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## Note

# The simultaneous determination of amitriptyline and nortriptyline in *post mortem* blood and urine using gas–liquid chromatography

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Amitriptyline hydrochloride is a tricyclic drug prescribed in the treatment of mental depression. It is mainly metabolized by N-demethylation to nortriptyline, and the simultaneous determination of these two compounds in biological samples is achieved by spectrophotometry after separation on thin-layer plates[1] or by gas chromatography[2]. In order to obtain satisfactory separation on the chromatograph, nortriptyline is allowed to react with trifluoroacetic anhydride.

In this paper, a gas chromatographic method is described for the simultaneous determination of amitriptyline and nortriptyline without the formation of derivatives. The method is suitable for forensic chemical analysis, but can also be modified for other purposes. Promazine is used as the internal standard.

## MATERIALS AND METHODS

### *Chemicals and reagents*

Amitriptyline, nortriptyline and promazine hydrochlorides were obtained through the Norwegian drug monopoly. All chemicals used in the procedure were of p.a. quality (E. Merck, Darmstadt, G.F.R.).

### *Stock solutions*

Amitriptyline, nortriptyline and promazine hydrochlorides were dissolved in methanol. The concentration of the internal standard was 1.0 mg/ml. The solutions were kept in the dark and refrigerated.

### *Extraction procedure*

Five-millilitre samples of blood or urine were diluted to about 30 ml, made strongly alkaline with potassium hydroxide and hydrolyzed for 10 min on a boiling water-bath. After being cooled, the samples were transferred to a separating funnel and extracted three times with 80 ml of diethyl ether. The ether extract was stored in the dark overnight and, after being washed with 10 ml of water, was re-extracted twice with 40 ml of 0.1 *N* sulphuric acid. The acid extract was made alkaline with concentrated ammonia solution and extracted twice with 150 ml of chloroform. This extract was evaporated to a small volume, transferred to a centrifuge tube with a ground-glass stopper and carefully evaporated to dryness under a stream of air. The residue

was dissolved in 100 $\mu$l of methanol containing 100 $\mu$g of promazine hydrochloride as internal standard and 2 $\mu$l were injected into the gas chromatograph.

*Gas–liquid chromatography*

The gas chromatographic analysis was carried out on a Varian Aerograph Series 2700 chromatograph equipped with a flame ionization detector. The column was a 6 ft. × 2 mm I.D. glass spiral and the packing consisted of 3% OV-17 on Chromosorb G, 80–100 mesh. The flow-rate of the nitrogen carrier gas was adjusted to 16 ml/min and the column oven was maintained at 280°. The injector and detector temperatures were both 300°. The detector sensitivity was $32 \cdot 10^{-11}$ or $64 \cdot 10^{-11}$ A/mV at full scale.

*Calibration graphs*

Standard calibration graphs were established by adding amitriptyline and nortriptyline in amounts from 5 to 150 $\mu$g to 100 $\mu$g of promazine, all dissolved in 100 $\mu$l of methanol. A 2-$\mu$l volume was injected into the gas chromatograph. The peak height ratios of amitriptyline and nortriptyline to that of the internal standard were calculated and the standard calibration graphs were drawn as in Fig. 1.

The nortriptyline calibration graphs were established for different amounts of amitriptyline, but no variation was observed.

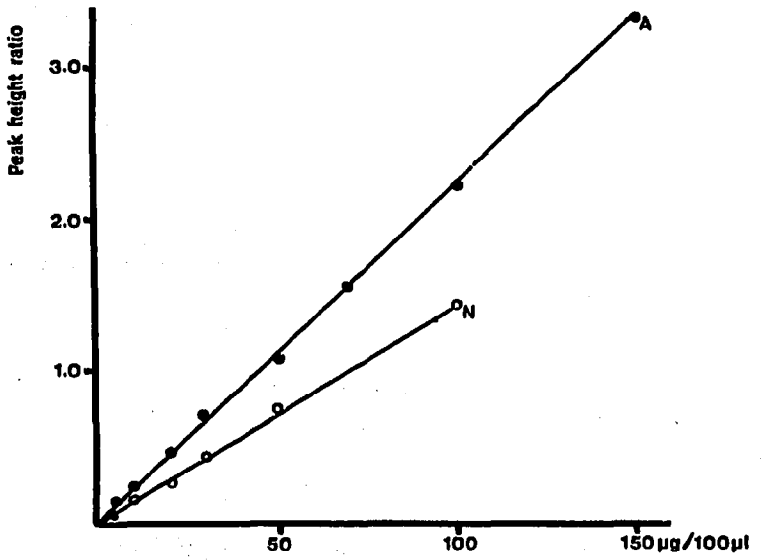


Fig. 1. Standard calibration graphs for amitriptyline (A) and nortriptyline (N). Promazine was used as the internal standard.

*Recovery studies*

To 5 ml of blood or urine, 100 $\mu$g of amitriptyline and nortriptyline hydrochlorides were added and the mixture was extracted as described above. The final residue was dissolved in 100 $\mu$l of internal standard and 2 $\mu$l was injected into the gas chromatograph.

## RESULTS AND DISCUSSION

The present method was developed for the simultaneous determination of amitriptyline and nortriptyline in blood and urine following fatal intoxications, but the extraction procedure can be used to isolate many basic drugs. It is important that the ether extract is allowed to stand overnight because this gives a cleaner extract.

The calibration graphs cover the concentration range from about 0.1–3 mg per 100 ml, which is suitable in most intoxications[3–5]. It was found that the peak height of nortriptyline was independent of even large amounts of amitriptyline and the calibration graph is a straight line. The amitriptyline calibration graph shows no deviation from a straight line.

The OV-17 column is often used in toxicological analysis and it is of importance to establish the most suitable conditions for the simultaneous determination of amitriptyline and nortriptyline on this column. When the gas flow-rate is reduced, a better separation is obtained. In order to compensate for the increased retention time, the temperature is also increased, and although this gives a slight decrease in detector response, the limit of detection for nortriptyline in the present method is lowered. With a flow-rate of nitrogen gas of 16 ml/min, a satisfactory separation is obtained and at a temperature of 280° the analysis is performed in less than 15 min. Chromato-

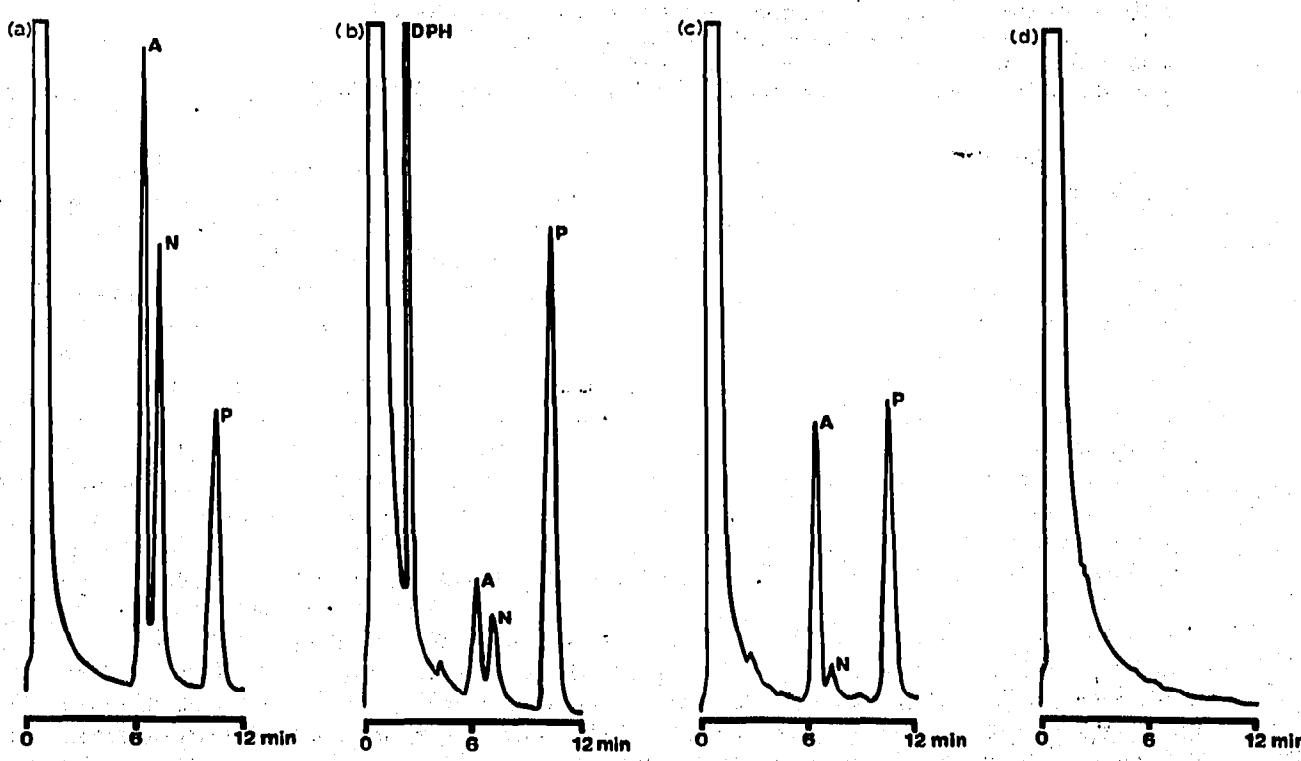


Fig. 2. Chromatograms of amitriptyline (A), nortriptyline (N), promazine (P) and diphenhydramine (DPH). (a) Standard solutions; (b) extract from blood; (c) extract from urine; (d) extract from blank blood.

grams of a standard solution and different extracts are shown in Fig. 2. Blank blood and urine samples had no interfering peaks.

In order to determine the recovery achieved with the present method, amitriptyline and nortriptyline hydrochloride were both added to blood and urine and the samples were extracted as described above. No differences were found between the blood and urine extracts. The mean recovery from six determinations was 89 ± 3.8% (S.D.) for amitriptyline and 90 ± 2.3% (S.D.) for nortriptyline. The detector response was the same whether the hydrochloride salt or the free base was injected into the gas chromatograph.

In the present method, the internal standard may be added to the samples before the extraction. In some instances, however, additional drugs are found (see Fig. 2b) and these can be quantified with the same internal standard. Without extraction, it is then easy to establish a new standard calibration graph, which makes the method more flexible. Promazine was separated well on the gas chromatograph from most other drugs found in the extract described above. Fatal intoxications from promazine have not been investigated in our laboratory.

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