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Sensitive high-performance liquid chromatographic method for the determination of a benzonaphthazepine antipsychotic agent, SCH 39166, and its active metabolite, SCH 40853, in human plasma and its cross-validation with a gas chromatographic method

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Abstract

A sensitive high-performance liquid chromatographic (HPLC) method was developed for the determination of a benzonaphthazepine antipsychotic agent, SCH 39166, and its active metabolite, SCH 40853. The HPLC method required a single-step organic extraction at alkali pH followed by HPLC analysis utilizing a CN column with UV detection at 205 nm. The limit of quantitation was 1 ng/ml for SCH 39166 and 0.5 ng/ml for SCH 40853. The HPLC method was cross-validated with a previously reported GC method by the analysis of 73 plasma samples spiked with various concentrations of SCH 39166 and SCH 40853. The correlation coefficient was 0.9969 for SCH 39166 and 0.9984 for SCH 40853. Both GC and HPLC methods were used for the determination of plasma concentrations and yielded similar pharmacokinetic parameters for SCH 39166 and SCH 40853 in man following oral administration of SCH 39166 (100 mg).

Keywords: Benzonaphthazepine antipsychotic

1. Introduction

SCH 39166, *trans*-(−)-(6aS,13bR)-11-chloro-6,6a,7,8,9,13b-hexahydro-7-methyl-5H-benzo[*d*]-naphth[2,1-blazepine-12-ol] (I, Fig. 1) is a benzonaphthazepine selective dopamine D_1-receptor antagonist [1–3] which produces reduced extrapyramidal effects in comparison to standard neuroleptics [4–8]. Quantitation of I in rat brain and plasma by a gas chromatographic (GC) method was first described by Hietala et al. [9]. In rats, I undergoes rapid N-demethylation to form SCH 40853 (II) a pharmacologically active metabolite. Tedford et al. [10] reported a modified GC method for I and II in rat plasma and brain. Both I and II are converted extensively into glucurono-conjugates [11–13]. Following oral administration of I in rats, plasma concentrations of I glucuronide, II glucuronide, I and II account for 89.1%, 6.8%, 3.0% and 1.0% of total plasma AUC, respectively [10]. This report describes a sensitive high-performance liquid chromatographic (HPLC) method and its cross-validation with the GC

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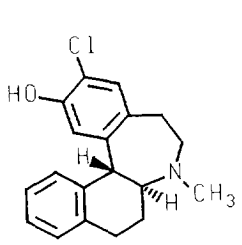
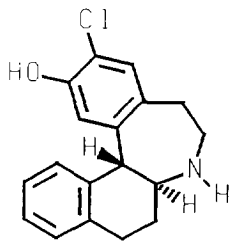


SCH 39166



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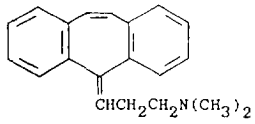

Cyclobenzaprine

Fig. 1. Structures of SCH 39166 (I), SCH 40853 (II) and cyclobenzaprine (internal standard).

method previously described by Tedford et al. [10] for the determination of I and II in human plasma.

2. Experimental

2.1. Chemicals

Compounds I·HCl and II (Fig. 1) were provided by Schering-Plough (Kenilworth, NJ, USA). Cyclobenzaprine·HCl (internal standard) was obtained from Sigma (St. Louis, MO, USA).

2.2. Drug administration and plasma sample collection

Six male volunteers between 19 and 40 years of age in Study I, and another six between 24 and 40 years of age in Study II each received one 100 mg capsule of I·HCl. All subjects were determined to be in good health through medical history, physical examination, electrocardiogram and laboratory tests (hematology, blood chemistry and urinalysis). Written informed consent was obtained upon enrolment in the study. Blood samples were collected at various time periods up to 48 h and 96 h for Studies I and II, respectively.

2.3. Sample preparation

A 50-μl aliquot of internal standard (cyclobenzaprine) solution (0.5 ng/μl), 0.2 ml of 4.5 *M* NH_4OH and 5 ml of 30% dichloromethane–hexane were added to a 1-ml sample of plasma, vigorously mixed on a vortex-mixer, and centrifuged at 1400 *g* for 10 min. After freezing the aqueous layer in dry ice–acetone bath, the organic layer was transferred to another tube and evaporated to dryness at 45°C under nitrogen. The residue was reconstituted in 0.2 ml of mobile phase (0.02 *M* ammonium phosphate, pH 6.5 acetonitrile, 55:45, v/v), and 0.1 ml of the mixture was injected onto the HPLC system.

2.4. HPLC chromatography

The HPLC system consisted of a TOSO HAAS autosampler, a Waters pump (Model 510), a Waters Lambda Max UV detector (Model 486) with the wavelength set at 205 nm, and a Hewlett-Packard Integrator (Model 3396 A). The analytical separation was accomplished on a Spherisorb CN column (15 cm×4.6 mm I.D.) fitted with an on-line pre-column filter. The isocratic mobile phase was 0.02 *M* ammonium phosphate (pH 6.5)–acetonitrile (55:45, v/v), delivered at a flow-rate of 1.5 ml/min.

2.5. Gas chromatography

The GC method used in this study was that of Tedford et al. [10] as previously described. The limit of quantitation was 0.5 ng/ml for both I (C.V.=3.5%, bias=1.2%) and II (C.V.=3.6%, bias=7.6%).

2.6. Calculations

Chromatographic peaks were identified on the basis of their respective retention times. A standard curve was constructed for I using the peak-height ratio of I to its respective internal standard. A separate standard curve was constructed for II using the peak-height ratio of II to its internal standard. The peak-height ratio for each analyte was plotted

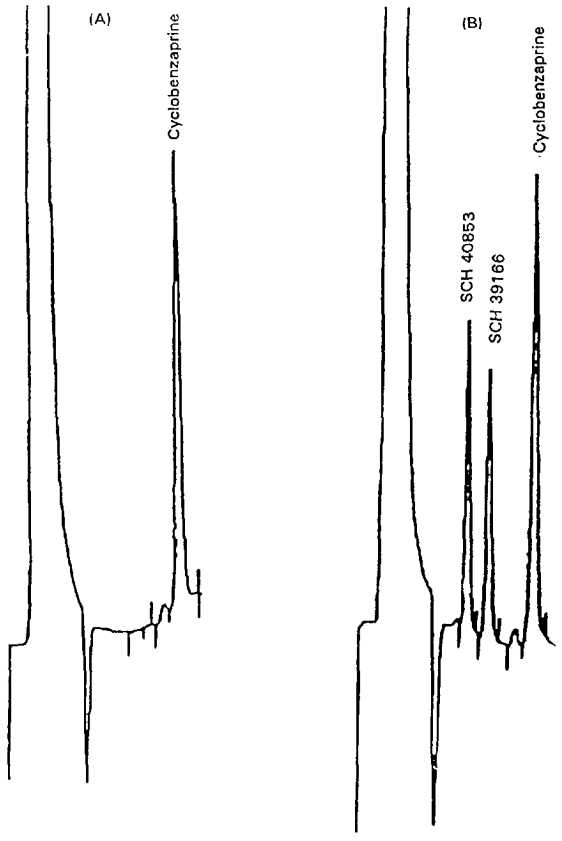

Fig. 2. Typical HPLC chromatogram of SCH 39166 (I), SCH 40853 (II) and cyclobenzaprine. (A) Control human plasma spiked with internal standard (cyclobenzaprine); (B) control human plasma spiked with I, II and internal standard.

against the known analyte concentration (ng/ml) and a standard curve generated using the linear regression equation, $y=mx+b$, where y is the peak-height ratio, x is concentration (ng/ml), m is the slope and b is the intercept.

3. Results

3.1. HPLC analysis

A typical HPLC chromatogram for I, II and the internal standard (cyclobenzaprine) extracted from plasma is shown in Fig. 2. The retention time was 6.0 min for I, 5.1 min for II and 8.2 min for the internal standard. The standard curves were obtained by plotting the peak-height ratio of I (or II) vs. concentrations of I ($y=0.0625x-0.0145$) or II ($y=0.0716x-0.0106$). There was a good linear relationship between peak-height ratio and plasma concentration with a correlation coefficient (r) of 0.9997 for I and 0.9995 for II. The limit of quantitation (LOQ) was 1 ng/ml for I (C.V.=7.45%, bias=6.0%) and 0.5 ng/ml for II (C.V.=6.47%, bias=2.0%) (Table 1). There were no interfering endogenous peaks in control plasma that occurred at the retention times of either I, II or the internal standard, indicating that HPLC method was selective for I and II.

Inter-day precision of the HPLC method was validated at nine concentrations of I and seven concentrations of II. The HPLC method was accurate

Table 1
Inter-day reproducibility for HPLC analysis

Concentration added (ng/ml)	I		II	
	Concentration found (ng/ml) (mean ± S.D., $n=26$)	C.V. (%)	Concentration found (ng/ml) (mean ± S.D., $n=26$)	C.V. (%)
0.5	–	–	0.51±0.033	6.47
1	1.06±0.079	7.45	0.99±0.052	5.25
2	2.03±0.075	3.69	1.99±0.104	5.23
5	4.95±0.150	3.03	4.96±0.222	4.48
8	7.71±0.301	3.90	7.93±0.266	3.35
10	9.83±0.191	1.94	10.1 ±0.246	2.44
20	19.7 ±0.781	3.96	20.1 ±0.418	2.08
50	49.8 ±1.24	2.49	–	–
75	75.6 ±1.39	1.84	–	–
100	101 ±2.51	2.49	–	–

Table 2
Intra-day reproducibility for HPLC analysis

Concentration added (ng/ml)	Concentration found (ng/ml) (mean ± S.D., $n=3$)	C.V. (%)
Compound I		
2	2.27±0.167	7.4
8	8.15±1.14	13.9
18	16.8 ±0.20	1.2
50	48.0 ±1.73	3.6
Compound II		
1	1.08±0.11	10.3
8	8.00±1.15	14.4
18	16.8 ±0.06	0.3

(bias less than 7%) and reproducible (C.V. less than 8%) (Table 2). Intra-day precision of the method was validated using replicate analysis of plasma samples spiked with either I or II. Intra-day precision was determined to be acceptable with C.V. of less than 14% for I and less than 15% for II.

3.2. Correlation between the GC and HPLC methods

The GC and HPLC methods were cross-validated by the analysis of 73 plasma samples spiked with various concentrations of I and II. Linear regression analysis of the data yielded an equation of $y=0.9058x+0.0986$ for I and $y=0.9775x-0.0561$ for II where y and x were the concentrations (ng/ml) obtained by HPLC and GC analysis, respectively. The correlation coefficient (r) of 0.9969 for I and 0.9984 for II indicated excellent correlation, hence interchangeable use between the two analytical methods.

3.3. Feasibility of GC and HPLC methods

The GC and HPLC methods described above were used in the analysis of plasma samples from Study I and II, respectively. In both studies, six male subjects received single 100 mg capsule of I·HCl. Mean plasma concentration-time curves for I and II gave similar profiles for I and II. Compound I was rapidly absorbed with a C_{max} of 52–64 ng/ml at a T_{max} of 2 h followed by a decline in plasma concentration with a $t_{1/2}$ of 14–15 h. Plasma concentrations of II, however, were low with a C_{max} of 1.5 ng/ml. The AUC of II accounted for only 5% of that for I.

4. Discussion

Both I and its N-demethylated metabolite, II, are potent dopamine D_1-receptor antagonists. However, glucuronidation at the free hydroxyl group of I or II leads to substantial losses in affinity for the D_1 dopamine receptor and renders the I and II glucuronides inactive. In clinical pharmacokinetic evaluation, only the concentrations of the parent drug and its active metabolite(s) are determined. We have, therefore, developed sensitive HPLC methods for I and II, but not for their inactive glucuronide metabolites.

Hietala et al. [9] reported a GC method for I in rat plasma and brain using a capillary column with temperature programming and a nitrogen–phosphorus detector. This method was modified by Tedford et al. [10] for both I and II using a megabore column at isothermal conditions with a similar nitrogen–phosphorus detector. The limit of quantitation was 2 ng/ml for I in rat plasma for both GC methods. In the present study, we have been able to obtain a limit of quantitation of 0.5 ng/ml for both I and II in human plasma using the GC procedure described previously by Tedford et al. [10].

However, the plasma samples required extensive and tedious sample preparation prior to injection onto the GC system, which included (a) alkalization of plasma followed by organic solvent extraction, (b) back extraction into 0.15 *M* HCl followed by washing with cyclohexane as a clean-up step, (c) alkalization of the 0.1 *M* HCl extract followed by re-extraction with organic solvent and (d) addition of 1-octanol followed by concentration in a Savant Speed Vac in which the organic solvent was evaporated leaving the samples concentrated in 1-octanol which has a very high boiling point (183°C). Since only 2 μl of the 1-octanol solution can be injected into capillary GC, the dilution factor of 50 (injection of 2 μl from approximately 100 μl of solution) greatly reduced the sensitivity of the GC method.

On the other hand, the HPLC method required only minimal sample preparation which involved alkalization of the plasma sample with NH_4OH

followed by extraction into dichloromethane–hexane, evaporation to dryness and reconstitution. The limit of quantitation for HPLC (1 ng/ml for I and 0.5 ng/ml for II) was similar to the GC method. This was achieved by a combination of UV detection at 205 nm and injection of 50% (100 μl out of 200 μl) of the sample solution. Since most organic endogenous compounds exhibit absorbance at 205 nm and can contribute significant background interference in the detection of I and II, this was circumvented by selecting a Spherisorb CN column fitted with an on-line precolumn filter, which enabled us to obtain a limit of quantitation of 0.5–1 ng/ml without interference from endogenous biological components. The HPLC assay also yielded higher sample throughput which is always an advantage in biomedical analyses.

The results of present study clearly indicate excellent correlation between HPLC and GC methods for determination of plasma concentrations of I and II. Both methods yielded similar pharmacokinetic parameters for I and II in man following oral administration of I. Thus, use of HPLC and GC methods are interchangeable in pharmacokinetic evaluation of I in man. However, HPLC method is the preferred choice of technique due to its much simpler sample preparation procedure and much higher sample throughput than the GC method.

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