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Note

Simultaneous determination of propoxyphene and norpropoxyphene in biological samples by gas chromatography using an on-column injection technique with a fused-silica capillary column

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Propoxyphene (PPX) is a mildly effective narcotic analgesic. It is metabolized primarily via N-demethylation to norpropoxyphene (NPX), which is 25–50% as active an analgesic as PPX. A number of procedures have been reported for the determination of PPX and NPX, including UV spectrometry [1,2], thin-layer chromatography (TLC) and high-performance liquid chromatography (HPLC) [3–5]. The HPLC–UV methods are inefficient at the wavelengths commonly used. On the other hand, most of the methods described for the determination of PPX and NPX in biological specimens have utilized gas chromatographic (GC) separation with either flame ionization [6–8] or nitrogen-selective detection [9,10].

The use of a nitrogen–phosphorus detector has been the most convenient method in toxicological analysis owing to its outstanding sensitivity for the detection of traces of drugs and the negligible interference from non-nitrogenous compounds, both endogeneous and exogeneous. However, a few investigators have mentioned the severe problems of decomposition of PPX and NPX on the GC column [7,8,11–13]. Conventional sample introduction on to capillary columns included evaporation of the sample in the injection port as the important step. This step was the primary cause of the poor precision and accuracy of quantitative analyses performed with capillary columns. Settlage

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and Jennings [14] illustrated how performance can vary with the injection technique used, and showed how on-column injection eliminated this variation.

This paper describes the simultaneous determination of PPX and NPX without derivatization using an on-column injection technique on a fused-silica capillary column. This technique eliminates the problem of the decomposition of PPX. Also, treatment with strong base before the extraction improves the chromatographic properties of NPX [1,8]. The sensitivity is sufficient for measuring trace levels of PPX and NPX, and their reproducibility is reasonable.

EXPERIMENTAL

Chemicals

All solvents and reagents were reagent grade. Stock solutions of propoxyphene, norpropoxyphene and pentazocine (all from Eli Lilly, Indianapolis, IN, U.S.A.) were prepared in methanol solution.

Drug administration

For the sample preparation two capsules containing PPX were pulvarized and the mass of the powder was determined. The constituent ratio of PPX in the powder was determined by GC, and the equivalent of 38.2 mg of PPX was administered orally to a 30-year-old man. Urine samples were collected at 0 (blank), 2, 4, 6, 8, 10, 13, 19, 25, 31, 37, 46, 49, 53 and 58 h.

Extraction procedure

For each analysis, 1 ml of human plasma, 2 ml of urine or 500 mg of lung, heart or kidney (each homogenized with three parts water) were used. To each sample 100 mg of a solution of pentazocine (internal standard) and 0.5 g of $NaHCO_3$–K_2CO_3 (1:2) were added in a 15-ml glass centrifuge tube. This mixture was extracted with 5 ml (10 ml for urine) of pentane–isoamyl alcohol (98:2) by shaking for 20 min and centrifuging for 5 min at 752 *g*. The organic layer was transferred to a 15-ml glass centrifuge tube. Then 0.5 ml of 0.2 *M* HCl was added, and extraction was performed by mixing for 10 min in a mechanical shaker. The solution was centrifuged for 5 min at 752 *g*, and the organic layer was aspirated and discarded. The aqueous layer was alkalinized with 50 μl of 5 *M* KOH to pH 14, reextracted with 5 ml of pentane–isoamyl alcohol (98:2) by shaking for 20 min and centrifuged for 5 min at 752 *g*. The organic layer was transferred to another tube and evaporated to dryness at reduced pressure, and then dissolved into 100 μl of 2-propanol.

Gas chromatography

All GC experiments were performed with a Hewlett-Packard (HP) 5890A gas chromatograph with a nitrogen–phosphorus detector, connected to a HP 3392A integrator. All extracts were injected into an HP on-column capillary

TABLE I

GC CONDITIONS USED FOR THE DETERMINATION OF PPX AND NPX

Parameter	Condition
Column	HP fused-silica capillary, cross-linked 5% phenylmethylsilicone (SE-54); 10 m × 0.33 mm I.D.; film thickness 0.52 μm
Detector temperature	300°C
Initial temperature	70°C (for 1 min)
Programme rate	35°C/min
Final temperature	300°C (for 3 min)
Carrier gas (helium) flow-rate	3.0 ml/min
Auxillary gas (helium) flow-rate	20 ml/min
Hydrogen flow-rate	4.0 ml/min
Air flow-rate	100 ml/min

inlet designed for fused-silica columns. The GC conditions for the biological sample analyses are shown in Table I.

RESULTS AND DISCUSSION

Fig. 1 shows chromatograms obtained from human plasma analysis. Similar chromatograms, with no interfering peaks, were obtained from human urine, rat heart, rat lung, rat liver or rat kidney.

The results shown in Fig. 2 indicate that the mode of injection affected the quality of chromatography for PPX. Use of an injection port caused breakdown of the substrate during its transit through the insert liner, as is indicated by peaks A, B, C and D. In the presence of glass wool, the amounts of the decomposition products increased relative to unchanged PPX. Symmetrical peaks with no evidence of decomposition and good baseline characteristics were obtained when on-column injection was used. Mass spectra of the PPX and its degradation products are in good agreement with the mass spectra of degradation peaks previously reported [12,15].

In this procedure NPX was treated with strong base before the final extraction, converting it into an internal amide.

When the initial column temperature exceeded the boiling point of the solvent during on-column injection, prevolatilization of solvent occurred which resulted in a loss of peak area. Grob and Neukom [16] described the relationship of the initial column temperature and the boiling point of the solvent for on-column injection. By using a solvent with a high boiling point (2-propanol), we obtained quantitative results.

Plots of the standard curve of the two drugs in the 1–500 ng range are linear. Similar curves are obtained when other biological samples are analysed. The

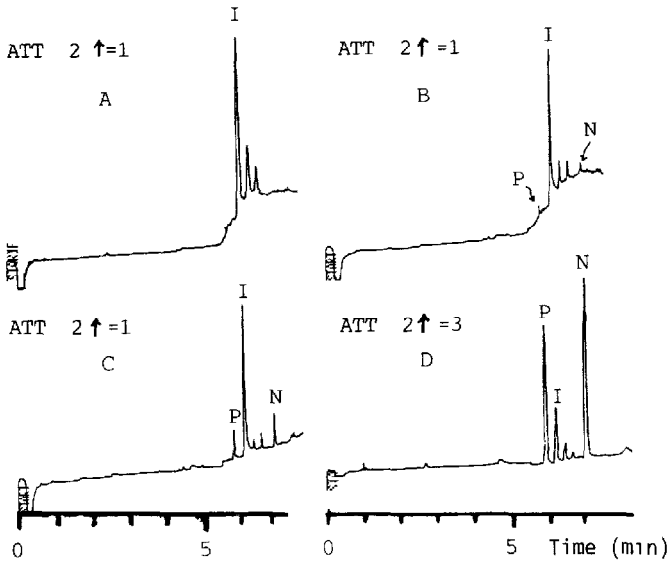

Fig. 1. Chromatograms of human plasma extracts. (A) Blank plasma; (B) plasma to which 1 ng/ml propoxyphene and norpropoxyphene were added; (C) plasma to which 5 ng/ml propoxyphene and norpropoxyphene were added; (D) plasma to which 70 ng/ml propoxyphene and norpropoxyphene were added. Peaks: P = propoxyphene; N = norpropoxyphene; I = internal standard.

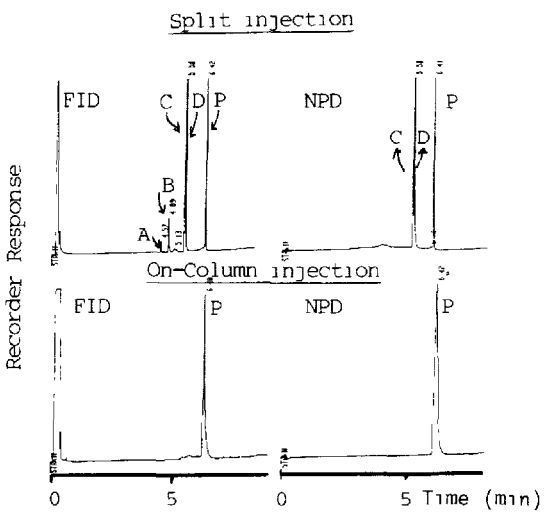

Fig. 2. Gas chromatograms of propoxyphene by split and on-column injection methods under the same conditions: 2 μg injected; split ratio, 1·20. Peaks: A, B, C and D = the degradation products of propoxyphene; P = propoxyphene.

line of best fit for PPX, using the internal standard pentazocine is $y=0.006x+0.013$ ($n=5$, $r=0.999$) and for NPX it is $y=0.007x+0.016$ ($n=5$, $r=0.999$), where x is the analyte concentration and y the peak-area ratio. A near-zero intercept for this curve also indicated that essentially no decomposition of the substrate occurred.

The lower limits of detection in urine for PPX and NPX were ca. 0.5 ng/ml and detection limits in other biological samples were ca. 1 ng/ml. The detectability in the instrument for PPX and NPX was 3 pg. One of the great advan-

TABLE II

DETECTION LIMITS OF PPX AND NPX COMPARED WITH VALUES FROM OTHER SOURCES

Ref.	Detection limit (ng/ml)	
	PPX	NPX
5	100	100
8	10	50
18	85	85
19	300	300
This report		
Plasma	1.0	1.0
Urine	0.5	0.5

TABLE III

PRECISIONS OF PPX AND NPX COMPARED WITH VALUES FROM OTHER SOURCES

Ref.	Coefficient of variation (%)			
	Within-day		Day-to-day	
	PPX	NPX	PPX	NPX
5	4.5	4.8	4.7[a]	6.8[a]
9	5.6	3.7[b]	11.3[c]	11.4[d]
17	49	64	–	–
18	8.5[e]	4.6[f]	–	–
This report	0.9	1.4	5.3[g]	3.4[g]

[a]At 1.0 ppm.
[b]At 0.462 ppm.
[c]At 0.289 ppm.
[d]At 0.458 ppm.
[e]At 0.340 ppm.
[f]At 0.326 ppm.
[g]At 0.5 ppm.

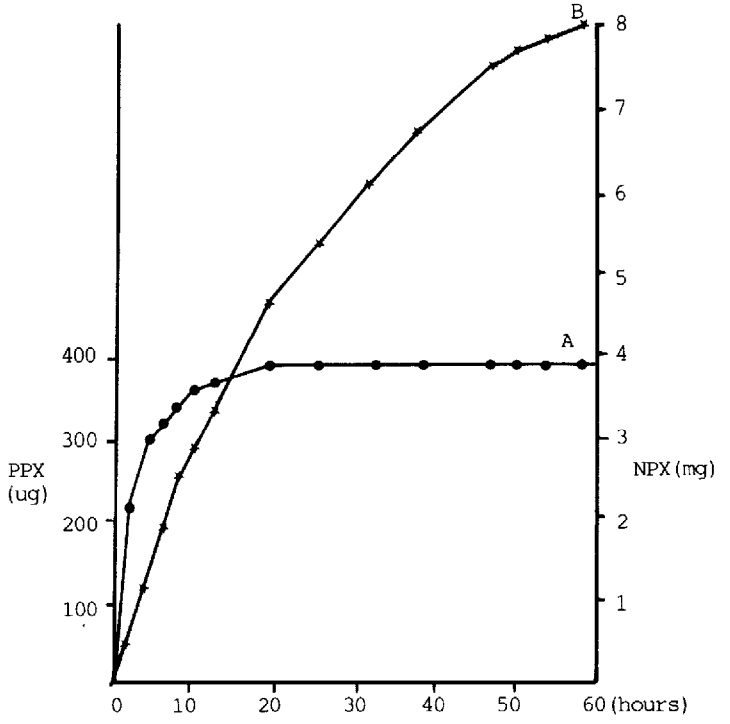

Fig. 3. Cumulative excretion of PPX (A) and NPX (B) over 58 h.

tages of this method is this low detection limit, which is due to the use of a nitrogen–phosphorus detector and the on-column injection technique.

The within-run precision was evaluated by analysing eight aliquots from a urine sample within the same analytical run. The within-run precisions (coefficient of variation, C.V., %) evaluated at a drug concentration of 0.5 μg/ml were 0.90% for PPX and 1.40% for NPX. The day-to-day precisions determined by analysing prepared urine samples at a drug concentration of 0.5 μg/ml on five different days were 5.26% for PPX and 3.44% for NPX.

Because of the instability of PPX and NPX, reported C.V. values for their GC analysis have been very large. But a number of problems associated with their GC determination were improved by our method for extraction and quantification. Tables II and III compare our values with those from other sources. Our method also eliminates the problem of decomposition.

The recoveries evaluated at a drug concentration of 2 μg/ml were $83.1 \pm 1.7\%$ ($n=5$) for PPX and $84.8 \pm 1.0\%$ ($n=5$) for NPX.

Urine samples of a man treated with PPX were studied. PPX and NPX were confirmed by gas chromatography–mass spectrometry (GC–MS) and quantitatively determined by GC.

Fig. 3 compares the cumulative excretion curves of both substances. The

total amounts of PPX and NPX recovered during the 58-h period were ca. 1.02 and 22.3%, respectively.

This method is thus sufficiently precise and sensitive for the quantitation of PPX and NPX in biological samples.

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