ISSN : 0974 - 7419 Volume 11 Issue 11,12

Trade Science Inc.

Analytical CHEMISTRY

An Indian Journal

Full Paper

ACAIJ, 11(11,12), 2012 [347-352]

Determination of (3aRS,4SR,7RS,7aSR)-2-(Tricyclo-[3.3.1.13,7] decan-1-yl)-4,5,6,7-tetrahydro-4,7-epoxyisoindoline-1, 3-dione (SU2162) in rat plasma by high-performance liquid chromatography with UV detection

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Received: 20th April, 2012 ; Accepted: 20th May, 2012

ABSTRACT

A selective and sensitive high-performance liquid chromatography method has been developed and validated for determination of (3aRS,4SR,7RS,7aSR)-2-(Tricyclo-[3.3.1.13,7]decan-1-yl)- 4,5,6,7-tetrahydro-4,7- epoxyisoindoline-1,3-dione (SU2162) in rat plasma. The method of plasma disposal involved extraction and deproteinization by acetonitrile, dryness under a stream of nitrogen, and reconstituted with acetonitrile. Chromatographic separation was achieved on a reversed-phase C18 column using acetonitrile and water (40:60) as mobile phase at a flow rate of 1.0 ml/min. The assay was linear in the range of 0.200-16.00μg/ml with a correlation coefficient (r2) of 0.9955. Limits of detection and quantification were 0.204μg/ml and 0.816μg/ml, respectively. The precision and accuracy of the method were within the acceptable limits. The absolute recoveries of SU2162 in plasma were acceptable. The method was successfully applied to study the pharmacokinetics of SU2162 in rats.

KEYWORDS

(3aRS,4SR,7RS,7aSR)-2-(tricyclo-[3.3.1.13,7] decan-1-yl)-4,5,6,7-tetrahydro4; 7-epoxyisoindoline-1; 3-dione; SU2162; HPLC; Rat;

INTRODUCTION

Cancer is the major disease which seriously hazard human health and lives, and the main cause of death in the world. According to the American Cancer Society (ACS), in 2008, there were 12.7 million new cancer cases and 7.6 million deaths. By 2030, the World Health Organization (WHO) predicts we'll face more than 21 million new cancer cases and 13 million deaths[1].

(3aRS, 4SR, 7RS, 7aSR)-2-(Tricyclo-[3.3.1.13,7]decan-1-yl)- 4,5,6,7- tetrahydro- 4,7-epoxyisoindoline-1,3-dione (SU2162) is a novel anticancer agent, and has been made from norcantharidin and amantadine by a new synthesis method. Its structure was shown in Figure 1. The preliminary study of SU2162 has proved that SU2162 has remarkable inhibitory effect on

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glioma growth, and shown a good dose effect relationship between the effect of inhibiting glioma growth and the concentration of SU2162, IC50=0.721umol/l[2]. In addition, the chemical synthesis now is available[2] and its crystal structure was reported on our other article[3,4]. Therefore, the development of a sensitive and simple method to determine SU2162 is necessary and valuable for further research. In this paper, a high-performance liquid chromatography with UV detection was developed for determination of SU2162 in rat plasma.

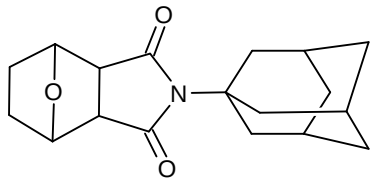

Figure 1 : Chemical structures of SU2162.

EXPERIMENTAL

Chemical regents and animals

SU2162 was supplied by the school of pharmacy, Guangdong Pharmaceutical University. Acetonitrile (high performance liquid chromatography grade) was purchased from Tianjin Kermel chemical reagent Co, Ltd.

Sprague-Dawley rats, weighing 275±25 g, were obtained from the Experimental Animal Center of Guangzhou University of Chinese Medicine. They were fed by a standard laboratory diet, and water was available ad libitum.

Chromatography

The HPLC system consists of a Shimadzu LC-10ATvp pump, a SPD-10Avp detector, and a manual injector with a 20µl fixed loop. Chromatographic separations of the compound SU2162 were achieved at ambient temperature, using a reversed-phase C18 column (Luna C18 column; 250mm×4.6mm; 5µm particle size; purchased from Phenominex Co.Ltd, Guangzhou, China) with a Phenominex prodigy analytical guard column (12.5mm×4.6 mm). The mobile phase consisted of a mixture of acetonitrile and water (40:60) and was daily prepared and filtered. The column was eluted for 80min under isocratic conditions, using a flow-rate of 1.0 ml/min at ambient temperature. Eluted peaks were detected at 211 nm. Chromatographic data were recorded and processed using a HW Chromatography Station.

Preparation of standard solutions

The standard stock solution of SU2162 was prepared in mobile phase (a mixture of acetonitrile and water (40:60)) at concentration of 40.0×10-2 mg/ml. The SU2162 stock solution was diluted stepwise with mobile phase to form working standard solutions of 5.0×10-3, 10.0×10-3, 25.0×10-3, 50.0×10-3, 100.0×10-3, 200.0×10-3 and 300.0×10-3 mg/ml for SU2162. These solutions were stored at ambient temperature.

Blanks and plasma standards for validation

Blood rat samples (about 300µl) were collected into heparinized centrifuge tubes, and then centrifuged at 13000rpm for 6 min. The plasma samples obtained were stored at -20℃ until analysis.

Blank rat plasma was extracted and analyzed for the assessment of potential interference by endogenous substances. To prepare plasma standards for validation, blank plasma (120µl) was spiked with known concentrations of SU2162 solutions (5µl), and vortexed for 2min. A standard curve consisted of plasma samples containing 0.200, 0.400, 1.00, 2.00, 4.00, 8.00, 12.00 and 16.00µg/ml of SU2162. To evaluate the precision, accuracy and absolute recovery of the method, plasma standards were prepared at concentrations of 0.408, 4.08 and 12.24µg/ml for SU2162. To investigate the stability of the residue after dryness, plasma standards were prepared at concentrations of 0.408, 12.24µg/ml for SU2162, and stored frozen at -20℃ after dryness under a stream of nitrogen. To determine the stability of the extracted plasma samples in reconstitution solutions, plasma standards were prepared at concentrations of 0.408, 12.24µg/ml for SU2162, and five freeze-thaw cycles were adopted.

Sample preparation

To each of centrifuge tubes with 120 µl plasma samples, 5µl of known concentrations of SU2162 solution was added except the blank plasma sample. After vortexed for 2min, 375µl

acetonitrile was added to each of these plasma samples for protein precipitation. The mixture was vortexed for 5min , and centrifuged at 13000rpm for 6 min. The supernatant were transferred to clean 0.5ml centrifuge tubes and dried under a stream of nitrogen. The residue was reconstituted with 125μl acetonitrile by vortex mixing and ultrasound for 5 min, respectively. Then a 25μl of this reconstitution solution was injected into the HPLC system for analysis after filtration by φ22 microporous membrane.

Validation of the method

Specificity

The specificity of the assay was evaluated by two criteria. One is comparing between the rat blank plasma sample and rat plasma sample spiked with the SU2162 standard solution for the assessment of interference by endogenous substances. Second is comparing between plasma sample after administration and rat plasma sample spiked with the SU2162 standard solution for the assessment of interference by metabolites.

Linearity

Standard curve in the range of 0.200-16.00ug/ml, in rat plasma, was plotted by the peak-area of SU2162 (axis y) against the respective nominal concentration (axis x). The equation of Calibration curve was obtained by least-squares regression analysis, and the correlative coefficient should be greater than 0.99[5].

Limits of detection and quantification

The limit of detection (LOD) was the lowest analyte concentration that could be detected with a signal-to-noise ratio of 3. The limit of quantification (LOQ) was the lowest analyte concentration that could be measured with a signal-to-noise ratio of 10. The LOQ was determined by following two criteria: (1) the signal-to-noise ratio is 10. (2) The values for accuracy and precision were less than 20%[5].

Accuracy, precision and absolute recovery

The accuracy of the method was evaluated with plasma samples at concentrations of 0.408, 4.08 and 12.24μg/ml for SU2162. Each concentration was 5 samples, and determined in parallel. The accuracy was evaluated by relative recovery (RR). The relative recovery (RR) was calculated by following formula:

$$RR = \frac{\text{average measured concentration}}{\text{added concentration}} \times 100\%$$

The value of RR should be in the range of 85%~115% except LOQ (low concentration) , which should be in the range of 80%~120%[5].

The intra-day and inter-day precision were determined with plasma samples at concentrations of 0.408, 4.08 and 12.24μg/ml for SU2162. The intra-day precision was determined 5 times within the same day. The inter-day precision was determined on three consecutive days. According to the reference, the precision was evaluated by the coefficient of variance (used the relative standard deviation (% R.S.D) in some references, actually the value of CV equals to the value of RSD). The value of CV must be less than or equal to 15%, except for LOQ, whose value of CV must be no larger than 20%[6]. The coefficient of variance was calculated by following formula[7]:

$$CV(\%) = RSD(\%) = \frac{SD}{\text{average calculated concentration}} \times 100\%$$

Where SD stands for standard deviation

Absolute recovery was calculated by comparing the respective peak areas of the chromatograms of the extracted samples at concentration of 0.408, 4.08 and 12.24μg/ml with the untreated standards solution containing an equivalent amount of the compounds. The calculated absolute recovery should larger than 50%, and the value of CV at high, middle concentration should be less than or equal to 15%, except for low concentration, whose value of CV must be no larger than 20%[5,7].

Stability

The plasma samples were assayed at concentrations of 0.408 and 12.24μg/ml for SU2162 under two different conditions to assess the stability of SU2162 in plasma. Each concentration was 9 samples, and divided in 3 groups on average. First group was determined at initial time. Second group was stored in -20℃ for 4days after dryness under a stream of nitrogen. And then the residue was reconstituted with 125μl acetonitrile and in-

jected into the HPLC system for analysis. Third group was the residue of plasma samples after extracted and dryness, reconstituted with 125µl acetonitrile, and then subjected to five freeze-thaw cycles (each freeze-thaw was 24 hours). The average measured concentration was 95%~105% of the initial concentration[5].

Application of method

The method described above was applied to the pharmacokinetic studies of SU2162 in rat after intravenous administration of a single dosage of SU2162 (5.5mg/kg) dissolved in a mixed solution of ethanol, propylene glycol and water (1:4:5). Six rats, weighing 290±30 g, were employed for this study (equal males and females). Blood samples (about 300 µl) were collected into heparinized tubes at the time points of 0.05, 0.1, 0.2, 0.25, 0.33, 0.67, 1.33, 1.67, 2.0 and 2.5h. The plasma samples were separated by centrifugation at13000rpm for 6 min and stored at -20℃ until analysis.

RESULTS

Specificity

Typical chromatograms are shown in Figure 2. Observed retention times are 70.133 min for SU2162. Blank plasma was tested for endogenous interference. A representative chromatogram of the blank plasma is shown in Figure 2A. No additional peaks of endogenous substances were observed. Figure 2B shows the chromatograms of blank plasma spiked with SU2162. Typical chromatograms of plasma samples are shown in Figure 2C, which were collected 6 min after intravenous administration of 5.5mg/kg of SU2162 to a rat.

Linearity

Linear standard curves with correlation coefficients greater than 0.99 were obtained over the concentration range 0.200- 16.00µg/ml for SU2162 in rat plasma and showed in Figure 3. The typical equation of the standard curve is as follows: y=5864.7 x+ 571.69, r2=0.9955. The regression equation and correlative coefficient (r2) showed a good linearity in the concentration range.

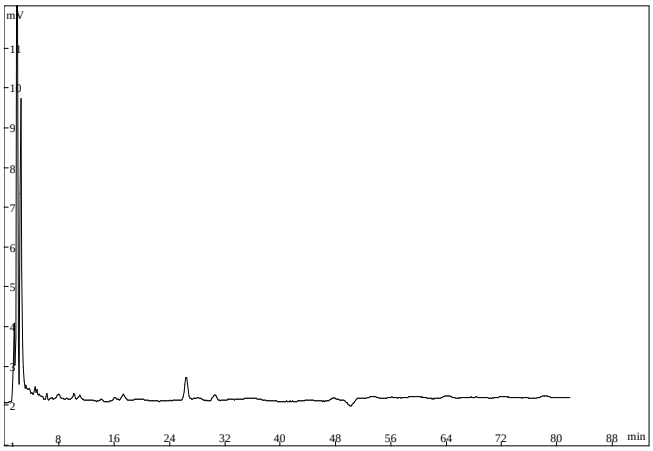

Figure 2a : HPLC trace of SU2162 using ultraviolet detection at 211 nm. (A) Blank plasma sample.

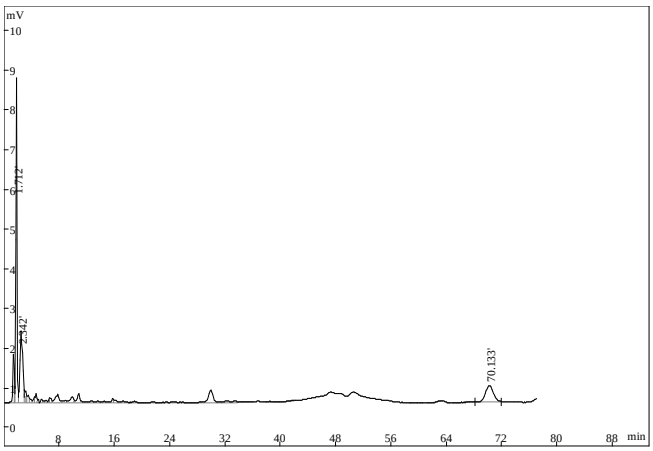

Figure 2b : HPLC trace of SU2162 using ultraviolet detection at 211 nm. (B) blank plasma spiked with SU2162 (4.08 µg/ml).

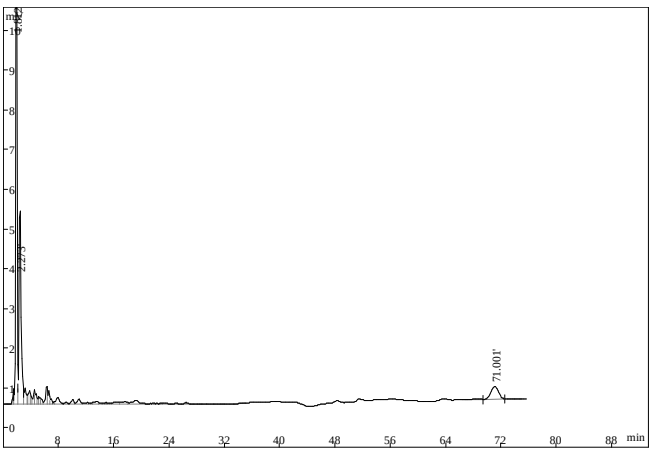

Figure 2c : HPLC trace of SU2162 using ultraviolet detection at 211 nm.(C) an extract of plasma obtained from a rat 6 min after intravenous injection of SU2162 (5.5mg/kg).

Limits of detection and quantification

Under the above Chromatography conditions, the limit of detection was 0.204µg/ml. The limit

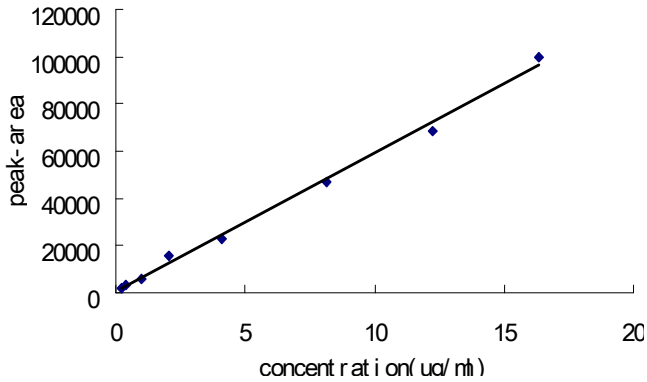

Figure 3 : Linear calibration curves was over the concentration range 0.200- 16.00μg/ml for SU2162 in rat plasma

of quantification was 0.816μg/ml with the coefficient of variation (CV) of 14.5% and an accuracy of 92.2%.

Precision, accuracy and absolute recovery

The precision and accuracy of the method are shown in TABLE 1 and 2. The results obtained were within the acceptable limits to meet the"2.6.4 Accuracy, precision and absolute recovery". The absolute recoveries of SU2162 in plasma are presented in TABLE 3. All the recoveries were over 50.0%.

TABLE 1 : Intra-day precision and accuracy of the method for the determination of SU2162 in rat plasma (n=5).

Added concentration (μg/ml)	Mean Measured Concentration (μg/ml)	SD	CV (%)	RR (%)
0.408	0.4621	0.0571	12.36	113.26
4.08	4.6612	0.2306	4.95	114.24
12.24	11.8057	1.0163	8.61	96.45

TABLE 2 : Inter-day precision of the method for the determination of SU2162 in rat plasma (n=3).

Added concentration (μg/ml)	Mean Measured concentration (μg/ml)	SD	CV (%)
0.408	0.4729	0.0298	6.31
4.08	4.4797	0.3865	8.63
12.24	10.5069	0.7608	7.24

Stability

The stability of SU2162 in residue after dryness under a stream of nitrogen, were found to be stable when the residue was stored in -20℃ for 4days. Extracted plasma samples in reconstitution solutions were shown to be stable after five freeze-thaw cycles. All the average measured concentration was 95%~105% of the initial concentration. The results were shown in TABLE 4 and 5.

TABLE 3 : The absolute recovery of SU2162 in rat plasma (n=5).

Concentration (μg/ml)	Absolute recovery (%)	SD	CV (%)
0.408	98.26	10.03	10.21
4.08	84.49	4.09	4.85
12.24	65.95	5.63	8.54

TABLE 4 : The stability of SU2162 in residue after stored in -20℃ for 4days.

Concentration (μg/ml)	Mean initial concentration (mean±SD)(μg/ml)	Mean Measured concentration (mean±SD)(μg/ml)	(Mean Measured concentration/ Mean initial concentration)(%)
0.408	0.3857±0.0579	0.3789±0.0183	98.25%
12.24	13.2806±1.2857	13.9000±1.2729	104.66%

TABLE 5 : the stability of extracted plasma samples in reconstitution solutions after five freeze-thaw cycles.

Concentration (μg/ml)	Mean initial concentration (mean±SD)(μg/ml)	Mean Measured concentration (mean±SD)(μg/ml)	(Mean Measured concentration/ Mean initial concentration)(%)
0.408	0.3857±0.0579	0.4042 ±0.0694	104.79%
12.24	13.2806±1.2857	13.4768 ±1.7973	101.48%

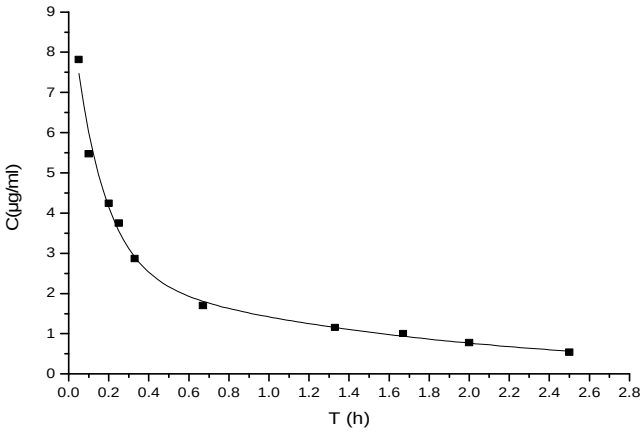

Figure 4 : Mean plasma concentration–time profile of SU2162 in rat plasma after intravenous administration of single dosage of 5.5mg/kg (*n* = 6).

Application of the method

The method was applied to the pharmacoki-

netic study of SU2162 in rats. The mean plasma concentration–time curve after intravenous administration of 5.5mg/kg SU2162 is shown in Figure 4. The basic pharmacokinetic parameters were calculated by kinetica 5.0 software. The results shows SU2162 eliminates from the plasma according to a two compartment model. The values of T1/2α and T1/2β for SU2162 were 0.1066 h and 1.1407 h, respectively. The details of the pharmacokinetics study will be described in a further publication.

CONCLUSION

In conclusion, a simple reversed-phase HPLC method has been described for the determination of SU2162 in rat plasma. The specificity, precision, accuracy, absolute recovery and linearity of the method were acceptable for the determination of SU2162 in rat plasma. Due to the lower absolute recovery when the SU2162 at high concentration in plasma sample, added more extraction solvent or two-step extraction method can be used to improve the method. The metshod was successfully applied to study the pharmacokinetics of SU2162 in rats after intravenous administration.

ACKNOWLEDGEMENTS

The authors acknowledge financial support from the Science and Technology Project of the Government of Guangdong Province, China (grant No. 2009B080701025).

REFERENCES

[1] Tiffany O'callaghan; J.Nature, 471 **(2011)**.
[2] Z.Tan; China, Patent No.200710029736.**1 (2008)**.
[3] Z.Y.Tan; L.Luo, E.J.Zhu, R.S.Yan, Z.H.Lin; J.Acta Cryst., **66**, 1374 **(2010)**.
[4] Z.Y.Tan, L.Luo, E.J.Zhu,, Lin Z.H.Lin, R.S.Yan, H.Zhang, K.Liang; Journal of Guangdong Pharmaceutical College, **26**, 441-444**(2010)**.
[5] H.Z.Li (Ed); 'The Analysis Method of Drugs in Vivo', 1st Edition'; Zhong Guo Yi Yao Ke Ji Publishers; Beijing, **(2003)**.
[6] U.S.Food, Drug Administration; Guidance for Industry Bioanalytical Method Validation **(2001)**.
[7] J.R.Zhang; Design and Evaluation of The Analysis Method of Drugs in Vivo, in J.R.Zhang, H.C.Zang; Hua Xue Gong Ye Publishers, Beijing, 11-18 **(2002)**.