Optimized HPLC Method for TX & FV Estimation in Plasma & Brain: Pharmacokinetics & Biodistribution

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INTRODUCTION

Glioblastoma multiforme (GBM) is an aggressive brain tumor with limited treatment options. This study explores a novel therapeutic strategy by combining the anti-mitotic drug TX and natural compound FV, known for their synergistic anti-cancer effects. Both drugs were loaded into nanostructured lipid carriers (NLC) for enhanced delivery. A validated HPLC method was developed for precise estimation of TX and FV in plasma and brain. Pharmacokinetic and biodistribution study showed improved bioavailability and distribution via intranasal delivery, highlighting potential for targeted GBM treatment.

OVERVIEW

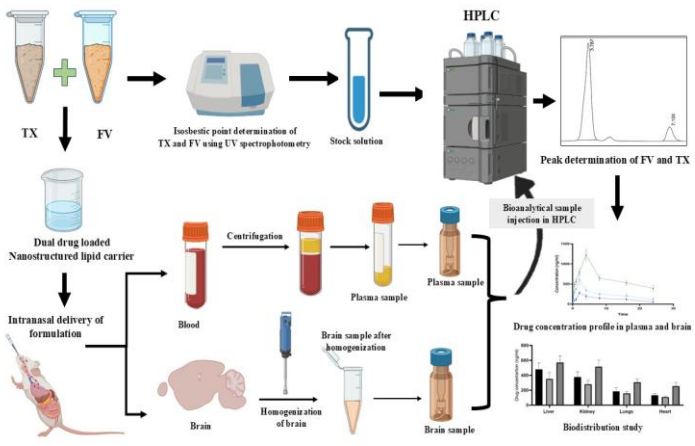

Figure 1: Workflow for Bioanalytical Method Development and Biodistribution Study of Intranasally and Intravenously administered Dual Drug-Loaded NLCs and Suspension.

LEARNING OBJECTIVES

- To establish a robust and efficient HPLC method for the simultaneous estimation of TX and FV in pharmaceutical formulations.
- To ensure the method's accuracy, precision, linearity, and robustness for regulatory compliance and reliable application.
- To apply the validated method for assessing the pharmacokinetic parameters of developed formulations.

DISCUSSION

The developed HPLC method proved to be a sensitive and reliable tool for simultaneous estimation of TX and FV in plasma and brain, enabling accurate pharmacokinetic and biodistribution assessment. I.N. administration of the optimized NLC formulation significantly enhanced brain drug levels, with 2.8–6.4-fold higher Cmax compared to suspensions, indicating efficient nose-to-brain targeting. The NLC also prolonged the half-life of both drugs, supporting sustained release and reduced systemic exposure. In contrast, I.V. delivery led to higher peripheral distribution and rapid clearance. Overall, the study demonstrates the effectiveness of the validated analytical method and highlights the potential of I.N. NLCs for targeted glioblastoma therapy by improving brain bioavailability and minimizing off-target effects.

CONCLUSION

The optimized chromatographic method demonstrated high sensitivity, precision, and recovery for TX and FV in plasma and brain. PK studies showed that I.N. NLC formulation significantly enhanced brain drug delivery, providing controlled, targeted release, while minimizing systemic exposure, highlighting its potential for GBM treatment.

REFERENCES

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ACKNOWLEDGEMENT

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METHOD

- Chromatographic analysis was performed using a Shimadzu LC-20 HPLC with a Shim-pack Solar C18 column.
- The mobile phase was Acetonitrile (ACN): water (60:40) with a flow rate of 1 ml/min at 30±0.2°C and a 10-minute run time. Detection was at 246 nm, based on the isosbestic point from a UV-vis spectrophotometer.
- The method was validated in plasma and brain per ICH Q2(R1) guidelines, evaluating system suitability, linearity, LOD, LOQ, accuracy, precision, and robustness. NLC formulations were optimized using CCRD.
- Pharmacokinetic (PK) and biodistribution studies assessed TX-FV suspension (SUS) and NLC formulations via intravenous (I.V.) and intranasal (I.N.) routes, focusing on absorption, distribution, metabolism, and organ localization.

RESULTS

- The chromatographic method effectively resolved drug peaks for FV (3.7 min), TX (7.1 min), with linear calibration curves (R^2>0.9900) in Wistar rat plasma and brain **(Figure 2, and Figure 3).**
- Sensitivity was confirmed by LOD and LOQ values for TX (plasma: 62.24 ng/ml, brain: 55.11 ng/ml) and FV (plasma: 68.58ng/ml, brain: 60.11ng/ml).
- The method showed >98% recovery with high precision, accuracy, and robustness.
- PK studies showed that I.N. NLC administration enhanced Cmax for TX (1579.54±356.09ng/ml) and FV (1740.90 ± 552.72 ng/ml) in brain, achieving 2.8–6.4-fold increases over I.N. or I.V. suspension administration **(Figure 4 and Figure 5).**
- The I.N. route provided controlled, targeted delivery with longer half-lives for TX and FV while I.V. administration showed rapid systemic availability with higher plasma Cmax.
- Biodistribution studies showed I.V. administration led to higher drug concentrations in peripheral organs, while I.N. administration, especially with NLC, enhanced brain accumulation and reduced systemic exposure, supporting targeted brain delivery for GBM

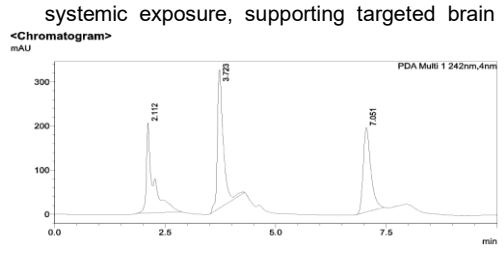

Figure 2: Chromatogram of plasma, FV, and TX recorded at 242 nm with a Rt of 2.17 min, 3.7 min, and 7.05 min respectively.

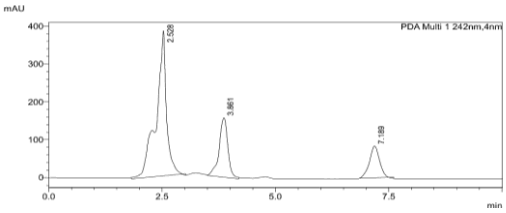

Figure 3: Chromatogram of brain homogenate, FV, and TX recorded at 242 nm with Rt of 2.52 min, 3.86 min, and 7.18 min respectively.

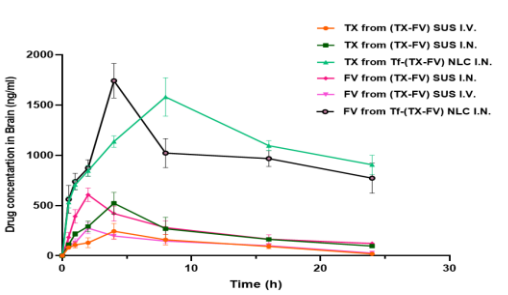

Figure 4: Brain concentration–time profiles of TX and FV showed significantly higher levels with I.N. Tf-(TX–FV) NLC compared to I.V. and I.N. suspensions, confirming enhanced brain targeting and retention.

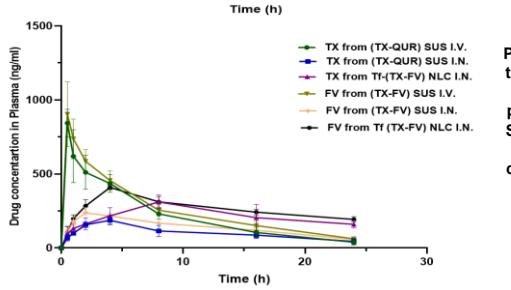

Figure 5: Plasma concentration–time profiles of TX and FV showed higher plasma levels with I.V. SUS, while I.N. Tf-(TX–FV) NLC exhibited controlled release and reduced systemic exposure.

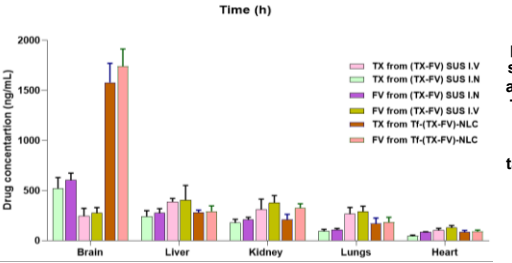

Figure 5: Biodistribution profiles of TX and FV showed highest brain accumulation with I.N. Tf-(TX–FV) NLC at 4h and 8h, confirming superior brain targeting over I.V. and I.N. suspensions (mean ± SD, n = 3).