Asenapine Loaded Nanostructured Lipid Carriers for Intranasal Delivery

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6. Summary and conclusions

6.1. Summary

- The method was validated according to the ICH guidelines with respect to system suitability, linearity, limit of quantitation and detection, precision, accuracy, robustness, and specificity.
- Accuracy was found to be 98.9-101.12 % for all samples. Intraday precision (repeatability) was found to be in the range of 1.04 – 1.44% RSD while the inter-day precision (intermediate precision) was in the range of 0.13-1.73% RSD for the samples studied.
- The calibration curve was linear with linear regression coefficient of 0.9988 over the range of 10.0-100.0 µg/ml. The LOD and the LOQ were determined to be 1.0 and 5.0 µg/ml respectively.
- Robustness studies confirmed that peak area was unaffected by small changes in flow rate, pH of mobile phase and composition. The above finding suggested that the proposed HPLC procedures could be used for routine quality control and dosage form assay of asenapine.
- Quality target product profile for the preparation of nanostructured lipid carriers were defined based on the literature and dosage form.
- Based on the critical material attributes of nanostructured lipid carriers, glyceryl monostearate, oleic acid and Tween 80 were selected as solid lipid, liquid lipid and surfactant, respectively.

- In critical process parameters, homogenization speed and sonication time were selected for the preparation and optimization of batches.
- Box-Behnken design was used for optimization of asenapine loaded nanostructure lipid carriers. This design was specifically selected for exploration of complete design space with reduced experimental runs, without aliasing interaction factors.
- Five independent variables were selected in which three were composition variables and two were process variables. The variables (A) liquid lipid to solid lipid ratio, (B) drug to solid lipid ratio, (C) aqueous surfactant concentration, (D) homogenization speed and (E) sonication time were selected as independent factors. Particle size (Y1) and entrapment efficiency (Y2) were selected as dependent variables (Response).
- A total of 46 batches were prepared and best model was selected on the basis of lack of fit test and model summary statistics. The risk assessment was measured by checking linearity in predicted *vs* actual response and symmetrical distribution pattern in residual *vs* predicted, residual *vs* run graph for both particle size, entrapment efficiency.
- This risk assessment suggested that model is fit and the possibilities of other missing variables which may be determinant of nanostructured lipid carriers critical quality product profile are low.
- The particle size, zeta potential and entrapment efficiency of optimized asenapine loaded nanostructured carriers (ANLC) was found to be 167.30 ±

7.52 nm, -4.34 ± 1.27 mV and 83.50 ± 3.48%, respectively at set experimental condition (A = 0.2 w/w, B = 0.10 w/w, C = 1.5 %w/v, D = 16000 rpm and E = 5 min).

- Glycol chitosan coated asenapine loaded nanostructured carrier (GC-ANLC) was developed with some modification. The optimized batch demonstrated particle size, zeta potential and entrapment efficiency as 184.20 ± 5.59 nm, 18.82 ± 1.18 mV and 83.52 ± 2.59%, respectively.
- *In-vitro* drug release studies showed that pure drug (ASM) release 50%, 90% and 100% drug in 3.0, 12 and 24 hours, respectively. This drug release was sufficiently sustained by both ANLC and GC-ANLC which exhibits 50% in 12 hours and approximate 90% in 24 hours.
- Solid state characterization by FTIR, DSC and XRD indicated that drug has been changed to amorphous state from crystalline state after incorporation of drug into nanostructured lipid carriers without interaction with excipients.
- Morphological study by TEM and AFM revealed the spherical structure of both ANLC and GC-ANLC without any aggregations. A thin surrounding layer was observed in TEM image of GC-ANLC.
- Stability data up to three months of ANLC and GC-ANLC indicated that change in particle size, zeta potential and entrapment efficiency were not significant difference (p>0.05) at studied time point. However, difference

factor (f_1) and similarity factor (f_2) determined in dissolution studies showed similarity in drug release pattern during 90 days of stability period.

- The cell viability in MTT assay demonstrated no significant difference in % viability in group treated with ASM, ANLC and GC-ANLC (p>0.05) compared to control. It suggested that chemical composition of ANLC and GC-ANLC were well tolerated and do not form any toxic product.
- In pharmacokinetic study, ANLC showed significantly higher (p<0.05) C_{max} (74.13 ± 6.73 ng/ml), $AUC_{0\text{-}24h}$ (560.93 ± 27.85 h.ng/ml) and MRT (7.1 ± 0.13 h) in brain compared to pure drug (ASM) when both were administered by i.n. route. All these contributed to 1.34 and 2.68 times higher bioavailability of drug in plasma and brain, respectively after i.n. administration of ANLC.
- Similarly, C_{max} (94.93 ± 11.73 ng/ml) and $AUC_{0\text{-}24h}$ (826.81 ± 78.29 h.ng/ml) of GC-ANLC was significantly higher (p<0.05) than those observed in ASM after i.v. and i.n. administration. So these results in 1.41 and 4.07 times higher bioavailability of GC-ANLC in plasma and brain compared to compared to ASM (i.v.).
- The drug targeting efficiency of ANLC and GC-ANLC were found to be 2.07 and 2.88, respectively. It indicates that glycol chitosan coating enhance brain targeting potential of nanoparticles.
- In comparing AUC and drug targeting potential, GC-ANLC demonstrated superior brain availability over ANLC.

- In locomotors activity test, the antagonistic activity was achieved by ASM, ANLC and GC-ANLC. Further significant reduction of count in ANLC and GC-ANLC at same dose confirmed that nanocarriers have ability to cross blood brain resulting in higher antagonistic activity.
- In paw test response of HRT, increase in the HRT value in ANLC and GC-ANLC compared to ASM indicates better antipsychotic potential of nanocarriers which was stabilized after 14 day of treatment.
- Catalepsy test of ANLC and GC-ANLC showed insignificant difference on the 1^{st} day of response and significant difference in 7^{th}, 14^{th} and 21^{th} day compared to ASM.
- ANLC and GC-ANLC demonstrated reduction in the catalepsy compared to ASM and response was stabilized after 14 day of treatment.
- Overall, GC-ANLC with significant reduction in cataleptic behavior, higher induced locomotor antagonistic activity and increased FRT compared to ANCL justified better carrier for asenapine delivery.
- Nasal toxicity study indicates that GC-ANLC exhibited intact epithelial layer similar to nasal mucosa treated with physiological saline. This micrograph also suggested that GC-ANLC is biocompatible with nasal mucosa and seems to be safe for intranasal administration.
- In embryo fetal toxicity study, the percentage of fetal birth defects was found substantially deficient in GC-ANLC exposed group when compared to pure

ASM administered group. In the vehicle of ASM or blank nanosuspention treated groups, birth defects were detected almost negligible.

- In comparison test indicate that there was substantial decrease in total number of the live fetuses and litter size in ASM exposed group only.
- Overall, exposure of GC-ANLC was found to be safer than ASM in relevance to developmental birth defects and litter size in rat fetuses.
- The present study revealed that prenatal administration to GC-ANLC during the critical period of organogenesis (GD 6-21), induced limited number of minor birth defects as developmental embryo-fetotoxicity whereas these ailments were more pronounced in ASM treated embryo-fetuses, thus elucidates the better safety concern of asenapine nanostructured lipid carriers for teratogenic safety point of view.

6.2. Conclusions

In this study, an alternate delivery system for asenapine with improved bioavailability and sustained delivery was successfully developed. The present study demonstrated the systematic development of ANLC and GC-ANLC by QbD approach with predetermined properties of formulation ideal for brain delivery. Pharmacokinetic study revealed that developed both nanocarriers (ANLC and GC-ANLC) are able to reach brain in greater extent compared to pure drug. The drug targeting efficiency of ANLC and GC-ANLC were found to 2.07 and 2.88, respectively. It indicates that glycol chitosan coating enhance the brain targeting potential. The animal behavioural study further revealed that GC-ANLC significantly reduced cataleptic side effect and locomotor activity with increased the FRT compared to ANCL. This justifies that GC-ANLC better carrier for asenapine delivery. Nasal toxicity and embryo fetal toxicity studies indicate that GC-ANLC is safe for administration through nasal route even during pregnancy. Thus, overall data suggest that GC-ANLC is an efficient nanoformulation for asenapine delivery to the brain via intranasal route.