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THE DETERMINATION OF HYDRALAZINE IN PLASMA BY GAS–LIQUID CHROMATOGRAPHY

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SUMMARY

A description is given of a gas chromatographic method for the determination of hydralazine in plasma. On treatment with nitrous acid, hydralazine is converted into tetrazolo[1,5-*a*]phthalazine, a stable compound that can be extracted from biological material with organic solvent and determined quantitatively by gas–liquid chromatography. The 4-methyl analogue of hydralazine serves as internal standard for derivatization, extraction and gas chromatography. The sensitivity (10 ng per ml of plasma) is sufficient to monitor plasma levels in man after administration of single oral doses of 50 mg of Apresoline®. The known metabolites do not interfere.

INTRODUCTION

Hydralazine (1-hydrazinophthalazine), the active ingredient of Apresoline® (CIBA-GEIGY, Basle, Switzerland) is well established as a therapeutic agent for the treatment of hypertension[1–3], and there have been a number of publications on its absorption and excretion[4–6]. In these studies, hydralazine concentrations were determined by colorimetric measurements of the hydrazone formed with either *p*-hydroxybenzaldehyde or *p*-methoxybenzaldehyde. Neither of these methods is sufficiently sensitive or specific to monitor plasma levels after single, oral doses of 50 mg.

Hydralazine is unstable and not extractable from biological material with organic solvents. It has been known for some time that hydralazine (1-hydrazinophthalazine; I in Fig. 1) reacts with sodium nitrite at acidic pH to yield tetrazolophthalazine[7] (II in Fig. 1). 1-Hydrazino-4-methylphthalazine (III in Fig. 1) undergoes the same reaction to form 6-methyltetrazolophthalazine (IV in Fig. 1), and thus can be used as analytical internal standard. Both hydralazine and the internal standard can be transformed into their tetrazolophthalazine derivatives directly in the biological sample. The derivatives can be extracted by organic solvents and display favourable gas chromatographic properties to permit quantitation by electron-capture detection.

Tetrazolophthalazine is also strongly fluorescent (excitation at 276 nm; emission at 344 nm). The native fluorescence permits quantitative determination of concentrations down to 2 ng/ml, but high blank values from biological material and the very similar fluorescent properties of the known metabolites prevent the specific

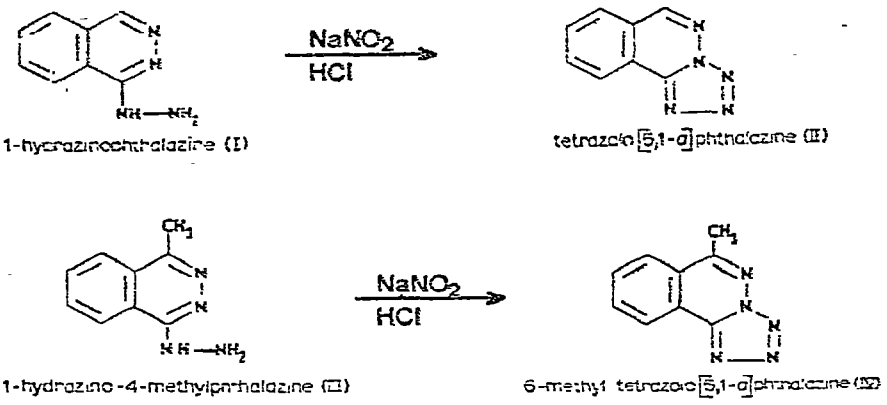

Fig. 1. Transformation of hydralazine (I) and 1-hydrazino-4-methylphthalazine (III) to tetrazolophthalazine (II) and 6-methyltetrazolophthalazine (IV).

quantitative determination of hydralazine by measuring the native fluorescence of its tetrazolophthalazine derivative without further purification procedures.

This paper describes the use of gas–liquid chromatography for the quantitative and specific determination of hydralazine in concentrations down to 10 ng per ml of plasma.

EXPERIMENTAL

Reagents

All solvents were distilled before use, and standard solutions of hydralazine and 1-hydrazino-4-methylphthalazine were prepared freshly in 0.1 *N* hydrochloric acid each time before use.

Procedure

The internal standard, 1-hydrazino-4-methylphthalazine (50 ng in 0.1 ml of 0.1 *N* hydrochloric acid) is added to 1 ml (or less) of plasma, then 1 ml of 2 *N* hydrochloric acid and 0.1 ml of 50% aqueous sodium nitrite solution are added. The solution is mixed and left to react for 15 min at room temperature, then the pH is adjusted to 10 (± 1) by adding 2 ml of 1 *N* sodium hydroxide and 4 ml of buffer of pH 10 (0.03 *M* borax and 0.04 *M* sodium hydroxide). The derivatives are then extracted with 3 ml benzene by shaking for 5 min; after centrifugation, the organic phase is removed and evaporated under a stream of dry nitrogen at 45°. Immediately after all the benzene has evaporated, the vials are removed from the water bath and refrigerated until gas chromatographic analysis can be carried out. (Excessively long evaporation results in loss of the derivatives.) The dry residue is redissolved in 300 to 900 μl of toluene (depending on concentration), and approximately 5 μl of this solution are injected.

Gas chromatographic conditions

The instruments used were a Pye Unicam Model 74, Series 104 gas chromato-

graph with a pulsed (150-μsec) electron-capture detector (^{63}Ni; 10 mCi) and a W+W Model 1100 recorder. The column used (5 ft. × 2 mm I.D.) was made of borosilicate glass and was packed with 3% of OV-225 on Chromosorb W-HP (80–100 mesh). The temperature of the column oven was 220°, that of the detector was 300° and that of the injector was 220°. The carrier gas was nitrogen (flow-rate 30 ml/min). The column was conditioned by heating at 250° with a nitrogen flow-rate of 15 ml/min for 24 h.

The retention times of the derivatives under the conditions described were 6.4 min for tetrazolophthalazine and 9.1 min for tetrazolomethylphthalazine. Chromatograms of a plasma blank and an extract from a sample containing 100 ng of hydralazine and 50 ng of internal standard are shown in Fig. 2.

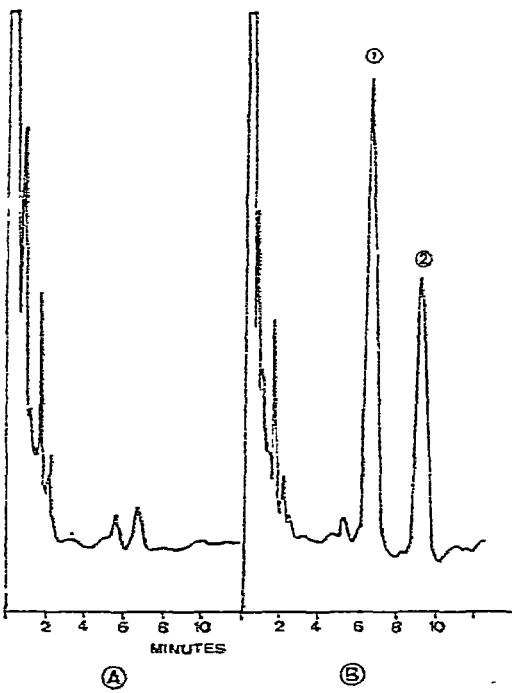

Fig. 2. Chromatograms of (A) blank extract of 1 ml of plasma (sample dissolved in 300 μl of toluene, and 5 μl injected); and (B) extract from 1 ml of plasma containing 100 ng of hydralazine (1) and 50 ng of internal standard (2) (sample dissolved in 300 μl of toluene and 5 μl injected).

Application

Plasma samples from a healthy human male volunteer (75 kg; 45 years) were analyzed following the ingestion of 50 mg of hydralazine (as a tablet of Apresoline). Blood samples were collected at intervals of 0, 0.5, 1, 2, 3, 4, 6, 8, 10 and 24 h after ingestion of the dose, and immediately after collection, the blood samples were mixed with heparin and centrifuged; 0.5-ml aliquots of the plasma were immediately processed through the nitrite reaction and extraction. The final benzene extract was kept at −20° until the following day, when the samples were chromatographed (see Table I and Fig. 3).

TABLE I

CONCENTRATION OF UNCHANGED DRUG IN PLASMA AFTER A SINGLE ORAL DOSE OF HYDRALAZINE (50 mg) TO A HUMAN MALE (75 kg) AS DETERMINED BY THE PROPOSED METHOD

Time after application (h)	*Unchanged hydralazine (ng/ml)*
0.0	0.0
0.5	266
1	226
2	155
3	115
4	67
6	44
8	32
10	21
24	8
32	0.0

RESULTS AND DISCUSSION

Derivatization of hydralazine was optimized by studying the reaction conditions using [^{14}C]hydralazine. In water and plasma, yields of tetrazolophthalazine varied only slightly (85–95%) over a wide range of reaction conditions (sodium nitrite solution concentration ranging from 10 to 50%; time 5 to 60 min; hydrochloric acid

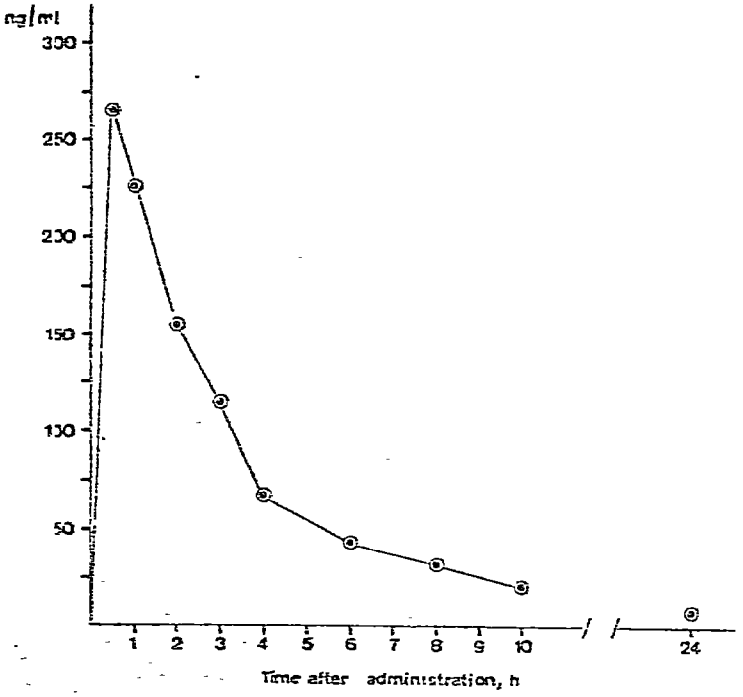

Fig. 3. Concentrations of unchanged hydralazine determined in plasma after a single oral dose of 50 mg to a human subject (male, 75 kg).

TABLE II

CALIBRATION GRAPH FOR COMPLETE PROCEDURE FOR DETERMINING HYDRALAZINE IN PLASMA

The 200-ng sample was dissolved in 900 μl of toluene, the 100-ng sample in 600 μl, and all samples below 100 ng in 300 μl; 5 μl of each solution were injected.

Hydralazine concentration in plasma (ng/ml)	*Value of H_x* for single determinations*	*Mean value of H_x**
12.5	0.25	0.25
	0.25	
	0.26	
25.0	0.38	0.38
	0.37	
	0.38	
50.0	0.70	0.68
	0.69	
	0.65	
100.0	1.33	1.36
	1.37	
	1.39	
200.0	2.52	2.50
	2.53	
	2.44	

* H_x is the ratio of the peak heights for tetrazolophthalazine and methyltetrazolophthalazine.

concentration 0.5 to 10 *N*). Yields in blood were generally lower (approx. 70%), and lower yields were observed when using dilute hydrochloric acid (< 2 *N*).

The derivative tetrazolophthalazine was found to be extractable with several organic solvents over a wide range of pH (2 to 14), but the cleanest extracts from a chromatographic viewpoint were obtained when the reaction mixture was extracted with benzene at pH 10 to 10.5.

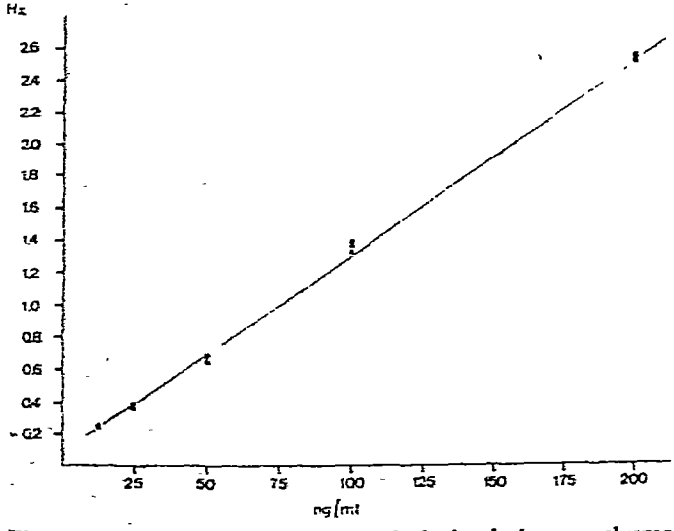

Fig. 4. Quantitative analysis for hydralazine in human plasma: calibration curve for complete analytical procedure. H_x is as defined in Table II, and the volumes of solutions and injections are as in Table II.

Owing to the instability of unchanged hydralazine in the biological material, the samples must be derivatized and extracted immediately after collection. The tetrazolophthalazines of both hydralazine and the internal standard are reasonably stable, but care must be taken when evaporating the final benzene extract; losses occur when the samples are left under the stream of nitrogen at 45° longer than is necessary. The samples redissolved in toluene for gas chromatography may be stored for about 48 h at −20°. After 4 days, losses up to 7.5% were observed in samples containing 12.5 to 200 ng/ml.

A linear relationship was observed between the peak-area ratios of tetrazolophthalazine and the internal standard 4-methyltetrazolophthalazine and the hydralazine present in the biological sample in the range of 10 to 200 ng/ml (see Table II). The calibration graph is presented in Fig. 4. The reproducibility was good; calibration graphs prepared at later dates proved identical.

The accuracy and precision were tested by analyzing samples containing amounts of hydralazine unknown to the analyst (Table III); both were satisfactory in the range of 12 to 160 ng per ml of plasma. Plasma samples from a healthy volunteer could be monitored for 24 h after ingestion of 50 mg of hydralazine (see Fig. 3).

TABLE III

ANALYSIS FOR HYDRALAZINE IN SPIKED PLASMA SAMPLES

Plasma samples containing amounts of hydralazine unknown to the analyst were analyzed by three independent determinations; each solution was injected once.

Hydralazine present (ng/ml)	*Hydralazine found (ng/ml)*			*Error, as percentage of theory*
	$\bar{x}$ ($n = 3$)	$s(x)$	*Coeff. of variation (%)*	
12.5	14.5	0.85	5.86	+ 13.8
20.0	23.4	1.27	5.42	+ 14.5
40.0	39.3	0.85	2.16	− 1.75
80.0	80.1	0.52	0.65	0.0
160.0	152.4	3.37	2.21	− 4.75

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REFERENCES

1 H. A. Schroeder, J. D. Morrow and H. M. Perry, Jr., *Circulation*, 8 (1953) 672.
2 E. S. Orgain, C. A. Munroe and G. L. Donnelly, *J. Amer. Med. Ass.*, 166 (1958) 2103.
3 P. Blaquier and S. W. Hoobler, *Amer. J. Med. Sci.*, 238 (1959) 740.
4 A. R. Schulert, *Arch. Int. Pharmacodyn.*, 132 (1961) 1.
5 J. M. Lesser, Z. H. Israili, E. Davis and P. G. Dayton, *Clin. Pharmacol. Ther.*, 14 (1973) 140.
6 S. B. Zak, M. F. Bartlett, W. E. Wagner, T. G. Gilleran and G. Lukas, *J. Pharm. Sci.*, 63 (1974) 225.
7 J. Druey and B. H. Ringier, *Helv. Chim. Acta*, 34 (1951) 195.