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Note

Determination of loxtidine in human serum by capillary column gas chromatography with nitrogen–phosphorus detection

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Loxtidine (Fig. 1), a phenoxypropylaminotriazole, is a potent, long-acting, selective H_2-antagonist [1]. Its pharmacologic activity has been well established in numerous animal models involving H_2-receptors [2–4]. In humans, single oral doses of 20, 40 and 80 mg of loxtidine effectively increase the median 24-h intragastric pH from 1.6 to 4.1, 5.4, and 5.5, respectively, emphasizing its potential role in peptide ulcer disease therapy [5].

A single method for quantitating loxtidine in serum has been published [6]. The method utilizes an automated liquid–liquid extraction coupled with a radioimmunoassay (RIA) procedure. The limit of detection is 5 ng/ml and the extraction efficiency is approximately 59%.

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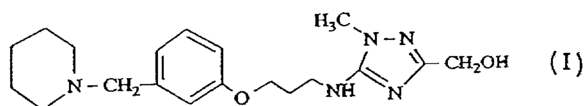

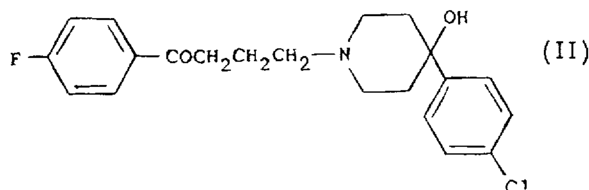

Fig 1 Structures of loxtidine (I) and haloperidol (II)

This paper describes a capillary column gas chromatographic (GC) method with nitrogen–phosphorus detection (NPD). The procedure employs an internal standard using a liquid–liquid extraction in the sample preparation. Haloperidol (Fig. 1) was used as the internal standard because of its extractability, stability and detection response. The limit of quantitation is 2.5 ng/ml with an 83% extraction efficiency The method has been shown to be sensitive, accurate and reproducible, and is suitable for routine analyses to support pharmacokinetic studies.

EXPERIMENTAL

Instrumentation and conditions

The chromatographic system consisted of a gas chromatograph (HP 5890A) equipped with a split–splitless injection port (HP 18740B), a nitrogen–phosporus detector (HP 18789A) and an autopsampler (HP 7673A). Separation was performed on a DB 5 (J&W) fused-silica capillary column (30 m × 0.32 mm I.D., 0.25 μm film thickness). The capillary inlet was operated at 325°C in split mode (1:20) with a split liner packed with 1% OV-1 on Chromosorb W HP, 80–100 mesh. The detector was set at 300°C and the column temperature was kept isothermal at 280°C.

Reagents and materials

Loxtidine succinate standard (Batch No C786/118/1) was acquired from the Biochemical Pharmacology Department, Glaxo Group Research (Ware, U.K.). Haloperidol was purchased from Sigma (St Louis, MO, U.S.A). Methanol and ethyl acetate were obtained from Caledon. Dichloromethane was purchased from Fisher Scientific.

Standard solutions

Stock solutions of loxtidine and haloperidol were prepared in methanol at a concentration of 100 μg/ml. Spiking solutions (500, 400, 250, 150, 100, 50, 25, 15, 10, 5 and 2.5 ng/ml) for standards and quality control samples were prepared by dilution of the loxtidine stock solution.

Preparation of calibration standards and quality control samples

Standards were prepared by adding 100 μl of the appropriate spiking solution to 9.9 ml of pooled drug-free serum. The resulting loxtidine standard curve ranged from 2.5 to 500 ng/ml. Three concentrations of quality control serum samples were prepared in a similar manner at 15.0, 150.0 and 400.0 ng/ml.

Preparation of serum samples

Aliquots (1 ml) of serum standard or quality control sample and 100 μl (2 μg/ml) of haloperidol were transferred to a 100 mm × 16 mm screw-cap glass tube containing 5 ml of dichloromethane. Sample tubes were capped and mixed using a reciprocating shaker for approximately 10 min at high speed. After centrifugation for 5 min at 1850 *g*, the organic phase was transferred to another tube and evaporated to dryness in the presence of nitrogen at room temperature. The residue was re-dissolved in 50 μl of ethyl acetate, placed in a 0 2-ml autosampler vial, and 3 μl were injected into the chromatographic system.

RESULTS AND DISCUSSION

Using the described capillary column GC procedure, loxtidine and haloperidol are chromatographically resolved with retention times of 7.44 and 6.14 min, respectively. Representative chromatograms of an extracted blank and spiked serum samples are shown in Figs. 2 and 3. The absolute recovery for loxtidine and haloperidol was investigated and found to be reproducible using a single extraction. Absolute recoveries for loxtidine at 15 and 400 ng/ml were found to be 83 and 89%, respectively The absolute recovery for the internal standard, haloperidol, was found to be 100.6% at 2 μg/ml. Complete results are shown in Table I.

A calibration curve for loxtidine was obtained using a least-squares parabolic regression: $\ln y = a(\ln x)^2 + b(\ln x) + c$ with x corresponding to the loxtidine concentration, y the peak-height ratio of loxtidine to haloperidol and a, b and c being curve coefficients. The quadratic equation was used because a non-linear detector response resulted in curvature of the regression line and in high errors at the low concentrations [7]. Standard curves consisted of eight concentrations (2.5, 5, 10, 25, 50, 100, 250 and 500 ng/ml). The between-run reproducibility for the standard concentrations resulted in coefficients of variation (C.V.) less than or equal to 6.3% for standards (Table II) and less than or equal to 8.5% for quality controls (Table III). Correlation coefficients were ≥ 0.9992 for all the standard curves.

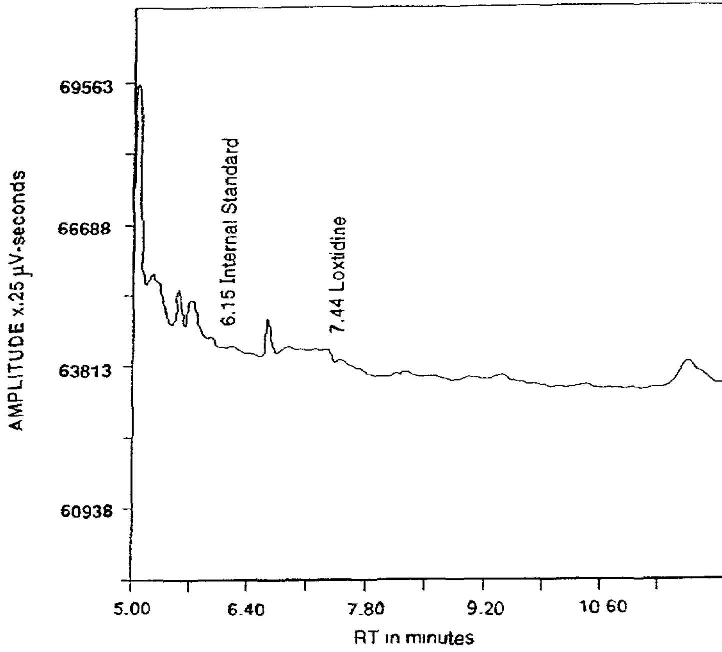

Fig 2 Chromatogram of extracted serum sample with no loxtidine present.

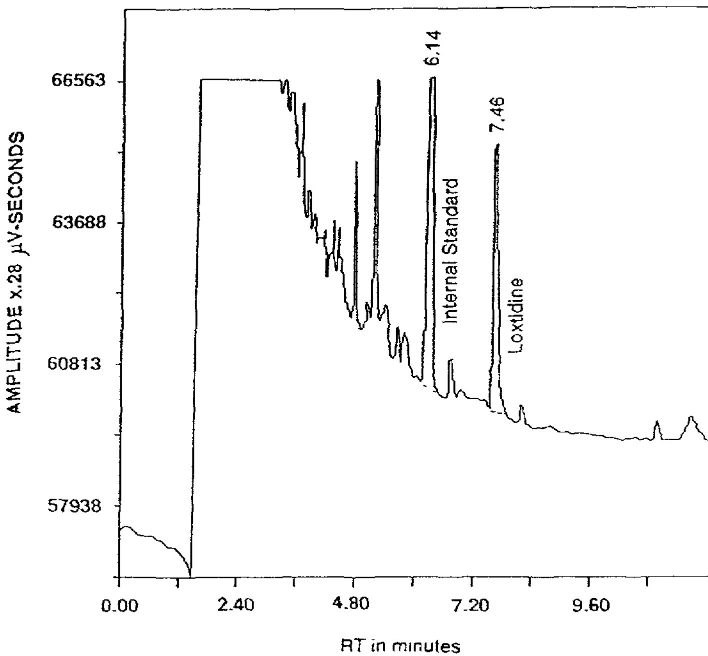

Fig 3. Chromatogram of extracted serum sample fortified with loxitidine (15 ng/ml)

TABLE I

RECOVERY OF LOXTIDINE AND HALOPERIDOL

Compound	Concentratıon (ng/ml)	*n*	C V (%)	Recovery (%)
Loxtıdıne	15	8	7 1	83 1
Loxtıdıne	400	8	4 0	89 0
Haloperidol	2000	8	4 6	100 6

The within-run reproducibility for the standards was determined by examining the coefficients of variation and the averages of batch replicate quality control samples. Two additional spiked concentrations (2.5 and 5.0 ng/ml), representing the limit of quantitation and two tımes this level, were also tested for accuracy and precision. The coefficients of variation ranged from 1.8 to 10.2% and the mean values from 93.5 to 118.8% of the nominal concentrations. These results are shown in Table IV.

TABLE II

BETWEEN-RUN REPRODUCIBILITY OF STANDARDS

Concentratıon added (ng/ml)	*n*	Concentration found (mean ± S D.) (ng/ml)	C V (%)	Error (%)
2.5	6	2 5 ± 0 049	2.0	−0.9
5 0	5	4 8 ± 0 104	2.2	−4 0
10 0	6	11 0 ± 0.229	2 1	10 2
25 0	6	24 3 ± 1.407	5 8	−2 8
50 0	6	47 1 ± 2 947	6 3	−5 8
100.0	6	102.9 ± 2 531	2 5	2.9
250 0	6	253 9 ± 8 491	3 3	1 6
500 0	6	497 1 ± 11.272	2.3	−0 6

TABLE III

BETWEEN-RUN REPRODUCIBILITY OF QUALITY CONTROLS

Concentratıon added (ng/ml)	*n*	Concentratıon found (mean ± S.D) (ng/ml)	C V (%)	Error (%)
15 0	12	13 9 ± 1 187	8 5	−7 3
150 0	11	157 4 ± 2 932	1 9	4 9
400.0	12	385.0 ± 11 419	3 0	−3 8

TABLE IV

WITHIN-RUN PRECISION AND ACCURACY

Concentration added (ng/ml)	n	Concentration found (mean ± S D) (ng/ml)	C V (%)	Error (%)
2.5	7	2.8 ± 0.221	7 9	11.3
5.0	8	5 6 ± 0 303	5.5	11.3
15.0	8	17 8 ± 1 824	10.2	18.8
150 0	8	163 6 ± 6 368	3 9	9 1
400 0	8	374 1 ± 6.874	1 8	−6 5

The interpolated quality control concentrations from six validation curves were used to ascertain the between-run accuracy and precision of the method. The coefficients of variation were 8.5, 1.9 and 3.0% for the 15, 150 and 400 ng/ml concentrations, respectively. The mean values ranged from 92.7 to 104.9% of their actual concentrations. These results are shown in Table III.

In conclusion, this new GC–NPD method for quantitating loxtidine in human serum is simple, accurate, sensitive and reproducible. It offers advantages over the referenced RIA procedure in that it is more sensitive, has a better extraction efficiency, is specific for the parent compound, uses readily available materials and is easily exportable to other laboratories. The procedure has been validated in the range 2.5–500 mg/ml and should be suitable for supporting clinical pharmacokinetic studies.

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