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## A simple and fast liquid chromatography tandem mass spectrometry method to determine cyclosporine A concentrations in endomyocardial biopsies

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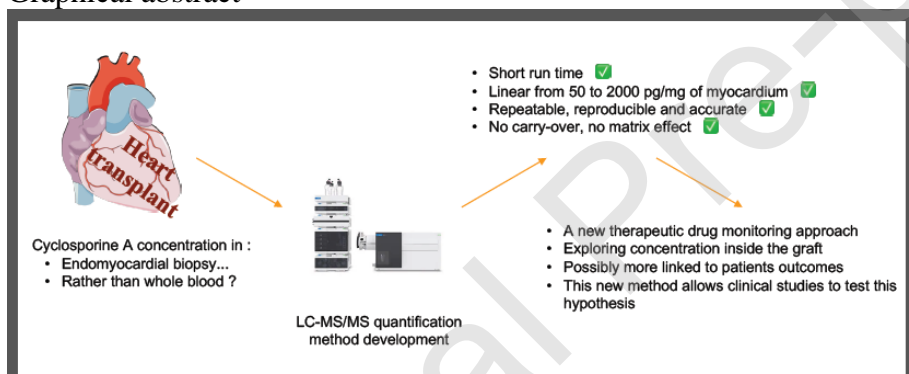
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### Graphical abstract



### Highlights

- A method to determine cyclosporine A concentration in heart tissue was developed
- This method is precise, accurate, reliable, and fast
- No matrix effect, carry-over, or interference with co-prescribed drugs was found
- The method was applied to analysis of human endomyocardial biopsies
- This method can be applied to clinic research protocols

## Abstract

Measuring cyclosporine A (CsA), an immunosuppressive drug used to prevent heart transplant rejection, concentrations in myocardial biopsies might be more informative than its measurement in whole blood. Therefore, a fast, accurate and reproducible method to determine CsA concentration in this complex matrix is needed. We report the validation of a liquid chromatography tandem mass spectrometry method to measure CsA concentration in heart parenchyma, applicable to everyday practice. The method was found to be precise, accurate, reproducible, specific of CsA, and without any matrix effect or carry-over. The lower limit of quantification was 50 pg of CsA in myocardium. The method was linear up to 2000 pg of CsA in myocardium. Samples were found stable for one year at – 80°C. At last, 40 drugs which could be prescribed to heart transplant recipients were tested with the method and showed no interference with CsA signal. The method was suitable to quantify CsA in endomyocardial biopsies from heart transplanted patients. This method allows designing clinical studies aiming at exploring the relationship between CsA intra-graft concentrations and outcome.

Abbreviations: acute rejection (AR); adverse events (AE); centre de ressources biologiques (CRB); coefficient of variation (CV); cyclosporine A (CsA); European Medicine Agency (EMA); endomyocardial biopsies (EMB); heart transplantation (HT); high performance liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS); immunosuppressive drugs (ISD); interleukine-2 (IL-2); internal standard (IS); lower limit of quantification (LLOQ); mass spectrometry (MS); matrix factor (MF); peripheral blood mononuclear cells (PBMC); quality controls (QC); relative error (RE); standards (Std); therapeutic drug monitoring (TDM).

**Keywords:** Heart transplantation; cyclosporine A; mass spectrometry; therapeutic drug monitoring; graft concentration

## 1. Introduction

Heart transplantation (HT) is the best treatment for terminal heart failure condition. It is also a life-saving procedure<sup>1</sup>. Without immunosuppressive drugs (ISD) treatment, the natural history of a transplanted graft is to undergo immunologic rejection. The use of calcineurin inhibitors, such as cyclosporine A (CsA), as a treatment aiming at preventing graft rejection has nowadays widely reduced the rate of rejection.

CsA is a potent ISD but also displays a large inter-individual variability of its pharmacological effect, together with a narrow therapeutic index, leading to frequent adverse events (AE). Thanks to the established relationship between whole blood CsA concentrations and their effect, a therapeutic drug monitoring (TDM) of patient trough concentrations is mandatory to mitigate these AE. However, despite this intensive TDM aiming at improving outcomes of heart transplant patients, some of them still experience acute rejection (AR) or AE while having adequate whole blood CsA exposure.

Moreover, patient and graft survival are not improving anymore<sup>2</sup>. Alternative TDM methods are therefore needed to bridge the gap toward optimal ISD treatment.

CsA inhibits an enzyme located into the lymphocyte cytosol, the serine-threonine phosphatase calcineurin, which activates the nuclear factor of activated T cells, resulting in transcription of immunity-enhancing genes such as interleukine-2 (IL-2) and interferon  $\gamma$ . Calcineurin inhibition by CsA leads to decrease IL-2 synthesis, hence inhibiting T-cell mediated immunity<sup>3</sup>. In transplant recipient patients, Concentration of CsA in peripheral blood mononuclear cells (PBMC), leucocytes fraction enriched in lymphocytes, is not well related to whole blood trough concentrations<sup>4</sup>. Thus, intracellular drug concentrations of CsA, might be more relevant than whole blood as a surrogate of ISD effect in transplanted patients<sup>5</sup>. It has also been shown, in liver transplantation, that ISD intracellular concentrations and graft tissue concentrations (measured into biopsies) are tightly correlated together as well as with the onset and severity of AR<sup>6</sup>. In HT, patients benefit from several endomyocardial biopsies (EMB) but to date, very few works has been conducted on evaluating tissular ISD concentrations and a simple and fast method aiming at measuring CsA concentrations in EMB is still lacking. Such a method should be developed, and TDM of CsA concentrations inside the graft could then be explored as a potential biomarker of ISD effect.

The objective of this work was to develop and to validate a fast and simple method to determine tissular CsA levels into EMB. Ultimately, TDM of ISD inside the graft could, as a complementary method to whole blood measurement, help reducing even more the rate of patients experiencing heart transplant AR or AE.

## 2. Materials and methods

### 2.1. Regents and chemicals

CsA and ascomycin were purchased from Sigma Aldrich (Saint-Louis, MO, US). Their chemical structures are shown in figure 1<sup>7,8</sup>. Acetonitrile were obtained from Carlo Erba (Val-de-Reuil, France). Zinc sulfate was purchased from Merck (Darmstadt, Germany) and ammonium sulfate provided from EMSURE (Batch, Germany). Water used for the experiments was of mass spectrometry (MS) grade and was provided by Thermo Fisher Scientific (Waltham, MA, US).

Due to the rarity of primary matrix and because of its similarity with human heart, heart tissues obtain from pig was used for preparation of standards (std) of calibration and quality controls (QC). A whole pig heart, weighting approximately 300g, was bought at a local delicatessen. Although we do not accessed precise information about the animal, it was a human consumption-destined pig, i.e. a young and healthy animal. Twelve hours after the animal was sacrificed, EMB biopsies were prepared and accurately weighted with an analytical balance (sensitivity of 0.1 mg) (Sartorius, Göttingen, Germany). Biopsies were crushed in water (10 mg of myocardium/ml of water) with a Polytron PT-

MR 2100, 230V (Kinematica, Lucerne, Switzerland) to prepare blank matrix for calibration curves and QC. Aliquots of crushed myocardium (10mg/mL) were then stored at  $-80^{\circ}\text{C}$ .

## 2.2. HPLC–MS/MS equipment and conditions

CsA quantification was performed using a high-performance liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) method. Apparatus was composed of a surveyor autosampler, a surveyor pump and a triple quadrupole TSQ Quantum Discovery max from Thermo Scientific (Waltham, MA, US). A C18 Hypersil™ Gold column (30 x 2.1 mm, 3  $\mu\text{m}$ ) fitted with a guard column (10 x 2.1 mm, 3  $\mu\text{m}$ ) (Thermo Electron, Dreieich, Germany) was used and maintained at a constant temperature of  $60^{\circ}\text{C}$ . The autosampler was set at  $10^{\circ}\text{C}$  during analysis. To measure CsA and ascomycin, elution was performed with a mobile phase of acetonitrile-ammonium acetate (pH=7; 10 mM in aqueous solution) (95:5 v/v). Isocratic elution was used and was set as follows: equilibration at initial conditions with a flow rate of 100  $\mu\text{L}/\text{min}$ , increase to 200  $\mu\text{L}/\text{min}$  from 0 min to 1 min, increase to 600  $\mu\text{L}/\text{min}$  from 1 min to 1.5 min, decrease to 200  $\mu\text{L}/\text{min}$  from 1.5 min to 2.5 min. The total run time was 2.5 min.

Parameters of the positive electrospray ionization interface were set as follow: the spray voltage was 4.5 kV, the sheath gas and auxiliary gas ( $\text{N}_2$ ) were 35 and 10 arbitrary units, respectively, and the capillary was heated at  $250^{\circ}\text{C}$ . The MS run was performed in the multiple reaction monitoring scanning mode. The adduct  $[\text{M} + \text{NH}_4^+]$  transitions  $m/z$  1219.98  $\rightarrow$   $m/z$  1203.01 (collision energy at 18 eV and Tube lens value at 185 V) was used to quantify CsA and the transition 1219.98  $\rightarrow$  1184.66 (collision energy at 37 eV and Tube lens value at 209 V) was monitored as confirmation. The adduct  $[\text{M} + \text{NH}_4^+]$  transitions  $m/z$  809.55  $\rightarrow$   $m/z$  756.52 (collision energy at 27 eV and Tube lens value at 129 V) was monitored to quantify ascomycin. Data acquisition and analysis were performed using LCquan software, version 2.5.6 (Thermo Fisher, San Jose, CA, USA).

## 2.3. Preparation of standards and QC samples

A stock solution of CsA was prepared in acetonitrile at a concentration of 1  $\mu\text{g}/\text{mL}$  and stored at  $-80^{\circ}\text{C}$ . The day of the analysis, an aliquot of this solution was diluted in acetonitrile to get working solution for each Std and QC level. Samples of 200  $\mu\text{L}$  of “blank” homogenized EMB (10 mg of biopsy /mL water) were then spiked with 50  $\mu\text{L}$  of the corresponding CsA working solutions to reach the following amounts of CsA: 50, 100, 200, 500, 1000, 2500 pg in calibration Std and 50, 125, 500, 2000 pg in QC samples. A lower limit of quantification (LLOQ) of 50 pg was targeted.

## 2.4. Sample preparation

Myocardium is a complex and protein-rich biological matrix. Although it is not biochemically similar to blood (a very common type of sample used in everyday TDM laboratory practice), we managed to adapt the method used for whole-blood CsA quantification to measurement of CsA in EMB. An aliquot of 250  $\mu\text{L}$  of spiked homogenized EMB was mixed with 50  $\mu\text{L}$  of internal standard (IS)

(ascomycin in acetonitrile (20 ng/mL). Then, CsA was extracted by protein precipitation with 800  $\mu$ L of acetonitrile-zinc sulphate in water (0.05 M) (1:1 v/v). Then, 200  $\mu$ L of ammonium sulfate in water (3 M) were added. Sample was vortex for 1 min and centrifuged at 3000 g for 10 min at 4°C. The supernatant (12  $\mu$ L) was then injected in the LC-MS/MS device.

## **2.5. Method validation**

The method was validated according to the European Medicine Agency (EMA) recommendations on bioanalytical methods validation<sup>9</sup>.

### **2.5.1. Selectivity**

Selectivity was investigated using blank samples and samples spiked with CsA at the expected LLOQ. The lack of endogenous interferences was checked by thoroughly examining the chromatograms of blank samples in the retention window containing the peaks of the CsA or ascomycin. For each sample, signal in blank should be less than 20 % of the LLOQ area. The LLOQ was defined as the lowest amount of CsA in a sample determined with precision and accuracy within  $\pm 20$  %. In addition, interferences coming from possible co-administered medications were evaluated for 40 drugs (table 1) by spiking blank samples with these compounds at high concentrations (using standard solutions corresponding to the upper limit of quantification of these compounds in human plasma). No significant peak should be observed at the MS transitions and the retention times of cyclosporine and ascomycin.

### **2.5.2. Calibration model**

Method calibration model was assessed by analyzing calibration samples spiked with CsA at 6 concentration levels. Calibration Std analysis was performed over 4 different days. Calibration curves were established by plotting the peak area ratio of the target compounds to the IS versus the nominal concentrations. A minimum of 75 % of the Std calibration samples had to be in the  $\pm 15$  % range (LLOQ:  $\pm 20$  %) of the nominal value.

### **2.5.3. Accuracy and precision**

Accuracy and precision were determined by replicate analysis of samples containing known amounts of CsA at each concentration level (LLOQ, low, medium and high). Within-day accuracy and precision were determined by analyzing QC in six replicates in a single validation batch. Between-day accuracy and precision were determined by repeating analysis of six replicates over three different days. Precision was assessed by calculating the coefficient of variation (CV). Between-day precision data were compared using one-way ANOVA for each of the four concentration levels (LLOQ, QC low, QC medium, QC high). Accuracy was reported as the relative error (RE) or bias to the theoretical value. The acceptance criteria for precision and accuracy were  $\pm 15$  % except for LLOQ which should not deviate more than 20 %.

#### 2.5.4. Carry-over

Carry-over was evaluated by sequentially injecting an upper limit of quantification sample (highest calibration Std) immediately followed by an extracted blank sample. The response in the blank matrix injection at the retention time region of CsA should be less than 20 % of the mean response at the LLOQ.

#### 2.5.5. Matrix effect

Matrix effect was evaluated by comparing signals from two types of samples, i) extracted matrix (EMB) samples spiked with CsA and ii) matrix-free samples (water) spiked with CsA at the same concentration. Matrix effect was evaluated at three QC levels in six replicates, by the formula  $ME (\%) = \frac{\text{CsA peak signal area in matrix sample} - \text{CsA peak signal area in matrix-free sample}}{(\text{CsA peak signal area in matrix-free sample})} * 100$ . For each concentration level, mean ME should be below 15 % to conclude that matrix effect was negligible. Due to the rarity of the matrix, samples from only one individual (the pig whose heart was used) were used to assess matrix effect. Additionally, a test of Mann & Whitney was performed to compare CsA signal in both groups (CsA areas in EMB matrix versus matrix-free).

#### 2.5.6. Stability

Long term stability of CsA in EMB was evaluated at four QC levels by comparing response ratios (Peak area of CsA normalized by IS) between QC samples stored for 1 year at – 80°C and QC samples prepared extemporaneously the day of the analysis.

### 2.6. Clinical application

The present method was applied to quantify CsA in EMB of heart transplant recipients. Samples were collected as part of INTRACAR study on-going in our center. The study was approved by local ethical committee (notice n° 19.148). All patients provided informed consent. Briefly, EMB were performed regularly as part of routine follow-up of heart transplant recipients. After histopathological analysis, instead of being discarded, EMB were kept at – 80°C for further CsA measurement. The day of the analysis, EMB were weighted and crushed and homogenized in HPLC grade water. CsA was extracted from 250µL of the homogenate according to the above described protocol. Amount of CsA in samples from patient was expressed in pg per mg of EMB.

## 3. Results

### 3.1. Selectivity

First, in the absence of CsA (blank sample), no peak was detected at the retention time and for the transition parameters set for CsA (fig. 2A). Secondly, no interfering chromatographic peak was detected at the retention time and for the transition parameters set for CsA, in samples spiked with



selected potentially co-administered drugs (table 1). An area that lower than 10000 arbitrary units was considered as background noise and no signal over this threshold was detected in these samples. The method therefore displayed excellent selectivity for CsA.

### 3.2. Calibration model and sensitivity

Subsequently to calibration curve analysis, a quadratic model was chosen with a weighing factor of  $1/x$ . Using this model, the obtained curves presented a mean  $r^2$  of  $0.9995 \pm 0.0004$  ( $n = 4$  on different days) and a minimal bias between observed and theoretical values (less than 15 % at each concentration levels) (fig S1, table 2). The LLOQ, represented by the lowest point of the calibration curve, was 50 pg of CsA. At this concentration, coefficients of variation (CV) did not exceed 20 %, as recommended.

### 3.3. Accuracy and precision

The method was found to be repeatable, reproducible and accurate. Indeed, within-day and between-day CV and RE were inferior to 15 % for every QC levels and less than 20 % for the LLOQ (table 3). CsA and the internal standard, ascomycine, both showed with clean peaks (fig. 2B and 2C).

### 3.4. Carry-over

Carry-over was found to be negligible as illustrated by the absence of signal obtained in a blank sample, analyzed just after the highest calibration point ( $n = 6$ ) (data not shown).

### 3.5. Matrix effect

Matrix effect, assessed by the relative difference between CsA signal in samples without or with EMB matrix, was lower than 15% (table 4). Moreover, Mann and Whitney tests performed on signals obtained with matrix or without matrix emphasized that there was no difference, for the 3 concentration levels tested. Thus, matrix effect was found to be negligible with this bioanalytical method.

### 3.6. Stability

CsA was found to be stable for one year at  $-80^\circ\text{C}$  in homogenized EMB. Relative bias between “old” samples and freshly prepared samples were all within acceptance criteria (13.5 % for LLOQ,  $-11.8$  % for QC low, 3.7 % for QC medium and  $-13.5$  % for QC high). Thus, study samples could be stored at  $-80^\circ\text{C}$  up to one year before analysis.

### 3.7. Clinical application

Nine EMB samples were successfully analyzed with the analytical method presented in this work. After normalization by the weight of the EMB, CsA concentration ranged from 124.4 to 883.5 pg/mg of biopsy (mean = 265.3 pg/mg of EMB). Results are presented in table 5.



#### 4. Discussion

We presented here a validated, fast, accurate and reliable method allowing determination of CsA concentrations in EMB. This method is also selective, as demonstrated by the absence of interference with numerous drugs usually co-prescribed to heart transplant recipients. The calibration model of the method is quadratic and not linear probably because we chose concentrations levels on a large range of concentrations. Despite this quadratic curve, repeatability on accuracy and precision fulfilled the acceptance criteria of the EMA guidelines. In our method, CsA and ascomycin elute very early. On the one hand, it is an advantage to perform high throughput analysis but on the other hand it could increase the risk of co-elution with matrix components. Fortunately, matrix effect was found to be negligible, meaning that matrix myocardium would not affect CsA detection. In addition, during the method development, we monitored MS signal from 0 to 3 min and observed that a lot of matrix was eluted before 0.5 min so before retention times of analytes. However, only one tissue source has been used for these experiments, despite the fact that EMA guidelines recommend to preferably evaluate relative matrix effect with biologic samples from at least 6 individuals. This is the principal limitation of this study. For practical and ethical purpose, only one pig heart was used for all the experiments, but the matrix effect was evaluated using six lots of the same matrix at three different concentrations, as it is alternatively proposed by the guidelines of the EMA. Consequently, assessment of relative matrix effect on different sources of matrix is lacking in the present work. In order to provide additional data, we are considering collecting residual human heart tissue on micro-biopsies from the removed heart of transplanted patient as an ancillary part of the clinical study which is going to start in our center.

Other detection methods of CsA have been reported<sup>10,11,12</sup>, however, we choose to use MS detection because of its high performances (sensitivity and selectivity), its suitability to measure immunosuppressive drugs and because of the experience of our team in LCMS technology.

Hence, it has been shown that ISD graft tissue concentrations can be tightly related to treatment outcome<sup>13</sup>. In a study published in liver transplant patients, Capron *et al.* have shown that intra-biopsy concentrations of tacrolimus were correlated with the onset of rejection and its severity<sup>6</sup>. Additionally, our team correlated tacrolimus concentration in bile (peri-tissular fluid) with neurologic side-effects in liver transplant recipients as well<sup>14</sup>. Intratissular ISD measurement can thus be used as an exploratory tool to evaluate drugs effects and represent a potential complementary test to usual whole blood concentration determination. Particularly appealing is the evaluation of the relationship between intra-graft and PBMC concentrations of ISD as the latter has also already been shown to be related to patient outcome<sup>5,6</sup>. A simple determination of ISD concentrations in PBMC could then be a surrogate of intra-graft concentrations but has still to be investigated in depth in heart transplant recipients.

EMB procedure is part of the usual follow up of heart transplant recipients and offers the possibility of accessing tissue materials available for drug monitoring<sup>15</sup>. Not to mention that a direct correlation could be made between the concentrations measured in tissue and the anatomopathological analysis.

To our knowledge, only one method aiming at determining CsA concentrations in EMB has been published to date<sup>16</sup>. In the study conducted by Robertsen and colleagues, the authors have developed a combined method to measure CsA and two of its metabolites in EMB biopsies. Due to the measurement of CsA metabolites, this method has supplemental preparation steps making it longer than ours but the main difference is the duration of the runs. Our method is substantially shorter with a total run of 2.5 minutes, versus 36 minutes in Robertsen's method. As we do not intend to measure metabolites, the present method is very simple and quite fast, allowing an easy implementation in a research program. In the work from Robertsen *et al.*, CsA amounts in patients EMB ranged from 216 to 833 pg/mg of heart biopsy. In the application of our method to analyze EMB samples from transplanted patients, we measured EMB-CsA amounts consistent with these values reported by another team. Thus, our method appears reliable to be used in future ambitious clinical programs aiming at improving the standard of care in TDM of cardiac transplant recipients.

At last, CsA is not the only calcineurin inhibitor used in transplantation. Tacrolimus, the other one, can also be prescribed to heart transplant recipients<sup>17</sup>. Very recently, Molinaro *et al.* developed a method to determine tacrolimus concentration in EMB, with an enzyme-digestion step. It can be an alternative procedure to mechanic crushing phase reported in our work<sup>18</sup>. Thus, it could be relevant in the future to develop multiplex analytical method to measure several immunosuppressive drugs in EMB as it is usually done in whole blood<sup>19</sup>.

## 5. Conclusion

A LC-MS/MS method has been developed and validated to determine CsA concentration in EMB as a tool for ISD monitoring optimization. The method is fast and easy, and can be used in further research program exploring the interest of intra-graft drug measurement in HT.

## CRedit author statement

CT : Conceptualization, Validation, Writing-Original Draft, Visualization, Project administration.  
GC : Formal analysis, Writing-Original Draft, Visualization. SL : Writing - Review & Editing. AB : Investigation, Visualization. YGJ : Investigation, Visualization. MJF : Investigation, Visualization.  
MCV : Writing - Review & Editing. EB : Writing - Review & Editing. FL : Conceptualization, Writing-Original Draft, Validation, Supervision.

## Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Disclosures

The authors declare no conflict of interest regarding this study.

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Fig 1: Chemical structure of cyclosporine A and ascomycin (according to <sup>7,8</sup>)

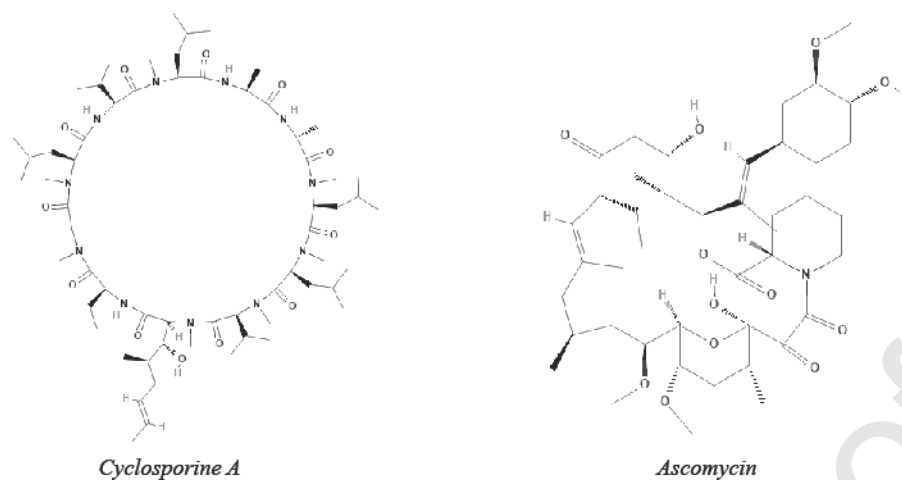
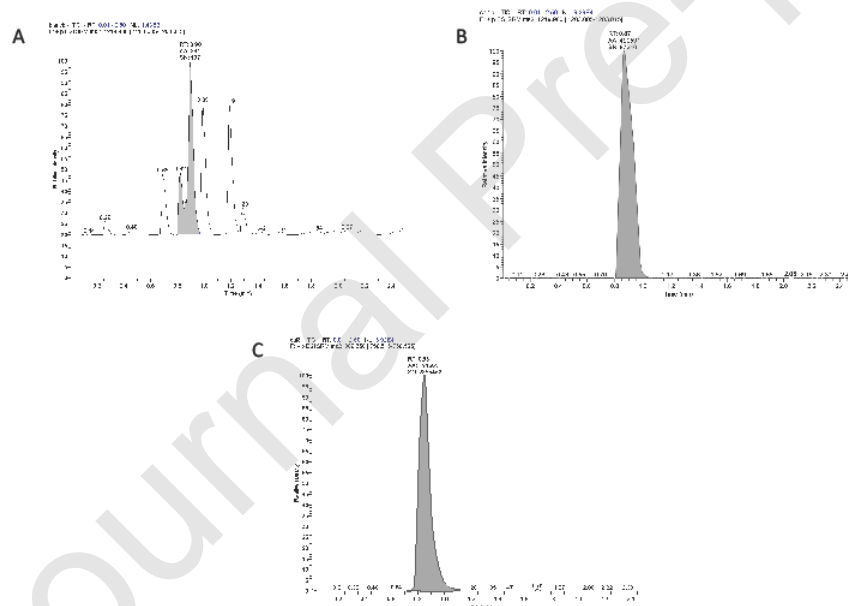


Fig 2: Example of a signal obtained with a blank sample, containing no cyclosporine (A), a sample containing cyclosporine 50 pg of CsA (LLOQ) (B), and a sample containing ascomycin, the internal standard, at the concentration mentioned in the material & methods part (C).



## Tables

**Table 1: List of drug used to test the method selectivity**

$\beta$ -lactams antibiotics	Antifungals	Antivirals	Antiretrovirals
<b>Penicillins:</b> Amoxicillin, Cloxacillin, Oxacillin, Penicillin G, Piperacillin, Temocillin	Fluconazole, Itraconazole, Posaconazole, Voriconazole, Isavuconazole	Aciclovir, Ganciclovir, Daclatasvir, Elbasvir, Grazoprevir, Ledipasvir, Sobosfuvir, Velpastasvir	Atazanavir, Darunavir, Dolutegravir, Efavirenz, Elvitegravir, Etravirine, Lopinavir, Maraviroc, Raltegravir, Rilpivirine, Ritonavir
<b>Cephalosporins:</b> Cefazolin, Cefepime, Cefotaxime, Ceftaroline, Ceftazidime, Ceftriaxone, Cefuroxime,			
<b>Carbapenems:</b> Ertapenem, Imipenem, Meropenem			

**Table 2: Calibration of quantification of CsA in EMB by HPLC-MS/MS (n = 4).**

	Theoretical CsA value (pg)						
	<b>50</b>	<b>100</b>	<b>200</b>	<b>500</b>	<b>1000</b>	<b>2500</b>	<b>r<sup>2</sup></b>
Mean (pg)	45.1	90.6	185.7	515.3	1018.6	2490.5	1.00
CV (%)	17.2	5.9	9.4	4.4	2.5	0.4	0.04
Bias (%)	-9.8	-9.4	-7.1	3.1	1.9	-0.4	

CsA: cyclosporine A; EMB: endomyocardial biopsy; LC-MS/MS: high performance liquid chromatography coupled with tandem mass spectrometry; r<sup>2</sup>: coefficient of determination of the calibration curve; CV: coefficient of variation. Bias is indicated compared to nominal concentration



**Table 3: Within-day and between-day accuracy and precision at 4 concentration levels on 3 different days** (n = 6 each).

Theoretical CsA value (pg)	Within day precision (CV%, n = 6)	Between day precision (CV%, n = 18)	Overall bias (% , n = 18)
LLOQ (50)	11.2	11.7	10.4
QC low (125)	7.6	8.2	2.5
QC medium (500)	7.6	8.8	0.1
QC high (2000)	7.1	9.6	-2.3

CsA: cyclosporine A; CV: coefficient of variation; LLOQ: low limit of quantification; QC: quality control. Overall bias is indicated compared to nominal concentration.

**Table 4: Matrix effect at 3 concentration levels (125, 500 and 2000 pg) (n = 6).**

	Theoretical CsA concentration (pg)	Matrix effect (%)	Mann-Withney test p-value
QC low	125	7.0	0.59
QC medium	500	7.3	0.59
QC high	2000	10.7	0.48

Either extracted blank matrix, or matrix-free sample were spiked with CsA at different concentrations (QC low, medium, high). CsA: cyclosporine A; QC: quality control.

**Table 5: Clinical application of the method to measurement of CsA in heart transplant EMB**

<b>Sample number</b>	<b>CsA pg / mg of EMB</b>	<b>Daily dose of CsA (mg)</b>
1	224.2	125
2	164.9	175
3	126.4	225
4	218.6	250
5	308.2	250
6	883.5	250
7	131.0	100
8	142.8	100
9	188.0	200

CsA: cyclosporine; EMB: endomyocardial biopsy