IJCRT.ORG ISSN : 2320-2882

INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)

An International Open Access, Peer-reviewed, Refereed Journal

Bioanalytical Method Development And Distribution Of Verapamil In Rat Body And Its Bioavailability Study By Using HPLC

KA EMMANUEL[1*], JALABABU R[1], PAUL DIVAKAR P[2], NAGALAKSHMI T[3], SUBRAHMANYAM TALARI[4]

[1] Professor, [2]Professor, [3] Professor, [4] Associate Professor

[1]Department of Chemistry, YVNR Government Degree College, Kaikaluru, Andhrapradesh, India-521333

[2] Department of Physics, YVNR.Govt.Degree College, Kaikaluru, AP, India-521333

[3] Basic Engineering Department, DVR & Dr. HS MIC College of Technology, Kanchikacherla, AP, India 521180

[4]Department of Chemistry, Sir CRReddy College, Eluru, AP, India-534007

Abstract: Verapamil is used to treat high blood pressure, and there is interest in creating a new HPLC method to measure the drug concentration and distribution in rat bodies. Using a gradient elution technique with a buffer containing 1 mL of FA in 1 L of HPLC-grade water and a mixture of two components, such as buffer and ACN, in a 60:40 ratio as the mobile phase at a flow rate of 1 mL/min over a good linear concentration range, chromatographic separation was accomplished on a Symmetry C18 column. The outcomes of the recovery study, precision, matrix effect, and extraction recoveries are all within an acceptable range. This technique was used to distribute verapamil throughout the rat's head, body, and tail. In accordance with UFDA rules, we discovered that the medications remained stable throughout the stability tests. Verapamil bioavailability experiments have been conducted effectively for the first time using the proven technique.

Keywords: Verapamil, Bioanalytical method, validation, ICH guidelines, Bioavailability study.

1. INTRODUCTION

1.1Profile of the drugs

Verapamil, sold under various trade names, it is a medication [1-2] used for the treatment of high blood pressure [3-5], and supra ventricular tachycardia [6-7]. It may also be used for the prevention of migraines [8-9] and cluster headaches [10-11]. It is given by mouth or by injection into a vein. Common side effects include head ache, low blood pressure, nausea and constipation. Other side effects include allergic reactions and muscle pains. It is not recommended in people with a slow heart rate or heart failure. It is believed to cause problems for the baby if used during pregnancy. It is in the non-dihydropyridine calcium channel blocker family of medications [12-22].The molecular structure of Verapamil is shown in Figure **1**.

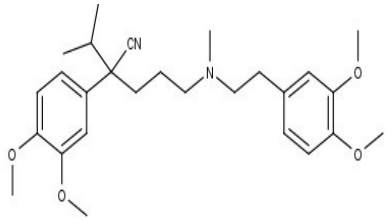


Fig. 1: Structural representation of Verapamil

1.2 Literature Survey

In recent years, there have been very few reports of verapamil measurement by HPLC. We saw several issues, such as lengthy runtimes, expensive sample preparations, and expensive mobile phases in earlier techniques. However, our created approach has been proven in accordance with USFDA criteria and has a great recovery rate, strong linear calibration curves, a shorter run time, and more precision at a lower cost. Successful application of the bioanalytical test was made to the verapamil bioavailability investigation.

1.3 Aim and objectives of the Present Investigation

To develop a new HPLC technique for figuring out how much verapamil is in rats' bodies and where it is distributed. Using a gradient elution technique with a buffer containing 1 mL of FA in 1 L of HPLC-grade water and a mixture of two components, such as buffer and ACN, in a 60:40 ratio as the mobile phase at a flow rate of 1 mL/min over a good linear concentration range, chromatographic separation was accomplished on a Symmetry C18 column. The findings of the extraction recoveries, precision, matrix effect, and recovery study fall within the permissible range.

II.RESEARCH METHODOLOGY

2.1Reagents and Chemicals

Merck Ltd. in Mumbai, India, was where the HPLC-marked Sodium Bisulphate, Methanol, and water were bought. APIs of verapamil (99.9% purity) are bought from Cipla Pharmaceutical Ltd. in Mumbai, India.

2.2 Instrumentation

A Waters Alliance e2695 HPLC with a quaternary pump, a Photodiode Array detector, and boost 2.0 software was used.

2.3 Analytical Method development

To find a satisfactory resolution on verapamil, a number of studies were carried out. To create the method, a variety of buffers and mobile phases were employed. The active component could not be successfully separated from the related compounds using any mobile phase. The chosen mobile phases enhanced verapamil resolving power and gave it superior results. ACN and buffer are the mobile phases. The mobile phase flow rate was maintained at 1.0 mL/min during the development trials, which employed the Develosil, Waters, and Grace columns (Table. **1**). Under ideal chromatographic conditions, the active medicinal component was effectively separated. Every result fell inside the parameters.

Table.1: Results of Method Optimization**.**

LC Parameters	
HPLC	Waters 2695
Isocratic Mobile Phase	ACN: 0.1% FA 60: 40 v/v
	Flow rate: 1.0ml/min
	Injection volume: 10µl
Waters Symmetry C18	150 mm Length
	4.6mm ID
	3.5 µm PS
Analyte	Verapamil

2.4 Method Validation

By examining factors including system suitability, linearity, LOD, LOQ, robustness, and accuracy, among others, the HPLC technique was validated, and the results were found to be within the ICH's acceptable range.

2.4.1. System suitability: The System suitability parameter predicts the perfectness and efficacy of the chromatographic status under various analytical parameters. This can be studied by operating the chromatogram for about six replications like MQC-1 to MQC-6. It passed the system suitability (Table.2)

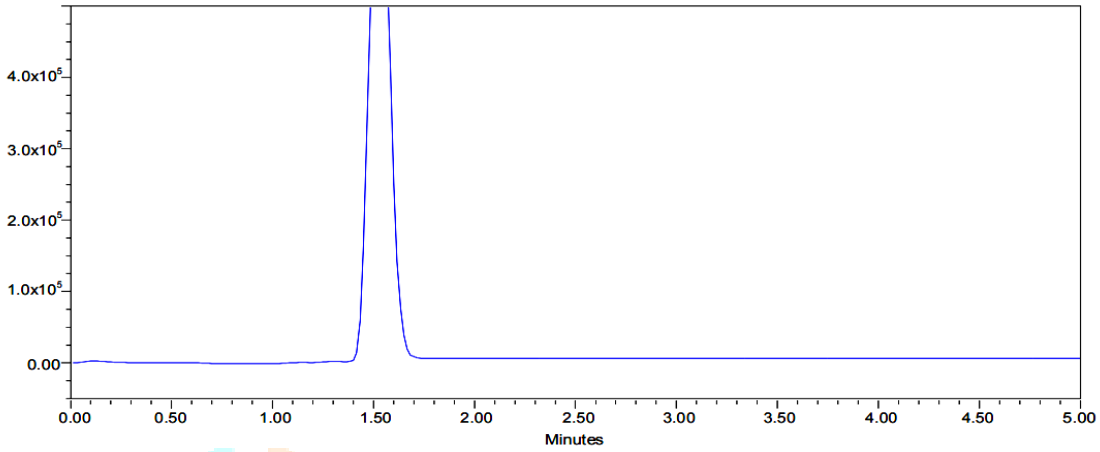
Table 2. System suitability Results of Verapamil

Sample Name MQC (24ng/mL)	Analyte Region(cps)	Analyte RT(min)	ISTD Region (24ng/mL)	ISTD RT (min)	Region Ratio
MQC-1	$2.845x10^5$	1.973	$2.854x10^5$	1.975	0.9968
MQC-2	$2.857x10^5$	1.967	$2.862x10^5$	1.964	0.9983
MQC-3	$2.838x10^5$	1.964	$2.847x10^5$	1.971	0.9968
MQC-4	$2.841x10^5$	1.976	$2.855x10^5$	1.972	0.9951
MQC-5	$2.859x10^5$	1.972	$2.862x10^5$	1.962	0.999
MQC-6	$2.847x10^5$	1.978	$2.851x10^5$	1.974	0.9986
Mean	$2.848x10^5$	1.9717	$2.855x10^5$	1.9697	0.9975
SD	0.00850	0.005	0.00598	0.005	0.00147
%CV	0.30	0.27	0.21	0.27	0.15

2.4.1 Selectivity

Selectivity was performed by analysing the rat plasma samples from six lots of different rats to test for interference from unknown components at retention time of verapamil (Fig.**2**)

Figure 2. Blank rat plasma Chromatogram



2.4.2 Matrix Effect

Matrix effect for Verapamil was evaluated by comparing the peak area ratio in the post extracted plasma sample from 6 different drug-free blank plasma samples and neat reconstitution samples. Experiments were performed at MQC levels in triplicate with six different plasma lots with the acceptable precision (%CV) of ≤15%. .

2.4.3 Dilution Integrity

Dilution integrity should be demonstrated by spiking the matrix with an analyte concentration above the ULOQC and diluting this sample with blank matrix.

2.4.4 Precision and Accuracy

Quality control samples at the lower limit of quantification (LLOQ), low quality control (LQC), medium quality control (MQC), and high quality control (HQC) levels were analyzed in duplicate. With the exception of LLOQ, where it should be within 20%, the accuracy and CV percentages should be within 15% and less than 15%, respectively (Table **3**).separated under ideal chromatographic conditions. Every result fell inside the parameters.

Table.3: Results of Precision and Accuracy**.**

Matrix	Sample	Verapamil		
		Accuracy bias (%)	Precision RSD (%)	
			Intra-day	Inter-day
Plasma	LLOQC	-0.88	0.82	0.79
	LQC	1.23	0.66	0.58
	MQC	0.47	0.59	0.42
	HQC	0.06	0.43	0.31

2.4.5 Carry over

The analyte that is maintained by the chromatographic system when a sample is injected and shows up in later blank or unidentified samples.

2.4.6 Recovery

Six replicates at each quality control concentration were analyzed to evaluate the extraction efficiencies of verapamil. By contrasting the peak regions of extracted and non-extracted standards, the % recovery was calculated(Table.4).

Table 4: Recovery of analyte of Verapamil

% Recovery			
LQC	HQC	Mean	% RSD
98.45	99.95	99.2	0.64
98.12	99.66	99.89	0.78

2.4.7. Stability

By contrasting the analytes area response in the stability sample with the sample made from the fresh stock solution, stock solution stability was assessed. Six replicates were used for stability tests in plasma at the LQC and HQC concentration levels. According to USFDA criteria, an analyte was deemed stable if the change was less than 15%. For 24 hours, the stability of spiked rat plasma samples kept at room temperature (also known as bench top stability) was assessed. For duration of 24 hours, the auto sampler stability of spiking rat plasma kept at 2–8°C was assessed. By contrasting the extract plasma samples that were reinjected after being stored in the auto sampler at 2–8°C for 24 hours with the samples that were injected right away, the stability of the auto sampler was assessed. The stability samples that had been frozen at -30°C and thawed three times were compared to newly spiked quality control samples in order to perform the freeze-thaw stability. For the study of freeze-thaw stability, six aliquots of each concentration level of LQC and HQC were utilized. The concentration measured 24 hours later was compared to the original concentration for the purpose of evaluating long-term stability.

2.5. Bioavailability Study

Verapamil was injected orally into the rat's body using pure HPLC-grade water in order to examine its bioavailability characteristics. At various intervals, such as 0.5, 1, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, and 24 hours after the medications were supplied to the rat body, we took samples from the body. Following that, a test sample was made, put into the chromatographic apparatus, and the results were recorded. The pharmacokinetic parameters that were evaluated were t1/2 (terminal half-life as determined by the quotient 0.693/Kel), Kel (apparent first order terminal rate constant calculated from a semi-log plot of the plasma concentration versus time curve using the method of the least square regression), Tmax (time to observed maximum drug concentration), and Cmax (maximum observed drug concentration-time measured, using the trapezoidal rule). The appropriate range of 80%–120% is identified for the test/reference ratios for Cmax, AUC0–12, and AUC. The pharmacokinetic parameters for the assessment of verapamil are displayed in Table 5 and Figure 3. The concentration measured 24 hours later was compared to the original concentration for the purpose of evaluating long-term stability.

Pharmacokinetic parameters	Verapamil
AUC_{0-t} (ng h/ml)	24±0.5
C_{max}(ng/ml)	19.011±0.237
$AUC_{0-\infty}$ (ng h/ml)	19.72±1.154
K_{el}	1.491±0.895
$T_{1/2}$(h)	7±0.5
T_{max} (h)	7±0.5

Table 5: Pharmacokinetic studies of Verapamil

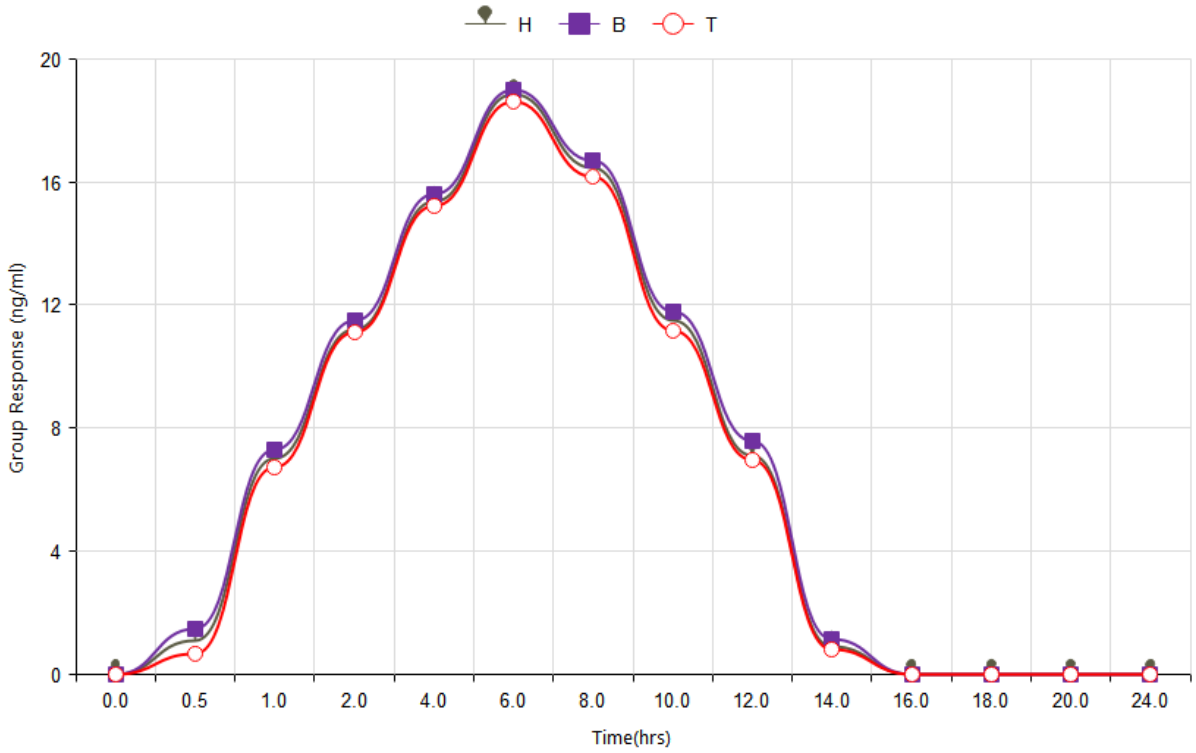


Fig. 3: Mean plasma concentration-time plot for Verapamil after oral administration

III.RESULTS AND DISCUSSION

To acquire the best chromatographic conditions, we used different buffers with acetonitrile as mobile phase in different ratios for isocratic and gradient mode was tested. The mobile phase composition was modified at each trial to enhance the resolution and also to achieve acceptable retention times. Finally, 0.1% formic acid and acetonitrile in isocratic mode at 60:40 v/v ratios was selected as mobile phase because it gives a maximum response of the selected drugs. In the optimization method we used different stationary phases like $C_{18,}$ C_8 and CN-propyl was used. From the different trials we get good peak shapes of verapamil and trandolapril by using symmetry C_{18} column of dimensions 150mmx4.6mm, 3.5 µ connected to a PDA detector. The flow rate of mobile phase was done at 1 mL/min.By applying the above conditions. We get 0.30 as % CV of six replicate injections; it indicates that the proposed method is highly accurate. The developed method was validated according to the USFDA guidelines. The process was validated and the results fell within the permitted range as per ICH standards.

IV.CONCLUSIONS

In this study, a high pressure liquid chromatography method for the determination of verapamil in rat body and bioavailability study was developed. It was quick, cheap, sensitive, and easily accessible. Shorter run times, lower costs, assessibility, sensitivity, and reproducibility are all advantages of this approach.

V. ACKNOWLEDGMENT

The authors would like to express special thanks and gratitude to RVR labs, Visakhapatnam to providing laboratory facilities.

References:

[1] Singh BN, Ellrodt G, Peter CT (March 1978). "Verapamil: a review of its pharmacological properties and therapeutic use". Drugs. 15 (3): 169–197.

[2] Schroeder JS, Frishman WH, Parker JD, et al. (2013). "Pharmacologic Options for Treatment of Ischemic Disease". Cardiovascular Therapeutics: A Companion to Braunwald's Heart Disease. Elsevier. pp. 83–130

[3] Merison K, Jacobs H (November 2016). "Diagnosis and Treatment of Childhood Migraine". Current Treatment Options in Neurology. 18 (11): 48

[4] Srinivasan V, Sivaramakrishnan H, Karthikeyan B (2011). "Detection, isolation and characterization of principal synthetic route indicative impurities in verapamil hydrochloride". Scientia Pharmaceutica. 79 (3): 555–568

[5] James PA, Oparil S, Carter BL, et al. (February 2014). "2014 evidence-based guideline for the management of high blood pressure in adults: report from the panel members appointed to the Eighth Joint National Committee (JNC 8)". JAMA. 311 (5): 507–520

[6] Beck E, Sieber WJ, Trejo R (February 2005). "Management of cluster headache". American Family Physician. 71 (4): 717–724.

[7] Jun P, Ko NU, English JD, et al. (November 2010). "Endovascular treatment of medically refractory cerebral vasospasm following aneurysmal subarachnoid hemorrhage". American Journal of Neuroradiology. 31 (10): 1911–1916.

[8] ICH (2003) Q1A (R2) Stability testing of new drug substances and products. https://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q1A_R2/Step4/Q1A_R2_Guideline.pdf.

[9] Young JR, Sternbach S, Willinger M, et al. (17 December 2018). "The etiology, evaluation, and management of plantar fibromatosis". Orthopedic Research and Reviews. 11: 1–7

[10] Dutta D, Nagendra L, Raizada N, et al. (May–June 2023). "Verapamil improves One-Year C-Peptide Levels in Recent Onset Type-1 Diabetes: A Meta-Analysis". Indian J Endocrinol Metab. 27 (3): 192–200

[11] Giannini AJ, Houser WL, Loiselle RH, et al. (December 1984). "Antimanic effects of verapamil". The American Journal of Psychiatry. 141 (12): 1602–1603.

[12] Giannini AJ, Taraszewski R, Loiselle RH (December 1987). "Verapamil and lithium in maintenance therapy of manic patients". Journal of Clinical Pharmacology. 27 (12): 980–982

[13] Giannini AJ, Nakoneczie AM, Melemis SM, et al. (February 2000). "Magnesium oxide augmentation of verapamil maintenance therapy in mania". Psychiatry Research. 93 (1): 83–87

[14] Krevsky B, Maurer AH, Niewiarowski T, et al. (June 1992). "Effect of verapamil on human intestinal transit". Digestive Diseases and Sciences. 37 (6): 919–924

[15] Steele RM, Schuna AA, Schreiber RT (April 1994). "Calcium antagonist-induced gingival hyperplasia". Annals of Internal Medicine. 120 (8): 663–664.

[16] Wilimowska J, Piekoszewski W, Krzyanowska-Kierepka E, et al. (2006). "Monitoring of verapamil enantiomers concentration in overdose". Clinical Toxicology. 44 (2): 169–171.

[17] Ahmad F, Abu Sneineh M, Patel RS, et al. (June 2021). "In The Line of Treatment: A Systematic Review of Paroxysmal Supraventricular Tachycardia". Cureus. 13 (6): e15502.

[18] Delaney B, Loy J, Kelly AM (June 2011). "The relative efficacy of adenosine versus verapamil for the treatment of stable paroxysmal supraventricular tachycardia in adults: a meta-analysis". Eur J Emerg Med. 18 (3): 148–52

[19] Soohoo MM, Stone ML, von Alvensleben J, et al. (2021). "Management of Atrial Tachyarrhythmias in Adults with Single Ventricle Heart Disease". Current Treatment Options in Pediatrics. 7 (4): 187–202

[20] Wang SP, Wang JA, Luo RH, et al. (September 2008). "Potassium channel currents in rat mesenchymal stem cells and their possible roles in cell proliferation". Clinical and Experimental Pharmacology & Physiology. 35 (9): 1077–1084

[21] Petersen AS, Barloese MC, Snoer A, et al. (September 2019). "Verapamil and Cluster Headache: Still a Mystery. A Narrative Review of Efficacy, Mechanisms and Perspectives". Headache. 59 (8): 1198–1211

[22] Bellamy WT (1996). "P-glycoproteins and multidrug resistance". Annual Review of Pharmacology and Toxicology. 36: 161–183