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DETERMINATION OF DIPIPANONE BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

S. CATHAPERMAL* and B. CADDY
Forensic Science Unit, University of Strathclyde, 204 George Street, Glasgow G1 1XW (U.K.)
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SUMMARY

A reversed-phase high-performance liquid chromatography method is described which is capable of determining the drug dipipanone in urine and plasma at concentrations down to 20 ng/ml.

INTRODUCTION

With the exception of heroin, dipipanone has probably been the most extensively abused narcotic analgesic in the United Kingdom in recent times[1–5]. It is a drug most frequently encountered in the preparation of Diconal, which also contains the anti-emetic cyclizine whose metabolism and pharmacokinetics, unlike that of dipipanone, have been explored in detail following administration to dogs and rats[6,7]. However, the influence of either of these drugs on each other when administered to humans has not been investigated. Apart from the obvious toxicity of narcotic analgesics taken in overdose, dipipanone has a certain inherent toxicity, in particular, the alarming fall in blood pressure which can occur following intravenous administration[8]. For most of the cases in which Diconal poisoning has been reported, cyclizine can be identified in the blood but not dipipanone, although both may be present in other biological samples[9]. Clinical and forensic toxicologists consequently have difficulty in interpreting such findings. As the first part of our investigation into the pharmacokinetics of dipipanone and cyclizine, it became important to develop a specific and reliable method for their assay in biological fluids. Thin-layer chromatography[1] and gas–liquid chromatography[5,6,10] have been used mainly as screening techniques for these drugs in the body fluids of suspected addicts but little has been reported on the routine application of these techniques in pharmacokinetic studies. High-performance liquid chromatography (HPLC) offers a means of analysing for these drugs in body fluids, since such procedures possess adequate sensitivity but are also able to accept samples which are thermally unstable, polar and non-volatile. Such materials may be the product(s) of metabolism of Diconal and for any pharmacokinetic studies it is important to take this into account. What follows is a discription of a method for the analysis of dipipanone by HPLC.

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EXPERIMENTAL

Apparatus

HPLC was performed with an Altex 100 pump, a Rheodyne 7125 injector valve fitted with a 20-μl loop and a Pye Unicam LC3 variable-wavelength ultraviolet detector operated at 230 nm and a sensitivity of 0.08 a.u.f.s. The column was 25 cm × 6 mm O.D. × 4.5 mm I.D. stainless steel, packed with Spherisorb octadecylsilane (ODS) C_{18} reversed phase. The flow-rate was 2 ml/min at a pressure of 3000 p.s.i.

Materials

Acetonitrile, HPLC grade, was obtained from Rathburn Chemicals, Walkerburn, U.K. Deionised distilled water was prepared as required and all other chemicals were of analytical grade. Dipipanone, obtained as the hydrochloride from Wellcome and codeine sulphate from McFarlane-Smith, were both used, as provided, without further purification.

Eluent

A series of eluting system were examined, some being chosen on the basis of their relative polarities, while others were based upon previous HPLC reports[11,12] on drugs having similar or related structures or pharmacological function to dipipanone. The eluting systems examined in the study are listed in Table I.

Limit of detection

Pure dipipanone isolated from the hydrochloride was accurately weighed and then dissolved in methanol to give a solution of 1 mg/ml. The solution was serially diluted by a factor of ten and 20 μl of each dilution injected on to the HPLC system. The limit at which dipipanone could be detected as represented by a peak height twice the size of background noise on this HPLC system was found to be 1 μg/ml. This is equivalent to 20 ng injected onto the column. For this reason all calibration curves were constructed, such that the smallest amount of drug injected was 40 ng.

TABLE I

HPLC SOLVENT SYSTEMS USED IN THE PRESENT STUDY

Solvent system	*Composition*	*Reference*
Methanol–water	(90:10), (80:20), (70:30), (60:40)	
Acetonitrile–water	(90:10), (80:20), (70:30)	
0.01 *M* Aqueous sodium pentanesulphonate–acetonitrile–orthophosphoric acid	(69.95:30:0.05)	11
Methanol–0.75% aqueous ammonium acetate	(80:29)	12
Acetonitrile–0.75% aqueous ammonium acetate	(80:20)	12
Acetonitrile–1% aqueous ammonium acetate	(80:20)	12
Acetonitrile–1% aqueous ammonium acetate, containing 0.05 *M* diethylamine (the pH of this mixture was brought to 7 with glacial acetic acid)	(80:20)	14

Preparation of a standard calibration graph for dipipanone in methanol

Pure dipipanone, isolated from the hydrochloride, and codeine base were accurately weighted and dissolved in methanol to give dipipanone concentrations in the range 2–10 μg/ml and codeine, as internal standard, at a concentration of 5 μg/ml. Aliquots of 20 μl of each concentration were injected into the HPLC apparatus and the relative peak height of dipipanone to codeine recorded in each case. this procedure was repeated ten times for statistical evaluation (Table II). Note that 20 μl of each concentration is equivalent to the range 40–200 ng.

Preparation of a calibration graph for dipipanone in aqueous solution

A stock solution of dipipanone hydrochloride in water at a concentration of 200 ng/ml was prepared. From this stock solution, a range of dilutions were made to give solutions containing 200, 160, 120, 80 and 40 ng/ml of dipipanone hydrochloride. A stock solution of codeine sulphate (internal standard) at a concentration of 100 ng/ml was also prepared. To 1 ml of each of the dipipanone hydrochloride solutions was added, 1 ml of internal standard solution. The mixtures were made alkaline with 1 ml of 1 *M* sodium hydroxide and extracted twice with 10 ml of anhydrous diethyl ether. Each extract was evaporated to dryness on a water bath at 30°C in a current of dry nitrogen, the residues redissolved in 20 μl methanol, and injected into the HPLC apparatus. The relative peak heights of dipipanone to codeine were recorded in each case. This procedure was repeated ten times for statistical evaluation.

Preparation of calibration graphs for dipipanone in urine

Samples were prepared in the same way as for the aqueous solution except that the combination of dipipanone hydrochloride and internal standard were first acidified with 1 ml of 1 *M* hydrochloric acid, extracted with 10 ml of diethyl ether, the ether layer discarded, and the solutions made alkaline with 2 ml of 1 *M* sodium hydroxide before the final extraction with diethyl ether. This process was repeated six times for statistical evaluation.

Preparation of a calibration graph for dipipanone in plasma

A stock solution of dipipanone hydrochloride was prepared in water at a concentration of 2 μg/ml. From this stock solution, a series of dilutions were prepared in water ranging from 0.4 to 2 μg/ml. To 1 ml of each of these diluted solutions contained in a 10-ml graduated flask were added plasma to the mark, to give plasma containing 40–200 ng/ml of dipipanone hydrochloride. A stock solution of the internal standard, codeine sulphate, in water was prepared at a concentration of 100 ng/ml. To 1 ml of each of the plasma solutions containing known amounts of dipipanone hydrochloride, 1 ml of internal standard solution was added and the mixture acidified with 1 ml 1 *M* hydrochloric acid. Each mixture was shaken with 10 ml of ether and the ether layers discarded. The aqueous phases were then made alkaline with 2 ml 1 *M* sodium hydroxide, extracted twice with 10 ml of diethyl ether and the extracts evaporated to dryness on a water bath at 30°C in a stream of nitrogen. Each residue was then dissolved in 20 μl methanol and injected in turn in to the HPLC apparatus and the relative peak height of dipipanone to internal standard recorded. This process was repeated six times for statistical evaluation.

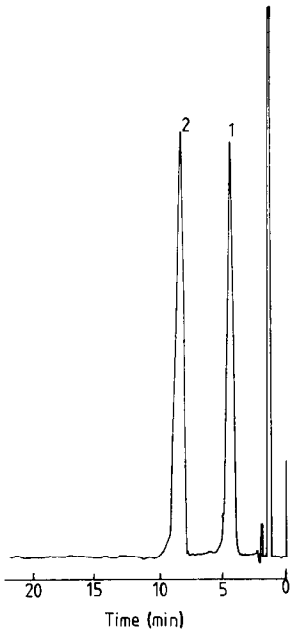
TABLE II

VARIATION IN PEAK HEIGHT RATIO OF PURE DIPIPANONE TO CODEINE OVER THE CONCENTRATION RANGE 2–10 μg/ml OF METHANOL (40–200 ng INJECTED)

Dipinanone concentration (μg/ml)	*Equivalence (ng)*	*Peak height ratio of dipipanone to codeine (internal standard)*										*Mean*	*Standard deviation*
		Determination											
		1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*		
10	200	1.02	1.02	1.03	1.04	1.02	1.05	1.05	1.04	1.04	1.03	1.034	0.01
8	160	0.8	0.8	0.82	0.82	0.82	0.82	0.82	0.85	0.84	0.86	0.825	0.02
6	120	0.64	0.63	0.64	0.61	0.6	0.61	0.6	0.61	0.6	0.61	0.615	0.02
4	80	0.42	0.41	0.43	0.43	0.42	0.43	0.43	0.42	0.41	0.41	0.42	0.01
2	40	0.22	0.21	0.23	0.22	0.2	0.23	0.23	0.21	0.21	0.2	0.21	0.01

RESULTS AND DISCUSSION

Methanol and water, as well as acetonitrile and water mixtures as mobile phases have proved successful for the reversed-phase separation of many compounds[13]. Unfortunately in the present work such mixtures did not give the required chromatographic performance. According to Deeks *et al.*[11] a mixture of 0.1 *M* aqueous sodium pentanesulphonate, acetonitrile and orthophosphoric acid in the ratio 69.95:30:0.05 is a good eluting system for the analysis of morphinan derivatives. Unfortunately, the same solvent mixture did not prove to be useful for the chromatographic analysis of dipipanone. Using ammonium acetate buffered with acetonitrile[12] gave reasonable separation of dipipanone from the internal standard codeine, but the tailing behaviour was unacceptable. Addition of diethylamine to such solvent mixtures has proved to be a very effective way of reducing peak tailing[14–21], but the pH can approach 10, a value usually considered deleterious for ODS columns. Addition of glacial acetic acid to reduce the pH does not appear to affect the tailing



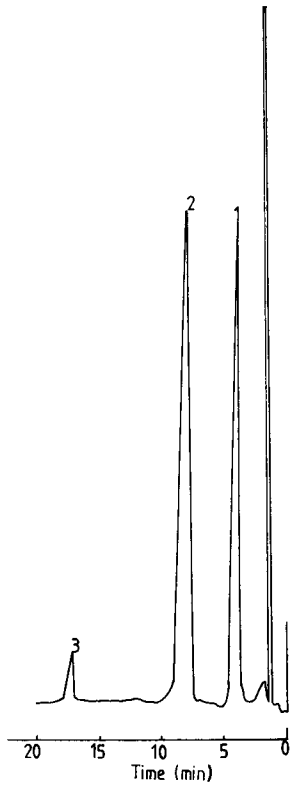


Fig. 1. HPLC profile of dipipanone with codeine as internal standard. Peaks: 1 = codeine; 2 = dipipanone. Chromatographic conditions given in text.

Fig. 2. HPLC profile of dipipanone and codeine prepared from aqueous solutions of dipipanone hydrochloride and codeine sulphate. Peaks: 1 = codeine; 2 = dipipanone; 3 = unknown. Chromatographic conditions given in text.

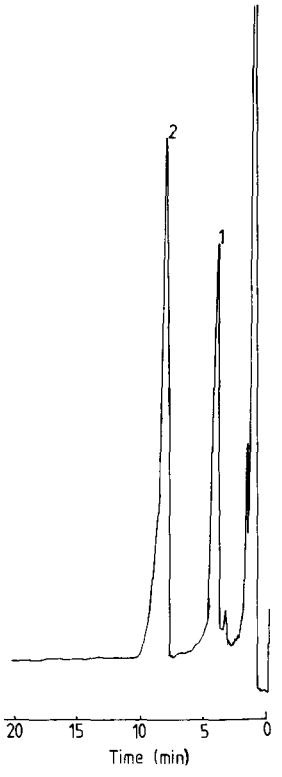


Fig. 3. HPLC profile of dipipanone extracted from urine, using codeine as internal standard. Peaks: 1 = codeine; 2 = dipipanone. Chromatographic conditions given in text.

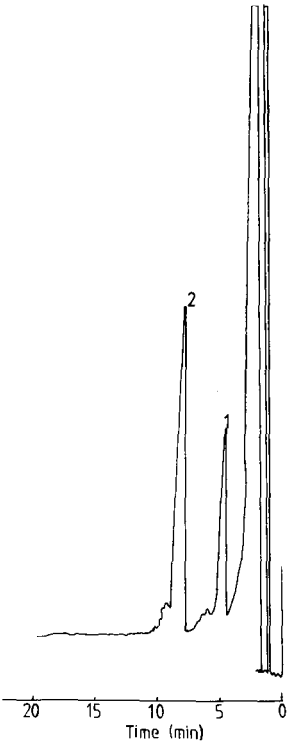


Fig. 4. HPLC profile of dipipanone extracted from serum, with codeine as internal standard. Peaks: 1 = codeine; 2 = dipipanone. Chromatographic conditions as in text.

improvement produced by the amine. Optimum resolution of dipipanone and codeine (internal standard) was achieved using a mobile phase composed of acetonitrile–1% aqueous ammonium acetate (70:30) to which was added diethylamine, so that its concentration was 0.05 *M*. The pH of this mixture was then brought to 7 with glacial acetic acid (Fig. 1).

It is almost impossible to obtain complete coating of the silica packing with the organic octadecyl phase. Any unbonded silica provides residual silanol groups which interact with polar molecules. It is suggested that tailing encountered in the present work, was due to the interactions between dipipanone and free silanol groups on the packing material. The addition of diethylamine ensures that the surface of the reversed phase material is uniformly hydrophobic by blocking the residual silanol groups, hence improving peak shapes and separations.

Maximum UV absorption for dipipanone occurs at 220 nm, but because of the increasing noise from the detector and absorbance by the eluting solvent the best sensitivity was attained by tuning to 230 nm. The sensitivity at this wavelength was higher than at 254 nm which is commonly used in a single wavelength. UV spectrum for morphine compounds[22].

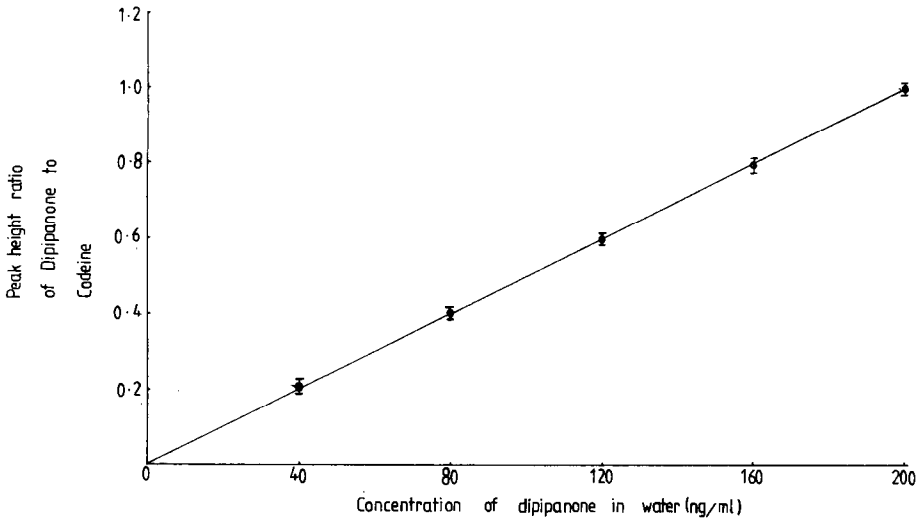


Fig. 5. Calibration graph for dipipanone extracted from aqueous solution.

The mean percentage extraction of dipipanone from aqueous solution was 96.1%, from urine 95.5% and serum 92.5%. With aqueous solutions, a single extraction with diethyl ether was enough (Fig. 2), but urine and serum, (Figs. 3 and 4), required a preliminary back extraction. Without back extraction many unwanted peaks appeared on the HPLC trace. The correlation coefficient of the calibration curves from aqueous, urine and plasma samples (Figs. 5–7) were 0.998, 0.996, 0.99

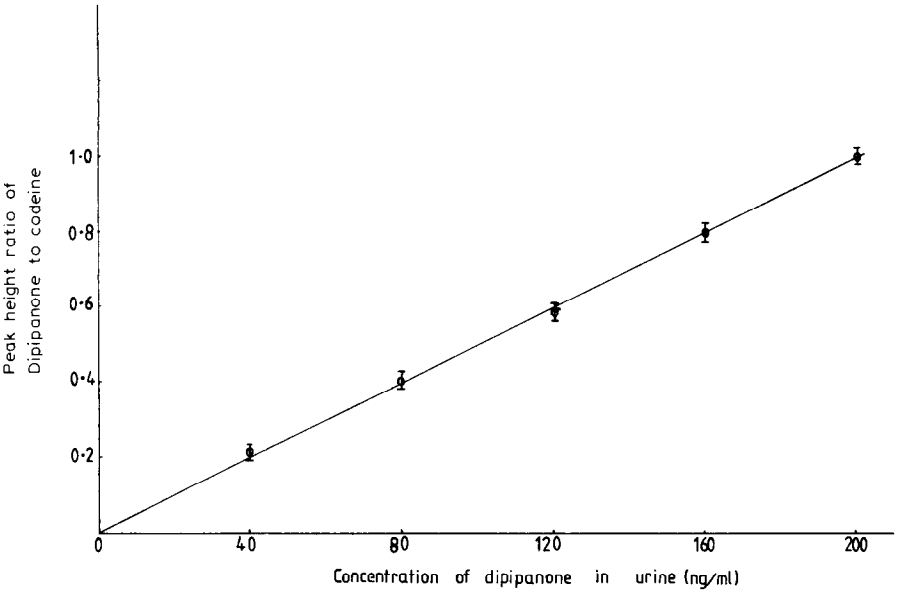


Fig. 6. Calibration graph of dipipanone extracted from urine.

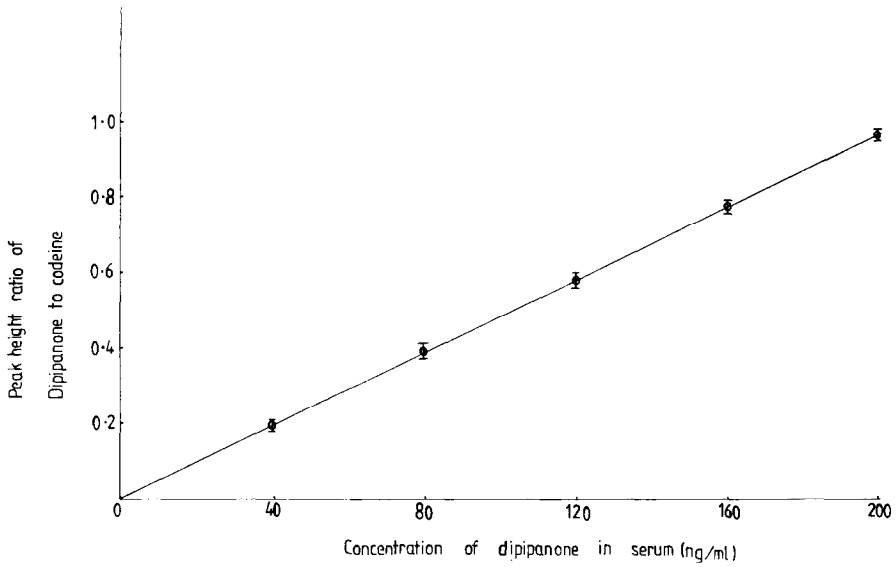


Fig. 7. Calibration graph of dipipanone in serum.

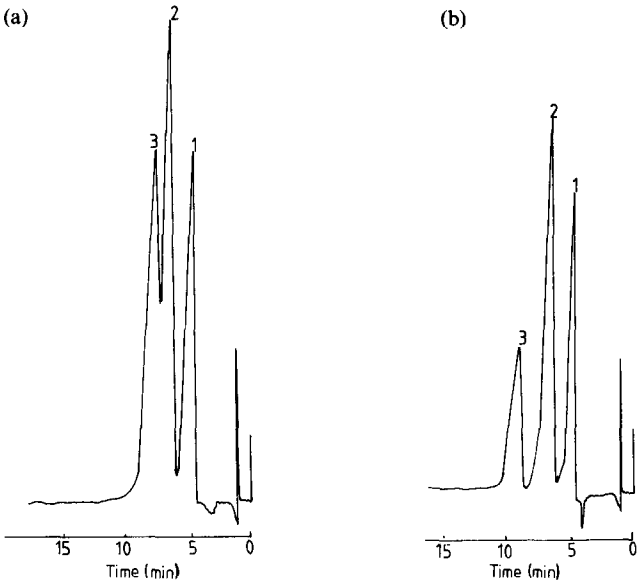


Fig. 8. (a) HPLC of dipipanone, cyclizine and internal standard codeine. Peaks: 1 = codeine; 2 = cyclizine; 3 = dipipanone. Chromatographic conditions as in text. (b) HPLC of dipipanone, cyclizine and internal standard codeine. Peaks: 1 = codeine; 2 = cyclizine; 3 = dipipanone. Chromatographic conditions as in text, with exception that the ratio of acetonitrile: ammonium acetate has changed from 70:30 to 60:40.

respectively. This indicates there is a strong relationship between the concentration of dipipanone and the relative peak height ratio of dipipanone to internal standard.

Statistical data

The regression equations are, for aqueous samples: $y = 0.0025 + 0.005x$; for urine samples: $y = 0.0092 + 0.005x$; and for plasma samples: $y = 0.00167 + 0.005\ x$, where y is the peak height ratio of the dipipanone peak to that of codeine, and x is the concentration of dipipanone in ng/ml. All the calibration graphs were shown to be linear over the concentration range 40–200 ng/ml, with the curve almost passing through the origin.

Since dipipanone is prescribed as a preparation with cylizine, it is necessary to establish if cyclizine interferes in this analytical system. Fig. 8a shows some overlap of the cylizine peak with that of dipipanone. When the ratio acetonitrile: ammonium acetate was changed from 70:30 to 60:40, good separation of all three compounds was obtained (Fig. 8b) but with some peak broadening and loss in sensitivity.

CONCLUSION

Although dipipanone and internal standard (codeine) can be resolved on reversed-phase HPLC, using acetonitrile–1% ammonium acetate (70:30) as eluting solvent, peak tailing is still a problem. Addition of diethylamine to the eluting solvent, sharpened both the dipipanone and internal standard peaks, and also produce a satisfactory separation of the two compounds. Maximum sensitivity of the system, occurs at a wavelength of 230 nm. Using the above condition it is possible to detect dipipanone at a level of 20 ng, hence this method is capable of detecting the drug in therapeutic and overdose situations. If pharmacokinetic monitoring[23] over an extended time period is required, then the method is not sufficiently sensitive.

The resolution of cylizine from dipipanone using a solvent mixture of acetonitrile–ammonium acetate (70:30) is sufficient to establish interference from the former drug, but should this interference be serious then a change in the binary mixture to 60:40 is recommended.

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