Journal of Chromatography, 140 (1977) 280–283

CHROM. 10,305

Note

Gas chromatographic method for the determination of dextropropoxyphene and nordextropropoxyphene in human plasma, serum and urine

HELLE RIIS ANGELO and JYTTE MOLIN CHRISTENSEN

The Department of Clinical Chemistry, Bispebjerg Hospital, DK-2400 Copenhagen NV (Denmark)

(Received April 8th, 1977)

Cases of acute poisoning by overdosing of dextropropoxyphene, which is a widely used analgesic, have appeared with growing frequency during the past few years. A method for the relatively rapid determination of dextropropoxyphene and its major metabolite nordextropropoxyphene is thus required. Previously described gas chromatographic (GC) methods[1–3] are based on determining dextropropoxyphene and nordextropropoxyphene (Fig. 1) without derivatization, and great problems with on-column decomposition have been described[4–6].

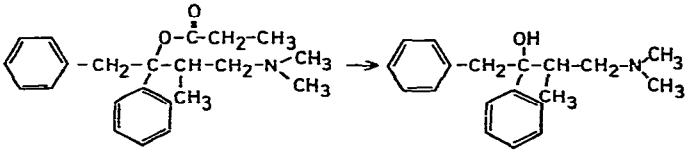

Fig. 1. Structural formulae of dextropropoxyphene and its hydroxy derivative.

This paper describes a method which solves these problems by reduction of the drugs before gas chromatography, using the easily handled and non-explosive sodium bis-(2-methoxyethoxy)aluminium dihydride[7]. The method is specific for the determination of dextropropoxyphene and nordextropropoxyphene, and has a reasonable reproducibility and sensitivity.

MATERIALS AND METHODS

Materials

Dextropropoxyphene hydrochloride was a gift from A/S Alfred Benzon Copenhagen, Denmark), nordextropropoxyphene hemicitrate and *α-d*-pyrroliphene hydrochloride (internal standard) were gifts from the Lilly Research Laboratories (Indianapolis, Ind., U.S.A.). The reagents were of analytical quality except for the sodium bis-(2-methoxyethoxy)aluminium dihydride, which was Merck zur Synthese (Merck, Darmstadt, G.F.R.). This reagent was diluted 1:10 with dry toluene just before use. The toluene was dried over sodium-lead alloy (Merck) for at least 12 h before use.

Extraction and reduction procedure

To 2.5 ml of plasma was added 1.0 ml of 1 *M* carbonate buffer, pH 10, 200 μl of pyrroliphene solution (0.13 mmole/l) and 5.0 ml of *n*-butyl chloride. The mixture was shaken for 5 min, and after centrifugation for 5 min at 1300 *g* and cooling in a dry ice–acetone bath for 1 min, the *n*-butyl chloride phase was easily decanted into a fresh glass vessel with 2.0 ml of 0.6 *N* HCl. After shaking for 5 min followed by centrifugation the *n*-butylchloride phase was aspirated off and discarded. The acid phase was made alkaline by the addition of 600 μl of 20% NaOH. After mixing, 5.0 ml of chloroform was added. The chloroform–NaOH mixture was shaken for 5 min, and after centrifugation for 5 min the aqueous phase was aspirated off and discarded. The chloroform phase was filtered through phase-separating paper and evaporated to dryness at room temperature under nitrogen. The residue was dissolved in 3 ml of dry toluene. Under a weak stream of nitrogen it was reduced by 2 ml of 6% (0.3 mole/l) sodium bis-(2-methoxyethoxy)aluminium dihydride. This reagent was added dropwise, and the mixture was heated to dryness at 100° (*ca.* 15 min) while being frequently shaken. After cooling, 3 ml of water was added slowly, and subsequently 5 ml of methylenechloride, and the mixture was shaken for 5 min. After centrifugation the water phase was aspirated off and discarded. The methylene chloride phase was filtered through phase-separating paper and evaporated to dryness at room temperature under nitrogen. The residue was dissolved in 25 μl of ethylacetate (containing 1% of triethylamine). Samples of 0.7 μl were analysed by GC. Quantitation was by peak height ratio.

Gas chromatography

A Model 900 gas chromatograph equipped with a nitrogen–phosphorus detector (Perkin-Elmer, Norwalk, Conn., U.S.A.) with the following operating conditions: a 1.8 m × 2 mm I.D. glass column was packed with 2.8% OV-210 and 3.2% OV-1 on 80–100 mesh Chromosorb W HP; carrier gas (helium) flow-rate, 40 ml/min; air flow-rate, 135 ml/min; hydrogen flow-rate, 2.7 ml/min; injector and detector temperatures, 300°; the oven was programmed from 210° to 230° at 10°/min. The rubidium bead heating was set at 6.5–7.5.

RESULTS

Evaluation of the analytical procedure

Fig. 2 shows chromatograms obtained from plasma analysis. Exactly the same chromatograms are seen when serum or urine is analysed and in no cases are there interfering peaks from the blank (Fig. 2A).

Plots of the standard curves of the two drugs over the range 1–30 μmole/l were linear and passed through the origin. The same curves are obtained when urine or serum is analysed.

The reproducibility of replicate analyses of plasma samples containing different concentrations is recorded in Table I. Table II compares these results with those from a mass fragmentographic method[8].

Specificity

The following drugs were investigated for possible interfering peaks in the

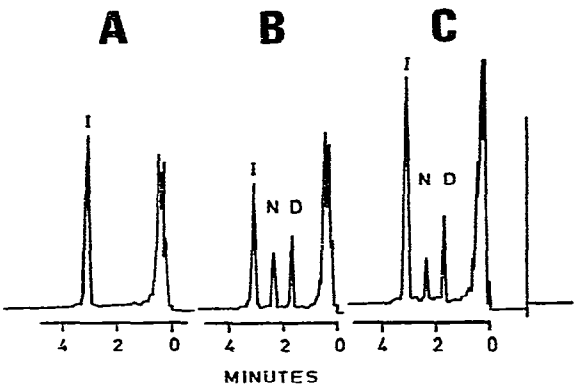

Fig. 2. Chromatograms of human plasma extracts, analysed as described in the text. A, Blank plasma; B, plasma to which dextropropoxyphene and nordextropropoxyphene were added (5.3 and 3.9 μmole/l, respectively); C, plasma from a patient overdosed with dextropropoxyphene. D = Dextropropoxyphene; N = nordextropropoxyphene; I = internal standard.

TABLE I

REPRODUCIBILITY OF REPLICATE SIMULTANEOUS ANALYSIS (MEANS FROM FIVE SAMPLES OF EACH CONCENTRATION) OF DEXTROPROPOXYPHENE HYDROCHLORIDE AND NORDEXTROPROPOXYPHENE HEMICITRATE ADDED TO HUMAN PLASMA

Compound	*Plasma concentration (μmole/l)*	*Coefficient of variation (%)*
Dextropropoxyphene	1.0	8.5
	4.9	2.2
	16.0	4.0
Nordextropropoxyphene	1.0	4.6
	4.9	4.9
	11.7	8.3

TABLE II

DETERMINATIONS OF STANDARDS PREPARED BY ADDING DEXTROPROPOXYPHENE AND NORDEXTROPROPOXYPHENE TO HUMAN PLASMA

A, Expected value; B, our results; C, results from a mass-fragmentographic method[8].

Sample No.	*Dextropropoxyphene (μmole/l)*			*Nordextropropoxyphene (μmole/l)*		
	A	*B*	*C*	*A*	*B*	*C*
1	1.2	1.3	1.4	0.83	0.8	0.88
2	0	0	0	0.89	1.0	0.86
3	6.1	6.2	6.6	4.1	4.4	4.2
4	0	0	0	4.4	4.7	4.2
5	12	11.8	13	8.3	8.9	8.5
6	0	0	0	8.9	9.6	9.1

same region as dextropropoxyphene, nordextropropoxyphene and pyrroliphene: benzodiazepines, barbiturates, common phenothiazines, common thioxanthenes, common tricyclic antidepressants and the most common drugs of abuse (codeine, methadone, morphine, ketobemidone, dextromoramide, pethidine and cocaine).

Only one of these drugs, chlordiazepoxide, showed peaks which might interfere with the determination of dextropropoxyphene. However, by changing the temperature programming, it was possible to separate the peaks.

DISCUSSION

The great advantage of this method is that it overcomes all problems of on-column decomposition. It has been performed without trouble by different technicians during the past 18 months. The time taken to process 18 samples (extraction and reduction procedure) is *ca.* 6 h, and a little less for fewer samples.

The lower limit of detection in our earlier published method [6] was *ca.* 5 μmole/l, because of interfering peaks. Use of a nitrogen–phosphorus detector led to the disappearance of these peaks and the limit was lowered to 0.25 μmole/l. Changing the column from 3% OV-17 to a mixture of 2.8% OV-210 and 3.2% OV-1 improved the linearity from 0.25–15 μmole/l to 0.25–30 μmole/l. This range of detection is sufficient for toxicological plasma (serum) determinations. Urine samples, however, frequently require dilution before the determination.

In 25 patients who had taken overdoses of dextropropoxyphene, the plasma levels of dextropropoxyphene and nordextropropoxyphene were determined to 0.4–9.2 μmole/l and 0.7–8.6 μmole/l, respectively.

ACKNOWLEDGEMENT

The authors thank Bengt Gustafsson, N.-O. Bodin and R. Nygren, Astra Läkemedel AB for their great help with the correlation results (Table II).

REFERENCES

1 K. Verebely and C. E. Inturrisi, *J. Chromatogr.*, 75 (1973) 195.
2 J. M. Manno, N. Jain and R. Forney, *J. Forensic Sci.*, 15 (1970) 403.
3 R. L. Wolen and C. M. Gruber, *Anal. Chem.*, 40 (1968) 1243.
4 C. M. Sparacino, E. D. Pellizzari, C. E. Cook and M. W. Wall, *J. Chromatogr.*, 77 (1973) 413.
5 R. K. Gilpin, J. A. Korpi and C. A. Janicki, *J. Chromatogr.*, 107 (1975) 115.
6 H. R. Angelo, J. M. Christensen and M. Cleemann, *Scand. J. Clin. Lab. Invest.*, 35 (Suppl. 143) (1975) 74.
7 M. Černý, J. Malek, M. Capka and V. Chavalovský, *Collect. Czech. Chem. Commun.*, 34 (1969) 1025.
8 N.-O. Bodin and R. Nygren, to be published.