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Note

Determination of oxeladin in human sera by gas—liquid chromatography with thermionic detection

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Oxeladin citrate [2-(2-diethylaminoethoxy)ethyl-2'-ethyl-2'-phenyl butyrate] (Pectamol®, Tussimol®) is a cough suppressant which depresses the cough reflex. It does not act on the higher centres and has no hypnotic effect in therapeutic doses. It has local anaesthetic properties, so a combined local and central depressant effect might be possible [1, 2]. So far, no method has been described for the determination of oxeladin in biological materials. Therefore blood levels have not as yet been correlated to cough suppressant activity.

This paper describes a gas—liquid chromatographic (GLC) method developed for the determination of oxeladin in serum samples in bioavailability tests of oxeladin from tablet and linctus formulations. Pharmacokinetic results are presented elsewhere [3].

EXPERIMENTAL

Standards and reagents

Oxeladin citrate was a gift from Apothekernes Laboratorium for Specialpræparater A/S (Oslo, Norway). Oxeladin base (OX) was supplied by E. Merck (Darmstadt, G.F.R.).

An aqueous stock solution of OX hydrochloride (1.0 mg/ml) was prepared by dissolving the base in 2 ml of ethanol, adding 0.1 mol/l hydrochloric acid to a pH of 2.5—3.0 and diluting to correct volume (100 ml) with redistilled water. Working standards (1 μg/ml) were made by diluting aliquots of the stock solution with redistilled water.

Tripelennamine hydrochloride (Pyribenzamine®, pharmacopoeial quality) was used as internal standard (I.S.); aqueous working standards of 1 μg/ml were prepared from an aqueous stock solution (1.0 mg/ml).

The stock solutions were stable for at least two months kept at 4°C. The working standards were prepared weekly.

All reagents were analytical grade. *n*-Hexane, chromatography grade, was supplied by Rathburn Chemicals (Walkerburn, Great Britain). Sodium hydroxide (1 *N*) was ether-washed.

Glassware

Conical glass-stoppered centrifuge tubes (15 ml) were silanized with 5% dimethyldichlorosilane in toluene for 4 h, then washed with methanol and acetone. Used extraction tubes were washed in hot soapy water, rinsed with tap water twenty times and then three times with ethanol.

The evaporating tubes were 15-ml Quickfit tubes finely tapered at the bottom (100 µl). After use they were kept overnight in 2 mol/l hydrochloric acid, rinsed with water twenty times, and then three times with ethanol. During the washing procedure the solvents were withdrawn from the bottom tip with a Pasteur pipette by suction with water vacuum. In order to prevent adsorption of OX to the glass walls, they were rinsed with acetone just before use. The injection syringes (Hamilton 10 µl) were rinsed with methanol which was a better solvent for OX than ethyl acetate. Three 200-µl volumes of methanol were washed through the syringe using a larger syringe (Hamilton 250 µl) as a supply.

Chromatography

The instrument used was a Varian Aerograph Series 1400 equipped with a nitrogen-sensitive Varian TSD detector (Basle, Switzerland) and a W + W recorder 1011 (Basle, Switzerland). The column, 180 cm × 2 mm I.D., was made of Pyrex glass and hand-packed with 3% SP-2100 (Supelco, Bellefonte, PA, U.S.A.) to which was added 0.1% HIEFF-8 BP (Applied Science Labs., State College, PA, U.S.A.). The support was Chromosorb G AW DMCS 70—80 mesh (Johns-Manville, Denver, CO, U.S.A.). Temperatures were injector 265°C, column 211°C and detector 280°C. The detector was used in the nitrogen mode under the following conditions: hydrogen 4.5 ml/min, air 162 ml/min, bias voltage —4.0 V, bead current adjust 5.20. A nitrogen carrier flow-rate of 25.2 ml/min was used.

Retention times were OX 110 sec, and I.S. 60 sec.

Extraction procedure

Aliquots (1.0 ml) of the serum samples were placed in 15-ml centrifuge tubes to which were added 100 µl of the internal standard solution (100 ng), two drops of 2 mol/l sodium hydroxide to a pH of 12, and 6 ml of *n*-hexane. The tubes were shaken horizontally for 5 min in a mechanical shaker (90 times/min), centrifuged for 5 min at 1000 *g*, and 5.5 ml of the hexane layer were then withdrawn. The serum samples were extracted a second time with 6.0 ml of *n*-hexane and the hexane layers were collected in the tapered evaporation tubes. Hexane was evaporated at 60—65°C in a water-bath. A stream of nitrogen was blown from above on to the hexane layer. The residual dry extracts were dissolved in 600 µl of methanol, the tube walls being thoroughly rinsed. After a renewed cautious evaporation, the residual extract was dissolved

in 25 μl of ethyl acetate by whirlmixing for 10 sec. A 1-μl aliquot was injected.

Calibration curve

Pooled blank serum samples (Red Cross Bloodcentre, Oslo, Norway) were spiked with fixed amounts of OX. Five concentrations (10, 50, 100, 200, and 250 ng/ml) were chosen and ten spiked samples of each concentration prepared. To each sample were added 100 ng of internal standard (100 μl of the working internal standard solution) and the samples were treated as above.

RESULTS AND DISCUSSION

Chromatograms of extracts from 1 ml of serum after single-dose administration of oxeladin linctus to a volunteer are shown in Fig. 1a and b. Ap-

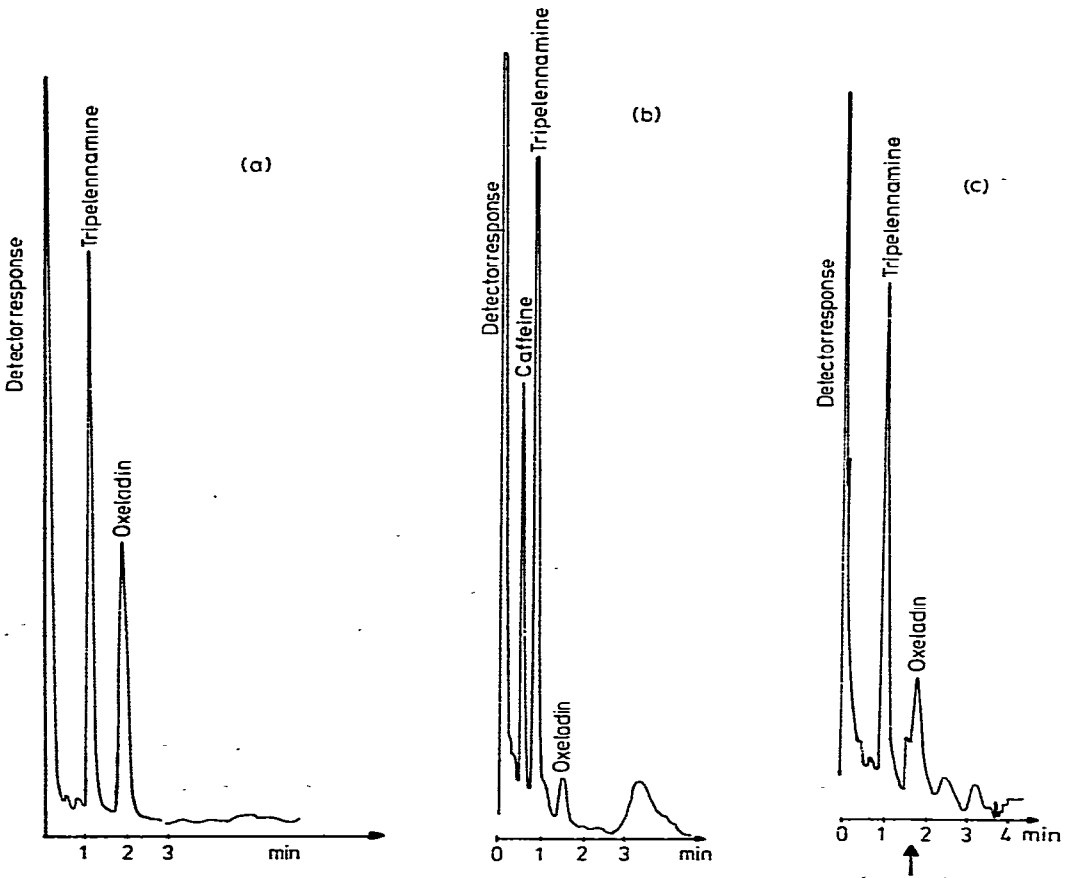

Fig. 1. Chromatograms of serum extracts from volunteers given a single dose of a cough linctus: (a) 1 h after administration and without coffee intake; (b) 6 h after administration and following coffee drinking. (c) A blank serum sample spiked with 10 ng/ml oxeladin base. Arrow marks attenuator shift.

parently caffeine does not interfere in the analysis. However, the caffeine peak interferes with the baseline when very small amounts of OX are being analyzed. In Fig. 1c is shown a chromatogram obtained from a serum spiked with 10 ng/ml OX. (Injected 1 μl = 0.4 ng.) After attenuator shift the OX peak is well above twice the noise.

Based upon the pooled blank serum samples spiked with OX and internal standard, a calibration curve was constructed, where 100 times peak height ratios of OX/IS were plotted as ordinates versus concentration of OX (in ng/ml) as abscissa. The calibration graph was linear over the concentration range 10—250 ng/ml with a regression line $y = ax + b$, where $a = 0.219$ and $b = -0.0846$. The correlation coefficient was 1.00. $S.D._{rel}$ at each concentration is shown in Table I.

TABLE I

RELATIVE STANDARD DEVIATION ($S.D._{rel}$) FOR EACH POINT OF THE CALIBRATION GRAPH

$n = 10$.

Concentration (ng/ml)	$S.D._{rel}$ (%)
10	7.9
50	6.2
100	6.5
200	5.2
250	5.0

Stability of solutions

Stock solutions with a pH of 2.5—3.0 were stable for at least two months at 4°C. The aqueous working standards with a pH of 6.5 were stable for at least two months, kept in a refrigerator at 4°C.

The stability of serum samples containing oxeladin was checked. A blank serum sample was spiked with 167 ng/ml OX and kept at —20°C for four months. Aliquots were analyzed at different times. A patient serum sample was kept for several weeks at —20°C. Replicate analyses were performed. In spite of repeated thawing and freezing of the samples, all analytical data were within the standard deviation range of the method.

Recovery

Recovery of oxeladin and tripelennamine was approximately 100%. About 95% of OX and 99% of internal standard was extracted into the first 6-ml hexane layer.

Injection precision

Ten injections of a given serum extract (spiked with 250 ng/ml OX and 100 ng/ml of internal standard were performed. $S.D._{rel}$ was 2.0%.

Applications

During bioavailability experiments blood was sampled into Venoject tubes without heparin, centrifuged after cooling and kept at —20°C until analysis. The quality of the serum samples varied and some samples were not easily mixed with the hexane layer in a rotating mixer. So a more heavy, horizontal shaking was necessary.

Although caffeine did not interfere in a normal chromatogram, the presence of a large caffeine peak during the analysis of very small amounts of oxeladin presented difficulties in drawing a correct baseline.

The flameless nitrogen-selective thermionic detector proved to be very stable and selective towards nitrogen under the operating conditions given.

In Fig. 2 are presented serum concentration—time data obtained from volunteers given a single dose of Pectamol® linctus.

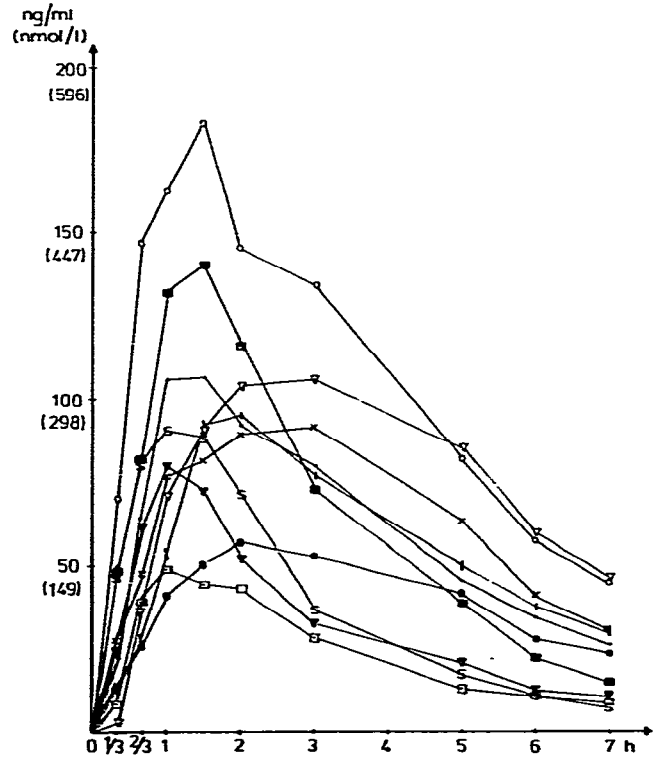

Fig. 2. Typical serum level—time profiles from healthy volunteers after oral administration of 80 mg of oxeladin citrate. Abscissa values represent hours after administration, ordinate is oxeladin concentration.

CONCLUSIONS

The GLC method described above for the determination of oxeladin in 1.0-ml serum samples using a flameless nitrogen-selective thermionic detector

was precise and sensitive. Mean $S.D._{rel}$ in the concentration range 10—250 ng/ml was better than 6.2%. Detection limit was 5 ng/ml of serum or 0.2 ng (1 μl) injected. Twenty serum samples can be analysed in duplicate a day.

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