Stability-Indicating HPLC Method for the Determination of Impurities in Meprobamate with Refractive Index Detection

K. Karthikeyan[1,2,*], T.S. Balaji[1], P. Shanmugasundaram[1], and **K. Chandrasekara Pillai[2]**

[1]Analytical Development, Shasun Research Center, Chennai - 600048, India and [2]Department of Physical Chemistry, University of Madras, Guindy Campus, Chennai -600025, India

Abstract

The purpose of this study is to develop and validate a simple, sensitive, and robust high-performance liquid chromagraphic (HPLC) method for the determination of impurities ca. 2-methyl-2-propyl-1,3-propane diol (MP0) and 2-hydroxymethyl-2-methyl pentyl carbamate (MP1) in meprobamate (MEP) drug substance with refractive index (RI) detection. This method utilizes a Zorbax Eclipse XDB C_{18} HPLC column, a mobile phase of 80:20 (v/v) 10 mM KH_2PO_4,–acetonitrile, respectively. The stability-indicating capability of the method has been established by performing stress studies under acidic, basic, oxidation, light, humidity, and thermal conditions. The major degradation products of acid and base hydrolysis are identified as MP0 and MP1. The recovery data obtained for impurities are between 96.0–109.8%. The detection and quantitation limits of this method ranges from 0.009 to 0.017 mg/mL and 0.029 to 0.055 mg/mL, respectively. The relative standard deviation (RSD) for the area at QL is less than 6.1%. Good linearity ($r^2 > 0.99$) and precision (RSD < 2.2%) have been obtained for MEP, MP0, and MP1. This method has been applied successfully to determine the content of impurities in MEP bulk drug.

Introduction

Meprobamate, (2-methyl-2-propyl-1,3-propanediol dicarbamate) is the most well-known member of a family of propane diol dicarbamates possessing tranquilizing and skeletal muscle relaxant properties. Meprobamate (MEP) is currently licensed only as an anxiolytic drug (1) and is administrated orally. It has been shown in animal studies to have effects at multiple sites in the central nervous system, including the thalamus and limbic system. MEP binds to GABA A receptors, which interrupt neuronal communication in the reticular formation and spinal cord, causing sedation and altered perception of pain. MEP is used for the treatment of anxiety disorders and for short-term relief of anxiety.

Literature shows that there are several methods developed for the estimation of MEP drug substance (2–19). Official monographs available for MEP in British, European, United States Pharmacopoeia (USP) and Analytical Profiles for drug substances (2–5) refer only to a titrimetric assay method based on the chemistry of the carbamate moiety, which includes aqueous hydrolysis followed by volumetric estimation. Spectral methods based on infrared (IR) and nuclear magnetic resonance (NMR) absorption also have been reported (6,7). Several gas chromatographic (GC) methods are described for the assay of MEP in drug substance, drug product, human plasma, urine, and water (8–11). Colorimetric assay procedures are also reported for MEP determination in pharmaceutical products (12,13). It has also been assayed by high-performance liquid chromatography (HPLC) and GC after alkaline hydrolysis to 2-methyl-2-propyl-1,3-propanediol and preparing the benzoyl ester of the diol (14). Also, a validated reversed-phase HPLC method using indirect photometric detection to determine MEP in pharmaceutical dosage forms has been reported (15). In addition to these, sensitive gas chromatography–mass spectrometry (GC–MS) assay methods using electron impact ionization also have been published (16,17). Reports are available, where MEP has been determined by normal phase HPLC using differential RI detection (18). The major degradation impurities of MEP are primarily MP0 (5,14) and MP1, which is also a known process impurity

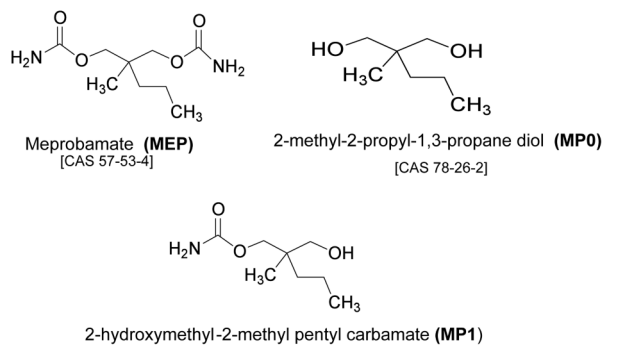

Figure 1. Chemical structure of meprobamate and impurities.

*Author to whom correspondence should be addressed: email karthi_kkn@yahoo.co.uk.

(Figure 1). It is important to note that while several sophisticated methods have been developed and employed for the drug estimation (2–18), there appears to be only a thin layer chromatography (TLC) method reported so far for the impurities determination of MEP drug substance (2–5,19)

The present investigation has been, therefore, initiated with the objective to develop a simple, sensitive, and stability-indicating method for the impurity analysis of MEP drug substance by reversed-phase (RP)-HPLC. Based on the maximum daily dosage (> 2 g) of MEP, the related impurities must be controlled below 0.05% as per International Conference on Harmonization (ICH) and Food and Drug Administration (FDA) regulatory guidelines (20,21). Hence, a sensitive and robust HPLC method is required to detect and quantitate the impurities of MEP. An important and critical aspect about MEP, MP0, and MP1 is their poor chromophoric property towards UV, and hence it is practically difficult to develop a sensitive HPLC method using direct UV detection. To overcome this issue, RI detection has been utilized in this study for the direct estimation of impurities in MEP. The proposed RP-HPLC method with RI detection has been validated using USP (4) and ICH (22) guidelines as references.

Experimental

Reagents and materials

HPLC-grade methanol, acetonitrile, and AR-grade KH_2PO_4 were purchased from Merck (Mumbai, India). The water used was from a Milli-Q purification system (Millipore, Bedford, MA). MEP and its impurities, prepared and characterized by Shasun Chemicals and Drugs (Chennai, India), were used in this study.

Equipments

The HPLC system consisted of a Waters Alliance separation module 2695 equipped with a Waters 2414 RI detector (Milford, MA). Waters Empower software (Build 1154) was used for the data acquisition and processing. A Shimadzu HPLC system having LC-10ATvp pump equipped with RID-10A detector, autosampler, and Class-VP software (Kyoto, Japan) was used for degradation studies, intermediate precision, and also for method development purpose. The peak purity studies were carried out on a Thermo Finnigan Surveyor LC system coupled with LCQ DECA XP Plus ion-trap mass spectrometer (San Jose, CA). The photolytic stress studies were carried out in a Thermolab stability chamber (Mumbai, India) equipped with both UV and fluorescent lamps having exposure capacity of 150 µW/cm^2 and 1000 lux, respectively.

Chromatographic conditions

Agilent Zorbax Eclipse XDB C_{18} HPLC column (250 mm length × 4.6 mm id, 5 µm particle diameter) (Palo Alto, CA) was used. The column was kept at 30 ± 2°C. The mobile phase was 80:20 (v/v), 10 mM KH_2PO_4 and acetonitrile, respectively. The injection volume was 40 µL, and the flow rate was 1.0 mL/min. The total run time was 30 min. A mixture of methanol and mobile phase in the ratio of 8:2 (v/v), respectively, was used as diluent for sample, standard, and system suitability preparations. The methanol–water (8:2 v/v) mixture was used for needle wash in the autosampler. Chromatograms were obtained from RI detector at positive polarity with the cell temperature of 30°C. Detector settings used for Waters RI detector were sampling rate, filter time, and sensitivity at 2, 3, and 16, respectively. Similarly, settings used for Shimadzu were auxiliary range and response at 4 and 5, respectively.

Solution preparation

System suitability stock solution was prepared by dissolving 6.25 mg each of impurity MP0 and MP1 using 8 mL methanol in a 10-mL volumetric flask and diluted to volume with diluent. System suitability solution was prepared by dissolving 1250 mg of MEP sample in a 5-mL volumetric flask using diluent with sonication followed by the addition of 1 mL of system suitability stock solution. This solution was then diluted to volume with diluent. The resolution (R) between MEP and MP1 was evaluated as part of system suitability with the acceptance criteria of not less than 0.35. Standard preparation was made by dissolving 25 mg each of impurity MP0, MP1, and MEP using 160 mL of methanol in a 200-mL volumetric flask with sonication and diluted to volume with mobile phase (0.125 mg/mL). The relative standard deviation (RSD) for the area of meprobamate peak from six replicate injections was evaluated with the acceptance criteria of not more than 5.0%. Sample preparation was made by dissolving 1250 mg of meprobamate sample using diluent in a 5-mL volumetric flask with sonication and diluted to volume (250 mg/mL).

Stress conditions

Acid hydrolysis

The MEP bulk drug sample was treated with 6 N HCl at a concentration of 250 mg/mL; the solution was heated at 70 ± 2ºC for a period of 2 h. Before carrying out the HPLC analysis, samples were suitably diluted and neutralized with 10 N NaOH.

Base hydrolysis

Base hydrolysis was performed in 1 N NaOH at a drug concentration of 250 mg/mL. The solution was then subjected to heating at 70 ± 2°C for 5 min and neutralized with 1 N HCl.

Oxidation

Hydrogen peroxide was utilized for the oxidative degradation study. The drug substance was treated with 15% hydrogen peroxide solution at 70 ± 2°C for a period of 2 h.

Thermal stress

A thin layer of MEP bulk drug was spread on a petri dish and subjected to heat at 60 ± 2°C in a dry heat oven for 72 h.

Humidity

MEP sample was spread on a Petri dish and exposed to 95 ± 5% relative humidity at 25 ± 2°C in a humidity chamber for 72 h.

Photolytic stress

Photolytic studies were conducted by exposing the drug in solution (250 mg/mL) and solid state to UV and fluorescent light separately. Samples were withdrawn after 72 h and analyzed.

Results

Method development

In preliminary work, experiments were performed using octadecyl silane (C_{18}) stationary phase to achieve separation between MP0, MP1, and MEP by RP-HPLC. MEP has good solubility in methanol, ethanol, and acetonitrile, but methanol was selected as organic solvent in mobile phase. When methanol was used, the retention factor (23) was found to be high (k > 10) with broad peaks. This suggested to use a stronger solvent, hence mixture of methanol–acetonitrile at various composition was used to have a good peak shape with reasonable retention factor (k). But the presence of only acetonitrile (20%, v/v) as organic solvent in the mobile phase yielded better peak shape with acceptable separation and retention. Other stationary phases, namely, cyano, phenyl, and octyl silane were also tried, but C_{18} (Zorbax Eclipse XDB) was found to be a robust stationary phase with very good precision. In all the experiments, KH_2PO_4 buffer (pH 4.5) without any adjustment in pH was used; however, effect of pH on k and R was studied. Figure 2 represents the effect of pH (at 2.5, 4.5, 6.5, and 7.5) on the k of MP0, MP1, and MEP. No significant change was observed in the R between MP0 and MP1 whereas little decrease in the R between MP1 and MEP at pH 6.5 and 7.5 was noticed. This concludes that pH of the buffer solution did not have any significant effect on the k and R, hence, KH_2PO_4 buffer without any adjustment in pH was finalized.

To achieve appropriate detection and quantitation limit (≤ 0.03%) for MP0 and MP1, an injection volume of 40 µL with the sample concentration of 250 mg per mL was used. Due to high sample load, peak width of MEP became large; however, the

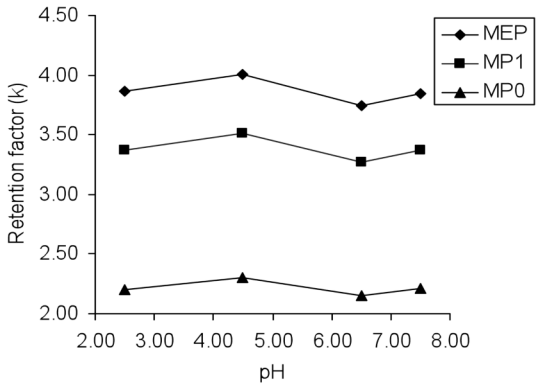

Figure 2. Influence of buffer pH in the mobile phase on retention of MP0, MP1, and MEP.

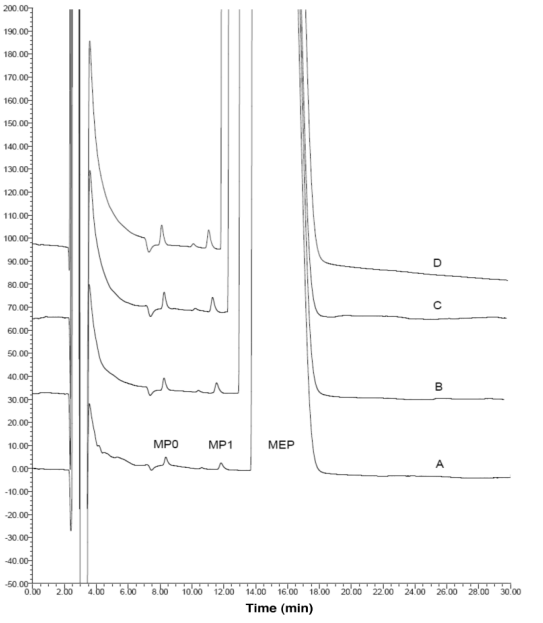

Figure 3. Overlaid chromatograms of MEP, containing MP0 and MP1 at 0.05% each, analyzed using (A) 20, (B) 30, (C) 40, and (D) 50 µL as injection volume.

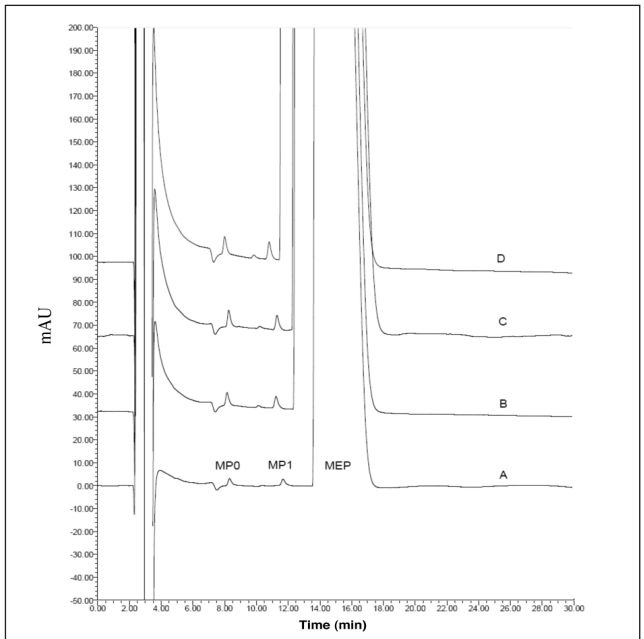

Figure 4. Overlaid chromatograms of MEP, containing MP0 and MP1 at 0.05% each, analyzed using (A) 100, (B) 200, (C) 250, and (D) 300 mg/mL as sample conc.

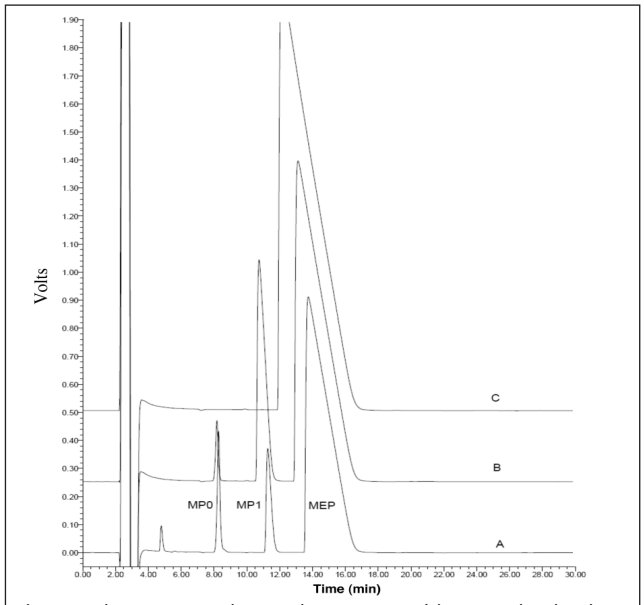

Figure 5. Chromatograms showing the separation of the (A) acid and (B) base degraded products of meprobamate along with (C) unstressed sample.

baseline separation between the impurities and MEP remained unaffected (23). As a consequence of the larger peak width of MEP, the R calculated (4) between MP1 and MEP was found to be about 0.5, even though the separation was very good. To demonstrate the separation and to ensure the robustness of the proposed method, studies on different injection volumes and concentrations were carried out. Figure 3 and 4 represent the overlaid chromatograms of MEP along with MP0 and MP1 analyzed at different injection volume and concentration, respectively.

The variation in acetonitrile content (± 2%) of the mobile phase and temperature of the column oven were also studied as a part of robustness, but no remarkable change in R and α (separation factor) was noticed. Because MEP sample did not contain MP0 and MP1 significantly, MEP sample spiked with MP0 and MP1 at 0.05% (0.125 mg/mL) level, relative to the test concentration (250 mg/mL), was used in all the previous studies. The effect of variation in pH, injection volume, and sample concentration on the chromatographic performance parameter (k, R, and α) is summarized in Table I. Typical retention time of MP0, MP1, and MEP was 8, 11, and 12 min, respectively. The k values for MP0, MP1, and MEP were 2.3, 3.5, and 4.0, respectively.

Table I. Influence of Variation in pH, Concentration, and Injection Volume on Chromatographic Performance Parameters

	k						
Variation	MP0	MP1	MEP	α[1]*	α[2]†	R[1]*	R[2]†
pH							
2.5	2.20	3.37	3.86	1.53	1.15	7.83	0.51
4.5	2.30	3.51	4.01	1.53	1.14	8.38	0.51
6.5	2.15	3.27	3.74	1.52	1.14	7.70	0.50
7.5	2.21	3.37	3.84	1.52	1.14	7.94	0.49
Concentration (mg/mL)							
100	2.32	3.67	4.56	1.58	1.24	8.14	1.22
200	2.26	3.49	4.07	1.54	1.17	8.03	0.62
250	2.30	3.51	4.01	1.53	1.14	8.38	0.51
300	2.20	3.32	3.71	1.51	1.12	7.66	0.38
Injection volume (µL)							
20	2.34	3.73	4.61	1.59	1.24	8.78	1.11
30	2.30	3.61	4.30	1.57	1.19	8.45	0.74
40	2.30	3.51	4.01	1.53	1.14	8.38	0.51
50	2.24	3.42	3.85	1.53	1.13	8.25	0.40

* Separation factor between MP0 and MP1; resolution between MP0 and MP1.
† Separation factor between MP1 and MEP; resolution between MP1 and MEP.

Table II. Stress Studies

		After degradation					
Stress conditions	Drug amount*	MP0*	MP1*	Unk. peak†	Total imps.†	Remaining drug amount*	Degradation (%, w/w)
Oxidation	256.3	‡	‡	‡	‡	257.1	§
Heat	251.4	‡	‡	‡	‡	250.6	§
Humidity	249.2	‡	‡	‡	‡	250.2	§
Hydrolysis							
Base	248.8	2.9	30.7	‡	14	200.9	19
Acid	250.0	10.8	18.5	‡	12	208.7	17
UV light							
Solid	250.5	‡	‡	‡	‡	249.9	§
Solution	259.1	‡	‡	‡	‡	260.2	§
Fluorescent light							
Solid	252.5	‡	‡	‡	‡	252.6	§
Solution	258.5	‡	‡	‡	‡	259.0	§

* Concentration in mg/mL.
† Impurities concentration in %.
‡ Not detected/not applicable.
§ No significant degradation.

Method validation

Specificity

Stress Studies. To demonstrate the stability indicating capability of the method, MEP sample was subjected to stress by acid, base, hydrogen peroxide, UV light, fluorescent light, heat, and humidity. The stressed samples were assayed to determine the percentage of degradation. Major degradation happened under acid and base hydrolysis whereas no significant degradation was observed in all other stress conditions. Major degradation products corresponded to MP0 and MP1. Overlaid chromatograms of the degraded samples along with MEP (unstressed) showing the degradation product are presented in Figure 5. The homogeneity of MEP peak in each stressed sample was examined through mass spectral studies. Due to the poor chromophoric property of MEP towards UV, peak purity testing by photodiode array (PDA) is practically difficult and would not be informative. Hence, mass spectra were collected from upslope and downslope of the peak and compared with the peak apex spectrum (24). No significant change in mass spectra was found across MEP peak in all the stressed samples. Also, the degradation products formed during the stress study were well-separated from each other and from MEP. Table II summarizes the results along with stress conditions.

Repeatability. MEP, MP0, and MP1 were prepared at 0.05% (0.125 mg/mL) each, relative to sample concentration (250

Table III. Repeatability of MEP, MP0, and MP1

Injections	Area of MEP	Area of MP0	AREA of MP1
1	93048	88044	82472
2	90432	88735	84143
3	89926	89308	85866
4	91367	91131	82802
5	88124	89006	87269
6	88459	90177	84750
Mean	90226	89400	84550
RSD (%)	2.0	1.2	2.2

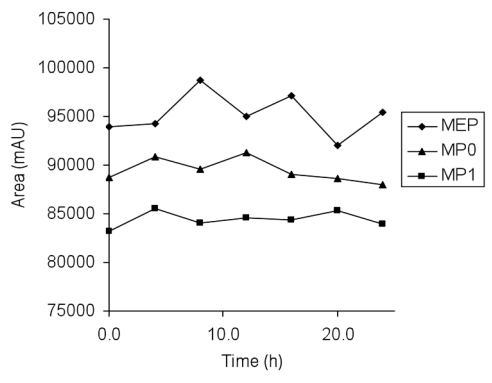

Figure 6. Stability of MP0, MP1, and MEP solution at 25 ± 2°C.

mg/mL) and injected in six replicates. The RSD (n = 6) values obtained for the area of MEP, MP0, and MP1 were 2.0, 1.2, and 2.2%, respectively. The repeatability results are listed in Table III.

Linearity. The linearity was established by measuring area responses for each impurity and MEP over the range of 0.012–0.060% (0.029–0.150 mg/mL) relative to sample concentration (250 mg/mL). Six concentrations (n = 6) were prepared across the range and injected in triplicate. The mean area (n = 3) calculated was plotted against the concentration. The squared regression coefficient obtained for MEP, MP0, and MP1 are 0.9978, 0.9997, and 0.9985, respectively (Table IV). The slope of the calibration curve for MEP was 1.05 and 1.06 times the slope values of MP0 and MP1, respectively.

Accuracy. Accuracy was validated through recovery experiments by spiking known amount of each impurity at 0.025, 0.05, and 0.06% with MEP relative to sample concentration (250 mg/mL). Each preparation was analyzed in triplicate (n = 3) and percent recovery was calculated. Table V–VI represent the recovery results of MP0 and MP1, respectively. For each preparation, the area response of the matrix interference was subtracted, and the corrected area response was used to calculate recovery. The recovery was found to be between 96.0 and 109.8% with the RSD of less than 3.3%.

Detection and quantitation limit. The detection limit (DL) and quantitation limit (QL) for MEP, MP0, and MP1 were determined by signal-to-noise (S/N) ratio method. The minimum concentration at 3:1 S/N ratio was established as its DL, and the concentration at 10:1 S/N ratio was considered as QL. The DL was 0.009, 0.012, and 0.017 mg/mL for MP0, MP1, and MEP, respectively, which correspond to 0.004, 0.005, and 0.007% relative to sample concentration (250 mg/mL). A solution containing impurities and MEP was prepared around their QL concentration and injected in six replicates. The QL values obtained for MP0, MP1, and MEP were 0.029 (0.012%), 0.040 (0.016%), and 0.055 mg/mL (0.022%); the RSD of area at QL was 5.9, 6.1, and 4.6, respectively (Table VII).

Stability of analyte solution. The stability of MP0, MP1, and MEP was studied by measuring the area response of standard preparation injected over a period of 24 h at 25 ± 2°C. Figure 6 represents the relationship between area response and time. The RSD values for the area responses of MP0, MP1, and MEP were 1.4, 0.98, and 2.3%, respectively.

Intermediate precision. The ruggedness of the method was evaluated by performing the sample (MEP bulk drug) analysis in six replicates using two different columns, different HPLC instruments and different analysts on different days. The results are summarized in Table VIII. The overall RSD values were 1.5, 1.6, and 1.1% for MP0, MP1, and total impurities, respectively.

Robustness. This study was performed by making small but deliberate variations in the method parameters. The effect of variation in flow rate, mobile phase composition, and column oven temperature was studied. The results pertaining to system suitability test and impurity levels are presented in Table IX. Under all the variations, system suitability requirements were found to be well within the acceptance criteria.

Table IV. Linearity

Amount (mg/mL)	Concentration (%)*	Mean area	Slope	r^2
MEP				
0.055	0.022	36682		
0.063	0.025	42990		
0.094	0.038	68294	761504	0.9978
0.125	0.050	87809		
0.138	0.0055	101295		
0.150	0.060	109594		
MP0				
0.029	0.012	20136		
0.062	0.025	44447		
0.094	0.038	67993	726243	0.9997
0.125	0.050	89139		
0.137	0.055	99656		
0.150	0.060	107894		
MP1				
0.040	0.016	25274		
0.061	0.025	42116		
0.092	0.037	65011	715260	0.9985
0.135	0.054	95536		
0.147	0.059	101734		

* Relative to 250 mg/mL of MEP sample.

Discussion

The HPLC method with RI detection was selected in this work because of the poor chromophoric property of MEP, MP0, and MP1 towards UV. RI is a physical property of a chemical compound, and any substance can be detected in principle at moderate levels (23). In this study, it was observed that the area percent results by RI detection were very much closer to weight percent values of MP0 and MP1, which in turn provided an additional advantage to determine the unknown peaks, more reliably by area normalization itself. Several experimental variables were considered, including pH, concentration, injection volume, mobile phase composition, etc. to establish appropriate chromatographic parameters and to demonstrate the robustness of the method. The variation study results suggested that R and k were influenced by concentration; however, MEP and impurities

Table V. Accuracy of MP0

Amount of sample (mg)*	Added amount (mg)*	Found amount (mg)*	Recovery (%)
1253.2	0.3212	0.3469	108.00
1252.3	0.3212	0.3340	103.99
1252.1	0.3212	0.3469	108.00
1250.2	0.6423	0.6675	103.92
1251.7	0.6423	0.6423	100.00
1251.3	0.6423	0.6549	101.96
1250.2	0.7708	0.8087	104.92
1250.4	0.7708	0.8466	109.84
1250.5	0.7708	0.8213	106.55
		Mean	105.24
		RSD	3.0

* Prepared in 5 mL.

were clearly separated from each other in all the conditions. Also, no remarkable impact on α was found (Table I). From the investigations carried out, the optimal chromatographic conditions established were 80:20 (v/v) 10 mM KH_2PO_4 and acetonitrile, respectively. The proposed HPLC–RI detection method was validated for specificity, repeatability, linearity, accuracy, detection limit, quantitation limit, stability of analyte solution, intermediate precision, and robustness. The stress studies indicated that MEP was sensitive to acid and base hydrolysis but quite stable in all other conditions. The degradation products of MEP were identified as MP0 and MP1 through comparison with standard. It is significant to note that MP0 and MP1 were well-resolved from each other and from MEP. The mass spectral data also revealed that MEP peak was homogeneous in all the stress experiments, which suggest that the method adopted is specific and stability indicating. Very good repeatability and linearity was obtained for MP0, MP1 and MEP. The overall RSD of less than 1.61 % for intermediate precision demonstrates the ruggedness of the method. The DL, QL, and recovery results indicate the accuracy and capability of the method to detect impurities at very low levels (< 0.01%). The proposed method is far better in terms of specificity, repeatability, and stability-indicating capability compared to the published reports (19) where TLC method was used to determine impurities in MEP. Moreover, the presently developed method is cost-effective as it utilizes the most commonly used HPLC column and reagents with moderate run time.

Table VI. Accuracy of MP1

Amount of sample (mg)*	Added amount (mg)*	Found amount (mg)*	Recovery (%)
1253.2	0.3162	0.3036	96.02
1252.3	0.3162	0.3415	108.00
1252.1	0.3162	0.3289	104.02
1250.2	0.6325	0.6704	106.00
1251.7	0.6325	0.6578	104.00
1251.3	0.6325	0.6578	104.00
1250.2	0.7590	0.8096	106.67
1250.4	0.7590	0.8096	106.67
1250.5	0.7590	0.7969	105.00
		Mean	104.49
		RSD	3.3

* Prepared in 5 mL.

Table VII. QL Data of MEP, MP0, and MP1

Amount (mg/mL)	Concentration (%)*	Area at QL	RSD (%)
MEP			
		36926	
		37363	
0.055	0.022	35757	4.6
		34879	
		32965	
		34481	
MP0			
		21011	
		19730	
0.029	0.012	19667	5.9
		21036	
		22054	
		18761	
MP1			
		23621	
		25251	
0.040	0.016	26950	6.1
		27198	
		28082	
		26557	

* Relative to 250 mg/mL of MEP sample.

Conclusion

The HPLC method described in this study was proved to be an ideal tool for the determination of impurities (MP0 and MP1) in MEP bulk drug at 0.05% and below levels to comply with the regulatory requirement. Method validation data demonstrated that the developed method is sensitive as well as accurate for the estimation of impurities and robust to the minor variation in the chromatographic parameters. The proposed method is simple and also cost-effective with moderate analysis time. The specificity and stability-indicating capability of the method was demonstrated through forced degradation studies as per ICH guidelines. Meprobamate was found to be acid and base labile compound, forming MP0 and MP1 as major degradation

Table VIII. Intermediate Precision

	Impurity MP0 (%)		Impurity MP1 (%)		Total impurities (%)	
	Set 1*	Set 2†	Set 1*	Set 2†	Set 1*	Set 2†
	0.053	0.052	0.057	0.057	0.110	0.109
	0.051	0.051	0.056	0.056	0.107	0.107
	0.052	0.051	0.056	0.055	0.108	0.106
	0.052	0.051	0.055	0.057	0.107	0.108
	0.053	0.052	0.056	0.054	0.109	0.106
	0.052	0.051	0.056	0.056	0.108	0.107
RSD (%)	1.44	1.01	1.13	2.09	1.08	1.09
Overall RSD (%)	1.46		1.61		1.14	

* Set 1 = analyst 1, column 1, HPLC 1, and day 1.
† Set 2 = analyst 2, column 2, HPLC 2, and day 2.

Table IX. Robustness

Variation	R*	RSD (%)	Impurities (%) MP0	MP1	Total
No variation	0.53	2.04	0.052	0.056	0.108
Flow rate (1.10 mL/min)	0.53	2.63	0.053	0.054	0.107
Flow rate (0.90 mL/min)	0.52	2.20	0.051	0.054	0.105
Column temp. (35°C)	0.41	2.83	0.050	0.056	0.106
Mobile phase comp.†	0.55	2.38	0.050	0.057	0.107
Mobile phase comp.‡	0.50	2.73	0.060	0.052	0.112
RSD (%)			7.17	3.35	2.26

* Resolution between MP1 and MEP.
† Buffer–ACE (v/v) for 82:18. ‡ Buffer–ACE (v/v) for 78:22.

product; however, it was stable under heat, light, humidity, and oxidative stress. The proposed RP-HPLC method with RI detection can be used conveniently for the stability monitoring and routine quality control of MEP drug substance.

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