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Spectrophotometric method for determination of midodrine hydrochloride using ninhydrin in pharmaceutical formulations

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ABSTRACT

A simple and fast spectrophotometric method has been developed for the determination of midodrine (MD) hydrochloride in pure form and in pharmaceutical formulations. The method is based on the reaction of the primary amino group of the drug with ninhydrin in methanol producing a Ruhemann purple coloured complex which absorbs maximally at 576.5 nm. The effects of variables such as reagent concentration, reaction time, reaction temperature, and Ruhemann purple color stability period, were investigated to optimize the procedure. The proposed method has been applied successfully to the analysis of the bulk drug and its pharmaceutical tablets and drops. No interference was observed from common pharmaceutical adjuvants. © 2008 Trade Science Inc. - INDIA

KEYWORDS

Midodrine hydrochloride;
Spectrophotometry;
Ninhydrin.

INTRODUCTION

Midodrine (MD) hydrochloride, 2-amino-N-(β -hydroxy-2,5-dimethoxyphenethyl)acetamide hydrochloride, is a direct-acting sympathomimetic with selective α -agonist activity. It acts as a peripheral vasoconstrictor so, it used in the treatment of hypotensive states particularly orthostatic hypotension^[1].

A review of literature revealed that several HPLC methods have been described for the determination of MD in biological fluids^[2-5], chiral chromatography^[6] and gas chromatography-mass spectrometry^[7]. These methods have enough sensitivity to determine the drug and hence, it is justified to use them for assay of MD in biological fluids. However, it is always required to develop analytical methods using low cost techniques. UV-Vis spectrophotometry is still considered a convenient

and economical technique for routine analysis of the drug in pharmaceutical formulations.

MD is a drug which having relatively low ultra violet absorbance. Using of derivatization technique and formation of coloured product with high absorbance, in turn increase method sensitivity. In addition, the formation of coloured derivative has the advantage of bathochromic shift to the longer wavelength of the visible region; this helps to get rid of the interfering noise and excipient effect in the shorter wavelength.

The present paper describes a rapid, simple and sensitive visible spectrophotometric method for the determination of MD. The determination is based on the reaction of the primary amino group of the MD with ninhydrin in methanol and producing a Ruhemann purple color. Furthermore, factors that influence the reaction process were identified and optimized. Also, the colo-

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rimetric method was validated.

EXPERIMENTAL

Instrumentation

A double-beam Shimadzu UV-VIS spectrophotometer, model UV-1601 PC, equipped with 1 cm quartz cells and connected to an IBM-compatible computer HP 600 inkjet printer were used. The bundled software was UVPC personal spectroscopy software Version 3.7 (Shimadzu). The spectral bandwidth was 2 nm, and the wavelength scanning speed was 2800 nm/min.

Materials and reagents

Pharmaceutical grade of MD hydrochloride was used and certified to contain 99.8%. Methanol HPLC grade (BDH, Poole, UK) was used. Sodium hydroxide (Sigma-Aldrich, Inc, St. Louis, USA) and ninhydrin (Sigma-Aldrich, Inc, St. Louis, USA) were used.

The pharmaceutical formulations of MD {Midodrine® tablets and drops batch no. 05263 and 16286 respectively} used were manufactured by The Nile company for pharmaceuticals and chemical industries (Cairo, Egypt). Each tablet was labeled to contain 2.5 mg of MD. The drops were labeled to contain 1% of MD. Also, {gutron® Tablets and drops batch no. L 610906 and L 530706 respectively} used were manufactured by October Pharma (6 of October city, Egypt). Each tablet was labeled to contain 2.5 mg of MD. The drops were labeled to contain 1% of MD.

Standard solutions and calibration graph

The drug base prepared using an accurately weighed amount of the drug hydrochloride salt equivalent to 50 mg base of MD, dissolved in a 100 ml of distilled water and render alkaline to reach pH 10.0 with 2 M sodium hydroxide solution. The liberated base of MD was extracted 3 times, each with 20 ml chloroform. The collected chloroformic extract was evaporated using rotary evaporator. The free base was then dissolved in 100 ml methanol.

The diluted standard solutions were prepared by appropriate dilutions with methanol to reach concentration range of 3-20 µg ml⁻¹ of MD. One ml of diluted standard solutions was pipetted into a series of boiling

test tubes. To each test tube 3 ml of 6 mM ninhydrin solution (freshly prepared in methanol daily) was added, mixed well and heated on a thermostatic oven at 100 ± 1 °C for 30 min. After heating the solution, the tubes were cooled to room temperature. The content of each tube was transferred to a 10 ml volumetric flask and diluted to volume with methanol. The absorbance was measured at 576.5 nm against reagent blank treated similarly. The absorbance values were plotted against corresponding concentrations. Linear relationship was obtained.

Sample preparation

1. For tablets

Forty tablets were weighed and finely powdered. A portion of the powder equivalent to about 50 mg of MD base was weighed accurately, dissolved in 100 ml of distilled water and render alkaline to reach pH 10.0 with 2 M sodium hydroxide solution. The powder was stirred for 30 min., then the procedures for the extraction of MD base, and reaction with ninhydrin described above was followed and the concentration was calculated.

2. For drops

A volume of MD drops, equivalent to about 50 mg of drug base was measured and render alkaline with 2 M sodium hydroxide solution. The same procedure done for tablets are followed for MD drops and concentration of MD was calculated using regression equation.

RESULTS AND DISCUSSION

MD exhibits relatively low UV absorption (Figure 1a) as a consequence; poor sensitivity will be achieved by conventional UV spectrophotometric methods. MD contains a primary aliphatic amino group, which is known to react with many color reagents.

Ninhydrin reagent is used for the determination of an aliphatic primary amine or an amino acid group^[8]. The reaction is usually carried out by heating for a short time in an organic solvent (2-propanol, butanol, methanol, DMF^[9]). The reaction product is measured between 550 and 580 nm depending on the reaction condition^[10]. MD reacts with ninhydrin reagent in methanolic

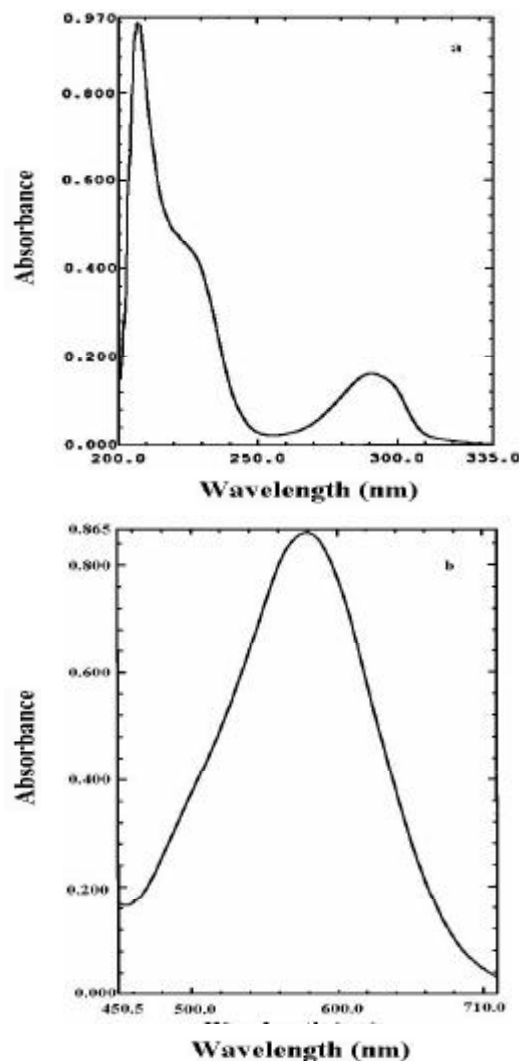


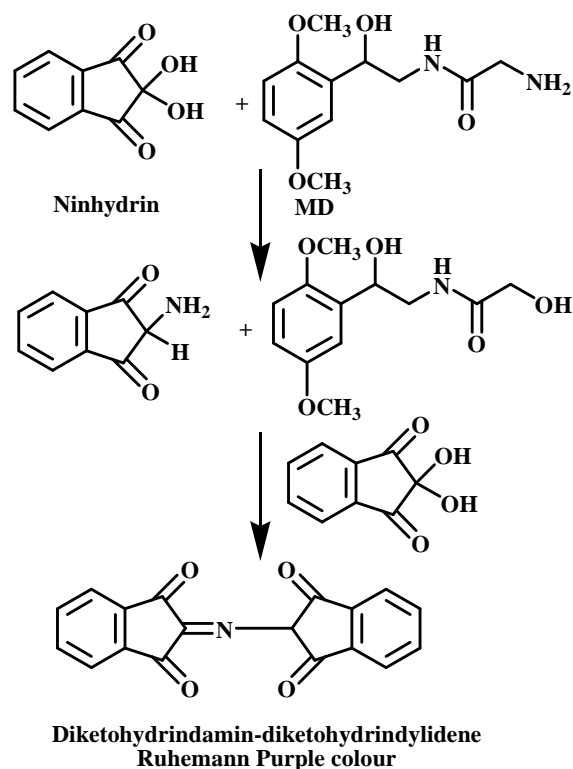
Figure 1: Absorption spectra for (a) 10 µg ml⁻¹ MD and (b) 10 µg ml⁻¹ MD after reaction with 3 ml of 6 mM ninhydrin in methanol

medium through oxidative deamination of the primary amino group followed by the condensation of the reduced ninhydrin to form the /Ruhemenn purple colored reaction product (diketohydrindylidene-diketohydrind amine) with maximum absorbance at 576.5 nm (Figure 1b, and SCHEME 1).

The different experimental parameters influencing the intensity of the developed color were extensively investigated to determine the optimal conditions for the assay procedure. All conditions studied were optimized in methanol via a number of preliminary experiments.

Effect of ninhydrin concentration

To a series of one ml methanolic solution containing 10 µg MD base, different volumes (0.5-5 ml) of 6 mM



SCHEME 1: The suggested reaction pathway between MD and ninhydrin in methanol

ninhydrin were added. The reaction mixtures were heated for 30 min on a thermostatic oven at $100 \pm 1^\circ\text{C}$. The colored product was diluted to 10 ml with methanol and the absorbance was measured against a reagent blank at 576.5 nm. The results showed that the highest absorbance was obtained with 3 ml, which remained unaffected with higher amounts of ninhydrin (Figure 2a). A 3 ml of the reagent, therefore, was chosen as optimum volume for the determination.

Effect of heating time

To a series of one ml methanolic solution containing 10 µg MD base, was mixed with 3 ml of 6 mM ninhydrin solution. The reaction mixture was heated on a thermostatic oven at $100 \pm 1^\circ\text{C}$ for different time up to 120 min and the colour product was diluted to 10 ml with methanol. The intensity of the colour was reached to maximum after 30 min of heating and remained constant up to 2h. Hence, the absorbance was measured after 30 min of heating. The results are shown in figure 2b.

Effect of reaction temperature

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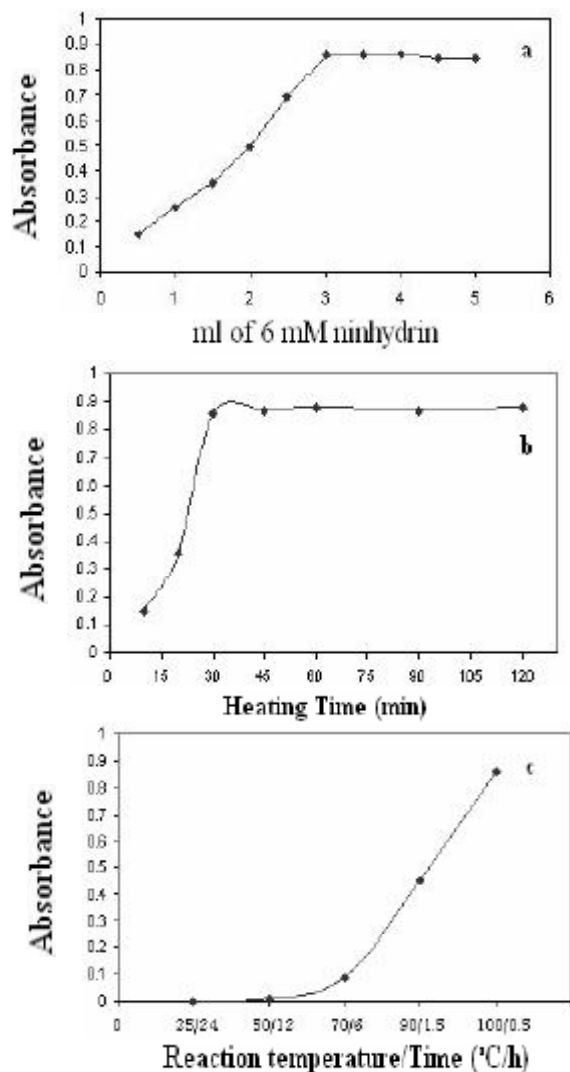


Figure 2: Effect of (a) ml of ninhydrin, (b) heating time, and (c) reaction temperature on the ninhydrin- MD reaction (MD conc. is $10\mu\text{g ml}^{-1}$)

It is known that the reaction temperature is very important. Majority of the reported work on ninhydrin reactions have been performed at elevated temperature. In this study, no color was formed at room temperature within 24h. Only slight purple color was observed after heating the samples at 50°C for 12h, and 70°C for 6 h. The intensity of the produced color increased at 90°C for 1.5h, as with the increase of reaction temperature, the time for the color formation was significantly reduced. At 100°C and exposure time of 30 min, dark Ruhemann purple color was rapidly formed as shown in figure 2c. It was found that, the reaction rate between ninhydrin and MD as primary amine is slower than that with amino acids^[9]. Therefore, in or-

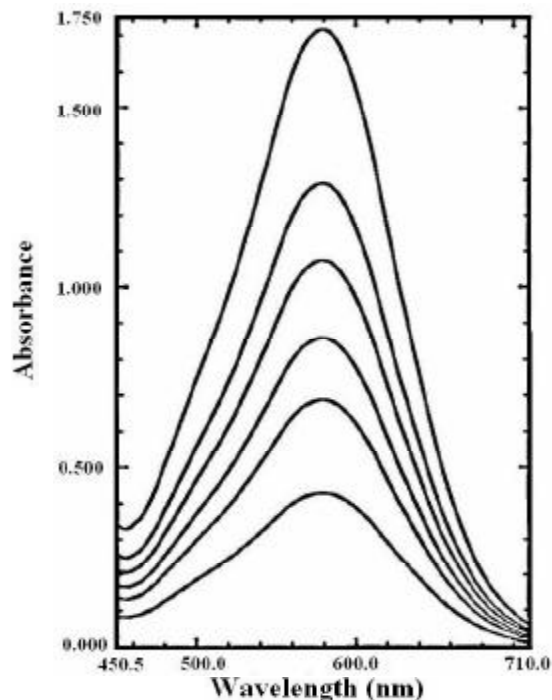


Figure 3: Absorption curves of the colored derivative resulted from ninhydrin-MD reaction for different concentrations of MD ($5\mu\text{g ml}^{-1}$ - $20\mu\text{g ml}^{-1}$)

der to assure completion of the reaction process, 100°C for 30 min was selected.

Validation

1. Linearity

The linearity of the proposed method was evaluated by analyzing seven concentrations of MD ranging between 3 - $20\mu\text{g ml}^{-1}$. Each concentration was repeated three times. The assay was performed according to experimental conditions previously established. The calibration graph was constructed by plotting absorbance of the reaction product at 576.5nm against corresponding concentrations of MD (Figure 3).

The regression plot showed a linear dependence of the absorbance over calibration range given in TABLE 1. The table also shows the results of the statistical analysis of the experimental data, such as the slopes, the intercepts, the correlation coefficients obtained by the linear least-squares treatment of the results.

The linearity of the calibration graph was validated by the high value of the correlation coefficient and the intercept value, which was not statistically ($p = 0.05$) different from zero (TABLE 1).

2. Precision

For evaluation of the precision estimates, repeatability and intermediate precision were performed at three concentration levels for MD. The data for each concentration level were evaluated by one-way ANOVA. A 8 days x 2 replicates design was performed. Statistical comparison of the results was performed using the P-value of the F-test. Three-univariate analyses of variance for each concentration level were made. Since the P-value of the F-test is always greater than 0.05, there is no statistically significant difference between the mean results obtained from one level of day to another at the 95 % confidence level (TABLE 2).

3. Range

The calibration range was established through consideration of the practical range necessary, according to MD concentration after administration of therapeutic dose of MD to give accurate, precise and linear results. The calibration range of the proposed method is given in TABLE 1.

4. Detection and quantitation limits

According to ICH recommendations^[12], the approach based on the S.D. of the response and the slope was used for determining the detection and quantitation limits. The theoretical values were assessed practically and given in TABLE 1.

5. Accuracy

The validity of the proposed method for the determination of drug in pharmaceutical formulations was tested by applying the standard addition technique.

This study was performed by addition of known amounts of the studied compound to a known concentration of the commercial pharmaceutical products. The

results obtained were reproducible with low relative standard deviations (0.37-0.72%) and the mean re-

TABLE 1: Characteristic parameters of the calibration equations for the proposed spectrophotometric method for the determination of MD

	MD
Calibration range ($\mu\text{g ml}^{-1}$)	3-20
Detection limit ($\mu\text{g ml}^{-1}$)	4.77×10^{-2}
Quantitation limit ($\mu\text{g ml}^{-1}$)	15.89×10^{-2}
Regression equation(Y) ^a :	
Slope (b)	8.47×10^{-2}
Standard deviation of the slope (S_b)	1.43×10^{-2}
Relative standard deviation of the slope (%)	1.69
Confidence limit of the slope ^b	$8.33 \times 10^{-2} - 8.61 \times 10^{-2}$
Intercept (a)	9.06×10^{-3}
Standard deviation of the intercept (S_a)	1.70×10^{-2}
Confidence limit of the intercept ^b	$(-7.41 \times 10^{-3}) - (2.55 \times 10^{-2})$
Correlation coefficient (r)	0.9998
Standard error of estimation	7.8×10^{-3}

^a $Y = a + bC$, where C is the concentration of MD in $\mu\text{g ml}^{-1}$ and Y is absorbance; ^b95% confidence limit

TABLE 2: Analysis of variance for repeatability and intermediate precision for MD by the proposed spectrophotometric method

Comp.	Conc. Level ($\mu\text{g ml}^{-1}$)	Source of variation	Mean squares	D.F	F-ratio*	P-value
MD	3	Between	1.58	7	0.30	0.94
		Within	6.01	8		
		Total	7.59	15		
	15	Between	1.91	7	0.58	0.76
		Within	3.77	8		
		Total	5.69	15		
	20	Between	3.17	7	0.45	0.85
		Within	8.08	8		
		Total	11.25	15		

Where D.F is the degree of freedom and MS is the mean square.

*The critical value of F- ratio is 3.5 and P -value is 0.05

TABLE 3: Application of standard addition technique on different pharmaceutical formulations of MD using the proposed spectrophotometric method

Sample no.	Claimed conc. ($\mu\text{g ml}^{-1}$)	Added conc. ($\mu\text{g ml}^{-1}$)	% Recovery of added			
	MD	MD	Midodrine tablets	Midodrine drops	Gutron tablets	Gutron drops
1	10	4	99.5	98.7	99.4	100.1
2	12	5	98.7	98.6	99.5	100.3
3	6	10	100.1	100.3	100.2	99.4
4	10	5	100.0	100.4	100.2	99.7
5	7	7	100.2	100.0	100.0	99.8
6	15	3	99.6	99.6	100.4	100.5
7	10	10	99.8	99.8	100.0	100.2
	Mean		99.70	99.63	99.96	100.0
	S.D.		0.51	0.72	0.37	0.38

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coveries were in the range of 99.63-100.00%. The results are reported in TABLE 3 suggested that good accuracy of the proposed method.

6. Robustness

Variation of the reaction temperature by $\pm 1^\circ\text{C}$, and heating time by ± 2 minutes did not have a significant effect on ninhydrin- MD reaction.

7. Analytical solution stability

The MD solution in methanol exhibited no spectrophotometric changes for 8 hours, when kept at room temperature, and for 3 days, when stored refrigerated at 5°C .

The colored Ruhemann purple product of ninhydrin-MD derivative is very stable and no changes on the color intensity for 3days at room temperature.

Analysis of pharmaceutical products

In order to investigate the applicability of this spectrophotometric method to the determination of MD in pure form or in pharmaceutical formulations, the effect of the presence of some common excipients such as starch, talc, lactose and magnesium stearate that may present in tablets or ethanol in drops was studied. It was found that the common excipients did not interfere in the determination.

Commercial formulations containing MD were assayed successfully by the proposed method. The results were compared to those obtained by the published HPLC method^[5]. Seven replicates determinations were made. Satisfactory results were obtained for MD in good agreement with the label claims in each formulation (TABLES 4). The performance of the proposed method was judged with regards to accuracy and precision by calculating student's t- test and F-values^[13]. At 95% confidence judged level; the calculated t- and F-values do not exceed the theoretical values indicating no significant difference between the proposed method and the reference method.

CONCLUSION

It is concluded that the proposed method is sensitive, simple, rapid and selective for determination of MD in bulk and commercial formulations with good accuracy and precision. The reaction conditions were

optimized and critical factors identified. The short analysis time and low cost are the main advantages of the developed method for routine analysis. This encourages its successful use in routine analysis of these MD in quality control laboratories.

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