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Short communication

Identification, isolation, characterization and response factor determination of process-related impurity in meprobamate drug substance

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ABSTRACT

This paper describes identification and characterization of a process-related impurity of meprobamate drug substance observed in HPLC-UV method. Forced degradation studies were carried out under acidic, basic, oxidation, light and thermal conditions to assess the nature of the impurity. The pure impurity was obtained by preparative LC isolation and analyzed by NMR and mass. Structural elucidation by spectral data and formation of this impurity were discussed in detail. The structure of the process-related impurity was established as *carbamic acid-2-carbamoyloxymethyl-2-methyl-pent-3-enyl ester* (olefin). Also, the relative response factor, linearity, detection limit (DL), quantitation limit (QL) and recovery were determined for meprobamate and the impurity. Good linearity was obtained for the impurity over the concentration range of 0.03–0.20% (w/w) with the coefficient of determination (r^2) of 0.999. The DL and QL of olefin impurity were 0.0003 and 0.001% (w/w), respectively. The isolated impurity was co-injected with meprobamate sample to confirm the retention time in HPLC.

1. Introduction

Meprobamate, chemically known as 2-methyl-2-propyl-1,3-propanediol dicarbamate (Fig. 1), 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 administrated orally. 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.

Several methods were reported based on titrimetry, infrared, nuclear magnetic resonance (NMR), gas chromatography, liquid chromatography (LC) and gas chromatography–mass spectrometry (GC–MS) for the assay determination of meprobamate [2–19]. Official monographs available for meprobamate drug substance refer to TLC method for impurity estimation [2–5]. Recently, a HPLC with refractive index detection (HPLC-RI) method, far better in terms of specificity, repeatability, and stability-indicating capability compared to TLC method, has been reported by us for the determination of impurities (2-methyl-2-propyl-1,3-propane diol and 2-hydroxymethyl-2-methyl pentyl carbamate) in MEP drug substance [20].

During the analysis of different MEP batches by HPLC-UV method, i.e., similar to USP assay method for MEP tablet [4], one unknown impurity was detected at about 0.6%. Till date, no report is available in the literature regarding this impurity. Hence, the present work was initiated to investigate the nature and origin of the impurity and to characterize it by NMR and mass. The structure of the impurity was established as *carbamic acid-2-carbamoyloxymethyl-2-methyl-pent-3-enyl ester* (olefin) (Fig. 1).

The maximum daily dose of meprobamate is >2 g/day and hence related impurity must be controlled to less than 0.05% as per ICH/FDA/EMEA regulatory guidelines and any impurity at or above 0.03% (reporting threshold) should be reported [21–23]. This investigation deals with identification, isolation, structure elucidation, relative response factor determination and formation of the impurity. Also, the linearity, detection limit (DL), quantitation limit (QL) and accuracy for olefin impurity were established utilizing USP and ICH guidelines as references [4,24].

2. Experimental

2.1. Reagents and samples

HPLC grade acetonitrile and methanol were purchased from Merck (Mumbai, India). The water used was from a Milli-Q purification system, Millipore (Bedford, USA). MEP (purity = 99.5%) and

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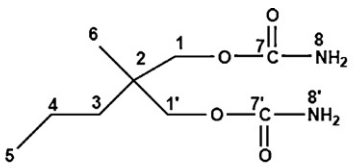
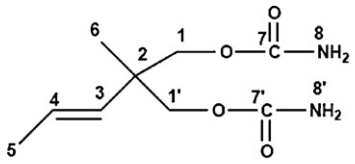


Meprobamate (MEP)



Carbamic acid 2-carbamoyloxymethyl-2-methyl-pent-3-enyl ester

(Olefin impurity)

Fig. 1. Chemical structure of meprobamate (MEP) and olefin impurity.

impurity, prepared and characterized by Shasun Chemicals and Drugs Ltd. (Chennai, India) were used in this study.

2.2. Equipments and chromatographic conditions

The HPLC system consisted of a Waters Alliance separation module 2695 equipped with Waters 2487 dual wavelength absorbance detector and 2996 photodiode array (PDA) detector (Milford, USA). Waters Empower 2 software (Build 2154, Waters) was used for the data acquisition and processing. The LC–MS studies were carried out on a Thermo Finnigan Surveyor LC system coupled with LCQ DECA XP Plus ion-trap mass spectrometer (San Jose, USA). A Shimadzu GC system coupled with quadrupole mass spectrometer (GCMS-QP2010) and equipped with direct sample inlet device (DI-2010) was used to collect electron impact (EI) mass spectra with the ionization voltage at 70 eV. GC–MS solution software (Kyoto, Japan) was utilized for mass spectral analysis. The LC-8A preparative liquid chromatograph from Shimadzu, equipped with SPD-10A VP, UV-VIS detector (Kyoto, Japan) was used. The ^{1}H and ^{13}C NMR experiments were performed on a Bruker Avance DPX-300 MHz NMR spectrometer (Faellanden, Switzerland) using $CDCl_3$ as solvent and TMS as internal standard. Agilent Zorbax Eclipse XDB C18 HPLC column of 250 mm length × 4.6 mm id, 5 μm particle size (Palo Alto, USA) was used. The column was kept at 30 ± 2 °C. The mobile phase was 8:2 (v/v) water and acetonitrile. Chromatograms were obtained with ultraviolet detection at the wavelength of 200 nm. The injection volume was 20 μL and the flow rate was 1.0 mL/min. The total run time was 30 min. For preparative isolation, Phenomenex Luna C18 (2) of 250 mm length × 50 mm id, 15 μm particle size (Torrance, CA) column was employed. The flow rate was 75 mL/min and UV detection was carried out at 200 nm.

2.3. Solution preparation

For analytical purpose, sample preparation was made by dissolving 100 mg of MEP sample using 1 mL of acetonitrile in 5 mL volumetric flask with sonication and diluted to volume with water (20 mg/mL). For preparative isolation, sample was dissolved in methanol.

2.4. Stress conditions

The MEP bulk drug sample was treated with 6 N HCl for acid stress, and the solution was heated at 70 ± 2 °C for a period of 2 h.

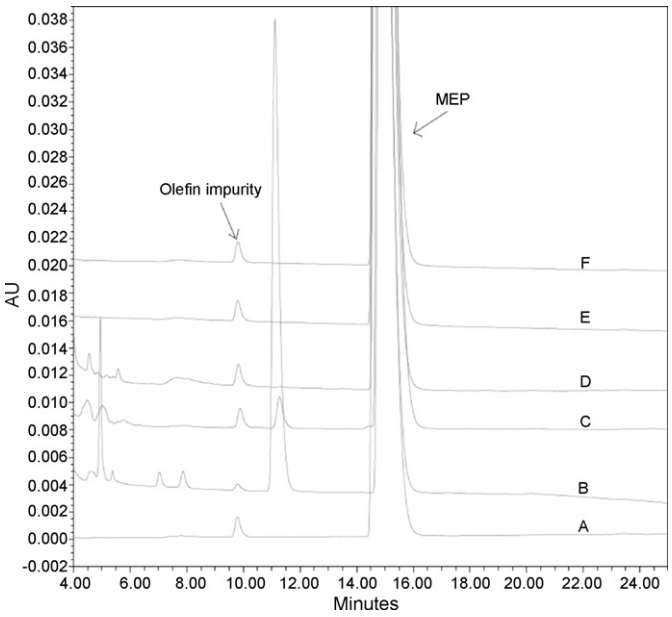


Fig. 2. Overlaid chromatograms of MEP showing the separation and response of olefin impurity under (A) unstressed, (B) base, (C) acid, (D) oxidation, (E) photolytic and (F) thermal stress conditions.

Base hydrolysis was performed in 1 N NaOH, and the solution was then subjected to heating at 70 ± 2 °C for 5 min. The drug substance was treated with 15% hydrogen peroxide solution at 70 ± 2 °C to a period of 2 h for oxidative degradation. A thin layer of MEP bulk drug was spread on a petri glass dish and subjected to thermal stress at 60 ± 2 °C in a dry heat oven for 72 h. Photolytic studies were conducted by exposing the drug both in solution (20 mg/mL) and in solid state to UV and fluorescent light for 72 h.

3. Results and discussion

3.1. Method optimization, LC–MS analysis and stress studies

The HPLC-UV method available in USP for MEP tablet analysis [4] was employed initially to detect the unknown impurity. The level of impurity observed in the sample was about 0.6%. In this method, MEP eluted at about 5.5 min and the impurity at about 4.5 min, i.e., with a relative retention time (RRT) of 0.8 for impurity with respect to MEP. To have better retention and resolution, the mobile phase composition of USP method was modified to 8:2 (v/v) water:acetonitrile instead of 7:3 (v/v). In the optimized method in which MEP eluted at about 15 min and impurity at 10 min (RRT of 0.65 for impurity with respect to MEP), the level of impurity obtained remained the same at about 0.6%. The MEP sample was subjected to LC–MS analysis to identify the mass of 0.65 RRT impurity. The mass obtained in ESI positive ion mode was *m*/*z* 216 which is two mass units less than MEP drug. To investigate the nature of this impurity, whether it was a degradation product or process-related impurity, MEP sample was subjected to various stress conditions, including acid, base, oxidation, thermal and light. Major degradation of MEP occurred under acidic and basic stress, whereas no significant degradation was observed in other conditions. However, in all the forced degradation conditions, no increase in the level of 0.65 RRT impurity was found, suggesting that the impurity could not be originated due to degradation of MEP but could be a process-related one. The overlaid HPLC traces of stress study are presented in Fig. 2.

3.2. Isolation of impurity by preparative HPLC

MEP sample was subjected to preparative HPLC as per the conditions described in Section 2.2. The preparative LC fractions were initially analyzed by analytical LC and then pooled together. The fractions were concentrated on rotavapor and then freeze-dried to remove solvent and water, respectively. The 0.65 RRT impurity was obtained as an off-white solid with the purity of 94% by HPLC-UV method.

3.3. Structural elucidation

The ESI mass spectrum recorded for the isolated (0.65 RRT) impurity in positive ion mode by LC–MS showed a molecular ion peak at *m*/*z* 217 $[M+H]^+$ indicating the molecular weight of the impurity as 216. Also, the EI mass spectrum obtained for the isolated impurity by GC–MS confirmed the molecular weight. The EI mass spectrum showed a parent ion peak at *m*/*z* 217 $[M+H]^+$ corresponding to the molecular weight 216. Also, it contained characteristic fragments at *m*/*z* values 156, 142, 112 and 95. The mass spectrum of MEP showed a parent ion peak at *m*/*z* 219 $[M+H]^+$ corresponding to the molecular weight 218 with fragments at *m*/*z* values 158, 144, 114 and 97. The EI mass spectra of the isolated impurity and MEP drug are presented in Fig. 3. The ^1H and ^{13}C NMR spectral assignments for MEP and olefin impurity are presented in Table 1.

The olefin proton at 5.41 ppm (position 3) appeared as doublet of a quartet with coupling constant of 16.0 and 1.6 Hz. The value 16.0 Hz is due to the coupling of proton at position 3 with adjacent olefin proton (position 4) which confirms the stereochemical relationship as a trans isomer. The value 1.6 Hz is due to the coupling of proton at position 3 with methyl proton at position 5. Similarly the other olefin proton at 5.56 ppm (position 4) appeared as quartet of a doublet with coupling constant of about 6.3 and 16.0 Hz. The value 6.3 Hz is due to the coupling of proton at position 4 with methyl proton at position 5, and 16.0 Hz is due to the coupling of a proton at position 4 with adjacent olefin proton at position 3 which also confirms the trans isomer relationship. The above spectral data confirms the structure (Fig. 1) of the impurity as *carbamic acid-2-carbamoyloxymethyl-2-methyl-pent-3-enylester* (olefin impurity) with the molecular formula and molecular weight of $C_9H_{16}N_2O_4$ and 216, respectively.

3.4. Relative response factor (RRF) determination and validation

3.4.1. Specificity

To demonstrate the specificity of HPLC-UV method, MEP sample was subjected to stress by acid, base, hydrogen peroxide, heat, and light. The homogeneity of MEP peak in each stressed sample was examined by peak purity testing utilizing PDA detector [25]. In all the degraded samples, the purity angle obtained for MEP peak was less than purity threshold, demonstrating spectral homogeneity. Also, the mass spectra (LC–MS) were collected under positive electrospray ionization (+ESI) mode and no significant change in mass spectra was found across MEP peak in all the stressed samples. The degradation products formed during the stress study were well separated from each other and from MEP which proved that the adopted method is specific.

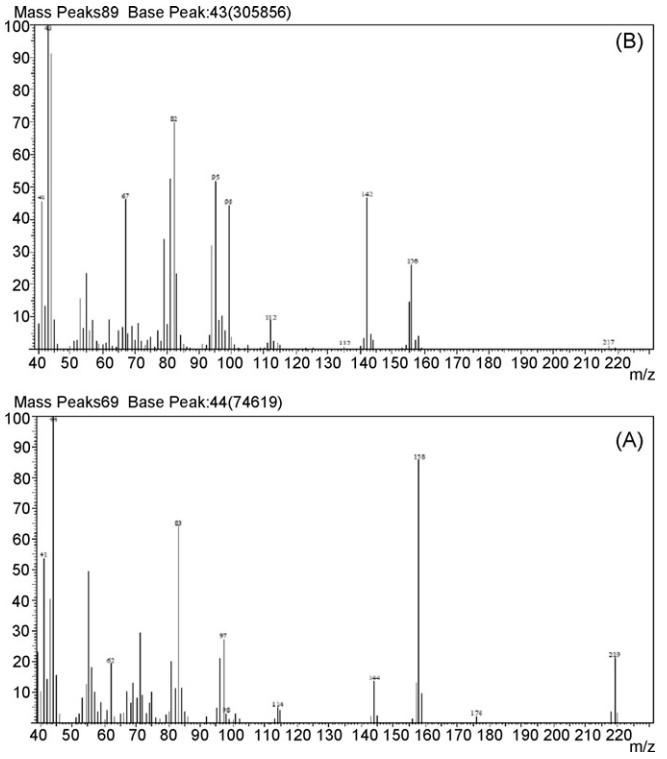


Fig. 3. Electron impact (EI) mass spectrum of (A) meprobamate and (B) olefin impurity.

3.4.2. Linearity, RRF, accuracy, detection (DL) and quantitation limit (QL)

The linearity was established by measuring area responses for olefin impurity and MEP over the range of 0.03–0.20% (w/w) relative to sample concentration (20 mg/mL). Seven concentrations ($n = 7$) were prepared across the range and injected in triplicate. The mean area ($n = 3$) calculated was plotted against the concentration. The coefficient of determination (r^2), slope and intercept are presented in Table 2. The slope of the calibration curve for MEP was 0.038 times the slope value of olefin impurity. This indicated that the response of olefin impurity was about 26 times higher than MEP. Hence, the RRF of olefin impurity was 0.038 with respect to

Table 1
Comparative ^1H and ^{13}C NMR assignments for MEP and olefin impurity.

Position[a]	MEP			Position[a]	Olefin impurity		
	^1H (ppm), multiplicity	^{13}C (ppm)	DEPT		^1H (ppm), multiplicity	^{13}C (ppm)	DEPT
1, 1′	3.91(s, 4H)	68.6	2× CH_2	1, 1′	3.96(s, 4H)	68.7	2× CH_2
2	–	37.4	–	2	–	40.1	–
3, 4	1.28(m, 4H)	36.9, 16.4	2× CH_2	3	5.41(dq, 1H)	132.5	CH
5	0.91(t, 3H)	14.9	CH_3	4	5.56(qd, 1H)	125.7	CH
6	0.92(s, 3H)	18.9	CH_3	5	1.69(dd, 3H)	18.4	CH_3
7, 7′	–	157.0	–	6	1.05(s, 3H)	19.4	CH_3
8, 8′	4.79(brs, 4H)	–	–	7, 7′	–	156.7	–
				8, 8′	4.67(brs, 4H)	–	–

s, singlet; d, doublet; t, triplet; m, multiplet; dd, double doublet; brs, broad singlet; dq, doublet of quartet; qd, quartet of doublet.
[a] Refer structures (Fig. 1) for numbering.

Table 2
Method validation data for MEP and olefin impurity.

Validation parameter	Results			
	MEP	Olefin impurity		
Linearity				
Calibration range (%, w/w)	0.028–0.170	0.033–0.197		
Calibration points	7	7		
Slope	44,716	1,191,726		
Intercept	−27	−105		
Coefficient of determination (r^2)	0.990	0.999		
Relative response factor (RRF)	1.000	0.038		
DL, QL				
Detection limit (%, w/w)	0.009	0.0003		
Quantification limit (%, w/w)	0.028	0.001		
Precision at QL (n = 6, % R.S.D.)	6.9	2.4		
Repeatability				
Concentration (%, w/w)		0.05	0.10	0.20
Standard precision (n = 6, % R.S.D.)		0.9	0.5	0.3
Intermediate precision				
Concentration (%, w/w)		0.05	0.10	0.20
Set-I[a] (n = 6, % R.S.D.)		1.2	1.1	0.7
Set-II[b] (n = 6, % R.S.D.)		1.5	0.8	1.1
Overall % R.S.D. (n = 12)		1.8	2.6	1.1
Accuracy				
Added (%, w/w)		0.055	0.109	0.164
Recovered (%, w/w)		0.050	0.099	0.151
% Recovery		91.5	91.1	92.3
% R.S.D.		2.3	1.5	0.4

[a] Set-I = Analyst 1, Column 1, HPLC 1, and Day 1.
[b] Set-II = Analyst 2, Column 2, HPLC 2, and Day 2.

MEP. The content of olefin impurity obtained by HPLC-UV method against MEP should be multiplied with 0.038 (RRF) to get the actual content to avoid overestimation. The DL and QL for MEP and olefin impurity were determined by signal to noise (S/N) ratio method. The DL and QL data obtained are presented in Table 2. Accuracy was validated through recovery experiments by spiking known amount of olefin impurity at 0.05, 0.10 and 0.15% with MEP relative to sample concentration (20 mg/mL). Each preparation was analyzed in triplicate (n = 3) and percent recovery was calculated (Table 2).

3.4.3. Repeatability and intermediate precision

To determine repeatability, olefin impurity standard solution was prepared at three different concentration levels and the results are presented in Table 2. The intermediate precision of the method was evaluated by performing MEP sample analysis in six replicates using two different columns, different instruments and different analysts on different days. MEP sample spiked with olefin impurity at 0.05, 0.10, and 0.20% (w/w) levels were used to establish intermediate precision over the whole concentration range and the results are represented in Table 2.

3.5. Formation of impurity

MEP was synthesized utilizing 2-methyl-2-propyl-propane-1,3-diol (MPPD) as the starting material. MEP is formed by the bis-carbamylation of MPPD in the presence of sodium cyanate and hydrochloric acid. It is proposed that the presence of 2-hydroxymethyl-2-methyl-pentane-1,3-diol as impurity in MPPD can undergo simultaneous bis-carbamylation and dehydration to give carbamic acid-2-carbamoyloxymethyl-2-methyl-pent-3-enyl ester which is the olefin impurity found at 0.65 RRT in the HPLC-UV method.

4. Conclusion

The process-related impurity (olefin) observed in MEP by HPLC-UV method was identified by LC–MS, isolated by preparative LC and characterized by NMR and MS. The RRF value of 0.038 obtained for olefin impurity due to its very high response in UV reveals that the content of this impurity observed based on area normalization against MEP must be corrected with RRF to obtain the true value and to avoid overestimation. Additionally, this RRF value can also corroborate with the published method [20], where, MEP sample showing 0.6 (% area) of olefin impurity by HPLC-UV method was found to have only about 0.02 (% area) by HPLC-RI method [20]. This parameter thus substantiates the high response of olefin impurity in UV method. Hence, HPLC-UV method with the obtained RRF from this investigation can be applied conveniently for the routine monitoring and quality control of olefin impurity in MEP drug substance.

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