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ORIGINAL RESEARCH

Ultra-fast chromatographic micro-assay for quantification of diphenidol in plasma: application in an oral multi-dose switchability trial

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ABSTRACT: Pharmacokinetics of diphenidol (DPN) is limited due to the lack of analytical methodology. Here, a micro-assay for DPN quantification was developed, by coupling ultra-performance liquid chromatography with tandem mass spectrometry. The procedure involved plasma precipitation and injection of supernatant into UPLC with an Acquity™ C_{18} column. Detection was in positive electrospray, following transitions of *m/z* 310.3 → 292.3 and *m/z* 275.3 → 230.2 for DPN and chlorphenamine (internal standard), respectively. The method was linear with a range of 4–400 ng/mL, and a 2 min run time. This method was applied in a switchability trial, where both formulations of DPN were bioequivalent. Copyright © 2008 John Wiley & Sons, Ltd.

KEYWORDS: CAS 3254-89-5; diphenidol pharmacokinetics; UPLC–mass spectrometry; multi-dose switchability trial

INTRODUCTION

Diphenidol (DPN; 1,1-diphenyl-4-piperidino-1-butanol) is an anti-emetic agent widely used in the Latin American market that is administered orally, rectally and parenterally. It has been proposed that the anti-emetic effect could consist of blunting the chemo-receptor trigger zone in the *area postrema*. Additionally, DPN is prescribed to control vertigo due to its specific effect on the vestibular apparatus (Bryfield *et al.*, 1999). Although new drugs are used at present for the prophylaxis of some forms of chemotherapy-, radiotherapy- and surgery-associated nausea and vomiting, DPN continues to be used in such circumstances because of cost–benefits issues.

Pharmacokinetic data of DPN are scant and limited, due in part to the long use of the molecule and the lack of sensitive methodology. The pharmacokinetics has been previously reported in an oral multi-dose trial in healthy female Mexican volunteers, in which peak plasma concentrations were reported between 0.5 and 1.5 h post-dose, and an elimination half-life *ca* 3 h was calculated during steady state (Hernández *et al.*, 2005). These data were obtained with the unique method previously mentioned: an HPLC technique coupled with UV detection; although this was validated and applied in a bioequivalence trial, it is time-consuming and requires the processing of 2 mL of plasma to reach a moderate sensitivity level (40 ng/mL).

The aim of the present work was to develop a rapid and more sensitive micro-volume assay for specific quantification of DPN in human plasma by coupling ultra-fast liquid chromatography with tandem mass spectrometry, and applying this in a biopharmaceutical switchability trial.

EXPERIMENTAL

Chemical and standard solutions. DPN chloride (purity 99.8%) was from Spectra Lab Products, Inc. (Gardena CA, USA). The chlorphenamine maleate (CLP) used as internal standard (purity 100.6%) was obtained from Retecma S.A. de C.V. (Mexico City, Mexico), while acetonitrile, methanol and acetic acid (HPLC-grade) were purchased from Tecsiquim (Toluca, Mexico). Formic acid (purity 98%) was purchased from Fluka (Hanover, Germany). Water (HPLC-grade) was obtained through a Milli-Q system (Millipore, Bedford MA, USA). The reference product was 25 mg DPN tablets (Vontrol®, SANFER S.A. de C.V., Mexico) and the test product was also 25 mg DPN tablets (Lansenol®, Landsteiner Scientific S.A. de C.V., Mexico).

Stock solutions of DPN (200 µg/mL) and CLP (200 µg/mL) were prepared in methanol–water (1:1 v/v) and protected from light exposure. Working standard solutions of DPN (40–4000 ng/mL), as well as working solution of IS (CLP 100 ng/mL) were prepared in methanol–water (4:1 v/v) and stored at 4°C. For chromatographic purposes, a weak washing solution

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Abbreviations used: CLP, chlorphenamine maleate; DPN, diphenidol; UPLC, ultra-performance liquid chromatography.