International Journal of Pharmacy and Biological Sciences-IJPBS™ (2019) 9 (2): 765-773
Online ISSN: 2230-7605, Print ISSN: 2321-3272
Research Article | Pharmaceutical Sciences | Open Access | MCI Approved
UGC Approved Journal

Analytical Method Development and Validation of Rapid and Simple Stability-Indicating RP-HPLC Method for Determination of Fluindione in Bulk and Tablet Dosage Forms

J.P. Srinivasa Reddy*[1,2], T. Parthasarathy[3], Chetan M. Bhalgat[2]

[1]Department of Chemistry, Dravidian University, Kuppam, Andhra Pradesh, India
[2]Analytical Development, Rubicon Research Pvt Ltd, Thane, MedOne House, Wagle Estate, Thane West, Maharashtra, India
[3]Department of Chemistry, Osmania University, Hyderabad, Telangana, India

Received: 15 Jan 2019 / Accepted: 11 Mar 2019 / Published online: 1 Apr 2019
Corresponding Author Email: srinivasjp777@yahoo.com

Abstract

Fluindione is an oral anticoagulant drug. A rapid, simple, selective and precise method has been successfully developed and validated for the determination of Fluindione in bulk form and tablet dosage formulations. This method is based on HPLC separation followed by UV detection at 285 nm. HPLC method was developed on a Symmetry ODS (4.6 x 150 mm, 5µ) column with a mobile phase consisting of sodium phosphate buffer pH 3.5: Acetonitrile, 50:50 v/v, pumped at 1.0 mL/min flow rate. The pH of buffer was adjusted to 3.5 with ortho phosphoric acid. The column was maintained at ambient temperature and 20µl of solutions were injected. The eluted compound was detected by using PDA detector. Fluindione was eluted at 3.5 minutes. Stress degradation study shows that sample degraded with acid hydrolysis, base hydrolysis, Oxidation and thermal stress conditions. The method was validated in accordance with requirement of ICH guidelines for specificity, linearity, accuracy, precision, robustness and ruggedness.

Keywords

Fluindione, Stability indicating RP-HPLC, Method development, Forced degradation, Validation and ICH Guideline

*****

1. INTRODUCTION

Fluindione is an oral anticoagulant with half-life of about 69 hours that works by inhibition of synthesis of vitamin K-dependent clotting factors. Fluindione is used in various cardiologic diseases for the prevention of thrombo embolism. It acts as a vitamin K antagonist to antagonize the effect of vitamin K required for the synthesis of active clotting factors II, VII, IX, and anticoagulant proteins C and S.

Chemically Fluindione is 2-(4-fluorophenyl) indene-1, 3-dione[1-5].

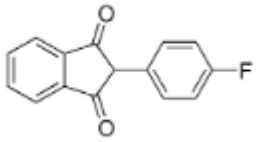

Figure 1: Chemical structure of Fluindione

Fluindione is a White amorphous powder with a molecular formula of $C_{15}H_9FO_2$ and a molecular weight of 240. Fluindione is freely soluble Water, Ethanol and Acetonitrile. It is available as tablet with strength 20 mg.

Literature survey reveals that very few analytical methods have been reported for Fluindione, by UV-spectrophotometer and HPLC. But, reported HPLC methods are either with higher run time or some complex mobile phase. Many methods used expensive solvents in their mobile phase. Hence, need to redevelop with economic solvents.[6-7]

2. MATERIALS AND METHOD

2.1. Chemicals and reagents

Analytically pure sample of Fluindione was kindly supplied by Toronto Research Chemical Inc., India. Acetonitrile (HPLC grade), Sodium hydrogen phosphate (Emparta Grade), Phosphoric acid (Emparta Grade) was purchased from Merck specialties Pvt. Ltd. (Mumbai, India).

2.2. Instruments

Chromatography was carried out by using Shimadzu LC 2010 and Agilent Technologies 1100/1200 series instruments equipped with column oven, PDA detector, and the data were processed using a computer program (Chromeleon 6.80 SR13 Build). An electronic balance (Mettler Toledo), MilliQ- water purification system was used in this study.

2.3. Selection of analytical wavelength

From the standard stock solution further dilutions were done using acetonitrile and scanned over the range of 200–400 nm.

2.4. Selection of mobile phase

The standard solution of Fluindione (16 µg/mL) was injected into the HPLC system and run in different solvent systems. Different mobile phases like acetonitrile and water, methanol and acetate buffer, methanol and phosphate buffer, acetonitrile and phosphate buffer in varying proportion of mobile phase components, varying conditions of pH were tried in order to obtain the desired system suitability parameters for the Fluindione.

2.5. Optimized chromatographic conditions

The mobile phase consisted of acetonitrile and 20mM sodium hydrogen phosphate buffer (pH 3.5) in the ratio of 50: 50 v/v. It was then filtered through 0.45 µ membrane filter paper using vacuum filtration assembly and then sonicated on ultrasonic water bath for 15 min. The flow rate of mobile phase was maintained at 1 mL/min. Different column were tried and found good peak shape on Symmetry ODS (4.6 x 150 mm, 5µ) column. The column and the HPLC systems were kept in ambient temperature. Mobile phase is also used as diluent.

2.6. Preparation of solutions

Preparation of standard stock solution

Prepared standard stock solution of Fluindione was prepared in methanol (400 µg/mL). Further dilution was made in methanol to get final solution of Fluindione 4, 8, 12, 16, 20 and 24 µg/mL for linearity. Concentration 16 µg/mL is selected for standard solution.

Preparation of buffer

2.85 gm of Sodium hydrogen phosphate was accurately weighed and dissolved in distilled water in a 1000 mL volumetric flask. The final volume was made upto the mark with purified water. pH of the buffer was adjusted to 3.5±0.05 with ortho phosphoric acid.

Preparation of 0.1 N hydrochloric acid solution

Hydrochloric acid (0.1N) was prepared by diluting 0.085 mL of concentrated hydrochloric acid solution to 10 mL with water.

Preparation of 0.1 N sodium hydroxide solution

Sodium hydroxide (0.1N) was prepared by diluting 0.4 g of sodium hydroxide flakes to 100 mL with water.

Preparation of 10 % v/v hydrogen peroxide solution

Hydrogen peroxide (10 % v/v) was prepared by appropriately diluting 33.5 mL of 30 % v/v hydrogen peroxide to 100 mL with water.

Preparation of sample solution of blend

Strength of marketed tablets is 20 mg of Fluindione per tablet. Twenty tablets were weighed, and average weight was calculated. The tablets were crushed to fine powder and used for assay. The excipient blend was spiked with Fluindione for accuracy.

2.7. Preparation of placebo blend

Blend equivalent to 250 mg Fluindione in tablet was prepared by blending 1.125 gm Starch, 1.125 gm Lactose. Geometric mixing method was used. Blend equivalent to 40 mg of Fluindione was used for accuracy evaluation.

2.8. Assay of tablet dosage form

Twenty tablets were weighed, and average weight was calculated. The tablets were crushed to fine

powder and a quantity of powder equivalent of 40 mg was accurately weighed and transferred to a volumetric flask of 100 mL capacity. To this 70 mL of diluent was added and sonicated for 15 minutes. Cooled and the final volume was made upto the mark with the diluent. The solution was filtered through 0.22 µ membrane filter. An aliquot of 4 mL was transferred to a 100 mL volumetric flask and diluted upto the mark with diluent. 20 µL of this solution was injected into the chromatograph and the chromatogram was recorded. The peak area was determined and the amount of Fluindione was calculated.

2.9. Accuracy for tablet dosage form

Fine powder of placebo blend equivalent of 40 mg of Fluindione was accurately weighed and transferred to a three volumetric flask of 100 mL capacity. To this 20 mg, 40 mg and 60 mg of Fluindione standard is added respectively. To each flask 70 mL of diluent was added and each flask sonicated for 15 minutes. Cooled to room temperature and the final volume was made upto the mark with the diluent. The solution was filtered through 0.22 µ membrane filter. An aliquot of 4 mL was transferred to a 100 mL volumetric flask and diluted upto the mark with diluent. 20 µL of this solution was injected into the chromatograph and the chromatogram was recorded. The peak area was determined and the amount of Fluindione was calculated.

2.10. Forced degradation studies

In order to determine whether the method is stability indicating, forced degradation studies were carried under condition of acid, alkaline, oxidation and dry heat photolysis as per ICH Q1A (R2) and Q1B[8-9]. The analysis was carried out by HPLC with a DAD detector. For each study, two samples were prepared: the blank subjected to stress in the same manner as the drug solution and working standard solution of Fluindione subjected to degradation condition. Dry heat and photolytic degradation were carried out in solid state[8-11].

2.10.1. Acid hydrolysis

For acid treatment 40 mg of fluindione sample was taken into a 100 mL round bottom flask, 10 mL of 0.1 M hydrochloric acid solution was added, and 50 mL of diluents is added. The contents were mixed well and kept for constant stirring for 3 hours at room temperature. To this 10 mL of 0.1 M sodium hydroxide added and sonicated for 5 minutes to neutralize the solution and volume is made upto 100 mL with diluent. From this 4 mL of solution was taken in 100 mL volumetric flask and diluted up to the mark with diluent. Similarly, blank is prepared without sample.

2.10.2. Base hydrolysis

For base treatment 40 mg of fluindione sample was taken into a 100 mL round bottom flask, 10 mL of 0.1 M sodium hydroxide solution was added, and 50 mL of diluents is added. The contents were mixed well and kept for constant stirring for 3 hours at room temperature. To this 10 mL of 0.1 M hydrochloric acid added and sonicated for 5 minutes to neutralize the solution and volume is made upto 100 mL with diluent. From this 4 mL of solution was taken in 100 mL volumetric flask and diluted up to the mark with diluent. Similarly, blank is prepared without sample.

2.10.3. Oxidation

For base treatment 40 mg of fluindione sample was taken into a 100 mL round bottom flask, 10 mL of 10 hydrogen peroxide solution was added, and 50 mL of diluents is added. The contents were mixed well and kept for constant stirring for 3 hours at room temperature and volume is made up to 100 mL with diluent. From this 4 mL of solution was taken in 100 mL volumetric flask and diluted up to the mark with diluent. Similarly, blank is prepared without sample.

2.10.4. Heat degradation

For base treatment 40 mg of fluindione sample was taken into a 100 mL round bottom flask and heated at 80°C for 6 hours. To this 50 mL of diluents is added. The contents were mixed well, and volume is made up to 100 mL with diluent. From this 4 mL of solution was taken in 100 mL volumetric flask and diluted up to the mark with diluent. Similarly, blank is prepared without sample. Similarly, blank is prepared without sample.

3. Result and Discussion

3.1. Selection of analytical wavelength

The UV spectrum was scanned over range of 200-400 nm. The drug showed considerable absorbance at 285 nm (Figure 2).

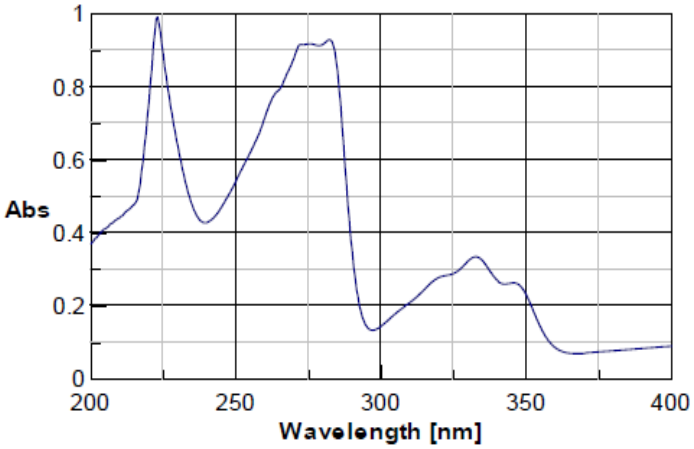

Figure 2: UV Spectrum of Fluindione (16 µg/mL)

3.2. Selection of mobile phase

After several trials, acetonitrile and 20 mM -sodium hydrogen phosphate pH 3.5 in the ratio of 50: 50 v/v was chosen as the mobile phase, which gave good resolution and acceptable peak parameters.

3.3. Chromatogram and system suitability parameter of drug

The column was equilibrated with the mobile phase (indicated by constant back pressure at desired flow rate). Working standard solution of drug (16 µg/mL) was injected into the system. The retention time for the drug was found to be 3.5 ± 0.2 minutes. System suitability parameters of Fluindione are summarized in (Table 1).

Table 1: System suitability parameters of Fluindione

S. No	Parameter	Result	Acceptance Limit
1.	Retention time (Rt)*	3.51 min.	--
2.	Number of theoretical plates (N)*	5500	More than 3000
3.	Tailing factor (T)*	1.3	Less than 2

3.4. Validation of Analytical Method

The method was validated as per ICH Q2 (R1) guidelines[10-11].

3.4.1. System suitability:

Preceding the validation, system suitability tests were conducted by measurement of general characteristics such as tailing factor, retention time, number of theoretical plates, and peak symmetry, etc. The outcomes were satisfactory and in accordance with guidelines. System suitability data was shown in Table 1.

3.4.2. Specificity

Specificity of an analytical method is its capability to measure the analyte precisely and particularly in presence of parts that may be likely to be present in the sample matrix. Chromatograms of blank, standard and sample (Figure 3-5) proves that the method was specific.

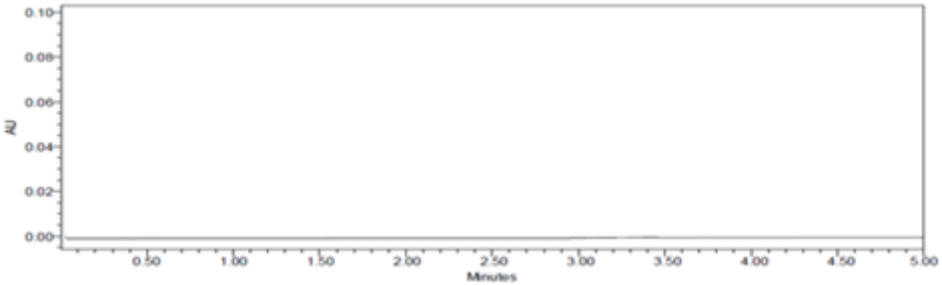

Figure 3: Chromatograms of Blank

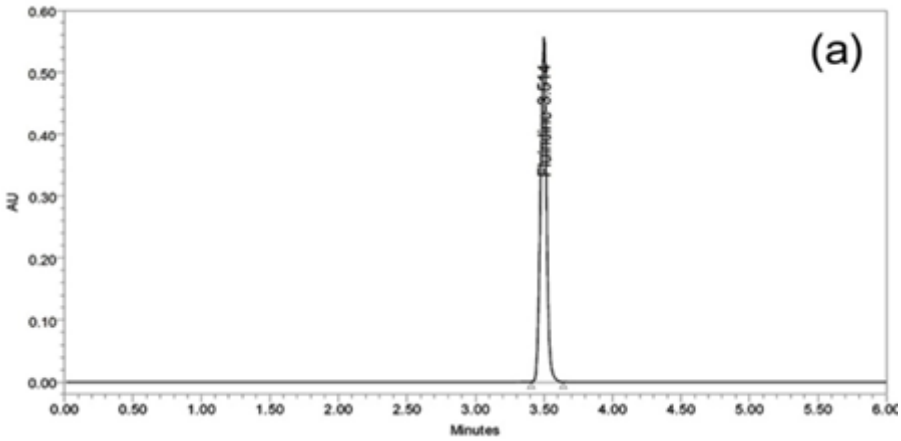

Figure 4: Chromatograms of Standard

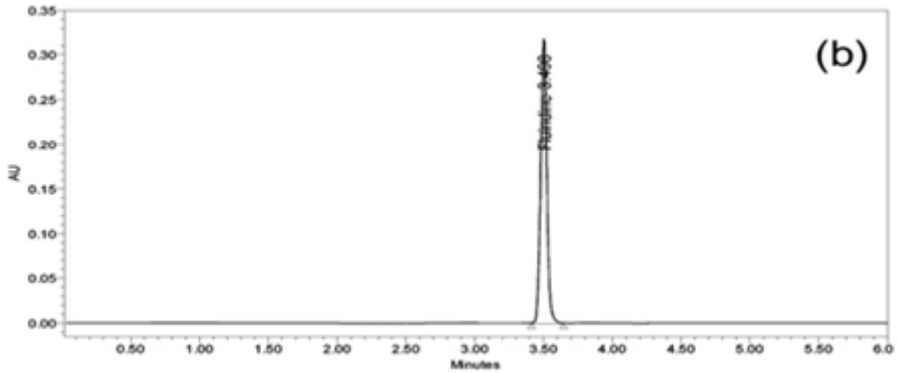

Figure 5: Chromatograms of Sample

3.4.3. Stress degradation studies of bulk drug

3.4.3.1. Acid hydrolysis

Under acid hydrolysis, 90.1 % Fluindione was recovered with the product of degradation at 8.4%. The chromatogram was showed in Figure 6 with degradation product.

3.4.3.2. Alkaline hydrolysis

After alkaline hydrolysis, 91.3 % Fluindione was recovered with the product of degradation at 7.2%. The chromatogram was showed in Figure 6 with degradation product.

3.4.3.3. Oxidative Hydrolysis

After Oxidative hydrolysis, 86.4 % Fluindione was recovered with the product of degradation at 12.1%. The chromatogram was showed in Figure 6 with degradation product.

3.4.3.4. Degradation under dry heat

After the dry heat degradation, 88.2 % Fluindione was recovered with the product of degradation at 10.3%. The chromatogram was showed in Figure 6 with degradation product. Forced degradation data is summarized in Table 2.

Table 2: Data of degradation studies of FLU

Stress condition	HPLC				
	Peak Area	% Assay of Fluindione	% Degradation	Purity Angle	Purity Threshold
Acid	3697026	90.1	8.4	0.57	0.62
Base	3744943	91.3	7.2	0.42	0.58
Peroxide	3532796	86.4	12.1	0.42	0.58
Thermal	3625764	88.2	10.3	0.35	0.48

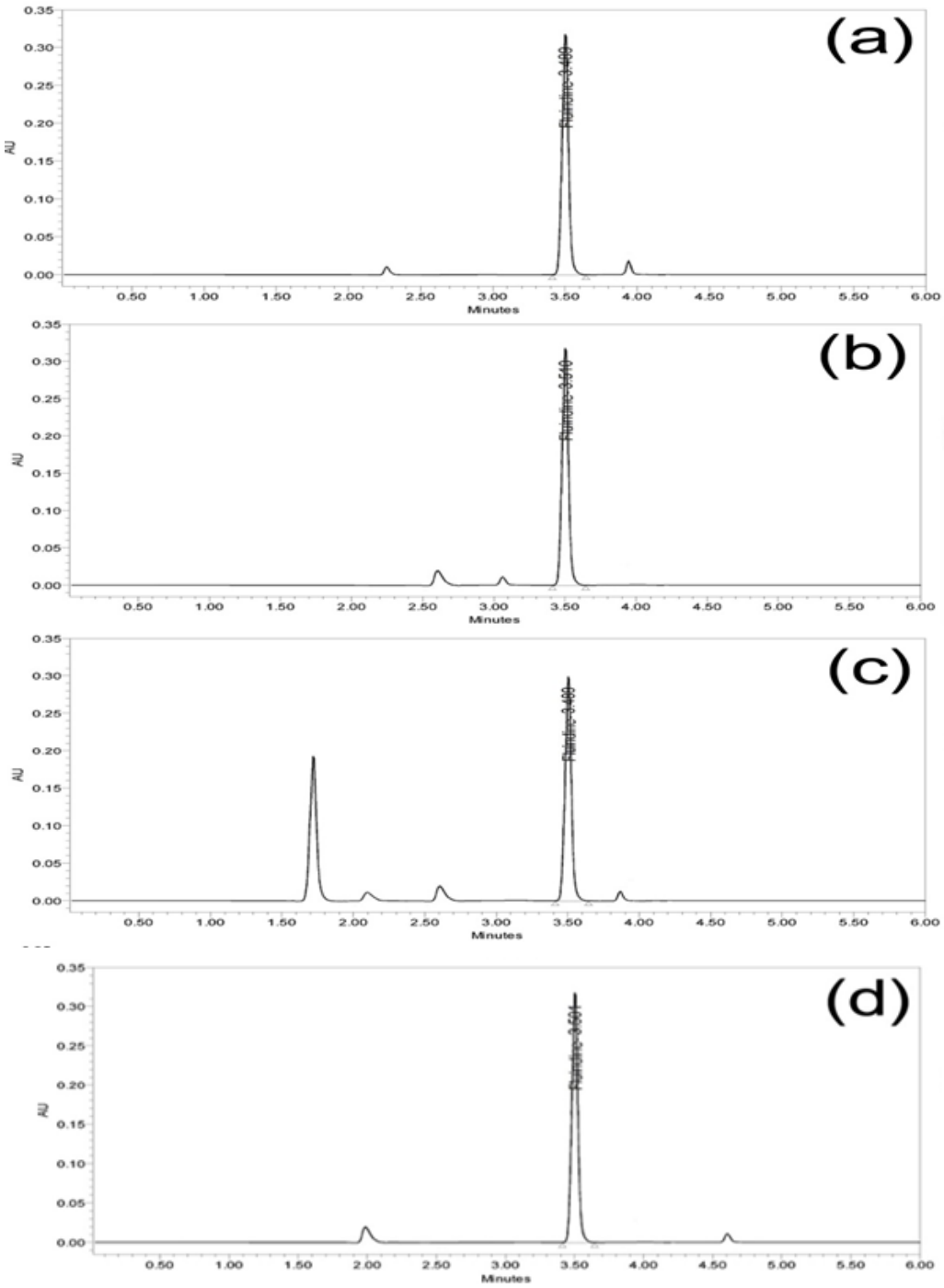

Figure 6: HPLC Chromatogram of (a) Acid Hydrolysis, (b) Base Hydrolysis, (c) Oxidation and (d) Thermal Degradation (80 ºC for 6 hours)

3.4.4. Linearity

The linearity plot was constructed with six concentrations at the level of 25-150% (4, 8, 12, 16, 20, and 24 µg/mL of Fluindione. The response of the drug was found to be linear in the studied concentration range and the linear regression equation was y = 255438x - 30988. The correlation coefficient was found to be 0.999. The linearity curve was shown in figure 7. Table 3 gives the Linearity data of FLU.

Table 3: Linearity data of Fluindione

Linearity Level	Concentration µg/mL	Area	Statistical Analysis	
L1-25%	4	1076185		
L2-50%	8	1944757	Correlation Coefficient (R^2)	255438
L3-75%	12	2919086		
L4-100%	16	4045333		
L5-125%	20	5048604	Slope	0.999
L6-150%	24	6156889		

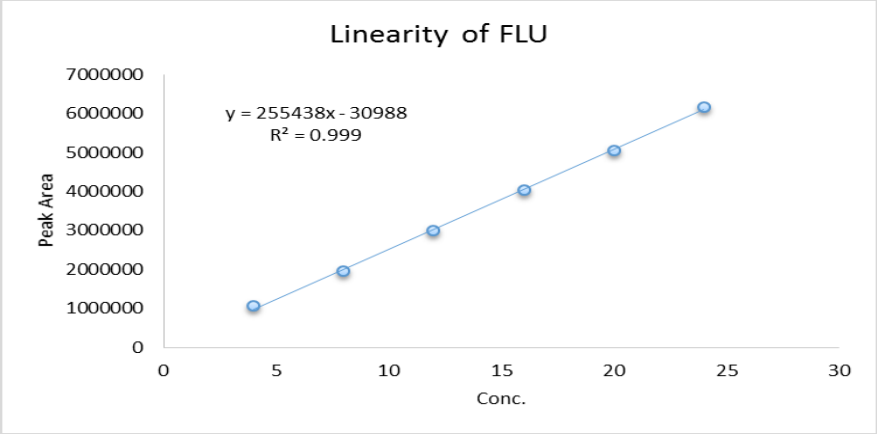

Figure 7: Linearity curve of Fluindione

3.4.5. Range

The linearity range of was found to be 4-24 µg/mL.

3.4.6. Assay

The proposed method was applied for the tablet of Fluindione and the mean % assay was found to be 100 %. The chromatograms show that no interference from excipients (Figure 5.18-7.19). The results of % assay was shown in Table 4.

Table 4: Assay data of Fluindione Tablets

Tablet	Label Claim (mg)	Amount found (mg/tablet)	% Label claim*± S.D.	% recovery
Previscan	20	19.70	98.5±0.0147	100.0

***average of three determinations**

3.4.7. Accuracy

The accuracy of the method was assessed by standard addition method. % Recovery for three concentrations (corresponding to 50, 100 and 150 % of test solution concentration) were determined. For each concentration three replicates were prepared. The mean recovery of Fluindione was between 97.0-100.0 % (Table 5).

Table 5: Result of accuracy study of Fluindione (n=3)

Spiked Level	Mean Sample Area	Amount added	Amount found	% Mean Recovery*
50%	2059980	200.00	200.07	100.0
100%	4032436	400.00	399.80	98.03
150%	6033497	600.00	598.54	97.70

3.4.8. Precision:

Inter and intra-day precision of the analytical method was determined. % RSD was premeditated and detected within the definite limits (<2 %). Table 6 shows the results of precision.

Table 6: Summary of precision study of Fluindione

S. No	Precision data of Fluindione			
	Intraday precision		Inter day precision	
	Peak Area	% Assay	Peak Area	% Assay
1.	4091234	99.70	4084593	99.60
2.	4090556	99.60	4086952	99.90
3.	4084067	99.40	4070766	99.10
4.	4088239	99.70	4078302	99.00
5.	4087654	100.0	4072752	99.00
6.	4087121	99.20	4072413	99.20
Average	4088145.2	99.60	4077629	99.30
STDEV	2580.05	0.28	6840.48	0.37
% RSD	0.06	0.28	0.17	0.37

3.4.9. Robustness study

The robustness was estimated by analysing test solutions after minor but thoughtful variations in the analytical environment like flow rate (± 0.1 mL/min), wavelength (± 2 nm) and the column temperature (± 2°C). System suitability data was detected to be satisfactory amid discrepancy of the analytical conditions. Results of system suitability show that the analytical method persistently modest by minor but thoughtful variations in the analytical settings. Table 7 depicts the outcomes of robustness study.

Table 7: Evaluation data of robustness study of Fluindione

Robust conditions	Rt (min)	Peak area	System-suitability parameters	
			Theoretical Plates	Asymmetry
Flow rate 0.9 mL/min	3.821	4041547	5654	1.4
Flow rate 1.0 mL/min	3.410	4056920	5500	1.3
Flow rate 1.1 mL/min	3.141	4045647	5654	1.4
Column temp at 23°C	3.542	4046589	5598	1.5
Column temp at 25°C	3.410	4056920	5500	1.3
Column temp at 27°C	3.578	4044567	5275	1.4
Wavelength 283 nm	3.414	4031546	5534	1.5
Wavelength 285 nm	3.410	4056920	5500	1.3
Wavelength 287 nm	3.422	4042356	5624	1.3

4. CONCLUSION

Results of all the method validation parameters shows that the developed RP-HPLC method was simple, sensitive, selective, accurate, precise, and economical. The relative standard deviation values of all the parameters were well within the limits, which prove that the method was validated. In the forced degradation studies, there was no interference of a degradation product. Assay results and non-interference of excipients indicates that the method can be used routinely for the quality control test of Fluindione tablets.

ACKNOWLEDGEMENT

The authors are thankful to principal and management of AISSMS college of Pharmacy, Pune for providing the research facilities. We also thankful to Mylan Laboratories Ltd. Hyderabad (India) for providing us Fluindione as gift sample.

REFERENCE

1. Kearon, C., Kahn, S.R., Agnelli, G., Goldhaber, S., Raskob G.E. and Comerota A.J., Chest, 133, 454S-545S (2008).
2. Holford, N., Clin. Pharmacokinet., 11, 483-504 (1986).
3. Fourel, I., Hugnet, C., Goy-Thollot, I. and Berny, P., J. Anal. Toxicol. 34, 95-102 (2010).
4. Lotfi, H., Dreyfuss, M.F., Marquet, P., Debord, J., Merle L. and Lachatre, G., J. Anal. Toxicol., 20, 93-100 (1996).
5. Imming, P., Sinning, C., Meyer, A., Nat. Rev. Drug Discov., 5(10), 821-834(2006).
6. Rao, N.M. and Gowrisankar, D., Eurasian J. of Anal. Chem. 10(2), 127-136 (2015).
7. Thimmaraju, M. and Hemanth, K., Der Pharm. Let., 7(2), 237-242 (2015).

8. International Conference on Harmonization (ICH), Stability testing of new drug substances and products, Q1A (R2), (2003).
9. International Conference on Harmonization (ICH), Photo stability testing of new drug substances and products, Q1B, (1996).
10. International Conference on Harmonization (ICH), Impurities in new drug product Q3B, (2006).
11. International Conference on Harmonization (ICH) harmonized tripartite guideline Validation of analytical procedures: text and methodology Q2 (R1) ICH, Geneva, (2005).