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Note

Determination of tetraprenylacetone in human plasma by high-performance liquid chromatography with fluorescence derivatization using dansylhydrazine

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6,10,14,18-Tetramethyl-5,9,13,17-nonadecatetraen-2-one (tetraprenylacetone, TPA) is a polyisoprenoid compound that has been shown to be effective against several experimentally induced ulcers in rats [1] and is currently in clinical use for the treatment of gastric ulcers.

Although a method for the determination of TPA in biological fluids has been established by gas chromatography–mass spectrometry (GC–MS) [2], the procedure is tedious and time-consuming, and a simpler method that would be suitable for routine analysis is required. For this purpose, we have established a high-performance liquid chromatographic (HPLC) method with fluorescence derivatization using dansylhydrazine (DH).

EXPERIMENTAL

Reagents and chemicals

The structures of TPA and the internal standard (I.S.) are shown in Fig. 1. TPA used in this study was a mixture of the *cis*-5-*trans*-9,13,17 isomer (39.8%) and the *trans*-5,9,13,17 isomer (60.2%). DH, a fluorescence labelling reagent (Tokyo Kasei, Tokyo, Japan), and acetonitrile, methanol and *n*-hexane were of HPLC grade. Bond Elut (200 mg, C_{18}), a disposable extraction column, was purchased from Analytichem International (Harbor City, CA, U.S.A.). All other reagents were of analytical-reagent grade.

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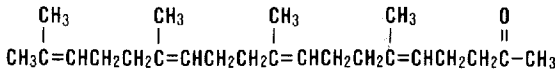
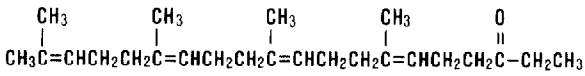


TPA



I.S

Fig. 1. Chemical structures of TPA and the internal standard (I.S.).

Plasma samples

Samples were prepared by dissolving TPA at the desired concentrations in human plasma. To confirm the usefulness of the established method, blood samples were obtained at intervals over 48 h after a single oral dose of 150 mg of TPA to eighteen healthy male volunteers 30 min after breakfast; plasma samples were obtained by centrifugation and stored at −20 °C until analysis.

Extraction of TPA from plasma

A 1-ml volume of water and 0.1 ml of I.S. in methanol solution (10 μg/ml) were added to 1 ml of plasma and mixed on a vortex mixer. After addition of 1 ml of ethanol and 0.1 ml of 50% potassium hydroxide solution, the mixture was saponified for 10 min in boiling water. After cooling, the mixture was shaken with 3 ml of hexane for 10 min and centrifuged for 5 min at 1670 *g*. This extraction procedure was repeated twice and the combined organic layer was evaporated at 45 °C by blowing a nitrogen gas stream over it.

Derivatization procedure

The extraction residue was dissolved in 0.2 ml of 0.5% hydrochloric acid in 2-propanol, and the solution was mixed with 0.2 ml of 0.2% DH in 2-propanol. This mixture was warmed at 35 °C for 60 min, then 1 ml of water was added.

The extraction column was attached to a vacuum manifold connected to a water aspirator. It was activated by washing with a packed-column volume of methanol and then two column volumes of water. The reaction mixture was transferred to the top of the activated extraction column, which was then washed twice with 3 ml of water and once with 1 ml of acetonitrile.

After the vacuum manifold had been disconnected, a collection tube was placed under the tip of the column, and elution was carried out with 0.5 ml of methanol–chloroform (1:1). After 30 s, the methanol–chloroform was drawn into the collection tube under vacuum. This extraction procedure was repeated twice, and the combined organic solvent was evaporated at 45 °C by blowing nitrogen gas over it. The residue was dissolved in 0.2 ml of ethanol, sonicated and, if necessary, centrifuged for 5 min at 1670 *g*. A 50-μl aliquot of the supernatant was then injected into the HPLC column.

HPLC analysis

The liquid chromatographic system consisted of a Beckman type 112 pump, a YMC-A312 C_{18} column (15 cm×6 mm I.D., 5 μm) (Yamamura, Kyoto, Japan) and a Hitachi F-1000 fluorescence spectrophotometer. The mobile phase was acetonitrile–water (92.5:7.5) containing 0.015% triethylamine. The flow-rate was 1.8 ml/min and the column temperature was 30°C. The column eluate was measured fluorometrically at excitation and emission wavelengths of 365 and 515 nm, respectively.

Calibration curves

Calibration curves were obtained by the use of TPA solutions in plasma in the concentration range 20–2000 ng/ml (*cis* isomer, 8–800 ng/ml; *trans* isomer, 12–1200 ng/ml). The ratio of the peak height of TPA-DH to that of I.S.-DH was plotted against TPA concentration.

RESULTS AND DISCUSSION

HPLC conditions

It has been reported that compounds containing a carbon–nitrogen double bond form *syn* and *anti* isomers [3]. TPA is a mixture of *cis* and *trans* isomers, and the derivatization of TPA with DH to the corresponding hydrazones thus gave four peaks on the chromatogram (Fig. 2). As the ratio of the peak heights of *syn* isomers and *anti* isomers was constant under all reaction conditions tested, the peaks of *anti* isomers were used for the determination of TPA.

The I.S. (all-*trans* isomer) was also converted into *syn* and *anti* isomers, and the peak of shorter retention time was used for the determination (Fig. 3).

Extraction and derivatization of TPA

The hexane extract from plasma was found to contain endogenous substances interfering with the HPLC analysis. These substances were removed by saponification of plasma under alkaline conditions prior to hexane extraction, and both *cis* and *trans* isomers of TPA could then be detected (Fig. 4). *Cis*- and *trans*-TPA in plasma were confirmed to be stable during the saponification procedure.

The effects of the concentration of hydrochloric acid [4,5] and DH, reaction temperature and reaction time on the derivatization of TPA were studied. On the basis of the experimental results, we selected a hydrochloric acid concentration of 0.5%, a reaction time of 60 min, a temperature of 35°C and a DH concentration of 0.2% for the standard procedure.

Calibration and precision

Linear calibration plots of fluorescence intensity versus amount of TPA (*cis*/*trans* = 2:3) were obtained in the following ranges: 8–800 ng/ml for the *cis* isomer, $y = 0.002761x + 0.0044$ ($r = 0.99986$); 12–1200 ng/ml for the *trans* isomer, $y = 0.002609x + 0.0047$ ($r = 0.99989$).

The within-day coefficients of variation were 0.3–13.7% for the *cis* isomer and

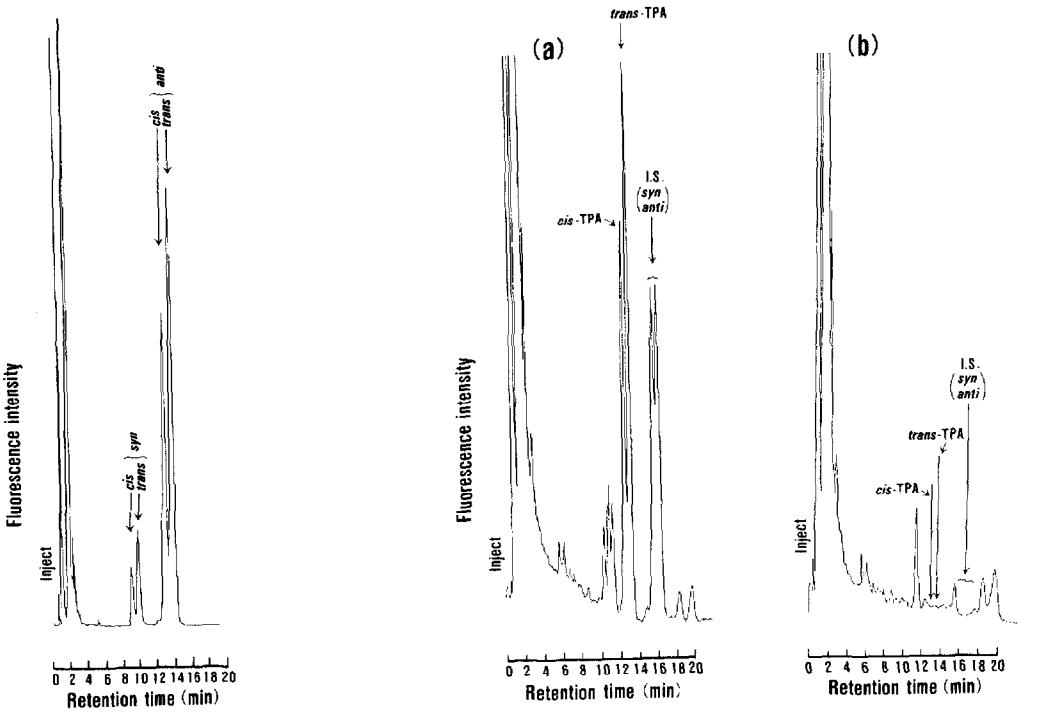


Fig. 2. Typical chromatogram of DH derivatives of TPA.

Fig. 3. Chromatograms of DH derivatives from plasma containing TPA (a) and blank plasma (b), obtained by the standard method. The arrows in (b) indicate the positions at which TPA and I.S. would appear, if present.

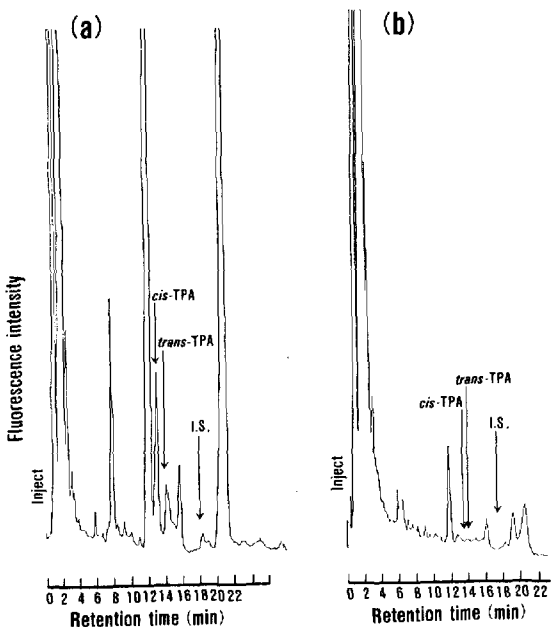


Fig. 4. Chromatograms of DH derivatives from blank plasma subjected to the DH derivatization procedure without saponification (a) and after saponification (b). The arrows indicate the positions at which TPA and the I.S. would appear, if present.

TABLE I

REPRODUCIBILITY OF THE PROPOSED HPLC METHOD FOR THE DETERMINATION OF TPA

	Added (ng)	Found (ng)	Recovery (%)	*n*	C.V. (%)
cis-TPA					
Within-day	8	8.9	111.5	4	13.7
	20	20.0	99.9	4	8.2
	100	101.3	101.3	4	2.5
	200	200.2	100.1	4	2.3
	400	394.4	98.6	4	2.6
	800	802.6	100.3	4	0.3
Day-to-day	80	82.6	103.3	20	7.8
trans-TPA					
Within-day	12	11.9	99.3	4	7.1
	30	31.3	104.3	4	3.4
	150	151.2	100.8	4	1.8
	300	301.7	100.6	4	1.8
	600	593.5	98.9	4	2.6
	1200	1202.6	100.2	4	0.3
Day-to-day	120	123.8	103.2	20	7.6

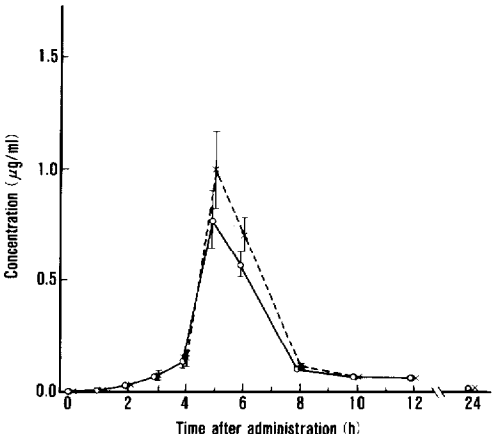


Fig. 5. Time courses of plasma levels of TPA after a single oral administration to healthy volunteers at a dose of 150 mg. Data points: ○ = *cis*-TPA; × = *trans*-TPA.

0.3–7.1% for the *trans* isomer, and the day-to-day coefficients of variation were 7.8% for the *cis* isomer and 7.6% for the *trans* isomer (Table I).

Application of the method

Plasma levels after an oral administration of 150 mg of TPA to eighteen healthy volunteers were determined. TPA could be detected up to 24 h after administration (Fig. 5). These results indicate that this method should be applicable to pharmacokinetic studies in humans.

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