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Short communication

High-performance liquid chromatographic method for the estimation of the novel investigational anti-cancer agent SR271425 and its metabolites in mouse plasma

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Abstract

A simple and reliable HPLC method was developed for the estimation of a new anti-cancer agent that belongs to the thioxanthone class, SR271425 in mouse plasma. SR271425, it's metabolites and internal standard (SR233377) were separated from plasma by liquid–liquid extraction using dichloromethane after quenching the plasma proteins with acetonitrile. Chromatography was performed on a reversed-phase C_{18} column using methanol–10 m*M* phosphate buffer, pH 3.5 (45:55) as mobile phase at a flow-rate of 0.8 ml/min for first 10 min and 1.4 ml/min for the next 15 min with UV–Vis detection at 264 nm and SR233377 as internal standard. The retention times of SR271425 and internal standard were 18.6 and 14.8 min, respectively. The limit of detection was 40 ng/ml and the limit of quantification was 78 ng/ml. This method was also able to detect the three metabolites of SR271425. The intra- and inter-day relative standard deviations were less than 13% at all concentrations. This analytical method was precise and reproducible for pharmacokinetics and metabolism studies of the drug in mice. SR271425 is proceeding to phase I clinical trials in 2001.

Keywords: SR271425

1. Introduction

SR271425, *N*-[1-{[2-(diethylamino)ethyl]amino}-7-methoxy-9-oxo-9H-thioxanthen-4-yl] methylformamide, belongs to the methylamino thioxanthone class, which has shown activity against a variety of subcutaneously growing solid tumors [1]. This agent is an analogue of SR233377, whose phase I pharmacokinetic trial was closed because of cardiac toxicity [prolongation of QT (QTc) interval] during a study by Svensson et al. [2]. This agent is equally active both by the intravenous (i.v.) and oral routes of administration. During the pre-clinical development of this molecule, an accurate and sensitive analytical method was needed for pharmacokinetic

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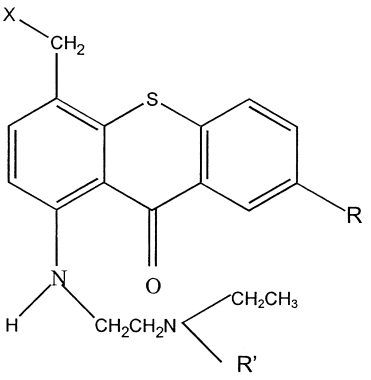
and metabolic evaluation. This study reports the development of an analytical method to monitor SR271425 and it's metabolites in mouse plasma using reversed-phase high-performance liquid chromatography (HPLC).

2. Experimental

2.1. Materials

SR271425 and its analogue, SR233377, used in this study as internal standard were provided by Sanofi-Synthelabo Research (Malvern, PA, USA). Previous studies indicated that the drug may potentially metabolize to three metabolites: SR200002, SR200019 and SR271904. The structures of these compounds are shown in Fig. 1. Methanol, acetonitrile and dichloromethane (all HPLC grade) and monobasic potassium phosphate (reagent grade) were obtained from Sigma (USA).



SR 233377 - R= H, R' = CH_2CH_3, X=$NH_2SO_2CH_3$

SR271425 - R=OCH_3, R' = CH_2CH_3, X= NHCHO

SR271904 - R=OCH_3, R' = CH_2CH_3, X= NH_2

SR200002 - R=OH, R' = CH_2CH_3, X=NHCHO

SR200019 - R=OCH_3, R' = H, X= NHCHO

Fig. 1. Structures of SR271425, its metabolites (SR271904, SR200002, SR200019) and internal standard (SR233377).

2.2. Sample preparation

Drug, internal standard and drug metabolites were separated from mouse plasma by liquid–liquid extraction. Briefly, to 250 μl of plasma, internal standard (1.6 μg of SR233377 in 100 μl of methanol) was added, and then the plasma proteins were precipitated by adding 250 μl acetonitrile. Then 4 ml of dichloromethane was added and the sample was rotated on a shaker for 15 min. The tubes were centrifuged at 2500 rpm for 15 min using a refrigerated centrifuge. The organic layer was separated and dried under nitrogen. The residue was reconstituted in 200 μl of acetonitrile and 100 μl of it was injected onto the column.

2.3. Calibration curve

A calibration curve was prepared by adding 0.078, 0.156, 0.312, 0.625, 1.25, 2.5 and 5 μg of SR271425 to 1 ml of plasma collected from naïve mice. A 250-μl volume of each of these samples was extracted in the same manner as described in the previous section. The peak area ratios (drug to internal standard) obtained at different concentrations of the drug were plotted against the drug concentrations. The slope of the plot, determined by the method of least-square regression analysis, was used to calculate the SR271425 concentration in the plasma samples obtained from mice treated with SR271425. Separately, triplicate samples of plasma were spiked with 1.25 μg of SR271425 for 7 days and extracted as described previously, and the peak area was compared with standards prepared from mobile phase to stability and recovery is estimated.

2.4. Instrumentation

A HPLC system, LC-HP 1100 series (Hewlett-Packard) equipped with a G1311A pump, a G1313A auto sampler, a G1315A diode array detector and Chem Station software was used.

2.5. Chromatographic conditions

Mobile phase consisted of methanol–10 m*M* phosphate buffer, pH 3.5 (45:55), the flow-rate was 0.8 ml/min for the first 10 min, then 1.4 ml/min for

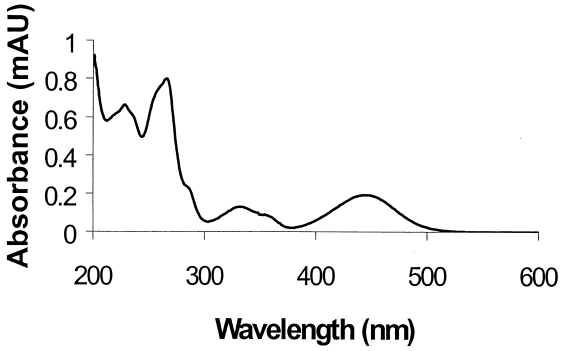


Fig. 2. UV spectrum of SR271425.

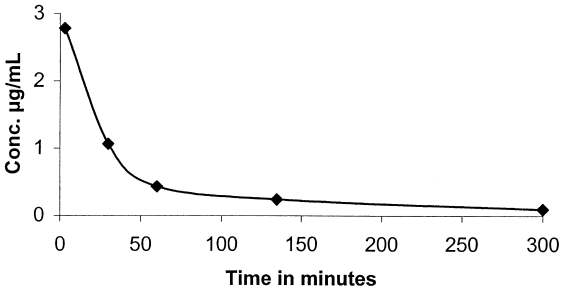


Fig. 4. Pharmacokinetic profile of SR271425 administered intravenously to mice at 30 mg/kg.

the next 15 min and the UV detector was set at 266 nm. An octadecyl silane reversed-phase column (Alltech Associates, USA) of 250×4.6 mm containing 5 μm size adsorbent as stationary phase was used. SR233377, an analogue of the investigational agent, was used as internal standard.

3. Results and discussion

The UV spectrum of SR271425 is shown in Fig. 2. SR271425 has maximum absorbance at 266 nm. The mobile phase [methanol–10 m*M* phosphate buffer, pH 3.5 (45:55)] was optimized for a rapid and interference-free chromatogram. The chromatogram of SR271425 and its metabolites and internal standard in plasma is shown in Fig. 3. The peaks were sharp, symmetrical and well resolved. The retention times of SR271425, SR200002, SR200019, SR271904 and SR233377 (internal standard) were 18.4, 9.5, 17.1, 5.9 and 14.7 min, respectively. The method used in this study is less complex and easily reproducible when compared to the column-switching high-performance liquid chromatographic assay reported for the internal standard SR233377 [3].

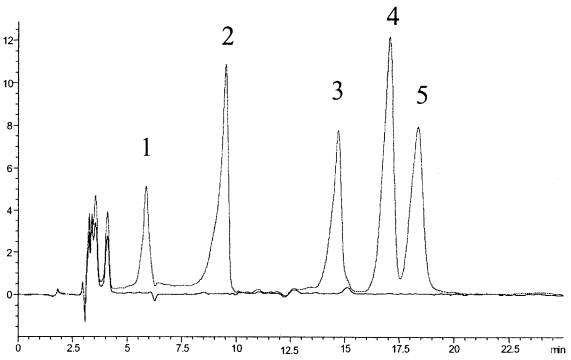


Fig. 3. Chromatogram showing the blank plasma and plasma containing SR271425 (5), SR20002 (2), SR200019 (4), SR271904 (1) and SR233377 (3).

3.1. Linearity and sensitivity

Least-square regression (without weighed averages) analysis has revealed that the peak area ratios (drug to internal standard) are linear in the concentration range 0.078 to 5.0 μg/ml. The liquid–liquid extraction efficiency was determined with different solvents such as chloroform, diethyl ether, ethyl acetate and dichloromethane. The recovery of SR271425 and it's metabolites from dichloromethane was more than 90% at all the concentrations. The extraction efficiency was less than 75% with other solvents. The minimum limits of detection and quantification were 40 ng/ml and 78 ng/ml, respectively. The recovery of SR271425 in plasma spiked with 1.25 μg of drug and stored for 7 days was found to be 95.0±3.0%, indicating that the drug was stable in plasma. These results are similar to the published data using internal standard SR233377 [3].

3.2. Accuracy and precision

The accuracy of the method was verified by

Table 1
Intra- and inter-day variations of the SR271425 HPLC method

Prepared concentration (μg/ml)	Measured concentration (μg/ml)	Accuracy[a] (% deviation)	Precision (RSD, %)	
			Intra-day[b]	Intra-day[c]
0.156	0.146	6.40	3.65	10.46
0.625	0.601	3.84	1.47	12.34
1.250	1.152	7.84	2.75	11.22
2.500	2.280	8.80	3.82	9.51
5.000	4.890	2.20	2.53	8.77

[a] $\%\ \text{deviation} = \frac{\text{prepared concentration} - \text{measured concentration}}{\text{prepared concentration}} \times 100$

[b] Intra-day variability is expressed by analyzing each concentration six times within a day.
[c] Inter-day variability is determined by analyzing each concentration on 5 consecutive days.

comparing the prepared concentrations with measured concentration and the data are shown in Table 1. The precision of this method was tested by analyzing the spiked plasma samples with different concentrations of the drug six times on 5 different days. The intra- and inter-day variations are listed in Table 1. This method was used for the analysis of the plasma samples of mice treated with 30 mg/kg of SR271425 intravenously. The plasma levels of SR271425 in mice are shown in Fig. 4.

In conclusion, this simple and reliable method can be used for the estimation of SR271425 in plasma and other organs to determine pharmacokinetics and tissue disposition, respectively.

Acknowledgements

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