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Selective determination of midodrine hydrochloride in the presence of its acidic degradation product

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ABSTRACT

Two stability-indicating methods were developed for determination of midodrine in the presence of its acidic degradation product (the metabolite). The degradation product was isolated *via* acid degradation and then characterized and structurally elucidated. The first method was the first derivative of the ratio spectra ^{1}DD. The second method was a high performance liquid chromatographic one.

Selective quantification of midodrine in pure form, pharmaceutical formulation and/or in the presence of its degradant was demonstrated. The indication of stability was done under condition likely to be expected at normal storage condition.

KEYWORDS

Midodrine;
Stability;
HPLC;
Spectroscopy.

INTRODUCTION

Midodrine hydrochloride (MD), is 2-amino-N-[2(2,5-dimethoxy phenyl)-2-hydroxyethyl] acetamide. It is a vasopressing agent used for the treatment of hypotension, figure 1[1].

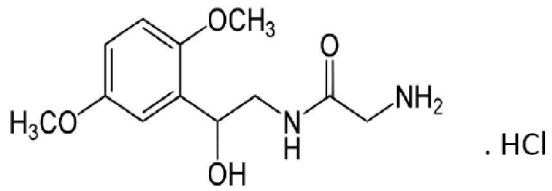

Figure 1: Structural formula of midodrine HCl [MD] M.W. [$C_{12}H_{19}N_2O_4Cl$ = 290.78]

There are few analytical methods for quantitative estimation of midodrine hydrochloride in dosage forms and in biological fluids. These methods include high performance liquid chromatography[2,3] and a colorimetric method[4].

Searching the literature, there is no published data concerning the stability indication of midodrine hydrochloride.

In modern analytical laboratory there is always a need for significant and simple stability-indicating method of analysis. The present work aimed to develop simple spectrophotometric and chromatographic methods for the quantification of MD in pure form or even in the presence of its degradant.

EXPERIMENTAL

Instruments

Spectrophometrer Shimadzu, 1601PC dual-beam UV-Vis spectrophotometer (Japan) with matched 1-cm quartz cells, connected to an IBM-compatible PC

and an HP-600 inkjet printer. Bundled, UV-PC personal spectroscopy software version 3.7 was used to process the absorption and the derivative spectra. The spectral bandwidth was 2 nm with wavelength-scanning speed of 2800 nm min^{-1}

- pH meter Jenway Model 3510(UK), Jenway Dunmow essex CM63LB.
- A liquid chromatograph consisted of Thermo Separation HPLC system, Molton Roy quaternary pump, UV variable wavelength detector model 3000 equipped with 20 µL injector loop. The separation was performed on a Zorbax C_{18} column (250 mm × 4.6 mm; 5 µm).

Materials and reagents

All chemicals and reagents were of analytical grade and water was bi-distilled.

(a) Materials

Reference MD standard was kindly supplied by El Nile Co., Cairo, Egypt. Its potency was found to be 99.60 ± 0.98% (n=6) according to a reference HPLC method[3].

(b) Pharmaceutical formulations

Midodrine tablet, B.N.18809 MD:1/09 ED:1/2013 manufactured by El Nile Co, Cairo-Egypt. Tablets were claimed to contain 2.5 mg of midodrine per tablet.

Midodrine drops B.N. 99395 MD:10/09 ED:10/2012 manufactured by El Nile Co, Cairo-Egypt.

(c) Standard solutions

MD standard solution 1 mg/mL in distilled water.

Durg degradant solution 1 mg/mL in distilled water.

All solution were freshly prepared on the day of analysis and stored in a refrigerator to be used within 24 h.

(d) Reagents

All solvents used were HPLC and spectroscopic grade.

Acetonitrile, HiPerSolv.®, HPLC-grade, E.Merck (Darmstadt, Germany). Hydrochloric acid and potassium dihydrogen phosphate: Adwic, El-Nasr Pharm. Co. (Cairo, Egypt). De-ionized water is bi-distilled from Aquatron automatic water still A4000, Bibby Sterling Ltd., UK.

Procedures

(a) Preparation of acid degradate of midodrine hydrochloride

Accelerated acid degradation was performed by refluxing 25 mg of pure MD powder with 25 mL of 2 molar hydrochloric acid for 13 hours till complete degradation that was confirmed by HPLC. The Completely degraded solution was neutralized using 2 molar sodium hydroxide solution. The solution was then evaporated to dryness under vacuum and the obtained degradation product was collected and characterized.

(b) First derivative of the ratio spectra method

(A) Spectral characteristics

Serial dilutions from both intact MD and its degradation product standard solutions were prepared and the spectra were scanned from 200 nm - 400 nm.

(B) Linearity

Aliquots (0.2-2 mL) from MD standard stock solution (1mg/mL) were transferred into series 25-mLvolumetric flasks. The volume was then completed with 0.01 molar hydrochloric acid to obtain a series with a concentration range of 8-80 µg/mL. A volume of 3 mL of the degraded standard solution (1mg/ ml) was transferred into a 25mL volumetric flask and the volume was completed with 0.01 molar hydrochloric acid to get a final concentration of 120 µg/mL of the degraded solution.

The spectra of the prepared standard solutions were scanned at 200-400 nm and the stored spectra of MD were divided (amplitude at each wavelength) by the spectrum of 120 µg/mL of the degradation product solution. The first derivative of the ratio spectra ^{1}DD with "A=4 nm with a scaling factor of 10 was obtained. The amplitude of first derivatives peaks of MD was measured at 268 nm and 308 nm. Two Calibration graphs were constructed relating the peak amplitude of ^{1}DD to the corresponding concentration. The regression equations were then computed for the studied drug at the two wavelengths and they were used for the determination of unknown samples containing MD.

(c) Liquid chromatography

(A) Chromatographic condition

Isocratic chromatographic separation was done at

ambient temperature using Stationary phase a C_{18} Zorbax® (250 x 4.6 mm, 5 µm) analytical column. The Mobile phase composed of 0.05M potassium dihydrogen phosphate: acetonitrile (80:20, v/v) the final pH value was adjusted to pH 7.0 ± 0.1 with sodium hydroxide. The mobile phase was filtered through 0.45 µm Millipore membrane filter and degassed for about15 min. in an ultrasonic bath prior to use. The mobile phase was run for 40 minutes at a flow rate of 1mL/min. to reach a good equilibrium. The analysis was usually performed just for conditioning and pre washing of the stationary phase. The eluent was detected with UV detector at 290 nm. The relative peak area ratio were then plotted versus the corresponding serial concentrations of MD (using MD standard solution of 30µg/mL as a divisor) to get the calibration graph and to compute the corresponding regression equation, concentration of unknown samples were determine using the obtained regression equation.

Samples were injected by the aid of a 25 µL Hammilton® analytical syringe.

(B) Linearity

Aliquots of 3-15 mL from MD standard solution (1mg/mL) were transferred separately into a series of 100-mL volumetric flask and the volume was completed with the mobile phase to get the concentration of 30-150 µg/mL of MD. The samples were then chromatographic using the mentioned chromatographic conditions.

(d) Analysis of laboratory prepared mixtures using the suggested methods

Aliquots of intact drug and the degraded drug were mixed to prepare different mixtures containing 10-90% (w/w) of the degradation product, and proceed as mentioned under each method. The concentration of the intact MD was calculated from the corresponding regression equation.

(e) Assay of pharmaceutical formulation

(A) Drops

The contents of five bottles were mixed then a volume of 10 mL (equivalent to one bottle) was transferred into a 100-mL volumetric flask. The volume was completed with the appropriate solvent as under each method.

(B) Tablets

Ten tablets were weighted to determine the average weight per tablet. They were then grinded very well. A weight equivalent to one tablet was then transferred into a 25-mL volumetric flask and then the volume was completed with with the appropriate solvent as under each method then filtered through a membrane filter of 0.45 µm diameter.

(f) Method validation

The developed analytical methods were validated according to ICH guidelines. Comparison of the results obtained by the proposed methods and the reference ones and statistical analysis of data was done.

RESULTS AND DISCUSSION

Degradation of midodrine

Midodrine is a prodrug which forms an active metabolite, desglymidodrine via metabolism or degradation. Complete degradation was achieved under acidic condition by refluxing with 2 molar hydrochloric acid for 13 hrs then the solution was neutralized. It was noticed that the main degradant is also the major metabolite of the drug inside the human body[2]. The main degradation product of MD, after cleavage of the amide bond, is the amine (desglymidodrine) and the glycine part. The degradation product was characterized and confirmed by GC/MS.

Derivative ratio spectroscopy

A hypochromic effect was observed during degradation of MD once complete degradation occur as the characteristic peak at 290nm was depressed, figure 2.

The absorption spectra of MD and its degradation product show sever overlap, that makes the use of direct measurement of MD in the presence of its degradant not applicable

When the first-derivative, figure 3 and the second-derivative, figure 4 were examined for both drug and its degradant, there was an interference that made the use of them inaccurate.

Derivatives ratio spectroscopy is a useful tool in quantification of the intact MD in the presence of its degradate.

The concentration of the divisor was studied; it was

found that upon dividing by the spectrum of 120µg/mL of the degradation product led to the best results in terms of sensitivity and repeatability. The first derivative of the ratio spectra was obtained.

Linear calibration graph were obtained for MD in concentration range 8-80 µg mL^{-1} by recording the peak amplitude at268 nm and 308 nm using120µg mL^{-1} of the degradate as a divisor, figure 5.

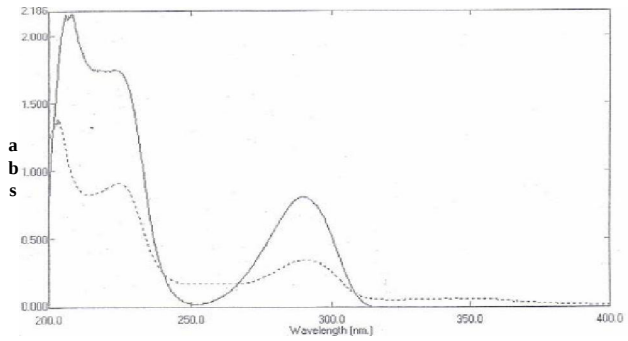

Figure 2 : Zero-order absorption spectra of intact midodrine (—) and degraded MD(…….) (each, 20 µg/mL in 0.01M HCl)

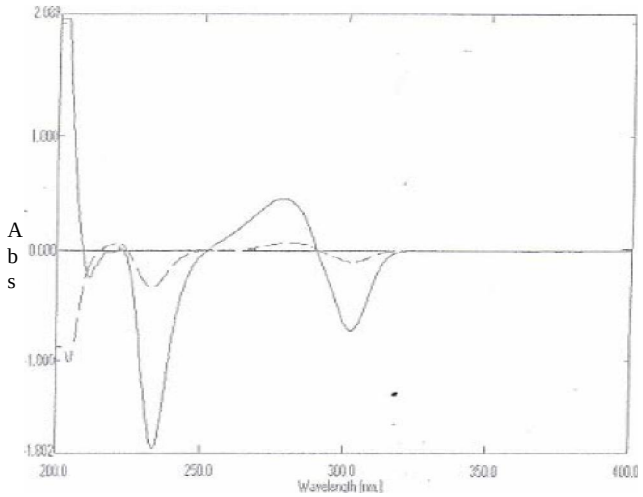

Figure 3 : The first derivative absorption spectra of intact midodrine (—) and degraded MD(…….) (each, 20 µg/mL in 0.01M HCl)

The regression equations were computed and found to be

Y = 5.914 X + 0.01559 at 268nm

Y = 8.829 X - 0.01192 at 308nm

Where, Y is the peak amplitude of the first derivative spectra. X is the concentration of MD in µg/mL and r is the correlation coefficient

The precision of the proposed method was checked by the analysis of pure MD sample in triplicates with good percentage recoveries.

High performance liquid chromatographic method

A simple isocratic high performance liquid chromatographic method was developed for the determination of MD in pure form and in pharmaceutical preparation using Zorbax® C_{18} (250 x 4.6mm, 5µm) analytical column. The mobile phase consisted of 0.05M potassium dihydrogen phosphate and acetonitrile (80:20, by volumes) and was adjusted to pH 7.0 ±0.1 using sodium hydroxide. The flow rate was 1 mL/min. and UV detection at 290 nm.

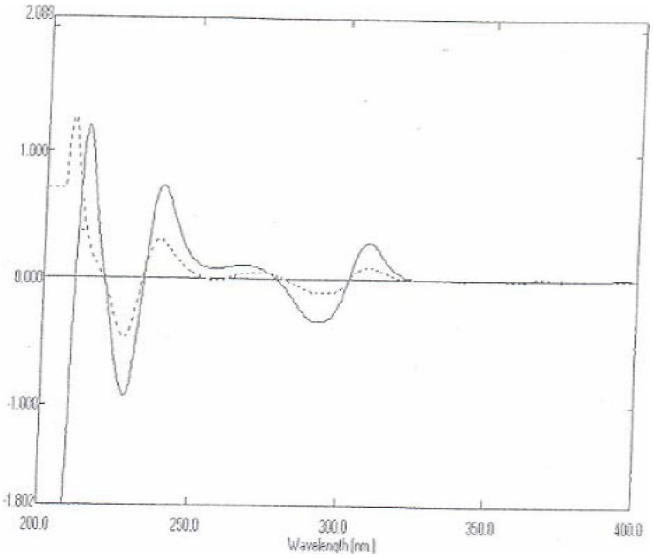

Figure 4 : The second derivative absorption spectra of intact midodrine (—) and degraded MD(…….) (each, 20 µg/mL in 0.01M HCl)

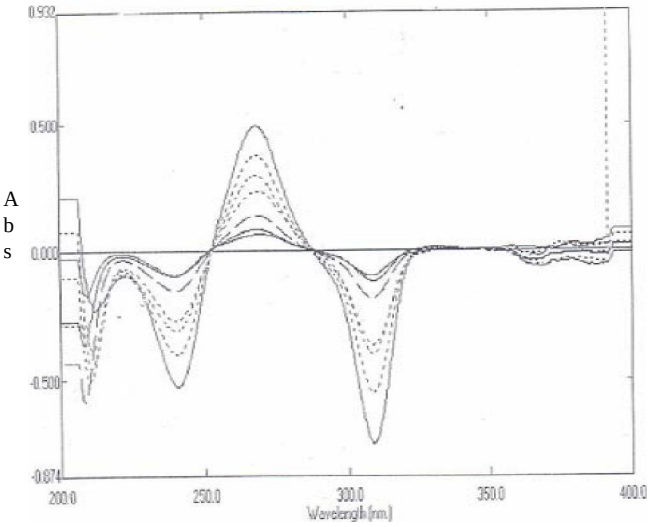

Figure 5 : First order of the ratio spectra of MD 8-80 µg/mL using the spectrum of 120 µg/mL of degradant as a divisor

The mobile phase was chosen after several trials to reach the optimum stationary and mobile phase matching. Using the described chromatographic conditions,

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MD and its degradant were well separated with average retention times of 2.1 min. and 3.7 min., for degradant and MD, respectively, one sample can be chromatographic in 5 minutes, figure 6.

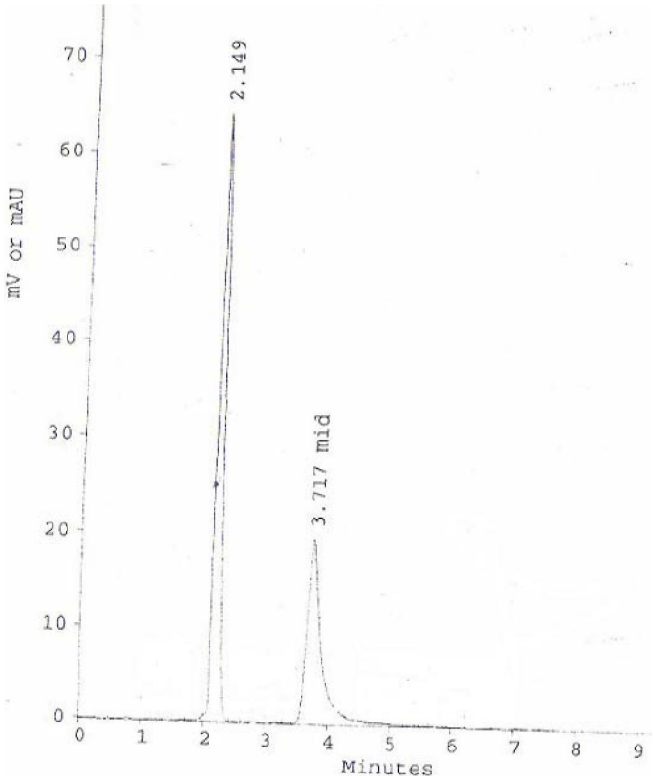

Figure 6 : Liquid chromatographic separation of MD(3.7 min) from its degradant (2.1 min), experimental conditions

The chromatographic system described in this work allows complete base line separation of MD from its degradation product. Peak purity was confirmed for the HPLC peaks of both intact MD and its degradant by a pilot run using a photodiode array detector.

By plotting the peak area ratio against concentration of MD, linearity was found to be 30-150 µg/ mL using the following regression equation

Y = 9.074 X - 0.006164

Where, Y is peak area ratio; X is the concentration of MD; and r is the relation coefficient.

System suitability parameters were tested by calculating the capacity factor, tailing factor, the sensitivity factor and resolution. The robustness of the HPLC method was investigated by analysisof samples under a variety of experimental conditions such as small changes in the pH (6.5-7.5), small changes in acetonitrile/buffer ratio of the mobile phase and changing the column using a C18 (250 x 4.6mm, 5µm) Inertsil™ column.

The effect on retention time and peak parameters was studied. It was found that the method was robust when the column and the mobile phase ratio were varied. During these investigations, the retention times were modified, however the areas and peaks symmetry were conserved.

Analysis of pharmaceutical formulations

The suggested methods were successfully applied for the determination of MD in its pharmaceutical formulations, showing good percentage recoveries, TABLE 1.

TABLE 1: Determination of MD in its pharmaceutical formulations by the proposed methods

Pharmaceutical Formulation	[1]DD method		HPLC method
	At 268	At 308 nm	
Midodrine drops	100.53	100.94	98.80
BN 99395 Mean ± SD	±1.17	± 1.03	±0.24
Midodrine tablets	100.24	100.68	99.64
BN 18809 Mean ± SD	±1.08	± 0.97	± 0.134

The validity of the suggested methods was further assessed by applying the standard addition technique, and the precision was also expressed in terms of relative standard deviation of the inter-day and intra-day analysis results.

TABLE 2 : The validation parameters of the proposed methods for the determination of MD

Parameter	[1]DD method		HPLC Method
	At 268 nm	At 308 nm	
Range (µg/mL)	8-80	8-80	30-150
Slope	5.914	8.829	9.074
Intercept	0.01559	-0.01192	-0.006164
Mean	100.94	99.83	100.43
SD	1.80	0.64	0.48
Variance	1.16	0.409	0.965
Coefficient of variance	1.78	0.64	0.48
Correlation coefficient	0.9998	0.9998	0.9998

The selectivity of the proposed methods was examined by analysis of different mixtures of the intact and degraded MD. The prepared mixtures were analyzed by the proposed methods and recoveries were 100.80 ± 0.98 and 99.87 ±0.63 for the spectrophotometric method at 268 nm and at 308 nm, respectively. Recovery was 98.85±0.52 for the HPLC method.

Results of the suggested methods for determina-

tion of MD were statistically compared with those obtained by applying the reference method[3]. The calculated t- and F values were less than the tabulated values[5].

TABLE 2 shows the validation parameters of the proposed methods for the determination of MD.

The kinetic order

Estimation of the kinetic order of the acid-degradation of MD could be done by calculating the percentage of the remaining drug concentration and its logarithmic value at different time intervals during the hydrolysis process. Assay of MD was carried out by adopting the developed HPLC method. The degradation process of MD follows pseudo-first-order kinetics during degradation as indicating by the straight line relationship between log the percentage of the remaining drug concentration versus time. The kinetics of the degradation process is pseudo-first order one as noticed for several drug substances[6].

CONCLUSION

The suggested methods of analysis are found to be simple, accurate, selective, cheap and equally sensitive with no significant difference of the precision compared with the reference method of analysis but being also stability indicating can detect degradation impurities in the intact molecule. Application of the proposed methods to the analysis of MD in its pharmaceutical formulation shows that neither the excipient nor the degradation product interferes with its determination.

REFERENCES

[1] M.J.O'Neil (Ed); The merck index, 14th Edition. Merck & Co., Inc., Whitehouse Station, New Jersey, **(2006)**.

[2] H.Yoshida, Y.Ohno, K.Yoshikuni, K.Todoroki, H.Nohta, M.Yamaguchi; Anal.Sci., **19**, 317 **(2003)**.

[3] W.Posch, W.Lindner; Biomedical Chromatography, **3**, 153 **(1989)**.

[4] A.El-Gindy, S.Sallam, R.A.Abdel-Salam; Anal.Chem.An Indian J., **7 (2008)**.

[5] M.R.Spiegel, L.J.Stephns; Schaum Outline of Theory and Problems of Statistics, Schaum Outline Series, NY, **(1999)**.

[6] N.A.Elragehy, E.M.Abdel-Moety, N.Y.Hassan, M.R.Rezk; Talanta, **77**, 26 **(2008)**.