Measurement of tacrolimus in dried blood spots: a fully automated sample preparation and LCMS method

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CONTEXT: The drug to measure

Pharmacokinetic models to assist the prescriber in choosing the best tacrolimus dose

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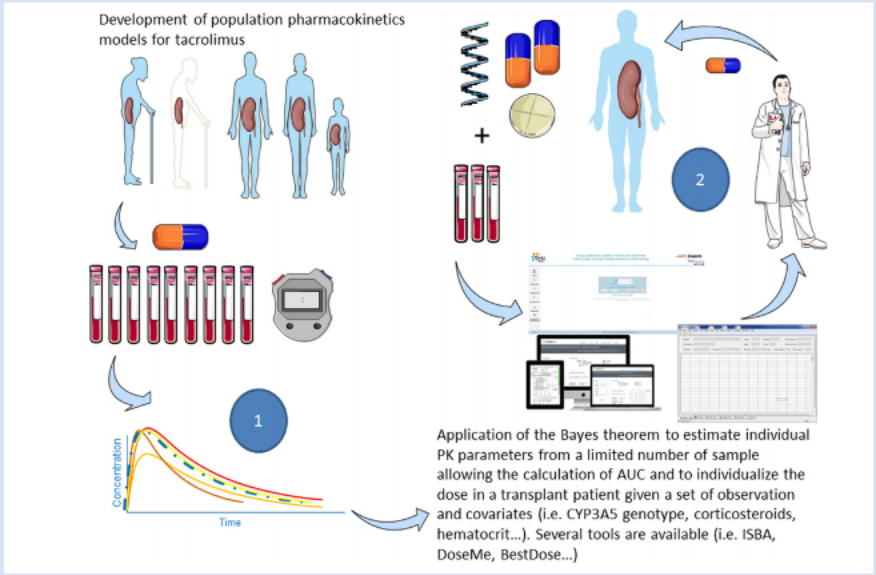

Pharmacol Res (2018), https://doi.org/10.1016/j.phrs.2018.02.016

Tacrolimus

- Most prescribed immunosuppressant in transplanted patients
- **Narrow therapeutic range**
- **Inter-individual variability**
- **Individual dose adjustment necessary**
- **TDM is mandatory**

- Quantification by LC-MS

Lessons From Routine Dose Adjustment of Tacrolimus in Renal Transplant Patients Based on Global Exposure

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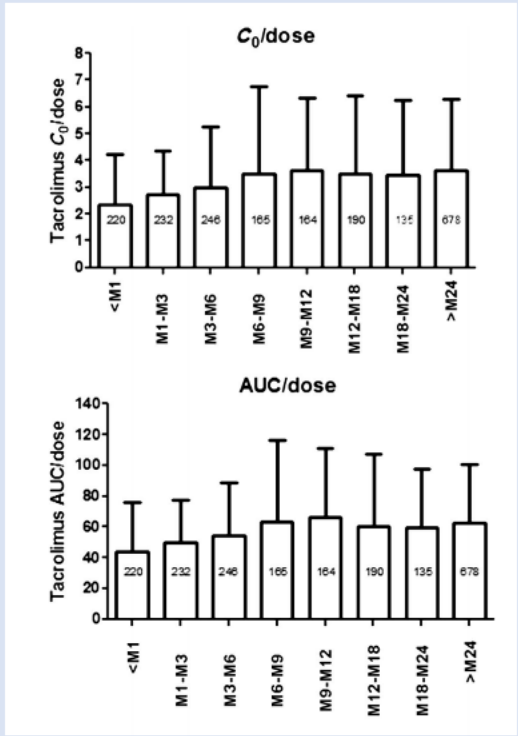


CONTEXT: The challenge

Objectives and global strategy

1

Development of an automated extraction procedure

Chromatographic and mass spectral conditions already available

2

Full validation of the whole procedure

3

Application in renal and hepatic transplant patients enrolled in a clinical trial

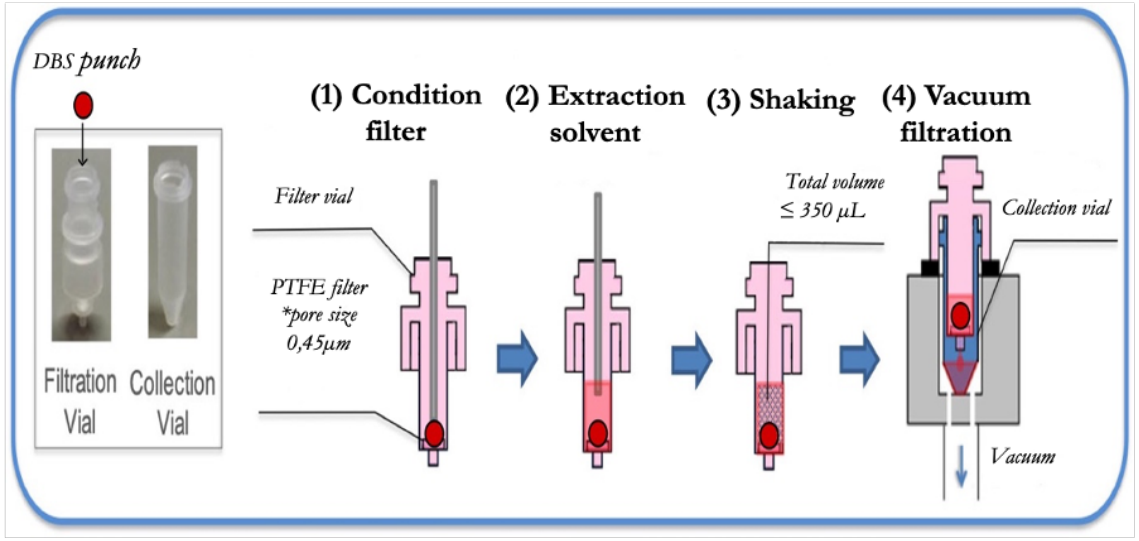
The validation procedure guidelines

Validation according to EMA and IATDMCT[1]

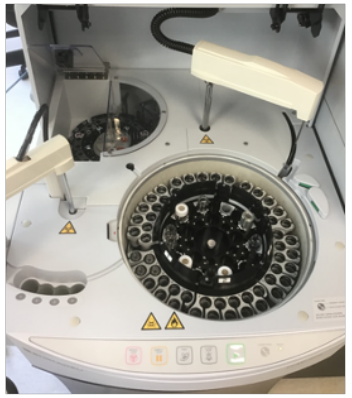
Evaluated parameter	Blood source	Repeat	Concentration levels	Duration
Intra-run precision and accuracy	6	/	4 levels (1; 3; 40 and 75 µg.L^{-1})	1 day
Inter-run precision and accuracy	1	Duplicate		3 days
Calibration	1	/	8 levels (1; 2,5; 5; 10; 15; 20; 50 and 100 µg.L^{-1})	4 days / 4 days
Selectivity	6	/	/	1 day
Recovery / Matrix effect	6	Quadruplicate	2 levels (3 and 75 µg.L^{-1})	1 day
Dilution integrity	1	Duplicate	1 level (150 µg.L^{-1})	3 days
Carry-over	1	/	/	4 days
Hematocrit effect	3 At (26.0, 43.9 & 60 %)	Quadruplicate	2 levels (3 and 75 µg.L^{-1})	1 day
Stability	1	Quadruplicate	2 levels (3 and 75 µg.L^{-1})	7 days at RT
				14 days at RT
				3 days at +60°C

1) Capiau et al, Ther Drug Monit 2019

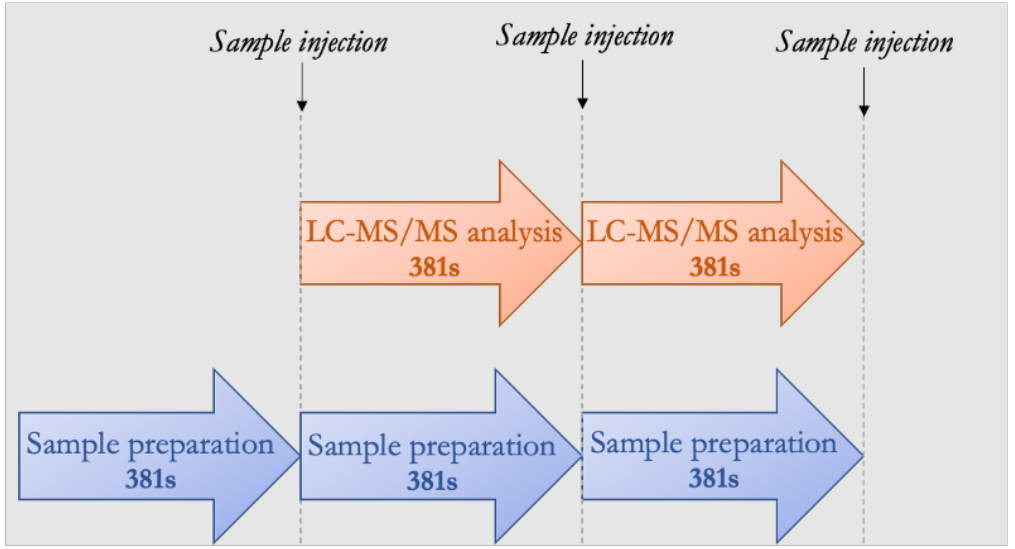
Results: The optimized fully-automated extraction procedure



Matrix preparation steps on the CLAM-2030



Parameters			
1	Reagent dispense	**Volume (µL)**	20
2	Reagent dispense	**Volume (µL)**	75
3	Shaking	**Time (s) / Speed (rpm)**	120 / 2000
4	Filtration	**Time (s)**	60



CLAM-2030 workflow

Results of the validation (1)

Intra-run accuracy and precision

Target concentration value (µg/L)		Results	Acceptance criteria (%)
1	**Accuracy** Mean relative error (%)	-12,1	≤ 20
3		-11,0	≤ 15
40		-4,6	
75		-2,2	
1	**Precision** CV (%)	9,3	≤ 20
3		5,4	≤ 15
40		5,8	
75		4,4	

Inter-run accuracy and precision

Target concentration value (µg/L)		Results	Acceptance criteria (%)
1	**Accuracy** Mean relative error (%)	-3,5	≤ 20
3		-7,7	≤ 15
40		-10,7	
75		-10,6	
1	**Precision** CV (%)	5,9	≤ 20
3		5,5	≤ 15
40		6,9	
75		3,2	

Results of the validation (2)

Evaluated parameter		Results		Acceptance criteria (%)
Dilution integrity	**Precision** CV (%)	1.0		≤ 15 ✓
	Accuracy Mean relative error (%)	- 12.8		
Selectivity	$\frac{\text{Mean signal}}{\text{Tacrolimus LLOQ signal}}$ (%)	No signal		≤ 20 ✓
	$\frac{\text{Mean signal}}{\text{Internal standard signal}}$ (%)	0.1		≤ 5 ✓
Matrix effect	CV (%)	/		≤ 15 ✓
Recovery	CV (%)	5.0 % QCL	4.0 (QCH)	
Carry-over	$\frac{\text{Mean signal}}{\text{Tacrolimus LLOQ signal}}$ (%)	4.6		≤ 20 ✓
	$\frac{\text{Mean signal}}{\text{Internal standard signal}}$ (%)	No signal		≤ 5 ✓

Results of the validation (3)

Parameter		Hematocrit level	Results		Acceptance criteria (%)
Hematocrit effect	**Accuracy** Mean relative error (%)	Low: 26.0 %	- 4.8 % (QCL)	- 11.6 % (QCH)	≤ 15 ✓
		Medium: 43.9 %	- 11.8 % (QCL)	- 11.1 % (QCH)	
		High: 60.0 %	- 10.8 % (QCL)	- 8.6 % (QCH)	

Stability parameter		Results		Acceptance criteria (%)
1 week at +4°C	**Accuracy** Mean relative error (%)	-9,86 (QCL)	-8,69 (QCH)	≤ 15 ✓
2 weeks at +4°C		1,69 (QCL)	6,51 (QCH)	
1 week at RT		9,6 (QCL)	12,2 (QCH)	
2 weeks at RT		14,7 (QCL)	0,09 (QCH)	
3 days at 60°C		5,06 (QCL)	8,66 (QCH)	

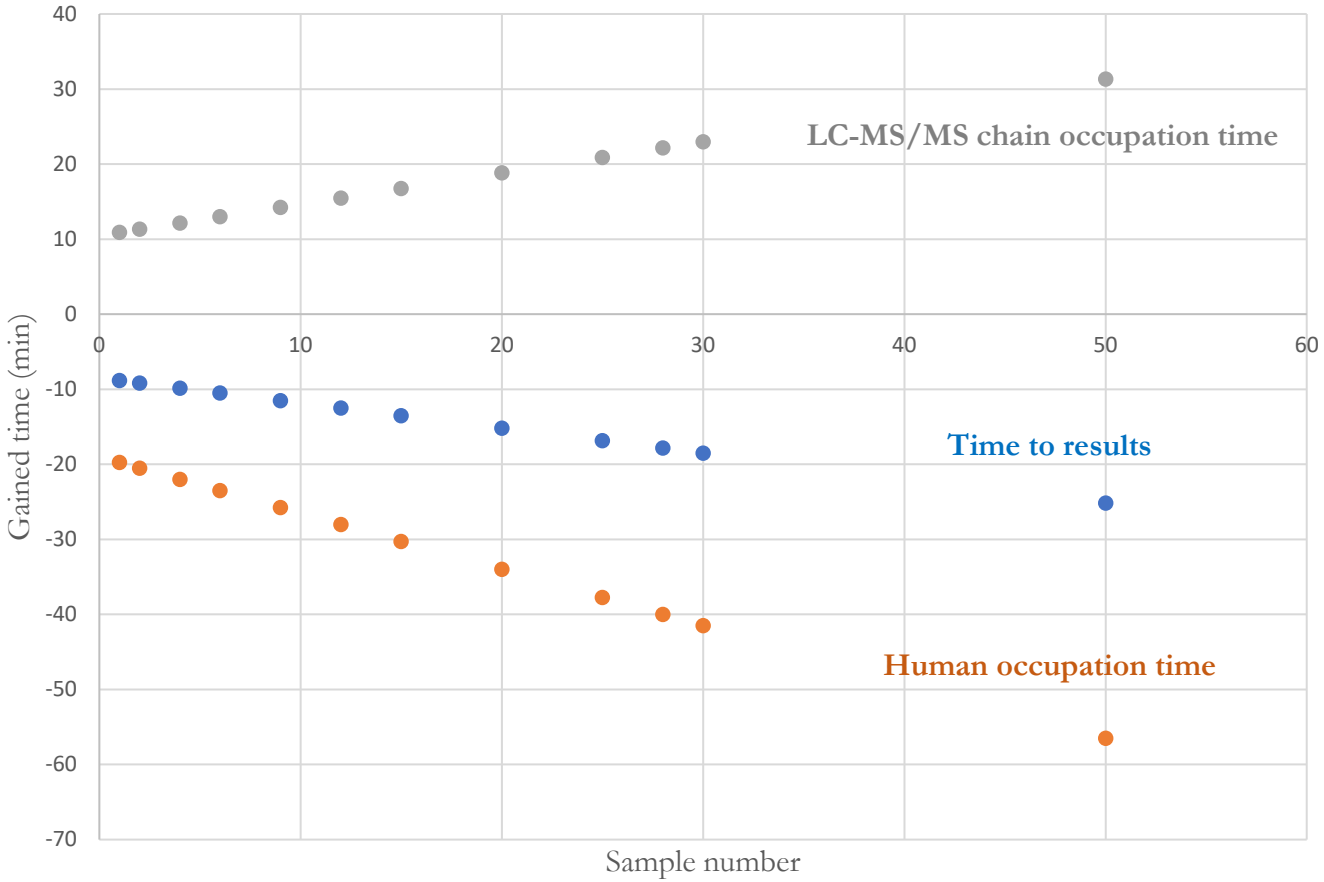
Time gain when compared to a manual extraction procedure of the DBS

(ie, LCMS with or without CLAM 2030)

Parameter	Automated method time gain
Human occupation time	**- 50/70 %**
LC-MS/MS chain occupation time	**+ 10/15 %**
Time to results	**- 10/20 %**

"Automation has to dominate where it is most effective, while creative tasks will remain the domain of humans"

JG Licouski 1985



Time gain for automated method preparation and analysis according to sample number

Application in transplant patients

(ie. transplant patients with a classical blood sample and a DBS collected simultaneously)

TAC-KIT-EASY
Version n° *1.0 du 11/05/2021*

Use of a microsampling kit for the therapeutic drug monitoring of tacrolimus in kidney and liver transplant patients

TAC-KIT-EASY

87RI21_0019

PROTOCOLE DE RECHERCHE NON INTERVENTIONNELLE IMPLIQUANT LA PERSONNE HUMAINE

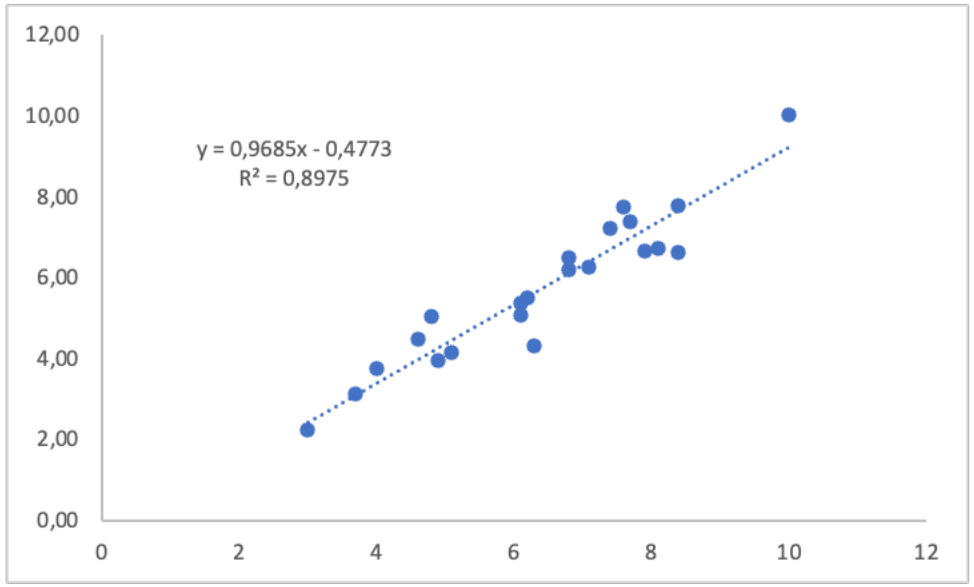
Version n°*1.0* du *11/05/2021*

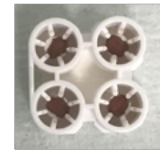
Numéro ID-RCB : 2021-A01361-40

Tacrolimus concentrations were measured after manual extraction of the DBS using a LCMS 8060

Preliminary results obtained in 21 renal and hepatic transplant patients

Venous sample





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CONCLUSION

1
Development of an automated extraction procedure

2
Full validation of the whole procedure

3
Application in renal and hepatic transplant patients enrolled in a clinical trial

Gracias por su atención

CHU
Centre hospitalier universitaire
Limoges