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Determination of tropisetron in human plasma by high performance liquid chromatographic method with UV detection and its application to a bioequivalence study

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Abstract

A simple and sensitive high performance liquid chromatography method with UV detection was described for the determination of tropisetron in human plasma. The prepared sample solution was injected onto BDS-C_8 reversed column using a mixture of ammonium acetate (100 mM, PH adjusted to 4.3 with glacial acetic acid) and acetonitrile (80:20, v/v) as mobile phase. The wavelength of UV detector was set at 285 nm. No interference from any endogenous substances was observed during the elution of tropisetron and internal standard (ondansetron hydrochloride). The lower limit of quantification was evaluated to be 1 ng/mL. The method was used in a randomized crossover bioequivalence study of two different tropisetron preparations in 20 healthy volunteers.

Keywords: Tropisetron; High performance liquid chromatography; UV detection; Bioequivalence study

1. Introduction

Tropisetron is a potent and selective 5-HT_3 receptor antagonist and it is used primarily for the treatment of postoperative nausea and emesis as well as of emetogenic side effects of cancer chemotherapy [1–4]. It is extensively metabolized to inactive metabolites by hydroxylation of the indole moiety and further conjugation to glucuronides and sulphates. The biotransformation is mediated by multiple cytochrome P-450 enzyme, among them the polymorphic CYP2D6 and CYP1A2 [5,6] resulting in a high interindividual variability in plasma concentrations and effectiveness. Accordingly, the terminal plasma elimination half-life of tropisetron varies between 6 and 8 h in extensive metabolizers and 30 and 40 h in subjects with CYP2D6 deficiency [7]. To our knowledge, most of Asians are CYP2D6 extensive metabolizers. Therefore, all the subjects included in the study were regarded to be CYP2D6 extensive metabolizers. The analytical methods used to determine tropisetron concentrations in biological samples include liquid chromatography with UV [8–10], fluorescence [11] and tandem mass spectrometric detector [12]. HPLC/MS method is sensitive and specific, but the devices may not be available in many pharmaceutical laboratories and need highly trained persons. Several HPLC methods using UV detection have been reported for analysis of tropisetron, however, there are noticeable shortages of methods described in the literature. In bioequivalence studies, the proposed method should be simple, accurate and reproducible for the quantification of the tested drug in biological fluids.

In this paper, a simple, accurate and sensitive HPLC method with UV detection was developed for the determination of tropisetron in human plasma, which was successfully used in a bioequivalence study of two different tropisetron preparations.

2. Experimental

2.1. Reagents and chemicals

Tropisetron hydrochloride and Ondansetron hydrochloride (I.S.) were purchased from Sigma (St. Louis, MO, USA). Sodium carbonate, dichlormethane, acetonitrile, methanol, glacial acetic acid, ammonium acetate were of HPLC grade or

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analytical grade and were obtained from Corncord Tech (Tianjin, China). Water was glass-double distilled and further purified for HPLC with a millipore purification system (Nihon Millipore, Tokyo, Japan). Blank human plasma was provided by the central blood bank of Shenyang (Liaoning, China).

2.2. Preparation of calibration standards and quality control samples

Stock standard solutions of tropisetron hydrochloride and ondansetron hydrochloride were prepared by dissolving accurately weighted tropisetron hydrochloride and ondansetron hydrochloride in methanol to yield final concentrations of 1.2 μg/mL and 50 μg/mL (both calculated as hydrochlorate). Tropisetron hydrochloride stock solution was further diluted with methanol to obtain the different working standard solutions ranging from 15 to 600 ng/mL. Working standard solution of ondansetron hydrochloride was prepared by diluting the stock solution with methanol to yield final concentration of 500 ng/mL. Calibration curves were prepared within the concentration range of 1–40 ng/mL. In disposable glass tubes, 100 μL of each working standard tropisetron hydrochloride solutions were evaporated to dryness under nitrogen at 40 °C. The residues were reconstituted in 1.5 mL human blank plasma and then mixed for 10 s on a vortex mixer. Quality control (QC) samples were prepared at low (1 ng/mL), medium (20 ng/mL) and high (40 ng/mL) concentrations in the same way as the plasma samples for calibration. The samples were subjected to extraction and analysis.

2.3. Instrumentation

The liquid chromatography system consisted of a LC-10A system (shimadzu, Kyoto, Japan) equipped with a LC-10AD pump, a fixed injection-loop of 100 μL, an ultra-violet detector model SPD-10A UV–vis operated at an wavelength of 285 nm, and a data processor of Anastar. (Tian Jin, China). The analytical column employed was a Hypersil BDS-C_8 column (150 mm × 4.6 mm I.D., 5 μm particle size) and protected by a ODS guard column (10 mm × 4.0 mm I.D., 5 μm particle size). The guard column was routinely changed to avoid excessive pressure built-up in the system. The mobile phase was comprised of acetonitril–100 mM ammonium acetate buffer solution adjusted to 4.3 with glacial acetic acid (20:80, v/v). The eluent was filtered through a 0.45 μm cellulose membrane filter (Auto Science, Tianjin, China) and degassed before use. Flow rate was set at 1 mL/min.

2.4. Extraction procedure

To each of these disposable glass tubes, 100 μL of the I.S. (ondansetron hydrochloride, 500 ng/mL) was added except for the blank plasma sample. The plasma samples were then mixed with 100 μL of saturated sodium carbonate and 5 mL of dichloromethane and extracted with a rotary mixer for 20 min at room temperature. The mixture was centrifuged for 10 min at 4000 × *g* and the organic phase was transferred into a new glass tube and evaporated to dryness at 40 °C under a gentle stream of nitrogen. The residue was reconstituted in 110 μL of mobile phase and a volume of 100 μL was injected into the HPLC for analysis.

2.5. Validation of the method

For method validation and linearity studies, blank plasma samples obtained from healthy volunteers were used. The specificity of the assay was evaluated by comparing between the human blank plasma sample and human plasma sample spiked with tropisetron hydrochloride and the I.S. The efficiency of the extraction procedure was observed at low (1 ng/mL), medium (20 ng/mL) and high (40 ng/mL) concentrations. Recovery was calculated by comparing the respective peak areas of the chromatograms of the extracted samples relative to the untreated standards containing an equivalent amount of the compounds in methanol. Calibration curves were constructed by linear least-squares regression analysis plotting of peak-area ratio (tropisetron/I.S.) versus the drug concentrations. The lower limit of quantification (LLOQ) was defined as the lowest concentration with a coefficient of variation (CV) of less than 20% and accuracy of 80–120%. The accuracy and precision of the method were evaluated with QC samples at concentrations of 1, 20, 40 ng/mL on three consecutive days, accompanying by a standard calibration curve on each analytical run. Stability of tropisetron in plasma samples was investigated by comparing of the determined concentration at different times up to 14 days after storage at −20 °C following three freeze–thaw cycles. During the stability study, a standard calibration curve was prepared on each analytical batch.

2.6. Application of the method

The present method was used to determine the plasma concentrations of tropisetron in a randomized crossover bioequivalence study. Twenty healthy volunteers received a single oral dose of 20 mg tropisetron from either ShuangLu (reference, Beijing, China) or DiSha (test, Weihai, China) pharmaceutical companies under fasting conditions. After 1 week wash-out period the subjects were crossed-over. The blood sampling was carried out before and 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 8, 12, 24, 36, 48 h after drug administration. Pharmacokinetic parameters were calculated and compared using paired Student's *t*-test. Statistical significance was defined at the level of $P < 0.05$.

3. Results

3.1. Specificity

No endogenous interference was found at the retention times of tropisetron and the I.S. Representative chromatograms for human blank plasma and human plasma spiked with tropisetron hydrochloride (20 ng/mL) and the I.S. (33.3 ng/mL) are shown in Fig. 1A and B, respectively. Tropisetron and the I.S. were well resolved with respective retention times of 10.8 min and 12.8 min. Fig. 1C and D represent the chromatograms of plasma

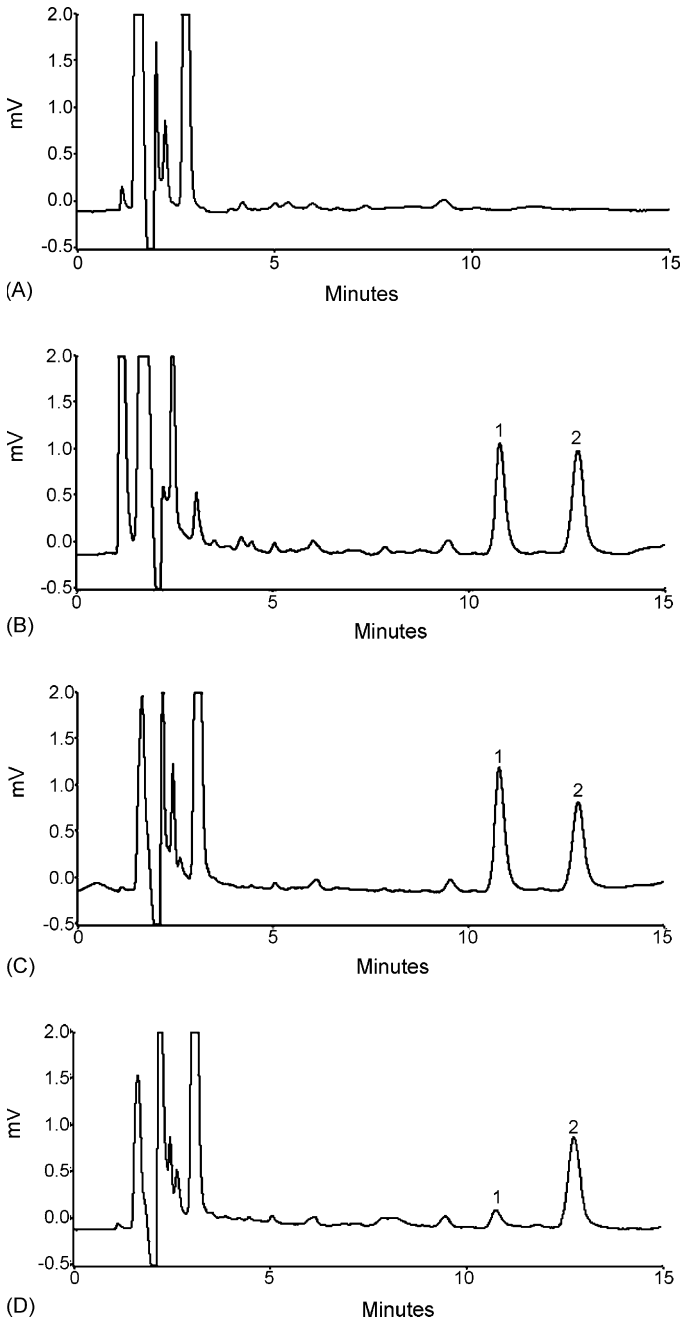

Fig. 1. Representative chromatograms of blank plasma (A); plasma spiked with tropisetron hydrochloride (20 ng/mL) and I.S. (ondansetron hydrochloride, 33.3 ng/mL) (B); plasma samples obtained at 3 h and 36 h after a single oral dose of 20 mg tropisetron from a healthy volunteer (C) and (D). Peak 1: tropisetron; Peak 2: ondansetron.

sample obtained at 3 and 36 h after administration from a healthy volunteer.

3.2. Linearity and LLOQ

The standard calibration curves were linear over the concentration ranges of 1–40 ng/mL for tropisetron hydrochloride with a mean correlation coefficient of 0.9995. The mean (±S.D.) regression equation from replicate calibration curves on different days was: $Y = (0.0453 \pm 0.00276)X + (0.006 \pm 0.0002)$, where Y was the peak area ratio of tropisetron to the I.S. and X, the plasma concentration of tropisetron hydrochloride. The lower limit of quantification with a coefficient of variation of less than 20% was 1 ng/mL using 1.5 mL plasma sample and 100 μL injection.

Table 1
Precision and accuracy of the method for the determination of tropisetron hydrochloride in human plasma ($n = 18$)

Concentration (ng/mL)		Relative error (%)	Intra-day R.S.D. (%)	Inter-day R.S.D. (%)
Added	Found			
1.00	0.97	−3.1	4.6	9.1
20.00	19.87	−0.6	4.3	6.4
40.00	40.82	2.0	2.9	4.1

3.3. Recovery, precision, accuracy and stability

The mean extraction recoveries of tropisetron hydrochloride at concentrations of 1, 20, 40 ng/mL were 92.8 ± 8.1, 92.1 ± 3.0 and 92.3 ± 3.2%. The extraction recovery of the I.S. was 94.3 ± 5.5%.

The precision and accuracy were investigated with QC samples at concentrations of 1, 20, 40 ng/mL. The results are shown in Table 1. The intra-day and inter-day precision of the QC samples were satisfactory with R.S.D.s less than 9.1%. The determined values deviated from the nominal concentration with R.E. less than −3.1%.

Extracted plasma sample was found to be stable at least 12 h when the samples were kept at room temperature (R.E.< − 3.7%). The concentrations of tropisetron hydrochloride in plasma which underwent three freeze–thaw cycles or storage at −20 °C for 14 days were found to be stable with relative errors less than −5.6%.

4. Discussion

Tropisetron is a compound of amine, thus end-capped base deactivated silica (BDS) column is better than octadecyl silica ODS for analysis. Since, in its manufacture a higher degree of silanization is achieved by the use of small alkyls to react with remaining silanol sites. The tailing of the chromatographic peaks on BDS column has been improved compared that obtained on ODS which had been employed in most of the previously published papers. The resolution between tropisetron and endogenous substances was more than 1.5, which met the requirements for quantification analysis. Plasma levels are relatively low after single dose administration of tropisetron, thus, for analysis of the drug using HPLC with UV detection, high efficient extraction procedures should be developed and the drug must be chromatographed without any interfering or coeluting peaks. Extraction efficacy of several organic solvents including ethyl acetate, diethyl ether, hexane and dichloromethane was investigated for extraction of tropisetron from plasma. As a result, more efficient extract was obtained with dichloromethane. Sample clean-up using back-extraction into hydrochloric acid [9] greatly decreased the interferences from plasma constituents, but underwent multi-steps and significantly reduced the efficacy

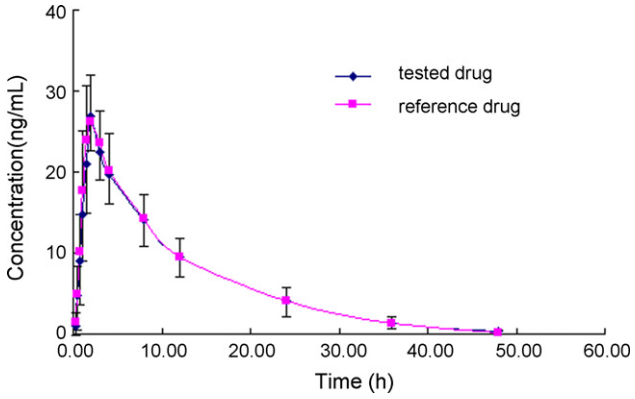

Fig. 2. Mean plasma concentrations-time profile of tropisetron hydrochloride in 20 healthy volunteers after administration of a single oral dose of 20 mg of the two formulations.

of the extraction. In this paper, we introduced a method for the determination of tropisetron in human plasma combining a simple liquid–liquid extraction procedure, although less sensitivity was obtained comparing to previously published LC methods with fluorescence or mass spectrometry detection, however, the resulted LOQ (1 ng/mL) was sufficient for human pharmacokinetic studies.

The mean plasma concentrations versus time curves of two preparations are shown in Fig. 2. Pharmacokinetic parameters of the two formulations obtained from the statistical calculation exhibited bioequivalent, however, a high interindividual variability in plasma concentrations was observed, the same result as obtained from Kim [9] which indicated that individual-administration should be considered under clinical circumstance.

5. Conclusions

In conclusion, a simple, accurate and sensitive reversed-phase HPLC method using UV detection has been described for the determination of tropisetron in plasma. The method was capable of estamating accurately tropisetron down to 1 ng/mL (calculated as tropisetron hydrochloride) which was enough for analysis of tropisetron up to 36 h after single dose administration in human plasma with high degree of reproducibility. The applicability of the described method was demonstrated in a randomized crossover bioequivalence study of two different tropisetron preparations in 20 healthy volunteers.

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