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Sensitive column-switching high-performance liquid chromatography method for determination of propiverine in human plasma

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Abstract

A sensitive column-switching high-performance liquid chromatography (HPLC) with ultraviolet (UV) detection was developed for the determination of propiverine in human plasma. Propiverine and internal standard, oxybutynin, were extracted from human plasma that had been made basic with 5N sodium hydroxide into methyl *tert*-butyl ether. The extracted plasma sample was injected onto the HPLC system consisting of a pretreatment column, a concentrating column, and an analytical column, which were connected with a six-port switching valve. The assay was linear in concentration ranges of 2–200 ng/ml for propiverine in human plasma. This method showed excellent sensitivity (a limit of detection of 0.5 ng/ml), good precision and accuracy. This method is suitable for bioequivalence studies following single dose in healthy volunteers.

Keywords: Propiverine; Column switching; High-performance liquid chromatography (HPLC)

1. Introduction

Propiverine hydrochloride (1-methyl-4-piperidyl diphenylpropoxyacetate hydrochloride, PPV) has been developed for the treatment of hypertonic functional states in the region of the bladder, such as pollakisuria, nocturia and nocturial enuresis [1]. The urospasmolytic action of drug is due to the inhibition of calcium influx into smooth muscle cells and competitive antagonism with acetylcholine at muscarinic receptor sites [2]. Thin-layer chromatography (TLC) [3], gas chromatography (GC)–mass spectrometry (MS) [4], high-performance liquid chromatography (HPLC) [5] and LC–tandem mass spectrometry (MS/MS) methods [6–8] have been reported for the quantification of PPV in biological fluids. Among these methods, TLC method has been limited by the lack of sensitivity and selectivity and GC–MS method is complicated because of time-consuming derivatization and extraction procedures. LC/MS/MS system is not readily available in most clinical research laboratory because of cost although LC/MS/MS method has been revealed to be a powerful technique for the analysis of PPV in biological fluids with high sensitivity. HPLC/UV is a common method used for the determination of drugs in biofluids and has been developed for the determination of PPV. However, this was inadequate in our hand for bioequivalence study of PPV because of low sensitivity and specificity. More sensitive and specific methods are therefore required for the analysis of PPV in plasma. Recently, a number of papers have been published on the subject of HPLC column-switching [9–13]. Some of these papers showed that HPLC column-switching method can increase the sensitivity and specificity [9,10]. Therefore, in the present study, we describe the development and validation of a sensitive and specific column-switching HPLC method for the detection of PPV in human plasma using liquid–liquid extraction to accomplish the bioequivalence study of PPV. In this study, PPV was only analyzed because the analysis of metabolites is not required in bioequivalence study. This analytical method was applied to determine the level of PPV following a single dose in human healthy volunteers.

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2. Experimental

2.1. Chemical and materials

Propiverine (PPV) was obtained from Hanmi Pharm Co. Ltd (Seoul, Korea). As an internal standard (IS), oxybutynin chloride was purchased from Sigma (St. Louis, MO, USA). The chemical structures are showed in Fig. 1. The purity of PPV and IS was more than 95%. Acetonitrile and methyl-*tert*-butyl ether (MTBE) were obtained from Burdick & Jackson (Muskegon, MI, USA). Water was purified by a Milli-Q system (Millipore Corp., Bedford, MA, USA). All other reagents were of analytical reagent grade and used without further purification.

2.2. Standard solutions

Stock solutions of PPV and IS were prepared by dissolving the drug in methanol to a final concentration of 50 μg/ml and further diluted into 2–200 ng/ml for the preparation of plasma calibration standards. All solutions were stored at −20 °C. Using these standard solutions, seven calibration standard (CS) solutions containing 2.0, 5.0, 10.0, 25.0, 50.0, 100 and 200 ng/ml and quality controls (QC) solutions at concentrations of 2.0, 25.0 and 200 ng/ml were prepared in human plasma.

2.3. Instruments

PPV was determined by column-switching HPLC with UV detector. All experiments were performed using an automated semi-microbore HPLC Nanospace SI-1 series (Shiseido, Tokyo, Japan) equipped with two 2001 pumps, a 2002 UV–vis detector, a 2003 autosampler, a 2004 column oven, a 2012 high-pressure switching valve and a 2009 degassing unit as schematically described in Fig. 2.

A Capcell Pak MF Ph-1 cartridge (20 mm × 4.0 mm i.d., 5 μm particles, Shiseido, Japan) was used for selective adsorption of PPV in plasma. A Capcell Pak C_{18} UG 120 V column

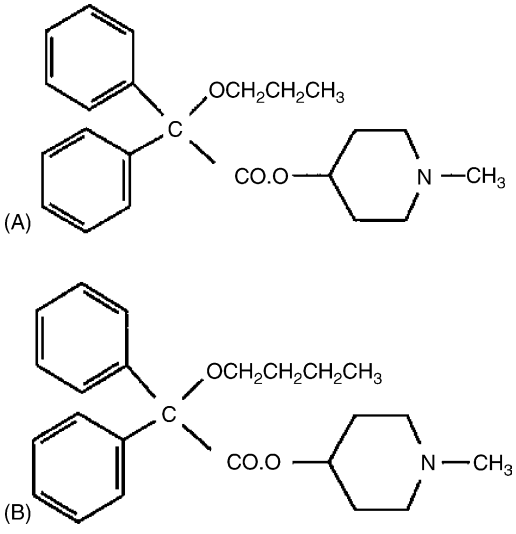

Fig. 1. Chemical structures of (A) propiverine (PPV) and (B) oxybutynin as an internal standard (IS).

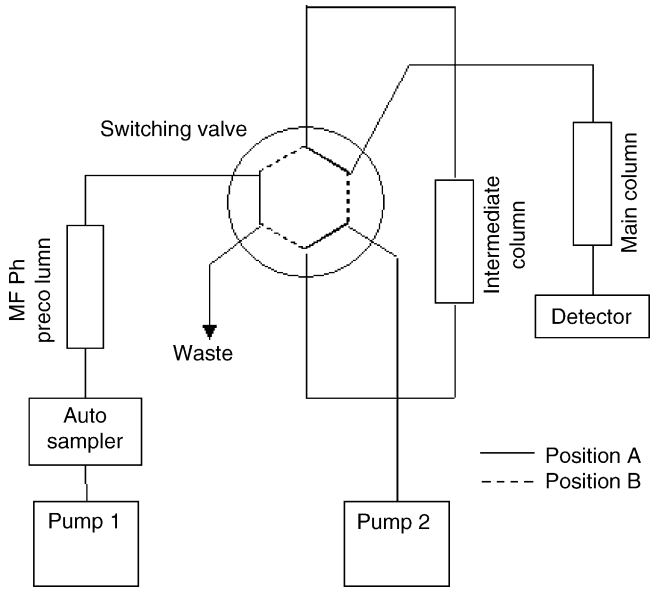

Fig. 2. Schematic diagram of a column-switching system.

(35 mm × 2.0 mm i.d., 5 μm particles, Shiseido) was used for concentrating PPV as an intermediate column. A Capcell Pak C_{18} MG II column (250 mm × 1.5 mm i.d., 5 μm particles, Shiseido) was used as a separation column. The pretreatment and separation column temperature was maintained at 30 °C during analysis. However, the concentration column was operated at room temperature.

2.4. Semi-microbore column-switching chromatographic conditions

The operation of this column-switching semi-micro HPLC consists of three main steps: sample loading and primary separation, enrichment of the analyte fraction and chromatographic separation.

When the column-switching valve was at the A position, an aliquot of extracted plasma sample was loaded to precolumn and primary separation of PPV and IS from plasma sample were performed using mobile phase 1 at a flow rate of 0.4 ml/min. Then, the valve was switched to the B position, and PPV and IS fraction were eluted from precolumn and concentrated in enrichment column by mobile phase 1 [28% (v/v) acetonitrile in 30 mM phosphate buffer (pH 7.5)] at a flow rate of 0.4 ml/min. Then, the valve position was returned to A, and PPV and IS concentrated in the enrichment column were separated on an analytical column using mobile phase 2 [40% (v/v) acetonitrile in 30 mM phosphate buffer (pH 2.5) and 0.02% TEA] at a flow rate of 0.15 ml/min. UV detection was performed at 220 nm using a chromatogram integration software dSChrom (Donam Instrument, Korea).

2.5. Extraction procedure

Extraction of PPV in plasma was conducted by the reported method [5] with slight modification. Briefly, a 20 μl of 5N NaOH solution and 5 μl of internal standard solution (50 μg/ml) were