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Short communication

Liquid chromatographic method for the determination of rizatriptan in human plasma

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Abstract

A high-performance liquid chromatographic (HPLC) method with fluorescence detection has been developed for the determination of rizatriptan in human plasma. Following a single-step liquid–liquid extraction with methyl tertiarybutyl ether, the analytes were separated using a mobile phase consisting of 0.05% (v/v) triethylamine in water (adjusting to pH 2.75 with 85% phosphoric acid) and acetonitrile (92:8, v/v). Fluorescence detection was performed at an excitation wavelength of 225 nm and an emission wavelength of 360 nm. The linearity for rizatriptan was within the concentration range of 0.5–50 ng/ml. The intra- and inter-day precisions of the method were not more than 8.0%. The lower limit of quantification (LLOQ) was 0.5 ng/ml for rizatriptan. The method was sensitive, simple and repeatable enough to be used in pharmacokinetic studies.

Keyword: Rizatriptan

1. Introduction

Rizatriptan, *N*,*N*-dimethyl-5-(1H-1,2,4-triazol-1-ylmethyl)-1H-indole-3-ethanamine, is an orally active serotonin 5-HT(1) receptor agonist that potently and selectively binds to 5-HT(1B/1D) subtypes. Earlier clinical trials demonstrated that rizatriptan 5 or 10 mg was more effective than placebo at providing pain relief and pain-free state, relieving associated symptoms of migraine, normalizing functional ability and improving patient quality of life. This clinical trials also showed that rizatriptan could provide faster freedom from pain and reduce nausea to a greater extent than oral sumatriptan [1]. In humans, the plasma concentration of rizatriptan was very low (less than 40 ng/ml) when rizatriptan was administered orally at a single dose of 10 mg. Recently, only liquid chromatography/tandem mass spectrometry method has been applied into the determination of rizatriptan in human plasma [2,3], the limit of quantification (LOQ) of this method was 0.5 ng/ml. However, the LC–MS machine is quite expensive and is not readily available in most clinical research laboratories. To our knowledge no HPLC method with commonly used fluorescence detection is available for rizatriptan determination at therapeutic concentrations in plasma. This paper describes a rapid, simple, selective and sensitive method for the determination of rizatriptan in human plasma using high-performance liquid chromatographic (HPLC)-fluorescence detection, and this method has been successfully used for clinical rizatriptan pharmacokinetic studies.

2. Experimental

2.1. Reagents and chemicals

Rizatriptan benzoate was obtained from Shanghai Institute of Pharmaceutical Industry (Shanghai, China). Zolmitriptan, used as internal standard (I.S.), was obtained from Shanghai Huatuo Pharmaceutical Ltd. (Shanghai, China). Chemical structures were presented in Fig. 1. The purity of Rizatriptan benzoate and Zolmitriptan were all >99.5%. Acetonitrile was purchased from BDH (Dorset, UK), methyl tertiarybutyl ether (HPLC/Spectro) was purchased from Tedia Company (Fairfield, OH, USA). Sodium hydroxide, triethylamine, phosphoric acid (analytical reagent grade) was purchased from Shanghai Chemical

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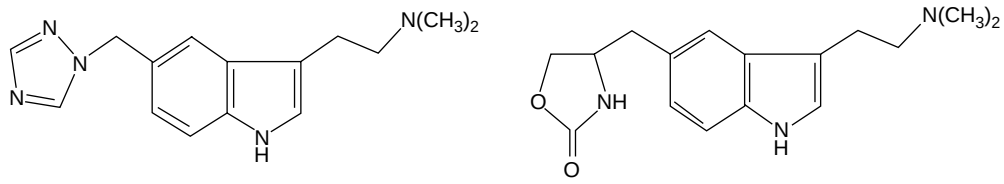

Fig. 1. Chemical structures of rizatriptan and zolmitriptan.

Reagent Company (Shanghai, PR China). Double distilled water was purified by Millipore Simplicity™ (Millipore, Bedford, MA, USA). The drug-free human heparinized plasma was obtained from Shanghai Blood Center (Shanghai, PR China).

2.2. Preparation of standard solution

Stock solution of rizatriptan was prepared by dissolving the appropriate amount of powder in a solution containing water, to yield the concentration of 1 mg/ml. Working solutions of rizatriptan were prepared by appropriate dilution. Standard solution of I.S. was prepared by dissolving the appropriate amount of zolmitriptan powder in a solution containing water, to yield the concentration of 0.2 μg/ml. All these solutions were stored at 4 °C and no change in stability over a period of 1 month was observed.

2.3. Apparatus and chromatographic conditions

A Shimadzu HPLC system consisting of LC-10AT VP pump, RF-10A XL fluorescence detector, CTO-10 AS VP column oven was utilized. Empire HS-2000 software was used for data acquisition. Analyses were performed on an Inertsil® ODS-3 reverse column (5 μm particle size, 4.6 mm × 200 mm i.d., GL Sciences Inc. Japan). The mobile phase was composed of 0.05% (v/v) triethylamine in water (adjusting to pH 2.75 with 85% phosphoric acid) and acetonitrile (92:8, v/v). The flow rate was set at 1.2 ml/min, and the total run time was 9 min. The column was maintained at 40 °C. Fluorescence detection was performed at an excitation wavelength of 225 nm and an emission wavelength of 360 nm.

2.4. Sample preparation

To a 1 ml aliquot of plasma in a 15 ml glass tube, 50 μl of I.S. solution (0.2 μg/ml of zolmitriptan solution) and 50 μl of 1 M sodium hydroxide solution were added. After vortex mixing, 4 ml of methyl tertiarybutyl ether was added. The mixture was then shaken for 2 min and centrifuged at 2000 × *g* for 10 min. The organic phase was decanted into a 10 ml conical glass tube. The organic phase was evaporated to dryness in a water bath at 50 °C under a stream of nitrogen. The residue was then reconstituted in 100 μl of mobile phase, and 20 μl of this solution was subsequently injected into the chromatographic system for HPLC analysis.

2.5. Validation of the assay method

2.5.1. Specificity

Chromatographic interference from endogenous plasma components was investigated using pooled blank plasma samples as well as samples from healthy subjects who participated in a clinical pharmacokinetic study of rizatriptan.

2.5.2. Linearity

Plasma samples were spiked in six replicates at concentrations of 0.5, 1, 5, 10, 25, 50 ng/ml. The samples were assayed using the method described above. The standard calibration curves for rizatriptan were constructed using the analyte/I.S. peak-area ratios versus the nominal concentrations of the analytes. Linear least-squares regression analysis with weighting factor of $1/x^2$ was performed to assess the linearity as well as to generate the standard calibration equation: $y = ax + b$, where y is the peak-area ratio, x the concentration, a the slope and b the intercept of the regression line.

2.5.3. Recovery

Spiked plasma samples were prepared in triplicate at concentrations of 0.5, 5 and 50 ng/ml, and assayed as described above. Recovery (extraction efficacy) was calculated by comparing the peak-area of the extracted sample to that of the unextracted standard solution containing the same concentration.

2.5.4. Precision and accuracy

The precision and accuracy of this method were evaluated using quality control samples at concentrations of 0.5, 5 and 50 ng/ml. For intra-day assay precision and accuracy, five replicates of quality control samples at each concentration were assayed all at once within a day. The inter-day assay precision and accuracy was determined by analyzing the quality control samples on five different days. Five replicates at each concentration were assayed per day.

2.5.5. Sensitivity

The lower limit of quantification (LLOQ) was determined for rizatriptan, based on the criteria that: (1) the analyte response at LLOQ is five times of baseline noise; (2) the

analyte response at LLOQ can be determined with sufficient precision and accuracy, i.e. precision of 20% and accuracy of 80–120%. The limit of detection (LOD) was determined as the lowest concentration, which gives a signal-to-noise ratio of 3 for rizatriptan.

2.5.6. Stability of analytes

2.5.6.1. Freeze-thaw stability. For freeze-thaw stability testing, the concentrations of the samples (in triplicate at each concentration, 0.5, 5 and 50 ng/ml) after three freeze-thaw cycles were determined and compared to the freshly prepared samples.

2.5.6.2. Reconstitution solution stability. The reconstitution solution stability of rizatriptan from plasma was assessed by spiking rizatriptan standard solution into blank plasma from healthy volunteer at three different concentrations (0.5, 5, 50 ng/ml). Extracted the sample from plasma following the sample preparation procedure to get the reconstitution solutions. Stored the reconstitution solution at room temperature. The reconstitution solutions of 0 and 24 h were analyzed on HPLC as described above.

2.5.6.3. Storage stability. The stability of rizatriptan plasma sample was assessed by spiking rizatriptan standard solution into blank plasma from healthy volunteer at three different concentrations (0.5, 5, 50 ng/ml). Stored the plasma samples in the freezer at −18 °C, on day 0 and day 30, extracted the sample from plasma following the sample preparation procedure to get the reconstitution solutions. The reconstitution solutions were analyzed on HPLC as described above.

3. Results

3.1. Separation and specificity

Fig. 2 shows the representative chromatograms of blank plasma, plasma samples spiked with rizatriptan at 25 ng/ml

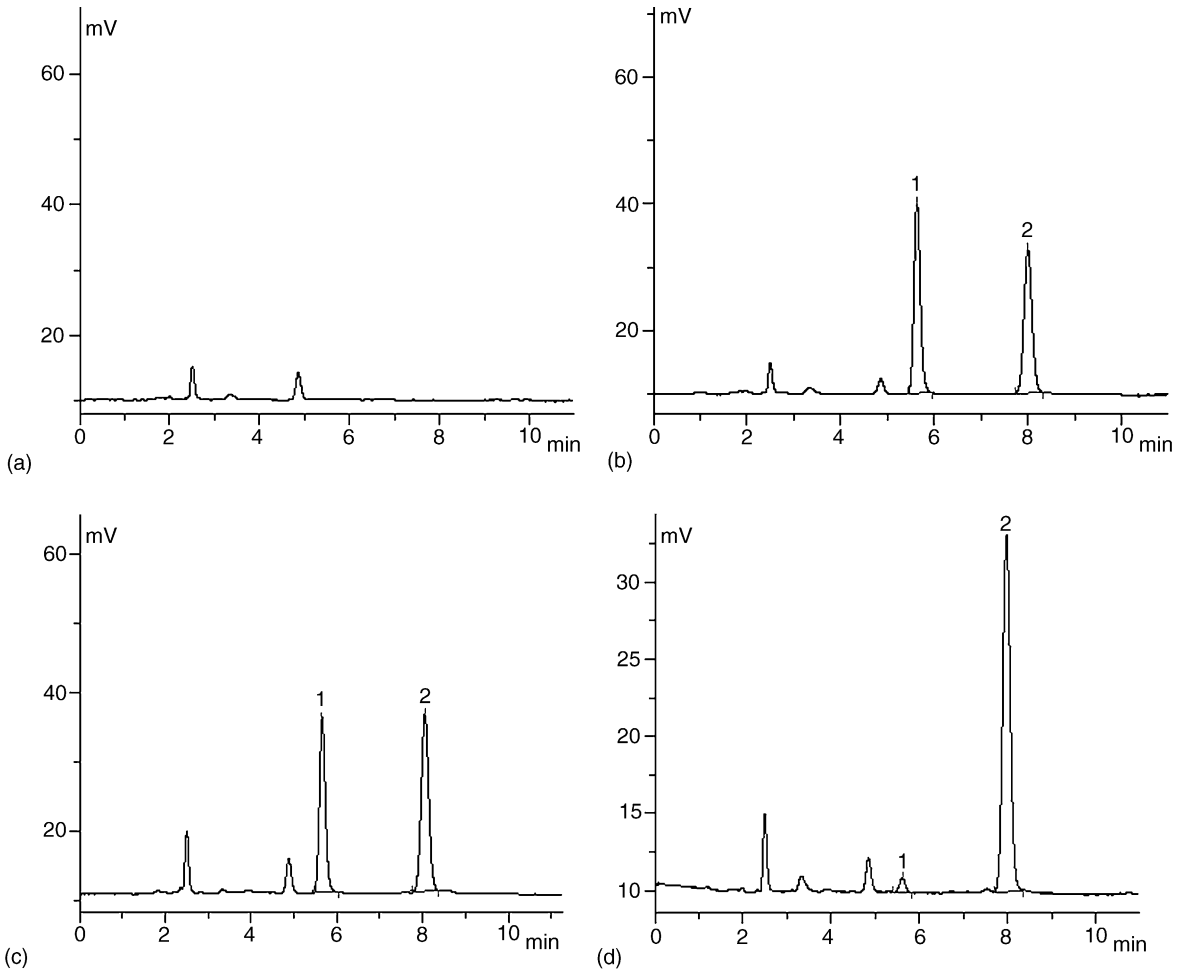

Fig. 2. Chromatograms of: (a) blank plasma; (b) plasma sample spiked with 25 ng/ml of rizatriptan and I.S.; (c) plasma sample from a healthy subject following a 10 mg oral dose of rizatriptan, the plasma concentration was determined to be 20.03 ng/ml for rizatriptan.; (d) spiked plasma sample at LLOQ (0.5 ng/ml).

Table 1
Inter-day precision in the slope and intercept of standard curves ($r = 0.9971$–0.9997)

Days	Slope	Intercept	Correlation
1	0.0197	0.0025	0.9993
2	0.0203	0.0016	0.9994
3	0.0204	0.0007	0.9997
4	0.0201	0.0026	0.9988
5	0.0208	0.0013	0.9971
Mean ± S.D.	0.0202 ± 0.0004	0.0017 ± 0.0008	
R.S.D. (%)	1.9	47.7	

and at LLOQ (0.5 ng/ml), and plasma sample obtained from a healthy subject following an oral 10 mg dose of rizatriptan. The analytes were well separated using the present chromatographic conditions. The retention times were 5.6 min for rizatriptan and 8.0 min for I.S. No interfering peaks from the endogenous plasma components were observed at the retention time of rizatriptan or I.S.

3.2. Calibration and linearity

Good linearity was observed over the concentration range of 0.5–50 ng/ml ($r = 0.9972$–0.9997). The R.S.D. ($n = 5$) of the slope calculated with calibration curve data was 1.9%, showing a good repeatability (Table 1).

3.3. Recovery

For plasma concentrations at 0.5, 5, 50 ng/ml, the mean recovery of rizatriptan were $87.01 \pm 3.52\%$, $84.40 \pm 4.52\%$ and $89.45 \pm 1.49\%$, respectively, and the mean recovery of I.S. was $83.14 \pm 1.53\%$.

3.4. Precision and accuracy

The precision and accuracy for measurement of rizatriptan was summarized in Table 2 . The R.S.D. of rizatriptan ranged from 3.3 to 7.7% for intra-day and 3.2 to 4.3% for inter-day, respectively. The relative error (R.E.) of rizatriptan ranged from −2.00 to 4.79% for intra-day and −1.60 to 0.94% for inter-day, respectively.

3.5. Sensitivity

The lower limit of quantification was 0.5 ng/ml for rizatriptan. The limit of detection (LOD) was 0.25 ng/ml for rizatriptan.

3.6. Stability

The results obtained after three freeze-thaw cycles demonstrated that 98.6–102.3% of the initial content of rizatriptan were recovered and that the analytes were stable under these conditions. Rizatriptan in reconstitution solution was found to be stable for approximately 24 h since the found concentrations of analystes were within 98.7–101.2% of the initial concentrations. The residual percentages of rizatriptan stored in plasma at −18 °C for 30 days ranged from 99.6 to 103.7%, indicating no stability problems occurred.

3.7. Dilution

The dilution study was also conducted to assess whether the upper concentration limit (50 ng/ml) can be extended. Quality control samples (in five replicates) at concentration of 100 ng/ml were diluted by two times with blank plasma, and the assay precision and accuracy were determined in a similar manner as described in Section 2.5.4. For rizatriptan, the R.S.D. was 3.7% and accuracy 103.21%. The results suggested that samples whose concentrations were greater than the upper limit of the standard curve could be re-analyzed by appropriate dilution.

3.8. Application to clinical study

The present HPLC method was for the first time employed to determine the pharmacokinetic parameters of rizatriptan in volunteers' plasma samples of clinical studies. After a single oral dose of 10 mg rizatriptan tablet in 20 healthy volunteers, concentration versus time profiles were constructed for up to 10 h for rizatriptan determination. Fig. 3 shows the representative concentration–time curves of rizatriptan in one subject following a 10 mg oral dose of rizatriptan under fasting condition. The maximum rizatriptan plasma concentration was 30.12 ng/ml, T_{max} was 2.0 h, and $t_{1/2}$ in the terminal elimination phase was 2.19 h. The parameter values are in good agreement with those reported previously [4,5].

Table 2
Intra- and inter-day precision and accuracy of rizatriptan spiked in human plasma ($n = 5$)

	Actual concentration (ng/ml)	Detected concentration (mean ± S.D.) (ng/ml)	Precision (R.S.D., %)	Accuracy (error, %)
Intra-day	0.5	0.4902 ± 0.0377	7.7	−2.00
	5	4.907 ± 0.285	5.8	−1.86
	50	52.39 ± 1.73	3.3	4.79
Inter-day	0.5	0.5047 ± 0.0171	3.4	0. 94
	5	5.027 ± 0.162	3.2	0.54
	50	49.20 ± 2.13	4.3	−1.60

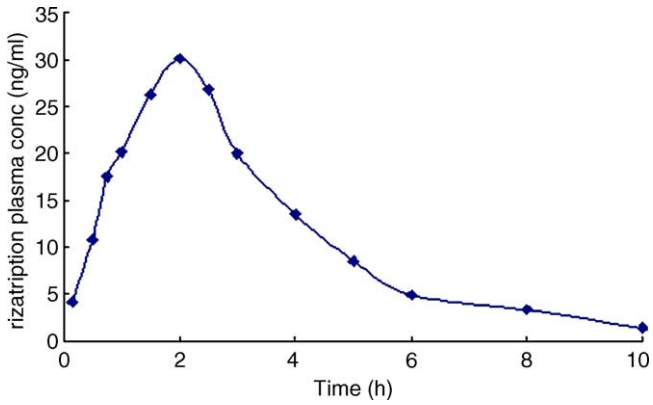

Fig. 3. Plasma concentration–time profiles of rizatriptan in a healthy subject following a 10 mg oral dose of rizatriptan.

4. Discussion

In the preliminary study, all the paper described a solid phase extraction using Oasis HLB to extract the rizatriptan from plasma; the extraction recovery was 82%. In order to develop a single step liquid–liquid extraction procedure with sufficient recovery, we investigated a large range of extraction solvents. The absolute recovery of rizatriptan after single extraction from plasma using chloroform, ethyl acetate, diethyl ether, dichloromethane were all <70%; however, when the methyl tertiarybutyl ether was used, the absolute recovery was quite high (>80% for both rizatriptan and I.S.). Alkalinization of the plasma with 1 M sodium hydroxide solution was found to be appropriate for the extraction of rizatriptan and I.S. Zolmitriptan was chosen as the I.S. for several reasons. First, Rizatriptan and I.S. exhibited almost same excitation and emission spectra and their molecular structure was also very similar. Second, the simultaneous prescription of two 5-HT(1) receptor agonists was not of usual medical practice.

5. Conclusion

The developed HPLC method employing liquid–liquid extraction for sample preparation is very simple and convenient for the determination of rizatriptan in plasma samples. The previously reported methods for the analysis of rizatriptan in biological fluids [2,3] were not too satisfactory because all of them were too expensive. The validation data also demonstrate good precision, accuracy and high extraction efficiency. In addition, this method has a short turnover time (<10 min) and is suitable for clinical pharmacokinetic studies. In conclusion, this paper describes a very simple and sensitive HPLC method for the determination of rizatriptan suitable to monitor plasma concentrations during clinical pharmacokinetic studies in humans.

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