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Application News

Fully automated sample preparation module: CLAM™-2030
Triple quadrupole mass spectrometer: LCMS-8060

Automated measurement of tacrolimus from hemaPEN dried blood spots using CLAM-2030

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User Benefits

- Micro-sampling technology such as hemaPEN allows more frequent and less invasive blood sampling
- Fully automated sample preparation for LC/MS/MS system
- The use of solid matrices with the CLAM-2030 beyond the current classic liquid samples

■ Introduction

The CLAM-2030 (Shimadzu) is a module that automates all analytical process steps and is directly connected to a liquid chromatography system to enable seamless analysis and management of samples (**Figure 1**).

The module can today autonomously handle various types of wet samples such as whole blood (WB), urine, serum, or plasma. The CLAM-2030's procedure results in an overall quicker analysis, while reducing the risk of human errors.(1) Furthermore, the module allows technicians to optimize their time during routine analysis, by removing key manual preparation steps. However, such automation has not been employed yet to prepare solid samples, such as dried blood spot (DBS).

Over the past few years, a growing interest in patient-centric micro-sampling has allowed DBS technology to position itself as an alternative substrate to wet samples.(2) Innovative patient-centric sampling solutions have been developed to address unmet markets needs that cannot be served by standard phlebotomy. These tools enable the collection of low volumes of blood samples remotely at the convenience and comfort of the patient, while being less invasive than venipuncture, and to provide physicians the confident results to longitudinally monitor markers of health.(3)

The hemaPEN (Trajan Scientific and Medical) is one such patient-centric devices that allows rapid and quality remote-sampling (**Figure 2**). With its intuitive pen like-design, the hemaPEN collects four samples from any single source, rapidly and with minimal volume collection. Its capillary based technology ensures accurate and precise volume collection, while pre-punched DBS substrates store the samples inside a contained embodiment. The hemaPEN enables the precise quantification of analytes with high confidence, by simplifying the sample collection procedure and by maintaining sample integrity throughout the complete analytical workflow, from patient to analysis.

The purpose of this study is to integrate hemaPEN DBS sample into a following workflow, demonstrating the possibility to use the CLAM-2030 with solid samples. Therefore, tacrolimus (TAC) was chosen as marker of interest due to its relatively complex and variable pharmacokinetics. Its narrow therapeutic index and usage in longitudinal monitoring of transplanted patients that require frequent and precise results to adjust their therapy makes TAC an ideal candidate for further clinical research implementation. In conclusion, TAC's analysis on WB is already well-established,(4) even with a CLAM-2030 preparation.(5)

Figure 1 – CLAM™-2030, fully automated sample preparation module, directly connected to LC/MS/MS system

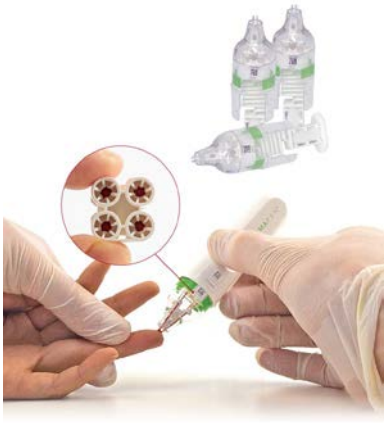

Figure 2 – hemaPEN and hemaPEN-QC patient-centric micro-sampling device

■ Method

A bioanalytical method for the quantification of TAC on volumetric dried blood spots (DBS) using liquid chromatography, positive electrospray ionization mode (ESI+), coupled with tandem mass spectrometry was developed and validated according to International Association of Therapeutic Drug Monitoring and Clinical Toxicology (IATDMCT) and European Medicines Agency (EMA) guidelines.[6], [7] Both the hemaPEN and hemaPEN-QC – a laboratory-centric version of the hemaPEN – were used to collect and store the blood sample. Each hemaPEN provided 4 DBS discs, each disc containing a volume of 2.74 µL of blood and located inside a plastic DBS cartridge. The analysis of TAC was performed using a fully automatic sample preparation module connected to a LC/MS/MS system (CLAM-2030 & LCMS-8060, Shimadzu). All automated workflow and MS parameters were first optimised and set before validating the extraction method. The treated samples were trapped on an online-SPE column using an isocratic charging phase and then separated at 60° C with an isocratic mode on an analytical column in 1.54 min. Conditions resulting in the more gaussian and intense peaks that allowed a lower limit of quantification of 1 µg/L were chosen (**Table 1**). Calibration ranged from 1 to 100 µg/L. Acquisition was performed in positive ionisation MRM mode using LC/MS/MS (**Table 2**). Quantification was done using stable isotope labelled internal standards (^{13}C-D_2 TAC). In this study, the validated parameters were: intra- and inter- assay, accuracy and precision, stability at different storage conditions, dilution integrity, selectivity, recovery, and carry-over. Matrix and hematocrit effects were evaluated in a parallel manual sample preparation, as they do not depend on the preparation method but on the matrix itself.

Table 1 – Liquid Chromatography Conditions

Liquid chromatograph	
System	: Nexera™ X2
Trap Column	: Reversed-Phase Column
Analytical Column	: Luna Phenyl-Hexyl (50 mm × 2 mm I.D., 5 µm)
Temperature	: 60 °C
Injection volume	: 10 µL
Mobile phase (Load)	: Water : MeOH= 90 : 10
Mobile Phase (Elution)	: MeOH : 3mM Ammonium formate in water =90 : 10
Flow rate (Load)	: 2.0 mL/min
Flow rate (Elution)	: 0.6 mL/min

Table 2 – Mass Spectrometry Conditions

Mass spectrometer	
System	: LCMS-8060
Nebulizing gas	: 3 L/min
Drying gas	: 10 L/min
Heating gas	: 10 L/min
DL temp	: 250 °C
Heat block temp	: 400 °C
Interface temp	: 300 °C
CID	: 250 kPa

■ Sample preparation for CLAM-2030

Collection of pre-spiked WB was performed using the hemaPEN-QC and left to dry for two hours minimum prior processing. Patient-centric hemaPEN were used to validate the stability parameters. For each sample, one DBS disc was pushed out manually from the hemaPEN DBS cartridge into the filtration vial, itself placed atop of a dedicated collection vial (see **Figure 3**). Then a sequential automated sample preparation and injection proceeded: briefly, a 20 µL of MeOH was added to condition the filtration vial's filter and 75 µL of extraction solvent MeOH / H_2O (80% / 20%), with internal standard at 2.5 mg/L was further added. Then, the vial was shaked for 120s at 2000 rpm prior a 60s vacuum filtration (**Figure 3**). The CLAM's automated arm discarded the filtration vial and moved the collection vial to the liquid chromatography's injector.

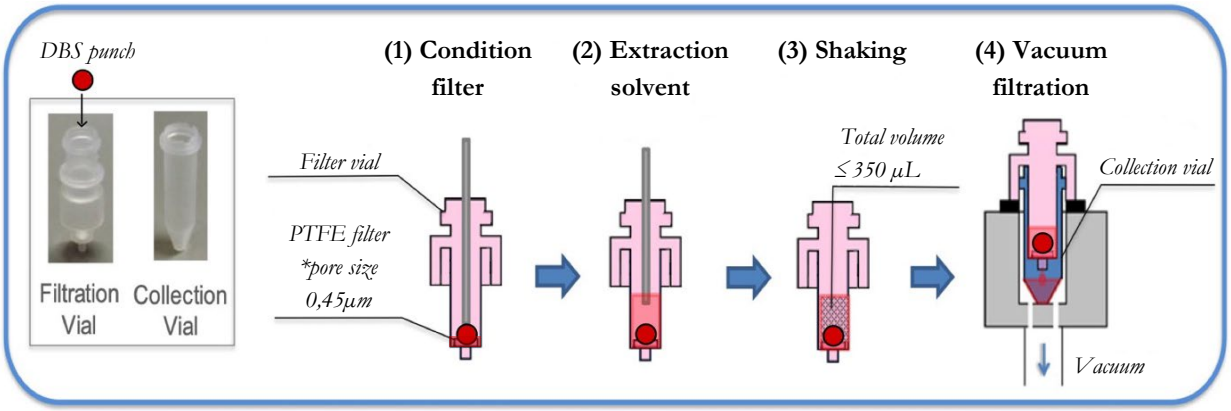

Figure 3 - Solid sample preparation steps on the CLAM-2030

■ Results and discussion

The assay was linear from 1 to 100 µg/L (**Figure 4**), with coefficient of determination (r^2) > 0.998. Intra-assay and inter-assay accuracies and precisions were all less than 15% (20% for the lower limit of quantification) (**Table 3**). TAC on hemaPEN DBS sample was stable for at least 14 days at 4° C, and at least 72h at 60° C (**Table 4**). No carry-over was observed. Selectivity and dilution integrity were validated as well. The mean recovery of TAC was 87.34% (**Table 5**). No matrix effects were observed with a manual preparation. Hematocrit had no effects on the results (data not shown).

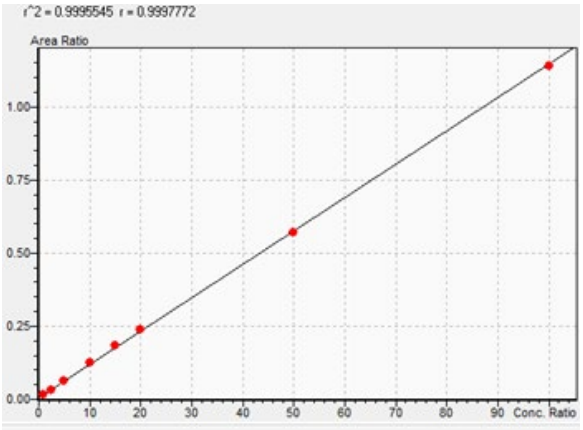

Figure 4 - Calibration curve for TAC, extracted on the CLAM-2030 and analysed on the LCMS-8060

Table 3 - Intra-run and inter-run accuracy and precision results.

Concentration (µg.L^{-1})	Intra-assay		Inter-assay		Acceptance criteria (%)
	Accuracy (%)	Precision (%)	Accuracy (%)	Precision (%)	
1	-12.10	9.47	-3.47	10.03	≤ 20
3	-10.97	5.46	-7.77	5.91	≤ 15
40	-4.63	5.88	-10.72	6.91	
75	-2.23	4.47	-10.63	3.67	

Table 4 - Stability evaluated with three different conditions: conservation at +60°C for three days and +4°C for one week and two weeks

Concentration (µg.L^{-1})	Accuracy			Acceptance criteria (%)
	7 days at +4° C (%)	14 days at +4° C (%)	3 days at +60° C (%)	
3	1.69	5.06	-9.86	≤ 15
75	6.51	8.66	-8.69	

Table 5 - Dilution integrity, selectivity, recovery, and carry-over evaluation

Parameter evaluated		CLAM validation results		Acceptance criteria (%)
Dilution integrity	Precision Coefficient of variation (%)	5.13		≤ 15
	Accuracy Average relative error (%)	-2.30		
Selectivity	$\frac{\text{Mean signal}}{\text{Tacrolimus signal at LLOQ}}$ (%)	No signal		≤ 20
	$\frac{\text{Mean signal}}{\text{Internal standard signal}}$ (%)	0.10		≤ 5
Recovery	Coefficient of variation (%)	4.61 (QCL)	4.45 (QCH)	≤ 15
Carry-over	$\frac{\text{Mean signal}}{\text{Tacrolimus signal at LLOQ}}$ (%)	4.56		≤ 20
	$\frac{\text{Mean signal}}{\text{Internal standard signal}}$ (%)	No signal		≤ 5

■ Conclusion

This study demonstrated the use of solid matrices with the CLAM-2030 beyond the current classic liquid ones. A reliable method has thus been established for the quantification of TAC, sampled by hemaPEN, with an automated extraction on CLAM-2030. Patient-centric micro-sampling technology such as hemaPEN allows more frequent and less invasive blood sampling for blood analysis, removing the necessity for the patient to go to the hospital or blood collection centre. This is more specifically relevant today in this new world of social distancing.

Such technology could also lead to an overall reduced cost on the healthcare system, since patient-centric micro-sampling device is cheaper than a day of hospitalisation or a home health nurse. The routine analytical laboratories would benefit the CLAM-2030 for its automated processes from pre-treatment to LC/MS/MS analysis, resulting in quicker data availability for the medical doctors and efficient resource allocation workflow for the laboratory. The laboratory would also benefit from reduced operator errors during sample manipulation. A clinical validation project, validated by an ethical committee, is going to clinically validate the overall benefits of the hemaPEN / CLAM-2030 workflow on transplanted patients.

■ Acknowledgement

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