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Determination of 10α-Methoxy-9,10-dihydrolysergol, a Nicergoline Metabolite, in Human Urine by High Performance Liquid Chromatography

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A specific method for the determination of 10α-methoxy-9,10-dihydrolysergol, a nicergoline metabolite (metabolite 2), in urine is described. Metabolite 2 was well separated from the urine components on a reversed phase column, Hypersil ODS 5 μm, using an acetonitrile : pH 3.5 phosphate buffer (40 : 60, v/v) as the mobile phase at a flow rate of 1 mL/min. UV detection was set up at 220 nm. After addition of a known amount of lysergamide as the internal standard, the compounds were extracted from alkalysed urine on a pre-packed glass column (Extrelut 1) with dichloromethane. With 0.5 mL urine, concentrations down to 0.56 μmol/L could be determined.

INTRODUCTION

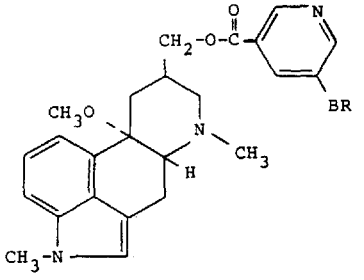
Nicergoline is an α-blocker vasodilator. After oral administration, the absorption and the elimination of nicergoline are rapid. The terminal half-life of unchanged nicergoline is around 1 h. Nicergoline is very unstable in blood, and it cannot be determined in human body fluids (Nieder and Jaeger, 1985). Due to a first-pass effect, lumilysergol (metabolite 1) (Fig. 1) appears rapidly in plasma. It reaches a concentration in the plasma of about 2 ng/mL after 40 min. The concentration of 10α-methoxy-9,10-dihydrolysergol (metabolite 2) (Fig. 1) is about 8 ng/mL after 4 h. 67% of the nicergoline dose is excreted in urine as free metabolite 2, whereas free metabolite 1 is only present in trace amounts. 18.5% of the dose is excreted as conjugated metabolite 2 and 1.1% as conjugated metabolite 1 (Nieder and Jaeger, 1985).

This paper describes a method for the determination of free metabolite 2 in human urine, this metabolite being a marker substance for the biopharmaceutical characterization of nicergoline.

EXPERIMENTAL

Solvents and reagents. Nicergoline, lumilysergol (metabolite 1) and 10α-methoxy-9,10-dihydrolysergol (metabolite 2) were supplied by Inverni della Beffa, Milan, Italy. Lysergamide (internal standard) was supplied by Sandoz, Rueil-Malmaison, France. The solvents and reagents used were acetonitrile (9017; Baker, Deventer, Holland), methanol (414816; Carlo Erba, Milan, Italy), dichloromethane (pestipur; SDS, Peypin, France), and a prepacked glass column (Extrelut 1, 15371; Merck, Darmstadt, Germany).

The 5 M sodium hydroxide was prepared by diluting 250 mL suprapur NaOH (5589; Merck) up to 500 mL of



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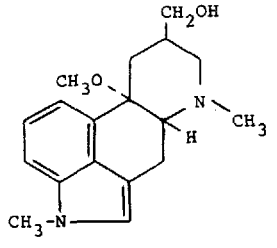

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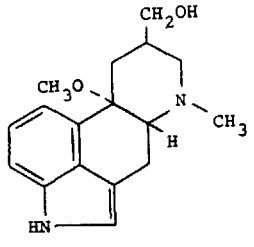

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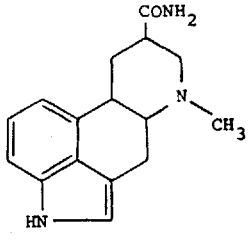

IV

Figure 1. Chemical structure of nicergoline (I), 10α-methoxy-1-methyl-9,10-dihydrolysergol (metabolite 1) (II), 10α-methoxy-9,10-dihydrolysergol (metabolite 2) (III) and lysergamide as internal standard (IV).

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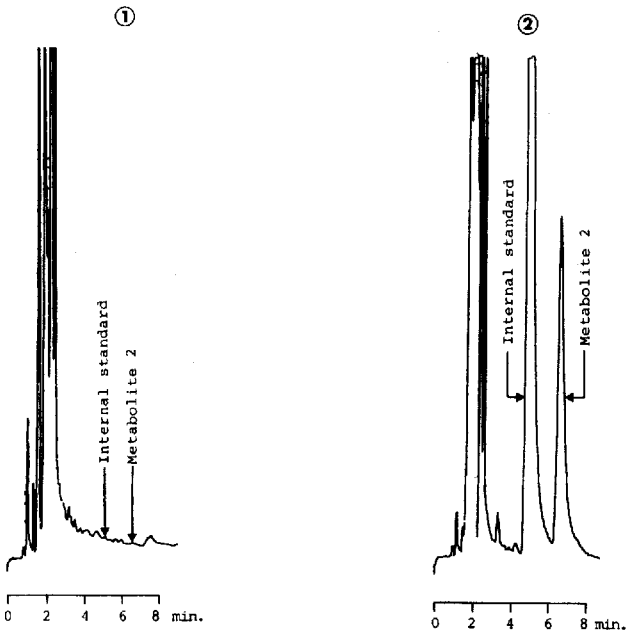

Figure 2. Examples of chromatograms: (1) Human urine blank (extract of 0.5 mL urine). (2) The same urine spiked to produce 3.49 μmol/L metabolite 2 and 14.91 μmol/L lysergamide (internal standard). Detector sensitivity: 10^{-3} aufs; integrator attenuation: 4 (160 μV/cm).

deionized water. The 10^{-2} mol/L pH 3.5 phosphate buffer was prepared by dissolving 2.72 g of anhydrous potassium dihydrogen phosphate in 2000 mL of deionized water. The pH value of this solution was then adjusted to pH 3.5 by addition of 350 μL phosphoric acid (573; Merck).

Chromatography. *Equipment and conditions.* The chromatography was performed on a system equipped with a Waters M510 solvent delivery pump and an Ultra Wisp Waters 715 automatic sampler. The column was connected to a Kratos 783 variable wavelength UV detector set at 220 nm. A Hewlett-Packard 3388 integrator recorded the chromatograms and calculated the peak heights. The chromatographic column (Hypersil ODS 5 μm, 20 cm long, 4.6 mm i.d.—No. 79916 OD, Option 574) was supplied by Hewlett-Packard. The compounds were eluted at a flow rate of 1 mL/min using a degassed mobile phase of acetonitrile: 10^{-2} M pH 3.5 phosphate buffer (40:60, v/v).

Preparation of urine calibration samples. Aliquots of metabolite 2 reference solutions and a constant amount of internal standard were added to 0.5 mL urine. The calibration samples corresponded to urine concentratons between 0.49 and 5.59 μmol/L.

Extraction procedure in urine. An aliquot of the internal standard solution (7.46 nmol in 50 μL), prepared in methanol, was introduced into a 10 mL glass tube and evaporated to dryness under a nitrogen stream at room temperature. After addition of 0.5 mL urine and 0.5 mL sodium hydroxide (5 M) this mixture was shaken on a Vortex mixer and immediately transferred into a prepacked glass column (Extrelut 1). Extraction was performed by elution with 5 mL dichloromethane. The organic phase was collected into a 10 mL glass tube and evaporated to dryness under a nitrogen stream at room temperature. The residue was dissolved in 1.5 mL of mobile phase and 30 μL were injected onto the column.

Calibration curve. This was obtained by plotting the peak height ratio of metabolite 2 and the internal standard versus metabolite 2 concentrations. Its equation was calculated by using weighted linear least-squares regression with a weighting factor of $1/(\text{concentration})^2$. The calibration range was 0.56–5.59 μmol/L. A calibration curve was established every working week. Its validity was checked by analysis, in duplicate, of samples spiked with metabolite 2. As soon as the duplicates gave results outside the accuracy interval of 90 to 110%, it was necessary to run a new calibration curve.

RESULTS

Selectivity

The metabolite 2 (retention time = 6.5 min) was well separated from nicergoline and metabolie 1 (retention time = 11.5 min). Owing to instability, two peaks were recorded for nicergoline (5.8 and 11.7 min). The method can be considered as selective since the available data indicate that the unchanged drug is not excreted in human urine (Nieder and Jaeger, 1985).

Urine interferences

Metabolite 2 was conveniently separated from the urine components. Urine from different volunteers was used to check the separation (Fig. 2).

Day-to-day accuracy

Six concentrations were analysed in duplicate on days 1 to 5 using the same calibration curve as obtained on day 1. The results are shown in Table 1.

Table 1. Day-to-day precision and accuracy of metabolite 2 in spiked urine samples

Given concentration (μmol/L)	Found concentration (μmol/L)					Mean found concentration (μmol/L)	Standard deviation	Accuracy (%)
	Day 1	Day 2	Day 3	Day 4	Day 5			
0.56	0.51	0.62	0.66	0.59	0.56	0.59	0.06	105.2
1.19	1.21	1.25	1.28	1.16	1.15	1.21	0.06	101.7
1.57	1.62	1.65	1.64	1.54	1.43	1.58	0.09	100.4
2.62	2.67	2.73	2.76	2.55	2.52	2.65	0.11	101.0
3.14	3.23	3.35	3.32	3.03	3.09	3.20	0.14	102.0
4.89	5.00	5.35	5.11	4.66	4.63	4.95	0.31	101.2
Mean (CV %)								101.9 (5.8)

CV = coefficient of variation.

Table 2. Within-day precision and accuracy of metabolite 2 in spiked urine samples

Given concentration (μmol/L)	Mean found concentration (μmol/L)	Standard deviation	Accuracy (%)
0.56	0.59	0.02	104.5
1.19	1.21	0.01	101.5
1.57	1.63	0.02	104.0
2.62	2.69	0.05	102.5
3.14	3.25	0.05	103.4
4.89	4.85	0.10	99.3
Mean			102.5
(CV %)			(2.5)

CV = coefficient of variation.

Within-day accuracy

Human urine samples containing metabolite 2 at six different concentrations were analysed repeatedly for every concentration on the same day. The results are shown in Table 2.

Limit of quantitation

The estimated limit of quantitation was 0.56 μM with a coefficient of variation of 9.7% and a mean accuracy of 105.2%.

Stability in urine

Metabolite 2 was stable for 23 h in urine at 5°C. Urinary fractions must be stored at 5°C immediately after urination. Metabolite 2 was stable in spiked urine samples after storage at −80°C for four months.

DISCUSSION

The described HPLC assay permits the determination of the 10 α-methoxy-9,10-dihydrolysergol, a nicergoline metabolite 2, in human urine down to 0.56 μM (160 ng/mL) with suitable precision and accuracy. This method is comparable to that of Nieder and Jaeger (1985), but it permits the rapid determination of the marker substance (metabolite 2) only for the biopharmaceutical characterization of nicergoline.

REFERENCE

Nieder, M. and Jaeger, M. (1985). *Z. Naturforsch. (B), Chem. Sci.* **42**, 1187.