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Short Communication

Simultaneous determination of dextromoramide, propoxyphene and norpropoxyphene in necropsic whole blood by liquid chromatography

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ABSTRACT

Dextromoramide, propoxyphene and its main metabolite, norpropoxyphene, were determined in blood after solid–liquid extraction by means of an HPLC method using photodiode-array detection. Two cases of fatal overdose resulting from abuse of the two drugs are presented. In case 1 the necropsic whole blood contained dextromoramide at toxic level (194 ng ml^{-1}) and propoxyphene (614 ng ml^{-1}) and norpropoxyphene (1100 ng ml^{-1}) within the therapeutic range; the death could be due to the combined effect of the two analgesics and, perhaps, other associated drugs. In case 2, the necropsic whole blood concentrations of propoxyphene and norpropoxyphene were 4330 and 3800 ng ml^{-1}, respectively, and could be considered as lethal.

INTRODUCTION

Dextromoramide and propoxyphene are two synthetic analgesic agents. When misused these drugs may induce a toxic overdose reaction involved in accidental and suicidal poisonings. The metabolic fate of dextromoramide is not clear; up to now, no information is available about the quantitation or the pharmacological activity of any metabolite. Regarding propoxyphene, its

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main metabolite, norpropoxyphene, is pharmacologically active.

Several methods have been reported for the determination of dextromoramide, propoxyphene and norpropoxyphene in biological fluids [1–9]. We recently described an HPLC procedure for the determination of dextromoramide only in plasma and whole blood [10]. This procedure was extended in the present study for determining the three compounds in necropsic whole-blood samples from fatal poisonings.

EXPERIMENTAL

Reagents and glassware

Reagents and washing conditions of the glassware were the same as described previously [10].

Apparatus and chromatographic parameters

These were also the same as for the determination of dextromoramide [10]. They are: HPLC Waters system. μBondapak C_{18} column (30 cm × 3.9 mm I.D., particle size 10 μm, ambient temperature) and μBondapak C_{18} Guard-Pak column (5 mm × 6 mm I.D.). Mobile phase: 75 ml of acetonitrile plus 25 ml of a mixture of 0.01 *M* ammonium acetate and acetic acid (100:0.1, v/v), flow-rate 1.5 ml min^{-1}. UV detection at 215 nm and spectral data between 200 and 350 nm with a photodiode-array detector.

Standards

Dextromoramide bitartrate was provided by Delalande Labs. (Courbevoie, France), propoxyphene by Houdé Labs. (Puteaux, France) and norpropoxyphene maleate and methadone hydrochloride by Sigma (La Verpillière, France).

In the method described previously [10], propoxyphene was used as internal standard (I.S.) for determining dextromoramide. In this study methadone was chosen as internal standard, because it is not a drug of abuse in France.

Stock solutions of the standards were made in methanol at a concentration of 1 mg ml^{-1} (expressed as the free bases). Working solutions were prepared by diluting to 0.1, 0.01 and 0.001 mg ml^{-1} with methanol. All standards were stored at 4°C. Quality remains good for at least one month.

Procedure

A 2-ml aliquot of whole blood was pipetted into a glass tube. A 20-μl volume of internal standard solution at 0.1 mg ml^{-1} in methanol (methadone: 2 μg) and 1 ml of Normex buffer solution pH 11 (from Carlo Erba, Milan, Italy) were added. After vortex-mixing for 20 s, the mixture was passed onto a 3-ml Extrelut cartridge (from Merck, Nogent sur Marne, France). After waiting for 10 min, elution was made with a diethyl ether–methylene chloride mixture (70:30, v/v). The eluate was collected into a 20-ml conical glass tube and evaporated in presence of 100 μl of 0.01 *M* hydrochloric acid under a stream of nitrogen in a 40°C water bath. The residual non-volatile acid solution remaining at the bottom was washed with 3 ml of diethyl ether (neutral drugs are thus almost completely eliminated). A 40-μl sample of the washed extract was injected into the chromatograph.

Calculation

The described chromatographic conditions showed a good efficiency to obtain sharp and Gaussian peaks. Thus it is preferable to choose peak heights rather than peak areas for calculation. The ratios between the peak heights of the analysed drugs and that of the internal standard were calculated and plotted against the concentrations of the drugs tested after analysis of blank whole-blood samples spiked with increasing concentrations of dextromoramide (20–1000 ng ml^{-1}), propoxyphene and norpropoxyphene (50–1000 ng ml^{-1}), and a constant amount of the internal standard. Within these concentration ranges the relations were linear for the three compounds. The equations of the curves and their correlation coefficients were $y = 0.0144x - 0.0018$ ($r = 0.999$) for dextromoramide, $y = 0.00186x + 0.004$ ($r = 0.998$) for propoxyphene and $y = 0.0019x + 0.0175$ ($r = 0.998$) for norpropoxyphene, where y is the ratio of the drugs to internal standard and x is the quantity of spiked drug. For concentrations higher than 1000 ng ml^{-1} the analysed sample must be diluted.

TABLE I

CAPACITY FACTOR (k'), QUALITY OF RESPONSE AND UV ABSORPTION CHARACTERISTICS (BETWEEN 200 AND 350 nm) OF DRUGS TESTED FOR POSSIBLE INTERFERENCE

Drug	k' and response	UV absorption characteristics (200–350 nm)	
		Maximum wavelength (nm)	Minimum wavelength (nm)
Barbiturates	Not extracted	–	–
Acetylsalicylic acid	Not extracted	–	–
Caffeine	0.10 medium	272	240, 300
Nitrazepam	0.12 poor	236, 320	290
Oxazepam	0.20 poor	230, 320	290
Triazolam	0.20 poor	240, 300	285, 325
Zolpidem	0.20 poor	245, 320	284
Paracetamol	0.22 poor	246	220, 270
Nordiazepam	0.28 poor	230, 320	290
Norflunitrazepam	0.28 poor	224, 260, 320	290
Flunitrazepam	0.34 poor	224, 260, 320	290
Benzoylecgonine	0.35 medium	233, 275	260, 295
Diazepam	0.40 poor	230, 320	290
Prazepam	0.40 poor	234, 324	290
Nalorphine	0.41 good	215, 285	262
Naloxone	0.43 medium	206, 282	260
Lorazepam	0.60 poor	230, 320	290
Normorphine	0.60 good	215, 285	262
Lidocaine	0.82 good	205, 263	250
Morphine	1.10 good	215, 285	262
Norcodeine	1.20 good	215, 285	262
Codeine	1.64 good	215, 285	262
Bupivacaine	1.64 good	205, 263	250
Glafenine	1.64 medium	226, 254	280
Ethylmorphine	1.82 good	215, 285	262
Dextromoramide[a]	2.28 good	Not exhibited	250
Cocaine	2.50 medium	233, 275	260, 295
3,4-Methylenedioxymetamphetamine	2.80 good	206, 245, 295	225, 262
Pentazocine	2.90 medium	225, 282	250
Buprenorphine	2.90 good	215, 282	262
Amphetamine	2.90 good	205, 250	230
Metamphetamine	2.96 good	205, 250	230
Norpropoxyphene[a]	3.32 good	Not exhibited	250
Pethidine	3.35 good	Not exhibited	250
Desipramine	4.00 good	210, 250	230, 320
Propoxyphene[a]	4.12 good	Not exhibited	250
Cyamemazine	4.15 medium	230, 270	250, 310
Nortriptyline	4.73 medium	250, 240	290
Levomepromazine	4.80 good	210, 254, 305	230, 280
Methadone (I.S.)[a]	5.32 good	Not exhibited	250
Alimemazine	5.84 good	200, 254, 305	220, 280
Imipramine	6.43 good	210, 250	230, 320
Amitryptiline	6.50 medium	205, 240	290
Clomipramine	7.70 medium (tailing)	220, 254	240, 320

[a] Analysed compounds.

RESULTS AND DISCUSSION

Under the conditions described above the capacity factor (k') of the analysed compounds (Table I) was 2.28 for dextromoramide, 3.32 for norpropoxyphene, 4.12 for propoxyphene and 5.32 for methadone (I.S.). The peaks of the four drugs were well separated (Fig. 1B). The spectra were characterized by a strong absorption below 220 nm, a minimum at 250 nm and an irrelevant

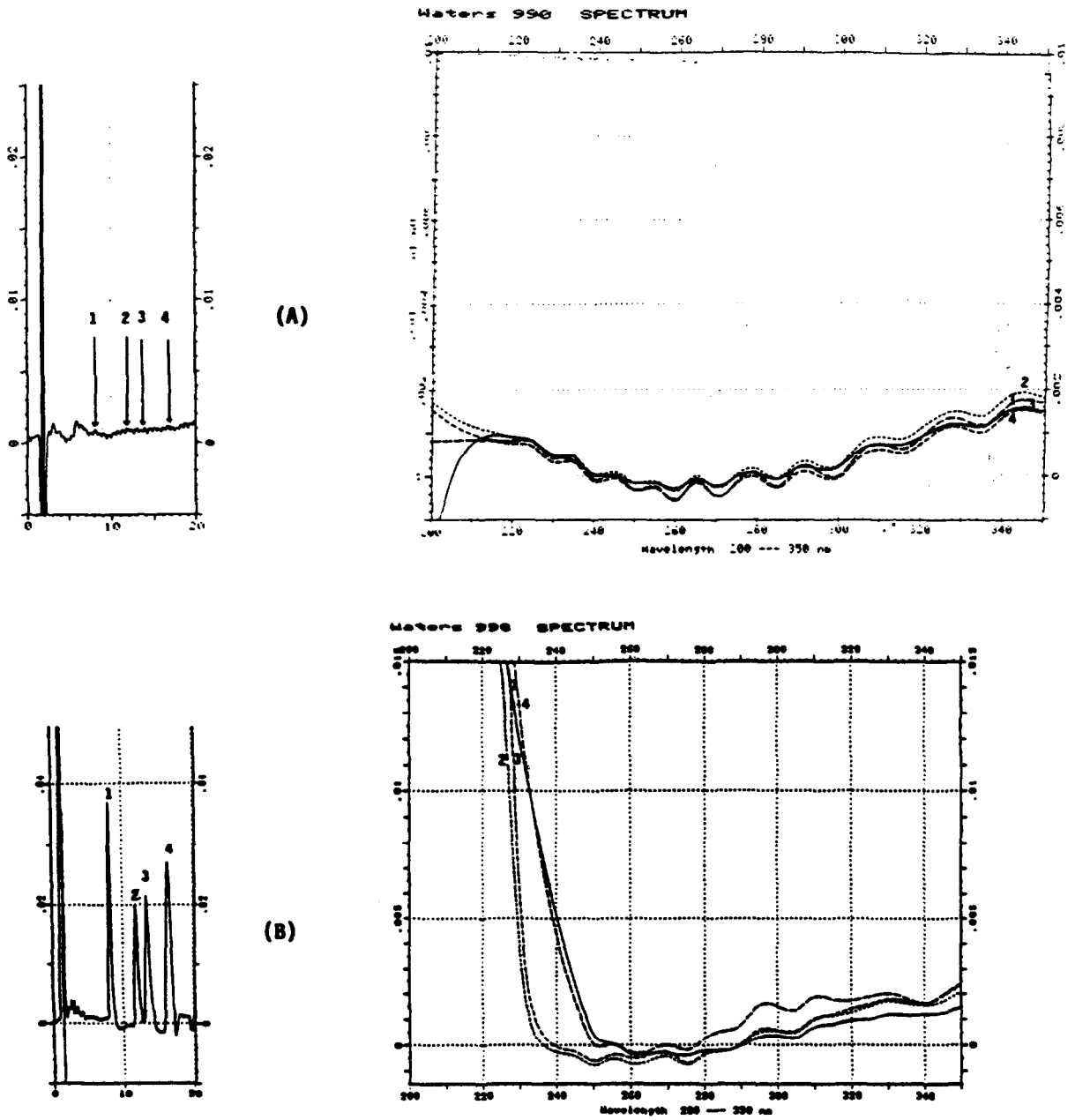

Fig. 1. Chromatograms and corresponding UV spectra of (A) a blank of whole blood (spectra drawn at retention times of 1 = dextromoramide 8.14 min, 2 = norpropoxyphene 11.92 min, 3 = propoxyphene 13.42 min, 4 = methadone I.S. 16.40 min) and (B) a blank of whole blood spiked with 100 ng ml^{-1} dextromoramide, 350 ng ml^{-1} norpropoxyphene, 400 ng ml^{-1} propoxyphene and 1 μg ml^{-1} methadone (I.S.).

TABLE II

REPRODUCIBILITY AND RECOVERY OF DEXTROMORAMIDE, PROPOXYPHENE AND NORPROPOXYPHENE

Added (ng ml^{-1})	Within-day (n = 10)		Day-to-day (n = 8)		Recovery (mean ± S.D., n = 6) (%)
	Found (mean ± S.D.) (ng ml^{-1})	C.V. (%)	Found (mean ± S.D.) (ng ml^{-1})	C.V. (%)	
Dextromoramide					
20	21 ± 2	9.5	19 ± 2	10.5	74 ± 2
50	47 ± 5	10.6	50 ± 5	10	75 ± 3
100	98 ± 8	8.1	102 ± 9	8.8	76 ± 3
250	249 ± 14	5.6	240 ± 18	7.5	77 ± 2
500	503 ± 22	4.4	493 ± 29	5.9	77 ± 2
1000	978 ± 20	2	980 ± 38	3.8	78 ± 2
Propoxyphene					
50	49 ± 5	10.2	48 ± 5	10.4	74 ± 1
100	104 ± 7	6.7	96 ± 7	7.3	76 ± 3
250	252 ± 10	4	250 ± 12	4.8	74 ± 3
500	496 ± 12	2.4	490 ± 14	2.9	75 ± 3
1000	1080 ± 22	2	996 ± 20	2	76 ± 2
Norpropoxyphene					
50	52 ± 5	9.6	49 ± 4	8.2	72 ± 2
100	96 ± 6	6.2	94 ± 8	8.5	73 ± 2
250	244 ± 10	4.1	241 ± 12	5	75 ± 2
500	488 ± 20	4.2	490 ± 26	5.3	74 ± 2
1000	1018 ± 24	2.3	995 ± 30	3	74 ± 3

absorption over 250 nm. Even at levels up to 1 μg ml^{-1} the drugs only exhibit a poor non-specific absorption in the 250–270 nm region. The characteristic absorbance above 280 nm in Fig. 1B can be attributed to blank blood rather than analysed drugs. Fig. 1A shows the spectra from a blank blood sample, drawn at the retention times of the analysed drugs.

The lower limits of detection were 5 ng for dextromoramide and 12 ng for propoxyphene and norpropoxyphene, injected directly in the chromatograph. For quantitation on whole blood the limits of determination were 14 ng ml^{-1} for dextromoramide and 40 ng ml^{-1} for propoxyphene and norpropoxyphene.

The reproducibility was tested on a pool of whole blood spiked with 20–1000 ng ml^{-1} of each drug. The within-day and day-to-day coefficients of variation were less than 11% (Table II).

It was previous indicated [11,12] that norpropoxyphene is unstable in alkaline medium. In this study the decomposition of norpropoxyphene was not considered, because in practice the pH of the mixture was about 9.5, at which pH Hackett *et al.* [6] obtained high recoveries for norpropoxyphene extraction. The recoveries, tested on whole blood, were greater than 70% for all the analysed compounds (Table II).

The tests were carried out on 2-ml samples spiked with 20–1000 ng ml^{-1} (dextromoramide) and 50–1000 ng ml^{-1} (propoxyphene and norpropoxyphene), extracted as described above. The internal standard was added to the eluates just before evaporating in the presence of 100 μl of 0.01 *M* hydrochloric acid. Peak-height ratios (drug/I.S.) of the extracts were compared with those obtained from injection of the residues of the methanolic solutions (containing drug and internal standard), after evaporating and dissolving in 100 μl of 0.01 *M* hydrochloric acid.

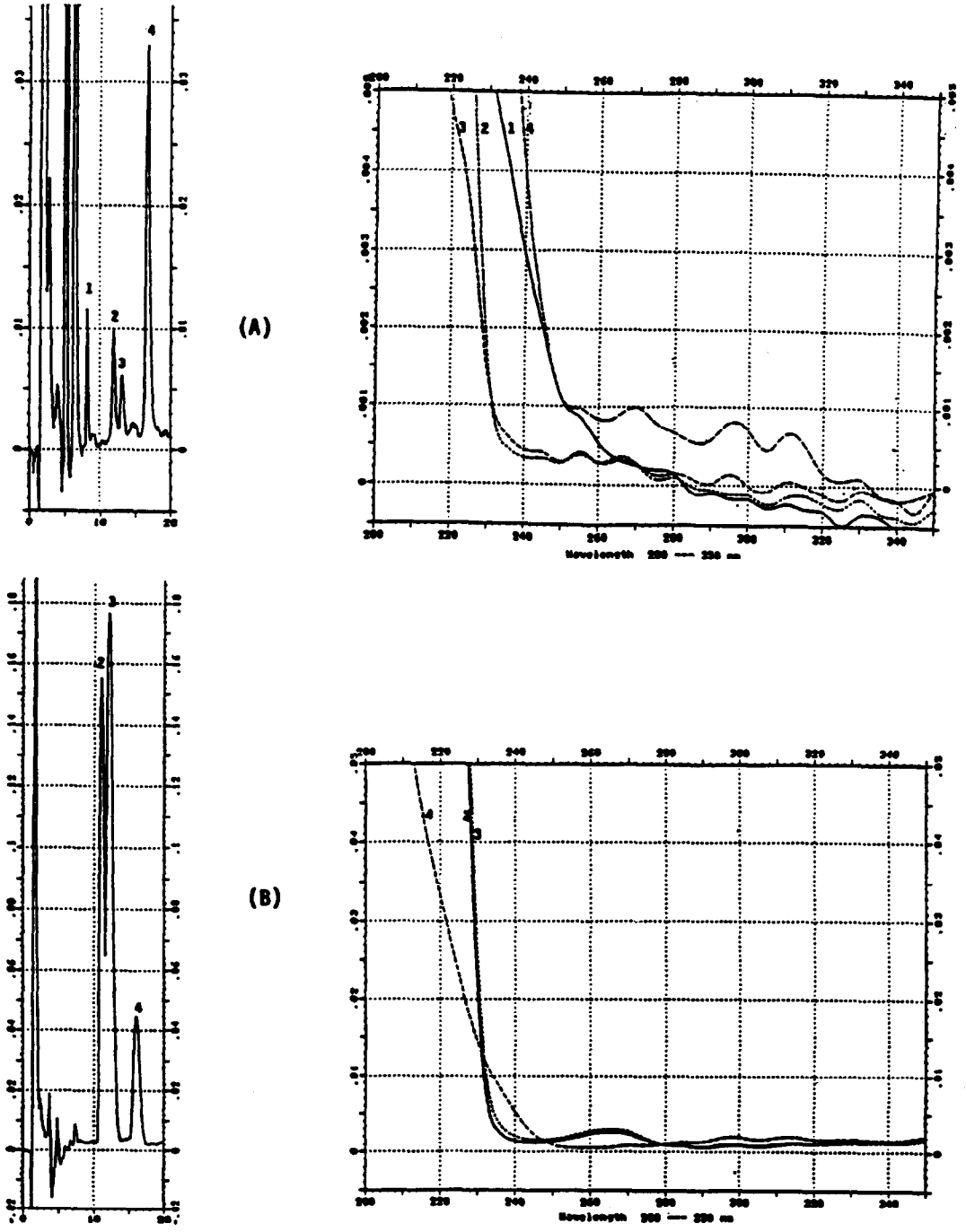

Fig. 2. Chromatograms and corresponding UV spectra of (A) a necropsic blood sample from the first case and (B) a necropsic blood sample from the second case. Peaks: 1 = dextromoramide; 2 = norpropoxyphene; 3 = propoxyphene; 4 = methadone (I.S.).

Several drugs were also tested with the proposed method for possible interference (Table I). Among these compounds pethidine might interfere with norpropoxyphene, and desipramine and cyamemazine with propoxyphene. Therefore propoxyphene and/or its metabolite cannot be

TABLE III

CONCENTRATIONS OF DEXTROMORAMIDE, PROPOXYPHENE AND NORPROPOXYPHENE IN COMPARISON WITH THOSE OF PREVIOUS REPORTS (ng ml^{-1})

P = Plasma; B = blood; Ther = therapeutic; Tox = toxic.

Compound	Present study		Levels from previous reports
	Case 1	Case 2	
Dextromoramide	194		Ther (P): 10–80 [7] Tox (B): >40 [5] Post-mortem (B): 280–984 [2,5,7]
Propoxyphene	614	4330	Ther (P): 50–570 [5] Tox (B): >2000 [5] Post-mortem (B): 1000–6000 [5]; 476–4284 or 1.4–12.6 μmol/l [9]
Norpropoxyphene	1100	3800	Ther (P): 600–3000 [5] Post-mortem (B): 1400–5900 [5]

quantitated in samples that also contain pethidine, desipramine or imipramine (a precursor of desipramine), or cyamemazine.

Application to necropsic whole-blood samples

Two cases of possible overdose were condidered. Only dextromoramide, propoxyphene and norpropoxyphene were determined by the described procedure. Nevertheless the possibly associated drugs encountered in these two cases and some of their metabolites were tested for possible interference (Table I). No interferences were observed.

Case 1. A 25-year-old man who had abused dextromoramide by intravenous injection was found dead in his home with syringes and empty phials of dextromoramide bitartrate (Palfium). He was previously treated with dextromoramide in association with propoxyphene plus acetaminophen (Diantalvic), codeine plus ethylmorphine (Neocodion), flunitrazepam (Rohypnol) and prazepam (Lysanxia). The results are presented in Table III and Fig. 2A[a]. The dextromoramide concentration in whole blood was found to be 194 ng ml^{-1}. It was about 2.5 times as great as the highest plasma therapeutic level, 10–80 ng ml^{-1} [7], but lower than blood post-mortem range (280–984 ng ml^{-1} [2,5,7]). The concentrations of propoxyphene (614 ng ml^{-1}) and norpropoxyphene (1100 ng ml^{-1}) were both in the therapeutic range. So this death could be attributed to the combined effect of dextromoramide and propoxyphene with possible intervention of other substances.

Case 2. An ex-abuser, 30 years old, was treated for addiction with propoxyphene (Antalvic), diazepam (Valium) and zolpidem (Stilnox). He was found dead in a hotel. At autopsy, regurgitation was observed. The results are presented in Table III and Fig. 2B. The concentrations of propoxyphene (4330 ng ml^{-1}) and norpropoxyphene (3800 ng ml^{-1}) in whole blood were greater than the highest therapeutic level and within the blood post-mortem range (Table III). This death was certainly caused by an overdose resulting from the abuse of propoxyphene.

[a] Figs. 1B and 2A seem to have different slopes. In fact, Fig. 2A was from a necropsic blood sample in which endogenous substances could affect the absorbance, whereas Fig. 1B was from a blank sample of whole blood without the same deterioration.

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