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GAS CHROMATOGRAPHIC DETERMINATION OF NOXYPTYLINE IN SUBSTANCE, TABLETS AND IN BIOLOGICAL MATERIAL

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SUMMARY

Noxyptyline, *i.e.* 5-(2-dimethylaminoethyloxyimine)-5*H*-dibenzo[*a*,*b*]cyclohepta-1,4-diene hydrochloride, is an antidepressant. A new, direct method for its determination in substance and in tablets by means of gas chromatography has been developed. The results were compared with those of the spectrophotometric method, and the systematic errors (coefficient of variation) were 2.19% and 2.49%, respectively.

Apropriate conditions were developed for the extraction of noxyptyline from plasma and urine, and the gas chromatographic method was applied for its determination. Within the concentration range 0.5–10 μg/cm^3, the systematic error after extraction from plasma was 4.61%, and after extraction from urine it was 2.08%. The recovery from plasma was 71.12 ± 8%, and from urine it was 90.94 ± 1.5%.

INTRODUCTION

Noxyptyline is administered[1] only in tablet form: the initial doses of 25–50 mg daily may be increased gradually to 100 and even 300 mg daily. Like other dibenzocycloheptadiene derivatives, it has been determined by titration and spectrophotometric methods described in pharmacopoeiae. Literature reports describe colorimetric methods for the determination of noxyptyline, involving the formation of complexes with bromothymol[2] and bromophenol[3], as well as two titration methods: one following precipitation with potassium titration methods: one following precipitation with potassium hexathiocyanogen chromate and ammonium tetrathiocyanogen chromate[4], the other applied after hydrolysis to dibenzocycloheptenone[2].

All the above methods for the determination of noxyptyline are not sensitive enough to be applied for determination of the same compound in body fluids (plasma, urine). It seems worth emphasising that no method for determination of noxyptyline in biological material has been quoted in literature.

Therefore, we have outlined the following aims of the present work:

(1) to work out conditions of determination of noxyptyline without synthesising its derivatives;

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(2) to develop conditions for the extraction of noxyptyline from human plasma and urine;

(3) to determine noxyptyline by gas chromatography of the extracts obtained.

These three problems are closely related to pharmacokinetic investigations of noxyptyline.

EXPERIMENTAL

Materials and reagents

The following chemicals were used: noxyptyline of 99.22% purity (Rzeszów Pharmaceutical Works, "POLFA"); noxyptyline of 99.42% purity (Bayer); noxyptyline tablets, 25 mg content of the active ingredient declared (Rzeszów); Oxazepam of 99.73% purity (Tarchomin Pharmaceutical Works, "POLFA"); 95° ethanol, hexane, silicone OV-17, and Gas-Chrom Q.

Apparatus

The following apparatus was used: Perkin-Elmer gas chromatograph, Model 3920 B; Mechanika Precyzyjna centrifuge, type 317a; Premed versatile shaker, type WV-3.

Qualitative determinations of noxyptyline

Noxyptyline used for the determinations melted in the range 188–189°C. The results of ultraviolet and infrared absorptiometric tests complied with the requirements of pertinent standards.

The substance was tested for purity with a thin-layer chromatography method in the following system: F_{254} Merck silica gel; chloroform–methanol (7:3). It exhibited one spot of R_F value 0.5, which complied with the standard. The active ingredient content determined with the titration method amounted to 99.22%.

Gas chromatographic determination of noxyptyline

The determination of noxypryline was conducted on a gas chromatograph equipped with a nitrogen detector. A glass column (1.8 m × 0.3 cm I.D.) filled with 1.5% OV-17 for Gas-Chrom Q qas used. The column was conditioned for 48 h at 280°C. The working temperatures were: column, 230°C; evaporator, 250°C; detector, 280°C. Nitrogen was used as a carrier gas at a flow-rate of 46 cm^3/min. The hydrogen flow-rate was 9.2 cm^3/min and the air flow-rate 150 cm^3/min.

The sensitivity of the measurement was 10 × 16. The internal standard was oxazepam, *i.e.* 7-chloro-1,3-dihydro-3-hydroxy-5-phenyl-2*H*-1,4-benzodiazepin-2-one.

Ethanolic solutions of the tested samples and of the standard, both containing 5 mg% of oxazepam, were prepared within the noxyptyline concentration range: 0.5–2 mg%. Portions (1 μl) of the standard and tested solutions were injected on to the column. The chromatogram obtained is shown in Fig. 1, and the results are listed in Table I. The retention time for noxyptyline was 1 min 50 sec.

The relation between the concentration and the peak height ratio of noxyptyline and the internal standard was linear within the range 0.5–5 μg/cm^3.

The same method was used for determination of noxyptyline in the model

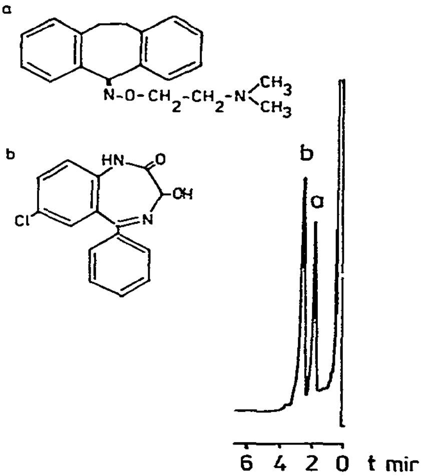

Fig. 1. Gas chromatograms of noxyptyline (a) and internal standard, oxazepam (b).

mixture containing talc, lactose, starch, magnesium stearate and polyvinylpyrrolidone in the amounts declared for the finished product (*i.e.*, for the tablets) by the producer.

A weighed portion of the model mixture, equivalent to one tablet, was placed in a 10-cm^3 measuring flask. Then 8 cm^3 of 95% ethanol was added. The flask was shaked on a shaker for 15 min then its contents were diluted with 95% methanol to 10 cm^3 and filtered. The filtrate was used for the preparation of a noxyptyline solution of 1 mg% concentration, to which 5 mg% of internal standard were added. Portions (0.001 cm^3) of the tested solution and the standard one of the same concentration were injected on to the column.

Noxyptyline was also determined in the tablets of declared 25 mg active substance content. The extraction of noxyptyline from the crushed tablets was conducted

TABLE I

PERCENTAGE OF NOXYPTYLINE IN SUBSTANCE, MODEL MIXTURE AND TABLETS

Materials	*Method*				
	Gas chromatography				*UV*
	$\bar{x}$	*S.D.* *(n = 5)*	*Confidence interval for p = 0.95*	*C.V.*	$\bar{x}$ *(n = 5)*
Noxyptyline in substance	99.09	2.17	99.09 ± 2.68	2.19	–
The model mixture (25 mg of noxyptyline and auxiliary substances)	97.19	2.42	97.19 ± 2.99	2.49	95.00
Noxyptyline in tablets of the declared 25 mg active substance content	99.64	1.74	99.64 ± 2.16	1.75	103.92

similarly to the process described above. Simultanously, noxyptyline extracted from the model mixture and from the tablets was determined with spectrophotometric methods. The results are presented in Table I.

In vitro determination of noxyptyline in human plasma

The determination of the tested compound in human plasma was conducted under the conditions worked out for the substance.

Two stock solutions were made up as follows: (1) 10 mg% noxyptyline ethanolic solution; (2) 2.5 mg% oxazepam ethanolic solution. From the stock solutions five standard solutions were prepared, containing 0.5, 1, 2, 3.5 and 5 μg of noxyptyline and 25 μg of oxazepam in 1 cm^3.

Preparation of calibration curves

From the five standard solutions the calibration curve I was constructed. After an extraction from plasma to which respective amounts of noxyptyline were added, the calibration curve II was prepared. In each of five centrifugal tubes 1 cm^3 of plasma was placed, then the following volumes of noxyptyline stock solution were added: 0.005, 0.01, 0.02, 0.35 and 0.05 cm^3. The contents of each tube were stirred and extracted three times with 4 cm^3 of hexane while being shaken for 15 min on a shaker. After 5 min centrifugation (4000 *g*) the upper layer was pipetted out into a conical flask and dried with anhydrous sodium sulphate. The mixture was filtered, and hexane was evaporated under vacuum below 30°C. The residue was dissolved in 0.5 cm^3 of the internal standard solution and 0.001 cm^3 of thus prepared solution was injected on to the column. The chromatogram obtained is shown in Fig. 2.

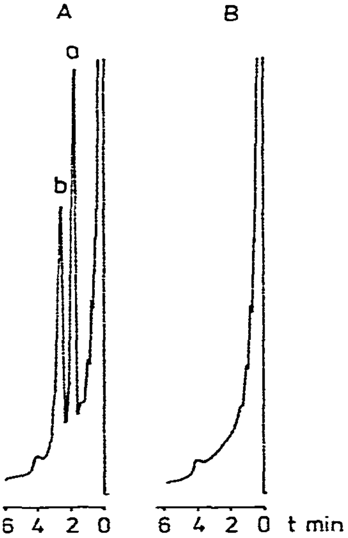

Fig. 2. Gas chromatograms of (A) extract from plasma containing noxyptyline (a) and internal standard, oxazepam (b); (B) extract from blank plasma.

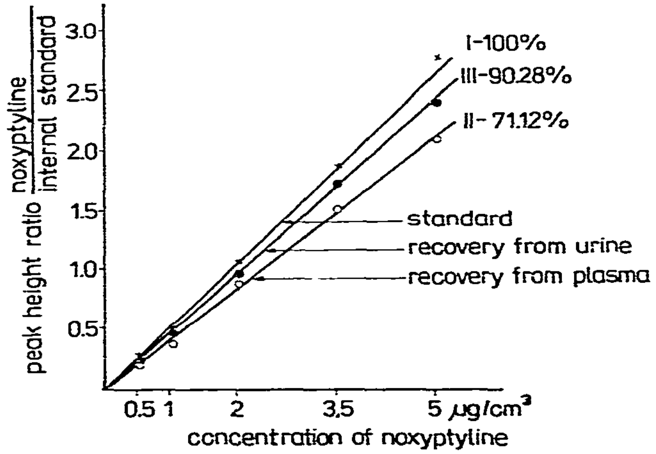

Fig. 3. Calibration curves of noxyptyline. I, Standard; II, after extraction from plasma; III, after extraction from urine.

The calibration curve I was used for determination of detector linearity. The calibration curves I and II show the relationship between the noxyptyline concentration and the peak height ratio of noxyptyline and the internal standard (Fig. 3).

Based on the curves obtained, the recovery of noxyptyline from human plasma was determined. The content of noxyptyline may be determined in 1–4 cm^3 of plasma under extraction conditions similar to those described for the construction of the calibration curve II. The concentration of noxyptyline was calculated from the calibration curve II prepared separately for each series of determinations.

Determination of noxyptyline in human urine

The human urine used for the determination was adjusted to pH 7 with borate buffer of pH 9.

Into five centrifugal tubes, each containing 1 cm^3 of human urine of pH 7, the following volumes of 10 mg % noxyptyline ethanolic solution were added: 0.005, 0.01, 0.02, 0.035 and 0.05 cm^3. The extraction process and the determination itself were conducted precisely as described for the determination of noxyptyline in human plasma. The chromatogram of the extract from urine is shown in Fig. 4.

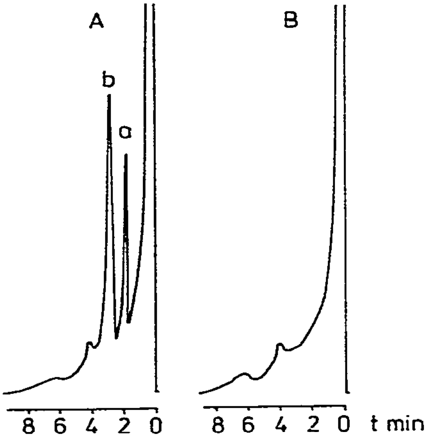

Fig. 4. Gas chromatograms of (A) extract from urine containing noxyptyline (a) and internal standard, oxazepam (b); (B) extract from blank urine.

Based on the results obtained the calibration curve I was constructed, and the calibration curve III was prepared after the extraction from urine (Fig. 3).

For determination of unknown amounts of noxyptyline 1–4 cm^3 of urine may be used, and the extraction should be conducted according to the instructions given for the calibration curve III preparation.

The noxyptyline content was read out from the calibration curve III prepared separately for each series of determinations.

RESULTS AND DISCUSSION

The gas chromatographic determination of noxyptyline was conducted with use of a nitrogen detector, which proved to be more sensitive to the tested compound than the flame ionization detector.

The results listed in the Table I show that the gas chromatographic method described can be used to determine noxyptyline in substance and in a model mixture with an error lower than that of the pharmacopoeial spectrophotometric method.

The use of hexane for extraction of noxyptyline from human plasma and urine was very convenient because the components of plasma and urine were only slightly soluble in the organic layer.

The extraction method worked out for isolation of noxyptyline from urine gave optimal results at pH 7 and therefore required adjustment of urine to neutral with borate buffer of pH 9. The retention times for the impurities from plasma and urine are different from those for noxyptyline and oxazepam.

The determination of noxyptyline in plasma and urine is possible in the concentration range 0.5–10 $\mu g/cm^3$. The recovery of noxyptyline from plasma amounted to 71.12% ± 8%. Statistical evaluation: $\bar{x} = 72.9\%$; S.D. = 3.36; confidence interval 72.9% ± 6.68 for $p = 0.95$ and $n = 8$; coefficient of variation (C.V.) = 4.6%.

The recovery of noxyptyline from urine amounted to 90.28% ± 7%. Statistical evaluation: $\bar{x} = 90.94\%$; S.D. = 1.82; confidence interval 90.94% ± 1.51 for $p = 0.95$ and $n = 8$; C.V. = 2.08%.

CONCLUSIONS

The gas chromatographic method described permits the identification and determination of noxyptyline in substance and tablets, with an error (coefficient of variation) of 2.19% and 2.49%, respectively.

The determination of noxyptyline in plasma and urine is possible in the concentration range 0.5–10 $\mu g/cm^3$. The sensitivity of the method is 0.1 $\mu g/cm^3$; the systematic error (coefficient of variation) is 4.61% for extraction from plasma and 2.08% for extraction from urine.

The method developed for extraction of noxyptyline from plasma and urine, and the method of its direct determination by gas chromatography, may be applied in bioavailability and pharmacokinetic investigations.

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