

PREFORMULATION STUDIES FOR A PARENTERAL SOLUTION OF MEMANTINE

MARCELA ACHIM^{1*}, LAURIAN VLASE¹, IOAN TOMUȚĂ¹, DANA MUNTEAN¹, CRISTINA IUGA², RADU GEORGESCU³, SORIN LEUCUȚA¹

¹*Department of Pharmaceutical Technology and Biopharmaceutics, University of Medicine and Pharmacy "Iuliu Hațieganu", Cluj-Napoca, 8 V.Babeș, 400012, Romania;*

²*Department of Drug Analysis, University of Medicine and Pharmacy "Iuliu Hațieganu", Cluj-Napoca, 8 V.Babeș, 400012, Romania;*

³*Department of Pharmacology, University of Medicine and Pharmacy "Iuliu Hațieganu", Cluj-Napoca, 8 V.Babeș, 400012, Romania*

**corresponding author: machim@umfcluj.ro*

Abstract

Preformulation studies regarding a physico-chemical evaluation of memantine, a non-competitive N-methyl-D-aspartate (NMDA) receptor antagonist, are presented in this work. This characterization of memantine is pre-required for the formulation of a parenteral solution. The water solubility, the thermal behavior and the chemical stability in aqueous solution, depending on the pH, temperature, UV light and the presence of oxidizing or isotonicising agents were determined.

A LC/MS/MS analytical method for memantine hydrochloride quantification was developed and validated. It was determined that memantine hydrochloride has a water solubility of 47.035 mg/mL (20°C). Thermal analysis revealed that it is an anhydrous substance, presenting a crystalline form, with the melting point at 297.16°C. In aqueous solution memantine hydrochloride is stable at room temperature (20°C), regardless the pH of medium, UV light, isotonicising agent or oxidizing agent and at 80°C, at acidic pH value. These results indicate that memantine hydrochloride has appropriate physico-chemical properties for a parenteral solution formulation.

Rezumat

În această lucrare sunt prezentate studii de preformulare constând într-o evaluare fizico-chimică a memantinei, un antagonist necompetitiv al receptorilor N-metil-D-aspartat (NMDA). Această caracterizare a memantinei este necesară în vederea formulării unei soluții parenterale. S-a urmărit determinarea solubilității în apă, a comportamentului termic și a stabilității chimice în soluție apoasă, în funcție de pH, temperatură, lumină UV și prezența unor agenți oxidanți sau izotonizanți.

S-a dezvoltat și validat o metodă analitică LC/MS/MS pentru dozarea clorhidratului de memantină. S-a determinat o solubilitate în apă de 47.035 mg/mL (20°C). Analiza termică a relevat faptul că memantina clorhidrat este o substanță anhidră, cristalină, cu punctul de topire la 297.16°C. În soluție apoasă, clorhidratul de memantină este stabil la temperatura camerei, indiferent de pH, lumină UV, prezența unor agenți oxidanți sau izotonizanți, ca și la 80°C, la pH acid. Aceste rezultate indică faptul că memantina are proprietăți fizico-chimice adecvate pentru formularea unei soluții parenterale.

Keywords: memantine, LC/MS/MS, preformulation

Introduction

Memantine hydrochloride (MMH), 1-amino - 3,5 - dimethyl adamantane hydrochloride is an adamantine derivative administered orally for many neurologic disorders. It is a low-affinity to moderate-affinity, uncompetitive N-methyl-D-aspartate (NMDA) receptor antagonist [14]. MMH was first synthesized in the 1960s, and was found in the 1980s to inhibit NMDA receptors [11]. Orally administered MMH's neuroprotective effect was intensely studied and demonstrated in patients with vascular dementia, Alzheimer disease, hemorrhagic stroke and neuropathic pain [13, 15]. MMH is available as tablets (5 and 10 mg) and oral solution (20 mg/mL).

NMDA receptors are also involved in the neuronal degradation process in cases of deprived oxygen supply (ischemia) [9]. Due to similar mechanisms of neuronal injury in Alzheimer disease and in cerebral ischemia, arose the idea of testing MMH in providing neuroprotection in cerebral ischemia after parenteral administration. In order to prepare a parenteral solution, preformulation studies are required to evaluate the specific physico-chemical properties of MMH for this type of formulation.

The aim of this work was the physico-chemical evaluation of MMH regarding the water solubility, thermal behavior and chemical stability in aqueous solution in different conditions. The development of a new high-throughput liquid chromatography coupled with mass spectrometry (LC/MS/MS) direct method for quantification of MMT in pharmaceutical formulations was also an important objective.

Materials and methods

Materials

MMH was purchased from Sigma-Aldrich (Germany). Methanol, formic acid, KH_2PO_4 , sodium citrate, H_2O_2 , NaCl, glucose were Merck products (Germany). All other chemicals used were reagent grade.

MMH assay

The quantification of MMH was performed in multiple reaction monitoring (MRM) mode using an ion trap mass spectrometer equipped with an atmospheric pressure electrospray ionization ion source (ESI), positive mode. The HPLC system was an Agilent 1100 series (binary pump, autosampler, thermostat) (Agilent Technologies, USA) and was coupled with a Bruker Ion Trap SL (Bruker Daltonics GmbH, Germany). The analyte was separated on a reversed phase column (Zorbax SB-C18, 100

mm x 3.0 mm I.D., 3.5 μ m) under isocratic conditions using a mobile phase of a 55:45 (v/v) mixture of methanol and 0.1% formic acid in water [17,6,12,8,16,10]. The flow rate was 1 mL/min at the column temperature 45°C. Preparing for this study, a series of calibration solutions containing the appropriate standards or spiked *placebo* were made by adequate dilutions of concentrated stock solutions. The typical concentration range of these calibration solutions was from 0.1 to 1.1 μ g/mL that covered the 20-120% range of concentrations in the dissolution study, after a 1:10 dilution.

In order to perform the validation of the method, the following statistical tests were used: Cochran test, Fisher test and Student t test [18]. The Cochran test estimates the variance in the group divided by sum of variances of the entire group. If $C_{calc} < C_{theoretical}$ the test is true, i.e. the variance is homogeneous. This test was applied to evaluate the linearity, the accuracy, and the reliability of the method. The Fisher test is mainly used for variance comparison. The ratio of two variances is compared with the $F_{theoretical}$ value. If $F_{cal.} < F_{theoretical}$ the difference is not significant, the two variances are coherent. This test is performed to control the least square regression (linearity): the slope must be significantly different to 0 ($F_{cal.} > F_{theoretical}$) and the linearity adjustments must be non-significant ($F_{cal.} < F_{theoretical}$). It is also applied to evaluate the validity of average recovery (accuracy) [18].

Solubility determination

Saturated solutions of MMH were prepared at 20°C, 48 hours (the solutions were homogenized every 12 hours). Filtered samples were successively diluted and analyzed by the LC/MS/MS method.

Thermal analysis

The thermal properties were studied by thermogravimetry analysis (TGA) and differential scanning calorimetry (DSC). Samples were analyzed using Mettler Toledo equipments, model TGA/SDTA851e and model 822e/700/984 DSC (Suisse). Experimental conditions applied for TGA analysis were: temperature range from 25 to 800°C with a heating rate of 10°C/min; DSC analysis was performed in a temperature range from 25 to 400°C with a heating rate of 2.5°C/min.

Stability studies in aqueous solution

The stock solution of MMH of 1 mg/mL was prepared by dissolving appropriate quantities of reference substance in methanol. One subsequent 1/100 dilution of stock solution with distilled water gave a working solution of 10 μ g/mL MMH concentration. The last 1/100 dilution was made using working solution and different solvents (table I) in order to finally obtain a

100 ng/mL MMH solution, which was subjected to exposure to five different types of stress conditions: pH (pH 1, 3, 5, 7, 9, 11), temperature (20°C and 80°C), light (darkness and UV light), isotonicising agent (sodium chloride and glucose) and oxidizing agents (H₂O₂) (Table I).

Table I
The experiments run for the stability study of MMH

Stress factor / Solution			Stress factor / Physico-chemical conditions		
			temperature 20°C	temperature 20°C	temperature 80°C
			darkness	UV light	darkness
pH	1	buffer	E01	E11	E18
pH	3	buffer	E02	E12	E19
pH	5	buffer	E03	E13	E20
pH	7	buffer	E04	E14	E21
pH	9	buffer	E05	E15	E22
pH	11	buffer	E06	E16	E23
pH	6-7	distilled water	E07	E17	E24
pH	7	glucose 5% in water	-	-	E25
pH	3	glucose 5% in buffer	-	-	E26
pH	9	glucose 5% in buffer	-	-	E27
pH	7	sol NaCl 0.9% in buffer	-	-	E28
pH	3	sol NaCl 0.9% in buffer	-	-	E29
pH	9	sol NaCl 0.9% in buffer	-	-	E30
pH	6-7	sol H ₂ O ₂ 1.5 mM in water	E08	-	-
pH	3	sol H ₂ O ₂ 1.5 mM in buffer	E09	-	-
pH	9	sol H ₂ O ₂ 1.5 mM in buffer	E10	-	-

At these solutions we added samples represented by MMH solutions in distilled water containing NaCl 0.845% and maintained 20 minutes at 120°C (steam sterilization conditions).

Results and Discussion

MMH assay

Memantine hydrochloride (MMH) is a primary amine (figure 1) possessing neither prominent UV absorption, nor fluorescence properties, nor even electroactive groups. Sample pretreatment with derivatization techniques coupled with gas chromatography (GC), high performance liquid chromatography (HPLC), capillary electrophoresis (CE) are frequently used for enhancing sensitivity of quantification methods [7].

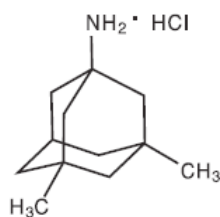


Figure 1
Chemical structure of MMH

A new high-throughput liquid chromatography coupled with mass spectrometry (LC/MS/MS) direct method (without derivatization) for quantification of MMH in pharmaceutical formulations was elaborated and validated. The ion transition monitored was m/z 180 $\rightarrow$ m/z 136, corresponding to a loss of ammonia from the protonated pseudomolecular ion of MMH (figure 2). In the described chromatographic conditions, the retention time of MMH was 1.80 minutes (figure 3).

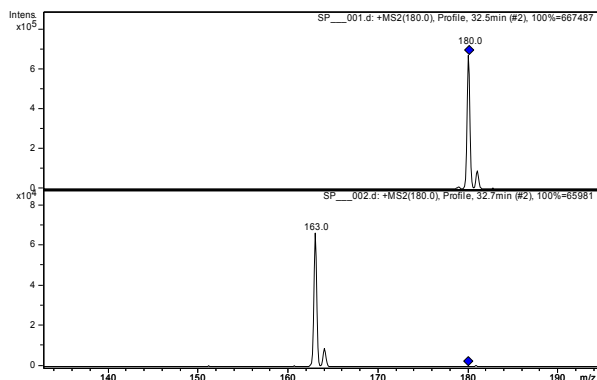


Figure 2
Mass spectra of MMH; upper- full scan spectra, lower- MS/MS spectra

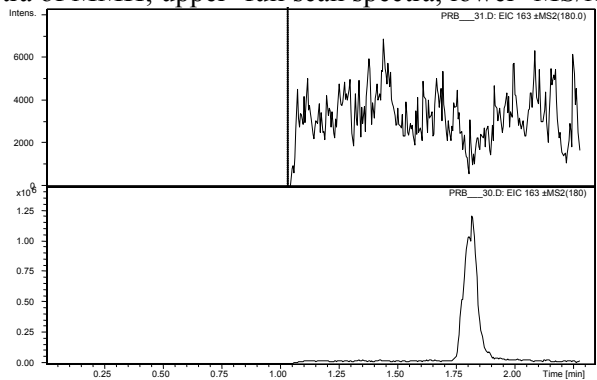


Figure 3
Chromatograms for a blank(upper trace) and a sample with MMH (lower trace)

The statistical evaluation of the linearity study is presented in table II. The obtained statistical parameters demonstrated that the method had a good linearity over the considered concentration range. The study of accuracy was realized for the reconstituted dosage forms, on 5 concentrations (the same concentrations used in the linearity study). The analyses were repeated in 3 days (table III).

Table II
Linearity

Tests	Standard sample (S.R.)	Spiked placebo sample	Theoretical values
Curve regression equation	$y = 7116853x + 113166$	$y = 6969766.x - 150971$	
Correlation coefficient	0.998	0.992	
Cochran Test - homogeneity of variance (C_{calc})	0.434	0.411	$Ct(0.05; 5; 2) = 0.68$
Fisher Test - validity of regression (F_{calc})	-2.255	-0.878	$Ft(0.05; 3; 10) = 3.71$
Fisher Test - significant slope (F_{calc})	3389.353	2293.531	$Ft(0.05; 1; 13) = 4.67$
Student t Test - comparative test of intercept with 0	3.498	1.403	$t(0,05; 13) = 2.160$
Student t Test - comparative test of slope (t_{calc})	0.774		$t(0,05; 26) = 2.056$
Student t Test - comparative test of intercept (t_{calc})	-1.159		$t(0,05; 26) = 2.056$

Table III
Accuracy

Test	Calculated values	Theoretical values
Cochran Test - comparative test of the homogeneity of variance	$C_{calc} = 0.392$	$C(0,05;5,2) = 0.68; n_j = 3$
Fisher test - validity of average recovery	$C_{calc} = 3.152$	$F(0,05;4;10) = 3.48$
Confidence interval	94.41 - 102.77	$t(0,05; 14) = 2.145$

Table IV
Reliability

Test		Value
Cochran test - comparative test of the homogeneity of variances intra-group	$Ct(0.05; 5; 2) = 0.68$	0.801
Intra - day precision	CVr	5.63%
Inter - day precision	CVR	7.32%

Reliability was appreciated by calculating the intra-day and inter-day variation at 100% theoretical concentration in 6 replicates. The results were shown in table IV.

Solubility

The solubility is an essential property for solutions preparation. The oral solution of MMH contains 20 mg/mL and a previously published paper [4] indicates a MMH water solubility of 35 mg/mL. We determined the water solubility of MMH at 20°C of 47.035 ± 2.83 mg/mL. The same solubility can be expressed as 1: 21.26 (21.26 mL water required to dissolve 1g MMH). In accordance with 6th European Pharmacopeia [1], this value indicates that the descriptive term adequate for MMH solubility is „soluble” (for soluble, 10-30 mL water required to dissolve 1 g substance).

Thermal analysis

The TGA and DSC techniques can provide qualitative information regarding the physico-chemical state of the drug. As TGA thermogram shows (figure 4), in the interval 25-110°C MMH is stable, without weight loss, which demonstrates the presence of the anhydrous form. A complete weight loss is occurring between 190-300°C and this corresponds to the complete degradation of MMH. In the described experimental conditions, the DSC thermogram (figure 5) indicates the melting point at 297.16°C followed by a slowly thermal decomposition, in two steps: the first endothermic peak at 304.13°C and the second one at 307.20°C. In some previously published papers, reported values for the melting point varied between 258 and 332°C [2,3,4]. The endothermic peak at 316.05°C indicates the final oxidative degradation. Due to the presence of a fixed melting point value, we can conclude that MMH is being in the crystalline form.

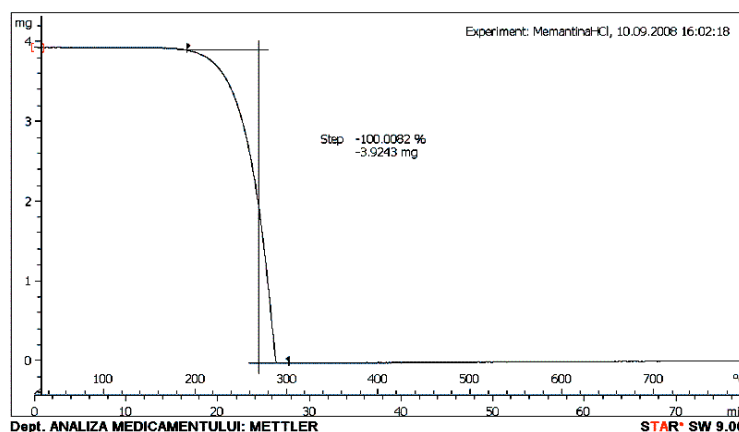


Figure 4

Memantine hydrochloride TGA thermogram

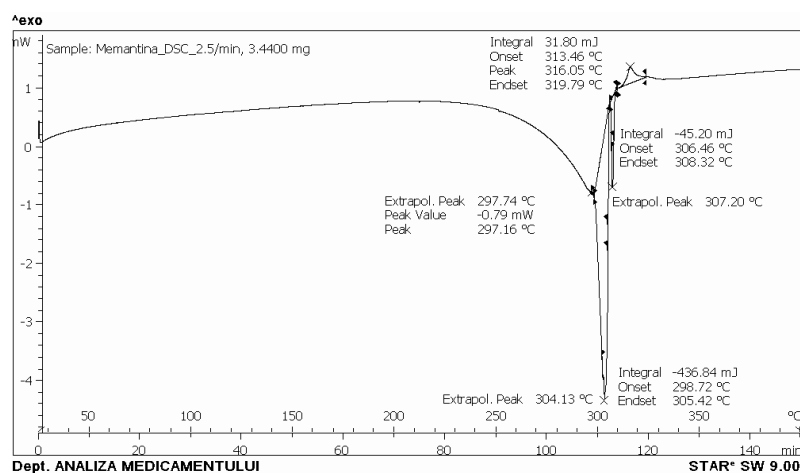


Figure 5
Memantine hydrochloride DSC thermogram

Stability studies in aqueous solution

The objective of these stability studies was to evaluate the MMH chemical stability in aqueous medium depending on the pH, temperature, light, isotonsing and oxidizing agents. Thirty experiments were carried out in the solutions the MMH assay was performed by the LC/MS/MS method previously presented. MMH concentrations were normalized using the percentage concentration (at the beginning of the experiments, MMH was considered 100%). Significant results were considered only the results situated outside the interval 94-106%, because the accuracy of the analytical method was $\pm 6\%$ (table III).

Table V
Results of MMH stability study in aqueous solution

	% MMT remains	Time (hr)		% MMT remains	Time (hr)		% MMT remains	Time (hr)
E01	96.05	6	E11	94.65	5	E21	101.08	45
E02	96.33	6	E12	98.38	5	E22	98.67	45
E03	98.91	6	E13	103.45	5	E23	19.62	45
E04	100.63	6	E14	99.04	5	E24	97.73	45
E05	102.76	6	E15	92.05	5	E25	98.21	23
E06	95.88	6	E16	89.65	5	E26	103.67	23
E07	96.41	6	E17	96.04	5	E27	97.12	23
E08	92.12	8.75	E18	97.43	45	E28	101.35	23
E09	93.42	8.75	E19	96.06	45	E29	98.7	23
E10	76.45	8.75	E20	98.53	45	E30	99.17	23

The results presented in table V indicate that, generally, MMH proved a good chemical stability, especially at room temperature regardless the pH of medium, UV light, isotonsing agent or oxidizing agent and at

80°C, at acidic pH value. These results are in accordance with other previously published data [5].

At 20°C, MMH stability was not influenced by the pH. The presence of the oxidant agent (H_2O_2) determined the MMH chemical degradation, depending on the pH. An acidic pH (pH 3) (E9) or neutral (distilled water) (E8) led to a moderate degradation of MMT (7-8 % degradation in 8.75 hrs), while in alkaline solution (pH 9) (E10) the degradation was accentuated (24% degradation in 8.75 hrs). This different behavior function of pH may be easily explained using the chemical structure of MMH. In acidic or neutral medium, the pH value is smaller than MMH pKa value and the MMH is protonated at the amino group. The protonation of the amino group increases the molecule resistance to oxidation, due to the loss of nucleophilic character of the nitrogen atom. At pH 9, a high proportion of MMH is under the base form and the nitrogen atom has an increased nucleophilicity which favors the oxidation of the amino group by the oxygen radical species resulted from the H_2O_2 .

The UV light (254 nm) has no significant effect on MMH stability at neutral or acidic pH. However, at pH 9 and 11 (E15 and E16), some degradation of MMH was observed (8 % degradation at pH 9 and 11 % degradation at pH 11, in 5 hrs). The explanation of this behavior is based on the same nucleophilic character of MMH in alkaline solution. In these conditions, MMH molecules interact with the free radicals resulting by the water irradiation with UV light.

The combination, high temperature (80°C) and high pH (pH 11) (E23) determined the most important degradation of MMT (81% degradation in 45 hrs).

The isotonic agents (glucose and sodium chloride, in isotonic concentrations) had no influence on MMH stability during 23 hrs at 80°C (E25-E30), regardless of pH, although it was expected a chemical interaction between MMH and glucose at alkaline pH, when there are the favorable condensation conditions between the two substances, with the possibility to result a Schiff base.

In steam sterilization conditions (120°C, 20 minutes), in an isotonic solution (sodium chloride isotonicising agent), MMH was also stable, the percentage of MMH recovered being 99.72%.

Conclusions

The elaborated LC/MS/MS method is accurate, precise and more rapid than the other published analytical methods and can be useful for the rapid quantification of memantine hydrochloride (MMH) in pharmaceutical

dosage forms. The physico-chemical evaluation of MMH carried out to formulate a parenteral solution revealed that MMH has appropriate properties for this objective. So, MMH is a water soluble drug, has a crystalline form and a high melting point. MMH is highly stable and only degrades at alkaline pH under oxidative conditions and elevated temperature. These results suggest that MMH may be formulated as a sterile solution for parenteral administration.

Acknowledgments

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