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Quantification of dapaconazole in human plasma using high-performance liquid chromatography coupled to tandem mass spectrometry: Application to a phase I study

Fernanda C. de Moraes[a,b], Samara F. Bittencourt[a,b], Elisa Perissutti[c], Francesco Frencentese[c], André M.M. Arruda[a], Lu Shi Chen[b], Tainah Babadópulos[a], Gilberto De Nucci[a,b,d,*]

[a] Galeno Research Unit, Latino Coelho St., 1301, Parquel Taquaral, 13087-010 Campinas, SP, Brazil
[b] Department of Pharmacology, Faculty of Medical Sciences, State University of Campinas (UNICAMP), Campinas, SP, Brazil
[c] Department of Pharmaceutical and Toxicological Chemistry, Federico II University, Naples, Italy
[d] Department of Pharmacology, Institute of Biomedical Sciences, University of Sao Paulo, Sao Paulo, SP, Brazil

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ABSTRACT

A simple, selective and sensitive method based on high-performance liquid chromatography coupled to tandem mass spectrometry (HPLC–MS/MS) has been developed for the determination of dapaconazole in human plasma using tioconazole as internal standard. The drugs were extracted from plasma by liquid–liquid extraction with ether/hexane (80/20, v/v). The chromatography separation was performed on a Genesis® C18 reversed phase analytical column 4 μm (100 × 2.1 mm i.d.) with a mobile phase of methanol/acetonitrile/water (80/10/10, v/v/v) + ammonium acetate (0.5 mM). Dapaconazole was quantified using a mass spectrometer with an electrospray source in the ESI positive mode (ES+) configured for multiple reaction monitoring (MRM) to monitor the transitions 415.1 > 159.2 and 387.0 > 131.0 for dapaconazole and tioconazole, respectively. The method had a chromatography run time of 3.8 min and a linear calibration curve over the range 0.2–100 ng/mL (r = 0.9998). The lower limit of quantification (LLOQ) was 0.2 ng/mL. The precision and accuracy values of the assay were within ±10%. The stability tests indicate no significant degradation under the conditions of the experiment. This method was used for a phase I study of topical administration of dapaconazole tosylate in healthy human male volunteers.

1. Introduction

Azole antifungal drugs are the most widely antifungal agents used in clinical practice. Woolley [1] described benzimidazole, the first imidazole antifungal compound. In the late 1960s, three new topical antifungal were introduced: clotrimazole, from Bayer AG (Germany) and miconazole and econazole both from Janssen Pharmaceutica (Belgium). With the introduction of clotrimazole researchers became more interested in antifungal activity of azole agents [2].

Until the 1990s, resistance to the azole antifungals was uncommon. However, because azoles are the most commonly antifungal agents used in clinical settings, resistance to these drugs has developed and this has become a significant problem in the treatment of fungal infections, including invasive and superficial mycoses [3,4]. The emergence of resistance generates a need for new drugs and their development involves the discovery of more selective targets, together with a reduction in drug toxicity, adverse effects and drug interactions [5,6].

Dapaconazole (Fig. 1A), chemical name (1-(2-(2,4-dichlorophenyl)-2-(4-(trifluoromethyl)benzyloxy)ethyl)-1*H*-imidazole, CAS 1269726-67-1) is a novel imidazole [7], with potent effects on a range of pathogenic fungi. The full clinical characterization of dapaconazole requires a robust and sensitive chemical assay, to allow adequate pharmacokinetic studies to be performed.

* Corresponding author at: State University of Campinas (UNICAMP), Department of Pharmacology, Faculty of Medical Sciences, 415 Jesuino Marcondes Machado Ave, Campinas 13092-320, SP, Brazil. Tel.: +55 19 3251 6928; fax: +55 19 3252 1516.
E-mail addresses: denucci@gilbertodenucci.com, denucci@dglnet.com.br (G. De Nucci).

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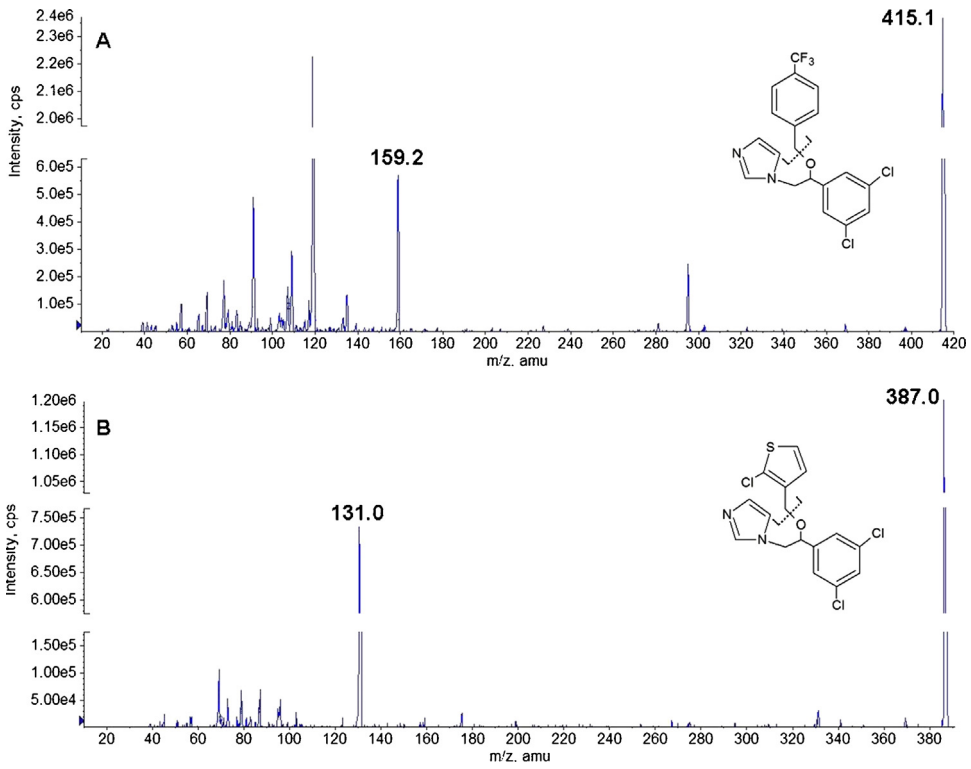

Fig. 1. Full scan mass spectrums of dapaconazole (A) and tioconazole (B) by electrospray ionization in positive mode. The dashed lines in the molecules indicate the point of fracture.

In this report we describe a selective and sensitive method for quantification of dapaconazole in human plasma using high-performance liquid chromatography coupled to tandem mass spectrometry (HPLC–MS/MS), with tioconazole as internal standard. Tioconazole (Fig. 1B) was chosen as the internal standard because of its structural similarity to dapaconazole. The method was subsequently used in a phase I study for quantification of dapaconazole in human plasma, after topical application of dapaconazole tosylate in healthy male volunteers.

2. Experimental

2.1. Chemicals and reagents

Dapaconazole was provided by Biolab Indústria Farmacêutica Ltda. and tioconazole, CAS 65899-73-2, was purchased from the United States Pharmacopeia (Rockville, MD, USA).Acetonitrile and methanol (HPLC grade) were purchased from J.T Baker (Phillipsburg, NJ, USA); ammonium acetate and hexane (analysis grade) from J.T Baker (Ecatepec, Mexico); ethyl ether (analysis grade) from Mallinckrodt (Phillipsburg, NJ, USA). Milli-Q water was obtained from a Millipore system. Blank human plasma samples were prepared from venous blood collected from healthy volunteers. Plasma was obtained by centrifugation and stored at −20 °C until use.

2.2. Calibration standards and quality controls

Stock solutions of dapaconazole and tioconazole (internal standard) were prepared in acetonitrile/water (50/50, v/v). Calibration curves for dapaconazole were prepared by adding dapaconazole to blank plasma to yield final concentrations of 0.2, 0.5, 1, 2, 20, 50, 75 and 100 ng/mL. The calibration curves were performed in duplicate for each day's assays. The quality control (QC) samples were prepared in blank plasma at low, middle, and high concentrations of 0.6, 40 and 80 ng/mL, respectively.

2.3. Sample preparation

Aliquots (0.2 mL) of each plasma sample were added to glass tubes followed by 0.05 mL of internal standard (tioconazole 300 ng/mL). The tubes were vortexed for 5 s and 0.3 mL of water Milli-Q were added. The samples were vortexed for 5 s again. Four mL of ether/hexane (80/20, v/v), were added and the samples were vortexed for 50 s. The samples were frozen and the organic phase transferred to another tube in which the organic solvents were evaporated under N_2 flow at 45 °C. Finally, the dry residues were dissolved in 0.2 mL of acetonitrile/water (50/50, v/v), vortexed for 10 s and transferred to microvials for analysis.

2.4. Instruments

2.4.1. Liquid chromatography

An aliquot (0.01 mL) of each plasma extracted was injected into a Genesis® C18 reversed phase analytical column 4 μm (100 × 2.1 mm i.d.) coupled to a Phenomenex®, Security Guard Cartridges C_{18}, 4 × 3 mm i.d. The temperature of column was maintained constant at 40 °C. The mobile phase used was methanol/acetonitrile/water (80/10/10, v/v/v) + 0.5 mM of ammonium acetate at a flow rate of 0.260 mL/min (split 1:2). The temperature of the auto-sampler was maintained at 8 °C.

2.4.2. Mass spectrometry

The mass spectrometer (Micromass model API 4000, Sciex/Applied Biosystems, Foster City, CA, USA) equipped with an electrospray source in the ESI positive polarity mode (ES+) was configured for multiple reaction monitoring (MRM) to monitor the transitions 415.1 > 159.2 and 387.0 > 131.0, for dapaconazole and tioconazole, respectively. The optimized values of declustering potential (DP), collision energy (CE) and collision cell exit potential (CXP) were respectively 86 (V), 41 (eV) and 24 (V) for dapaconazole and 91 (V), 37 (eV) and 10 (V) for tioconazole. Data acquisition and