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Note

Determination of 2-(5-benzoyl-2-thienyl)propionic acid (tiaprofenic acid) in the plasma of elderly patients with multiple diseases using high-performance liquid chromatography

W. RIECK* and D. PLATT

Institut für Gerontologie der Universität Erlangen-Nürnberg and 2. Medizinische Klinik, Klinikum Nürnberg, Nürnberg (F.R.G.)

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In performing a pharmacokinetic study of the drug Surgam® (tiaprofenic acid, TA) with geriatric, multimorbide patients (average age 78.8 years; S.D. 5.4), the plasma levels of tiaprofenic acid had to be measured by high-performance liquid chromatography (HPLC). However, the procedures described in the literature were either not sensitive enough [1,2] or they require lengthy sample preparation and are not suitable for use with an autosampler [3–5].

Though the HPLC method of Jamali et al. [2] is suitable for an autosampler, in our opinion it cannot be recommended for pharmacokinetic studies and it is not very economic, because the flow-rate (1.5 ml/min), retention time (7.8 min) and detection limit of tiaprofenic acid (250 ng/ml) can all be improved markedly.

We have developed a sensitive and selective method which enables us to process twenty plasma samples within 1 h and consecutively inject them into the chromatographic system by an automatic sampling device. A typical chromatogram obtained with the new method is presented.

EXPERIMENTAL

Reagents

TA and the metabolites MI (2-[5-(α-hydroxybenzyl)-2-thienyl]propionic acid; RU 19263 Lot 6) and MII (2-[5-(*p*-hydroxybenzoyl)-2-thienyl]propionic acid; RU 19585 Lot 2) were gifts of Albert-Roussel Pharma (Wiesbaden, F.R.G.). Water and acetonitrile (both HPLC grade) were purchased from Promochem (Wesel, F.R.G.). Phosphoric acid (85%), sodium dihydrogenphosphate 1-hydrate

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and dimethyl sulphoxide (DMSO) were obtained from Merck (Darmstadt, F.R.G.; p.a. quality).

The stock solution (solution A) was prepared by dissolving 100 mg of TA in 100 ml of acetonitrile. For the preparation of the plasma samples, the following two solutions were made. Solution B: 0.69 g of $NaH_2PO_4 \cdot H_2O$ were dissolved in ca. 10 ml of water in a 20-ml volumetric flask. After addition of 350 μl of phosphoric acid (85%), the flask was filled up with water to give 20 ml solution. For solution C, 3.06 g of $NaH_2PO_4 \cdot H_2O$ and 1.5 ml of phosphoric acid (85%) were dissolved in water to give 20 ml solution.

Instrumentation and chromatographic conditions

The HPLC system consisted of a single-piston pump (Model 410; Kontron, Eching, F.R.G.), a mixing chamber (Kontron), an autosampler (Model MSI 660, equipped with a 50-μl loop, Kontron), a UV detector (Model Uvicon 720 LC, 2.8-μl cell, Kontron), a programmer (Model 200, Kontron) and an integrator (Model SP 4100, Spectra-Physics, Darmstadt, F.R.G.).

An RP-300 column (particle size 10 μm; 10 cm $\times$ 4.6 mm I.D.) and a RP-18 guard column (particle size 5 μm; 3 cm $\times$ 2.1 mm I.D.), both supplied from Kontron, were used in combination with the Brownlee cartridge holder system.

The analyses were carried out isocratically with acetonitrile–water (40:60, v/v) as the eluent. The water, before use, was acidified by addition of 500 μl phosphoric acid (85%) per litre. The flow-rate of the mobile phase was adjusted to 1.0 ml/min. The HPLC system was operated at ambient temperature.

For technical reasons, the connection of the pre-column with the analytical column was not achieved by direct coupling of the Brownlee cartridge holders but by means of 20-cm steel capillary (0.12 mm I.D.). Likewise, a Rheodyne inlet filter (2-μm frit) was installed between the injection valve and the pre-column. In the chromatogram of TA, however, there was no evidence of any band-broadening effect caused by the connection capillary or by the inlet filter.

Sample solution volumes of 50 μl were injected by the autosampler, and the detection wavelength for TA was 303 nm. The external standard method was chosen for calibration.

Preparation of standard plasma samples

Venous blood (containing neither TA nor its metabolites) was centrifuged for 10 min at 3000 *g* in a heparinized test-tube. Then, to 500-μl portions of the separated plasma, 10, 20, 30 and 50 μl of the TA stock solution (solution A) were added in volumetric flasks, which, after shaking gently, were filled up with plasma to 1 ml each. After mixing for 1 min, the resulting concentrations were: 10, 20, 30 and 50 μg/ml of plasma.

When aliquots of 100, 50 and 20 μl, respectively, were taken from the spiked standard plasma sample that contained 10 μg/ml TA and filled up with plasma to 1 ml each, a new set of concentrations was obtained: 1 μg/ml, 500 ng/ml and 200 ng/ml. Of the spiked plasma samples 500 μl were transferred each into an Eppendorf micro-test-tube, together with 50 μl of DMSO and 100 μl of solution

B. After vortexing for 1 min and centrifugation for 2 min (Eppendorf centrifuge, Model 3200, 14 000 *g*), 500 µl of acetonitrile were added to each sample, which was mixed for 1 min and centrifuged for 2 min.

Finally, 800-µl portions of every upper layer obtained were once more transferred to Eppendorf test-tubes. To each sample 20 µl of solution C were added, and after vortexing for 1 min and centrifugation for 2 min, 600 µl of the upper liquid were placed in a 2-ml autosampler vial.

For the determination of the recovery rate at various concentrations, plasma was replaced by water and the samples were worked up as described above.

Patients' plasma samples

Patients' plasma was isolated as described for the standard plasma samples. It was stored at −20 °C, thawed at room temperature directly before use and prepared in the same way as the standard plasma samples.

RESULTS AND DISCUSSION

Sample preparation

Previously published methods utilize the extraction of TA from acidified plasma with an organic solvent [1,3–5]. For our purposes (more than 1000 samples), these procedures not only seemed to be lengthy but also might not deliver sufficient final sample volume [4] for two injections with our autosampler. On the other hand, our method makes use of centrifugation steps, only thus rendering the preparation of a large number of samples within a short time. After all, a sample can be prepared in less time than is necessary to outline the work-up procedure. Also, it is important to use the designated gradated concentrations of sodium dihydrogenphosphate. When solution B is applied exclusively, the TA signal in the chromatogram shows a shoulder. If only solution C is used, the solutions ready for injection of some of the plasma samples became highly viscous within 1–2 h.

Calibration curve

The calibration curve of TA from plasma was linear in the range 0.2–50.0 µg/ml (linear regression analysis) with a correlation coefficient (r) greater than 0.999.

Precision, recovery and recovery rate

The efficiency of our method is indicated in Table I, which lists data on precision and recovery. Both the recovery, which represents a measure of the accuracy at a given selectivity, and the good precision clearly demonstrate that the method can be used for pharmacokinetic investigations. The recovery rate of TA is found to be ca. 95% in the concentration range 0.2–50 µg/ml. The data determined for the concentrations 1.0, 10 and 30 µg/ml, respectively, are listed in Table II. They were calculated from the peak areas of TA in spiked plasma samples compared with those from special samples where, under the same preparation conditions, plasma was substituted by water.

TABLE I

PRECISION AND RECOVERY OF TIAPROFENIC ACID

Actual plasma concentration (μg/ml)	Measured plasma concentration (mean ± S.D.) (μg/ml)	Relative S.D. (%)	Recovery (%)	n
0.500	0.510 ± 0.032	6.28	102.0	20
1.000	0.954 ± 0.056	5.86	95.4	18
10.000	9.855 ± 0.383	3.89	96.6	18
30.000	29.463 ± 1.540	5.23	98.2	18

TABLE II

RECOVERY RATE OF TIAPROFENIC ACID ($n = 18$)

Tiaprofenic acid (μg/ml)	Area (mean ± S.D.) (arbitrary units)		Recovery rate (mean ± S.D.) (%)
	Water sample	Plasma sample	
1.0	87 961.0 ± 2455.6	83 497.3 ± 5502.5	94.9 ± 6.3
10.0	910 592.2 ± 15 503.6	856 296.9 ± 33 909.4	94.0 ± 3.7
30.0	2 688 156.1 ± 57 282.6	2 558 654.7 ± 134 329.4	95.2 ± 5.0

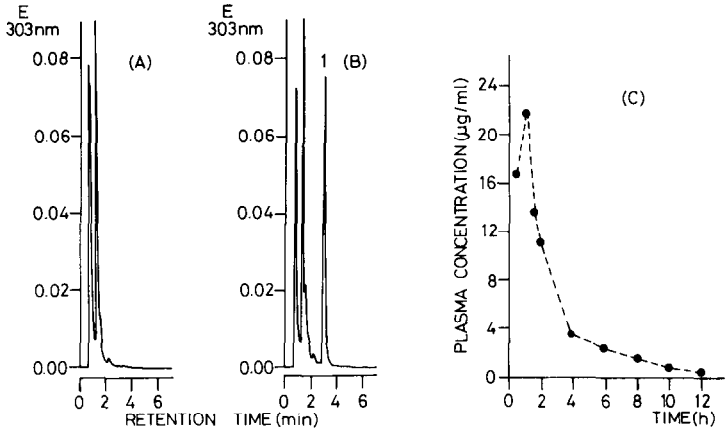

Fig. 1. Chromatograms of a patient's plasma samples. (A) Before administration of tiaprofenic acid; (B) after administration of tiaprofenic acid. Peak 1 = tiaprofenic acid (time 2.95 min; 13.6 μg/ml). (C) Profile of tiaprofenic acid plasma concentrations versus time after a single oral dose of 300 mg of Surgam.

Sensitivity, selectivity and practical application of the method

TA can be reliably quantified down to a minimum concentration of 100 ng/ml of plasma (R.S.D. = 9.1%; $n = 10$). With our method, metabolite I (time 2.2 min; detected at $\lambda = 248$ nm) and metabolite II (time 1.7 min) are clearly separated from TA in the chromatogram and, therefore, do not disturb the quantitative detection of TA. Moreover, it became obvious in the course of our study that

medicaments administered in addition to Surgam during therapy did not interfere with the analysis of TA. Fig. 1 shows a typical chromatogram, together with the individual kinetic curve. The medicaments administered additionally to this patient were Digimerck, Isoket, Trental, Lexotanil, Bepanthen, Nootrop, Vibramycin, Maaloxan and Spartocine. The patient was under long-term medication, which was started before this pharmacokinetic study.

Furthermore, it is worth mentioning that, with the method described, a very large number of samples could be analysed in a relatively short time (1800 samples in ten days) without any decrease in the separation power of the two columns used.

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