Research Article

Reverse Phase High Performance Liquid Chromatographic Determination of Flunarazine in Tablet Dosage Form

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ABSTRACT

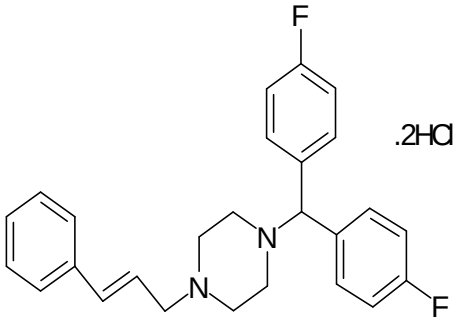
An accurate, simple, selective and economic Reversed Phase High Performance Liquid Chromatographic method (RP- HPLC) has been developed and validated for determination of Flunarazine in its tablet dosage form. Chromatographic separation was achieved using Welchrom C18 isocratic column, (250x4.6mm i.d. partical size 5µm.) Mobile phase containing a mixture of Methanol: Acetonitrile: Water in the ratio (50:30:20v/v) was prepared and flow rate set at 1.0ml/min. UV detection was set at 245nm. The developed method was validated as per ICH guidelines an d was revealed to be specific, rapid and reproducible.

Keywords: RP-HPLC, Flunarazine, Methanol,Water and Acetonitrile.

INTRODUCTION

Flunarazine is chemically 1-[bis (4-fluorophenyl)methyl 1]-4- [2E-3- phenyl prop-2—en 1yl piperazine] It is one of the latest calcium channel antagonist with proven antimigraine effect. It prevents cell damage due to calcium over load by selectively blocking the entry of calcium into the cells of tissues. It has been also found to inhibit the contraction of vascular smooth muscles and protect brain cells from effects of hypoxia.

The study revealed RP-HPLC analytical method for determination of Flunarazine in tablet dosage form to be novel, simple, efficient and reproducible. The established method has been validated with respect to specificity linearity, accuracy, precision and robustness according to ICH guidelines.



Flunarazine has molecular formula $C_{26}H_{26}N_2F_2$ 2HCl and molecular wt 477.4 It is widely used in treatment of cerebral and peripheral vascular insufficiency. It is white crystalline powder sparingly soluble in Methanol, Ethanol and slightly soluble in Chloroform, freely soluble in water and insoluble in ether and Isopropyl alcohol.

MATERIALS AND METHODS

Chemicals and Reagents

The reference sample of Flunarazine standard was kindly supplied as gift sample by Hetero Drugs Ltd., Hyderabad, Andhra Pradesh, India. All the chemicals were analytical grade. Acetonitrile (HPLC grade) and triethylamine (HPLC grade) were purchased from Merck Pharmaceuticals Private Ltd., Mumbai, India. Methanol and water used were of HPLC grade and purchased from Merck Specialties Private Ltd., Mumbai, India. Commercial tablets of FLN formulation was procured from local market.

Instruments and Chromatographic Conditions

Chromatographic separations were achieved by using Shimadzu LC-20AT Prominence Liquid Chromatograph comprising a LC-20AT VP pump, Shimadzu SPD-20A Prominence UV-detector and Welchrom C18 column (4.6 mm i.d. X 250 mm, 5 micron particle size). 20 µL of sample was injected into the HPLC system. The HPLC system was equipped with “Spinchrom” data acquisition software. Separations were performed on the reversed phase column using a mixture of methanol, acetonitrile and water (pH adjusted to 4.6 using o-phosphoric acid) in ratio of 50:30:20,

v/v as mobile phase. Triethylamine was used as column modifier. The mobile phase was delivered at a flow rate of 1 mL/min. Eluate was monitored at 245 nm. In addition, an electronic balance (Shimadzu TX223L), digital pH meter (Systronics model 802), a sonicator (spectra lab, model UCB 40), UV-Visible Spectrophotometer (Systronics model 2203) were used in this study.

Preparation of solvents and Reagent

Mobile phase

The mobile phase was prepared by mixing of methanol, acetonitrile and water (all of HPLC grade) in the ratio of 50:30:20, v/v. Then pH is adjusted to 4.6 with 0.1N o-phosphoric acid and 0.5ml triethylamine is added as column modifier. It is filtered through 0.45 µm nylon membrane filter and then sonicated for degassing.

Working Standard Solutions

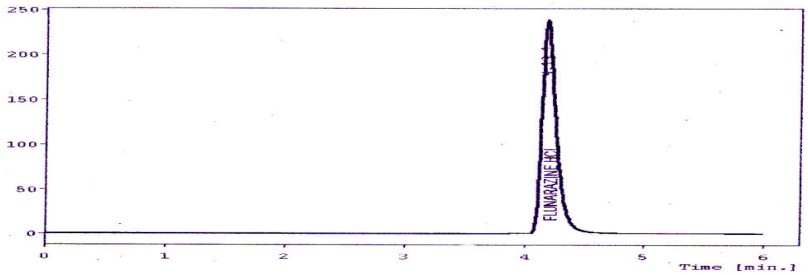
Accurately weigh and transfer about 10 mg of Flunarazine dissolve in a 100ml volumetric flask with mobile phase. This is stock standard solution of Flunarazine with concentration of 100 µg/mL. Prepare five working standard solutions for calibration by adding defined volumes of the stock standard solution and diluting with mobile phase. The concentrations of Flunarazine are 2.0, 4.0, 6.0, 8.0, 10.0 µg/mL, respectively.

Sample Preparation

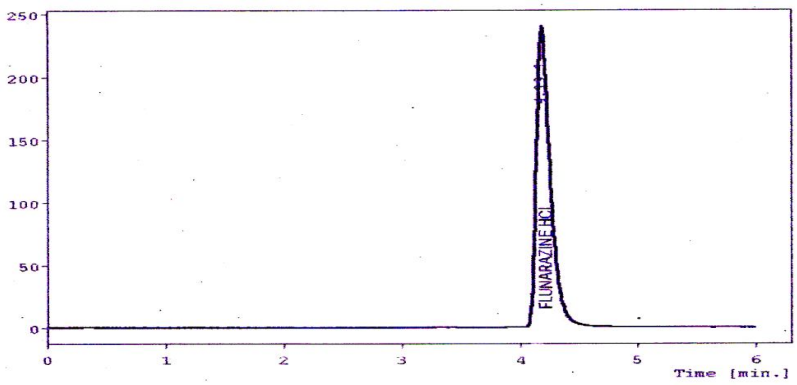
Weigh accurately not less than 20 tablets and determine average weight. Crush the tablets of Flunarazine into fine powder. Weigh equivalent to 10 mg of Flunarazine into 100 mL volumetric flask. Add 70 mL mobile phase and sonicate until dissolution is complete. Make up the volume to 100 mL. Pipette out 1.0 mL of solution into a 10 mL volumetric flask and dilute with mobile phase upto the mark. Mix well. The resulting solution was filtered using 0.2 µm filter and degassed by sonication.

Selection of Detection Wavelength

The UV spectrum of diluted solutions of various concentrations of Flunarazine in mobile phase was recorded using UV spectrophotometer. The wavelength of maximum absorbance was observed at 245 nm. This wavelength was used for detection of Flunarazine.

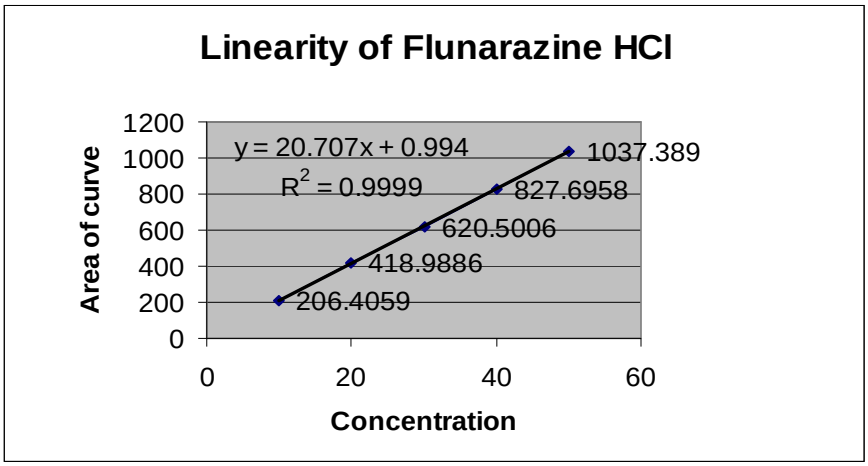


Standard chromatogram of Flunarazine



Chromatogram of Flunarazine for sample

R_t	Mean	Mean Deviation	Average Deviation	% RMD	S.D	Range of Precision	% RSD	Std. Error
4.199		0.007						
4.182		0.010						
4.194		0.002				4.192		
4.179	4.192	0.013	0.0092	0.219	0.011	±	0.2624	0.117
4.206		0.014				0.011		



Linearity Plot of Detector Response for Flunarazine

Linearity Regression Data of Flunarazine

Parameters	Flunarizine
Conc. Range(µg/ml)	10-50
m	20.707
b	0.994
r	0.9999

Recovery Data of Flunarazine

Amount of sample drug taken in mg	Amount of standard drug added in mg	Amount recovered in mg	% Of recovery	RSD
10.0	0.0	10.04	100.06	0.026
10.0	5.0	14.98	99.95	0.018
10.0	10.0	20.03	100.09	0.058
10.0	15.0	25.05	100.15	0.074

Observation table of Flunarazine

Graph	Retention time (min.)	Area (mV.s)	Height (mV)	W05 (min.)
LINEARITY				
1	4.199	206.4059	26.9554	0.1270
2	4.182	418.9886	53.5533	0.1268
3	4.194	620.5006	78.2127	0.1265
4	4.179	827.6958	102.8064	0.1262
5	4.206	1037.3886	131.4232	0.1275
RECOVERY				
1	4.196	2962.3178	355.0188	0.1267
2	4.190	3914.8376	464.9073	0.1333
3	4.199	4964.2043	589.6515	0.1333
STANDARD				
1	4.193	1995.2254	239.4890	0.1267
SAMPLE				
1	4.188	1985.0769	240.8215	0.1267

Result of Precision (Intraday)

Sample	Concentration in mg/ml	Injection No.	Peak Area	% RSD
Flunarazine	10	1	992.884	0.5947
		2	976.568	
		3	984.924	
		4	978.912	
		5	981.826	
		6	979.382	

Result of Precision (Interday)

Sample	Concentration in mg/ml	Injection No	Peak Area	% RSD
Flunarazine	10	1	980.458	0.7768
		2	968.324	
		3	982.964	
		4	970.562	
		5	962.746	
		6	972.862	

Recovery data of flunazarine

Recovery Level	Amount added	Total Amount	Amount found	Amount recovered	% Recovery	Mean Recovery	%RSD
80%	7.92	18.01	18	7.91	99.87	99.79	0.755
80%	8.01	18.1	18.02	7.93	99.00		
80%	7.97	18.06	18.1	8.01	100.50		
100%	10.03	20.12	20.07	9.98	99.50	99.89	0.458
100%	9.86	19.95	19.93	9.84	99.79		
100%	9.98	20.07	20.11	10.02	100.40		
120%	11.9	21.99	21.91	11.82	99.32	99.77	0.705
120%	12. 06	22.15	22.08	11.99	99.41		
120%	11.85	21.94	22.01	11.92	100.59		

Robustness Results of Flunarazine

S No.	Parameter	Optimized	Used	Retention time	Peak asymmetry	Remark
1	Flow rate	1.0ml/min	0.8 ml/min	6.384	1.098	Robust*
			1.2ml/min	5.872	1.084	Robust*
2	Detection wavelength	245nm	240nm	6.026	1.096	Robust*
			250nm	6.123	1.092	Robust*
3	Mobile Phase composition	50:50v/v	55:45 v/v	6.428	1.094	Robust*
			45:55v/v	5.898	1.085	Robust*

RESULTS AND DISCUSSION

The present study was aimed to develop a rapid, accurate and precise HPLC method for the determination of Flunarazine in pharmaceutical dosage form. Acceptance peak symmetry was achieved using a column of C18 column (250mmx4.6mm i.d, 5µm partical size) and mobile phase composed of methanol, acetonitrile and HPLC grade water in the ratio of 50:30:20, v/v with pH adjusted to 4.6 using O-phosphoric acid and triethylamine as column modifier at a flow rate of 1ml/min. The retention time for Flunarazine was found to be 6.023% RSD% for intra-day and interday precision variation studied at 10µg/ml obtained were 0.5947 and 0.7768 respectively. The mean recovery of Flunarazine was 99.07% to 100.35%

ACKNOWLEDGEMENT

The authors thank Dr. AD Taranalli, Principal, and Prof. Dr. V P Rsal as Vice-Principal, K.L.E.S's College of Pharmacy, Belgaum, for the providing necessary facilities and encouragement. And also to geno pharmaceuticals ltd. Goa for providing gift sample of Flunarizine.

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