Research in Pharmaceutical Sciences, June 2014; 9(3): 199-206
Received: Feb 2013
Accepted: Apr 2013

School of Pharmacy & Pharmaceutical Sciences
Isfahan University of Medical Sciences

Original Article

A stability indicating HPLC method for determination of mebeverine in the presence of its degradation products and kinetic study of its degradation in oxidative condition

E. Souri[1,*], A. Negahban Aghdami[1] and N. Adib[2]

[1]Department of Medicinal Chemistry, Faculty of Pharmacy and Drug Design and Development Research Center, Tehran University of Medical Sciences, Tehran, I.R. Iran.
[2]Department of Pharmaceutics, Food and Drug Laboratory Research Center, Ministry of Health, Tehran, I.R. Iran.

Abstract

An HPLC method for determination of mebeverine hydrochloride (MH) in the presence of its degradation products was developed. The degradation of MH was studied under hydrolysis, oxidative and photolysis stress conditions. Under alkaline, acidic and oxidative conditions, degradation of MH was observed. The separation was performed using a Symmetry C_{18} column and a mixture of 50 mM KH_2PO_4, acetonitrile and tetrahydrfuran (THF) (63:35:2; v/v/v) as the mobile phase. No interference peaks from degradation products in acidic, alkaline and oxidative conditions were observed. The linearity, accuracy and precision of the method were studied. The method was linear over the range of 1-100 μg/ml MH (r^2>0.999) and the CV values for intra-day and inter-day variations were in the range of 1.0-1.8%. The limit of quantification (LOQ) and the limit of detection (LOD) of the method were 1.0 and 0.2 μg/ml, respectively. Determination of MH in pharmaceutical dosage forms was performed using the developed method. Furthermore the kinetics of the degradation of MH in the presence of hydrogen peroxide was investigated. The proposed method could be a suitable method for routine quality control studies of mebeverine dosage forms.

Keywords: Mebeverine; HPLC; Stability indicating HPLC; Stress degradation

INTRODUCTION

Mebeverine hydrochloride (MH), 3, 4-dimethoxybenzoic acid 4-[ethyl-2-(4-methoxy-phenyl)-1-aminobutylveratrate hydrochloride (Fig. 1), is a nonspecific antispasmodic agent which acts directly on the smooth muscle of the gastrointestinal tract. Mebeverine hydrochloride is widely used as a relaxant agent for the treatment of gastrointestinal spasmodic disorders such as irritable bowel syndrome (1).

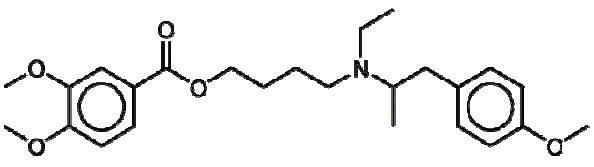

Fig. 1. Chemical structure of mebeverine.

A nonaqueous titration method is described in the British Pharmacopoeia (BP) (2008) and this procedure is used for determination of MH in bulk drug. Additionally spectrophotometric determination of MH in tablets is described in BP 2008. Literature survey showed several HPLC methods for determination of MH or its main metabolite in biological fluids (2-5). Also HPLC methods (6-9) and spectrophotometric methods (8, 10-12) are reported for the determination of MH in dosage forms.

According to the International Conference on Harmonization (ICH) guidelines, information on stability of drug substances under hydrolytic, oxidative, heat and photolytic conditions are required. Although the stability of MH in acidic and basic solutions has been reported before (6), to the best of our knowledge, no comprehensive

*Corresponding author: E. Souri
E-mail address: souri@sina.tums.ac.ir
Tel. 0098 21 66959065, Fax. 0098 21 66461178

investigation has been reported to undertake the stress degradation of MH under different conditions. The present study involves a stability indicating HPLC method for detailed examination of the factors that influence the stability of MH. Also, the kinetics of the degradation process of MH under oxidative conditions was investigated.

MATERIALS AND METHODS

Chemicals

Mebeverine hydrochloride was from Synthokem Labs Private Limited, India (Batch No: W.STD/MEB-003/2008). All other reagents, chemicals and HPLC grade solvents were from Merck (Darmstadt, Germany). HPLC grade water was obtained by a Milli-Q purification system (Millipore, Milford, MA, USA).

Instrumentation

A Waters HPLC system consisted of an isocratic pump (Model 515), an autosampler (Model 710 plus) and a variable UV-Vis detector (Model 480) was used. A multi-channel Chrom & Spec software for chromatography, version 1.5 x was used for data processing. A dry air oven (Melag, Germany) was used for heating the samples. The light sources were a l00 W Tungsten lamp (visible light) and a low-pressure Mercury lamp 200 W (UV light) with λ_{max} around 254 nm.

Chromatographic conditions

The analytical column used was a Symmetry® C_{18} 5 μm column (4.6 mm × 150 mm, Waters). The mobile phase consisted of a mixture of 50 mM KH_2PO_4, acetonitrile and tetrahydrfuran (THF) (63:35:2; v/v/v) eluted at a flow rate of 1 ml/min. The mobile phase was used after passing through a 0.45 μm filter and degassed under sonication for 10 min. UV-Vis detector was set at 263 nm. Separation was performed at ambient room temperature and the volume of solution injected on to the column was 30 μl.

Stock standard solution

By dissolving accurately weighed amount of MH in methanol a stock standard solution of 5000 μg/ml was prepared. The stock solution was kept refrigerated until the use. Standard solutions for calibration were prepared by serial dilution using mobile phase as diluent to acheive the desired concentrations.

Forced degradation studies

All degradation studies were performed at a MH concentration of 500 μg/ml. Neutral degradation was studied using a solution of MH in water and refluxed for 24 h. For acidic degradation 0.1 M or 1 M HCl was used at room temperature or reflux conditions. Alkaline degradation was also studied using 0.1 M NaOH at room temperature and reflux condition. The solutions of all acidic or alkaline degradations were neutralized by addition of appropriate amounts of sodium hydroxide or hydrochloric acid solution, respectively, and injected into the HPLC system after dilution with the mobile phase to yield the stated concentration of 25 μg/ml. Each experiment was performed in triplicate and mean data was calculated.

For oxidative conditions, different hydrogen peroxide concentrations (3%, 10% and 15%) were used. Moreover, to evaluate the kinetics of degradation under oxidative conditions, experiments were also performed in 30% hydrogen peroxide solution. One ml aliquots of MH solution (5000 μg/ml) were transferred into 10 ml volumetric flasks and mixed with 9 ml of H_2O_2. The flasks were placed in a dry air oven at different temperatures (70, 80, 85 and 90°C). The disappearance of MH was followed by injection of samples at different time intervals to the HPLC system and comparing the peak area with a standard solution. The experiment was performed in triplicate at each temperature and time interval.

Photostability studies were carried out by exposing a thin layer of MH powder and a methanolic solution of MH to visible and UV light. The samples were exposed to light at 20 cm distance from the light source in a 40 × 40 × 40 cm chamber for 5 days. Samples were withdrawn at different time intervals and analyzed after dilution with the mobile phase and compared with samples kept under the

same conditions but totally covered with aluminum foil to minimize the effect of heat produced from the light sources. After removal from the light chamber, all samples were analyzed as previously described.For thermal stress studies MH powder was exposed to dry heat at 90 °C in a dry air oven for 7 days.

Linearity

Standard solutions of MH in mobile phase at 1, 2, 5, 10, 20, 50 and 100 μg/ml were prepared and injected to the HPLC system. Calibration curves were constructed and statistical data calculated.

Precision and accuracy

The intra-day and inter-day repeatability and reproducibility were established using three separate sample solutions of MH at three different concentration levels (1, 10 and 100 μg/ml) on one day and three different days.

Recovery

To study the recovery of the developed method, a portion of powdered tablet or capsule content equivalent to 25% of one dosage form weight (equivalent to 50 mg or 33.75 mg of MH for capsule and tablet, respectively) was spiked with two different concentrations of MH standard solution in 100 ml volumetric flasks. After addition of 50 ml of the mobile phase to each flask and sonication for 20 min, mobile phase was added to volume. The resulted solution injected into the HPLC system after filtration through a 0.45 μm polypropylene syringe filter (Teknokroma, Spain) and diluted ten times. The peak area of the triplicate injections compared with the same concentrations of standard solutions of MH and the relative recovery was calculated.

Robustness

To study the robustness of the proposed method, the percent composition of organic solvent and the ionic strength of the buffer portion of the mobile phase were varied. The influence of the mentioned parameters on the chromatographic parameters (peak area and retention time) of a standard solution of MH was studied.

Application of the method to pharmaceutical formulations

To determine the MH in tablets, a portion of 20 tablets powder (Colofac 135 mg) equivalent to 100 mg of MH was accurately weighed and mixed with 50 ml of the the mobile phase in a 100-ml volumetric flask. The mixture was sonicated for 20 min and made up to volume with the mobile phase and injected into the HPLC system after filtration and ten times dilution. To determine the MH in capsules, the contents of 20 capsules (Colofac retard 200 mg) weighed and mixed and determined as described earlier for tablets.

RESULTS

Chromatographic conditions

Separation of MH and its degradation products was performed on a Symmetry C_{18} column using a mixture of KH_2PO_4, acetonirile and THF (63: 35: 2) as the mobile phase. Good chromatographic specificity and peak symmetry were achieved. As shown in typical chromatograms (Fig. 2), interfering peaks from degradation products in acidic, alkaline and oxidative conditions were not observed.The system suitability parameters (Table 1) indicate that the developed method could be suitably used in stability studies, as the tailing factors and injection repeatability are within the acceptable criteria.

Linearity and sensitivity

The linearity of the developed method was assessed based on the analysis of six calibration series in the range of 1-100 μg/ml. Acceptable linearity was observed with $r^2>0.999$ according to the statistical data in Table 2. The limit of quantification (LOQ) with CV<1.8 % was about 1 μg/ml. An estimate of the limit of detection (LOD) based on S/N ratio of 3 was found to be 0.2 μg/ml.

Precision and accuracy

Triplicates of MH samples at 1, 10 and 100 μg/ml on three separate days were used and the precision and accuracy were evaluated. Concentration of each sample was determined by constructing calibration standard curves prepared for each day.

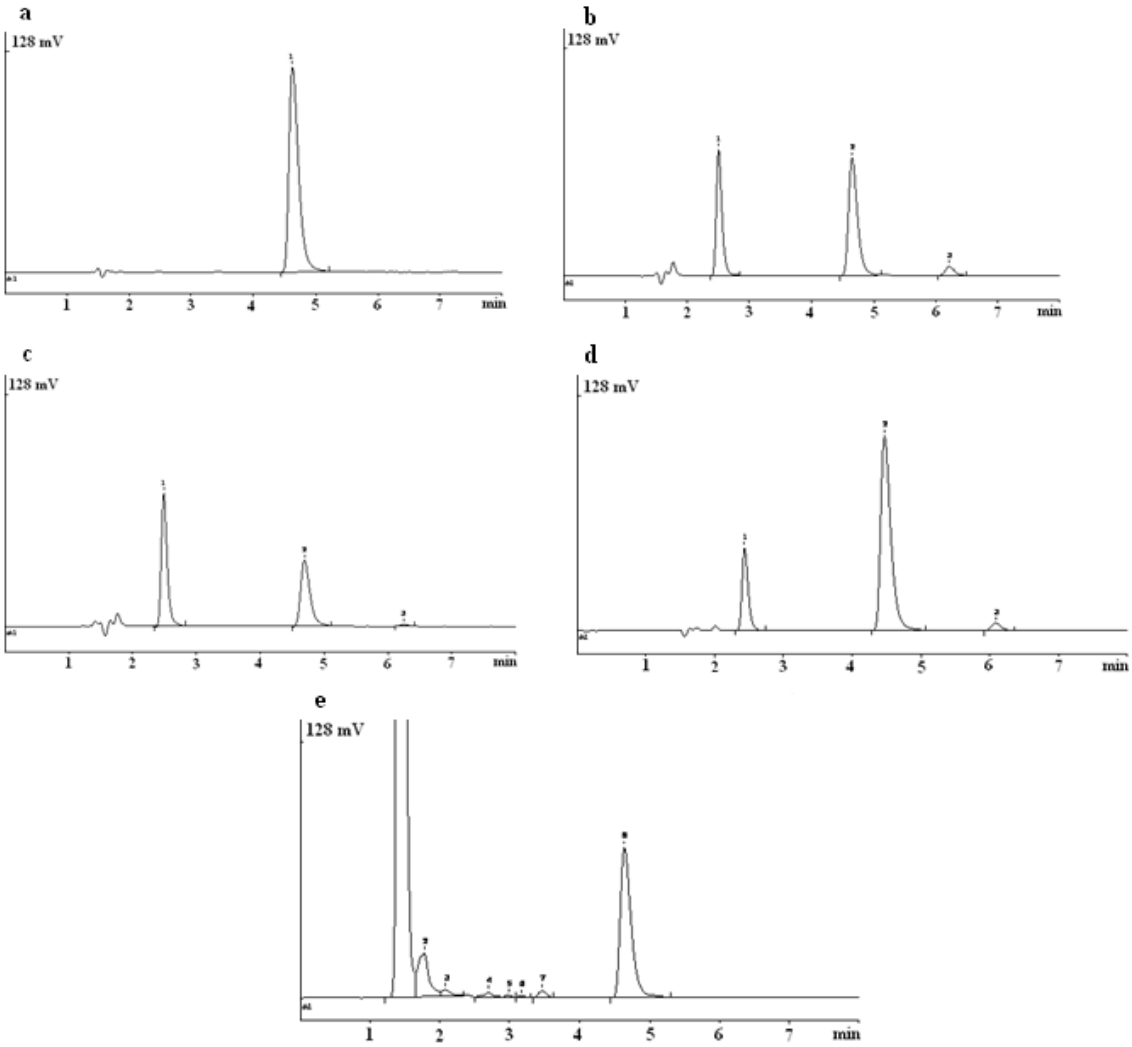

Fig. 2. Typical chromatograms obtained from stability studies of mebeverine hydrochloride. a; mebeverine hydrochloride standard solution (25 µg/ml), b; mebeverine hydrochloride solution in 1 M HCl after 1 h reflux, c; mebeverine hydrochloride solution in 0.1 M NaOH after 6 h in room temperature, d; mebeverine hydrochloride solution in water after 48 h reflux, e; mebeverine hydrochloride solution in 30% H_2O_2 after 24 h at 70 °C.

Table 1. System suitability parameters.

Parameters	Found	Acceptable limits
USP theoretical plates (n = 6)	5500	N>1500
USP tailing factor (n = 6)	1.3	T<1.5
Repeatability (t_R) (n = 6)	0.43	RSD<1%
Repeatability (peak area)(n = 6)	0.59	RSD<1%

t_R; Retention time (min), N; Theoretical plate, T; Tailing factor; RSD; Relative standard deviation.

Table 2. Statistical data of calibration curves of mebeverine hydrochloride (n=6).

Parameters	Results
Linearity range	1-100 µg/ml
Regression equation	y = 43.91 x-2.17
Standard deviation of slope	0.36
Relative standard deviation of slope (%)	0.81
Standard deviation of intercept	0.41
Correlation coefficient (r^2)	0.9997

The intra- and inter-day precision and accuracy are shown in Table 3. To study the intermediate precision, the assay method was performed by two chemists using two different HPLC systems. The CV values did not exceed 2%.

The robustness results are shown in Table 4. The peak area values were not influenced more than 0.5% under different conditions. The retention time was decreased by increasing the amount of organic solvent. Despite the variations in retention time, the developed method could be used for quantification of MH.

Relative recovery

The mean recovery of MH after standard addition of drug solution at two different concentrations (25 and 50 μg/ml) to tablet and capsule powder, ranged from 99.1 to 100.6% and no interfering peaks from tablet or capsule excipients was observed.

Solution stability

Methanolic stock solutions of MH were stable at 4 °C at least for 7 days. Also MH solution in mobile phase was stable at room temperature for at least 24 h with a recovery of 99%.

Analysis of pharmaceutical products

The developed method was used for assay determination of tablets and capsules containing MH. The obtained results showed satisfactory results (198.69 ± 2.06 mg per capsules and 134.11 ± 1.39 mg per tablets) which was in good agreement with the label claims.

Degradation studies

The obtained results of degradation studies upon the treatment of MH under neutral , alkaline (0.1 M NaOH), acidic (0.1 M and 1 M HCl), oxidative conditions (hydrogen peroxide 3%, 10% and 15%), and heat and light are summarized in Table 5.

Under these conditions after 7 days of heating at 90 °C and also after 5 days of visible or UV light exposure the samples were practically stable in solid form or in methanolic solution. Only a slight decrease in peak area, about 3.2 and 2.3%, was observed in methanolic solution of MH after 5 days exposure to the UV or visible light respectively.

Table 3. Precision and accuracy of the method for determination of mebeverine hydrochloride (three sets for 3 days).

Concentration added (μg/ml)	Concentration found (μg/ml)	CV (%)	Error (%)
Intra-day (n = 3)			
1.00	1.01 ± 0.02	1.81	1.00
10.00	9.99 ± 0.10	1.04	-0.10
100.00	99.65 ± 1.10	1.10	-0.35
Inter-day (n = 9)			
1.00	1.01 ± 0.02	1.71	1.00
10.00	10.02 ± 0.13	1.32	0.20
100.00	99.69 ± 1.09	1.09	-0.31

Table 4**.** The influence of small changes in mobile phase composition (method robustness).

Mobile phase composition	Retention time	Peak area
KH_2PO_4 45 mM/acetonirile/tetrahydrofuran (66:32:2)	5.71	1113.75
KH_2PO_4 45 mM/acetonirile/tetrahydrofuran (63:35:2)	4.37	1111.01
KH_2PO_4 45 mM/acetonirile/tetrahydrofuran (60:38:2)	3.55	1100.96
KH_2PO_4 50 mM/acetonirile/tetrahydrofuran (66:32:2)	6.16	1115.17
KH_2PO_4 50 mM/acetonirile/tetrahydrofuran(63:35:2)	4.45	1113.29
KH_2PO_4 50 mM/acetonirile/tetrahydrofuran (60:38:2)	3.44	1116.20
KH_2PO_4 55 mM/acetonirile/tetrahydrofuran (66:32:2)	5.98	1112.28
KH_2PO_4 55 mM/acetonirile/tetrahydrofuran (63:35:2)	4.45	1115.86
KH_2PO_4 55 mM/acetonirile/tetrahydrofuran (60:38:2)	3.64	1113.63

Table 5. The results of the stress degradation tests using different conditions.

Stress test condition	Solvent	Temperature	Time	% of mebeverine
Neutral	Water	Reflux	24 h	89.2
Acidic	0.1 M HCl	Room temperature	24 h	94.2
	1 M HCl	Room temperature	3 days	88.0
	1 M HCl	Reflux	3 h	20.8
Alkaline	0.1 M NaOH	Room temperature	4 h	61.4
	0.1 M NaOH	Reflux	1 h	70.3
Oxidative	3% H_2O_2	Room temperature	4 h	99.7
	3% H_2O_2	Reflux	3 h	79.2
	10% H_2O_2	Reflux	4 h	50.7
	15% H_2O_2	Reflux	12 h	46.7
Photolytic				
UV light	solid form	Room temperature	5 days	99.9
UV light	methanol	Room temperature	5 days	96.8
Visible light	solid form	Room temperature	5 days	100.0
Visible light	methanol	Room temperature	5 days	97.7
Heat	solid form	90 °C	7 days	99.3

Table 6. Apparent degradation rate constant (k) and half-life ($t_{1/2}$) for mebeverine hydrochloride in 30% H_2O_2.

Temperature (°C)	K (h^{-1})	($t_{1/2}$) (h)
70	0.017	40.765
80	0.038	18.237
85	0.052	13.327
90	0.073	9.493

The hydrolysis of MH was observed in neutral, acidic and basic conditions. The hydrolysis of MH was relatively mild under acidic or neutral conditions. On the other hand, the hydrolysis under basic conditions was fast. The main degradation product in all these conditions was the same and observed at retention time of 2.5 min. Also another peak at retention time of 6.2 min was generated in later stages (Fig. 2).

Using different strengths of hydrogen peroxide the instability of MH to oxidative conditions was observed. No major degradation product was detected. A mixture of unknown degradation products were observed at 1.8, 2.1, 2.7, 3, 3.2 and 3.5 min with small peak areas (Fig. 2).

The kinetics of degradation of MH in 30% hydrogen peroxide was also studied. Quantitative degradation showed that the MH concentration decreased with increasing time at studied temperatures. By plotting the *log* of percent remained MH versus time, a linear relationship was observed (Fig. 3) which indicates pseudo first order kinetics described by the following equation:

$$log\ C_t = log\ C_0 - kt/2.303$$

where, C_t is the percent remained MH peak area, C_0 is the initial percent of MH (100%), k is the apparent first order rate constant with a negative sign and t is the time (h). The correlation coefficients of all plots in different temperatures were more than 0.98. The apparent first order rate constants were calculated by using the slopes of the straight lines.

The half-life was also determined at each temperature. The results are shown in Table 6.

The Arrhenius plot constructed by plotting log k values versus 1/T in the temperature range 70-90 °C was also linear (Fig. 4). The activation energy of the oxidative degradation was also calculated by using the classical Arrhenius equation

$$k = Ae^{-Eact/RT}$$

where, k is the apparent first order rate constant, A is the pre-exponential factor, E_{act} is the activation energy and T is the temperature. The calculated activation energy from the linear Arrhenius plot was found to be 71.13 KJ/mole.

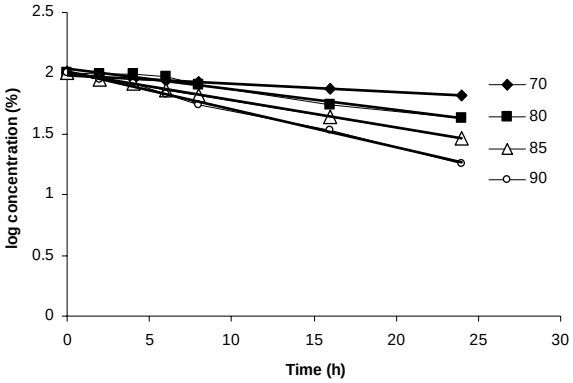

Fig. 3. Pseudo first order plots for the degradation of mebeverine hydrochloride in 30% H_2O_2 at various temperatures using HPLC method. Key; C_t, percent remained mebeverine hydrochloride at time t, and C_0, percent mebeverine hydrochloride at zero time.

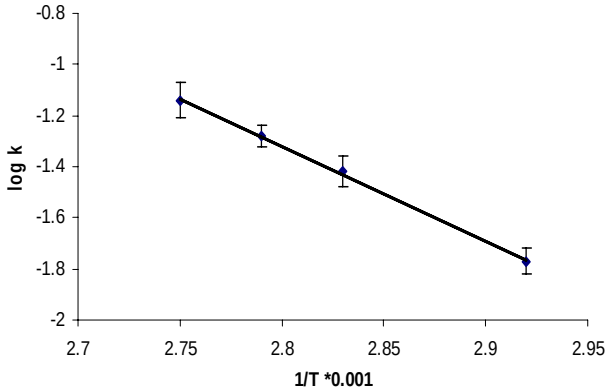

Fig. 4. Arrhenius plot for degradation of mebeverine hydrochloride in 30% H_2O_2 .

DISCUSSION

Few HPLC methods have been reported for the determination of MH in dosage forms. According to the ICH guidelines, stability indicating methods should be developed for determination of drug compounds in the presence of their degradation products. As no comprehensive stability indicating HPLC method has been reported for the determination of MH, in the current study an HPLC method was validated and the stability of MH under different conditions such as acidic, basic, oxidative, heat and light was studied. Mebeverine hydrochloride is relatively stable under visible and UV light and also heating. On the other hand, as shown in the result section, MH was degraded significantly in the presence of 1 M HCl, 0.1 M NaOH and H_2O_2. The degradation of MH under these conditions was dependent on the temperature.

At higher temperatures, more degradation was observed. The kinetics of degradation of MH was studied in 30% H_2O_2 since the degradation rate at lower strengths of hydrogen peroxide was too slow to obtain reliable results. At selected temperature pseudo first-order kinetics was observed. The reaction rate constant was calculated from the slope of the line produced from the relationship between the log of the percent of MH versus time. The Arrhenius plot was linear at the temperature range of 70-90ºC.

Using the optimized chromatographic conditions, acceptable chromatographic separation and peak shape for MH in the presence of its degradation products was observed. Also the HPLC method was linear over the range of 1-100 µg/ml and the intra-day and inter-day variability was very low. The precision and accuracy of the method was acceptable for quality control purposes. The validated HPLC method was used for the determination of MH in pharmaceutical dosage forms without any interference from excipients.

CONCLUSION

In this study stress degradation of MH was performed under different recommended conditions of ICH guidelines. The developed stability indicating method resolved all degradation products in a single isocratic run. The results showed that MH is unstable under alkaline, acidic and oxidative conditions. A pseudo first order kinetics was observed for oxidative degradation of MH in hydrogen peroxide.

The developed method was also validated and successfully used for the determination of MH in pharmaceutical dosage forms. Although no attempt was made for identification of degradation products, the proposed method could be used as a precise stability indicating method for determination of MH in pharmaceutical dosages forms.

ACKNOWLEDGMENTS

This study was part of a Pharm D thesis supported by Drug Design and Development Research Center, Tehran University of Medical Sciences (grant No:9706).

REFERENCES

1. Brunton LL, Lazo JS, Parker KL. Goodman and Gillman's. The Pharmacological Basis of Therapeutics, 11th edition, Mc Graw-Hill Medical Publishing Division, USA, 2005. p. 1000.
2. Moogewijs G, Massart DL. Development of a standardized analysis strategy for basic drugs using ion-pair extraction and high-performance liquid chromatography. VIII. Method construction for the determination of mebeverine in tablets and biological fluids. J Chromatogr. 1986;377:391-398.
3. Dickinson RG, Baker PV, Franklin ME, Hooper WD. Facile hydrolysis of mebeverine in vitro and in vivo: negligible circulating concentrations of the drug after oral administration. J Pharm Sci. 1991;80:952-957.
4. Stockis H, Guelen PJ, de Vos D. Identification of mebeverine acid as the main circulating metabolite of mebeverine in man. J Pharm Biomed Anal. 2002;29: 335-340.
5. Elliot S, Burgess V. Investigative implications of the instability and metabolism of mebeverine. J Anal Toxicol. 2006;30: 91-97.
6. De Schutter JA, De Croo F, Vander Weken G, Ven den Bossche W, De Moerloose P. Stability study and quantitative determination of mebeverine hydrochloride in tablets by means of reversed-phase high-performance liquid chromatography. Chromatographia. 1985;20:185-192.
7. Al-Deeb Q, Al-Hadiya BM, Foda NH. Quantitative analysis of mebeverine in dosage forms by HPLC. Chromatographia. 1997;44:427-430.
8. El Walily AFM, El Gindy A, Bedair MF. Application of first- derivating UV-spectrophotometry, TLC-densitometry and liquid chromatography for the simultaneous determination of mebeverine hydrochloride and sulpiride. J Pharm Biomed Anal. 1999;21:535-548.
9. Arayne MS, Sultana N, Siddiqui FA. A new RP-HPLC method for analysis of mebeverine hydrochloride in raw materials and tablets. Pak J Pharm Sci. 2005;18:11-14.
10. Shama SA, Amin AS. Spectrophotomertric microdetermination of nefopam, mebeverine and phenylpropanolamine hydrochloride in pharmaceutical formulations using alizarins. Spectrochim Acta A Mol Biomol Spectrosc. 2004;60:1969-1974.
11. Zayed SI. Simultaneous determination of mebeverine hydrochloride and sulpiride using the first derivatives of ratio spectra and chemometric methods. Anal Sci. 2005;21:985-989.
12. El-Didamony AM. Spectrophotometric determination of benzydamine HCl, levamisole HCl and mebeverine HCl through ion-pair complex formation with methyl orange. Spectrochim Acta A Mol Biomol Spectrosc. 2008;69:270-275.