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Determination of S 12024 enantiomers in human plasma by liquid chromatography after chiral pre-column derivatization*

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Abstract: S 12024-2 is a new drug in phase II development that possesses cognitive enhancing properties. As its molecular structure has a chiral centre, a stereoselective method for the analysis of both enantiomers in human plasma has been developed. The method involves pre-column derivatization of the amine moiety with a homochiral reagent (+)-1-(9-fluorenyl)ethyl chloroformate (FLEC), and chromatographic separation of the two diastereoisomers on an achiral reversed-phase cyanopropyl column, with fluorimetric detection (λ_{ex} = 260 nm; λ_{em} = 310 nm). A liquid–liquid extraction procedure with diethyl ether–dichloromethane (70:30, v/v) was used for sample preparation. This technique provides a linear response for both enantiomers over a concentration range of 10–500 ng ml^{-1} and the quantitation limit was set at 5 ng ml^{-1} in human plasma. Within-day and between-day precision and accuracy are within 9% limits for all concentrations assessed. This procedure was therefore used for determining both enantiomers in human plasma following oral administration of racemic S 12024-2 to elderly healthy subjects.

Keywords: *Enantiomers; HPLC; fluorescence; chiral derivatization; plasma.*

Introduction

S 12024-2 is a new original compound with cognitive enhancing properties. It is supposed to act via noradrenergic facilitation. The chemical structure of the drug is a substituted quinoline derivative with a chiral centre in the morpholinyl moiety (Fig. 1). A stereoselective method has therefore been developed for the analysis of both enantiomers of S 12024-2 in human plasma, in order to determine their pharmacokinetic profiles after oral administration of the racemic compound. Separation of both enantiomers could have been obtained on a chiral stationary phase, Chiralcel-OJ, but to increase the sensitivity and the chromatographic resolution, pre-column derivatization with a fluorescent homochiral reagent, FLEC, was developed. This paper describes the development of a reversed-phase HPLC chiral assay for S 12024-2 and its application to the determination of both enantiomers in human plasma.

Experimental

Materials and reagents

The racemic compound S 12024-2 ((*R*,*S*)-1-methyl-8-[(morpholin-2-yl)methoxy]-1,2,3,4-tetrahydroquinoline, monomethane sulphonate salt) was used for all the validation assays. Each enantiomer, (+)-(*S*)-S 12024 and (−)-(*R*)-S 12024, was used for determining the retention time of each chiral form of S 12024. The chiral compound, (+)-(*S*)-S 12027 (8-[(morpholin-2-yl)methoxyl]-1,2,3,4-tetrahydroquinoline, monomethane sulphonate salt) was used as the internal standard. All these compounds were obtained from Technologie Servier (Orléans, France). HPLC grade acetonitrile was purchased from Riedel-de-Haën (Seelze, Germany). Diethyl ether and sulphuric acid (95–97%) were obtained from E. Merck (Darmstadt, Germany). Dichloromethane was supplied by Solvants Documentation Syntheses (Peypin, France). Triethylamine, D,L-proline and (+)-1-(9-fluorenyl)-

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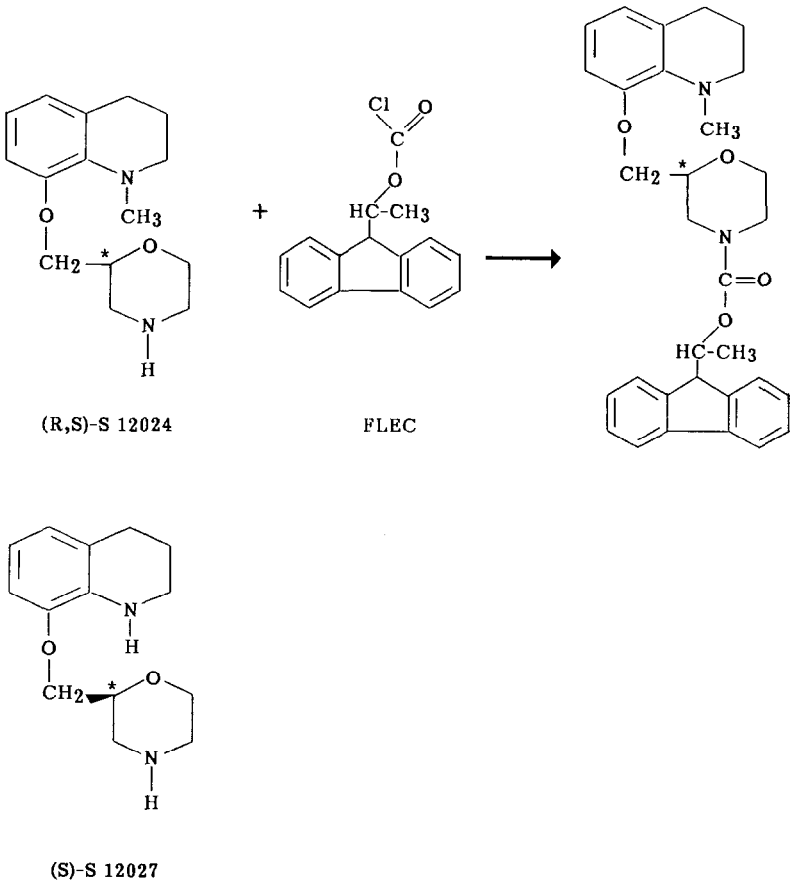

Figure 1
Chemical structures of (*R*,*S*)-S 12024, the internal standard (*S*)-S 12027, and the reaction scheme with FLEC.

ethyl chloroformate FLEC (18 mM in acetone) were purchased from Aldrich (Strasbourg, France). Sodium hydroxide (Normex 10 M) was obtained from Carlo Erba (Milan, Italy). The water was de-ionized reagent grade (Milli-Q).

Instrumentation and chromatographic conditions

The chromatographic system comprised a model P 4000 pump, a model AS 3500 auto-sampler thermostatted at +10°C (Spectra-Physics, Les Ulis, France) and a Perkin–Elmer LC 240 fluorimetric detector (Saint-Quentin-en-Yvelines, France) with an excitation wavelength set at 260 nm and an emission set at 310 nm. Data acquisition and reprocessing were carried out with Spectra Station Software (Spectra-Physics). Chromatographic separations were performed on a reversed-phase 3-μm Spherisorb Cyanopropyl column (15 cm × 4.6 mm i.d.) from Colochrom (Paris, France). The mobile phase was acetonitrile–water containing 0.05% (v/v) sulphuric acid (adjusted to pH 3 with sodium hydroxide 10 M) (25:75, v/v). The flow rate was 1.3 ml min^{-1} and the injection volume was 20 μl.

Standard solutions

Stock solutions of S 12024 and of each enantiomer were prepared in de-ionized water at a concentration of 1 mg ml^{-1}, expressed as the base form. Appropriate dilutions were made with de-ionized water at a concentration of 100 μg ml^{-1} and were kept for 1 week at 4°C. Other dilutions were prepared each working day in human plasma (10 and 1 μg ml^{-1}) and used to prepare spiked plasma standards at concentrations of 20, 50, 100, 400, 600 and 1000 ng ml^{-1} of racemic S 12024. Spiked plasma quality control samples were prepared in pools at final concentrations of 50, 200 and 1000 ng ml^{-1} of racemic S 12024. A stock solution of the internal standard (*S*)-S 12027 was prepared at a concentration of 1 mg ml^{-1} in de-ionized water, expressed as the salt form, and diluted to 5 μg ml^{-1} in de-ionized water. Stock solutions of reference compounds and

the internal standard can be stored for one month at 4°C since no significant degradation has been observed over this period.

Sample preparation

An aliquot of plasma (1 ml) was added to a disposable borosilicate tube, vortexed for 5 s, and spiked with 50 μl of the internal standard spiking solution (5 μg ml^{-1}). To the biological sample, 50 μl of 1 M sodium hydroxide was added and the sample was then extracted with 5 ml of a diethyl ether–dichloromethane (70:30, v/v) solution. The tubes were vigorously shaken for 3 min and then centrifuged at 1500*g* (3500 rpm) for 5 min at 5°C. The organic layer was transferred to another disposable tube and dried under a stream of nitrogen at 37°C.

Chiral derivatization

After evaporation, the residue was reconstituted in 250 μl of a mixture of de-ionized water–triethylamine solution (0.1% v/v in acetonitrile) (4:1, v/v). After vortexing for 1 min, 200 μl of a 61 μg ml^{-1} FLEC solution in acetonitrile was added. The reaction medium was stirred for 1 h at room temperature. A D,L-proline solution (10 mg ml^{-1} in water) was then added to the medium in order to remove the excess reagent. After 1 min of stirring, the reaction mixture was directly injected into the liquid chromatographic system.

In vivo *studies*

Blood samples were collected from elderly healthy subjects after receiving single oral doses of 50, 100 and 200 mg of racemic S 12024. Blood was collected at t = 0, 1, 4, 8, 16 and 36 h following oral administration. The plasma obtained was stored at −20°C until analysis, since the stability of the unchanged compound in human plasma has been confirmed over 6 months' storage.

Results and Discussion

Several chiral fluorescent reagents are available for the derivatization of amino acids and amines. *R*-Naphthylethyl isocyanate [1, 2] and *S*-naphthylethyl isothiocyanate [3] react selectively with primary and secondary amines in mild conditions but are less used because of their tendency to hydrolyse. Orthophthalaldehyde (OPA) reacts in a few minutes with primary amino acids in the presence of chiral thiols [4, 5] to give highly fluorescent isoindole derivatives. However, the reaction is selective for primary amines and fluorescent derivatives have limited stability. A new commercially available chiral fluorescent reagent FLEC was therefore used. This agent reacts in mild conditions with numerous compounds, like amino acids [6–8], primary and secondary amines [9] and also alcohols [6, 7] to give the corresponding carbamates. Depending on the compound assayed, the reaction takes place within a few minutes or longer (30–60 min) for secondary amines or alcohols. The operating conditions — basic medium, ambient temperature, aqueous or organic reaction medium — are adapted for the derivatization of drugs in a biological medium.

Pre-column derivatization of S 12024 with FLEC reagent was then investigated. A chiral internal standard was chosen for the validation assay in order to obtain a single diastereoisomer derivative in chromatography. The optimization of reaction conditions was studied. The organic base triethylamine proved to be more reactive than an inorganic buffer like borate or phosphate for total completion of the reaction with S 12024. Using a molar excess of FLEC to the internal standard of 20:1 for all the assays, the reaction efficiency was complete within 1 h for S 12024 and the internal standard in a biological medium. At the end of the reaction, the excess reagent was consumed with D-,L-proline [6, 7]. The polar proline carbamate derivative obtained was eluted in the solvent front of the chromatogram in a reversed-phase mode, and therefore did not interfere with chromatographic peaks of the drug. Typical chromatograms obtained from human plasma extracts after derivatization with FLEC are given in Fig. 2. No interference from endogenous compounds was observed. Both enantiomers were well resolved since a resolution R_S of 0.7 was obtained. The retention times of the internal standard, (*R*)-S 12024 and (*S*)-S 12024 were 18.5, 23.3 and 24.2 min, respectively.

Analytical validation

The absolute recovery of the liquid–liquid extraction procedure for the unchanged drug was evaluated at 80% for each enantiomer and at 63% for the internal standard by comparison of plasma extracts with aqueous standard solutions after derivatization. The linearity of the method has been assessed over the whole

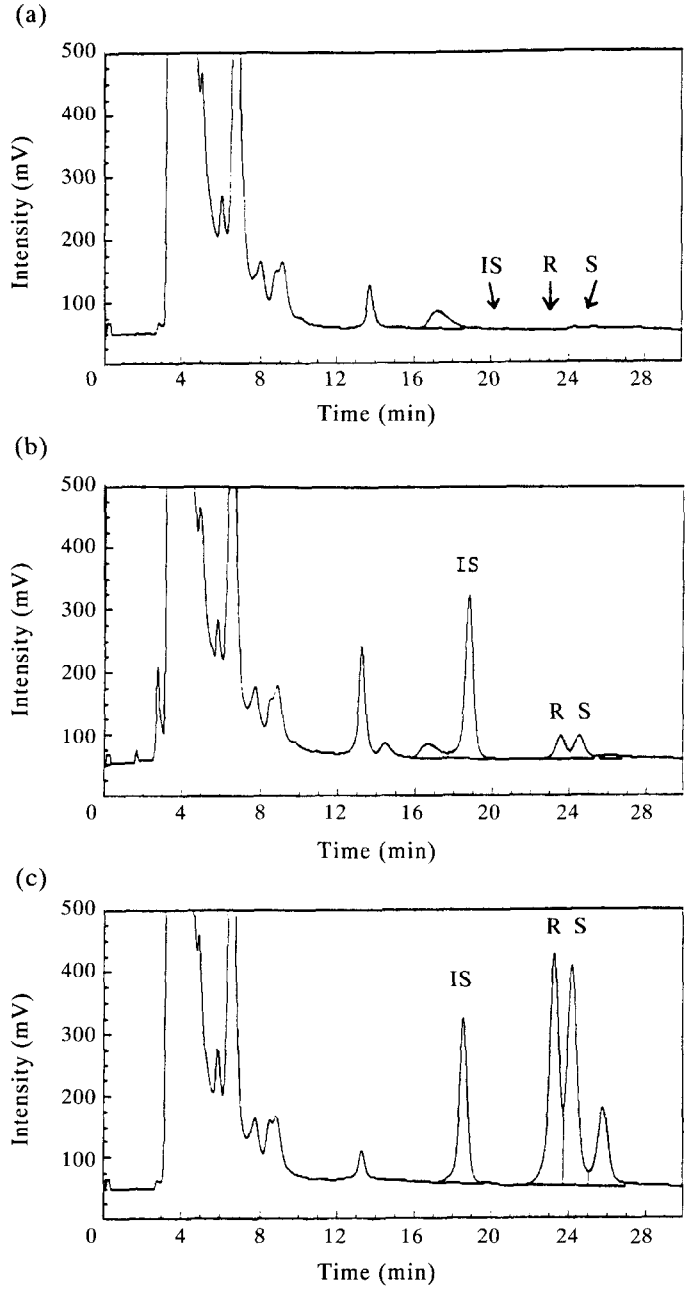

Figure 2
Typical chromatograms obtained from FLEC derivatization of (a) blank human plasma; (b) human plasma spiked with 10 ng ml^{-1} of each enantiomer of S 12024; and (c) human plasma extract obtained 8 h after a single oral 100 mg dose of racemic S 12024-2; ((*R*)-S 12024, R; (*S*)-S 12024, S; (*S*)-S 12027, IS).

concentration range 10–500 ng ml^{-1} for each enantiomer, based on the determination of the peak-height ratios of the (*R*)- and (*S*)-enantiomers relative to the internal standard as a function of the concentration of each enantiomer of S 12024. A weighting of $1/C^2$ was used in order to obtain a more precise fit of the regression lines. The mean values obtained for the regression parameters (Table 1) demonstrate the reliability of the calibration curves from day to day, with low relative errors. The limit of quantitation was set at 5 ng ml^{-1} for each enantiomer by determination of the relative standard deviation (RSD) and relative error (RE) on six replicates. For (*R*)-S 12024 the RSD = 4.2% and the RE = −4.0%. For (*S*)-S 12024 the RSD = 6.4% and the RE = −6.0%.

Table 1
Regression parameters for (*R*)- and (*S*)-enantiomers of S 12024 in the range 10–500 ng ml^{-1}

Compound	Slope (mean ± SD) (n = 3)	Intercept (mean ± SD) (n = 2)	Correlation coefficient (mean ± SD) (n = 2)
(*R*)-S 12024	0.00675 ± 0.0001	0.0037 ± 0.0004	0.9988 ± 0.0014
(*S*)-S 12024	0.00668 ± 0.0001	0.0042 ± 0.0008	0.9992 ± 0.0009

Table 2
Within-day assay and between-day assay precision and accuracy for (*R*)- and (*S*)-enantiomers of S 12024

		Within-day assays			Between-day assays		
Compound	Conc. added (ng ml^{-1})	Conc. found (n = 6) (mean ± SD)	RSD (%)	RE (%)	Conc. found (n = 8) (mean ± SD)	RSD (%)	RE (%)
(*R*)-S 12024	25.0	24.4 ± 0.6	2.5	−2.4	24.8 ± 2.1	8.5	0.5
	100	104 ± 0.9	0.9	3.7	102 ± 5	4.4	2.6
	500	506 ± 9	1.8	1.2	524 ± 38	7.2	4.1
(*S*)-S 12024	25.0	24.6 ± 0.4	1.6	−1.6	25.5 ± 1.7	6.7	3.7
	100	104 ± 2	1.5	3.7	104 ± 3	3.1	4.4
	500	500 ± 12	2.3	0.1	527 ± 31	5.8	4.8

Table 3
Results of racemization study on (*R*)- and (*S*)-enantiomers of S 12024

Compound	Conc. added (ng ml^{-1})	Conc. found (n = 6) (mean ± SD)	RSD (%)	RE (%)
(*R*)-S 12024	100	102 ± 2	2.4	2.0
(*S*)-S 12024	100	101 ± 2	1.7	1.2

The precision and accuracy of the method were determined by within-day (six replicates) and between-day (4 days, two replicates per day) assays at three concentration levels for each enantiomer (Table 2). The precision of the method, as measured by the per cent relative standard deviation, was within 3% for the within-day assays and within 9% for the between-day assays. The accuracy determined as the per cent relative error from the theoretical value was lower than 5% for all the assays.

Racemization study

In order to study the enantiomeric stability of (*R*)- and (*S*)-S 12024 during the extraction and derivatization procedures, a racemization test was done. Control samples of each enantiomer were therefore prepared separately at an intermediate concentration level (100 ng ml^{-1}) and analysed with a calibration curve established with racemic S 12024. The mean concentration found for each chiral form (Table 3) demonstrates the enantiomeric stability of both enantiomers, proving that neither racemization nor configuration inversion occurred over the whole sample pretreatment.

Application

The chiral method developed was applied to the stereoselective determination of (*R*)- and (*S*)-S 12024-2 following single oral administration of S 12024-2 to elderly healthy human volunteers. For each dose (50, 100 and 200 mg), plasma samples were analysed with the analytical technique. The pharmacokinetic profiles obtained for each enantiomer proved to be very similar at the three doses studied (Fig. 3), with an enantiomeric ratio of 50:50 at each time point.

Conclusions

A stereoselective HPLC method involving pre-column chiral derivatization with a chiral reagent FLEC has been developed for the

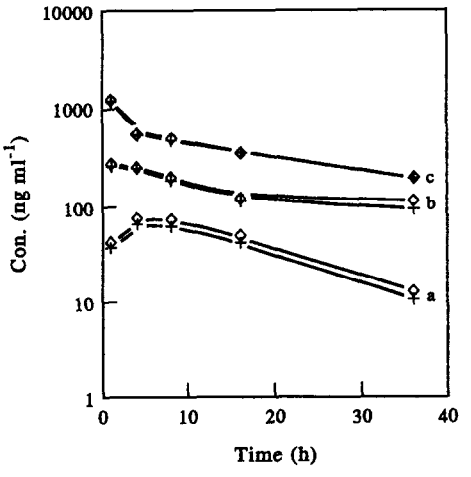

Figure 3
Typical plasma concentration–time curves for (*R*)-S 12024 (◇) and (*S*)-S 12024 (+) in elderly helathy subjects following single oral administration of racemic S 12024-2: (a) 50 mg; (b) 100 mg; and (c) 200 mg dose.

analysis of (*R*)- and (*S*)-enantiomers of S 12024 in human plasma. The method has been shown to be sensitive, selective and reliable, since low values for the precision and accuracy during within-day and between-day assays were obtained. The method was then successfully applied to the determination of both enantiomers following single oral administration of racemic S 12024-2 to elderly healthy subjects. The enantiomeric ratio of 50:50 obtained for each chiral form each time and for each dose showed similar pharmacokinetic profiles for (*R*)- and (*S*)-S 12024.

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