Journal of Chromatography, 424 (1988) 152–157
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMBIO. 3933

Note

Measurement of dipipanone using capillary gas chromatography

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(First received April 17th, 1987; revised manuscript received August 26th, 1987)

Dipipanone, a synthetic narcotic analgesic, is available only combined with the anti-emetic cyclizine in the preparation Diconal (10 mg dipipanone hydrochloride, 30 mg cyclizine hydrochloride). Diconal is used clinically for the treatment of moderate to severe pain, particularly in terminal illness. Diconal has been widely abused by drug addicts [1–3].

Although dipipanone has been in clinical use since the early 1950s and abused by addicts since the early 1970s, no pharmacokinetic studies of the drug have been performed, probably because no method of sufficient sensitivity existed for measuring its concentration in plasma and urine. Methods published previously had a limit of detection of 20 ng/ml [4,5] and the peak plasma level of dipipanone after a single oral dose of Diconal is only in the range 26–86 ng/ml [4]. The method described here, which has a limit of detection of at least 5 ng/ml, is four times more sensitive than previously published methods.

EXPERIMENTAL

Apparatus

The apparatus used consisted of a Varian 3400 series gas chromatograph (Varian Assoc., Walnut Creek, CA, U.S.A.) fitted with a split/splitless capillary injector system and a thermionic specific detector, a Varian Series 8000 autosampler and a Spectra-Physics 4270 integrator (Spectra-Physics, San Jose, CA, U.S.A.). The gas chromatograph was fitted with a 15 m × 0.32 mm I.D. BP1 (bonded phase dimethylsiloxane) silica capillary column with 0.5 μm film thickness (Thames Chromatography, Maidenhead, U.K.).

0378-4347/88/$03.50

Reagents and materials

Dipipanone hydrochloride (4,4-diphenyl-6-piperidinoheptan-3-one hydrochloride) was a gift from the Wellcome Foundation (Berkhamstead, U.K.). Diphenylpyraline hydrochloride (4-benzhydryloxy-1-methylpiperidine hydrochloride) was a gift from Smith Kline and French (Welwyn Garden City, U.K.).

Tris (Trizma base) was obtained from Sigma (St. Louis, MO, U.S.A.). 0.1 *M* Tris, pH 10 was prepared and the pH checked on a pH meter using a Russell TR-CW78-TB electrode (Russell pH, Fife, U.K.). No adjustment of pH was found necessary.

Propan-1-ol (Aristar grade), dichlorodimethylsilane (laboratory reagent) and 1,1,1-trichloroethane (Analar) were obtained from BDH Chemicals (Poole, U.K.); methanol (Pronalys AR) and diethyl ether (peroxide-free) were obtained from May and Baker (Dagenham, U.K.).

Chromatographic conditions

The gas chromatograph was set with an injector temperature of 250 °C and a detector temperature of 280 °C. The injector was used in the splitless mode. The voltage on the detector was set to give a 25% deflection with the attenuation set at 32×12. The column was temperature-programmed as follows: starting at 80 °C, holding for 2.5 min, to 230 °C at 40 °C/min without holding, to 250 °C at 5 °C/min, holding for 1.5 min. The split ratio valve was opened at 1.5 min with a flow-rate of 100 ml/min. Helium was used as the carrier gas and was set to give an average linear flow-rate of 60 cm/s.

Preparation of standard curves and extraction

Drug-free plasma and drug-free urine were spiked with dipipanone hydrochloride to give concentrations of 0–1 μg/ml dipipanone base. Using these solutions standard curves were prepared covering two concentration ranges, 0–100 ng/ml and 0–1 μg/ml. An aqueous solution of diphenylpyraline hydrochloride was used as the internal standard at a concentration of 50 ng/ml for the lower-range standard curve and 0.25 μg/ml for the higher-range standard curve.

To 1 ml plasma or urine spiked with dipipanone, 1 ml of internal standard solution and 1 ml of 0.1 *M* Tris buffer pH 10 were added. These solutions were prepared in 75×10 mm soda glass tubes (Downswood Products, Knebworth, U.K.).

Extraction was performed using octyl Bond-Elut cartridges (Jones Chromatography, Mid Glamorgan, U.K.) placed in a Vac-Elut separator (Jones Chromatography) to which a vacuum was applied. The vacuum was adjusted to give a flow-rate through the cartridge of about 5 ml/min. The cartridge was primed using 2×1 ml of methanol followed by 2×1 ml of 0.1 *M* Tris buffer pH 10. The sample prepared for extraction was then applied to the cartridge. This was followed by 2×1 ml water as a wash. Finally the isolate was eluted using 2×1 ml of diethyl ether. The diethyl ether was collected into tubes which had been silanised using a 2% solution of dichlorodimethylsilane in trichloroethane. This was to ensure that no drug was lost onto the glass during evaporation. The diethyl ether was evaporated to dryness at room temperature under nitrogen in a fume cup-

board. The residue was dissolved in 100 μl propan-1-ol, and 1 μl was injected onto the gas chromatograph.

Recovery experiments were carried out using the following procedure. A solution containing both 1 ng/μl dipipanone free base and 1 ng/μl diphenylpyraline free base in propan-1-ol was prepared. Eight injections of 1 μl of this solution were made onto the gas chromatograph. Eight 1-ml samples of drug-free plasma which had been spiked with dipipanone hydrochloride to give a concentration of 100 ng/ml dipipanone free base were extracted and dried down. These were each dissolved in 100 μl of propan-1-ol containing 1 ng/μl diphenylpyraline free base, and 1 μl from each of these solutions was injected onto the gas chromatograph. The recovery of dipipanone was calculated by comparing the average peak-area ratio of dipipanone to diphenylpyraline from the extracted solutions with the non-extracted solutions. This procedure was used to calculate the recovery of dipipanone at 25 and 100 ng/ml from both plasma and urine.

RESULTS AND DISCUSSION

Fig. 1 shows traces after extraction from plasma and from urine. The integrator was programmed with a chart speed of 0.5 cm/min for the first 6.5 min and increased to 4 cm/min while the peaks of interest were eluting from the column. This made it much easier to distinguish peaks and to check that the integrator had defined the limits of each peak correctly.

Samples were initially extracted using a liquid phase, diethyl ether followed by back-extraction into 0.1 *M* hydrochloric acid. Good recoveries were found. Cathapermal and Caddy [5] also had good recoveries using liquid-phase extraction. However, as liquid extraction is relatively non-specific, a large number of endogenous compounds were coextracted. Solid-phase extraction was more selective and much cleaner gas chromatographic (GC) traces were obtained thereby allowing a higher attenuation to be used and therefore lowering the limit of detection. The results for recoveries are shown in Table I. These were reproducible as shown by the coefficients of variation.

As solid-phase extraction was used, simple disposable glass tubes could be used throughout. This meant there was no chance of contamination from glassware. Solid-phase extraction was also quicker than a lengthy liquid-phase extraction involving a clean-up stage.

At the lower concentration range the best results were obtained if the GC system was cleaned before use. This was done by leaving the injector at 260°C, the detector at 290°C and the oven at 250°C and leaving the split ratio valve open for several hours, usually overnight before use.

Standard curves were prepared and the resulting coefficients of variation for each point are shown in Table II. The calibrations were linear from both plasma and urine for both concentration ranges. This is shown by the following correlation coefficients (r): plasma: 0–100 ng/ml, $r=0.9997$; 0–1.0 μg/ml, $r=0.9993$; urine: 0–100 ng/ml, $r=0.9987$; 0–1.0 μg/ml, $r=0.9999$. This shows that the method is suitable both for single-dose pharmacokinetic studies where a very sensitive

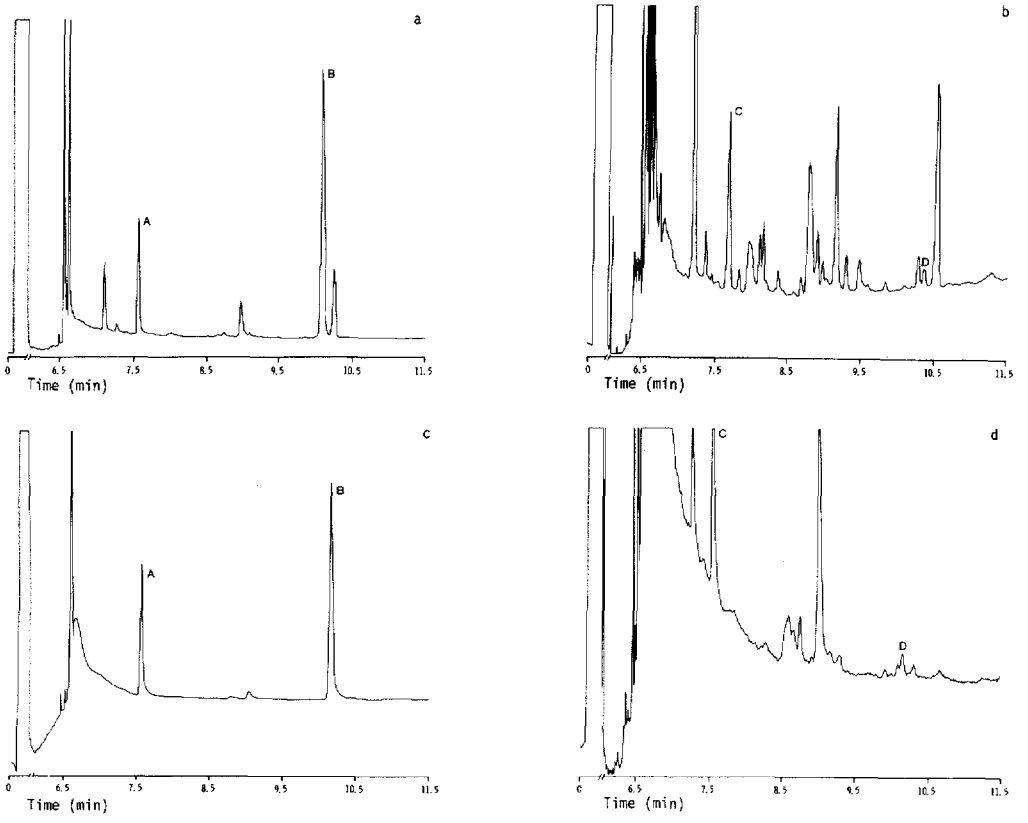

Fig. 1. Typical chromatograms (a,b) from plasma and (c,d) from urine. Peaks: A = diphenylpyraline (0.25 μg/ml); B = dipipanone (1.0 μg/ml); C = diphenylpyraline (50 ng/ml); D = dipipanone (5 ng/ml).

method is needed, and for work where less sensitivity is required such as the measurement of steady-state therapeutic levels.

Retention times for other drugs commonly abused by addicts are shown in Table III. These data show that none of the compounds would interfere with the analysis of samples from addicts for dipipanone. They also show that both dipipanone and the internal standard used separate from cyclizine, the other drug found in Diconal.

This method has been used to measure the levels of dipipanone in urine and plasma samples from drug addicts suspected of abusing Diconal. The levels of

TABLE I

RECOVERY OF DIPIPANONE FROM PLASMA AND URINE ($n = 8$)

Concentration (ng/ml)	Plasma		Urine	
	Recovery (%)	C.V. (%)	Recovery (%)	C.V. (%)
25	73.3	12.5	49.8	4.8
100	75.6	4.3	49.8	4.9

TABLE II

REPRODUCIBILITY OF THE ASSAY ($n=8$)

Concentration (μg/ml)	C.V. (%)		Concentration (ng/ml)	C.V. (%)	
	Plasma	Urine		Plasma	Urine
1.0	7.0	5.3	100	12.4	12.2
0.5	5.3	3.0	50	8.7	17.1
0.25	4.2	5.8	25	10.6	16.1
0.1	6.9	11.1	10	11.0	15.6
			5	13.1	29.6

TABLE III

RETENTION TIMES FOR DRUGS OF ABUSE

Drug	Retention time (min)
Pethidine	6.13
Cyclizine	7.22
Diphenylpyraline	7.57
Methadone	7.83
Propoxyphene	8.05
Cocaine	8.07
Pentazocine	8.61
Codeine	9.27
Dihydrocodeine	9.30
Dipipanone	10.11
Morphine	N.P.*
Dextromoramide	N.P.*

*N.P. = no peak after 15 min.

TABLE IV

LEVELS OF DIPIPANONE IN SAMPLES FROM DRUG ADDICTS

Sample No.	Urine level (μg/ml)	Plasma level (μg/ml)
1	0.78	
2	2.40	
3	1.60	
4	0.54	
5	0.09	
6	2.54	
7	4.60	0.43
8	0.05	
9	0.29	

dipipanone found are shown in Table IV. The level of dipipanone in these urine samples ranged from 0.05 to 4.6 μg/ml. The usual method of screening urine from addicts for drugs of abuse is thin-layer chromatography. Typically a 10-ml sample is used and the limit of detection is in the region of 1 μg/ml. Using such a method the dipipanone would not have been detected in five of these urine samples. Only one plasma level has been measured due to difficulty in obtaining such samples.

REFERENCES

1 M.G. Kelly, J. Irish Med. Assoc., 65 (1972) 415.
2 I.P. James, H.G. Morgan and M. Gay, Br. Med. J., 2 (1976) 1448.
3 B.P. Bishop, G.C. Cave, M.J. Gay and H.G. Morgan, Br. J. Psychiatry, 129 (1976) 465.
4 S.C. Paterson, G.T. Smith and K. Fieldstead, in N. Dunnett and K.J. Kimber (Editors), Proceedings of the 21st International Meeting of the International Association of Forensic Toxicologists, Brighton, Sept. 13–17, 1984, TIAFT, Newmarket, 1985.
5 S. Cathapermal and B. Caddy, J. Chromatogr., 351 (1986) 249.