ride (0.1 μl). When this inlet system was used with the C_{18} *n*-alkane in C_{12} *n*-alkane (5 μl), N_{eff} = 40,000 was obtained. However, GLC of cannabinoids in the higher alkanes produced very poor resolution with considerable peak tailing. Alternative solvents were tested (*e.g.*, *n*-alkanes up to C_{20}, branched chain alkanes up to C_{10}, higher boiling alkenes, alcohols, and ketones), and only fatty acid esters provided acceptable resolution. The best was methyl laurate, which yielded N_{eff} = 25,000 (Fig. 2).

The temperature of the inlet port is critical for good resolution. If the temperature is too low—by as little as 10–15°—no recognizable chromatogram will be achieved. Operating conditions for the cannabinoids in methyl laurate were: oven temperature, 230°; and inlet temperature, 250°.

The N_{eff} for cannabinoids is only 63% that achieved for alkanes. This reduction in efficiency apparently is due, at least in part, to support effects (11); the possibility exists that even more efficient separations of cannabinoids can be achieved with a different stationary phase in combination with another solvent. Different stationary phases may, of course, also provide more convenient elution orders to suit particular problems.

The main point to be noted is that the described device now permits the application of capillary columns (with its attendant 10-fold plus improvement in resolution over conventional columns) to cannabinoid analyses; this system should be of considerable use for biological as well as plant extracts. Other stationary phases and solvents possibly will be applicable to GLC of derivatized cannabinoids. This variety would allow further improvements in the sensitivity of detection of cannabinoids when utilizing electron-capture (12) and flame-ionization (13) detectors.

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Dihydralazine Sulfate Analysis Using 2-Methyl-3-nitropyridine-6-carboxaldehyde

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Abstract □ A sensitive, selective colorimetric assay was developed for the quantitative analysis of dihydralazine sulfate. The method is based on the interaction of buffered (pH 4) dihydralazine sulfate with a methanolic solution of 2-methyl-3-nitropyridine-6-carboxaldehyde upon heating to give an orange color. This color can be quantified spectrophotometrically at 450 nm, with a lower limit of detection of 1 μg/ml. The color is stable for at least 24 hr. There is no interference from other drugs likely to be present along with dihydralazine sulfate and common excipients. The method was used successfully for the determination of dihydralazine sulfate in combination with other drugs in different commercial tablets. The developed method was applicable as a stability-indicating assay.

Keyphrases □ Dihydralazine sulfate—colorimetric analysis using 2-methyl-3-nitropyridine-6-carboxaldehyde, prepared samples □ Colorimetry—analysis, dihydralazine sulfate in prepared samples □ 2-Methyl-3-nitropyridine-6-carboxaldehyde—color reagent in analysis of dihydralazine sulfate in prepared samples □ Antihypertensive agents—dihydralazine sulfate, colorimetric analysis in prepared samples

Various methods for the analysis of dihydralazine sulfate (1,4-dihydrazinophthalazine) have been reported (1–11). The bromometric assay for pure dihydralazine sulfate used potassium dichromate (1) or potassium permanganate (2) as the titrant in the presence of potassium bromide. The dihydralazinebenzoic acid–bivalent metal complexes were estimated by titration with edetic acid (3, 4); the dihydralazine–copper thiocyanate complex was estimated by titration with silver nitrate (5).

The reported argentimetric procedures depend on either the estimation of excess silver nitrate added to the buffered solution of dihydralazine at pH 5–6 (6) or stoichiometric determination of metallic silver separated after the addition of ammoniacal silver nitrate (7). Direct potentiometric titration of pure dihydralazine sulfate and other hydrazine derivatives, using chloramine-T in an acidic medium, was reported (8). A coulometric method for dihydralazine, using electrolytically generated bromine, also was reported (9).

Colorimetric methods were developed for the estimation of dihydralazine sulfate in the pure form and in tablets, depending on the formation of a chromogenic product with ammonium molybdate (6) and ferric ion (10); the lower limits of detection were 70 and 150 μg, respectively. Also, a colorimetric method was reported

Table I—Effect of pH on the Intensity of the Developed Color

pH	McIlvaine's Citric Acid–Phosphate Buffer, A^a	Acetic Acid–Sodium Acetate Buffer, A	Phosphate Buffer, A
2.20	0.410	—	—
3.00	0.680	—	—
3.45	—	0.075	—
3.60	0.680	—	—
3.72	—	0.670	—
3.80	0.690	—	—
4.00	0.780	—	—
4.05	—	0.760	—
4.20	0.720	—	—
4.27	—	0.710	—
4.40	0.710	—	—
4.45	—	0.680	—
4.99	—	0.660	—
5.00	0.660	—	—
6.00	Turbidity	—	0.380
7.00	Turbidity	—	0.270
8.00	Turbidity	—	0.160

$^a A$ = absorbance of solution containing 10 μg of dihydralazine sulfate/ml.

for the analysis of dihydralazine sulfate using 4,4′-dimethoxydiquinone as the chromogen-developing reagent.

It has been observed in these laboratories that 2-methyl-3-nitropyridine-6-carboxaldehyde interacts with hydrazine derivatives to form chromogenic products. The reaction can be quantified to be used as a simple analytical tool for selective estimation of hydrazine derivatives. This report describes the use of 2-methyl-3-nitropyridine-6-carboxaldehyde as a chromogenic reagent for sensitive and selective estimation of dihydralazine sulfate in the pure form and in combination with other drugs in commercial pharmaceutical formulations. The method also was investigated as a stability-indicating assay.

EXPERIMENTAL

Instrumentation—Spectra and absorbance measurements were made[1] using matched glass cells with 1-cm optical path.

Reagents and Chemicals—The color reagent (2-methyl-3-nitropyridine-6-carboxaldehyde) was prepared according to Banas and Skrowaczewska (12), bp_4 115°. Dihydralazine sulfate[2], hydrochlorothiazide, reserpine, glucose, sucrose, lactose, maize starch, gum acacia, and magnesium stearate were pharmaceutical grade and used as supplied. All solvents and other reagents were reagent grade.

Standard Solution of Dihydralazine Sulfate—Weigh accurately 10 mg of dihydralazine sulfate, transfer to a 10-ml volumetric flask, and dilute to volume with McIlvaine's citric acid–phosphate buffer (pH 4) (13).

Color Reagent—Weigh accurately 25 mg of 2-methyl-3-nitropyridine-6-carboxaldehyde, transfer to a 50-ml volumetric flask, and dissolve in, and dilute to volume with, methanol.

Preparation of Assay Solutions—*For Pure Dihydralazine Sulfate*—Use appropriate volumes of standard dihydralazine sulfate as the assay solution.

For Synthetic Mixture—Transfer an accurately weighed amount of the powdered synthetic mixture, equivalent to 1 mg of dihydralazine sulfate, to a 10-ml volumetric flask. Dissolve as completely as possible in, and dilute to volume with, McIlvaine's citric acid–phosphate buffer (pH 4). Filter and discard the first portion of the filtrate. The clear solution obtained is the assay solution.

For Tablets Containing Dihydralazine Sulfate—Weigh and powder 20 tablets. Transfer an accurately weighed quantity of the powder, equivalent to 10 mg of dihydralazine sulfate, to a 10-ml volumetric flask and proceed as for the synthetic mixture, starting from: "dissolve as completely . . . . "

Recovery Experiment—Add an accurately weighed amount of dihydralazine sulfate to an accurately weighed amount of the powdered tablets, equivalent to a known amount of dihydralazine sulfate, in a 10-ml volumetric flask and proceed as for the synthetic mixture, starting from: "dissolve as completely . . . . "

Development of Color—Into 10-ml volumetric flasks, each containing 1 ml of the color reagent, pipet 1 ml of the assay solution of appropriate dilution. Then heat on a boiling water bath for 5 min and cool. Dilute to volume with methanol and mix well. Determine the absorbance at 450 nm against the blank.

Application of Developed Method as Stability-Indicating Assay—Prepare a 0.1% solution of dihydralazine sulfate in McIlvaine's citric acid–phosphate buffer (pH 8). Titrate an aliquot of this solution with potassium iodate, using the NF XIII method (14). For the colorimetric assay, measure accurately suitable volumes of the prepared solution, make the appropriate dilution with McIlvaine's citric acid–phosphate buffer (pH 4), and carry out the assay as already described. Repeat the analysis by both methods after heating aliquots of the prepared solution on a boiling water bath for 15, 30, and 60 min.

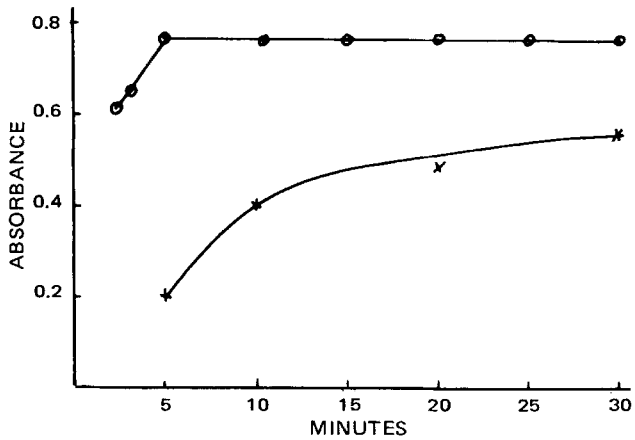

Figure 1—*Effect of the reaction time on the intensity of color. Key:* O, *at the temperature of the boiling water bath; and* ×, *at room temperature.*

RESULTS AND DISCUSSION

The interaction between hydrazino derivatives and aldehydes to yield the respective hydrazones is well known and reported to be acid or base catalyzed (15). Initial trials to react a methanolic solution of 2-methyl-3-nitropyridine-6-carboxaldehyde with dihydralazine sulfate in the presence of trichloroacetic acid failed to yield the chromogenic hydrazone, even upon heating in a boiling water bath for 1 hr.

Accordingly, a rigorous investigation was carried out to develop suitable conditions for color development. Different buffer systems of various constitution and pH were investigated.

When phosphate buffer was used (pH 6–8) (16), an orange-colored product developed with maximum absorption at 440 nm. The extinction of the colored product increased with a decrease in pH (Table I). By using McIlvaine's citric acid–phosphate buffer (pH 2.2–8), the intensity of the color production reached its maximum value at pH 4 (Table I) with an absorption peak at 450 nm and a broad absorption band extending from below 400 to 550 nm. The use of acetic acid–sodium acetate buffer (pH 4.05) (17) instead of McIlvaine's citric acid–phosphate buffer did not significantly affect color intensity or the λ_{max}. However, the McIlvaine's citric acid–phosphate buffer was tentatively chosen as the reaction medium because of the appreciable reduction in color development time compared with the acetate buffer.

Investigations were carried out to determine the solvent and reagent concentration that would give the highest color intensity. Solvents tested include methanol and ethanol. At reagent concentrations greater than 0.4 mg/ml, the intensity of color was only slightly de-

[1] Spekol spectrocolorimeter, Carl Zeiss Jena.
[2] Nepresol, Ciba.

Table II—Determination of Dihydralazine Sulfate in Presence of Reserpine, Hydrochlorothiazide, and Common Excipients

Substances Added[a]	Amount, mg	Recovery[b], %	SD
Reserpine	0.5	97.9	0.85
Hydrochlorothiazide	2.0	99.8	0.50
Glucose	20.0	95.6	0.37
Starch	20.0	100.6	0.58
Sucrose	20.0	99.9	0.45
Lactose	200.0	100.02	0.050
Magnesium stearate	20.0	97.1	0.057
Acacia	20.0	99.6	0.75
Mixture of all ingredients	—	99.1	1.20

[a] Per milligram of dihydralazine sulfate. [b] Average of four determinations.

pendent upon the reagent concentration. A concentration of 0.5 mg/ml was selected.

The reaction time was determined by following the color development at room temperature and at the temperature of a boiling water bath (Fig. 1). The results indicated that the 30-min interval was not sufficient to bring the color to maximum intensity. Under the same pH, a heating time interval of only 5 min was required to obtain the maximum color intensity, which remained stable for at least 24 hr. This asset is important for a quality control method and for a series of determinations. The color reaction product showed a broad absorption peak with a maximum at about 450 nm and an apparent molar absorptivity of 37.3×10^3.

Beer's law was obeyed ($r = 0.9997$) up to an initial dihydralazine sulfate concentration of 14 μg/ml ($A \approx 1.07$). A typical linear regression line had a slope of 0.078, an intercept of −0.028, and SS of 7.25×10^{-4}.

The specificity of the method for the determination of dihydralazine sulfate in the presence of several frequently encountered excipients, additives, and certain drugs, such as reserpine and hydrochlorothiazide, was investigated. A synthetic mixture was prepared by mixing a known amount of dihydralazine sulfate with an excipient or drug, each approximating sample weights of commercial tablets, and the assay was performed. The average recovery ranged from 97 to 100% (Table II). The relatively low percentage recovery obtained when glucose was the additive may be due to the partial reaction of dihydralazine with nonchromogenic aldose.

Different marketed dihydralazine tablets were satisfactorily analyzed by this method (Table III).

The preliminary stability study showed that solutions of dihydralazine in distilled water and in McIlvaine's citric acid–phosphate buffer below pH 7 were stable at room temperature for at least 48 hr, as shown by the constant readings of samples analyzed by the developed method and by the reference iodate method (14). Upon heating in a boiling water bath for 15 min, the color intensity decrease was not significant. However, a solution of dihydralazine in McIlvaine's citric acid–phosphate buffer (pH 8) acquired rapidly a yellow coloration, which was intensified by heating.

Table III—Determination of Dihydralazine Sulfate in Commercial Tablet Formulations

Formula[a]	Amount Claimed, mg	Amount Found[b], %	SD	Amount Added, mg	Recovery[b], %	SD
A	10	107	0.00	10	99.3	1.15
B	10	97.6	1.03	10	99.6	1.10

[a] A = reserpine, 0.1 mg; dihydralazine sulfate, 10 mg; hydrochlorothiazide, 10 mg; and excipients. B = reserpine, 0.1 mg; dihydralazine sulfate, 10 mg; and excipients. [b] Average of three determinations.

Table IV—Effect of Time on the Stability of Dihydralazine Sulfate Solution at pH 8

Minutes	Recovery[a], % Iodometric Method	Proposed Method
0	100.0	100.0
15	90.0	85.0
30	89.0	78.7
60	87.1	75.0

[a] Average of two determinations.

The degradation of dihydralazine sulfate at the temperature of the boiling water bath was followed by the colorimetric and iodometric methods at 15, 30, and 60 min. The rather low selectivity of the iodometric method was shown by the pattern of higher results obtained compared to the proposed method (Table IV). Any degradation product susceptible to oxidation by potassium iodate would probably interfere in the iodometric method, but the proposed method allows interaction only with the intact hydrazine moiety. Consequently, the proposed colorimetric method is more selective and can be recommended as a stability-indicating assay.

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