

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

Prof. Franck SAINT-MARCOUX, Naïma TAFZI, Gauthier ROSÉ, Dr Caroline MONCHAUD

Department of pharmacology and toxicology

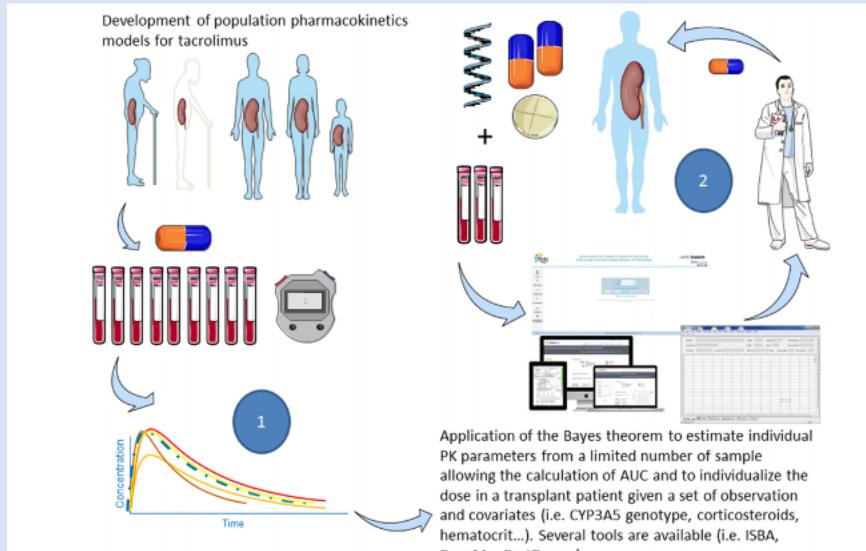
Limoges University Hospital, France

Dr. Florian LAPIERRE (Trajan Scientific and Medical®)
Stéphane MOREAU (Shimadzu Europa GmbH)

CONTEXT: The drug to measure

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

Jean-Baptiste Woillard ^{a,b,c,*}, Franck Saint-Marcoux ^{a,b,c}, Jean Debord ^{a,b,c},
Anders Åsberg ^{d,e}



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

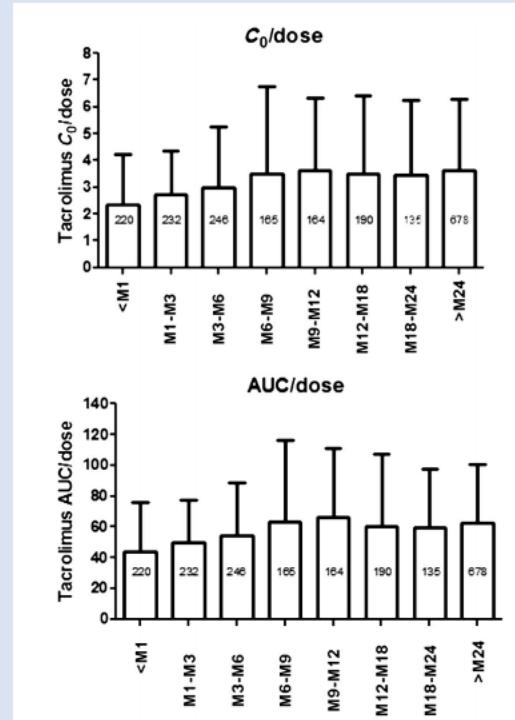
Tacrolimus

- 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

Franck Saint-Marcoux, PhD, PharmD,^{*†} Jean-Baptiste Woillard, PharmD, PhD,^{*†}
Camille Jurado, PharmD,^{*} and Pierre Marquet, MD, PhD^{*†}

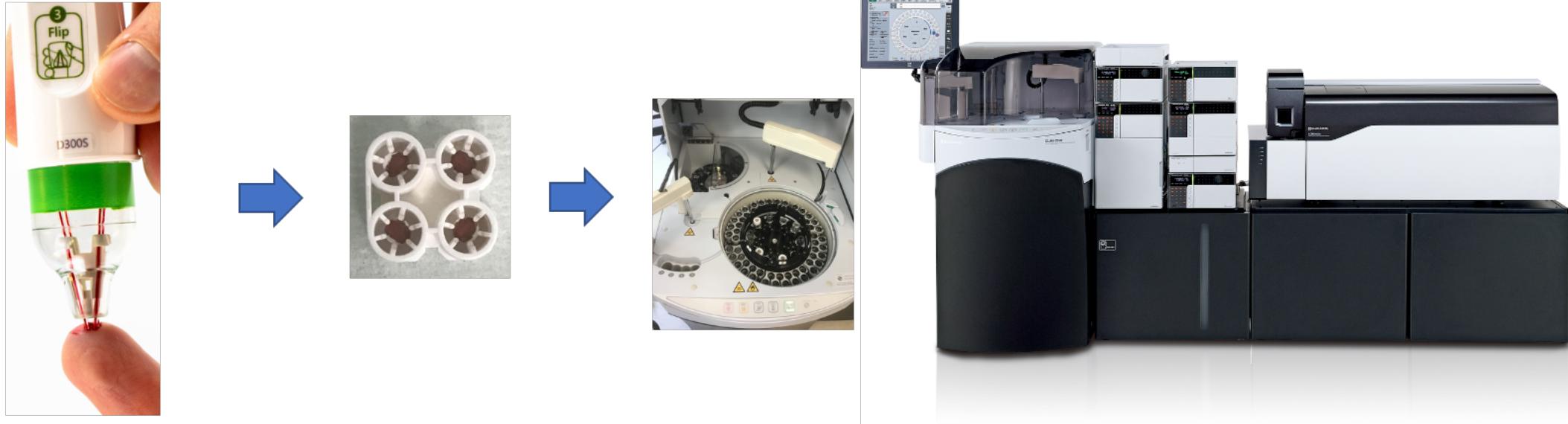
Ther Drug Monit • Volume 35, Number 3, June 2013



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

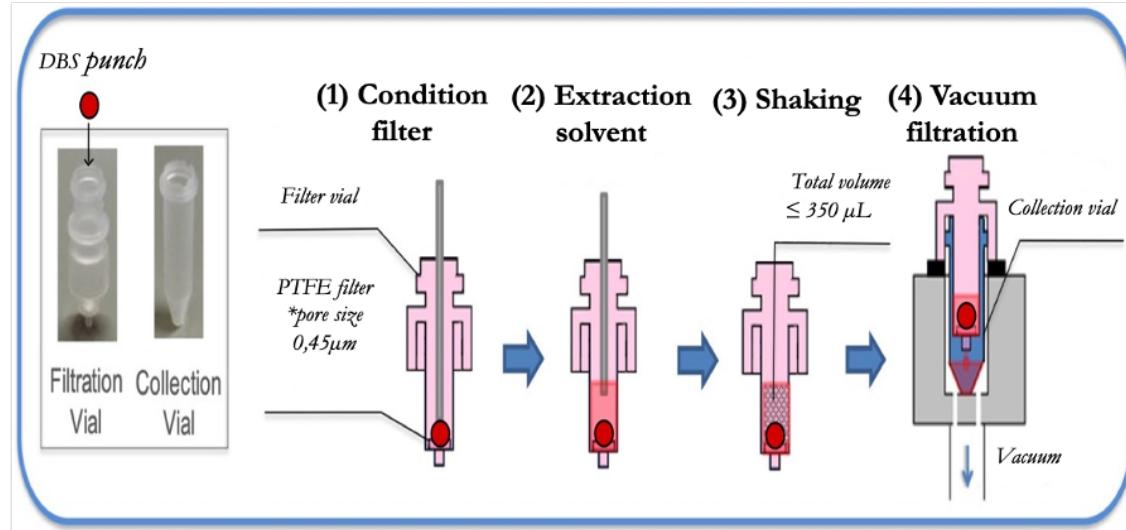
Application in renal and hepatic transplant patients enrolled in a clinical trial

The validation procedure guidelines

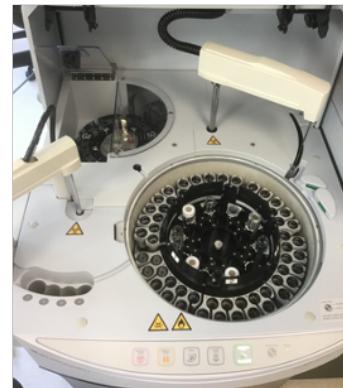
Validation according to EMA and LATDMCT¹

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

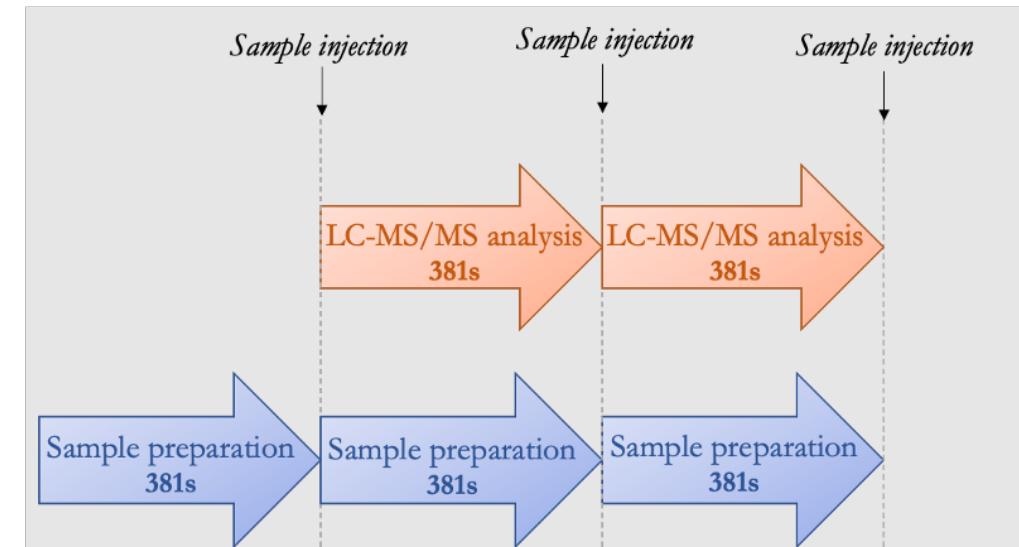
Results: The optimized fully-automated extraction procedure



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 ($\mu\text{g/L}$)		Results	Acceptance criteria (%)
1	Accuracy	-12,1	≤ 20
3		-11,0	
40		-4,6	≤ 15
75		-2,2	
1	Precision CV (%)	9,3	≤ 20
3		5,4	
40		5,8	≤ 15
75		4,4	



Inter-run accuracy and precision

Target concentration value ($\mu\text{g/L}$)		Results	Acceptance criteria (%)
1	Accuracy	-3,5	≤ 20
3		-7,7	
40		-10,7	≤ 15
75		-10,6	
1	Precision CV (%)	5,9	≤ 20
3		5,5	
40		6,9	≤ 15
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	
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	

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

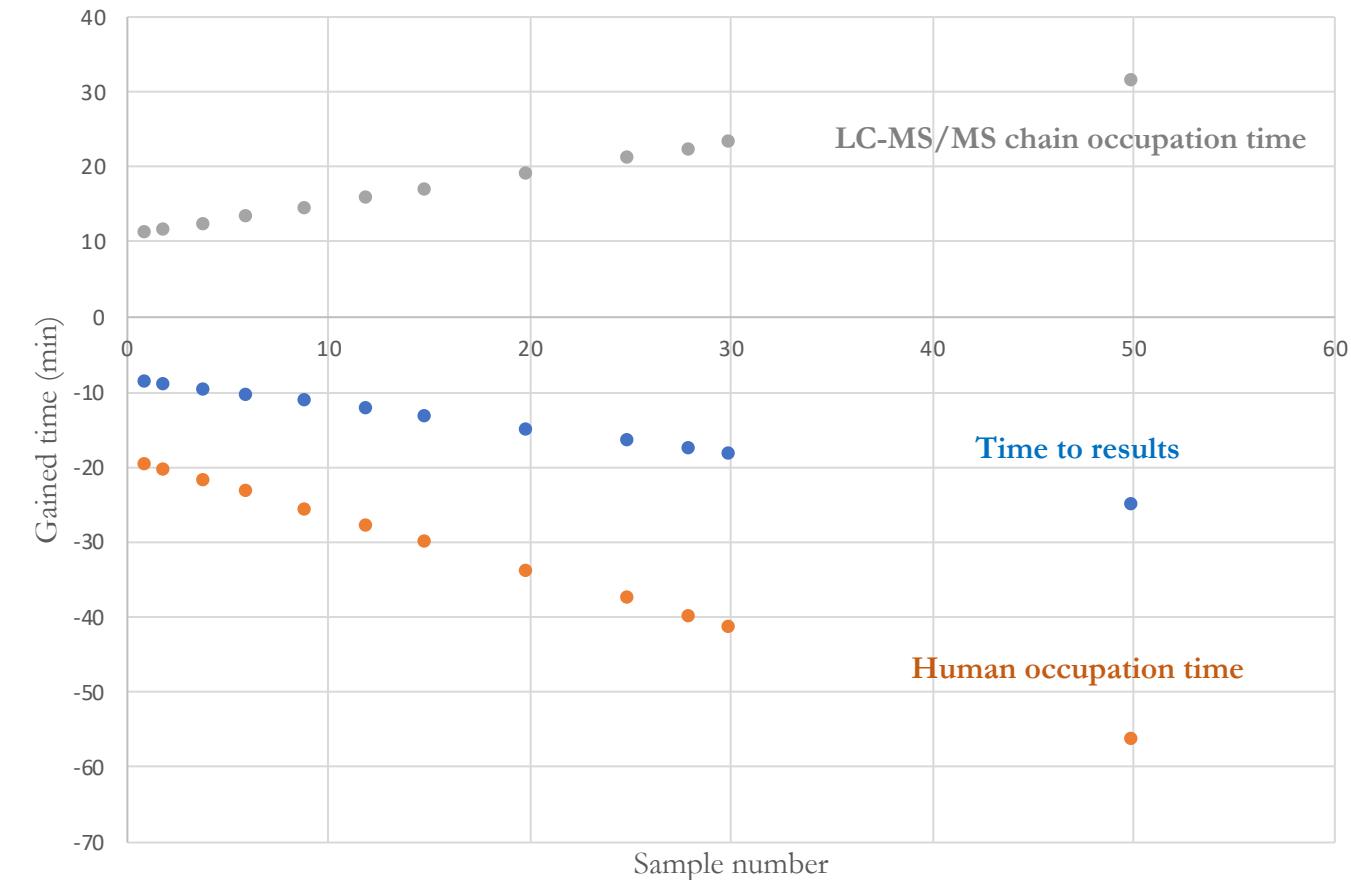
(ie, LCMS with or without CLAM 2030)

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)



GIRC SOH
GROUPE D'INVESTIGATION
SUD-OUEST OUTRE-MER HOSPITALIER

CHU
Centre hospitalier universitaire
Limoges

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

Instituts thématiques
Inserm
Institut national de la santé et de la recherche médicale

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

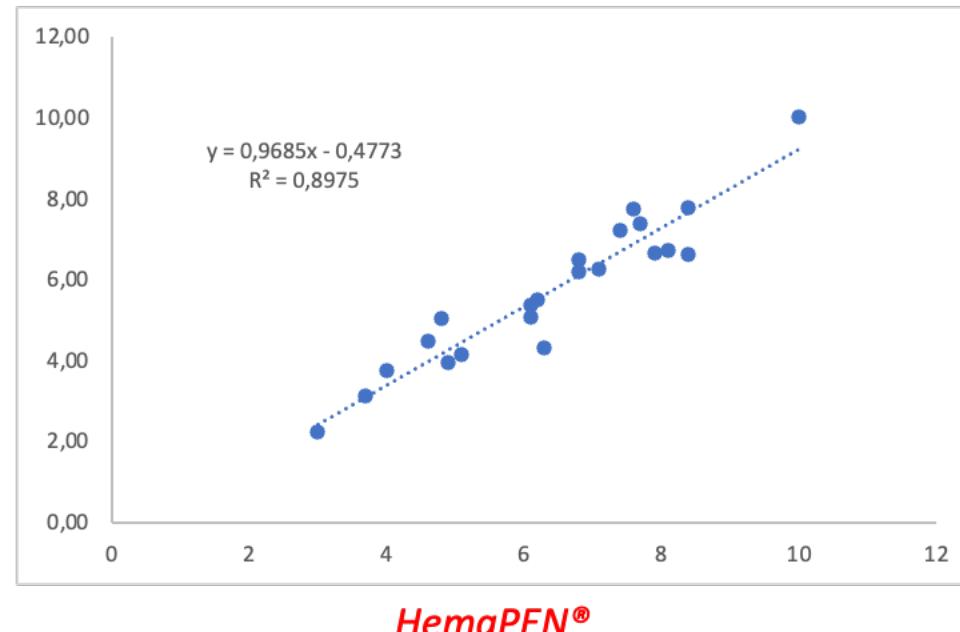
Version n°1.0 du 11/05/2021

Numéro ID-RCB : 2021-A01361-40



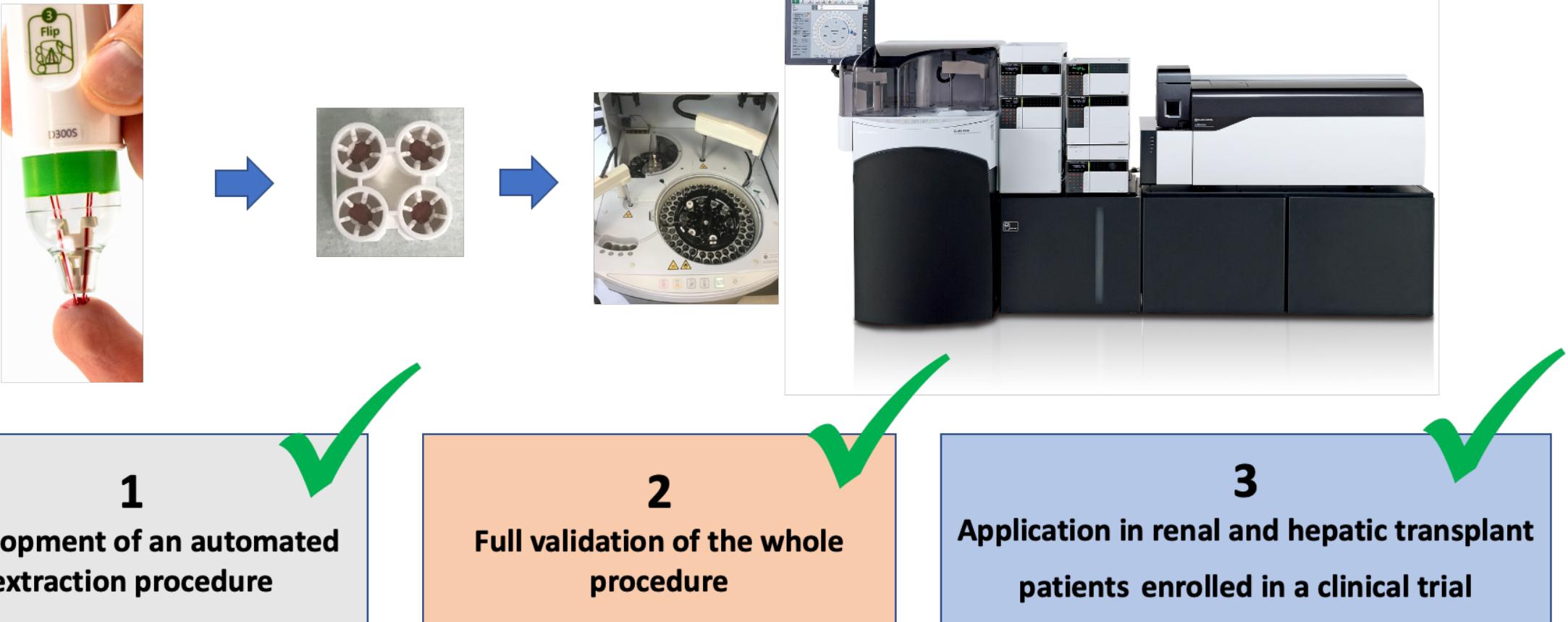
Preliminary results obtained in 21 renal and hepatic transplant patients

Venous sample

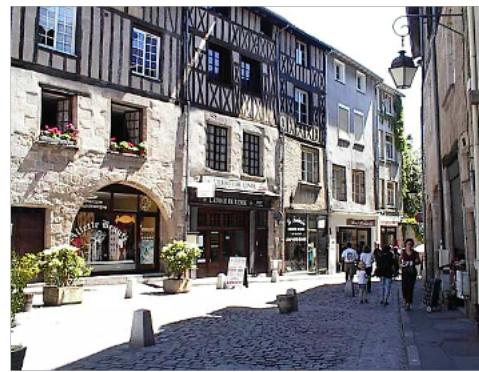


Tacrolimus concentrations were measured after manual extraction of the DBS using a LCMS 8060

CONCLUSION



Gracias por su atención



Supplemental data

Spectral conditions optimisation

- Parent ion: $[M + NH_4]^+$
- Analyte, Tacrolimus and isotope, [$^{13}C, D_2$ -Tacrolimus], infusion

Compound	Precursor m/z	Product m/z	Pause Time (msec)	Dwell Time (msec)	Q1 Pre Bias (V)	CE (collision energy, V)	Q3 Pre Bias (V)
Tacrolimus	821,25	768,40	1,0	20,0	-28,0	-22,0	-28,0
		786,40			-30,0	-18,0	-28,0
		576,30			-30,0	-25,0	-20,0
$^{13}C, D_2$ -Tacrolimus	824,45	771,40			-20,0	-22,0	-28,0
		789,40			-20,0	-18,0	-28,0

- Acquisition in MRM mode, positive ionisation in electrospray

Chromatographic conditions optimisation

Column	Characteristic	Column reference	Stationary phase composition	Granulometry (µm)	Porosity (Å)	Supplier
SPE	POROS 20 R1 2.1mm 30 mm	1102412	Poly[styrenevinyl benzene]	20	4000	Life technologies
Analytical	Luna Phenyl- Hexyl 5 µm; 50 x 2mm	00B 4257-B0	Hexyl linked phenyl with TMS endcapping	5	100	Phenomenex

Optimised phases for preparation and separation

Phase	Solvent A proportion	Solvent B proportion	Phase	Solvent proportion	Buffer proportion	Buffer molarity
Charging	90 % H ₂ O	10 % MeOH	Mobile	90 % MeOH	10 % Ammonium formate	3 mM

On-line SPE

