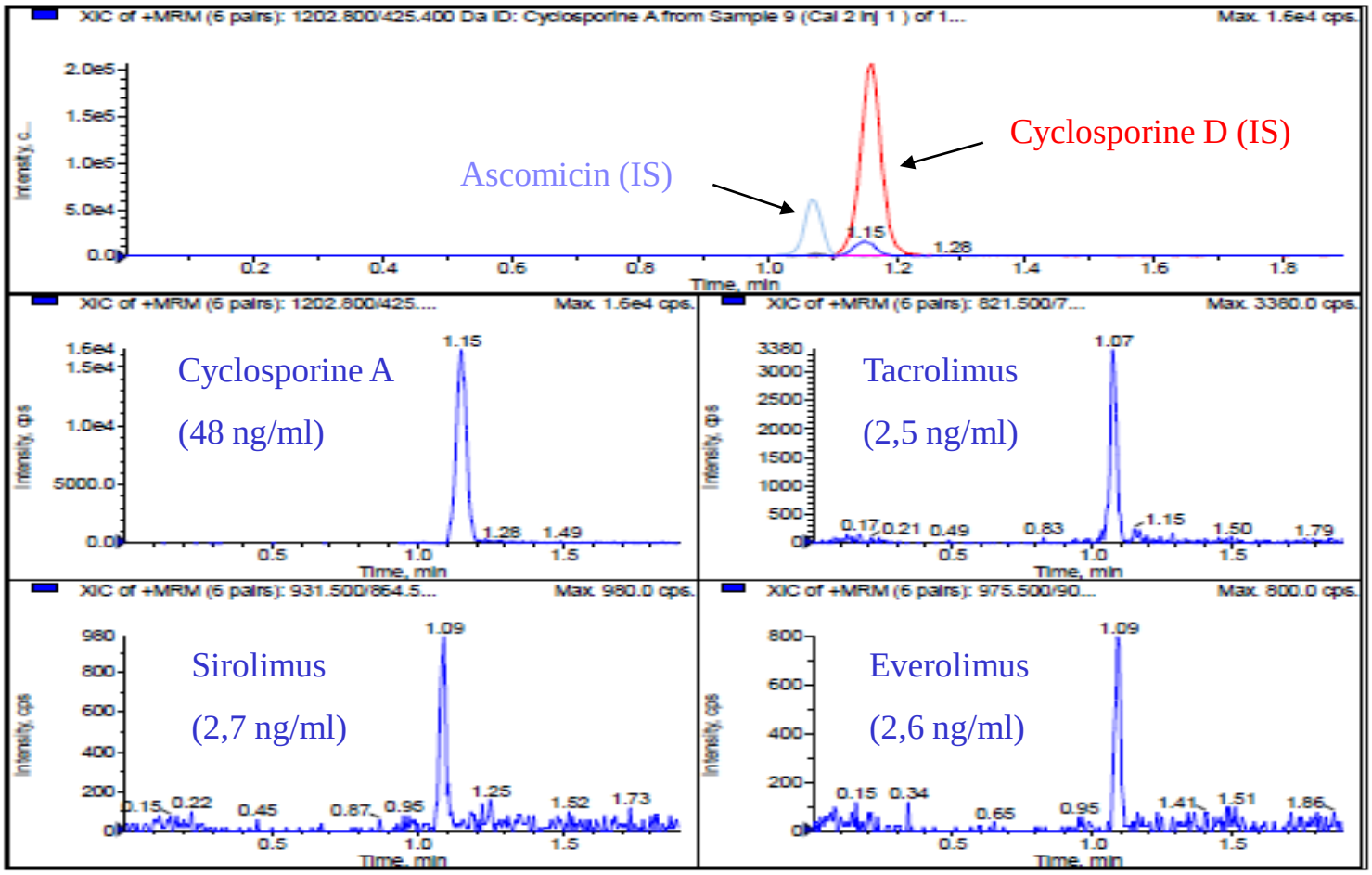
↓

12000 rpm / 10 min

↓

Inject supernatant

Results and discussion



Results and discussion

1. Linearity

Analyte	Calibration curve					
	Range (ng/mL)	R^2	CV_m(%) <10%	RIKILT Test (90-110)%	Mandel Test (PG<F)	
					PG	F
Cyclosporine A	48 - 1350	0.9999	1.4	96-102	0.7	18.5
Everolimus	2.6 – 44.9	0.9997	1.9	89-104	1.5	18.5
Tacrolimus	2.5 – 39.7	0.9998	2.1	98-102	1.3	18.5
Sirolimus	2.7 – 44.3	0.9996	2.5	96-107	-2.0	18.5

F: Fisher Snedecor distribution; (1, N-3);95%

Results and discussion

2. Analytical limits

Analyte	LOD (ng/mL)	LOQ (ng/mL)
Cyclosporine A	14	48
Everolimus	0.9	2.6
Tacrolimus	0.7	2.5
Sirolimus	1.0	2.7

Results and discussion

3. Repeatability

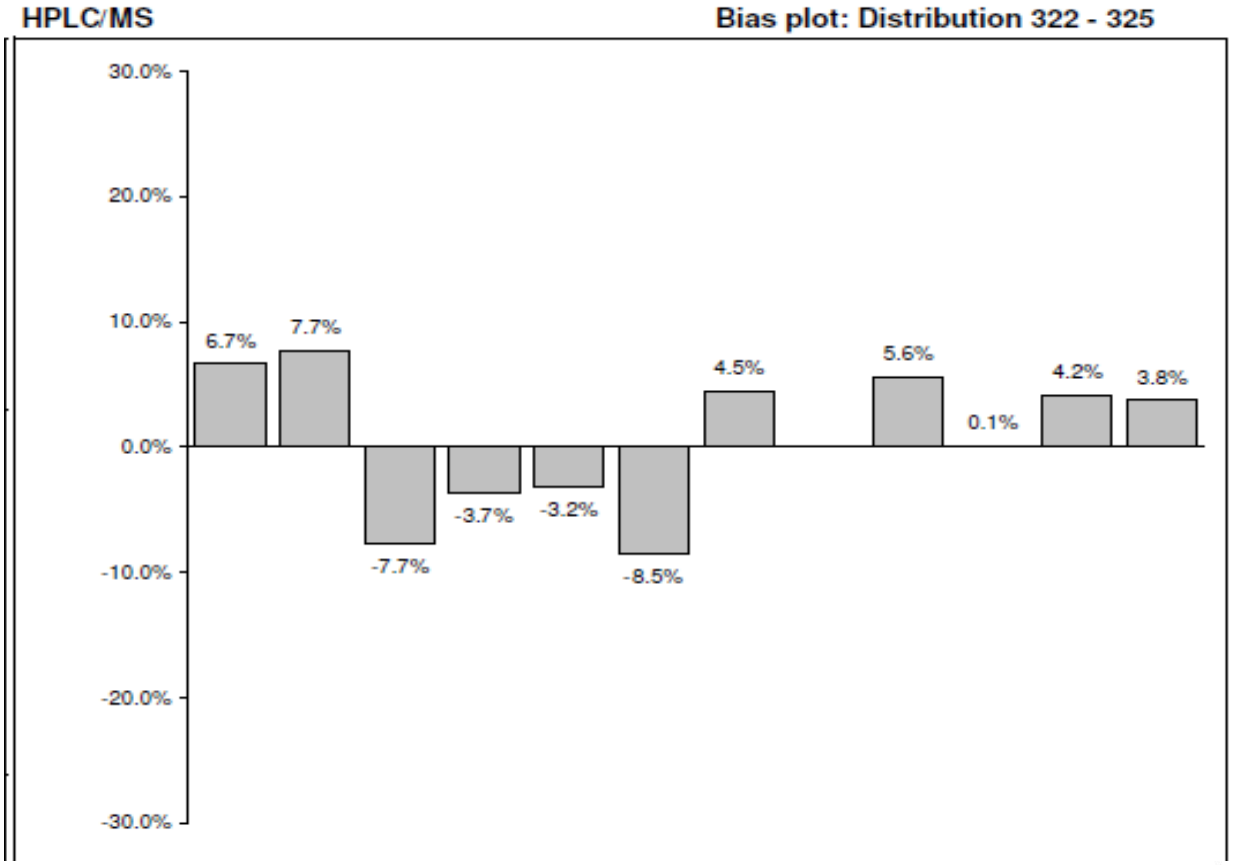
Analyte	RSD(%)		
	Low (QC I)	Medium (QC II)	High (QC III)
Cyclosporine A	3.8	2.7	1.9
Everolimus	11.2	10.8	8.8
Tacrolimus	7.9	5.4	5.4
Sirolimus	12.8	5.3	4.8

Results and discussion

4. Accuracy

Results from participation in UK NEQAS proficiency test

Cyclosporine A

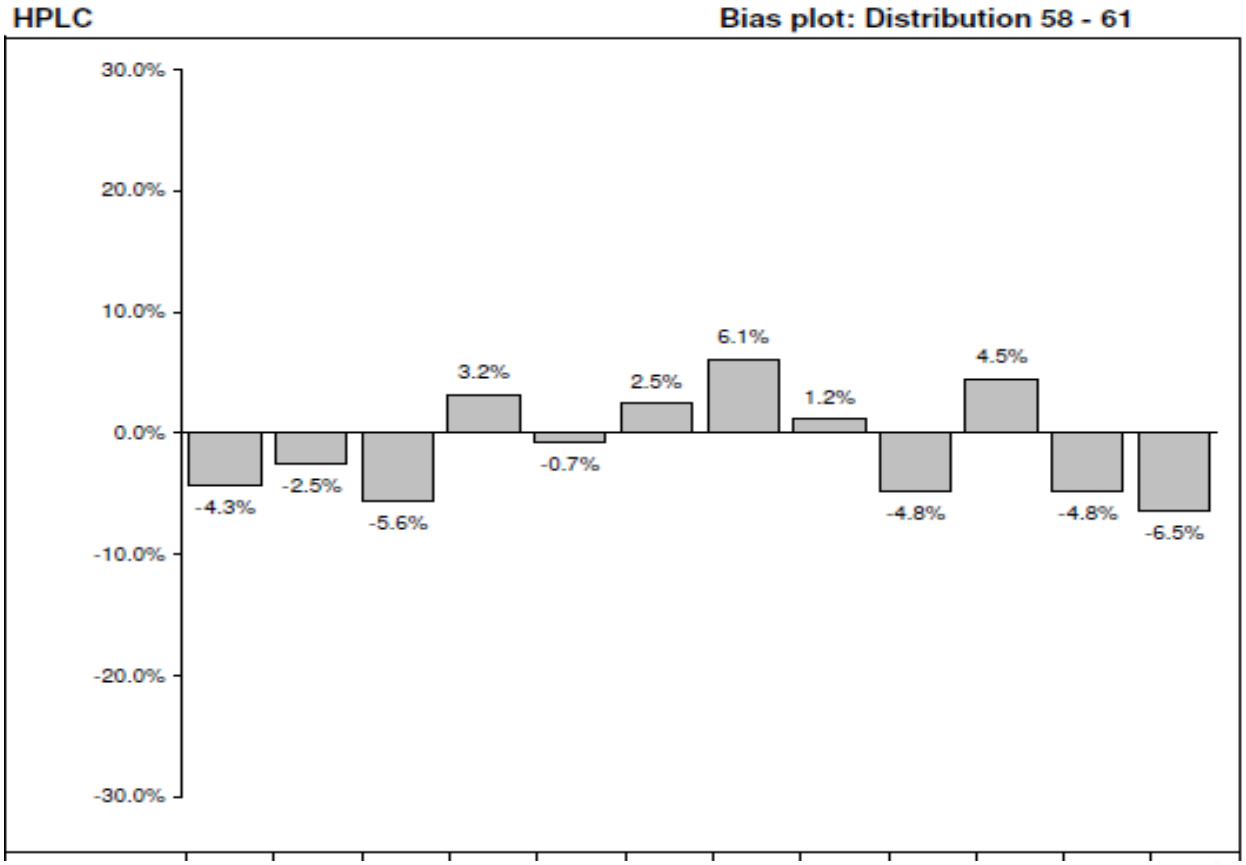


Results and discussion

4. Accuracy

Results from participation in UK NEQAS proficiency test

Everolimus

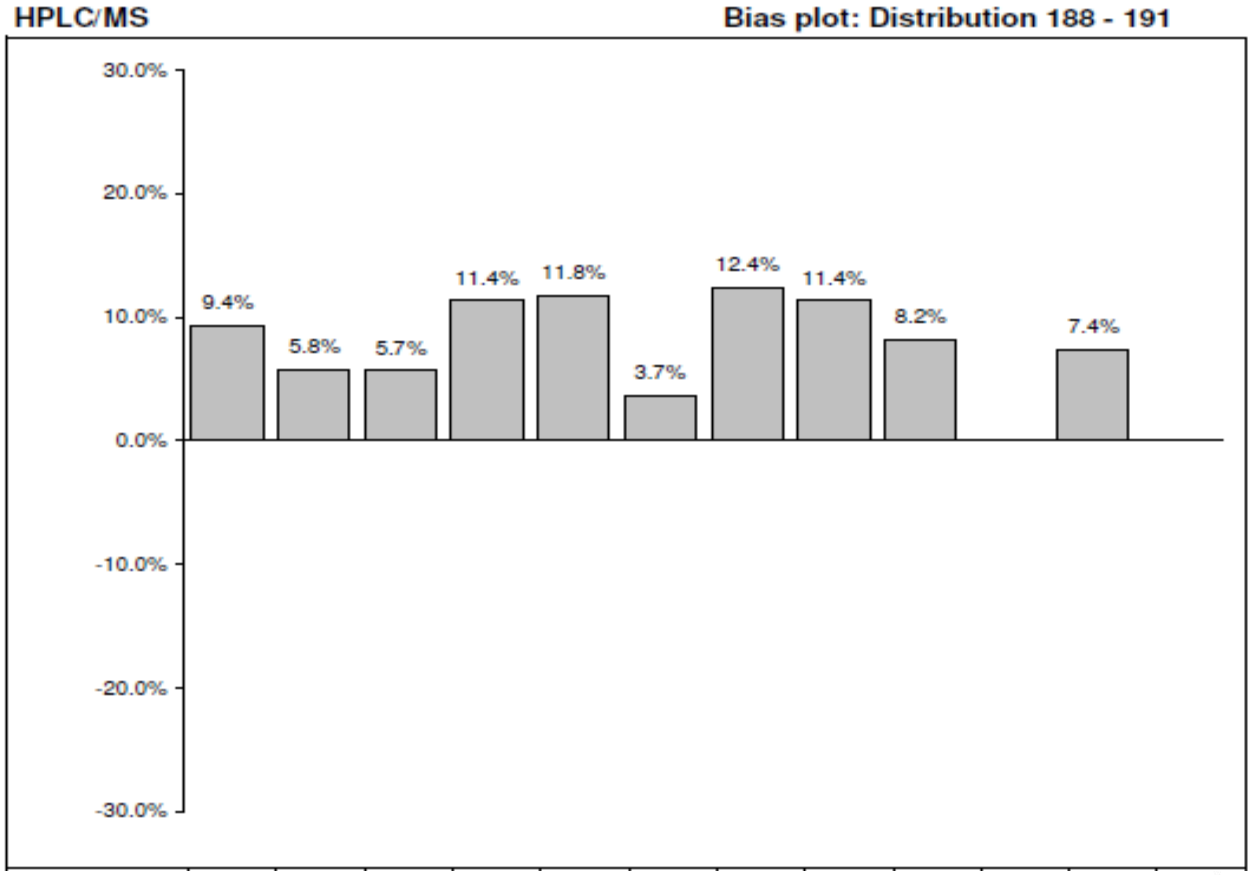


Results and discussion

4. Accuracy

Results from participation in UK NEQAS proficiency test

Tacrolimus

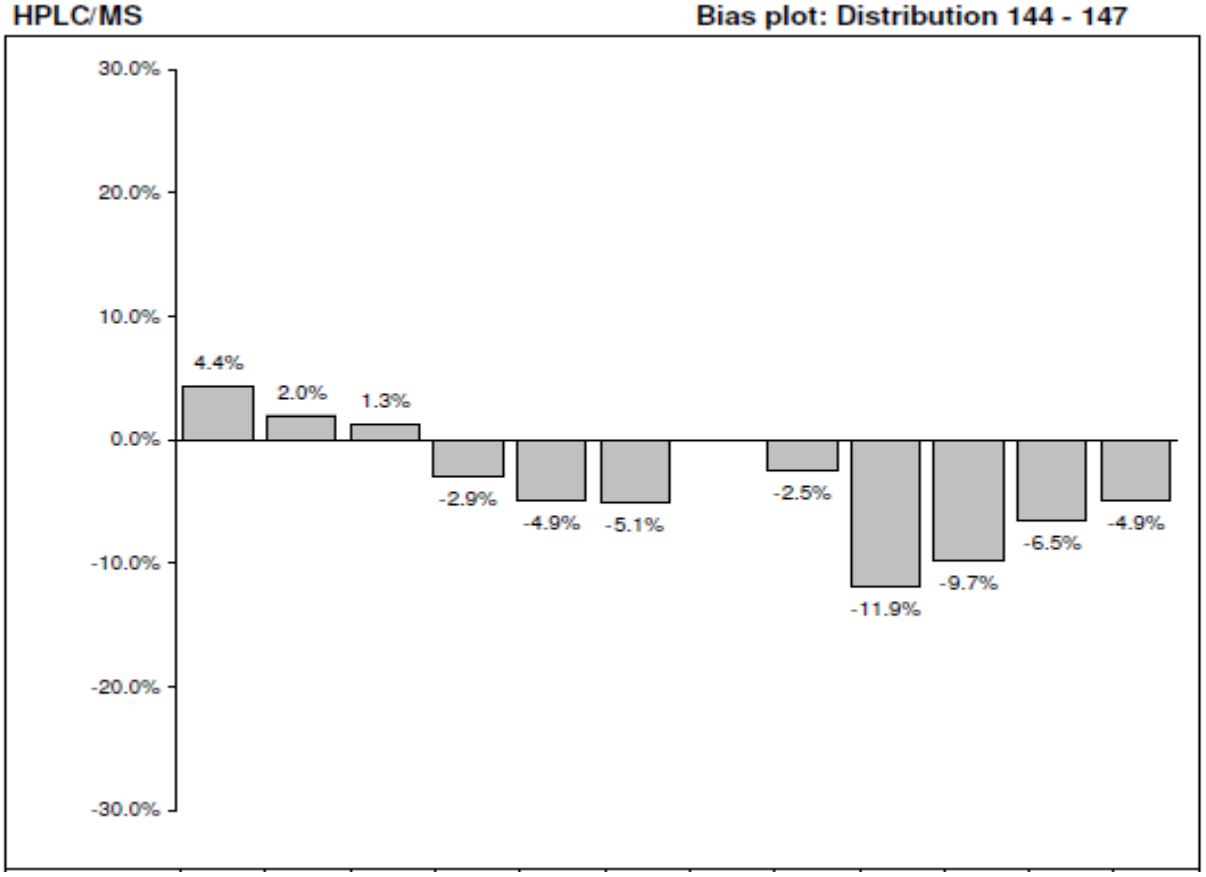


Results and discussion

4. Accuracy

Results from participation in UK NEQAS proficiency test

Sirolimus



Results and discussion

5. Samples

Results for everolimus blood levels in 35 samples

Everolimus (ng/mL)			
	Subtherapeutic range (<3)	Therapeutic range (3-8)	Toxic range (>8)
Nº of samples	22 (60%)	13 (37%)	1 (2.8 %)

Conclusions

- The LC-MS/MS method is linear for all target compounds within the range used. The limits of quantification are adequate for TDM of CsA, Tac, Ever and Sir. The relative standard deviation for repeatability is below 15% for all drugs and the method is accurate.
- Sample preparation is rapid and simple and run time is short which enables high throughput.
- The reported method provides a rapid, specific and accurate measurement of CsA, Ever, Sir and Tac over a wide analytical range making it suitable for routine use in therapeutic drug monitoring.

Acknowledgements

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