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Validated RP-HPLC method for the determination of Meprobamate in bulk and pharmaceutical formulations

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Abstract
Objective: The main objective of the present work is to develop a specific validated HPLC method for the determination of Meprobamate in bulk and pharmaceutical dosage forms.
Method: A reverse phase HPLC method was developed using Bondapak C18 10 µm (3.9 x 300 mm) Capillary column was employed for investigation and mobile phase of composition Acetonitrile: Water in the ratio of 50:50 v/v at a flow rate of 1.0 mL/min with UV detection at 240 nm for Meprobamate.
Results: The retention time of the drug was 7.0 minutes. The developed method was validated for specificity, linearity, precision, accuracy and robustness as per ICH guidelines. Linearity was found in the range of 0.0375-0.225mg/ml. The mean recovery of the drug was 99.6%. The proposed method could be used for routine analysis of Meprobamate in their dosage forms.
Conclusion: The proposed method is accurate, precise, simple, sensitive and rapid and can be applied successfully for the estimation of Meprobamate in bulk and in pharmaceutical formulations without interference and with good sensitivity.

Keywords: Liquid Chromatography; Meprobamate, dosage forms, determination, Validation

Introduction

Drug Profile

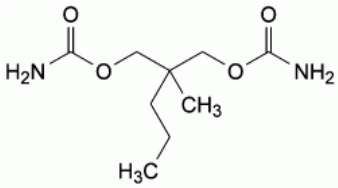

Fig 1: Meprobamate

Meprobamate{[2-(carbamoyloxymethyl)-2-methyl-pentyl] carbamate} binds to GABA receptors [1] [GABA receptors are a class of receptors that respond to the neurotransmitter gamma-aminobutyric acid] which interrupts neuronal communication in the reticular formation and spinal cord, causing sedation and altered perception of pain. It has been shown that meprobamate has the ability to activate currents even in the absence of GABA [2]. This relatively unique property makes meprobamate exceptionally dangerous when used in combination with other GABA-mediated drugs (including alcohol). It is also a potent adenosine reuptake inhibitor (Ado RI) [3, 4] which is most likely responsible for its lower degree of sedation compared to barbiturates. Related drugs include carisoprodol and tybamate (prodrugs of meprobamate), felbamate and mebutamate.

Materials and Methods

Instrument

Peak HPLC containing LC 20AT pump and variable wavelength programmable UV-Visible detector and Rheodyne injector was employed for investigation. A suitable Column - Bondapak C18 10 µm (3.9 x 300 mm) Capillary column was employed for investigation.
Degassing of the mobile phase was done using a Loba ultrasonic bath sonicator. A Denwar Analytical balance was used for weighing the materials.

Chemicals and reagents

The reference sample of Meprobamate was obtained from Ranbaxy, Mumbai. The Formulation Antipyn Forte Tablet was purchased from the local market. Acetone and Hydrochloric acid used were of HPLC grade and purchased from Merck Specialties Private Limited, Mumbai, India.

The mobile phase
A mixture of Acetonitrile: Water in the ratio of 50:50 v/v was prepared and used as mobile phase.

Preparation of Standard Solution
Accurately weigh 150 mg of Meprobamate into 100 ml volumetric flask. To this Add 10 ml of methanol and sonicate for 5 minutes. Dilute to the volume with solvent and mix well. Dilute 10 ml (accurately known) to a 100 ml with solvent

Preparation of Sample Solution
Place 900 ml of dissolution medium to each of 6 the vessels of the apparatus. Assemble the apparatus and allow the temperature of the dissolution medium to equilibrate to 37 °C ±0. 5 °C. Place one tablet into each of the vessels, taking care to exclude air bubbles from the surface of the tablet and immediately operate the apparatus at 50 rpm. At 30 minutes, withdraw 20 ml aliquot from zone midway between the surface of the dissolution medium and the top of the rotating blade, not less than 1 cm from the vessel wall. Filter the sample through a 0.45 µm filter before use, discarding the first few ml of the filtrate.

Method Development
For developing the method [5-10], a systematic study of the effect of various factors was undertaken by varying one parameter at a time and keeping all other conditions constant. Method development consists of selecting the appropriate wave length and choice of stationary and mobile phases. The following studies were conducted for this purpose.

Detection wavelength
The spectrum of diluted solutions of the Zopiclone in mobile phase was recorded separately on UV spectrophotometer. The peak of maximum absorbance wavelength was observed. The spectra of the both Zopiclone were showed that a wavelength was found to be 303 nm.

Choice of stationary phase
Preliminary development trials have performed with octadecyl columns with different types, configurations and from different manufacturers. Finally the expected separation and shapes of peak was succeeded on Bondapak C18 10 µm (3.9 x 300 mm) column.

Selection of the mobile phase
In order to get sharp peak, low tailing factor and base line separation of the components, we carried out a number of experiments by varying the composition of various solvents and its flow rate. To effect ideal separation of the drug under isocratic conditions, mixtures of solvents like methanol, water and Acetonitrile with or without different buffers indifferent combinations were tested as mobile phases on a C18 stationary phase. A mixture of Acetonitrile: Water: in the ratio of 50:50 v/v was proved to be the most suitable of all the combinations since the chromatographic peak obtained was better defined and resolved and almost free from tailing.

Flow rate
Flow rates of the mobile phase were changed from 0.5 – 1.5 mL/min for optimum separation. A minimum flow rate as well as minimum run time gives the maximum saving on the usage of solvents. It was found from the experiments that 1.0 mL/min flow rate was ideal for the successful elution of the analyte.

Optimized chromatographic conditions
Chromatographic conditions as optimized above were shown in Table these optimized conditions were followed for the determination of Meprobamate in bulk samples and its combined tablet Formulations. The chromatograms of standard and sample were shown in Figure

Validation of the Proposed Method
The proposed method [11-29] was validated as per ICH guidelines. The parameters studied for validation were specificity, linearity, precision, accuracy, robustness, system suitability, limit of detection, limit of quantification, Range and solution stability.

Specificity
Specificity of an analytical procedure is its ability to assess unequivocally the analyte in the presence of components that may be expected to be present. The solvent and placebo solutions must contain no components, which co-elute with the Meprobamate. The solutions were injected using the conditions specified in the method of analysis. No components are seen to co-elute with Meprobamate peaks can therefore be considered spectrally pure. The Chromatograms obtained are shown in the Fig: 2 to Fig: 5.

Linearity
The linearity of a dissolution method is its ability to elicit test results, which are directly proportional to the concentrations of drug actives in samples in a given range. Proof of linearity justifies the use of single-point calibrations. The correlation coefficient of the regression line for Meprobamate should be greater than or equal to 0.999. The Y-intercept of the line should not be significantly different from zero, i.e. the assessment value (z) falls within the specified limits only when $+5 > z > -5$. Six solutions containing 25, 50, 75, 100, 125, and 150% of Meprobamate relative to the working concentrations, were prepared and injected according to the method of analysis. A linear regression curve was constructed, and the correlation coefficients (R2) and assessment values calculated. The correlation coefficient (R2) for Meprobamate is 1.0000.The plot is a straight line, and the assessment value (z) for Meprobamate is 1.99. The linearity results are shown in the Fig: 6.

System Suitability
System suitability is a measure of the performance and chromatographic quality of the total analytical system – i.e. instrument and procedure. The requirements for system suitability for this method are: The % RSD of the peak responses due to Meprobamate for the six replicate injections must be less than or equal to 2.0%. Six replicate injections of working standard solution were injected according to the method of analysis. The percentage relative standard deviation (% RSD) for the peak responses was determined. The analytical system complies with the requirements specified by the system suitability. The results are tabulated in the Table 2.

Accuracy

The accuracy of an analytical method expresses the closeness of test results obtained by that method to the true value. The percentage recovery of the active compounds, for each solution prepared, must be within 95.0 – 105.0% of the actual amount. Sample solutions were spiked with known concentrations of Meprobamate to result in concentrations representing respectively 25, 50, 75, 100,125, and 150% relative to the working concentrations. The above samples were injected in duplicate according to the method of analysis. From the accuracy results above, the percentage recovery values for Meprobamate satisfy the acceptance criteria for accuracy across the range of 25% - 150%. The results are tabulated in the Table: 3.

Method Precision

The precision of an analytical procedure expresses the degree of agreement among individual test results when the method is applied repeatedly to multiple sampling of a homogenous sample.

Repeatability

This parameter determines the repeatability of assay results under the same operating conditions over a short period of time. The % RSD due to Meprobamate for the six samples must be less than or equal to 2.0%. Six separate sample preparations of batch 737968 were analysed according to the method of analysis. The % RSD due to Meprobamate concentration for the Dissolution meets the requirements for reproducibility at 1.1%. The results are tabulated in the Tables: 4, 5 and 6.

Range of an analytical procedure is the interval between the upper and lower concentration of analyte in the sample for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy and linearity. Based on the accuracy results, the range for the Dissolution of Antipyn tablets is 37.5 – 225 mg/tablet of Meprobamate which represents 25% to 150% of the working concentration for the meprobamate active.

Results and Discussion

To optimize the RP-HPLC parameters, several mobile phase compositions were tried. A satisfactory separation and good peak symmetry was found in a mixture of Acetonitrile: Water in the ratio of 50:50 v/v and 1.0 mL/min. Flow rate proved to be better than the other mixtures in terms of resolution and peak shape. The optimum wavelength for detection was set at 240nm at which much better detector responses for drug was obtained. As it was shown in Fig 3. the retention times were 7 min for Meprobamate. The number of theoretical plates was found to be indicates efficient performance of the column. A system suitability test was applied to representative chromatograms for various parameters. The results obtained were within acceptable limits and are represented in table: 2. Thus, the system meets suitable criteria.

The correlation coefficient of the regression line for Meprobamate should be greater than or equal to 0.999. The Y-intercept of the line should not be significantly different from zero, i.e. the assessment value (z) falls within the specified limits only when +5 > z > -5. Six solutions containing 25, 50, 75, 100, 125, and 150% of Meprobamate relative to the working concentrations, were prepared and injected according to the method of analysis. A linear regression curve was constructed, and the correlation coefficients (R^2) and assessment values calculated. The correlation coefficient (R^2) for Meprobamate is 1.0000.The plot is a straight line, and the assessment value (z) for Meprobamate is 1.99. The linearity results are shown in the Fig:

The calibration curve was obtained for a series of concentration in the range of 0.2-1.4µg/ml and it was found to be linear. Seven points graphs was constructed. The standard deviation of the slope and intercept were low. The data of regression analysis of the calibration curves are shown in figure 6.

The proposed method has been applied to the assay of T commercial tablets containing Meprobamate. Sample was analyzed for five times after extracting the drug as mentioned in assay sample preparation of the experimental section. The results presented good agreement with the labeled content. Low values of standard deviation denoted very good repeatability of the measurement. Thus it was showing that the equipment used for the study was correctly and hence the developed analytical method is highly repetitive. For the intermediate precision a study carried out by the same author working on the same day on two consecutive days indicated a RSD of 1.2 & 1.4. This indicates good method precision.

The system suitability parameter like capacity factor, asymmetry factor, tailing factor and number of theoretical plates were also calculated. It was observed that all the values are within the limits. The statistical evaluation of the proposed method was revealed its good linearity, reproducibility and its validation for different parameters and let us to the conclusion that it could be used for the rapid and reliable determination of Meprobamate in tablet formulation.

All these factors lead to the conclusion that the proposed method is accurate, precise, simple, sensitive and rapid and can be applied successfully for the estimation of Meprobamate in bulk and in pharmaceutical formulations without interference and with good sensitivity

Table 1: Optimized chromatographic conditions for estimation Meprobamate

Mobile phase	Acetonitrile : Water 50:50 v/v
Pump mode	Isocratic
Mobile phase P^H	4.5
Diluent	The mobile phase
Column	Bondapak C18 10 µm (3.9 x 300 mm)
Column Temp	Ambient
Wavelength	240 nm
Injection Volume	100 µl
Flow rate	1.0 mL/min
Run time	10 min
Retention Time	7 min

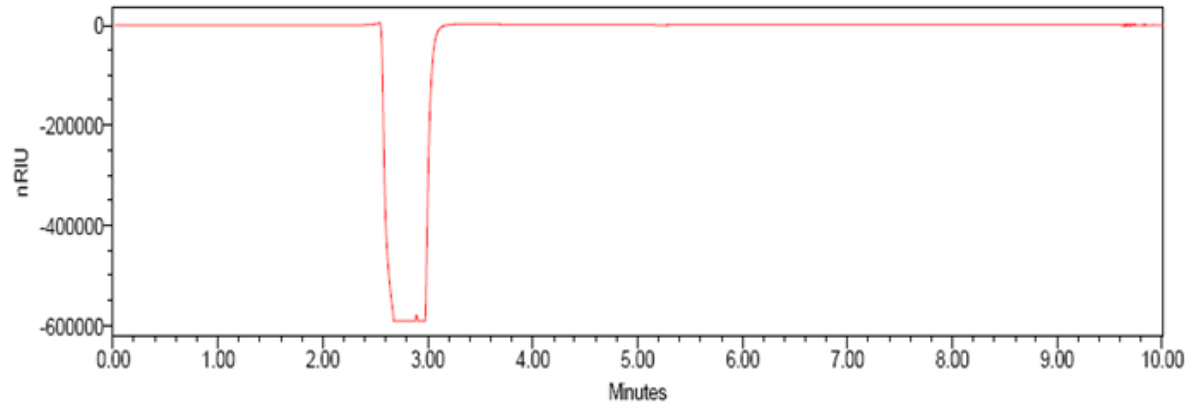

Fig 2: Solvent – No significant peak detected

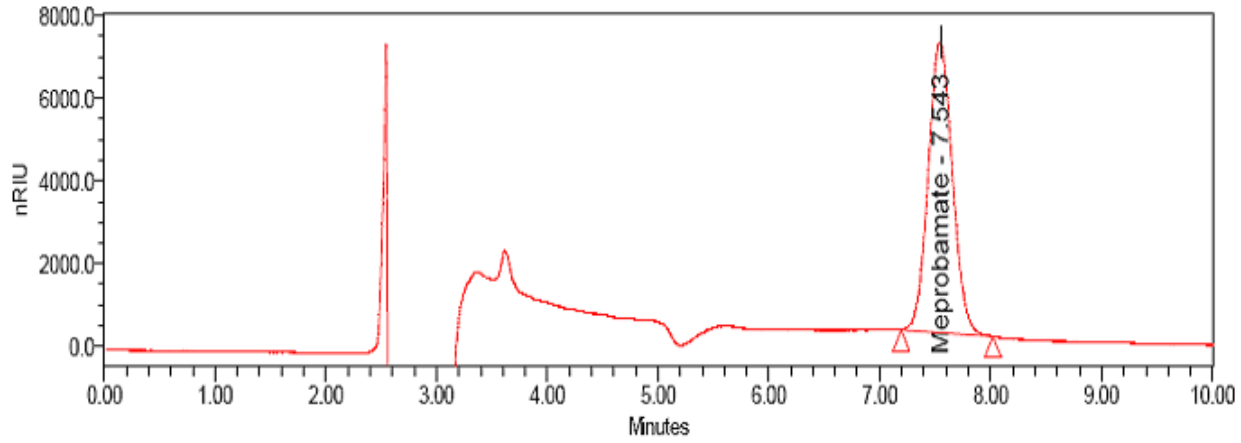

Fig 3: Drug actives – Peak due to Meprobamate.

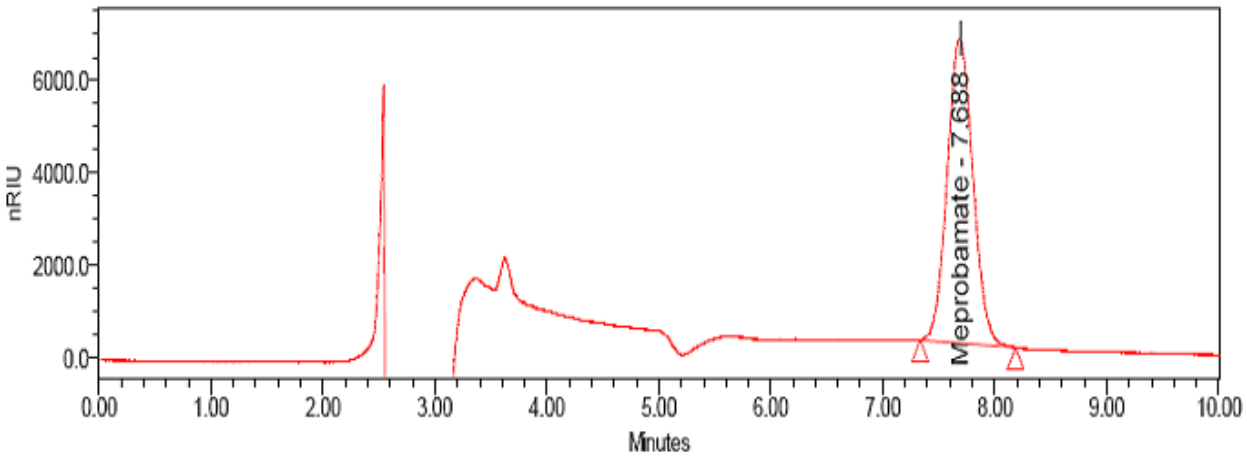

Fig 4: Product – Peak due to Meprobamate.

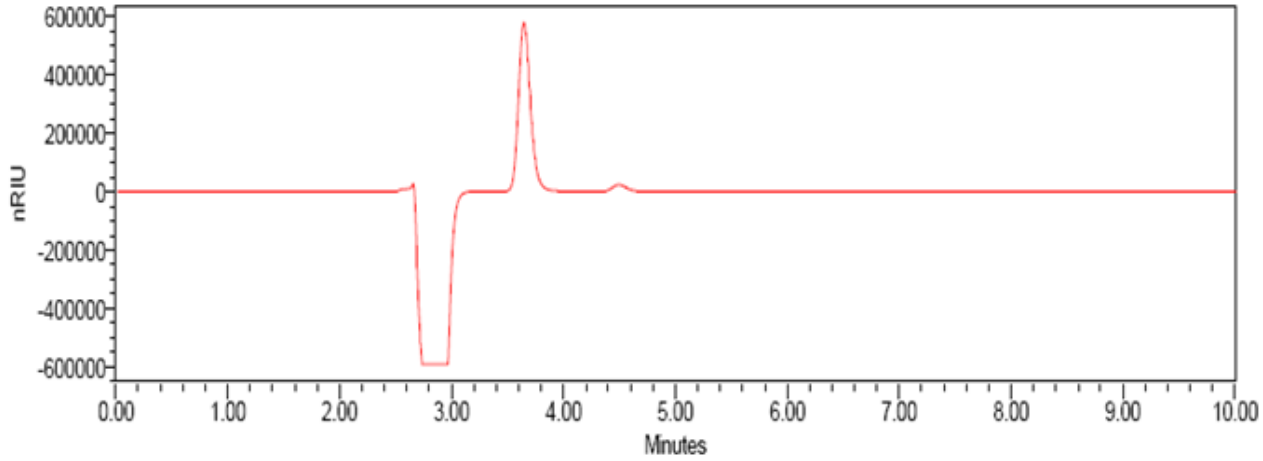

Fig 5: Placebo – No significant peak detected.

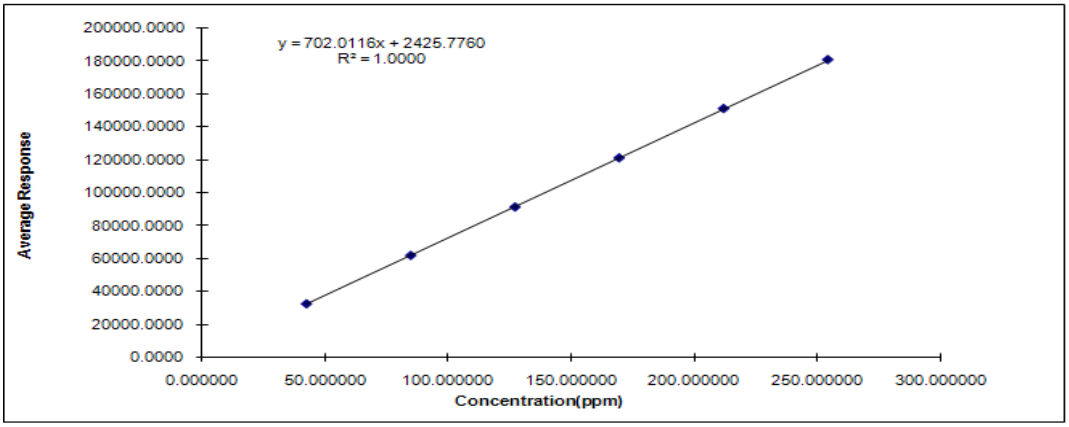

Fig 6: Linearity Results

Table 2: System Suitability Results

Sample	Meprobamate Area
1	108288
2	108258
3	108617
4	108323
5	108486
6	108299
Mean	108378
% RSD	0.1

Table 3: Accuracy Results

Sample	Theoretical	Actual	% Recovery	Average % Recovery
25%	97.8	101.5	103.8	104.1
25%	97.8	102.0	104.3	
50%	97.8	97.2	99.4	99.4
50%	97.8	97.1	99.3	
75%	97.8	97.3	99.5	99.4
75%	97.8	97.1	99.3	
100%	97.8	96.2	98.4	98.4
100%	97.8	96.1	98.3	
125%	97.8	95.7	97.9	98.2
125%	97.8	96.3	98.5	
150%	97.8	95.6	97.8	98.1
150%	97.8	96.2	98.4	

Table 4: Repeatability Results

Sample number	Results
	% Dissolution of Meprobamate
1	97
2	98
3	96
4	98
5	99
6	98
Mean	98
% RSD	1.1

Table 5: % RSD Results

Sample	Results
	% Dissolution of Meprobamate
1	98
2	100
3	101
4	99
5	100
6	101
Mean	100
% RSD	1.2

Table 6: Intermediate Precision and Repeatability Results

Sample	Mean Results
	% Dissolution of Meprobamate
Repeatability	98
Intermediate Precision	100
Mean	99
% RSD	1.4

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