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DETERMINATION OF HYDRALAZINE IN TABLETS BY GAS CHROMATOGRAPHY

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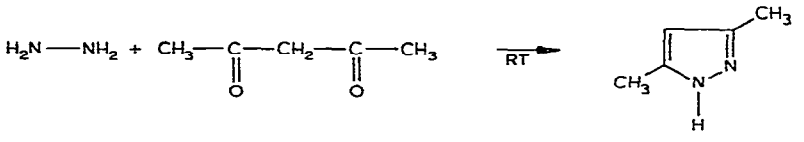
SUMMARY

A description is given of a gas chromatographic method for the determination of hydralazine in various tablet formulations based on the quantitative reaction of hydralazine with 2,4-pentanedione to yield 1-(3,5-dimethylpyrazole)phthalazine. The stable hydralazine derivative formed is extracted from aqueous solution and chromatographed resulting in a precise and specific determination of hydralazine which correlates well with the U.S.P. XIX titrimetric procedure.

INTRODUCTION

Methods for determining hydralazine (1-hydrazinophthalazine), an antihypertensive drug, have utilized ion-exchange chromatography[1,2], potentiometry[3,4], high-pressure liquid chromatography[5], fluorescence[6], polarography[7], titrimetry[8–11], and gasometry[12–14]. Spectrophotometric methods[15,16] for assaying hydralazine have also been developed, as have colorimetric methods based on reactions of hydralazine with various reagents such as tetrazolium chloride[17,18], 4-nitrobenzaldehyde[19], 4-methoxybenzaldehyde[20], 4-hydroxybenzaldehyde[21–23], cinnamaldehyde[24,25], 4-dimethylaminobenzaldehyde[26] and ninhydrin[27–29]. In addition a gas chromatographic (GC) method has been developed for determining hydralazine in which hydralazine was treated with aqueous sodium nitrite and the product, tetrazolo[5,1-*a*]phthalazine, was chromatographed[30].

Hydrazine (I) has been shown to react with 2,4-pentanedione (II) yielding 3,5-dimethylpyrazole (III)[31]:



I II III

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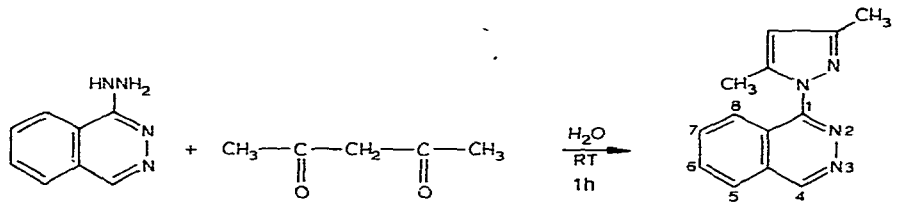
The reaction with 2,4-pentanedione was quantitative and represented a reproducible, sensitive GC method for determining hydrazine content in aqueous solution. Hydralazine (IV), which contains a hydrazino functional group, was found to undergo an analogous reaction yielding 1-(3,5-dimethylpyrazole)phthalazine (V):



IV II V

This paper presents a novel GC procedure for the determination of hydralazine in various tablet formulations based on the reaction of hydralazine with 2,4-pentanedione. The hydralazine derivative is extracted from aqueous solution, in the presence of tablet excipients, and chromatographed resulting in a precise and specific determination of hydralazine. Data are presented which show that the method is stability indicating and that it correlates well with the U.S.P. XIX titrimetric method[32].

EXPERIMENTAL

Reagents and standards

2,4-Pentanedione (Aldrich) was used without further purification. Hydralazine hydrochloride was an in house reference standard assayed at 100.0% by titrimetric[32] and UV methods.

1-(3,5-Dimethylpyrazole)phthalazine·HCl reference standard was prepared by gently warming hydralazine hydrochloride (10 g) in a solution of 100 ml methanol and 10 ml of 2,4-pentanedione. The solution was taken to dryness and the residue was dissolved in 150 ml of boiling isopropyl alcohol. Hexane (500 ml) was added slowly to the hot, filtered solution. The resulting crude product was recrystallized from a boiling mixture of 500 ml of ethyl acetate, 100 ml of isopropyl alcohol and 100 ml of chloroform yielding 4.5 g of the monohydrochloride salt [m.p. 181–183° (dec.); NMR (dimethylsulfoxide-d_6, $CDCl_3$):2.50(6H, d, CH_3–), 6.30(1H, s, C_4), 8.27–8.53 and 8.77–9.13(4H, multiplets, C_{5-8}), 10.68(1H, s, pyrazole-H), and 13.6(1H, s, HCl) ppm].

Sample preparation

The average tablet weight of ten tablets was obtained and the tablets were ground to a fine powder. A powdered amount equivalent to the tablet potency was weighed out and transferred quantitatively to a 35-ml screw cap centrifuge tube. An appropriate amount of water was added to the tube resulting in a hydralazine concentration of 2 mg/ml. The solution was sonified for 5 min and shaken for 30 min, then allowed to stand at room temperature for 10 min. A 10-ml aliquot of the suspension was removed and added to a 35-ml centrifuge tube containing 1.0 ml of 2,4-pentanedione. The resulting mixture was shaken for 30 min and left to react at room temper-

ature for an additional 30 min. 10.0 ml of internal standard solution (0.6 mg phenanthrene per ml of ethyl acetate) was then added and the tube was placed on a mechanical shaker for 10 min to extract the resulting hydralazine derivative. Approximately 1 μl of the ethyl acetate layer was injected without further treatment.

Standard preparation

Three reference standard solutions were prepared by weighing out 10 mg, 20 mg and 30 mg of hydralazine hydrochloride reference standard and transferring quantitatively to separate 35-ml screw cap centrifuge tubes. Water (10.0 ml) and 1.0 ml of 2,4-pentanedione were added to each tube and the resulting mixtures were shaken mechanically for 30 min and left to react at room temperature for an additional 30 min. The internal standard addition and extraction of the derivatized standards was identical to the extraction procedure for the samples.

Peak area ratios for the standards were calculated from individual areas measured by an AutoLab System 4 Chromatographic Data Analyzer and a calibration curve was constructed. The concentrations of hydralazine in the sample solutions were calculated from the standard curve.

GC–mass spectrometry of 1-(3,5-dimethylpyrazole)phthalazine

For characterization and identification purposes a mass spectrum of 1-(3,5-dimethylpyrazole)phthalazine was obtained via a gas chromatograph–mass spectrometer (Fig. 1). The spectrum supported the proposed structure (V), displaying a molecular ion at *m/e* 224 and a base peak at *m/e* 223 which was consistent with the pyrazole portion of the molecule losing a proton and rearranging to form a positively charged methyl substituted pyridazine ring[33].

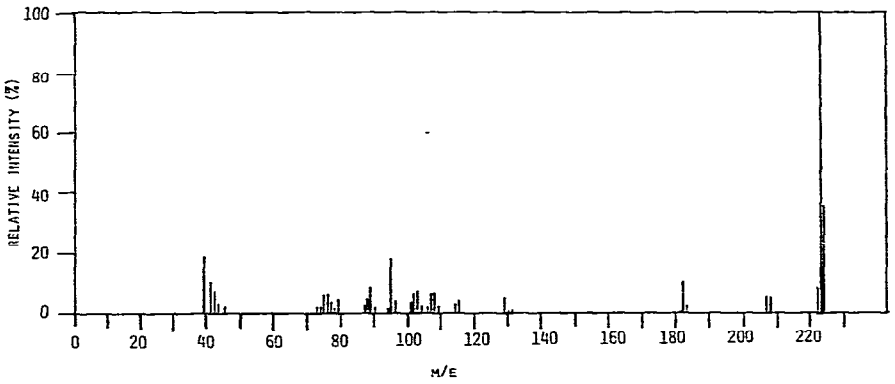


Fig. 1. Mass spectrum of 1-(3,5-dimethylpyrazole)phthalazine.

Gas chromatographic conditions

A Tracor MT-220 and a Varian 2100 gas chromatograph equipped with flame ionization detectors were used. The column was a 6 ft. × 4 mm I.D. U-shaped glass tube, packed with 10% SE-30 on 80–100 mesh Gas-Chrom Q. The temperature of the column oven was 200°C, the detector was at 250°C and the inlet at 210°C. The carrier gas was oxygen-free nitrogen at a flow-rate of 55 ml/min.

Fig. 2 shows a typical gas chromatogram illustrating the internal standard and 1-(3,5-dimethylpyrazole)phthalazine peaks corresponding to a 1-μl injection of the ethyl acetate extract which contained 0.6 mg/ml phenanthrene and 2.0 mg/ml hydralazine. A 3-ft. column as well as a 6-ft. column effected a satisfactory separation. However, response factors were not reproducible when the column support loading was less than 6%. Inlet temperatures were kept as close to the column oven temperature as possible to avoid any thermal decomposition of the product in the inlet.

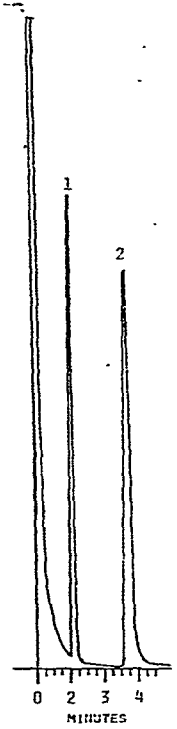


Fig. 2. Chromatogram of (1) internal standard and (2) 1-(3,5-dimethylpyrazole)phthalazine at a concentration of 2 mg/ml; sample size, 1.0 μl.

RESULTS AND DISCUSSION

The completeness of the reaction of 2,4-pentanedione and hydralazine was calculated on a percent yield basis by comparing the detector response for hydralazine carried through the method with that for a reference solution of 1-(3,5-dimethylpyrazole)phthalazine derivative. The following equation was used to show that the reaction went to 99% completion when carried out under the conditions previously described:

$$\% \text{ yield} = \frac{\text{Hyd}}{\text{IS}} \times \frac{\text{IS}_{\text{Ref.}}}{\text{Ref.}} \times \frac{W_{\text{Ref.}}}{W_{\text{Hyd}}} \times \frac{\text{MW}_{\text{Hyd}}}{\text{MW}_{\text{Ref.}}} \times 100$$

where Hyd = peak area of 1-(3,5-dimethylpyrazole)phthalazine associated with hydralazine sample, IS = peak area of internal standard associated with hydralazine

sample, $IS_{Ref.}$ = peak area of internal standard associated with 1-(3,5-dimethylpyrazole)phthalazine standard, Ref. = peak area of 1-(3,5-dimethylpyrazole)-phthalazine in standard, $W_{Ref.}$ = weight (mg) of 1-(3,5-dimethylpyrazole)phthalazine in standard, W_{Hyd} = weight (mg) of hydralazine in sample, MW_{Hyd} = molecular weight of hydralazine, $MW_{Ref.}$ = molecular weight of derivative.

Reaction mixtures of hydralazine and 2,4-pentanedione were shaken for varying amounts of time (5–180 min) at room temperature. After 30 min there was no observable increase in the peak area ratio of derivatized hydralazine to internal standard indicating that the reaction had achieved completion. A further indication that the reaction and extraction procedures were quantitative was observed when two placebos were spiked with a known amount of hydralazine and carried through the method. The percent recovery of the spiked material was 98.1 and 98.5. In addition to the reaction being facile, the product was stable for at least 24 h at room temperature when the organic layer was left in contact with the aqueous portion of the reaction mixture.

Various formulations and tablet strengths of hydralazine, subjected to stress conditions, were assayed by the U.S.P. XIX titrimetric method[32] and the described GC method using 2,4-pentanedione. A comparison of the results shows very close agreement between the two conceptually different methods with the values obtained by the GC method for hydralazine being approximately equal to or slightly less than the values obtained by the U.S.P. method (Table I). Since the GC method is considered stability indicating, perhaps some difference in assay values can be attributed to increased specificity by GC.

TABLE I

COMPARISON OF RESULTS OF U.S.P. XIX TITRIMETRIC METHOD[32] AND GC METHOD FOR DETERMINATION OF HYDRALAZINE

RH = relative humidity.

Sample	*Formulation*	*Storage conditions*	*Hydralazine (mg per tablet)*		
			Claim	*GC method*	*U.S.P. method*
1	A	Initial	50	47.7	47.4
2	A	6 weeks at 51°C	50	46.5	46.5
3	A	Initial	50	48.3	47.9
4	A	6 weeks at 51°C	50	47.1	47.1
5	B	Initial	25	25.3	25.9
6	C	Initial	50	48.9	51.3
7	C	80°F, 50% RH for 24 h	50	49.4	51.5
8	D	Initial	50	49.5	49.3
9	D	3 weeks at 4°C	50	49.3	48.7
10	D	4 weeks at 51°C	50	48.5	48.7
11	D	80°F, 50% RH for 24 h; 4 weeks at 51°C	50	45.0	45.0
12	E	Initial	100	102.3	102.0

Decomposed hydralazine tablets were also assayed by GC and titrimetry. Two samples of hydralazine tablets were stored at an elevated temperature for three weeks. At the end of this time, both sets of tablets were visibly discolored. When the titrimetric method was used for assaying one of these samples the chloroform layer

acquired a dark purple color obscuring the color change which needed to be observed and the sample could not be assayed. The other sample was less highly colored and a titration value was obtained (Table II). Satisfactory results for both samples were achieved by the GC method. A blank was run for the two decomposed sets of tablets by following the usual sample work-up, except no 2,4-pentanedione was added. Two injections, one isothermal, the other temperature programmed, were used to determine that no interferences from decomposition products or excipients were causing a bias in the results.

TABLE II

COMPARISON OF ASSAY RESULTS FOR DECOMPOSED HYDRALAZINE TABLETS USING GC, U.S.P.[32] AND UV[20] METHODS

— = U.S.P. method could not detect color change in chloroform layer.

Sample	*Hydralazine (mg per tablet)*			
	Claim	*GC method*	*U.S.P. XIX method*	*UV method*
1	50	36.0	40.2	37.8
2	50	13.2	—	17.1

In addition, good precision was obtained with the GC method when one sample was assayed in duplicate on four different days (Table III). An estimation of the assay variance between duplicates determined on the same day was calculated to be $s_1^2 = 0.12$ and the variance for the sample assayed on consecutive days was calculated to be $s_2^2 = 0.94$.

TABLE III

ASSAY VARIANCE BETWEEN DUPLICATES (s_1^2) AND VARIANCE FOR THE SAMPLE ASSAYED ON CONSECUTIVE DAYS (s_2^2)

$$s_1^2 = \frac{(\Sigma Y_1)^2 + (\Sigma Y_2)^2}{4} - \frac{(\Sigma Y_1 + \Sigma Y_2)^2}{8}; \quad s_2^2 = \Sigma \frac{(Y_1 + Y_2)^2}{2} - \frac{(\Sigma Y_1 + \Sigma Y_2)^2}{8}.$$

Day	*Duplicates*	
	Y_1 *(mg per tablet)*	Y_2 *(mg per tablet)*
1	48.74	48.92
2	49.69	49.29
3	48.61	48.62
4	49.29	49.21

The possibility of using 1-(3,5-dimethylpyrazole)phthalazine with electron capture detection for determining plasma levels of hydralazine is currently being investigated.

Also, early indications suggest that other compounds containing hydrazine functional groups, such as hydrazides, will undergo cyclization when treated with 2,4-pentanedione.

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