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Note

Determination of timelotem dihydrogenmaleate and its main metabolite N-desmethyltimelotem in plasma by capillary gas chromatography

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Timelotem dihydrogenmaleate (KC-7507, I) (10-fluoro-1,2,3,4,4a,5-hexahydro-3-methyl-7-(2-thienyl)-pyrazino[1,2-*a*][1,4]benzodiazepine dihydrogenmaleate, Fig. 1) is a representative of a new class of 1,2-anellated 1,4-benzodiazepines, which shows pronounced antipsychotic properties [1,2].

This paper presents a method for the determination of I that is sensitive, selective, easy-to-handle and rapid, thus fulfilling the requirements of a reliable analytical method needed for determinations in biological fluids (e.g. plasma) in clinical studies investigating pharmacokinetics, bioavailability and/or bioequivalence, etc. [3]. Besides the unchanged drug, its major biotransformation product N-desmethyltimelotem (KC-8122, II) can be quantified.

EXPERIMENTAL

Materials

Pure samples of timelotem dihydrogenmaleate, N-desmethyltimelotem and the internal standard (KC-7099, III, the 10-chloro analogue of timelotem) were provided by the Kali-Chemie, Sparte Pharma, Hannover, F.R.G. All other chemicals, reagents, etc. were of analytical grade (E. Merck, Darmstadt, F.R.G.). For extraction, 3-ml Extrelut columns (E. Merck) were employed.

Apparatus and chromatographic conditions

A Hewlett-Packard 5880A system, equipped with a nitrogen-selective detector and a fused-silica, cross-linked methylsilicone capillary (25 m×0.31 mm I.D., 0.17 μm film thickness from Hewlett-Packard, Palo Alto, CA, U.S.A.) were used.

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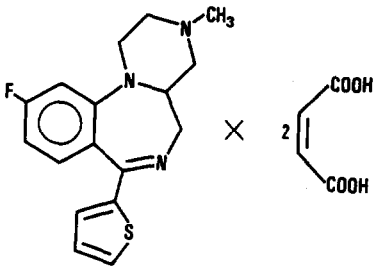
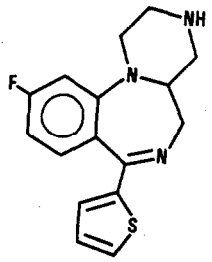
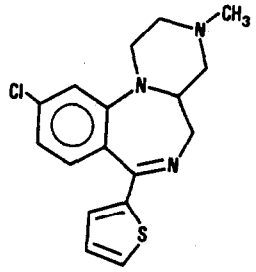


I



II



III

Fig. 1. Structural formulae of timelotem dihydrogenmaleate (I), its main metabolite (II) and the internal standard (III).

Samples of 1 μl were injected by an auto-sampler (HP 7673A) in the split mode [4]. The temperatures were: injector, 290°C; detector, 350°C; oven, 220°C for 1 min, 5°C/min to 260°C, then ballistic to 300°C as a post-run. The gas flow-rates were: carrier (helium), 6 ml/min; hydrogen, 3 ml/min; synth.air (mixture of 95% oxygen and 5% carbon dioxide), 80 ml/min; make-up, 16 ml/min; the split vent, 12 ml/min.

Preparation of samples/extraction

Ethanolic spiking solutions of I and II with concentrations of 16, 8, 4, 2, 1, 0.5, 0.25, 0.10 and 0.05 ng/μl and of III (1 ng/μl) were prepared, and 100 μl of the appropriate solutions were added to 2 ml of blank plasma in a round-bottom red glass tube to make the calibration standards. After a 10-s mixing (Mixomat; Bos-

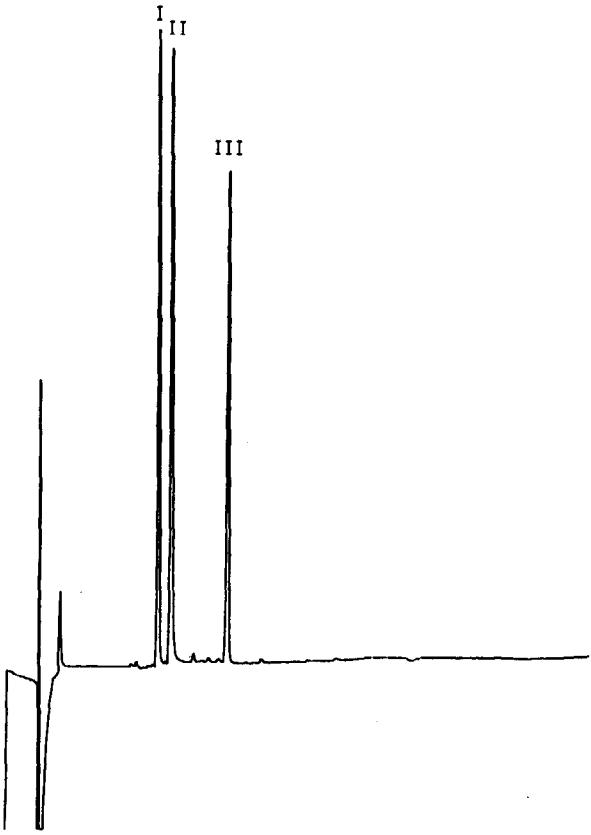


Fig. 2. Chromatogram of pure I, II and III (2 ng each) in ethanol.

kamp, Hersel, F.R.G.), 200 μl of an ammonia solution (25%) were added and mixed. The resulting sample was transferred (with a 2-ml pipette) onto a 3-ml Extrelut column. Following a diffusion time of 10–15 min, the emptied sample tube was washed with 5 ml of toluene, followed by another 10 ml, which were poured onto the column for elution. The eluate was evaporated to dryness at 45 °C under a gentle stream of nitrogen, and the dry residue was dissolved in 100 μl of ethanol of which 1 μl was injected in the GC apparatus.

RESULTS AND DISCUSSION

Under the chromatographic conditions described, all three substances showed well separated and resolved symmetric peaks with retention times of 3.05, 3.31 and 4.47 min for I, II and III, respectively, as depicted in Figs. 2 and 3. In the chromatograms of plasma blank samples there was no peak at the retention time of the substances under consideration (Fig. 4).

Linearity and sensitivity

For all three compounds the detector's linearity was checked first with ethanolic solutions, then with plasma blank samples (plasma was extracted and then

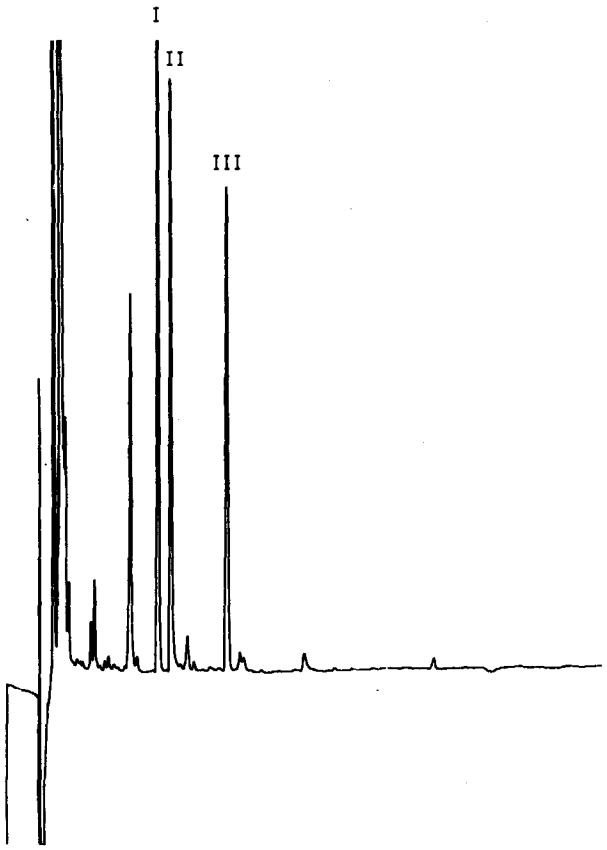


Fig. 3. Chromatogram of plasma sample spiked with 2 ng each of I, II and III.

spiked; these samples were also used as the 100% recovery samples for the recovery evaluations) and finally with spiked samples. For I and II the linearity range was 2.5–800 ng/ml, and for III 0.5–2 ng/ml; in this range the relation between concentration and peak-area ratio (versus internal standard) remained linear.

The limit of detection was calculated from the data in Table I [3]. For I and II coefficients of variation (C.V.) of 7.7 and 9.6% were calculated, and the differences between the amounts added and amounts measured of over 30% forced us to adopt 5 ng/ml as the limit of quantification in both cases.

Stability in plasma

Spiked plasma samples were several times analysed, deep frozen, thawed and analysed again. The results showed that all three compounds are stable for at least three months when frozen at $-15\,^{\circ}C$.

Precision (reproducibility) and accuracy

The accuracy of the method was checked by assaying six times each of the five concentrations. Table I shows that the assay was accurate in the concentration

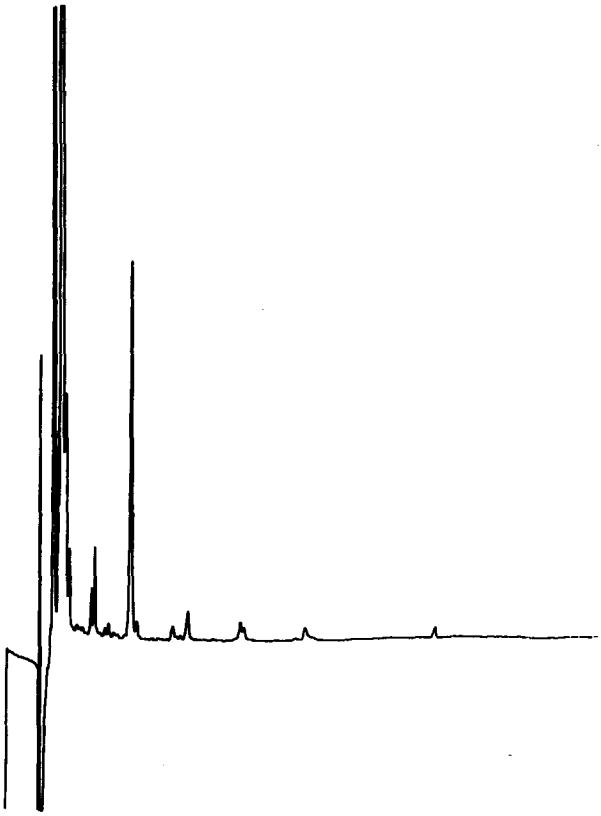


Fig. 4. Chromatogram of blank plasma sample.

range 5–800 ng/ml, and that the C.V. values varied from 7.7% at 5 ng/ml to 2.3% at 800 ng/ml for I and from 9.6% at 25 and 100 ng/ml to 1.3% at 5 ng/ml for II.

The C.V. values for the precision data ranged from 4.5% at 5 ng/ml to 5.4% at 800 ng/ml for I and from 12.1% at 5 ng/ml to 2.3% at 800 ng/ml for II.

Recovery

The recovery from human plasma was determined for I and II at 10, 50, 100 and 500 ng/ml, and for III at 25, 50 and 100 ng/ml. Each concentration was assayed six times. The mean recovery for I was ca. 99% (range, 95–104%; S.D., 3.8%), for II ca. 95% (range, 90–98%; S.D., 4.1%) and for III ca. 97% (range, 95–99%; S.D., 2.1%).

CONCLUSION

A method for the quantitative determination of I and II in human plasma by capillary gas chromatography with nitrogen-selective detection was developed and validated. The recovery from plasma was ca. 99% (I), 95% (II) and 97% (III), the precision generally below 5% (I), 9% (II) and 1% (III) and the accuracy ca. 10%. The linearity remained stable in the calibrated range 0–800 ng/ml

TABLE I

PRECISION AND ACCURACY OF THE ASSAY FOR TIMELOTEM DIHYDROGEN-MALEATE (I), ITS METABOLITE (II) AND INTERNAL STANDARD (III)

Compound	Amount added (ng/ml)	Amount measured (mean ± S.D.) (ng/ml)	C.V.
I	5	6.5 ± 0.5	7.7
	25	26 ± 1.4	5.4
	100	99 ± 3.4	3.4
	400	403 ± 11.7	2.9
	800	796 ± 18.7	2.3
II	5	6.9 ± 0.9	1.3
	25	24 ± 2.3	9.6
	100	89 ± 8.5	9.6
	400	408 ± 24.4	6
	800	811 ± 58.5	7.2
III	25	25 ± 0	0
	50	51 ± 0.5	1
	100	100 ± 0.5	0.5

and the limit of detection was below 5 ng/ml (I) and 12.5 ng/ml (II), respectively. The nitrogen-selective detector, the sample clean-up procedure and other precautions guarantee the necessary selectivity of the method. The presented data show that I and II can be quantitated in plasma reliably and rapidly by this method: it is selective, sensitive and accurate, and the sample clean-up procedure is rapid and simple.

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