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COMPARATIVE PHARMACOKINETICS OF LIQUIRITIN AND CYANOCOBALAMIN COMBINATION IN RAT SERUM: AN HPLC-BASED STUDY

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ABSTRACT
Liquiritin and cyanocobalamin (vitamin B12) were evaluated individually and in combination in a preclinical antidepressant research program, with fluoxetine included as a reference treatment. Healthy adult male Wistar rats were allocated to four pharmacokinetic groups (n=6): liquiritin 25 mg/kg, cyanocobalamin 100 µg/kg, liquiritin plus cyanocobalamin 25 mg/kg + 100 µg/kg, and fluoxetine 10 mg/kg, administered orally. Blood was collected at scheduled post-dose intervals, serum was separated, proteins were precipitated with methanol, and analytes were quantified using the reported RP-HPLC conditions with a C18 column. The consolidated HPLC dataset showed Cmax values of 21.8100 µg/mL for liquiritin, 23.0696 µg/mL for cyanocobalamin, 16.1558 µg/mL for the combination and 7.7428 µg/mL for fluoxetine. Tmax values were 4, 2, 4 and 4 h, respectively. Reported $AUC_{0–24}$ values were 137.16, 109.62, 147.95 and 73.70 µg·h/mL, respectively. The combination therefore showed the highest reported $AUC_{0–24}$ but a lower Cmax than liquiritin or cyanocobalamin alone. These findings provide a descriptive comparison of systemic concentration profiles; they do not by themselves establish absolute bioavailability enhancement or a pharmacokinetic interaction mechanism.
KEYWORDS: Liquiritin; Cyanocobalamin; Vitamin B12; Fluoxetine; Pharmacokinetics; Rat serum; RP-HPLC; Non-compartmental analysis

1. INTRODUCTION

Pharmacokinetic characterization provides quantitative information on systemic exposure, absorption and disposition and is an important complement to behavioral pharmacology. This is particularly relevant when bioactive compounds are administered as a combination because co-administration may alter the observed exposure profile.

Liquiritin is a flavonoid glycoside derived from Glycyrrhiza species. Experimental studies have reported antidepressant-like activity of liquiritin and related flavonoids in animal models, providing a pharmacological rationale for further investigation.[1,2]

Vitamin B12 is involved in neuronal function and one-carbon metabolism. Its neuropsychiatric relevance and relationship with methylation pathways have been described in the literature.[3–5]

The source doctoral thesis evaluates fluoxetine, liquiritin, vitamin B12 and their combination in a preclinical program. This manuscript focuses specifically on the HPLC-supported pharmacokinetic component and compares Cmax, Tmax and reported $AUC_{0–24}$ among the four treatment groups.

2. MATERIALS AND METHODS

2.1 Experimental animals and ethics

The study was approved by the Institutional Animal Ethics Committee (IAEC), Datta Meghe Institute of Higher Education & Research, under approval number DMHER/IAEC/25-26/83. The procedures were conducted according to applicable CPCSEA guidance.

2.2 Pharmacokinetic treatment groups

Table 1. Pharmacokinetic treatment groups (n=6/group).

Group	Treatment	Dose	Route
I	Liquiritin	25 mg/kg	Oral
II	Cyanocobalamin	100 µg/kg	Oral
III	Liquiritin + Cyanocobalamin	25 mg/kg + 100 µg/kg	Oral
IV	Fluoxetine	10 mg/kg	Oral

2.3 Blood sampling and serum preparation

The thesis reports blood collection from the retro-orbital plexus under mild anesthesia at 0, 1, 2, 4, 8, 12 and 24 h after oral administration, with approximately 1 mL blood collected at each point. Serum was separated by

centrifugation at 3000 rpm for 10 min and stored at −80 °C. Protein precipitation was performed using methanol, followed by vortex mixing and centrifugation at 10,000 rpm for 10 min. The clear supernatant was filtered through a 0.22-µm membrane before HPLC injection.

2.4 RP-HPLC analysis

Table 2. Reported chromatographic conditions.

Parameter	Reported condition
System	Shimadzu LC-20AD HPLC with UV detector and LC-Solution software
Column	RP-C18, 250 × 4.6 mm, 5 µm
Mobile phase	Methanol : acetonitrile: H2O(25:50:25)
Flow rate	1.0 mL/min
Detection wavelength	227 nm
Injection volume	20 µL
Column temperature	Ambient

Calibration standards were evaluated over 5–25 µg/mL. The consolidated HPLC report gives the following calibration relationships: liquiritin, $y = 16.248457x - 6.662381$ ($R^2 = 0.998681$); cyanocobalamin, $y = 80.482737x + 8.808952$ ($R^2 = 0.999746$); fluoxetine, $y = 57.632914x + 4.361905$ ($R^2 = 0.999747$); and the separate B12 + liquiritin combination calibration dataset, $y = 26.295x + 1.1941$ ($R^2 = 0.999$).

2.5 Pharmacokinetic analysis

Cmax and Tmax were obtained directly from the observed serum concentration–time data. $AUC_{0–24}$ values reported in the consolidated HPLC report were calculated using the linear trapezoidal method from the supplied concentration observations. The source report notes that the calculations are derived from the supplied numerical observations and have not been independently validated against raw chromatographic integration files.

3. RESULTS

3.1 Serum concentration–time profiles

Table 3. Consolidated serum concentration–time values from the HPLC report.

Time (h)	Liquiritin (µg/mL)	Vitamin B12 (µg/mL)	B12 + Liquiritin (µg/mL)	Fluoxetine (µg/mL)
1	10.850000	19.107120	2.622138	0.196755
2	12.000000	23.069590	6.512210	1.385719
4	21.810000	5.479635	16.155801	7.742825
6	—	—	8.620616	—
8	10.640000	5.280828	7.853785	6.970831
12	0.410000	2.568849	5.845417	2.156325
24	0.410000	1.224167	2.832864	0.526445

3.2 Pharmacokinetic summary

Table 4. Updated reported pharmacokinetic parameters from the consolidated HPLC dataset (n=6/group).

Parameter	Liquiritin	Cyanocobalamin	B12 + Liquiritin	Fluoxetine
Cmax (µg/mL)	21.8100	23.0696	16.1558	7.7428
Tmax (h)	4	2	4	4
$AUC_{0–24}$ (µg·h/mL)	137.16	109.62	147.95	73.70

3.3 Relative differences of the combination dataset

Table 5. Percentage difference for the B12 + liquiritin combination relative to each comparator, calculated from the consolidated HPLC values.

Comparator	Cmax difference vs combination	$AUC_{0–24}$ difference vs combination
Liquiritin	−25.9%	+7.9%
Cyanocobalamin	−30.0%	+35.0%
Fluoxetine	+108.7%	+100.7%

The supplied HPLC dataset is represented graphically in Figures 1–26. Figures 1–14 reproduce the principal source graphs, while Figures 15–26 reproduce the corresponding annotated source graphs with numerical data labels. The figures are supplied as separate stand-alone TIFF files in accordance with the current GMR figure-file requirements.

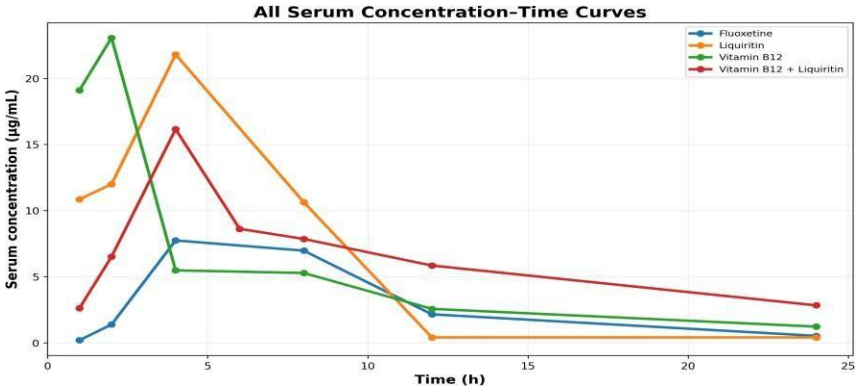


Figure 1. Combined serum concentration–time profiles for fluoxetine, liquiritin, cyanocobalamin (vitamin B12), and the vitamin B12 + liquiritin combination.

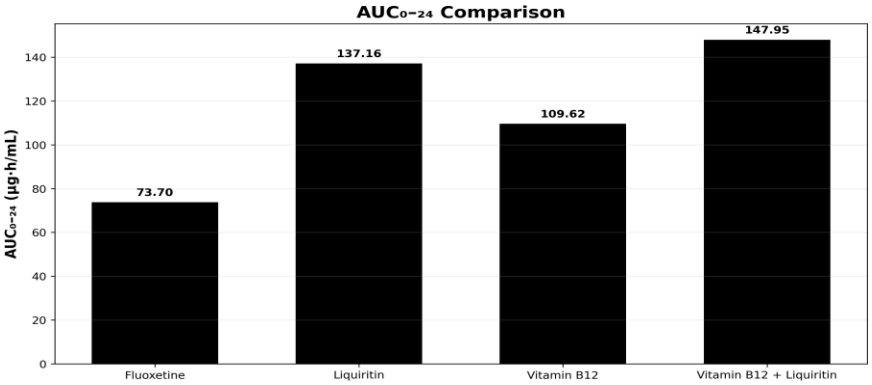


Figure 2. Comparison of reported $AUC_{0–24}$ values for fluoxetine, liquiritin, vitamin B12, and vitamin B12 + liquiritin.

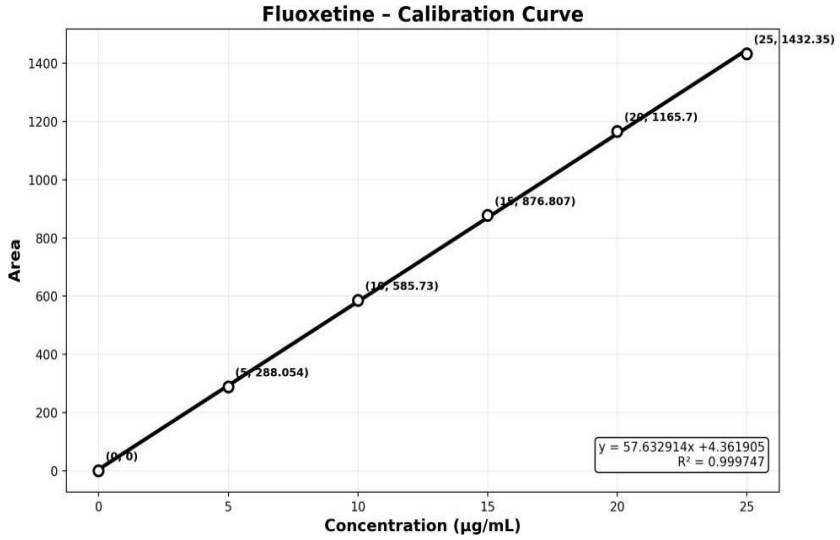


Figure 3. Calibration curve of fluoxetine over the reported 0–25 µg/mL concentration range.

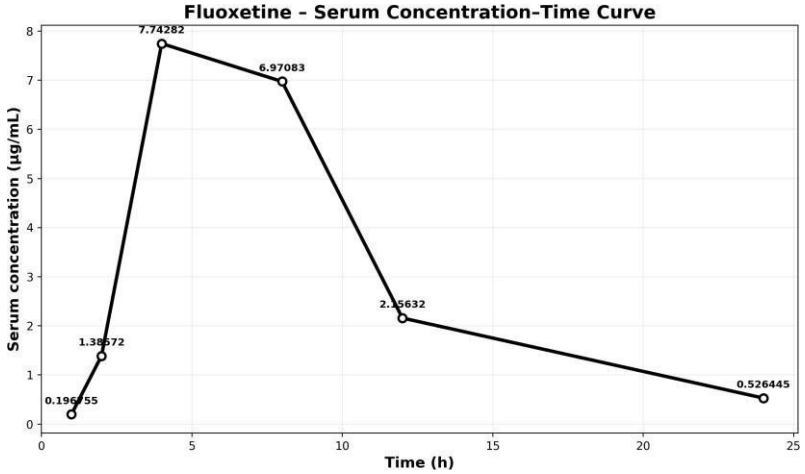


Figure 4. Serum concentration–time profile of fluoxetine.

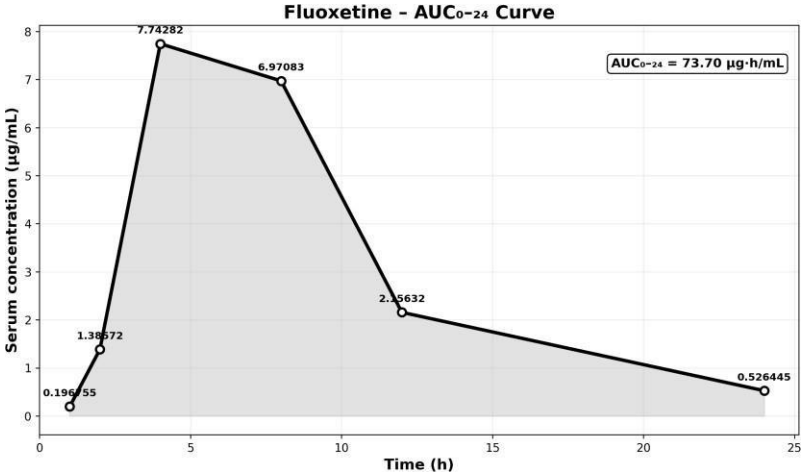


Figure 5. $AUC_{0–24}$ profile of fluoxetine calculated from the reported serum concentrations by the linear trapezoidal method.

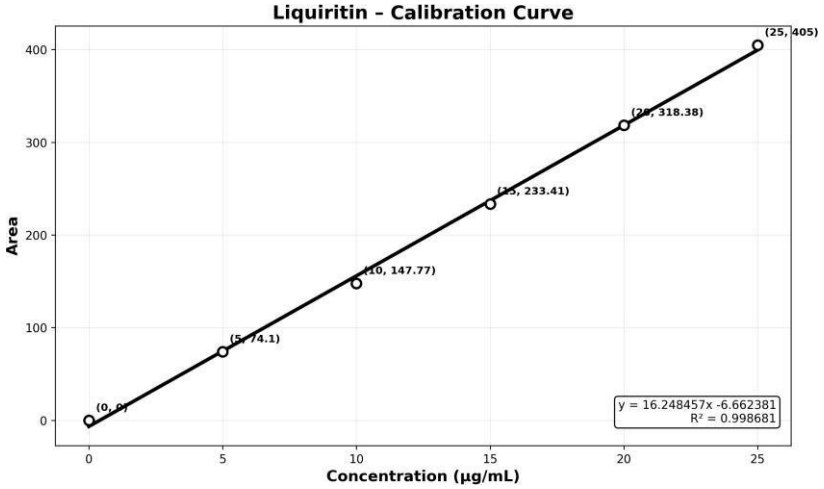


Figure 6. Calibration curve of liquiritin over the reported 0–25 µg/mL concentration range.

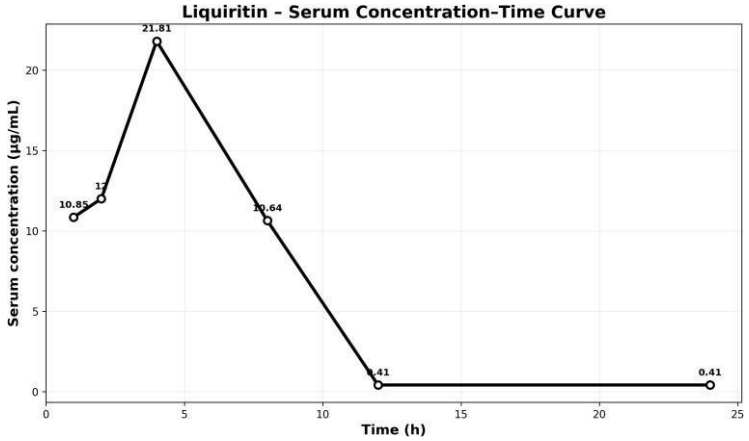


Figure 7. Serum concentration–time profile of liquiritin.

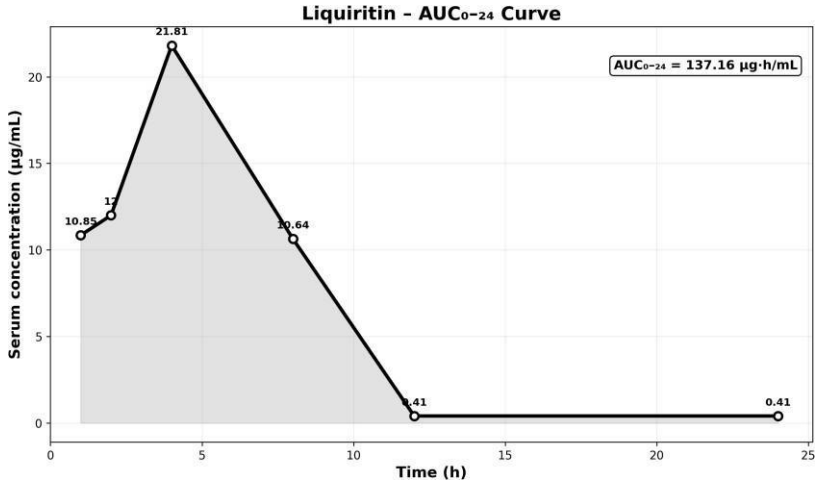


Figure 8. $AUC_{0–24}$ profile of liquiritin calculated from the reported serum concentrations by the linear trapezoidal method.

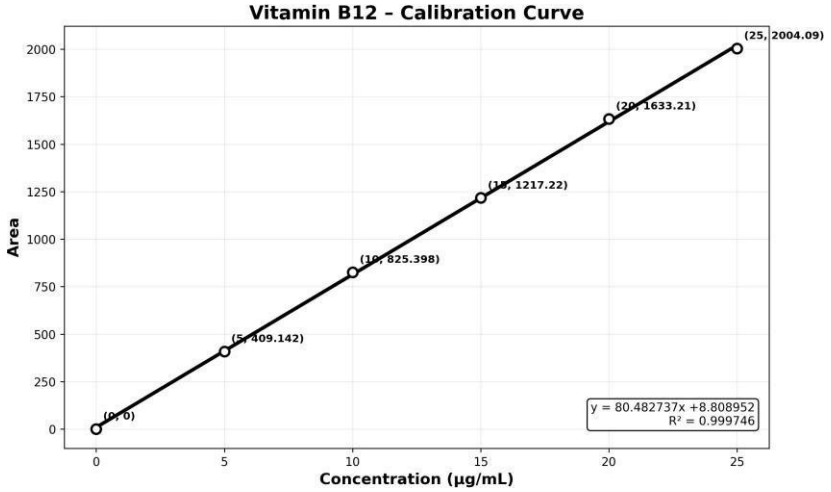


Figure 9. Calibration curve of cyanocobalamin (vitamin B12) over the reported 0–25 µg/mL concentration range.

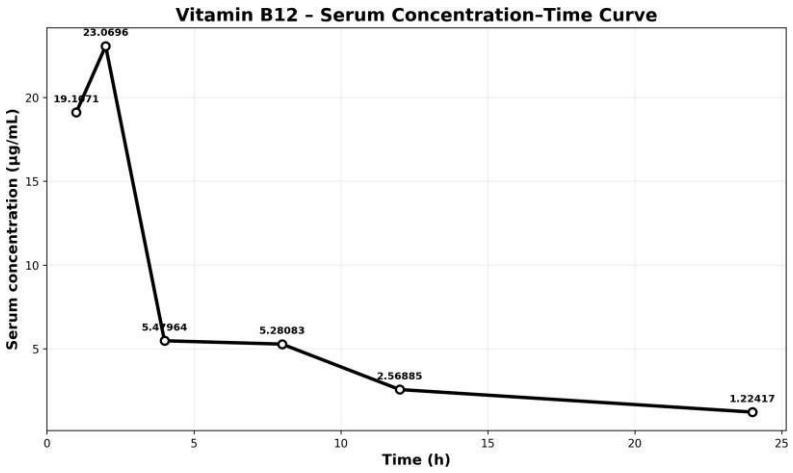


Figure 10. Serum concentration–time profile of cyanocobalamin (vitamin B12).

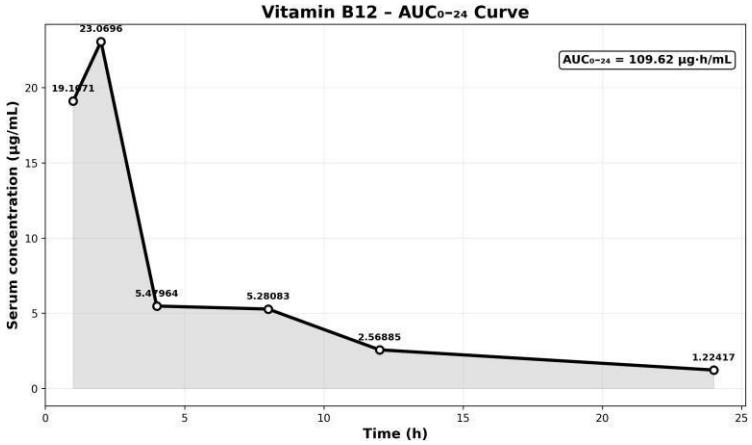


Figure 11. $AUC_{0–24}$ profile of cyanocobalamin calculated from the reported serum concentrations by the linear trapezoidal method.

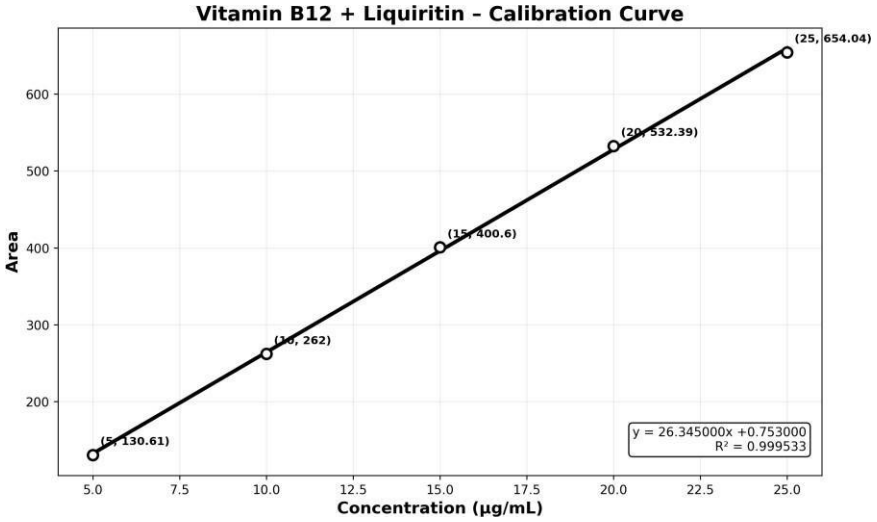


Figure 12. Calibration curve of the vitamin B12 + liquiritin dataset over the reported 5–25 µg/mL concentration range.

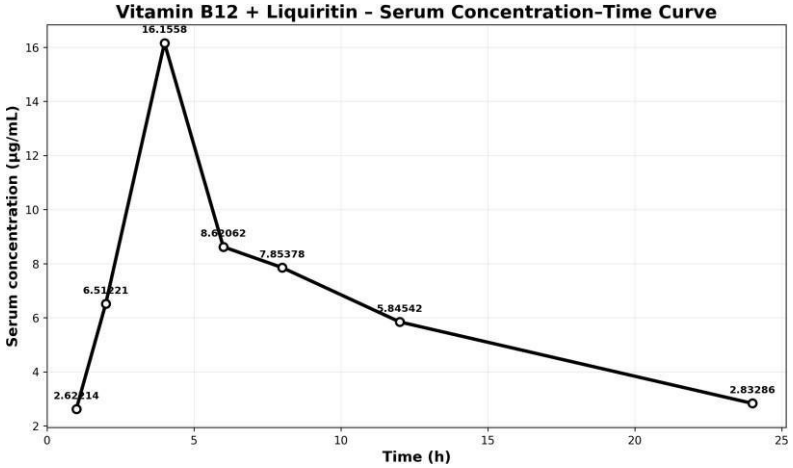


Figure 13. Serum concentration–time profile of the vitamin B12 + liquiritin combination.

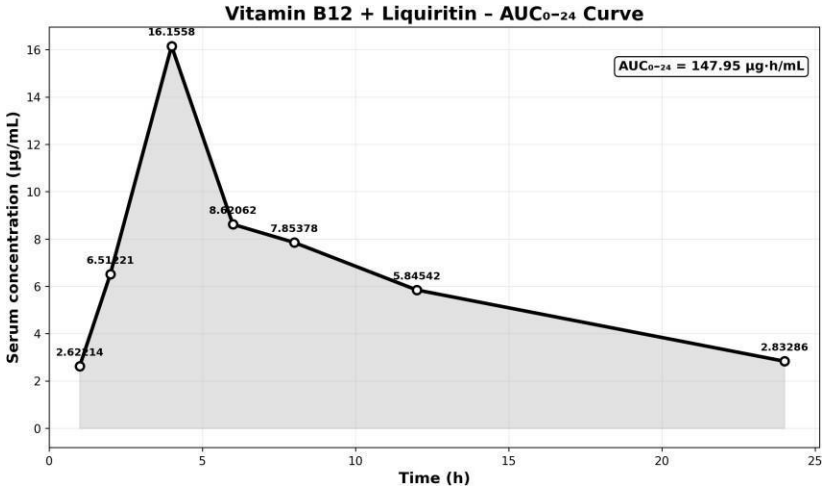


Figure 14. $AUC_{0–24}$ profile of the vitamin B12 + liquiritin combination calculated from the reported serum concentrations by the linear trapezoidal method.

3.4 HPLC chromatographic evidence

Representative chromatograms reproduced from the supplied thesis are included to document the reported bioanalytical method and serum analysis. The supplied records include blank-serum, spiked-serum, standard and serum chromatograms. The current HPLC optimization record reports retention times of approximately 2.653 min for fluoxetine, 3.717 min for liquiritin and 5.701 min for cyanocobalamin in the spiked-serum chromatographic assessment. These chromatographic figures are source-derived and have not been synthetically generated.

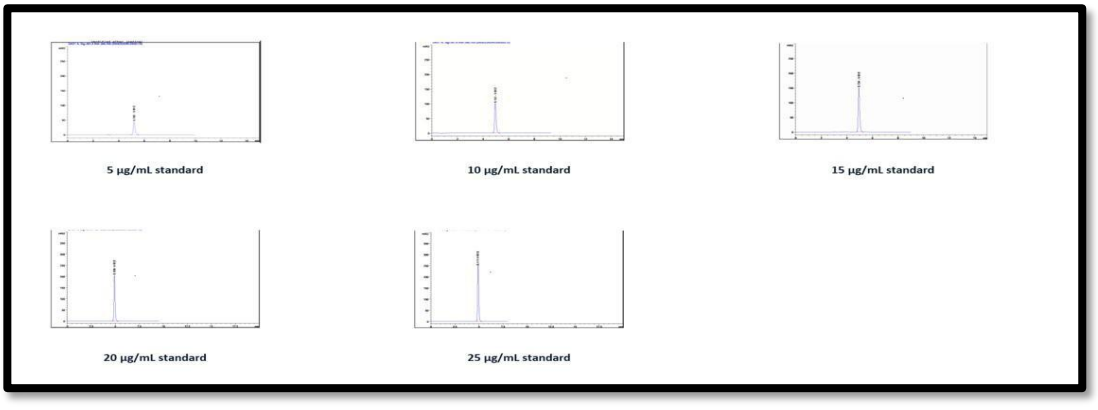


Figure 15. Vitamin B12 standard chromatograms at 5, 10, 15, 20 and 25 µg/mL.

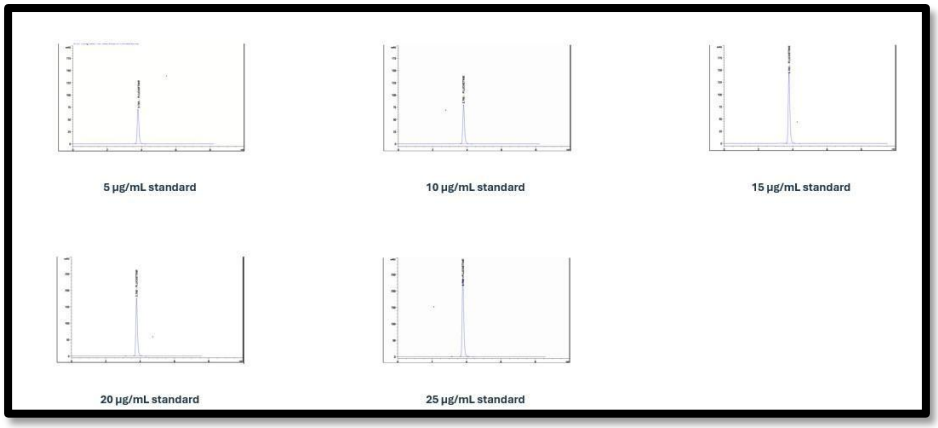


Figure 16. Fluoxetine standard chromatograms at 5, 10, 15, 20 and 25 µg/mL.

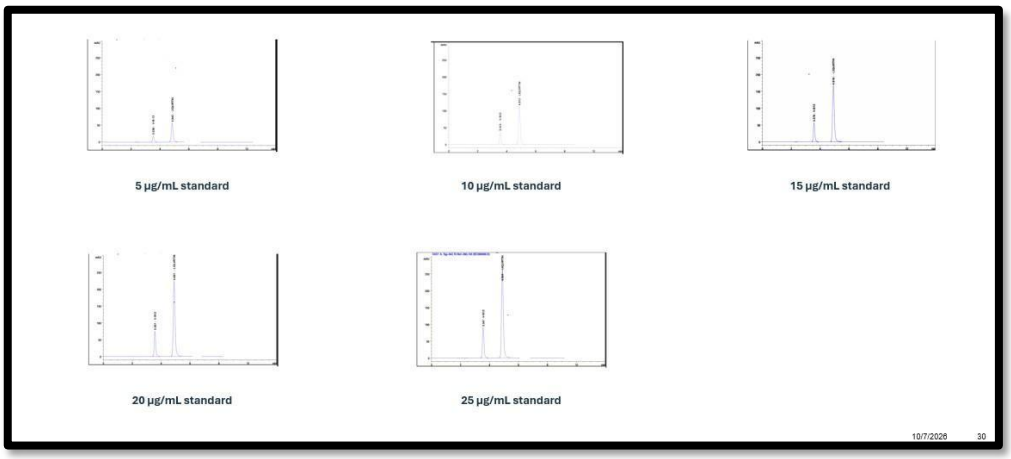


Figure 17. Combined Liquiritin and Vitamin B12 standard chromatograms at 5, 10, 15, 20 and 25 µg/mL.

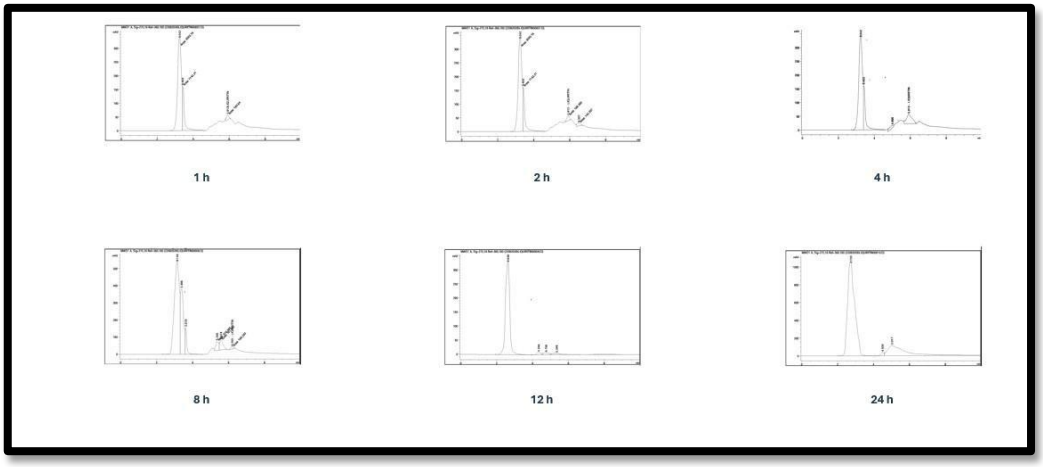


Figure 18. Representative Liquiritin serum chromatograms at 1, 2, 4, 8, 12 and 24 h after administration.

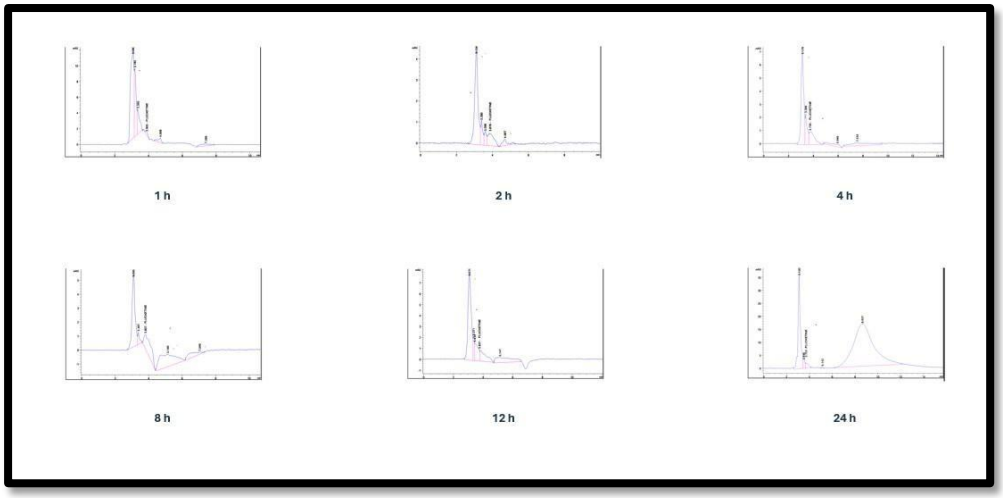


Figure 19. Representative Fluoxetine serum chromatograms at 1, 2, 4, 8, 12 and 24 h after administration.

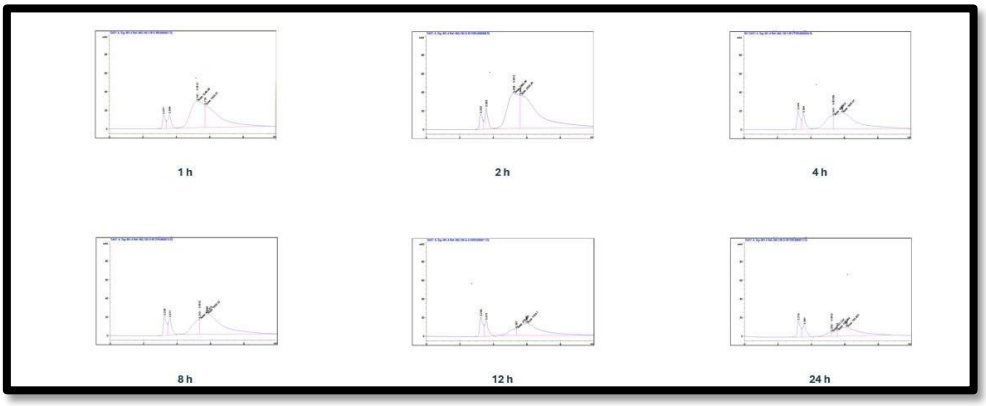


Figure 20. Representative Vitamin B12 serum chromatograms at 1, 2, 4, 8, 12 and 24 h after administration.

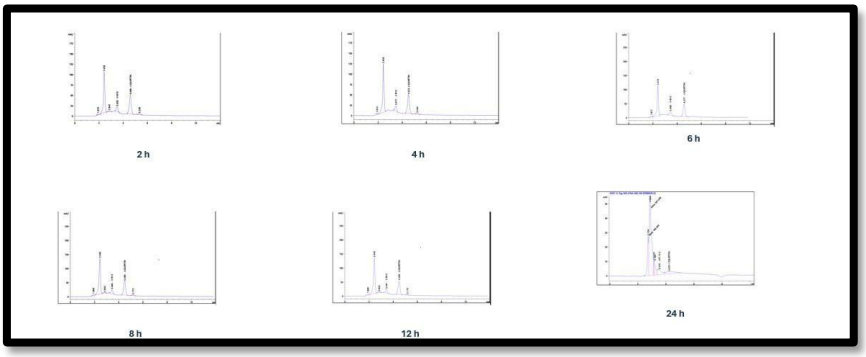


Figure 21. Representative Vitamin B12 + Liquiritin serum chromatograms at the reported sampling time points, including the 6 h combination sample.

Figure 22. Annotated fluoxetine calibration curve showing the supplied numerical calibration points and fitted regression.

4. DISCUSSION

The updated HPLC dataset shows distinct concentration–time profiles for all four treatment groups. Liquiritin reached an observed Cmax of 21.81 µg/mL at 4 h, while cyanocobalamin reached the highest individual Cmax of 23.0696 µg/mL at 2 h. The combination reached 16.1558 µg/mL at 4 h, which was lower than the Cmax of either liquiritin or cyanocobalamin alone. In contrast, the combination showed the highest reported $AUC_{0–24}$ (147.95 µg·h/mL), compared with 137.16 µg·h/mL for liquiritin, 109.62 µg·h/mL for cyanocobalamin and 73.70 µg·h/mL for fluoxetine.

The higher reported AUC of the combination indicates greater calculated exposure over the reported observation interval than either individual component or fluoxetine in this dataset. However, a higher AUC alone does not establish absolute oral bioavailability enhancement. Absolute bioavailability requires an appropriate reference-

route design, and a pharmacokinetic interaction or bioenhancement mechanism cannot be established solely from summary exposure values.
The combination reached its observed maximum concentration at 4 h, indicating that peak exposure occurred within the sampling schedule. The additional 6 h combination observation was retained in the concentration–time dataset and in the trapezoidal AUC calculation.
The source HPLC records provide calibration relationships and serum concentration data, but the compiled report does not independently establish a complete bioanalytical validation package covering accuracy, precision, recovery, matrix effect, LLOQ/ULOQ and stability. Therefore, the present analysis should be interpreted as a descriptive consolidation of the supplied HPLC data.

5. Data Integrity and Limitations
The present manuscript uses the numerical values from the consolidated HPLC report as the principal PK dataset. Earlier thesis sections contained a separate numerical PK dataset with different concentrations and PK parameters; those conflicting values have not been used in the updated tables or figures. The original animal-level HPLC worksheets, chromatograms and calculation sheets should be checked before final journal submission.
The reported $AUC_{0–24}$ values were calculated by the linear trapezoidal method from the supplied serum concentration observations. Because the displayed concentration series begin at 1 h, the exact time-zero handling and interval label should be verified against the original calculation sheet before final submission.
Terminal-phase parameters such as elimination half-life, Ke and MRT are not included in the consolidated HPLC report used for this update and therefore have not been carried forward as numerical results in the revised manuscript.

6. CONCLUSION
The updated HPLC dataset demonstrates measurable serum exposure for liquiritin, cyanocobalamin, the liquiritin–cyanocobalamin combination and fluoxetine. Liquiritin showed a Cmax of 21.81 µg/mL at 4 h, cyanocobalamin 23.0696 µg/mL at 2 h, the combination 16.1558 µg/mL at 4 h and fluoxetine 7.7428 µg/mL at 4 h. The reported $AUC_{0–24}$ values were 137.16, 109.62, 147.95 and 73.70 µg·h/mL, respectively. The combination therefore showed the highest reported $AUC_{0–24}$ but not the highest Cmax. These results support further investigation of systemic exposure and disposition, with final reconciliation against the original animal-level chromatographic records and validated bioanalytical calculations before journal submission.

7. Ethics Statement
The animal procedures were approved by the Institutional Animal Ethics Committee (IAEC), Datta Meghe Institute of Higher Education & Research, under approval number DMHER/IAEC/25-26/83 and were conducted according to applicable CPCSEA guidance.

8. Data Availability
The consolidated serum concentration and calibration data used for this manuscript are presented in the tables. Individual animal concentration–time data and chromatographic validation records should be retained and, where required, provided as supplementary material after reconciliation.

9. Conflict of Interest
To be completed by the authors before submission.

10. Funding
Not Applicable

11. ACKNOWLEDGMENTS
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