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High-performance liquid chromatographic method for the determination of RGH-5702 in plasma samples

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ABSTRACT

A quick and selective high-performance liquid chromatographic method has been developed for the determination of RGH-5702 in plasma samples. A simple one-step extraction is used followed by reversed-phase chromatography and UV detection. This method allowed the separation of the compound and internal standard within 7 minutes. Validation of the method was performed prior to the assay of samples and continued throughout the study. Acceptable accuracy and precision was achieved at all concentrations investigated. The quantitation limit was 20 ng/ml using 1 ml of plasma. The method has been applied to the analysis of plasma samples from toxicokinetic studies in dogs.

INTRODUCTION

3-(2-Nitrophenylmethyl)-2-thiazolidinone (RGH-5702) is a new cytoprotective agent synthesized by the Chemical Works of Gedeon Richter. According to pharmacological investigations the compound has an inhibitory effect against indomethacin-induced gastric ulcer and decreases the acid secretion in pylorus ligated rats [1]. As part of the development of the compound it was necessary to develop a simple and selective method for the determination of the compound in biological fluids in order to perform a toxicokinetic study on dogs at three dose levels corresponding to the 28-day toxicity study.

EXPERIMENTAL

Chemicals

RGH-5702 (Fig. 1) and the internal standard (Fig. 1) were supplied by the Chemical Works of Gedeon Richter. Ethanol (HPLC grade) and diethyl ether (spectroscopy grade) were purchased from Merck (Germany). Glycocoll and sodium hydroxide (analytical purity) were obtained from Reanal (Hungary) and methanol (HPLC grade) from Aldrich (U.S.A.).

Chromatographic equipment and conditions

Chromatography was carried out with a Model 303 solvent delivery system, a

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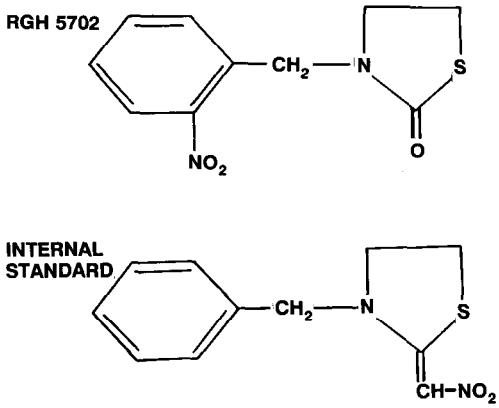

Fig. 1. Chemical structures of RGH-5702 [3-(2-nitrophenylmethyl)-2-thiazolidinone] and the internal standard (3-benzyl-2-nitro-3-methylene thiazolidine).

Model 116 variable-wavelength detector, a Model 231 sample injector, Dilutor 401, all from Gilson (France) and a COPAM PC-501 Turbo Computer (U.S.A.).

A LiChrosorb RP-8 column 200 × 4.6 mm I.D., particle size 5 μm (Hewlett-Packard) was used with methanol–doubly distilled water (60:40, v/v) as mobile phase at a flow-rate of 1.0 ml/min. Detection was carried out at 265 nm. High-performance liquid chromatographic (HPLC) analysis was performed at room temperature (*ca.* 25°C).

Preparation of plasma calibration samples

A stock solution of RGH-5702 was prepared in ethanol (10 mg/100 ml). A series of plasma calibration samples containing the compound in equal concentrations of 20, 40, 100, 200, 500 ng/ml were prepared from stock solution and pooled dog plasma. For example 10.0 ml of pooled dog plasma was spiked with 10 μl of stock solution to give a final concentration of 100 ng/ml for RGH-5702. These plasma calibration samples were divided into 1 ml aliquots stored at −18°C and used to construct the calibration curves.

General assay procedure

A stock solution of internal standard (3-benzyl-2-nitro-3-methylene-thiazolidine) was prepared in chloroform (10 mg/50 ml). A working solution of internal standard was prepared by diluting 1 ml of the stock solution with chloroform to a final concentration of 400 μg/ml.

A volume of 10 μl of the internal standard working solution was dried into a test tube into which 1 ml of dog plasma, 0.2 ml of gly–NaOH buffer (1.0 *M*, pH 10.0) were added and shaken with 7.0 ml of diethylether for 10 min. After refrigerated centrifugation (10 min at 1500 *g*, 4°C) the organic layer was removed to a new test tube and evaporated to dryness under a stream of nitrogen at approximately 40°C. The residue was redissolved in eluent (100 μl) by vortexing and sonication.

An aliquot (20 μl) was injected into the HPLC system.

RESULTS AND DISCUSSION

Chromatography

Chromatograms from the assay of dog plasma are given in Fig. 2. Significant interfering peaks of endogenous compounds were not recorded on the chromatogram. The described method allowed the separation of the compound and the internal standard within 7 min. The chromatographic system is very simple and requires

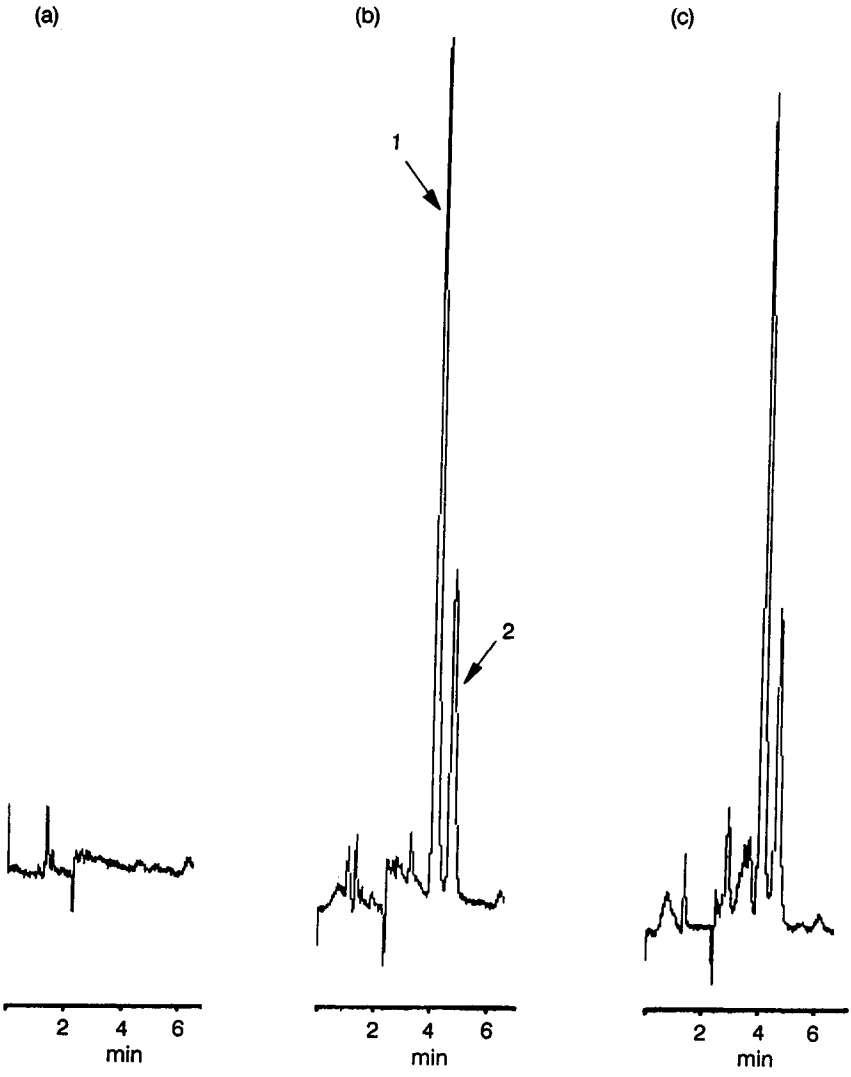

Fig. 2. Chromatograms of extracted dog plasma samples: (a) blank plasma, (b) extracted dog plasma calibration standard containing 200 ng/ml RGH-5702 (1 = internal standard, 2 = RGH-5702), (c) extracted plasma samples with internal standard 1 h after oral administration of 10 mg/kg of RGH-5702.

TABLE I

WITHIN-DAY AND BETWEEN DAYS PRECISION OF HPLC PROCEDURE FOR RGH-5702 IN DOG PLASMA

Concentration (ng/ml)	Peak area ratio (mean ± S.D. $n = 4$)	Coefficient of variation (%)
Within-day precision ($n = 4$)		
40	0.185 ± 0.005	3.1
200	0.520 ± 0.029	5.5
500	1.075 ± 0.068	6.3
Between-days precision ($n = 6$)		
20	0.127 ± 0.023	18.1
40	0.162 ± 0.013	8.0
100	0.275 ± 0.041	14.9
200	0.498 ± 0.039	7.8
500	1.015 ± 0.139	13.6

a minimum of maintenance. The column was regenerated once or twice a month by repacking the top. More than 600 plasma samples could be assayed on the same column.

Validation procedure

Validation of the method was performed prior to the assay of toxicokinetic samples and continued throughout the study. The summaries of within run precision and the between run precision of the method appear in Table I.

The percentage coefficient of variation of peak area ratios was taken as a measure of precision. The percentage coefficient of variation varied randomly over the concentration range. The accuracy of the HPLC method was determined by recalculating the concentration of the calibration plasma samples using the calibration curve (Table II).

Linearity of response was demonstrated by linear regression analysis from concentrations of 20–500 ng/ml ($n = 6$). The least-squares correlation values ranged from 0.9987 to 0.9999 (mean ± S.D. = 0.9993 ± 0.0005, $n = 6$) with slopes between

TABLE II

ACCURACY OF HPLC PROCEDURE FOR RGH-5702

Theoretical concentration (ng/ml)	Peak area ratio	Recalculated concentration
20	0.035 ± 0.006	22.35 ± 7.65
40	0.061 ± 0.011	41.48 ± 3.51
100	0.135 ± 0.015	98.48 ± 4.69
200	0.260 ± 0.023	200.80 ± 9.70
500	0.650 ± 0.033	500.00 ± 2.82

TABLE III

RECOVERY OF RGH-5702 IN DOG PLASMA

Concentration (ng/ml)	Recovery (% mean ± S.D., $n = 5$)	C.V. (%)
20	81.8 ± 9.5	11.6
40	81.0 ± 8.5	10.5
100	82.5 ± 5.7	6.9
200	78.5 ± 4.1	5.2
500	76.2 ± 10.2	13.4
1000	79.3 ± 7.4	9.3
2000	74.6 ± 5.1	6.8
Mean ± S.D.	79.1 ± 2.9	

$1.166 \cdot 10^{-3}$ and $1.360 \cdot 10^{-3}$ (mean ± S.D. = $1.284 \cdot 10^{-3} \pm 0.065 \cdot 10^{-3}$, $n = 6$). We also checked that the extension of the calibration range to 2000 ng/ml did not affect the linearity.

Plasma recovery

The recovery of the assay was determined as the detector response of pure authentic standard solutions compared to the response from extracted plasma calibration samples containing an equivalent amount of RGH-5702. The percentage of recovery of the compound was calculated as:

$$\text{recovery} = \frac{\text{peak area of extr. plasma calibration sample}}{\text{peak area of standard solution}}$$

(An aliquot of stock solution of RGH-5702 was further diluted and dried into vial, reconstituted in eluent then injected.) The results are shown in Table III and the overall recovery of the assay was 79.1 ± 2.9%.

CONCLUSION

A quick and selective liquid chromatographic method for the determination of RGH-5702 in dog plasma has been developed. The quantitation limit for the method is 20 ng/ml with a signal-to-noise ratio of > 5. The HPLC method described in this paper enables the monitoring of RGH-5702 during toxicokinetic and chronic toxicity studies.

REFERENCE

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