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Simultaneous quantification of cyclosporine, tacrolimus, sirolimus and everolimus in whole blood by liquid chromatography–electrospray mass spectrometry

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Abstract

Objectives: The aim of this work was to develop a selective method for the simultaneous quantification of cyclosporine, tacrolimus, sirolimus and everolimus in whole blood.

Design and methods: An automated on-line solid-phase extraction system coupled with liquid chromatography–mass spectrometry (LC–MS) was used. After a simple protein precipitation, the supernatant was load on a C8 column with a mobile phase composed of MeOH/H_2O (5/95 v/v), supplemented with formic acid 0.02% and sodium formate 1 μM. After column-switching, the analytes were transferred in the back-flush mode on a C18 column with MeOH/H_2O (65/35). The valve was then commuted to its initial position and the chromatographic separation was performed with a gradient of MeOH/H_2O (65/35–95/5). The sodium adducts $[M+Na]^+$ were monitored for quantification with an electrospray ionization-single quadrupole MS.

Results: The LC–MS assay was fully validated on a concentration range of 2.5–30 ng/mL for tacrolimus, sirolimus and everolimus and of 50–1500 ng/mL for cyclosporine, allowing a quantification of cyclosporine 2 h post-dose without sample dilution. Trueness, repeatability and intermediate precision were found to be satisfactory.

Conclusion: This method provided a selective, rapid and automated procedure that can be easily used for routine quantification of immunosuppressive drugs in most clinical laboratories.

Keywords: Immunosuppressants; LC–MS; Column-switching; Method validation

Introduction

Cyclosporine A (CsA), tacrolimus, sirolimus and everolimus (Fig. 1) are immunosuppressive drugs used in organ transplantation [1]. The calcineurin inhibitors (CsA and tacrolimus) block interleukin-2 production, leading to a decrease in T lymphocyte proliferation [2]. The mammalian target of rapamycin (sirolimus and everolimus) blocks interleukin-2 induced intracellular signal transduction, leading to an inhibition of T cell cycle progression [2]. Due to complementary mechanisms of action, these two classes of agents are often combined in the clinic, resulting in a synergistic effect [3]. Due to high pharmacokinetic variability, narrow therapeutic indexes and many potential drug–drug interactions, close monitoring of blood concentrations is required to prevent rejection and minimize toxicity [4]. It is currently recommended to quantify these drugs in whole blood, due to a high distribution in erythrocytes, between 40–60% for CsA [5], about 95% for tacrolimus [6] and sirolimus [7] and 75% for everolimus [8].

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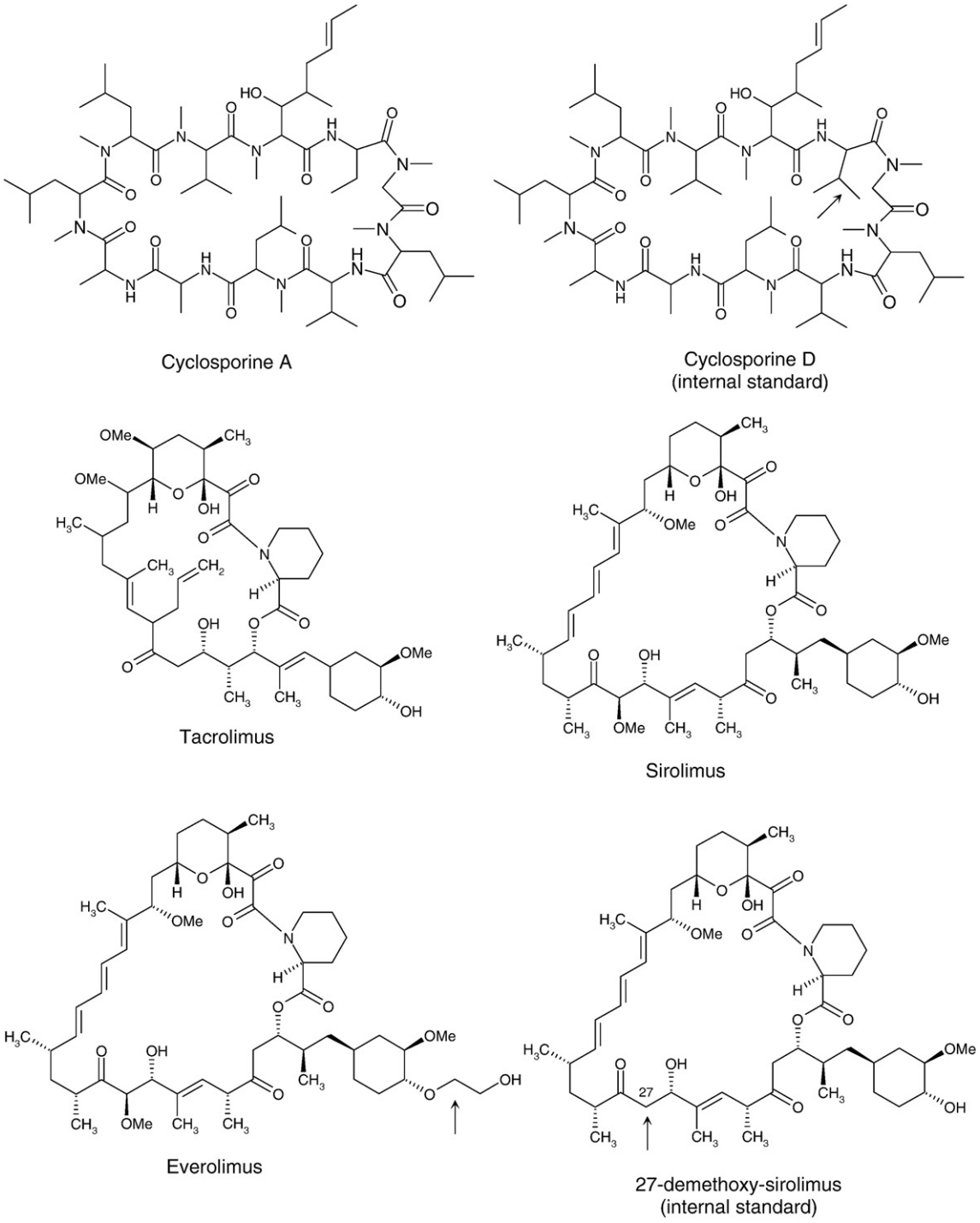

Fig. 1. Chemical structures of the studied drugs and the internal standards. The arrows indicate the structural differences.

Traditional monitoring of CsA used trough concentration (C_0) as the parameter of choice [9], however, it has been shown that the latter was not a good surrogate of the area under the concentrations time curve (AUC) and that a measurement 2 h after drug intake (C_2) correlated better with clinical outcome [10]. For the monitoring of tacrolimus [11], sirolimus [12] and everolimus [13], C_0 measurement currently remains the main approach. The usual whole blood therapeutic indexes, which depend on transplantation type, time after graft, association of other immunosuppressive agents and analytical methods, range between 100–400 ng/mL for CsA at C_0 [14], 600–1500 ng/mL for CsA at C_2 [15], 5–20 ng/mL for tacrolimus [14], 4–20 ng/mL for sirolimus [16] and 3–8 ng/mL for everolimus [17] at C_0.

Immunological methods are largely used in routine laboratories for quantification of immunosuppressive drugs. Despite an increase in the selectivity of these techniques, the disadvantage is that cross-reactions can occur with some metabolites, resulting in an overestimation of the measured drug