Journal of Chromatography, 497 (1989) 223–230
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMBIO. 5014

DETERMINATION OF OXIRACETAM IN PLASMA AND URINE BY COLUMN-SWITCHING HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

J.B. LECAILLON*, C. SOUPPART, F. LE DUIGOU and J.P. DUBOIS

Laboratoires Ciba-Geigy, Biopharmaceutical Research Center, B.P. 308, 92506 Rueil-Malmaison Cedex (France)

(First received June 26th, 1989; revised manuscript received August 28th, 1989)

SUMMARY

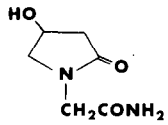
A column-switching high-performance liquid chromatographic method was developed for the determination of oxiracetam in plasma and urine. A sample of plasma (250 μl) or urine (10 μl) is mixed with the internal standard solution, 4.2 ml of acetonitrile–water (1000:4, v/v) and 0.8 ml of dichloromethane, and 1 ml of the clear solution is injected onto a first column filled with LiChrosorb NH_2. The sample is eluted with acetonitrile–water (95:5, v/v). The portion of the eluate (heart-cutting) from this column containing the compounds of interest is selected and loaded on a Nucleosil NH_2 column and eluted with acetonitrile–water (90:10, v/v). During this chromatography the first column (LiChrosorb NH_2) is rinsed with acetonitrile–water (50:50, v/v). Ultraviolet detection at 200 nm is used for quantitation. The limit of quantitation of oxiracetam is ca. 1.5 μM (240 ng/ml) in plasma and 76 μM (12 μg/ml) in urine. Oxiracetam was stable in plasma and urine samples kept frozen at $-20\,^{\circ}C$ for nine months and one year, respectively.

INTRODUCTION

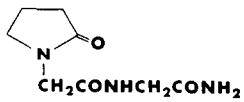
Oxiracetam is a new nootropic agent. In elderly patients with organic brain syndrome, oxiracetam exhibited a significant improvement of the memory function.

The molecular structures of oxiracetam and internal standard (I.S.) in the analytical method are shown in Fig. 1. Oxiracetam is a very polar compound of small molecular mass. Its solubility in water is 0.3 g/l. The partition coefficient in *n*-octanol–aqueous buffer (pH 7.4) is 0.005 and in *n*-octanol–0.1 *M* hydrochloric acid, it is 0.006. Attempts to extract oxiracetam from biological

0378-4347/89/$03.50



Oxiracetam



CGP 14 998
(Internal standard)

Fig. 1. Structures of oxiracetam and of the internal standard.

fluids by organic solvents were unsuccessful. Furthermore, its maximum absorption is close to 200 nm, a wavelength at which many endogeneous plasma and urine compounds absorb.

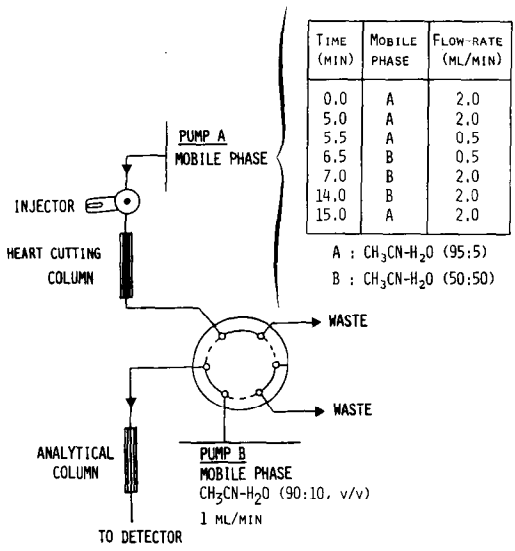
Several liquid chromatographic methods have been described for the quantitative determination of oxiracetam in biological fluids [1–3]. In two of them [1,2], the formation of a derivative followed by extraction, washing, evaporation of the organic phase and extraction of the reconstituted residue on a cartridge containing a silica phase were performed before high-performance liquid chromatography (HPLC). The present method is similar to that described in ref. 3. The advantage over the previous methods is that the formation of a derivative and an extraction step are not required before chromatography. One column was used for the analytical step instead of two as previously described [3], and the time necessary for sample purification on the first column was reduced.

EXPERIMENTAL

Chemicals and equipment

Oxiracetam ($C_6H_{10}N_2O_3$; MW 158.16) and the internal standard CGP 14 998 ($C_8H_{13}N_3O_3$; MW 199.21) were supplied by Ciba-Geigy (Basle, Switzerland). Acetonitrile was of HPLC quality (Art. 412412, Carlo Erba, France, Puteaux, France). Dichloromethane was of pestipur quality (Art. 2922 A21, SdS, Peypin, France). The stationary phases were LiChrosorb NH_2, 5 μm (Art. 9376, Merck, Darmstadt, F.R.G.) and Nucleosil NH_2, 5 μm (Art. 71220 Macherey-Nagel, Düren, F.R.G.).

The liquid chromatographic system (Fig. 2) included two pumps: (A) a Model



Time (min)	Mobile phase	Flow-rate (ml/min)
0.0	A	2.0
5.0	A	2.0
5.5	A	0.5
6.5	B	0.5
7.0	B	2.0
14.0	B	2.0
15.0	A	2.0

Fig. 2. Diagram of the column-switching system for the determination of oxiracetam. Time schedule: 0 min, injection and heart-cutting column (HCC) eluate to waste; 4.0 min, HCC and analytical column connected; 5.5 min, HCC eluate to waste; 20 min, end of determination. Time schedule should be adjusted for each new HCC. Time programme for pump A is shown on the diagram.

8700 elution gradient mode pump (Spectra-Physics, San Jose, CA, U.S.A.) and (B) a Constametric Model III isocratic mode pump (LDC Division of Milton Roy, Riviera Beach, FL, U.S.A.). The injection system consisted of a Model IV sampler (Technicon, Tarrytown, NY, U.S.A.), a Model III peristaltic pump (Technicon) and a Model AH-CV-6-UHPa-N60 air-actuated sample injection valve (Valco, Houston, TX, U.S.A.); an identical valve was used for column-switching. A Model 4270 computing integrator (Spectra-Physics) actuated the valves, the sampler and the pump (SP 8700) and recorded the chromatogram. Detection was performed with a Spectroflow Model 773 variable-wavelength UV detector (Kratos, Ramsey, NJ, U.S.A.) set at 200 nm.

Two columns were used. The 'heart-cutting' column (stainless-steel tube, 10 cm×4.7 mm I.D.) was filled with 1.5 g of LiChrosorb NH_2 (5 μm) suspended in 8.7 ml of 0.01 *M* aqueous sodium acetate solution–methanol (20:80, v/v). Packing was performed with a pneumatic Haskel pump using methanol as pressurizing solvent under 400 bar pressure for 30 min. The analytical column (stainless-steel tube, 30 cm×4.7 mm I.D.) was filled with 3.5 g of Nucleosil NH_2 (5 μm) suspended in 21 ml of 0.01 *M* aqueous sodium acetate solution–methanol (20:80, v/v). The pressure was maintained at 600 bar for 30 min.

Calibration standard solutions

The I.S. solution was prepared by dissolving 10 mg of I.S. in 200 ml of distilled water. The master calibration solution was prepared by dissolving 30 mg of oxiracetam in 10 ml of I.S. solution. Calibration standard solutions were prepared by dilution of the master standard solution with the I.S. solution.

Sample preparation

A 250-μl sample of plasma (or 10 μl of urine) was added under mixing to 4.2 ml of acetonitrile–water (1000:4, v/v) in a 12-ml polypropylene tube. Then 10 μl (or 40 μl for urine samples) of I.S. or calibration standard solution and 0.8 ml of dichloromethane were added. The tube was closed and shaken for a few seconds on a Vortex mixer.

For plasma samples, the tube was centrifuged for 5 min at 2000 *g*, and the supernatant was transferred to another 6.5-ml polypropylene tube, which was loaded on the autosampler.

For urine samples centrifugation was not necessary, and the sample was diluted as mentioned above and loaded on the autosampler.

Chromatography

The schematic outline of the column-switching system and the time programme for the flow-rate and mobile phase composition of pump A are shown in Fig. 2. The time programme for the valve connections is given in the legend of Fig. 2. The prepared plasma or urine sample was carried by the peristaltic pump into the injection valve equipped with a 1-ml loop. The sample was injected on the heart-cutting column. The compounds were eluted with the mobile phase acetonitrile–water (95:5, v/v) at a flow-rate of 2 ml/min. The retention times obtained on this column were ca. 4.3 and 4.5 min for I.S. and oxiracetam, respectively. The portion of the eluate was selected between ca. 4 and 5.5 min after injection (the time must be precisely adjusted: see section *Heart-cutting adjustment*) and loaded on the analytical column. The analytical column was eluted with acetonitrile–water (90:10, v/v) at a flow-rate of 1 ml/min.

During the analysis on the analytical column, the heart-cutting column (LiChrosorb NH_2) was flushed with acetonitrile–water (50:50, v/v) at a flow-rate of 2 ml/min.

Quantitative evaluation

Five or six biological calibration standards were worked up, and the results were used to calculate the calibration curve by weighted linear least-squares regression with a weighting factor of $1/(\text{concentration})^2$ [4]. The oxiracetam/I.S. peak-area ratios were plotted as a function of the oxiracetam concentration in the biological calibration standards. Correlation coefficients of the regression lines were higher than 0.999.

RESULTS AND DISCUSSION

Owing to its high polarity, oxiracetam was not or almost not retained on columns containing reversed-phase material used with acidic, neutral or slightly basic mobile phases with or without acetonitrile or methanol as organic modifier. Since oxiracetam could not be extracted from biological fluids by an organic solvent, it is not possible to inject the solute in a low-polarity medium. Therefore, the use of conventional normal-phase chromatography with an organic solvent of rather low polarity was not possible. As a compromise, the polarity of the injection medium was strongly decreased by the addition of acetonitrile and dichloromethane to plasma or urine. For plasma, the aqueous moiety in the injected solution represented ca. 5% in volume. The chromatographic system selected (amino polar phase and acetonitrile with water as modifier) reacted as for normal-phase chromatography with the retention time of oxiracetam which increased when the water content decreased.

Large differences of retention were found between bonded stationary phases produced by different manufacturers, as has already been reported for almost all kinds of bonded phase [3,5]. These differences were represented not only by proportional modification of the retention time of compounds but also by a modification of the separation factors of the compounds. In the present situation, the k' value of oxiracetam on LiChrosorb NH_2 was half the value recorded on Nucleosil NH_2, with acetonitrile–water (95:5, v/v) as mobile phase.

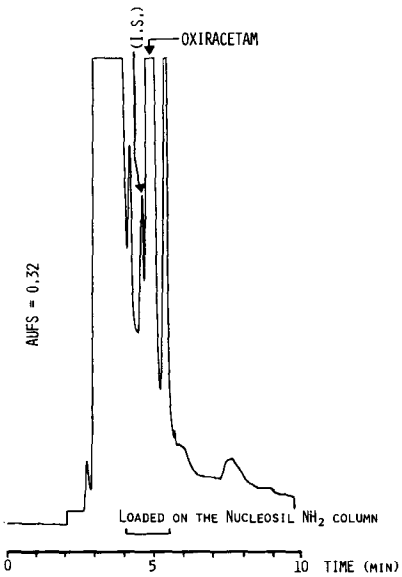


Fig. 3. Chromatogram of a sample of human urine spiked with oxiracetam and I.S. on the LiChrosorb NH_2 column (heart-cutting column) used for time schedule adjustment.

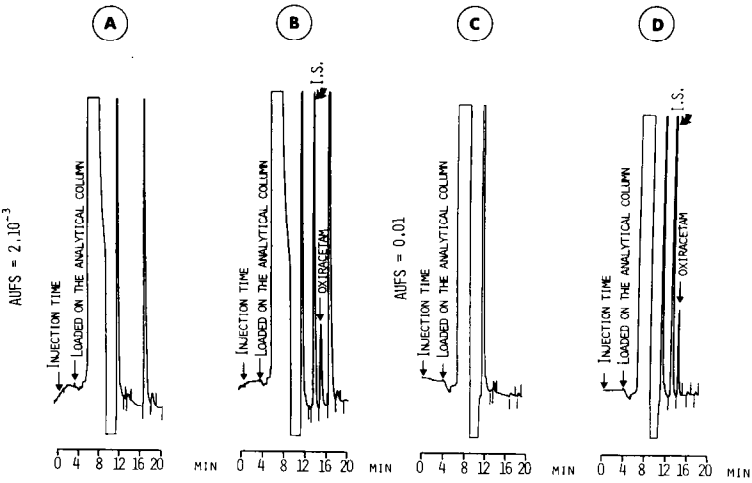


Fig. 4. Chromatograms obtained with the Nucleosil NH_2 analytical column after injection on the heart-cutting column and switching: (A) human plasma blank; (B) plasma spiked with oxiracetam (3.79 μM) and I.S.; (C) human urine blank; (D) urine spiked with oxiracetam (152 μM) and I.S.

The interfering compounds detected on the first column (Fig. 3) did not interfere with oxiracetam and I.S. on the analytical column (Fig. 4).

Heart-cutting adjustment

The retention times on LiChrosorb NH_2 may vary slightly from one column to another and also in the time course of use of the column. Therefore, the retention times on LiChrosorb NH_2 must be determined with each new column so as to adjust the eluate cut (containing I.S. and oxiracetam) that is loaded on the Nucleosil NH_2 column. We started the eluate cut (Fig. 3) 0.3 min before the retention time of I.S. and stopped it 1 min after the retention time of oxiracetam.

In routine analysis, the heart-cutting column keeps its good performances for 100 injections and the analytical column for ca. 600 injections of plasma or urine samples. After the heart-cutting, the first column is rinsed with a mobile phase of higher polarity to eliminate strongly retained polar endogenous components.

Selectivity

Oxiracetam and I.S. are conveniently separated from plasma and urine components (Fig. 4). Spiked plasma and urine samples from several different volunteers were tested. Oxiracetam is apparently not or almost not metabolized after drug administration to volunteers, since more than 90% of unchanged drug was recovered in urine after intravenous administration [2].

Accuracy and reproducibility

Series of plasma or urine control standards spiked with oxiracetam were determined on the same day and on different days. The recoveries and the coefficients of variation (C.V.) are given in Table I. The day-to-day reproducibility shows that the calibration curves need to be prepared once a month only. The limits of quantitation, established on the basis of the reproducibility results, are ca. 1.5 μM (240 ng/ml) in plasma and 76 μM (12 μg/ml) in urine. The difference between the limits of quantitation in plasma and urine was mainly due to the chromatographic background of the two fluids. Concentrations below these limits can be estimated, but with a C.V. greater than 10%.

Stability of oxiracetam in plasma and urine samples

At room temperature no loss of oxiracetam was detected when the plasma samples were left for 24 h (mean recovery $= 103 \pm 7\%$, $n = 8$) and when the urine samples were left for one week (mean recovery $= 98 \pm 5\%$, $n = 11$).

At $-20°C$ no loss of oxiracetam was detected from plasma samples after nine months (mean recovery $= 108 \pm 5\%$, $n = 4$) or from urine samples after one year (mean recovery $= 97 \pm 6\%$, $n = 9$).

Application

The procedure was applied to the quantitative determination of oxiracetam in plasma and urine. Fig. 5 shows the oxiracetam concentration curve in the plasma of a volunteer given a single oral dose of 800 mg of oxiracetam.

TABLE I

REPRODUCIBILITY OF THE METHOD

Sample	Concentration added[a] (μM)	Mean recovery[b] (%)	C.V.[c] (%)
Within-day ($n = 6$)			
Plasma	1.52	109	8
	3.79	103	6
	75.9	94	3
Urine	75.9	103	10
	7590	101	0.4
Day-to-day[d] ($n = 5$)			
Plasma	3.79	103	2
	75.9	100	4
Urine	75.9	93	11
	7590	103	1

[a]To convert into μg/ml, multiply the concentration by 0.15816.
[b]Recovery = (found/added) $\times$ 100.
[c]C.V. = (S.D./mean) $\times$ 100.
[d]Over 41 days for plasma and 29 days for urine.

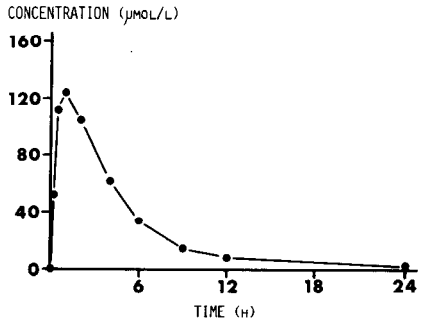


Fig. 5. Oxiracetam concentrations in the plasma of a volunteer after a single oral administration of 800 mg of oxiracetam.

CONCLUSIONS

The present method allows the quantitative determination of oxiracetam down to 1.5 μM in plasma and 76 μM in urine with convenient reproducibility and accuracy. It is sensitive enough to follow the plasma and urine concentrations recorded after administration of therapeutic doses of oxiracetam to human volunteers. More than 1000 plasma or urine samples have been analysed by the present method.

REFERENCES

1 M. Visconti, R. Spalluto, T. Crolla, G. Pifferi and M. Pinza, J. Chromatogr., 416 (1987) 433.
2 E. Perucca, A. Albrici, G. Gatti, R. Spalluto, M. Visconti and A. Crema, Eur. J. Drug Metab. Pharmacokin., 3 (1984) 267.
3 J.B. Lecaillon, N. Febvre and C. Souppart, J. Chromatogr., 317 (1984) 493.
4 D.A. Schoeller, Biomed. Mass Spectrom., 3 (1976) 265.
5 A.P. Goldberg, Anal. Chem., 54 (1982) 342.