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DETERMINATION OF XYLOMETAZOLINE IN PLASMA AND URINE BY GAS CHROMATOGRAPHY USING A FUSED-SILICA CAPILLARY COLUMN AND AN ELECTRON-CAPTURE DETECTOR

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SUMMARY

A sensitive method is described for the determination of unchanged xylometazoline in plasma and urine at concentrations down to 35 nmol/l. After addition of naphazoline as an internal standard, both compounds are extracted with dichloromethane–diethyl ether (20.80) at pH 10, back-extracted with an acidic solution and re-extracted from a sodium hydroxide solution with dichloromethane–diethyl ether (20:80). The compounds are then derivatized with heptafluorobutyric anhydride in the presence of pyridine. The derivatives are determined by capillary gas chromatography using electron-capture detection.

INTRODUCTION

Xylometazoline (I) has been used for several years as a nasal decongestant (Fig. 1), but little information on the concentrations of I in plasma and urine is available owing to the lack of suitable methods for its sensitive determination in biological media.

High-performance liquid chromatographic (HPLC) methods for the determination of I in pharmaceutical preparations have been reported [1,2], but none of these methods was extended to measurements in biological fluids. Auer et al. [3] described a general procedure for extracting basic drugs but their method was applied in antidoping analyses and no precision was given on the sensitivity of the procedure. Vanezis and Toseland [4] reported a case of poisoning with xylometazoline. In their study xylometazoline was extracted from blood at basic pH and quantitated by gas chromatography (GC) with flame ionization detection using packed columns.

We have now developed a GC procedure using a capillary column and an elec-

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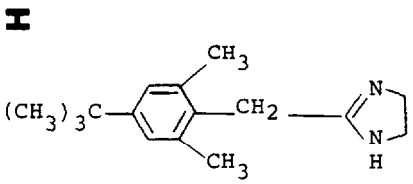
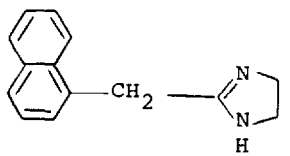


XYLOMETAZOLINE



NAPHAZOLINE
(Internal standard)

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

tron-capture detector to determine I in plasma and urine. The procedure is rapid and allows determinations at levels down to 35 nmol/l in plasma and urine with good accuracy and precision.

EXPERIMENTAL

Chemicals and reagents

The hydrochloride salt of xylometazoline, I·HCl ($C_{16}H_{24}N_2$·HCl; molecular mass 280.83) and the hydrochloride salt of naphazoline (internal standard), II·HCl ($C_{14}H_{14}N_2$·HCl; molecular mass 246.74) were supplied by Ciba-Geigy (Basle, Switzerland) (Fig. 1). A 1-pmol amount I·HCl is equivalent to 0.2808 ng.

Buffer of pH 10 was prepared by diluting two units of pH 10 Titrisol (Merck, Darmstadt, F.R.G.) in water and diluting to 500 ml.

The reagents used for the acylation were heptafluorobutyric anhydride in 1-ml glass ampoules (Pierce, Rockford, IL, U.S.A.) and distilled pyridine (Fluka, Buchs, Switzerland). All other chemicals were of analytical-reagent grade.

Chromatographic equipment

GC was performed on an HP 5890 A chromatograph (Hewlett-Packard, Palo Alto, CA, U.S.A.) equipped with an electron-capture detector and an HP 7673 A automatic sampler. The peak areas were obtained on an HP 5895 A GC workstation and the printer was an HP Thinkjet. The column was a 25 m × 0.31 mm I.D. fused-silica capillary column coated with cross-linked methylsilicone (Hewlett-Packard, No. 19091 A, option 112).

Extraction yield

The extraction yields of I and the internal standard II from plasma and urine were determined by comparison with directly derivatized samples based on the detector response. The calculation was based on the average of six samples each.

Calibration and validation standards

Stock standard solutions were prepared by dissolving I·HCl in methanol and working standard solutions were obtained by dilution of the stock standard solution with methanol. All solutions were stored at 4 °C and were stable for two weeks. The stock standard solutions were different for the preparation of the calibration graphs and for the method validation samples.

The concentration of the internal standard used for determinations in plasma and urine samples was 40.53 μmol/l II·HCl in methanol.

Preparation of plasma and urine calibration standards

Aliquots of standards of I·HCl were placed together with a constant amount of internal standard (II·HCl) in a 10-ml glass tube. Methanol was evaporated under nitrogen at room temperature and 1 ml of plasma or urine was added to produce calibration samples for plasma in the range 35.61–1780.4 nmol/l I·HCl and for urine in the range 34.19–1794.7 nmol/l I·HCl. Actual samples were processed using only the internal standard concentration of II·HCl.

Analytical procedure

To the plasma or urine sample, 2 ml of pH 10 buffer and 5 ml of dichloromethane–diethyl ether (20:80) were added. The tube was shaken for 15 min at 300 rpm and centrifuged for 5 min at 2500 *g*. An aliquot of the organic phase was transferred into another tube and extracted with 2 ml of 0.05 mol/l sulphuric acid by shaking for 10 min. After brief centrifugation the organic phase was discarded. To the aqueous phase 2.5 ml of 0.1 mol/l sodium hydroxide solution were added and the mixture was extracted with 5 ml of dichloromethane–diethyl ether (20:80) by shaking for 10 min. After brief centrifugation, the organic phase was transferred into a 10-ml tube and evaporated to dryness at 40 °C, then 20 μl of heptafluorobutyric anhydride and 1 ml of a 1% solution of pyridine in hexane were added to the residue and the reaction was performed for 30 min at 65 °C. Then 1 ml of hexane and 2 ml of water were added and the mixture was shaken for 15 min. After brief centrifugation, an aliquot of the organic phase was transferred into a conical vial of the automatic sampler and 2 μl were injected into the gas chromatograph.

Chromatographic conditions

Splitless injection was used with a 30-s splitless period. The inlet pressure of the carrier gas (helium) was 100 kPa; the septum purge-rate was 3 ml/min and the flow-rate of the auxiliary gas [argon–methane (90:10)] for the detector was 60 ml/min. The injection temperature was 220 °C and the detector temperature 300 °C.

After injection, the column was held at 50 °C for 1 min, then increased at 70 °C/min to 205 °C, with an isothermal hold at 205 °C for 10.5 min. To wash out plasma or urine residues, the column temperature was then increased at 70 °C/min to 300 °C and held at this temperature for 3 min.

Quantitative evaluation

Quantitation was based on the peak-area ratio y of substance and internal standard derivatives. Calibration graphs were obtained by plotting y against the concentration (x) of I in the sample and calculating the regression line. This was done by weighted linear least-squares regression analysis with a weighting factor of $1/x^2$.

Stability of xylometazoline in urine

In view of the low daily doses in man of 2–3 drops three times a day (50–60 μg of I·HCl per application), we checked the stability of xylometazoline in urine according to a recently published method [5]. The lower and upper limits of the percentage concentration after storage (Δ) were calculated. A degradation of $\Delta = -10\%$ is considered to be relevant.

RESULTS AND DISCUSSION

Choice of analytical column and derivative

The best chromatographic separation was obtained with fused-silica capillary columns coated with cross-linked methylsilicone. The optimum length of the column was 25 m. With shorter columns (15 m) the derivative of I was not well separated from plasma components and with longer columns (50 m) the analysis time was too long. In a screening procedure we tested the acylation of I and II with trifluoroacetic, pentafluoropropionic and heptafluorobutyric anhydride. The best resolution and sensitivity were obtained by derivatization with heptafluorobutyric anhydride. The maximum temperature that allowed the internal standard (II) derivative to be eluted was found to be 210°C. With column temperatures above 210°C, the internal standard derivative decomposed.

Extraction

Compound I was best recovered from plasma and urine at pH 10 using toluene, ethyl acetate and dichloromethane–diethyl ether (20:80). The cleanest chromatograms from plasma and urine extracts were obtained when using dichloromethane–diethyl ether (20:80) as the solvent for extraction. The extraction yield for plasma was 80% for I and 55% for II (internal standard); in urine the extraction yield was 85% for I and 75% for II.

Gas chromatography of plasma and urine extracts

Typical chromatograms of extracts from blank plasma and plasma spiked with I·HCl and II·HCl are shown in Fig. 2. The derivatives of I and II are conveniently separated from plasma components.

Typical chromatograms of extracts from blank urine and urine spiked with I·HCl and II·HCl are shown in Fig. 3. No interfering peaks derived from endogenous urine components were observed at the expected retention times for I and II. The retention times were around 9.5–10.5 min for I and around 12–14 min for II, depending on the different batches of capillary columns tested.

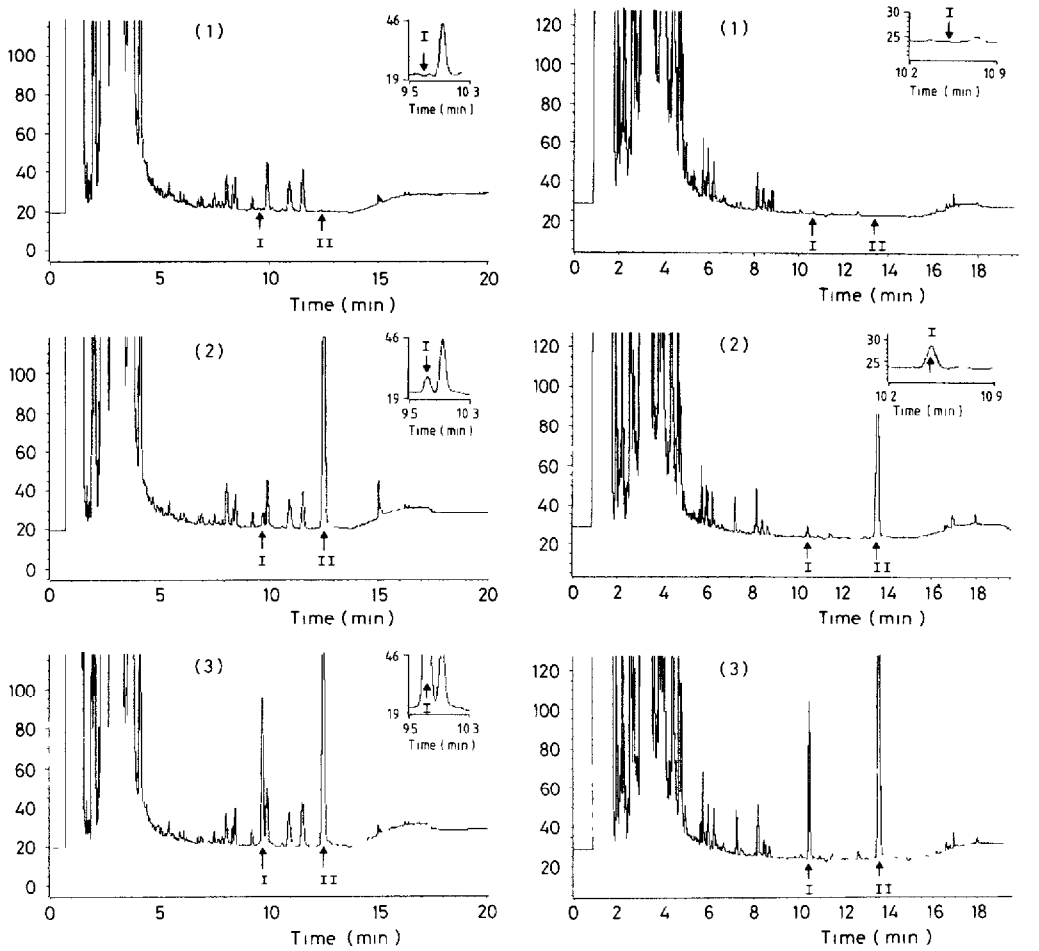


Fig. 2. Typical chromatograms of (1) a derivatized extract of blank human plasma sample, (2) a derivatized extract of a spiked plasma sample containing 35.61 nmol/l I·HCl and 2026.42 nmol/l II·HCl (internal standard) and (3) a derivatized extract of a spiked plasma sample containing 356.09 nmol/l I·HCl and 2026.42 nmol/l II·HCl (internal standard).

Fig. 3. Typical chromatograms of (1) a derivatized extract of blank human urine sample, (2) a derivatized extract of a spiked urine sample containing 34.19 nmol/l I·HCl and 2026.42 nmol/l II·HCl (internal standard) and (3) a derivatized extract of a spiked urine sample containing 356.10 nmol/l I·HCl and 2026.42 nmol/l II·HCl (internal standard).

Calibration graphs

Calibration graphs for I in plasma showed a linear response in the range 35.61–1780.4 nmol/l. Typical parameters for calibration graphs for plasma were $y=0.001058x+0.0057973$, $r=0.9982$, where y is the peak-area ratio, x the concentration of I and r the correlation coefficient. For urine the calibration graphs were linear in the range 34.19–1794.7 nmol/l. Typical parameters of the calibration graphs for urine were $y=0.00081742x-0.0037942$, $r=0.9990$.

Every day, the validity of the calibration graphs was checked by the analysis,

in duplicate, of samples spiked with a low and a high amount of I·HCl. If these spiked samples gave results that deviated too much, a new calibration graph was run. For routine analysis, the calibration graphs in plasma and in urine were valid for about one week.

Within-day precision

Human plasma and urine samples containing I·HCl at different concentrations were repeatedly analysed, six (for urine) or eight (for plasma) times for every concentration, on the same day. The relative standard deviation (R.S.D.) was used as a measure of the precision and the relative difference between the found and added amounts as a measure of the accuracy. The results obtained with the procedure described are given in Table I for plasma and in Table II for urine.

Between-day precision

Six concentrations were determined in duplicate on days 1–5 with the same validation curve obtained on day 1. The results obtained with the procedure described are given in Table III for plasma and in Table IV for urine.

TABLE I

WITHIN-DAY PRECISION AND ACCURACY OF THE DETERMINATION OF I IN SPIKED HUMAN PLASMA

Concentration added (nmol/l)	Mean concentration found (nmol/l)	R.S.D.[a] ($n=8$) (%)	Relative error (%)
35.61	35.85	5.2	+0.7
89.02	87.29	2.3	−1.9
178.04	172.30	2.4	−3.2
356.09	361.47	1.3	+1.5
890.22	887.95	3.8	−0.2
1780.4	1735.1	3.1	−2.5

[a]R.S.D. = (S.D./mean)·100%.

TABLE II

WITHIN-DAY PRECISION AND ACCURACY OF THE DETERMINATION OF I IN SPIKED HUMAN URINE

Concentration added (nmol/l)	Mean concentration found (nmol/l)	R.S.D.[a] ($n=6$) (%)	Relative error (%)
34.19	35.61	1.6	+4.1
91.16	87.86	9.6	−3.6
179.47	172.11	1.1	−4.1
356.10	349.83	1.0	−1.8
854.64	861.09	7.0	+0.8
1794.7	1819.3	2.4	+1.4

[a]R.S.D. = (S.D./mean)·100%.

TABLE III

BETWEEN-DAY PRECISION AND ACCURACY OF THE DETERMINATION OF I IN SPIKED HUMAN PLASMA

Concentration added (nmol/l)	Concentration found (nmol/l)					R.S.D. (%)	Relative error (%)
	Day 1	Day 2	Day 3	Day 4	Day 5		
35.61	34.23	34.02	32.80	37.09	37.88	6.2	−1.1
89.02	86.52	91.45	86.74	91.55	95.05	4.0	+1.4
178.04	176.34	180.05	177.93	175.81	178.99	1.1	0
356.09	360.00	345.18	343.59	359.47	359.47	2.4	−0.7
890.22	875.50	902.50	859.63	894.56	871.27	2.0	−1.0
1780.4	1694.3	1712.3	1713.3	1692.2	1702.2	0.6	−4.4

TABLE IV

BETWEEN-DAY PRECISION AND ACCURACY OF THE DETERMINATION OF I IN SPIKED HUMAN URINE

Concentration added (nmol/l)	Concentration found (nmol/l)					R.S.D. (%)	Relative error (%)
	Day 1	Day 2	Day 3	Day 4	Day 5		
34.19	34.63	35.93	38.50	36.53	34.05	4.9	−5.1
91.16	85.93	86.68	89.20	86.69	85.74	1.6	+4.7
179.47	170.55	164.18	178.45	178.67	172.83	3.5	−3.6
356.10	354.64	336.55	358.69	356.81	360.48	2.7	−0.8
854.64	846.32	850.75	891.25	869.14	870.47	2.1	+1.3
1794.7	1813.4	1756.3	1855.0	1764.7	1741.3	2.6	−0.5

Limit of quantitation

The limit of quantitation (coefficient of variation $\leq 10\%$) was calculated from the results in Tables I–IV. The limit of quantitation of xylometazoline (I) was 35 nmol/l in plasma and 34 nmol/l in urine. Lower concentrations in plasma and urine can be determined with a reduced precision.

Stability in urine

Twenty urine samples spiked with 356.10 nmol/l I·HCl were stored at −20°C. Four cycles of thawing and refreezing were applied to the samples. For each cycle, five of the thawed samples and five freshly prepared samples spiked with the same amount were analysed at the same time. The drug was stable in human urine during the four thawing–freezing cycles (Fig. 4).

The stability of xylometazoline in urine at 4°C spiked with 356.10 nmol/l was studied for various storage times (4, 24, 48 and 72 h). Xylometazoline is stable for at least 72 h when stored at 4°C (Fig. 4).

The stability of xylometazoline in urine at ambient temperature for the concentration range 34.19–1794.7 nmol/l was studied for various storage times (4, 24, 48 and 72 h). Xylometazoline is stable over the whole concentration range under these storage conditions (Fig. 5), so it would be possible to analyse 24-h urine from human subjects.

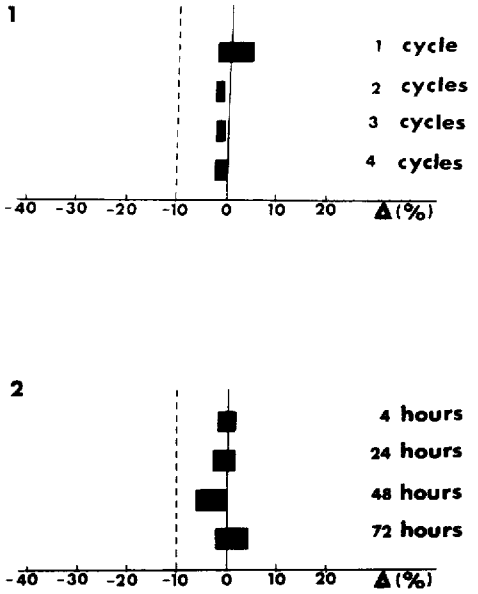


Fig. 4. (1) Stability of I in urine after several cycles of thawing–refreezing (added amount, 356 10 nmol/l); (2) stability of I in urine at 4°C (added amount 356.10 nmol/l).

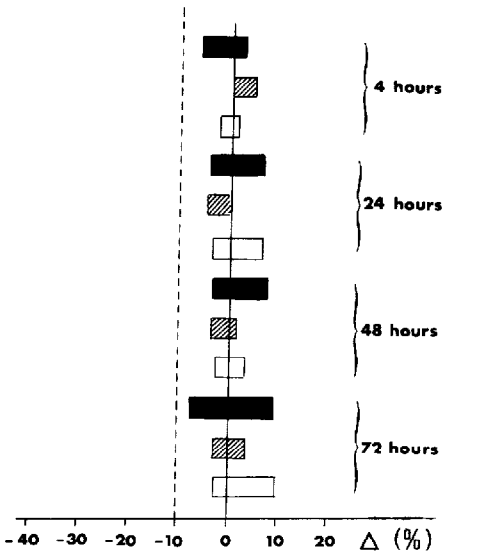


Fig. 5. Stability of I in urine at ambient temperature. Concentrations: black panels, 34.19 nmol/l; hatched panels, 356.10 nmol/l; white panels, 1794.10 nmol/l.

Application

The method was applied to the determination of concentrations of I in plasma from two dogs after an infusion of short duration at a dose of 0.25 mg/kg I·HCl. For illustration, plasma concentration–time profiles of I are shown in Fig. 6.

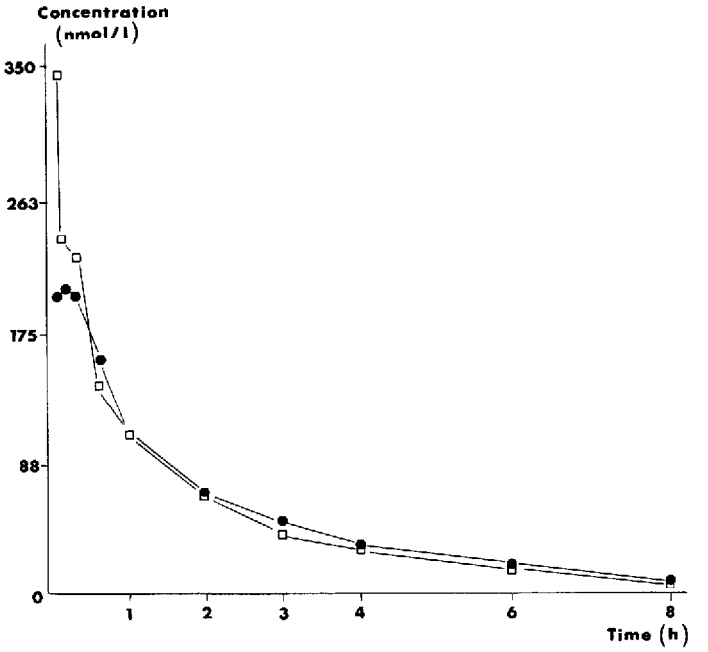


Fig. 6. Plasma concentrations of I in two dogs (●, □) after a single dose of 0.25 mg/kg (intravenous administration).

CONCLUSION

The capillary GC method described here permits the determination of unchanged I in plasma and urine with good accuracy, precision and sensitivity.

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