RESEARCH ARTICLE

Bioanalytical Method Development and Validation of MEM-PLGA-Nanoparticles *via* Nasal Route Administration

Bhavana Ashok Wani*, Sonali Paresh Mahaparale

Dr. D. Y. Patil College of Pharmacy, Akurdi, Pune, Maharashtra, India.

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ABSTRACT

The aim of this effort is to increase extended pharmaceutical release by systematizing PN elaboration for ME, a novel treatment for Alzheimer's disease. Bioanalysis is an essential part in drug discovery and development. Bioanalysis is related to the analysis of analytes (drugs, metabolites, biomarkers) in biological samples and it involves several steps from sample collection to sample analysis and data reporting. A simple, sensitive, and quick LC-MS technique for quantifying MEM in rat plasma was developed and validated. Chromatography was carried out on a C18 column (4.6 × 75 mm, 3.5 µm). ME and its internal standard, NLB, were extracted by direct protein precipitation and were chosen as the sample treatment method rather than liquid-liquid extraction techniques and evaluated with LC-MS/MS in multiple-reaction monitoring (MRM) mode. This approach demonstrated intra- and interday precision in the ranges of 2.1-3.7 and 1.4-7.8%. Furthermore, intra- and interday accuracy ranged from 95.6 to 99.8, and 95.7-99.1%, respectively. MEM and NLB showed mean recovery rates of 86.07 ± 6.87 and 80.31 ± 5.70%, respectively. The stated method was effectively applied in the bioequivalence study of MEM and evaluated its potential for treating Alzheimer's disease via the nasal route. MEM administered via the nasal route will demonstrate improved bioavailability and efficacy in the treatment of Alzheimer's disease compared to current therapies.

Keywords: Alzheimer's, MEM, NLB, LC-MS, PN.

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Conflict of interest: None

INTRODUCTION

Alzheimer's disease is a progressive neurodegenerative disorder that affects millions of people worldwide, and there is currently no cure[1-3]. The development of effective treatments for Alzheimer's disease is a critical area of research, as the aging population is expected to increase the prevalence of the disease, several drugs are currently available for the treatment of Alzheimer's disease, but they only provide temporary relief of symptoms and do not slow down the progression of the disease[4-8]. There is a lack of research on the potential of MEM, a novel compound, for the treatment of Alzheimer's disease via the nasal route. The nasal route has been shown to offer several advantages over other routes of administration, including increased bioavailability and reduced side effects[9-13].

Pathophysiology of Alzheimer Disease:

Figure 1 shows the pathophysiology of Alzheimer Disease Structure

Bioanalytical Method development

Figure 2 shows the fundamental parameters for Bioanalytical method development and validation

MATERIALS AND METHODS

Bioanalytical Method Development for MEM and MEM-PLGA NP:

Chromatographic conditions:

Utilizing a C18 column (100 ×3.0 mm) with a particle size of 2 µm and an isocratic flow rate of 0.4 mL min-1, a mobile phase system comprising 20% ammonium formate buffer pH 9.5 and 80% methanol was employed. MS detector in SIM (single ion monitoring) mode and positive polarity at m/z 289 (for MEM) and 359 (for NLB).

Schematic representation is given in Figure 3.

RESULT AND DISCUSSION

Calibration curve and Linearity Data for Memantine UV absorbance:

Figure 4 shows the Calibration Curve of MEM by UV

Mass Spectrum Fragmentation:

Base peaks which are also protonated molecular ion peaks are selected for additional single-ion monitoring (SIM) detection

*Author for Correspondence: bpkapse24@gmail.com

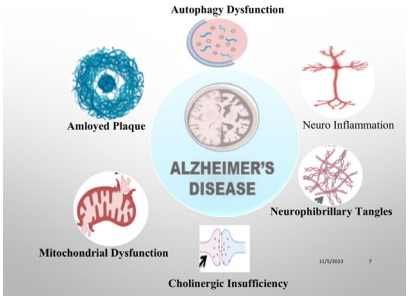

Figure 1: Pathophysiology of Alzheimer Disease Structure

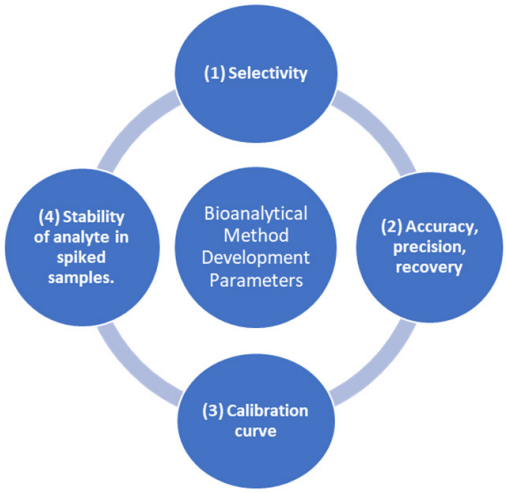

Figure 2: The fundamental parameters for Bioanalytical method development and validation

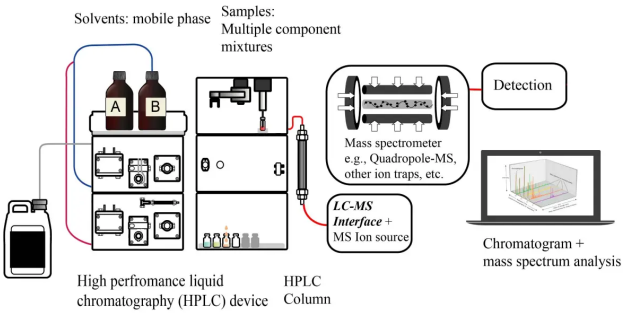

Figure 3: LC-MS system with the Shimadzu® UFLC series trademark was linked to a PC running Lab Solutions LCMS® Software.

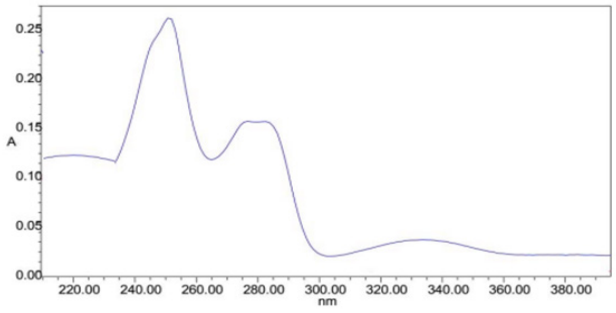

Figure 4: Calibration Curve of MEM by UV

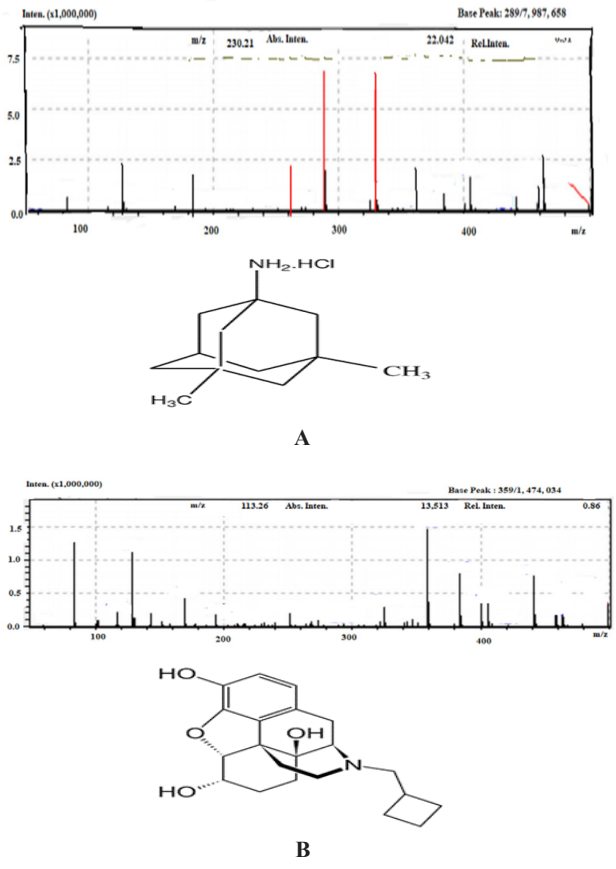

Figure 5: a) MEM and b) nalbuphine's structure and fragmentation pattern utilizing electrospray ionization (ESI) in positive mode from stock injection

as shown in Figure 5a and 5b

Preparation of Calibration Standards and Quality Control Samples

Figure 6 shows the chromatograms for brain homogenate samples taken from rats administered MEM-NP dosage form a without sample preparation using acidification treatment and b) with sample preparation using non acidification treatment; the latter exhibiting a peak at MEM retention.

Method validation

Selectivity and matrix effects:

Figure 7a & b show the blank and zero calibrator chromatograms in the brain homogenate matrix.

Linearity

Table 1 shows the linearity parameters for MEM response factor in different biological matrices

Sensitivity

Figure 8 shows the limit of detection (LOD) was established using an LC chromatogram of a 0.2 ng mL-1 injection that demonstrated a noticeable peak response at MEM retention with a signal-to-noise ratio greater than 3. **Table 2** shows the Intraday and Interaday Precision Data of MEM on Spiked Rat

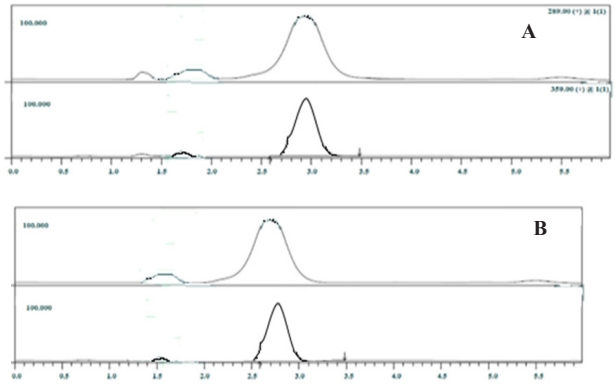

Figure 6: Chromatograms for brain homogenate samples taken from rats administered MEM-NP dosage form a without sample preparation using acidification treatment and b) with sample preparation using non acidification treatment; the latter exhibiting a peak at MEM retention

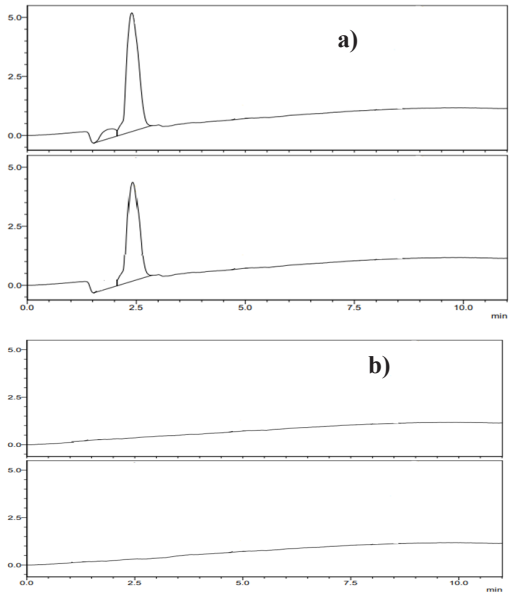

Figure 7a and b: show the blank and zero calibrator chromatograms in the brain homogenate matrix

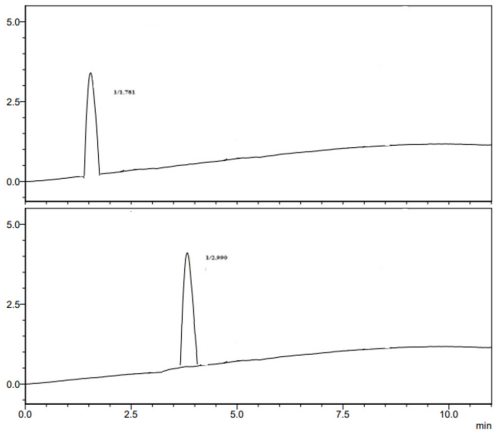

Figure 8: limit of detection (LOD) was established using an LC chromatogram of a 0.2 ng mL-1 injection that demonstrated a noticeable peak response at MEM retention with a signal-to-noise ratio greater than 3

Table 1: Linearity parameters for MEM response factor in different biological matrices

Parameter	*Plasma*	*CSF*	*Brain*
Range (ng mL-1)	0.5 – 300.0	0.5 – 300.0	0.5 – 300.0
Intercept (a)	−335.728	788.456	−743.288
Sa	3750.757	3724.873	5104.245
Slope (b)	3092.681	4674.896	3968.757
Sb	32.455	32.238	43.808
RSD% of the slope	1.018	0.669	1.080
Adjusted R square	0.9993	0.9997	0.9992
S y/x	9198.804	9136.307	12,520.147
F	9661.444	22,389.621	8592.246
Significance F	1.283 ×10-13	4.453 ×10-15	2.050 ×10-13

Table 2: Intraday and Interaday Precision Data of MEM on Spiked Rat Plasma

Conc (µg/mL)	*Intra-day*	*Inter day*
75	231456	232456
75	245412	246546
75	241455	241652
75	242156	241232
75	241562	241546
75	240215	240514
Avg	241254	241412
SD	881.935	2142.56

Table 3: Recovery of MEM

Conc. ng/mL	*% Recovery*	*% RSD (n= 3)*
50	77.85	6.94
100	78.96	3.11
150	83.68	6.98
200	82.56	6.10
250	79.71	3.47
300	82.65	4.34

Table 4: Summary of Validation Parameter

Parameter	*Observation*
Detection Wavelength	289nm
Linear Range (ng/ml)	0.5-300
Correlation coefficient R2	0.9992
Recovery (%)	77-83
LOD	0.2 ng mL-1

Plasma. **Table 3** shows the Recovery of MEM. **Table 4** shows the summary of validation parameter

CONCLUSION

After all, given their proven capacity to provide a more effective regimen, MEM-PLGA NPs are a more practical alternative for treating AD patients than free MEM. A bioanalytical method for ME has been developed and the method was validated as per USFDA guidelines. The proposed methods were found to be simple, accurate, precise, and reproducible and can be applied to the analysis of drugs in rat plasma. The proposed method was also applied for the estimation of bioavailability, bioequivalence, pharmacokinetic & toxicokinetic data of nanoparticulate formulation.

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