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Journal of Taibah University for Science 8 (2014) 54–63

Journal of Taibah University for Science

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Method development and validation for simulataneous estimation of albendazole and praziquantel in bulk and in a synthetic mixture

Shreya R. Shah [a,*], S. Dey [b], Prasanna Pradhan [a], H.K. Jain [a], Umesh M. Upadhyay [a]

[a] *Sigma Institute of Pharmacy, Bakrol, Vadodara 390019, Gujarat, India*

[b] *Dr B.C. Roy College of Pharmacy and Allied Health Sciences, Meghnad Saha Sarani, Bidhan Nagar, Durgapur 713206, West Bengal, India*

Received 3 June 2013; received in revised form 5 August 2013; accepted 14 August 2013

Available online 2 October 2013

Abstract

A simple, rapid, sensitive reversed-phase high-performance liquid chromatography method was developed and validated for simultaneous measurement of albendazole and praziquantel with an internal standard, simvastatin, at single wavelength of 225 nm. Chromatographic separation was performed on an Enable C_{18} column (250 mm × 4.6 mm, 5 μm: Spinco Biotech Pvt Ltd) and a mobile phase consisting of acetonitrile:water (60:40, v/v) with 10% orthophosphoric acid to adjust the pH to 3.2, at a flow rate of 1.0 ml/min. The calibration curve was linear ($r^2 \geq 0.999$) over the concentration range 0.05–8.0 μg/ml. The concentrations of simvastatin was 1.0 μg/ml. The limit of quantification was 0.05 μg/ml for both albendazole and praziquantel. No interference was found by the excipients in the synthetic mixture. The proposed methods were validated as per International Conference on Harmonisation guidelines for linearity, accuracy, precision and robustness for estimation of albendazole and praziquantel in bulk and in a synthetic mixture, and the results were found to be satisfactory.

Keywords: Albendazole; Praziquantel; Simvastatin; HPLC; UV detection; Validation

1. Introduction

Neurocysticercosis is the commonest helminthic disease of the nervous system and is considered a serious public health problem in developing countries of Latin America, Asia, and Africa [1–3]. Although the treatment of neurocysticercosis is restricted to palliative measures, it has advanced over the past 20 years with the use of praziquantel and albendazole, which are effective against the cystic larvae [2,4]. Albendazole is more effective than praziquantel, but cysts persist in some patients even after repeated use of albendazole [2]. For these cases, alternative treatment schedules such as simultaneous use of praziquantel and albendazole have been evaluated [4,5]. The combination of praziquantel with albendazole has also been extensively used in human hydatid disease [6–9]. Albendazole is extensively metabolized to its active metabolite albendazole sulfoxide, which is further metabolized to the inactive albendazole sulfone [10]. Because of this extensive metabolism, plasma concentrations of albendazole are usually low, and pharmacokinetics are studied by measuring the sulfoxide and sulfone concentrations [11–15]. Praziquantel is metabolized to several hydroxylated metabolites [16–18], mainly *trans*-4-hydroxypraziquantel, an active metabolite [19].

In order to evaluate the kinetics of albendazole and praziquantel, selective, sensitive, reproducible analytical methods are required for their quantification in plasma samples as well as for their metabolites. High-performance liquid chromatography (HPLC) [20–29] and capillary electrophoresis [30,31] have been used,

☆ Peer review under responsibility of Taibah University.

* Corresponding author. Tel.: +91 9274201130.

E-mail address: shah.shreya723@gmail.com (S.R. Shah).

Peer review under responsibility of Taibah University.

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http://dx.doi.org/10.1016/j.jtusci.2013.08.004

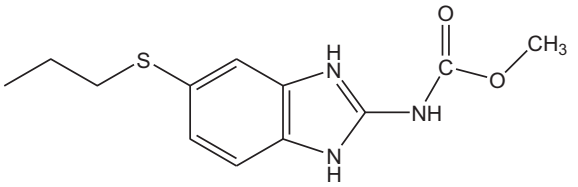

Fig. 1. Chemical structure of albendazole.

and the coupling of mass spectrometry to liquid chromatography (LC–MS and LC–MS–MS) has brought new insight into quantitative bioanalysis. Use of these techniques for the analysis of albendazole metabolites, praziquantel and *trans*-4-hydroxypraziquantel has been described only for isolated drugs. Bonato et al. [32] and Chen et al. [33] reported the use of LC–MS–MS for two methods, with quantification limits for albendazole sulfoxide of 5.0 and 4.0 μg/ml, respectively, and a quantification limit of 0.5 μg/ml for albendazole sulfone. LC–MS–MS was used only for qualitative analysis of praziquantel metabolites [16,34].

The aim of this work was to develop an HPLC method for simultaneous estimation of albendazole and praziquantel in bulk and in the synthetic mixture. The method was validated according to the International Conference on Harmonisation guidelines.

2. Materials and methods

2.1. Chemicals and reagents

Albendazole (Fig. 1) was obtained from Mercury Pharmaceutical Ltd, Vadodara, Gujarat, India, praziquantel (Fig. 2) from Micro Labs Ltd, Goa, India, and simvastatin (internal standard) (Fig. 3) from Dr Reddy's Lab, Hyderabad, India. Acetonitrile, methanol and water of HPLC grade were used. All the other reagents (including 10% *ortho*-phosphoric acid) were of analytical grade.

2.2. Chromatographic conditions

The high-performance liquid chromatograph (Shimadzu, Kyoto, Japan) was composed of an LC-20AT

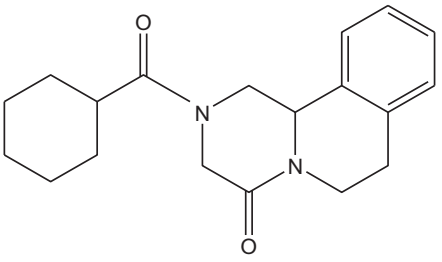

Fig. 2. Chemical structure of praziquantel.

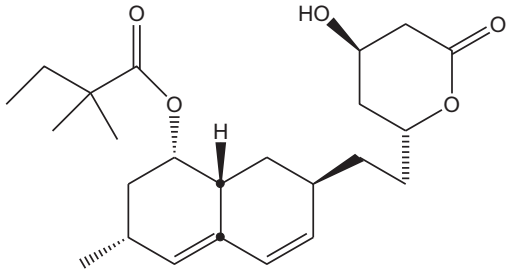

Fig. 3. Chemical structure of simvastatin (IS).

Prominence solvent delivery module, a manual rheodyne injector with a 20-μl fixed loop and a SPD-20A Prominence ultraviolet–visible detector. Separation was performed on an Enable C18 G column (particle size 5 μm; 250 mm × 4.6 mm) preceded by an ODS guard column (10 μm, 10 mm × 5 mm) at ambient temperature. Data were acquired on a Spinchrom Chromatographic Station® CFR Version 2.4.0.195 (Spinchrom Pvt. Ltd, Chennai, India). The mobile phase consisted of acetonitrile:water in a ratio of 60:40, the pH was adjusted to 3.2 with *ortho*-phosphoric acid, and the flow rate was 1.0 ml/min. Mo was vacuum filtered and degassed through 0.2 μm pore polymeric PTFE filters.

2.3. Method

2.3.1. Preparation of standard stock solutions

A sample of 25 mg of each drug is weighed and transferred to a 25-ml volumetric flask; 15 ml of methanol are added, and the solution is sonicated for 15 min. The volume is made up to the mark with methanol to obtain a stock solution of 1000 μg/ml. Simvastatin is also prepared in the diluent to obtain a working standard solution of 1000 μg/ml.

2.3.2. Preparation of working standard solutions

From the standard stock solutions, 2.5 ml are withdrawn and transferred to 25-ml volumetric flasks, and the volume is made up to the mark with diluent to obtain working standard solutions of 100 μg/ml. The working standard solution of simvastatin is diluted to a final solution of 10 μg/ml.

2.4. Validation

The method was validated by evaluating recovery, linearity, precision, accuracy, quantification limit and stability. Coefficients of variation and relative errors <2% were considered acceptable [35,36].