Solubility Enhancement and Pharmacokinetic Assessment of Chemically Modified Lamotrigine in Rat Blood Plasma by HPLC

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Authors’ contributions

This work was carried out in collaboration among all authors. Author KRG designed the study and wrote the protocol. Author ARG performed the study, carried statistical analysis and wrote the first draft of the manuscript and author AVG managed the study. Author MJU managed the literature searches. All authors read and approved the final manuscript.

ABSTRACT

Aims:
1. Synthesis/preparation of Lamotrigine (LMN) complexes with β-CD, Caffeine, Nicotinamide, EDTA and
2. Development of a new reverse phase liquid chromatographic (HPLC) method for the investigation of Lamotrigine in rat plasma after oral administration and pharmacokinetic assessment of Lamotrigine.

Study Design: The present work describes the formation of LMN drug complexes with β-Cyclodextrin, Caffeine, Nicotinamide and Disodium EDTA. Physical mixture, kneading and solvent evaporation methods were used to prepare LMN complexes (In ratios 1:1, 1:2, 2:1). Further characterization was performed by UV, FTIR, PXRD, and DSC. A reverse phase HPLC method was developed for the investigation of LMN in rat plasma using internal standardization method after oral administration of LMN and its complexes.

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1. INTRODUCTION

Solubility is one of the important parameters to achieve desired concentration of drug in systemic circulation for achieving required pharmacological response [4]. If drug product is to be administered orally in solid form, then it is mandatory that the drug product must solubilized within the gastro-intestinal fluid prior to absorption, and if drug product fails to do so then the patient will not achieve the required therapeutic effect [5]. Low aqueous solubility is the major problem encountered with formulation development of new chemical entities as well as generic development. Any drug to be absorbed must be present in the

Place and Duration of Study: Department of Pharmaceutical Chemistry, Smt. Kishoritai Bhoyar College of Pharmacy, RTMN University, Nagpur, between July 2018 and June 2019.

Methodology: LMN complexes with β-CD, Caffeine, Nicotinamide, EDTA was prepared in three ratios i.e. 1:1, 1:2 and 2:1 and characterized by UV, FTIR, PXRD, and DSC. In-vitro Solubility study was performed by saturation solubility study, further % practical yield, drug content, melting point was determined. In-vitro dissolution study of prepared complexes was performed in dissolution apparatus using the paddle method, according to USP Type II. Dissolution studies were carried out using 900 mL 0.1M HCl at 37± 0.5°C at 50 rev/min−1 (US FDA guidelines). The interaction of LMN with these hydrophilic complexing agents was characterized by UV, FTIR, PXRD and DSC.

A reverse phase HPLC bioanalytical method was developed and validated as per ICH guidelines for the quantitative determination of LMN in rat plasma using internal standardization method (HTZ) after oral administration of LMN and its complexes. The method was successfully applied for the pharmacokinetic study in rat. The pharmacokinetic parameter like AUC, AUC, MRT, Cmax, Tmax, t½, were calculated using pharmacokinetic software PK solver 2. The efficient separation was carried out for High Performance Liquid Chromatography (HPLC) method on Eclipse XDB-C18 (150×4.6×5 μ) column using a mobile phase consisting of filtered and degassed mixture of potassium dihydrogen orthophosphate buffer (pH 7.0) and Methanol in the ratio 65:35 v/v at a flow rate of 0.8 mL/min and UV detection at 307 nm.

Results: The LMN complexes were successfully prepared and characterized by UV, FTIR, PXRD, and DSC from which solvent evaporation method was found to be best as per result of in-vitro dissolution study. In-vitro dissolution study reveals that LMN-Caffeine (C1) and LMN-NTM (N1) complexes showed 100.14 and 100.01% drug release at 15 min and 20 min respectively as compared to pure drug (LMN) which shows only 50.56% drug release at 75 min. LMN concentration in blood plasma reached (Cmax) was found to be 19.4732 µg/mL at Tmax of 5h. Whereas Cmax of LMN complexes were found to be 48.4876 (B1), 72.2160 (C1), 62.2739 (N1) and 49.3170 (E1) µg/mL at Tmax of 5h out of which complex C1 and N1 in the present study resulted in a sharp increase in Cmax. All complexes showed 4 to 5 time enhancement of Cmax as compared to LMN. The results demonstrated that complexes of Lamotrigine were successful strategy to improve the solubility and dissolution behavior of Lamotrigine. The complex B1 shows maximum t1/2 and MRT of 36.224 h and 52.441 h as compared to C1, N1 and E1 having t1/2 of 14.1575, 16.258 and 21.702 h and MRT of 19.997, 22.994 and 30.883 h respectively. Hence B1 required lesser dosing frequency as compared to other complexes.

Conclusion: The Lamotrigine complexes were prepared and confirmation of prepared complexes was done by physical characterization (FTIR, DSC, PXRD and UV) and solubility determination by saturation solubility study. The bioanalytical method was developed for estimation of plasma drug concentration of LMN. The method was validated according to ICH guidelines to estimate the mean plasma concentration of LMN after oral administration using internal standardization method (HTZ). Method was reproducible and high recovery of LMN from its complexes was achieved. The method was found to be highly satisfactory sensitive, accurate, linear and specific.

Keywords: Lamotrigine; β-Cyclodextrin; caffeine; nicotinamide; disodium EDTA; solubility enhancement; pharmacokinetic assessment.
form of an aqueous solution at the site of absorption [6].

Lamotrigine [6-(2,3-dichlorophenyl)-1,2,4-triazine-3,5-diamine] is an anticonvulsant drug used in the treatment of epilepsy and bipolar disorder. For epilepsy it is used to treat partial seizures, primary and secondary tonic-clonic seizures, and seizures associated with Lennox-Gastaut syndrome [7]. Lamotrigine is a white to pale cream colored powder with a pKa of 5.7. Lamotrigine is very slightly soluble in water (0.17 mg/mL at 25°C) and slightly soluble in 0.1 M HCl (4.1 mg/mL at 25°C). Lamotrigine is classified as a BCS Class II drug. It is a well characterized small molecule with molecular formula C_{21}H_{17}N_{5}C_{2} and molecular weight 256.09 [8].

Various techniques are used for the enhancement of the solubility of poorly soluble drugs which include physical and chemical modifications of drug and other methods like particle size reduction, crystal engineering, salt formation, solid dispersion, use of surfactant, complexation and so forth [9]. Literature survey revealed that various reports are available on solubility enhancement of Lamotrigine by physical and miscellaneous methods. Literature on solubility enhancement using chemical method includes inclusion complexation of Lamotrigine with β-CD. But none of methods are available based on Stacking complexation and Chelation methods for solubility enhancement.

Cyclodextrins form an important group of pharmaceutical excipients. They are cyclic oligosaccharides composed of α, 1,4-linked D-glucopyranose units. The most common of these ring-shaped molecules are α, β and γ-CDs formed by six, seven, and eight glucose units, respectively [10]. Cyclodextrins have attracted the attention of many formulation experts because of their hydrophilic nature and ability to form stable inclusion complexes with properly sized guest molecules [11].

Stacking complexes are formed by the overlap of the planar regions of aromatic molecules. Some compounds that are known to form stacking complexes are as Nicotinamide, Anthracene, Pyrene, Methylene blue, Benzoic acid, Salicylic acid, Ferulic acid, Gentisic acid, Purine, Theobromine, Caffeine, and Naphthalene etc [12].

Caffeine has been proved to be a good complexing agent to improve the therapeutic efficacy of many drugs. Complexation with caffeine has been used successfully to improve the solubility, dissolution rate, and in vivo efficacy of many drugs [13].

The use of nicotinamidne as a solubilizing agent has been widely reported. The idea that nicotinamide undergoes stacking with drug molecules has been widely proposed. Nicotinamide is more effective than cyclodextrins for solubilizing some of the drugs [13].

Several methods have been reported for the quantitative determination of lamotrigine in bulk and pharmaceutical and biological samples. These methods include HPLC [14], UPLC-MS [15], TLC & HPTLC [16], UV-Visible spectrophotometric [17]. Literature survey revealed that there is no internal standard method has been reported for the quantification of lamotrigine in bulk and complexed form. So, developed a new internal standard method which is more precise and accurate.

2. MATERIALS AND METHODS

2.1 Materials

Lamotrigine and Hydrochlorothiazide were a gift samples from Alembic Pharmaceuticals Ltd. (Vadodra, Gujrat, India). β-CD, Caffeine and Disodium EDTA were supplied by Merck Specialities Private Limited. Nicotinamide was supplied by LOBA Chemie Private Limited. HPLC grade acetonitrile was purchased from Merck Life Science Private Limited. Double distilled water was used throughout the work. Solvents used were of HPLC grade and all other chemicals and reagents were of analytical grade.

2.2 Methods

2.2.1 Preparation of LMN complexes

2.2.1.1 Physical mixture method

The required molar quantities i.e. 10 mM of LMN and β-CD were weighted accurately and mixed in a ratio of 1:1, 1:2, 2:1 together thoroughly in a mortar, with vigorous trituration, for about 2-3 h. These mixtures were then passed through sieve No. 44 and finally stored in dessicator till further use.

2.2.1.2 Kneading method

The required quantities (10 mM) of LMN and β-CD were weighed accurately in a ratio of 1:1, 1:2, 2:1. A homogenous paste of cyclodextrin was prepared in a mortar by adding water: Methanol
mixture (1:1) in sufficient quantity to maintain suitable consistency of the paste. This paste was dried in a hot air oven at 45-50°C for 24 h. The dried complexes were then powdered and passed through sieve No. 44 and finally stored in dessicator till further use.

2.2.1.3 Solvent evaporation method

LMN was dissolved along with solubilizing additives like acetone at 25°C. The required quantity of 10mM LMN (1:1, 1:2, 2:1) with β-CD, Caffeine, Nicotinamide and Disodium EDTA in hot distilled water, separately added dropwise into LMN solution, with continuous stirring, for 2 to 3 h. The solution obtained, was evaporated on water bath at 50°C until the residue was obtained. The dried complexes were then powdered and passed through sieve No. 44 and finally stored in dessicator till further use.

2.2.2 Saturation solubility of LMN & prepared complexes

The saturation solubility of LMN & prepared complexes were determined at room temperature. An excess amount of sample was transferred to 25 mL of beaker containing 10 mL of distilled water. The sample was rotated on magnetic stirrer at 30 rpm for 24h. After that, solution was filtered rapidly through whatman filter paper no. 41 and suitably diluted. The absorbance was recorded spectrophotometrically at 307.0 nm.

2.2.3 Drug content determination

Drug content was determined by dissolving LMN complexes equivalent to 1 mg/mL and sonicated for 20 min. The volume was adjusted to 100 mL with distilled water. The solution was filtered through whatman filter paper no. 41, 1 mL aliquot was pipette out and suitably diluted with distilled water. Absorbance was measured at 307 nm using a double-beam UV spectrophotometer.

2.2.4 In-vitro dissolution study

The dissolution rate of LMN alone, LMN-β-CD, LMN-Caffeine, LMN-NTM, LMN-Disodium EDTA was measured in dissolution apparatus (USP Type II). Dissolution studies were carried out using 900 mL 0.1M HCl at 37±0.5°C at 50 rpm (US FDA guidelines). About 25 mg of LMN, or its equivalent amount of prepared complexes, were kept into muslin cloth and tied on paddle with the help of thread, and placed in 900 mL 0.1 M HCl.

About 10 mL of aliquot were withdrawn after 5, 10, 15, 20, 25, 30, 45, 60 and 75 min and replaced each time with 10 mL fresh 0.1M HCl. The solutions were suitably diluted and the concentration of LMN determined at 271.4 nm spectrophotometrically.

2.2.5 Characterization

2.2.5.1 Fourier Transform Infrared (FT-IR) spectroscopic studies

Moisture free powdered samples of LMN and prepared complexes were characterized using a FT-IR spectrometer (Thermo Nicolet, Avatar 370) by potassium bromide (KBr) pellet method. The scanning range was between 4,000 and 500 cm-1. An average of 32 scans is reported.

2.2.5.2 Differential scanning calorimetry (DSC) analysis

DSC spectra of samples were recorded using DSC (Mettler Toledo STAR® 10.00). The samples (6–7 mg) were accurately weighed in crimped aluminum pans and heated from 130°C to max 450°C, at a scanning rate of 10.00°C/min.

2.2.5.3 Powder X-ray Diffraction (PXRD) analysis

The PXRD patterns of pure LMN and prepared complexes were recorded using X-ray diffractometer (Bruker AXS D8 Advance) Vertical, Theta/2 Theta Geometry with Cu, at Wavelength 1.5406 A° using detector Si (Li) PSD.

2.2.6 HPLC method development

The HPLC (Shimadzu1100 series) system consist of a Binary gradient pump L-10ADVP, Rheodyne injector, PDA detector and LC solution software. The analytical column was Eclipse XDB-C18 (150×4.6×5µ) column. The mobile phase comprised potassium dihydrogen orthophosphate buffer (pH 7.0) and methanol in the ratio 65:35 v/v. The prepared mobile phase was filtered through a 0.45 µm membrane filter paper and each mobile phase was sonicated for 15 minutes. Chromatographic analysis was performed at a flow rate of 0.8 mL/min. The column eluate was monitored at 307 nm.

2.2.7 Pharmacokinetic assessment in rats

Either sex (200-250 g) wistar rats were housed in experimental groups according to the guidelines
for care and use of laboratory animals. Before the experiments, the rats were housed in a temperature and humidity controlled room (23°C; air humidity, 55%) with free access to water. Rats were randomly divided into six groups and were fasted for 12 h with free access to water before the experiments. They receive oral dosing LMN, LMN-β-CD (B1), LMN-Caffeine (C1), LMN-NTM (N1), LMN-Disodium EDTA (E1) at a dose equivalent to 10 mg/kg of LMN and blank (saline) using oral gavage. Blood samples (0.5mL) were collected into small plastic centrifuge tube through the eye vein at a interval of 1, 3, 5, 7 and 24 h after treatment. Then the plasma sample were separated by centrifugation at 15,000 rpm for 30 minutes and stored at -20°C until analysis. The protocol for this work was approved by the “Committee for the Purpose of Control Supervision of Experimental Animals (CPCSEA)”. 

2.2.8 Preparation of samples

Frozen plasma samples were thawed at room temperature and prepared for analysis. About 0.2 mL of plasma was pipette out and then added 100µL potassium phosphate buffer (0.01 M) and 1 mL of chloroform in isopropanol (95:5 v/v) and the mixture were vortex-mixed for 30sec to extract Lamotrigine after centrifugation at 8000 rpm for 15 minutes. After centrifugation the precipitate containing protein was discarded, the organic layer was collected and evaporated to dryness. Then 10 µL of internal standard (Hydrochlorothiazide) was added and reconstituted with 90 µL of mobile phase. A 20µL solution was injected to HPLC system for analysis. The same procedure followed for each sample.

2.2.9 Pharmacokinetic data analysis

The LMN concentrations were calculated using following formula.

\[ R = \frac{A_x}{A_{IS}} \times \frac{C_{IS}}{C_x} \]  

\[ C_x = \frac{A_x/A_{IS}}{R} \times C_{IS} \]

Where,

\[ A_x = \text{Area of Standard} \]

\[ A_{IS} = \text{Area of Internal standard} \]

\[ C_x = \text{Concentration of Standard} \]

\[ C_{IS} = \text{Concentration of Internal standard} \]

\[ R = \text{Response} \]

Drug concentration–time profile curve was plotted by utilizing the LMN concentrations in rat plasma at various time intervals to propose its PK profiles. The software “PK solver 2” was used for the calculation of PK parameters of LMN and values are expressed as the mean±standard deviation (SD). The non-compartmental analysis is frequently used method in PK analysis and this non-compartmental model was applied to calculate various PK parameters including maximum plasma concentration (\(C_{\text{max}}\)), time to reach maximum concentration (\(T_{\text{max}}\)), area under the curve (AUC), half-life (\(T_{1/2}\)) and mean residence time (MRT).

3. RESULTS AND DISCUSSION

3.1 Saturation Solubility of LMN & Prepared Complexes

Saturation solubility of LMN in water was found to be 0.1314 mg/mL, whereas saturation solubility of prepared complexes was found as shown in Table 1. 

Saturation solubility study showed that complexes of LMN with complexing agents like β-Cyclodextrin, Caffeine, Nicotinamide and Disodium EDTA resulted into increased water solubility. The enhancement of LMN solubility was due to the inclusion complex with β-Cyclodextrin, dipole-dipole interaction between the electrophilic nitrogen of Caffeine and Nicotinamide and chelates with Disodium EDTA.

Prepared complexes showed increase in solubility in following order:

\[ \text{LMN} < \text{C3} < \text{E3} < \text{N3} < \text{C2} < \text{B3} < \text{N2} < \text{E2} < \text{N1} < \text{B2} < \text{E1} < \text{B1} < \text{C1} \]

3.2 UV-spectral Analysis

The UV-spectrum of LMN and its complexes exactly overlap to each other at the region of 270-350 nm suggesting the intactness of basic moiety of LMN in its complexes. UV spectrophotometric characterization of LMN and its complexes are depicted in Fig.1 showing the maximum absorbance in methanol. However, the magnitude of absorbance was different for all the complexes. When the absorbance of prepared complexes compared with std. LMN showed the increased absorbance in same dilution and concentration using the same solvent.
Table 1. Saturation solubility data of lamotrigine and its prepared complexes

<table>
<thead>
<tr>
<th>System</th>
<th>Saturation solubility (mg/mL) mean ± s.d.</th>
</tr>
</thead>
<tbody>
<tr>
<td>LMN</td>
<td>0.1314 ± 0.010</td>
</tr>
<tr>
<td>LMN-β-CD (1:1) B1</td>
<td>0.6646 ± 0.030</td>
</tr>
<tr>
<td>LMN-β-CD (1:2) B2</td>
<td>0.5705 ± 0.076</td>
</tr>
<tr>
<td>LMN-β-CD (2:1) B3</td>
<td>0.4019 ± 0.026</td>
</tr>
<tr>
<td>LMN–Caffeine (1:1) C1</td>
<td>0.7791 ± 0.014</td>
</tr>
<tr>
<td>LMN–Caffeine (1:2) C2</td>
<td>0.3916 ± 0.006</td>
</tr>
<tr>
<td>LMN–Caffeine (2:1) C3</td>
<td>0.1408 ± 0.009</td>
</tr>
<tr>
<td>LMN–NTM (1:1) N1</td>
<td>0.5682 ± 0.009</td>
</tr>
<tr>
<td>LMN–NTM (1:2) N2</td>
<td>0.4992 ± 0.009</td>
</tr>
<tr>
<td>LMN–NTM (2:1) N3</td>
<td>0.3641 ± 0.010</td>
</tr>
<tr>
<td>LMN–EDTA (1:1) E1</td>
<td>0.6527 ± 0.009</td>
</tr>
<tr>
<td>LMN–EDTA (1:2) E2</td>
<td>0.5093 ± 0.013</td>
</tr>
<tr>
<td>LMN–EDTA (2:1) E3</td>
<td>0.3313 ± 0.009</td>
</tr>
</tbody>
</table>

Mean ± s.d. (n = 3)

Fig. 1. Overlaid UV-Spectra of LMN-β-CD (A), LMN-Caffeine (B), LMN–NTM (C), LMN-Disodium EDTA (D) complexes

3.3 Drug Content Determination

Percentage drug content was in the range of 78.29 to 83.30% for β-CD inclusion complexes, 82.24 to 83.40% for stacked Caffeine complexes, 85.95 to 97.29% for stacked Nicotinam ide complexes and 77.95 to 83.54% for Disodium EDTA complexes, which are in good agreement to theoretical drug content. The results of drug content determination are shown in Table 2.
3.4 *In-vitro* Dissolution Study

The dissolution profile curves of LMN, LMN-β-CD, LMN-Caffeine, LMN-NTM, LMN-Disodium EDTA in 0.1M HCl at 37±0.5°C are shown in Fig. 2. According to these results, all complexes of LMN show better drug release than the pure drug. The stacked Caffeine (C1) and Nicotinamide (N1) complexes showed 100% drug release at 15 min and 20 min respectively as compared to pure drug which shows only 50.56% drug release at 75 min., Inclusion complex (B1) and Disodium EDTA chelate (E1) also showed maximum drug release at 75 min i.e 99.69% and 98.08% respectively as compared to LMN.

The increase in dissolution rate was possibly due to a local solubilization action operating in the microenvironment or to the hydrodynamic layer surrounding the drug particles in the early stages of the dissolution process; enhanced dissolution from the complex was due to greater hydrophilicity, higher wetting effect and mechanical treatment, which increased the contact between the drug and the complexing agent and the ability to form a stable complex. The result of % drug release are shown in Table 3.

Prepared complexes showed increase in dissolution rate in following order:

E2 < LMN < C2 < E1 < B2 < B1 < N2 < N1 < C1

### 3.5 Characterization

#### 3.5.1 FTIR

Fig. 3 shows the FTIR spectra of LMN, B1, C1, N1 and E1. The IR spectrum of LMN was characterized by principal absorption peaks at 3448.87 cm⁻¹ (N–H aromatic); 3209.71 cm⁻¹ (C–H aromatic); 1620.62 cm⁻¹ (C=N); 1316.94 cm⁻¹ (C=C aromatic); 1050.44 cm⁻¹ (C–Cl); 748.60 cm⁻¹ (o substituted benzene). The IR spectrum of β-CD shows prominent peaks at 3353 cm⁻¹ (O–H); 1631.51 cm⁻¹ (H–O–H bending); 1155.06 cm⁻¹ (C–O). IR spectrum of the inclusion complex shows broad peak at 3353.87 cm⁻¹ due to combined OH and NH stretch indicating that β-CD form inclusion complex with LMN. The intense peaks in the spectra of LMN and β-CD are due to asymmetric stretching vibrations of the functional groups. The IR spectra of the stacking complexes i.e. Caffeine and Nicotinamide shows peak at 3450.82 cm⁻¹, 3367.53 & 3158.14 cm⁻¹ (N–H aromatic) and 1700.93 cm⁻¹, 1683.55 cm⁻¹ (C=O) respectively. The stacking complexation occurs due to dipole-dipole interaction between the electrophilic nitrogen of caffeine. The characteristic C=N stretching of caffeine shifted to 1631.51, 1630.76, 1631.30, 1624.69 cm⁻¹ respectively. In the complexes C1, N1 and E1 the characteristic N–H stretching of LMN (3448.87 cm⁻¹) shift to 3450.82, 3367.53 & 3158.14, 3448.60 cm⁻¹ respectively. The shifting in the vibrational frequencies of drug shows the formation of complexes.

![Fig. 2. Dissolution curves of LMN alone and from prepared complexes of LMN with β-CD, Caffeine, NTM, Disodium EDTA](image)

*Data are mean ± s.d. (n=3)*
Table 2. Result of drug content determination of lamotrigine complexes

<table>
<thead>
<tr>
<th>LMN Complexes</th>
<th>% Drug content (Mean ± s.d.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LMN–β-CD (1:1) B1</td>
<td>78.29 ± 0.165</td>
</tr>
<tr>
<td>LMN–β-CD (1:2) B2</td>
<td>79.90 ± 0.380</td>
</tr>
<tr>
<td>LMN–β-CD (2:1) B3</td>
<td>83.30 ± 0.325</td>
</tr>
<tr>
<td>LMN–Caffeine (1:1) C1</td>
<td>83.40 ± 0.050</td>
</tr>
<tr>
<td>LMN–Caffeine (1:2) C2</td>
<td>88.95 ± 0.417</td>
</tr>
<tr>
<td>LMN–Caffeine (2:1) C3</td>
<td>82.24 ± 0.170</td>
</tr>
<tr>
<td>LMN–NTM (1:1) N1</td>
<td>85.95 ± 0.225</td>
</tr>
<tr>
<td>LMN–NTM (1:2) N2</td>
<td>94.87 ± 0.091</td>
</tr>
<tr>
<td>LMN–NTM (2:1) N3</td>
<td>97.29 ± 0.307</td>
</tr>
<tr>
<td>LMN–EDTA (1:1) E1</td>
<td>82.62 ± 0.381</td>
</tr>
<tr>
<td>LMN–EDTA (1:1) E2</td>
<td>77.95 ± 0.242</td>
</tr>
<tr>
<td>LMN–EDTA (1:1) E3</td>
<td>83.54 ± 0.101</td>
</tr>
</tbody>
</table>

*Mean ± s.d. (n = 3)*

Fig. 3. FTIR spectra of LMN, LMN-β-CD (B1), LMN–Caffeine (C1), LMN-NTM (N1) and LMN-Disodium EDTA (E1)

3.5.2 PXRD

Powder x-ray diffractometer (PXRD) was the important technique used to characterize the complex formation. The new solid phase was formed, if the resulting PXRD pattern of the solid product after preparation by solvent evaporation method (API and complexing
agents) was different from the pure drug. The diffractionograms of the LMN complexes were different from the pure drug i.e. LMN. The characteristics peak of LMN and β-Cyclodextrin, Caffeine, Nicotinamide and Disodium EDTA (complexing agents) were disappeared, whilst new peaks appeared after preparation by solvent evaporation method. The change in the position of peaks after this process indicates the formation of new complexes. The peaks of LMN, B1, C1, N1 and E1 were shown in Fig. 4.

Table 3. The dissolution time of pure LMN, LMN-β-CD, LMN-Caffeine, LMN-NTM, LMN-Disodium EDTA in 0.1M HCL at 37±0.5°C

<table>
<thead>
<tr>
<th>Compound</th>
<th>DP&lt;sub&gt;15&lt;/sub&gt; ±</th>
<th>DP&lt;sub&gt;75&lt;/sub&gt; ±</th>
</tr>
</thead>
<tbody>
<tr>
<td>LMN</td>
<td>47.12 ± 1.04</td>
<td>50.56 ± 2.12</td>
</tr>
<tr>
<td>LMN-β-CD (1:1) B1</td>
<td>73.46 ± 3.58</td>
<td>99.69 ± 1.10</td>
</tr>
<tr>
<td>LMN-β-CD (1:2) B2</td>
<td>67.11 ± 2.87</td>
<td>91.99 ± 1.37</td>
</tr>
<tr>
<td>LMN-Caffeine (1:1) C1</td>
<td>100.14 ± 2.75</td>
<td>102.53 ± 1.40</td>
</tr>
<tr>
<td>LMN-Caffeine (1:2) C2</td>
<td>62.09 ± 3.53</td>
<td>101.69 ± 2.27</td>
</tr>
<tr>
<td>LMN-NTM (1:1) N1</td>
<td>98.92 ± 0.86</td>
<td>100.69 ± 2.19</td>
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<tr>
<td>LMN-NTM (1:2) N2</td>
<td>84.43 ± 2.95</td>
<td>101.36 ± 1.65</td>
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<tr>
<td>LMN-EDTA (1:1) E1</td>
<td>65.97 ± 0.84</td>
<td>98.8 ± 0.85</td>
</tr>
<tr>
<td>LMN-EDTA (1:2) E2</td>
<td>45.43 ± 2.07</td>
<td>88.02 ± 2.41</td>
</tr>
</tbody>
</table>

*% dissolved at 15 and 75 min; mean ± s.d. (n = 3)*

Fig. 4. PXRD pattern of LMN, LMN-β-CD (B1), LMN-Caffeine (C1), LMN-NTM (N1) and LMN-Disodium EDTA (E1)
It was observed that LMN exhibited characteristics sharp diffraction peaks (2θ) with 100% intensity at 13.857°. However LMN complexes i.e. B1, C1, N1 and E1 exhibited characteristics diffraction peaks (2θ) with peak intensity of 100% at 5.737°, 11.944°, 14.944° and 29.149° respectively indicating formation of new complex. The characteristics diffraction peaks (2θ) of LMN at the same angle with 100% peak intensity had disappeared in all the complexes. The shifting of 100% intensity for 2θ angle in comparison with std. LMN and complexes was mainly because of inter planer distance (d angle) indicating the different arrangement of molecules, hence confirm the development of new crystalline phase.

3.5.3 DSC

DSC experiments were carried to study the thermal behaviour of the prepared samples in relation to individual components. DSC thermogram of LMN demonstrated the endothermic peak at 217.47°C corresponding to the melting point of the crystalline form of LMN (phase transition from solid to liquid). The DSC thermogram of LMN complexes B1, C1, N1 and E1 shown in Fig. 5 exhibited the endothermic peaks at 140.77 & 215.56°C, 105.43 & 184.01°C, 122.66 & 161.66°C and 162.50 & 216.27°C respectively and one exothermic peak for LMN-β-CD at 240.25°C. This all complexes exhibited broad melting endotherm at temperature significantly different from those of the drug indicating a dehydration process. While the DSC thermogram of std. LMN show the sharp endothermic peak suggesting the formation of new crystalline phase. The sharp melting point peak of pure LMN at 217.47°C was not visible. The characteristic features of the LMN peak were lost. This indicated that LMN was molecularly complexed with complexing agents. In all the complexes i.e. B1, C1, N1 and E1, there are change in enthalpy of -4.65, -4.84 & 12.18 mJ, -46.60 & -155.59 mJ, -83.32, -57.98 & -73.95 mJ and -26.28, -126.75, -226.97 mJ.

Fig. 5. DSC thermogram of LMN, LMN-β-CD (B1), LMN–Caffeine (C1), LMN-NTM (N1) and LMN-Disodium EDTA (E1)
3.6 Validation

3.6.1 Specificity

Fig. 6 shows Specificity is expressed as the capability of method to distinguish the analyte from all potentially intrusive substance. The specificity of method was scrutinized by blank plasma detection, spiking blank plasma with pure std. compounds. Blank plasma had no interference when LMN and HTZ (internal std.) are eluted. At optimized conditions, the separation of LMN and HTZ was completed within 10 min.

3.6.2 Accuracy

The recovery experiments done at three concentration levels and the % accuracy values are given in Table 4.

The quantitative recovery of LMN achieved ranged from 99.00, 101.29 to 103.03% for three concentration levels respectively. Acceptable criteria – the mean value should not be deviated more than ± 15%.

3.6.3 Limit of detection and limit of quantitation

The LOD and LOQ of the method were evaluated for LMN by evaluating standard deviation and slope data from calibration curve parameter. LOD and LOQ were found to be 0.17656 µg and 0.5350 µg respectively.

3.6.4 Precision

Precision of any analytical method is expressed as SD an RSD of series of measurement. Precision of estimation by proposed method was ascertained by replicate analysis of homogeneous samples of std. LMN with constant concentration of HTZ internal standard. The result of precision study is shown in Table 6.

Table 4. Result of accuracy study

<table>
<thead>
<tr>
<th>Sr. no.</th>
<th>Observed concentration (µg/mL)</th>
<th>% Accuracy</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>19.80</td>
<td>99.00</td>
</tr>
<tr>
<td>2</td>
<td>25.32</td>
<td>101.29</td>
</tr>
<tr>
<td>3</td>
<td>30.91</td>
<td>103.03</td>
</tr>
</tbody>
</table>

Table 5. Result of LOD and LOQ

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>LOD</th>
<th>LOQ</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.17656</td>
<td>0.53503</td>
</tr>
</tbody>
</table>

Fig. 6. Chromatogram of blank plasma A) with internal standard B) and Std. lamotrigine C)
Table 6. Result of precision, intra day and inter day precision study

<table>
<thead>
<tr>
<th>Parameters</th>
<th>LMN</th>
<th>HTZ</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SD</td>
<td>% RSD</td>
<td>Mean ± SD</td>
<td>% RSD</td>
</tr>
<tr>
<td>Precision</td>
<td>1640241</td>
<td>11004.19</td>
<td>0.67</td>
<td>285689</td>
</tr>
<tr>
<td>intra day precision</td>
<td>1654170</td>
<td>2038.31</td>
<td>0.12</td>
<td>286836</td>
</tr>
<tr>
<td>Inter day precision</td>
<td>1576591</td>
<td>7270.66</td>
<td>0.46</td>
<td>277932</td>
</tr>
</tbody>
</table>

**Fig. 7. Chromatogram of LMN, LMN-β-CD (B1), LMN –Caffeine (C1), LMN-NTM (N1) and LMN-Disodium EDTA (E1) at T<sub>max</sub>.**
Table 7. The main pharmacokinetic parameter of lamotrigine after oral administration of lamotrigine and its prepared complexes in rat in dose of 10 mg/kg

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Pharmacokinetic parameter</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$C_{\text{max}}$ (µg/mL)</td>
</tr>
<tr>
<td>LMN</td>
<td>19.47 ± 0.36</td>
</tr>
<tr>
<td>LMN-β-CD (1:1) B1</td>
<td>48.48 ± 0.51</td>
</tr>
<tr>
<td>LMN-Caffeine (1:1) C1</td>
<td>72.21 ± 1.40</td>
</tr>
<tr>
<td>LMN-NTM (1:1) N1</td>
<td>62.27 ± 1.27</td>
</tr>
<tr>
<td>LMN-EDTA (1:1) E1</td>
<td>49.31 ± 1.24</td>
</tr>
</tbody>
</table>
3.6.5 Intra-day and Inter-day precision

Single-laboratory validation precision should include an intra-day (repeatability) and inter-day component. It is considered adequate to determine the intra- and inter-day precision of the analytical method as part of the validation procedure. The result of Intra-day and Inter-day precision study is shown in Table 6.

3.7 Pharmacokinetic Study in Rat

The validated method was successfully applied to investigate the content of prepared LMN complexes *in vivo*, after administered orally in rats.

Pharmacokinetic studies in rats were carried out to evaluate the oral absorption of LMN and its prepared complexes. The chromatograms are shown in Fig. 7. The mean plasma concentration-time profile curve of LMN after oral administration are shown in Fig. 8. The pharmacokinetic parameter like AUCt, AUCi, MRTi, Cmax, Tmax, t1/2, were calculated using pharmacokinetic software PK solver 2. The pharmacokinetic parameters are listed in Table 7. Compared with LMN its complexes B1, C1, N1 and E1 showed a significant improvement in Cmax, AUCt and AUCi. All the compounds achieved the maximum concentration at 5 h. There was no a shifting of Tmax value for every prepared complex. Oral administration of LMN alone resulted into low blood levels, with Cmax of 19.4732 µg/ml at 5 h. and AUCt of 258.84 µg/ml in contrast. Oral administration of prepared LMN complex C1 and N1 in the present study resulted in a sharp Cmax 72.2160 µg/ml, 62.2739 µg/ml at 5 h respectively. The compound B1 and E1 shows Cmax 36.224 and 21.702 µg/ml at 5 h respectively which was slightly lesser than the C1 and N1. The compound B1 shows maximum t1/2 of 36.224 h as compared to C1, N1 and E1 having t1/2 of 14.1575, 16.258 and 21.702 h respectively. Hence B1 required lesser dosing frequency as compared to other complexes.

The maximum drug concentration achieved in blood found to be following order of prepared LMN complexes and std. LMN.

LMN < B1 < E1 < N1 < C1
19.4732 < 48.4876 < 49.3170 < 62.2739 < 72.2160 µg/ml

4. CONCLUSION

The LMN prepared analogs were complexes. LMN complexes prepared using β-Cyclodextrin, Caffeine, Nicotinamide, and Disodium EDTA.
(complexing agents) by inclusion complex, staching complex and chelation by evaporation method. The prepared complexes were confirmed by physical characterization (UV, FTIR, DSC and PXRD) and solubility determination by saturation solubility study.

The results demonstrated that complexes of LMN were successful strategy to improve the solubility and dissolution behaviour of LMN. Further the bioanalytical method were developed and validated to estimate the mean plasma concentration of drug after oral administration using internal standard method. The developed method was successfully applied to measure the drug concentration of prepared LMN complexes in plasma after oral administration in rats, reproducible high recovery of LMN was achieved. Because of its highly satisfactory sensitivity, accuracy, linearity and specificity, this HPLC methodology could thus be an appropriate tool for further determination of LMN complexes in plasma samples in the pharmacokinetic studies.

From the result obtained it can be concluded that the solubility problem of LMN was overcome to some extent and the prepared complexes can be a better alternative to solubility enhancement.

5. SIGNIFICANCE OF STUDY

Solubility of drug substance is a major issue in Pharmaceutical area. If drug product is to be administered orally in solid form, then it is mandatory that the drug product must solubilized within the gastro-intestinal fluid prior to absorption, and if drug product fails to do so then the patient will not achieve the required therapeutic effect [5].

LMN is very slightly soluble in water (0.17 mg/mL at 25°C) [7]. Although LMN has gained widespread acceptance in the treatment of seizures, its poor aqueous solubility (0.17 mg mL⁻¹ at 25°C) limits its absorption and dissolution rate and thus delays onset of action and its therapeutic efficacy [19]. Due to variable absorption and consequently the difficulties in maintaining the desired concentration in the blood, determined the need for the enhancement of solubility. As Lamotrigine belongs to BCS class II [8], solubility enhancement is necessary so that the drug is released and gets solubilized immediately to produce the therapeutic effect.

Keeping in view the above aspect we have selected the drug LMN used in therapy but suffer from drawback of low aqueous solubility. Hence it was thought to prepare LMN complexes with β-CD, Caffeine, Nicotinamide and EDTA for enhancement of aqueous solubility and thereby dissolution rate. The result suggested that LMN complexes are the better alternative to LMN to overcome the problem of low aqueous solubility and dissolution and can be used as a better drug candidate.

COMPETING INTERESTS

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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