

**DEVELOPMENT AND VALIDATION OF A GAS  
CHROMATOGRAPHIC METHOD FOR THE ASSAY OF  
MEMANTINE HYDROCHLORIDE  
IN PURE AND TABLET DOSAGE FORMS<sup>†</sup>**

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**K. Siddappa<sup>\*</sup>, Metre Mallikarjun, Tambe Mahesh, Kote Mallikarjun,  
Reddy Chandrakanth**

Department of Post-Graduate Studies and Research in Chemistry, Gulbarga University,  
Gulbarga-585106, Karnataka, India

**Abstract.** *A gas chromatographic method has been developed and validated for the determination of memantine hydrochloride (MMT) in pure and pharmaceutical preparations. The detection was carried out using flame ionization detector. Separation was achieved on a DB-624 fused silica packed capillary column (30 m x 0.320 mm x 1.8 µm). Nitrogen was used as a carrier gas at a flow rate of 40 mL/min. The column temperature was maintained at 300 °C while the temperature of injection port and detector were maintained at 270 ° and 300 °C, respectively. Gabapentin (GPN) was used as an internal standard. The procedure gave a linear response over the concentration range of 0.5-3.5 mg/mL with sufficient reproducibility. The method has been applied successfully for the determination of MMT in pure and pharmaceutical formulations. The excipients present in the formulations did not interfere with the assay procedure. The recovery values were found to be in the range of 99.85-100.1% with RSD values less than 1%. The results obtained from this method were compared with the reference method (HPTLC) reported in literature and no significant difference was found statistically.*

**Key words:** *gas chromatography, memantine hydrochloride, pharmaceutical dosage forms*

## 1. INTRODUCTION

Memantine (1-amino-3,5-dimethyladamantane) is a tricyclic amine structurally and pharmacologically related to the antiviral amantadine. This drug is used to treat Parkinson's disease and movement disorders, as well as dementia syndrome. It is usually taken orally as the hydrochloride salt (MMT) [1].

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<sup>\*</sup> Corresponding author. E-mail: siddappa\_65@rediffmail.com

MMT is well absorbed after oral administration and has linear pharmacokinetics over the therapeutic dose range. It acts as a non-competitive inhibitor of the *N*-methyl-D-aspartate (NMDA) receptor complex. The principal mechanism of action of MMT is believed to be the blockade of current through channels of NMDA receptors (a glutamate receptor subfamily) broadly involved in brain function. MMT undergoes little metabolism, with the majority (57-82%) of administered dose excreted unchanged in urine. It is primarily eliminated by kidneys (75-90%) and remaining (10-25%) through bile and feces. The drug undergoes both renal tubular secretion and reabsorption and has a terminal elimination half life of about 60-80 h [2]. It is orally administered as capsule-shaped or film coated tablets.

The therapeutic importance of amantadine has promoted the development of many analytical methods for its quantitation. These methods include HPLC [3, 4] and LC [5], capillary GC [6], capillary electrophoresis [7], potentiometry [8] and fluorimetry [9]. However, only few spectrophotometric methods have been reported for amantadine quantification [10-12]. This is attributed to the absence of chromophores and/or auxochromes in the amantadine molecule. Thus, it shows no distinct absorption in the ultraviolet region above 200 nm, and direct UV spectrophotometry is not useful for its determination. Other techniques, such as HPTLC [13], GC/MS [14], LC [15] and LC/MS [16-18], have been previously used to determine MMT in a variety of matrices. Some studies have proposed potentiometric sensors to be used in the determination of MMT in urine and in its pharmaceutical preparations [19]. Leis *et al.* have reported a GC/MS method for the assay of this drug which involves a very tedious procedure [14]. Another GC method for the assay of amantadine in human plasma was described by Rakestraw [6]. However, this is not a direct method as it is based on capillary gas chromatography using electron-capture detection following derivatization with pentafluorobenzoyl chloride.

The reported analytical assays based on spectrophotometry or HPLC, as well as majority of other mentioned methods, are elaborate, time-consuming and involve sophisticated equipment that might not be available in most analytical laboratories. Literature survey revealed that no attempt has been made for the assay of pure MMT in pharmaceutical preparations using gapapentine (GPN) as an internal standard.

The present work describes a newly developed gas chromatography with flame ionization detection (GC/FID) method for the determination of MMT following a simple sample preparation without any derivatization of pharmaceutical preparations using an internal standard methodology. The developed method was validated *via* linearity, stability, precision, accuracy and sensitivity parameters according to International Conference on Harmonization (ICH) guidelines [20]. The result obtained through the use of this newly developed and validated method was statistically compared to the reference HPTLC method [13].

## 2. MATERIAL AND METHODS

### 2.1. Chemicals and reagents

All used solvents were of HPLC purity. Distilled water was used for the preparation of aqueous solutions. Reference standards of MMT were kindly provided from Sai Ram Organics Pvt Ltd. India. Tablets of MMT were procured from the local market.

## 2.2. Preparation of stock and standard solutions

The stock solutions of MMT and GPN (gapapentine) were prepared separately by dissolving 2.5 g of MMT and 1.0 g of GPN in 50 mL of methanol. These solutions were stored in amber bottles. Appropriate volumes of these solutions were combined and diluted with methanol to obtain standard solutions containing 0.5-3.5 mg/mL of MMT and 20 mg of GPN. Studies on the stability of analytes in standard working solutions showed that there were no decomposition products in the chromatogram during analytical procedure.

## 2.3. Preparation of tablet samples

Ten tablets of MMT were weighted and finely powdered. An accurately weighed quantity of the powdered tablet contents equivalent to 100 mg of the active ingredient was transferred into a 100 mL beaker. Using a mechanical stirrer, the powder was completely disintegrated in methanol. The solution was then filtered off and made up to 50 mL with the same solvent.

## 2.4. GC/FID analysis

A GC-17A (Shimadzu, Japan) equipped with a flame ionization detector (FID) and clarity software were used to analyze the samples. Separation was achieved using a fused silica capillary column DB-624 (30 m × 0.320 mm, film thickness 1.80 μm). Nitrogen was used as a carrier gas at a flow rate of 40 mL/min. The column temperature was maintained at 300 °C while the temperature of the injector and detector were maintained at 270 ° and 300 °C, respectively.

## 2.5. Recommended procedure

### 2.5.1. Chromatographic conditions

Chromatograms of MMT solutions containing 20 mg of internal standard (GPN) were recorded under the above described conditions by injecting 1 μL of the sample solutions into the column. The total time of analysis was found to be less than 20.0 min. Throughout the study, the suitability of the chromatographic system was monitored by calculating the capacity factor ( $k^1$ ), the resolution ( $R_s$ ), the selectivity ( $\alpha$ ) and the peak asymmetry (T).

### 2.5.2. Construction of the calibration graph

Working standard solutions of MMT (0.5-3.5 mg/mL) containing fixed concentrations (20 mg) of GPN were prepared in dichloromethane. Then 1 μL of the solution was injected and a chromatogram was recorded. The values ratio of peak areas of MMT to that of GPN was calculated and plotted against the concentration of MMT to obtain a calibration graph.

### 2.5.3. Assay procedure for pharmaceutical formulations

An aliquot of the drug obtained by following the procedure described for analysis of pharmaceutical preparations was taken and analyzed. The chromatogram at the above said conditions showed a good resolution between MMT and GPN.

### 3. RESULTS AND DISCUSSION

#### 3.1. Method development and optimization of GC conditions

The DB-624 fused silica packed capillary column has been widely used for the separation of a large number of pharmaceutical compounds. This column gave quantitative separation of analyzed compounds in our case as well. After examining the various drugs (mebeverine hydrochloride, flavoxate hydrochloride, gapapentine (GPN), ritodrine hydrochloride, hydralazine hydrochloride, bipyridine hydrochloride) as potential internal standards, it was found that GPN gave proper resolution, less time for analysis and good chromatographic behavior compared to other tested drugs. Various solvents (like ethanol, dichloromethane, methanol, *n*-heptane and etc.) were evaluated as well. The most satisfactory resolution and recoveries were observed with dichloromethane so it was used as the extracting and injecting solvent.

The method development for the assay of MMT was based on its chemical properties. The gas chromatography with flame ionization detection (GC/FID) parameters are used in the method development were based on the boiling point. Different temperature programs were investigated for exception of matrix interference. At the end of this investigation, the best temperature program was selected for a good resolution and thus for all experiments the oven temperature program described in section 2.4 was used. When the ramp rate was more or less 10 °C/min good resolution of the peaks (analyte peak and matrix interference peaks) were not obtained. As shown in Fig. 1, a good separation of MMT from internal standard was achieved and no further matrix interfering peaks at the retention times of MMT ( $t_R = 8.7$  min) and GPN ( $t_R = 5.0$  min) were observed. The reproducibility of the retention times of MMT and GPN was calculated based on the average of five determinations. Chromatograms of standard and drug sample gave very good peak shapes.

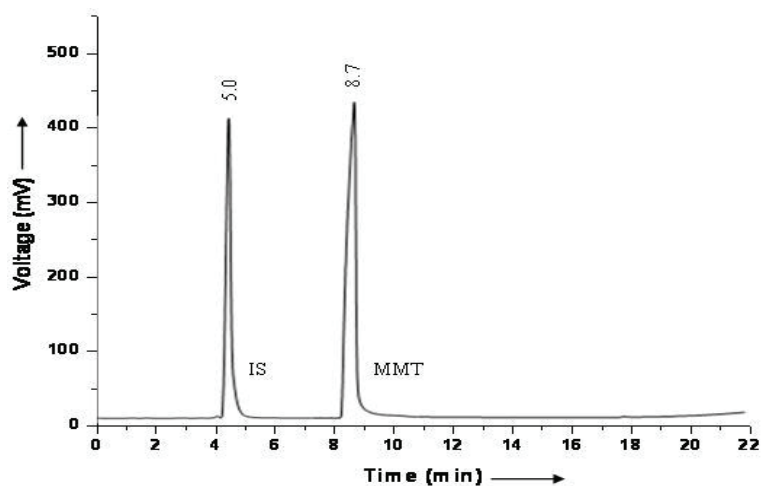


Fig. 1. A typical chromatogram showing the separation of GPN (20 mg/ml) and MMT (30 mg/ml) in pure form.

### 3.2. Suitability of the method

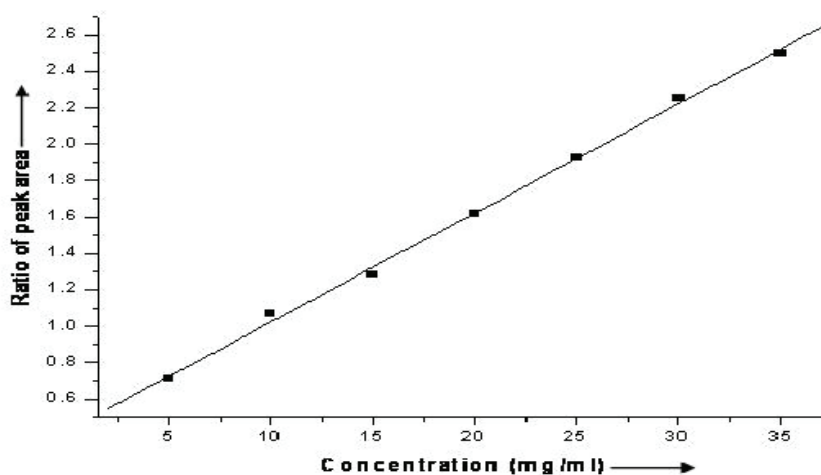
The chromatographic parameters such as resolution, selectivity, capacity factor and peak asymmetry were found to be satisfactory. The values of these parameters are tabulated in Table 1.

**Table 1.** System performance parameters for MMT and GPN

Parameters	GPN	MMT
Retention time ( $t_R$ )	5.0	8.7
Capacity factor ( $k^1$ )	2.75	1.12
Peak asymmetry (T)	0.4	1.0
Selectivity factor ( $\alpha$ )	0.567	
Resolution (Rs)	52.0	
Height equivalent to theoretical plate (HETP) in mm	$4.14 \cdot 10^{-6}$	

### 3.3. Linearity of detector response

Linearity and range of the developed method were determined by analyzing different concentrations of the mixed standard solution containing 0.3-3.0 mg/mL of MMT and 20 mg of GPN under the chromatographic conditions ( $n = 5$ ) mentioned above. The response factor of the standard solutions was calculated. The ratio of peak area of MMT to that of GPN was plotted against the concentration of MMT to obtain the calibration graph (Fig. 2) and was found to be linear over the concentration range of 0.5-3.5 mg/mL of MMT. The data were analyzed by linear regression least-squares method and the corresponding equation is given by  $Y = bX + c$ , where 'Y' is the ratio of the peak areas values of MMT and GPN, 'b' is the slope, 'c' is the intercept and 'X' is the concentration of the analyte. Linear regression least squares fit data are given in Table 2.



**Fig. 2.** Calibration graph for MMT.

### 3.4. Limits of detection and quantification

The limit of detection (LOD) was established at a signal-to-noise ratio (S/N) of 3 while the limit of quantification (LOQ) was calculated at a signal-to-noise ratio (S/N) of 10. The LOD and LOQ were calculated to be 0.79 mg/mL and 2.6 mg/mL, respectively. The results are shown in Table 2.

**Table 2.** Linear regression least squares fit data for the determination of MMT

Parameters	MMT
Linear dynamic range [mg/mL]	0.5-3.5
Regression equation (Y)*	
Slope (b)	0.059
Intercept (a)	0.425
Correlation coefficient (R <sup>2</sup> )	0.9990
RSD [%]	1.24
Error [%]	-0.78
LOD [mg/mL]	0.79
LOQ [mg/mL]	2.63

\*Y = bX + c, where 'Y' represents the ratio of MMT peak area to GPN peak area and 'X' represents the concentration of MMT.

### 3.5. Precision and accuracy

By analyzing five replicates of fixed amount of MMT, the precision and accuracy of the proposed method was examined. The precision of the method was calculated in terms of the relative standard deviation. Low values of percentage relative standard deviation and percentage error indicated high precision and accuracy of the proposed method. The results are summarized in Table 2.

### 3.6. Comparison of chromatographic (HPTLC and GC/FID) methods

The suggested GC/FID method was applied to the analysis of two dosage forms containing MMT without interference from excipients encountered in pharmaceutical preparations using internal standard methodology. The developed and validated GC/FID method was also statistically compared with reference HPTLC method [15]. The linear concentration range of the proposed and reference method was 0.5-3.0 mg/mL and 5-40 µg/spot, respectively. The average recovery value for MMT in 100 mg tablet composites ranged from -0.12 to 0.10% for the proposed method, while for the reference method it was found to be 0.07 to 0.08%. The RSD values obtained from recovery studies ranged from 0.03 to 0.12%, which indicated high accuracy and precision of our method. In the reference method, the average recovery value ranged from 0.08 to 0.25%. The results indicating that there was no significant difference between the proposed and reference method (Table 3).

### 3.7. Analysis of pharmaceutical preparations

The applicability of the proposed method was examined by analyzing MMT in various pharmaceutical preparations at different concentration levels and the results are shown in Table 3. Low values of relative standard deviation indicated high precision of the proposed method. Recovery values were found to be satisfactory.

**Table 3.** Analysis of MMT in pharmaceutical formulations

Method	Formulation	Labelled amount [mg/tablets]	Amount recovered*	RSD [%]	Recovery [%]
Proposed	Neaman <sup>†</sup>	100	99.90	0.03	-0.12
	Amantrel <sup>‡</sup>	100	101.10	0.12	0.10
Reference	Admantine [ $\mu\text{g}/\text{spot}$ ]	10	10.01	0.08	0.07
		15	14.81	0.25	0.08

\*Average of five determinations.

<sup>†</sup>Marketed by Nestor Ltd. India.<sup>‡</sup>Marketed by Protec. Pharmaceuticals, India.

#### 4. CONCLUSIONS

This study showed that the antiviral drug, MMT can be precisely and accurately determined in pure and pharmaceutical dosages. The proposed method is simple and requires less time for analysis. System performance parameters revealed that the method is ideal for the assay of MMT. For this reasons, it can be used for the determination of MMT in pharmaceutical preparations in routine quality control measurements.

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## RAZVIJANJE I VALIDACIJA GASNO-HROMATOGRAFSKE METODE ZA ODREĐIVANJE MEMANTIN-HIDROHLORIDA U ČISTOM OBLIKU I TABLETAMA

**K. Siddappa, Metre Mallikarjun, Tambe Mahesh, Kote Mallikarjun, Reddy  
Chandrakanth**

*Razvijena je i validirana gasno-hromatografska (GC) metoda za određivanje memantin hidrohlorida (MMT) u čistom obliku i farmaceutskim preparatima. Detekcija je vršena korišćenjem plameno-jonizujućeg detektora. GC razdvajanje je vršeno na kapilarnoj koloni DB-625 (30 m × 0.320 mm, debljina filma 80 μm). Kao noseći gas korišćen je azot, čiji je konstantni protok održavan na 40 ml/min. Temperature injektora i detektora su održavane na 270 °C i 300 °C, dok je temperatura kolone bila 300 °C. Gabapentin (GBN) je korišćen kao interni standard. Metoda je dala linearni odziv u opsegu koncentracija od 0,5 do 3,5 mg/mL sa zadovoljavajućom reproduktivnošću i uspešno je primenjena za određivanje MMT i u čistom obliku i u farmaceutskim formulacijama. Utvrđeno je da pomoćne supstance u farmaceutskim formulacijama ne smetaju pri određivanju MMT ovom metodom. Nađeno je da je rikaveri u opsegu od 99,85 do 100,1% sa RSD vrednostima manjim od 1%. Poređenjem rezultata dobijenih ovom metodom sa rezultatima referentne metode (HPTLC) nađeno je da među njima nema statistički značajne razlike.*

**Ključne reči:** *gasna hromatografija, memantin hidrohlorid, farmaceutske formulaacije*