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Research Article

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Development and validation of stability-indicating HPTLC method for determination of fluindione

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ABSTRACT

A simple, accurate, precise and rapid validated stability indicating HPTLC method of Fluindione was successfully developed. This method is based on HPTLC separation followed by UV detection at 285 nm. The separation was carried out on merck TLC aluminium sheets precoated with silica gel 60F_{254} using chloroform: methanol (9.9: 0.1% v/v) as a mobile phase. Fluindione gave well defined and sharp peak at R_f 0.47 ± 0.02. Calibration curve was linear in range 1000-3000 ng/band. Stress degradation study shows that sample degraded with acid and base hydrolysis, under oxidation, thermal and photolytic stress conditions. The peak purity parameter ensured noninterference by product of degradation. This method can be applied to determination of stability of Fluindione. The suitability of this HPTLC method for quantitative determination of Fluindione was proved by validation in accordance with requirements of ICH guidelines.

Keywords: Fluindione, HPTLC, Forced degradation, Validation

INTRODUCTION

Fluindione is an oral anticoagulant. It is used in various cardiologic diseases for the prevention of thromboembolism. It inhibits the synthesis of vitamin K-dependent clotting factors. Chemically it is 2-(4-fluorophenyl) indene-1, 3-dione [1].

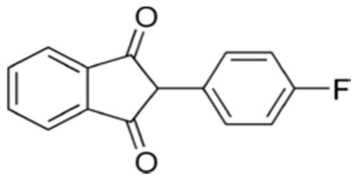

Fig 1.: Chemical structure of Fluindione

Literature survey reveals that very few analytical methods have been reported for Fluindione. Hence, considering inherent advantage of HPTLC over HPLC, the objective of current work was to develop SIM HPTLC method as per ICH Q1A (R2) guidelines. It was aimed to establish inherent stability of the Fluindione through stress studies under a variety of stress conditions and to develop a validated Stability-Indicating Assay method. There is no report yet on SIM by HPTLC. UV -Spectrophotometric method, HPLC SIM has been reported [2], [3].

EXPERIMENTAL SECTION

Chemicals and reagents

Analytically pure sample of Fluindione was kindly supplied by Mylan Laborotories Ltd. (Hyderabad) India. Chloroform and Methanol (HPLC grade) were purchased from Merck specialties Pvt. Ltd. (Mumbai, India).

Instrumentation and chromatographic conditions

Precise analytical weighing balance (Shimadzu AY120) was used to maintain weighing accuracy. Chromatographic separation of drug was performed using aluminium plate precoated with silica gel 60 F_{254} (10 ×10) with 250 µm thickness (E. MERCK, Darmstadt, Germany) using a CAMAG Linomat 5 sample applicator (Switzerland). Samples were applied on the plate as a band with 6 mm width using Camag 100 µL sample syringe (Hamilton, Switzerland). Photo stability was determined in photo stability chamber (Neutronic). Thermal degradation study was carried out in hot air oven (Kumar lab).

Linear ascending development was carried out in 10 x 10 cm twin trough glass chamber (CAMAG, Muttenz, Switzerland) by using Chloroform: Methanol (9.9:0.1 v/v) as mobile phase. The optimized chamber saturation time for mobile phase was 15 min. The length of chromatogram run was 9 cm and development time was approximately 15 min. TLC plates were dried in a current of air. Densitometric scanning was performed on CAMAG thin layer chromatography scanner at 285 nm operated by WINCATS software version 1.4.2. The source of radiation utilized was deuterium lamp emitting a continuous UV spectrum between 200 to 400 nm.

Preparation of Stock Solution

Standard stock solution of Fluindione was prepared by dissolving 10 mg of drug in 10 ml of methanol to get concentration of 1000 µg/ml from which 1 ml was further diluted to 10 ml with methanol to get concentration of solution 100 µg/ml.

Selection of Detection Wavelength

The UV spectrum of Fluindione (10 µg/ml) solution was obtained over the range of 200-400 nm. It was observed that drug showed considerable absorbance at 285 nm. So, wavelength 285 nm was selected as the wavelength for detection.

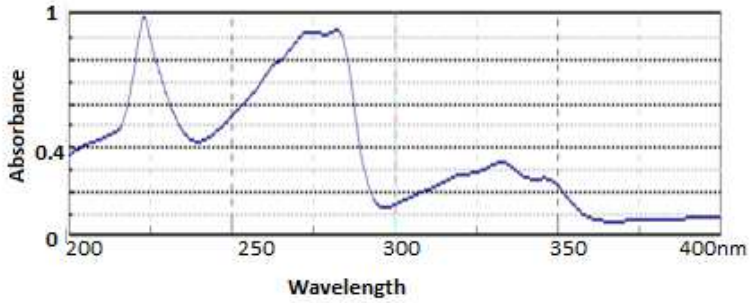

Fig. 2: UV Spectrum of Fluindione (10µg/ml)

Analysis of Drug in blend mixture

Accurately weighed quantity of Fluindione was mixed with blank blend containing starch and lactose. Spiked blend was assayed and used for accuracy studies. To determine accuracy, 2.250 gm BB and 250 mg drug were mixed properly and finally 2.5 gm SB were prepared. 100 mg were then collected and tested under 80% accuracy parameter; subsequently 100% and 120% accuracy levels were also determined.

Stress degradation studies of bulk drug

The forced degradation studies were carried out on bulk drug substance in order to prove the stability-indicating property and selectivity of the developed method. The degradation was carried out under acid, base and neutral hydrolytic, Oxidative, Thermolytic and Photolytic stress conditions [4], [5], [6].

Acid treatment
1 ml working standard solution of Fluindione (1000 µg/ml) was mixed with 1 ml of 0.1 N methanolic hydrochloric acid (HCl) and 8 ml of methanol. Solution was kept at room temperature for 3 days. The 20 µl of resulting solution was applied on TLC plate and developed under optimized chromatographic condition.

Alkali treatment
1 ml working standard solution of Fluindione (1000 µg/ml) was mixed with 1 ml of 0.01 N methanolic sodium hydroxide (NaOH) and 8 ml of methanol. Solution was kept at room temperature for 1 hour. The 20 µl of resulting solution was applied on TLC plate and developed under optimized chromatographic condition.

Neutral Hydrolysis
1 ml working standard solution of Fluindione (1000 µg/ml) was mixed with 1 ml of water and 8 ml of methanol. Solution was kept at room temperature for 1 hour. The 20 µl of resulting solution was applied on TLC plate and developed under optimized chromatographic condition.

Oxidative degradation
1 ml working standard solution of Fluindione (1000 µg/ml) was mixed with 1 ml of 0.3% v/v metabolic Hydrogen peroxide (H_2O_2) and 8 ml of methanol. Solution was kept at room temperture for 1 hour. The 20 µl of resulting solution was applied on TLC plate and developed under optimized chromatographic condition. .

Degradation under dry heat
Dry heat study was performed by keeping drug in oven at 60ºC for period of 6 hours. A sample was withdrawn at appropriate times, weighed and dissolved in methanol to get solution of 1000 µg/ml. 20 µl of the resulting solution was applied to HPTLC.

Degradation under Photolytic conditions
Photolytic degradation studies were carried out by exposure of drug to UV light up to 200 watt hours /square meter and subsequently to fluorescence light illumination not less than 1.2 million lux hours. Sample was weighed, dissolved in methanol to get concentration of 100 µg/ml. 20 µl of the resulting solution was applied to HPTLC.

RESULTS AND DISCUSSION

Optimization of chromatographic conditions
The primary objective in developing this stability indicating HPTLC method is to achieve the resolution of Fluindione and its degradation products. The chromatographic separation was achieved by linear ascending development in 10 cm × 10 cm twin trough glass chamber using Chloroform: Methanol (9.9: 0.1, v/v) as mobile phase and detection was carried out at 285 nm. The retention factor for Fluindione was found to be 0.47 ± 0.02. Representative Densitogram of standard solution of Fluindione is shown in figure: 3

Results of forced degradation studies
Forced degradation study showed that the method is highly specific and there was no interference of degradation products observed at retention factor of drug.

Acid treatment
In Acid hydrolysis condition, 19.27 % degradation of Fluindione was observed with the product of degradation at R_f = 0.51 ± 0.02. The representative densitogram obtained from acid treatment after 3 days and for a day is shown in figure 4 and 5 respectively.

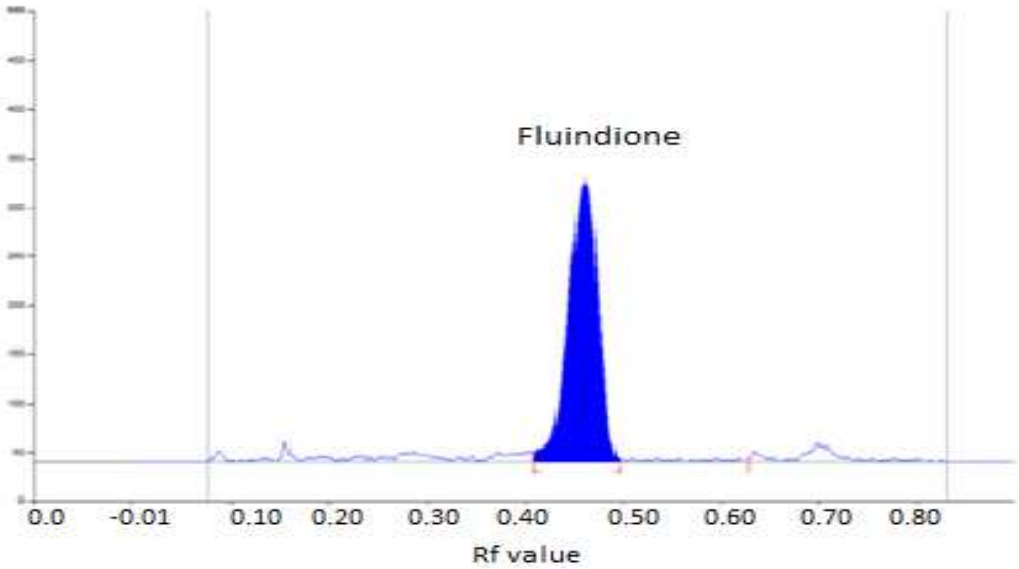

Fig. 3: Representative Densitogram of standard solution of Fluindione (2000ng/band, R_f = 0.47 ± 0.02)

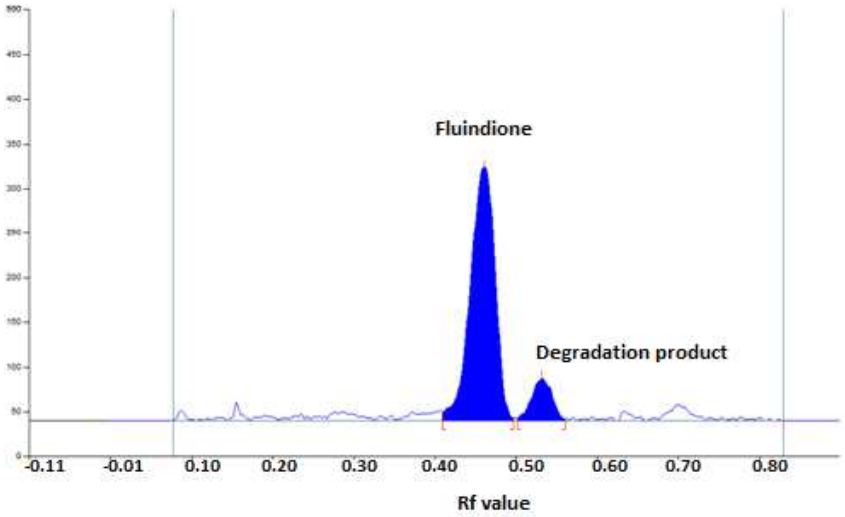

Fig. 4: Densitogram obtained from sample subjected to acid degradation with degradation product at R_f 0.51 (Duration 3 days)

Alkali treatment
In alkaline hydrolysis condition, 17.66 % degradation of Fluindione was observed.

Oxidative degradation
Fluindione when treated with 0.3% v/v H_2O_2 i.e. oxidative degradation, 12.98 % degradation was observed.

Neutral Hydrolysis
In neutral hydrolysis condition, 8.77 % degradation of Fluindione was observed.

Dry heat degradation studies:
When the drug substance was exposed to dry heat at 60º C for 6 hrs, 14.70% of degradation was observed.

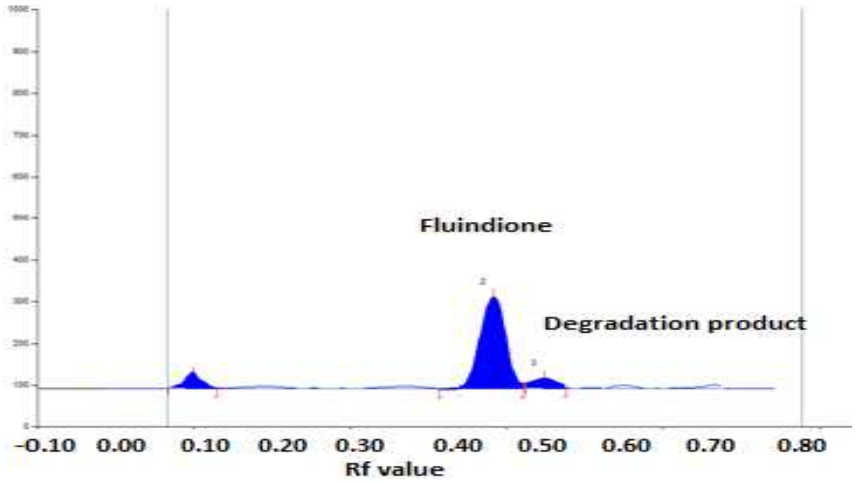

Fig. 5: Densitogram obtained from sample subjected to acid degradation with degradation product at R_f 0.51 (Duration 1days)

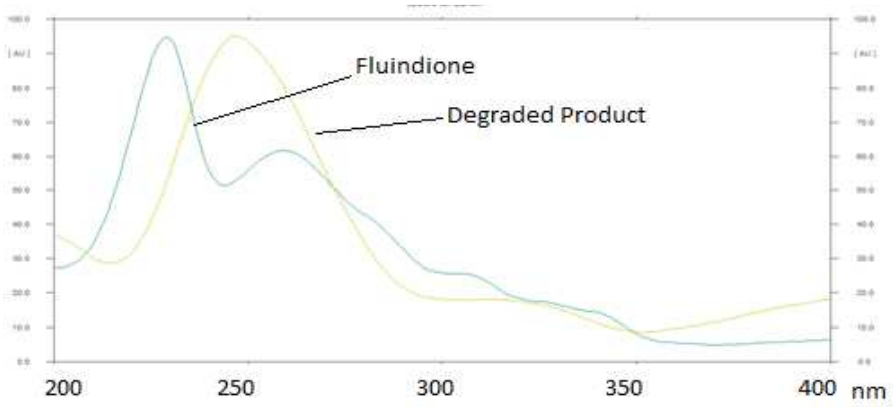

Fig. 6: Overlay of UV spectra of Fluindione drug and degradation product

Photo degradation Studies

Fluindione exhibited 18.10 % of degradation, when exposed to ultraviolet light (200 Watt hours/Square meter) and 19.41 % of degradation when exposed to fluorescence light (1.2 million lux hours).

The forced degradation studies data are summarized in Table 1.

Table 1: Data of forced degradation studies of Fluindione

Stress conditions/ duration	% Assay of Fluindione	Rf values of degraded Products
Acidic hydrolysis / 0.1 N HCl/ kept at room temperature for 3 days.	80.73 %	0.51
Alkaline hydrolysis /0.01 N NaOH/ kept at room temperature for 1 Hr.	82.34 %	-
Oxidative /0.3 % v/v H_2O_2 / kept at room temperature for 1 Hr.	87.02 %	-
Neutral hydrolysis /H_2O/kept at room temperature for 1Hr.	91.23 %	-
Dry heat/ 60ºC/ 6 hours	85.30 %	-
Photolysis UV fluorescent light (320 to 400 nm)	81.90 %	-
Cool white fluorescent light	80.59 %	-

Validation of the method

The method was validated for various parameters in accordance with ICH guidelines. [7]

Specificity

The developed method was specific for analyte.

Linearity and range

The standard stock solutions of Fluindione (100 µg/ml) were applied by spotting on TLC plate in range of 10, 15, 20, 25 and 30 µl. Straight-line calibration graphs were obtained in the concentration range 1000-3000 ng/band with high correlation coefficient > 0.99.

Accuracy

To check accuracy of the method, recovery studies were carried out by adding standard drug to sample at three different levels 80, 100 and 120 %. Basic concentration of sample was 1000 ng/band from SB. The drug concentrations were calculated from respective linearity equation. The results of the recovery studies indicated that the method is accurate for estimation of drug in tablet dosage form. The results obtained are shown in Table 2.

Table 2: Recovery studies of Fluindione

Drug	Amount taken (ng/ band)	Amount added (ng/ band)	Total Area found (ng/ band)	% Recovery	% RSD
Fluindione	1000	800	11120.49	99.73%	1.225
	1000	1000	12158.6	100.47%	1.701
	1000	1200	13089.9	100.07%	1.551

**Average of three determinations*

Precision

A set of three different concentrations in three replicates of standard solutions of Fluindione were prepared. All the solutions were analyzed on the same day in order to record any intraday variations in the results. Intra-day variation, as RSD (%), was found to be in the range of 0.64 to 1.77. For Inter day variation study, three different concentrations of the standard solutions in linearity range were analyzed on three consecutive days. Interday variation, as RSD (%) was found to be in the range of 0.61 to 1.51. The lower values of % R.S.D. (< 2) indicated that method was found to be precise.

Limit of detection (LOD) and Limit of quantitation (LOQ)

LOD and LOQ were calculated as 3.3 σ/S and 10 σ/S, respectively; where σ is the standard deviation of the concentration response (y-intercept) and S is the slope of the calibration plot. The LOD and LOQ were found to be 88.67 ng/band and 268.70 ng/band respectively.

Robustness Studies

Robustness of the method was determined by carrying out the analysis under conditions during which mobile phase composition, chamber saturation time was altered and the effect on the area of drug was noted. Robustness of the method checked after deliberate alterations of the analytical parameters showed that areas of peaks of interest remained unaffected by small changes of the operational parameters (% R.S.D. < 2). The results are given in Table 3.

Table 3: Robustness Data in Terms of Peak Area (% RSD)

Sr. No.	Parameter	(% RSD)
1	Mobile phase saturation time 10 min and 20 min	1.34
2	Mobile phase variation chloroform 9.8 and 10 ml	0.885

**Average of three determinations*

DISCUSSION

The percent degradation under various stress conditions in our study, match fairly with reported method but this literature report does not mention product of degradation, hence there is a need to investigate acid catalysed hydrolysis in detail.

CONCLUSION

The developed method is stability indicating, since the drug peak was found to be pure as confirmed by peak purity profiling study. This proves that there is no interference of degradation product in analytical peak. The method is specific, accurate, precise, and robust and can be used for routine quality control as well as assessing the stability of Fluindione.

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