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Development and validation of UV spectrophotometric method for fluindione

Manish Kumar Thimmaraju[1]*, Kyatham Hemanth[2], Swarnalatha P.[2], Vinayakumar J.[3] and Sagar Kasagoni[1]

[1]Central Analytical Laboratory, Department of Pharmaceutical Analysis, Balaji Institute of Pharmacy, Narsampet, Warangal, Telangana
[2]St'John College of Pharmacy, yellapur, Warangal- Telangana
[3]Kakatiya Institute of Pharmaceutical Sciences, Warangal- Telangana

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ABSTRACT

A simple, accurate, precise, specific and highly sensitive spectrophotometric method developed for the determination of Fluindione in bulk drug .The optimum conditions for the analysis of the drug were established. The λ max of the Fluindione was found to be 230nm in 10% methanol in 0.1N HCL. The method shows high sensitivity with linearity 1 to 5µg/ml. The regression of the curve was Y = 0.097x+0.1277.The apparent molar absorptivity was found to be 4.79x10^{4}mol−1 cm−1 in 10 % Methanol. The lower limit of detection and the limit of quantitation was found to be 0.09302µg/ml and 0.28186µg/ml respectively. All the calibration curves shows a linear relationship between the absorbance and concentration and correlation coefficient was 0.999.The proposed method will be suitable for the analysis of Fluindione in bulk form.

Key words: Fluindione, Methanol, apparent molar absorptivity, UV Spectroscopy

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INTRODUCTION

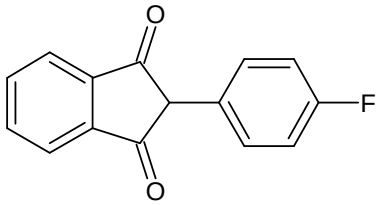

Fig-1.Fluindione

Fluindione (FLU) is an oral anticoagulant with a long half-life that inhibits the synthesis of vitamin K-dependent clotting factors. It is used in various cardiologic diseases for the prevention of thromboembolism.[1] Chemically it is 2-(4-fluorophenyl) indene-1, 3-dione[2] Fluindione (FLU) a vitamin K antagonist has been the essential key in deep venous thromboembolism treatment. 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. Antagonism of vitamin K reduces the amount of these clotting factors, thereby producing anticoagulation.[3] very few analytical methods were reported on Fluindione, Becquemont L[4] reported a pharmacokinetic-pharmacodynamic model to identify the genetic

predictors which are important for dose individualization. Berny P[5] reported a validated Liquid chromatography-tandem mass spectrometry method for the identification and quantification of anticoagulant, this method is based on ion-trap technology with electrospray ionization. Dariusz Pawelec,[6] synthesized and investigated 2-Substituted derivatives of indane-1, 3-dione as anticoagulant agents. Jérémie Sellam,[7] reported a research article which gave the information about the interaction between Dexamethasone (DXM) and oral coagulants. Laurent Pinede,[8] reported a review article emphasis on practical aspects of clinical management of oral anticoagulant for the treatment of venous thromboembolism. Aymard G[9] reported a new reversed phase High-performance liquid chromatographic (HPLC) method of assay which includes no extraction procedure for the quantification of Fluindione Debord.J[10] reported a simultaneous identification and quantitation of 13-Hydroxycoumarin and Iodandione anticoagulant drugs from human serum by RP-HPLC reported RP-HPLC method to describe the conjoint measurement in urine of the alcoholic catecholamine metabolites 3, 4-dihydroxyphenylglycol (DHPG) and 3-methoxy-4-hydroxyphenylgIycol (MHPG). The main objective of this study is to develop a simple, rapid, accurate, precise and inexpensive method which can be used for routine analysis of Fluindione (FLU) in bulk, pharmaceutical formulations and for dissolution studies.

MATERIALS AND METHODS

Fluindione (FLU) is obtained from Clearsynth Labs, Mumbai. Analytical grade Methanol, hydrochloric acid and triple distilled water (TDW) were purchased from SD fine chemicals, Mumbai, India.

Instrument:
UV-visible spectrophotometer Lab India (UV 3092), connected to digital system loaded with UV win software 5.2.0.1104 having a wavelength accuracy of ±5.0nm with quartz cells of 1cm path length

Absorption Maximum
In order to ascertain the wavelength of maximum absorbance (λ_{max}) the solution with particular concentration of drug 3.0µg/ml in 10% methanol in 0.1N Hydrochloric acid was scanned within the wavelength range of 200-400nm against a corresponding reagent blank. The resulting spectrum shows absorption curve with unique characteristic absorption maximum at 230 nm. The absorption and overlay spectra of Fluindione (FLU) are shown in Fig-2 &3.

Preparation of Stock Solutions
10 mg of Fluindione (FLU) was accurately weighed and dissolved in 100 ml of 10% methanol in 0.1NHydrochloric acid in a100ml volumetric flask to get the concentration about 100 µg/ml stock solution.

Preparation of Calibration curve
From the above stock solution prepare serial dilutions from 0.1ml to 0.5 ml and transfer it to 10ml volumetric flasks. Dilute it with 10% methanol in 0.1N Hydrochloric acid to get the concentrations ranging from 1µg/ml to 5µg/ml respectively. The absorbances were measured at λ_{max} 230 nm aganist 10% methanol in 0.1N Hydrochloric acid as a blank. Results are shown in (Table-1).The calibration curve was shown in Fig-4.

Method validation
Specificity
Fluindione (FLU) solutions (3.0µg/ml) were prepared in both the selected media along with and without common excipients (starch, dextrose, benzalkonium chloride, magnesium separate) separately. All the solutions were scanned from 450 to 200 nm and checked for change in the absorbance at respective wavelengths (Table-2).

Accuracy
As a part of determining accuracy of the proposed method, drug concentrations were prepared from independent stock solution and analyzed (N = 9). Accuracy was assessed as mean percentage recovery (Table-3).

Precision
Repeatability was determined by using different levels of drug concentrations (same concentration levels taken in accuracy study), prepared from independent stock solution and analyzed (N = 9) (Table-4). Inter-day and intra-day variation and instrument variations were taken to determine intermediate precision of the proposed methods.

Different levels of drug concentrations in triplicates were prepared three different times in a day and studied for intra-day variation. Same protocol was followed for three different days to study inter-day variation (*N* = 27).

Linearity

The linearity is established for the proposed method; nine separate series of solutions of the drug (2.0 to 10.0µg/ml in 25% Methanol medium) were prepared from the stock solutions and analyzed. Least square regression analysis is done for the obtained data. ANOVA test (one-way) was performed based on the absorbance values observed for each pure drug concentration during the replicate measurement of the standard solutions (Table-2)

Detection limit (DL) and Quantitation limit (QL)

Detection limit (DL) and quantitation limit (QL) for the proposed method is determined by using calibration standards. DL and QL were calculated as 3.3r/S and 10r/S, respectively, where S is the slope of the calibration curve and r is the standard deviation of y-intercept of regression equation (Table-5)

Robustness

Robustness of the proposed method is determined by (a) changing strength of the media by ± 2% and (b) stability of the Fluindione (FLU) in selected medium at room temperature for 8 hrs. Three different concentrations (LQC, MQC and HQC) were prepared in both media with different strength. Mean percentage recovery was determined (Table-6).

RESULTS AND DISCUSSION

For media optimization various aqueous media like acetate buffers (pH 3.6–5.8), phosphate buffers (pH 5.8–8.0) and 0.1N sodium hydroxide were investigated. Fluindione (FLU) showed UV absorption spectra in 10% methanol in 0.1N hydrochloric acid. The final decision of 10% methanol in 0.1N hydrochloric acid (pH-1.2) as a media is based on criteria like sensitivity of the method, cost, and ease of preparation and applicability of the method to dissolution studies. . The spectra of Fluindione (FLU) in the 10% methanol in 0.1N hydrochloric acid medium are shown in Fig. 1 the lamda max of Fluindione (FLU) in 10% methanol in 0.1N hydrochloric acid was found to be230 nm, respectively. Absorption spectra of Fluindione (FLU) at different concentrations are overlaid in media (Fig.2). Apparent molar absorptivity of drug was found to be a $4.79x10^4$ mol^{-1} cm^{-1} in 10% methanol in 0.1N hydrochloric acid (Table-2). In hydrochloric acid medium, the linear regression equation obtained was: absorbance at 230 nm = [0.097×concentration in µg/ml] + 0.1227; with a regression coefficient of 0.9999 (Table-2).

Table 1: Calibration data of the developed method
(Each value is result of nine separate determinations)

Sno	*Concentration (mcg/ml)*	*Absorbance at 230nm (±S.D)*	*%RSD*
1	1.0	0.315±0.0045	1.4188
2	2.0	0.518±0.0090	1.7473
3	3.0	0.731±0.0079	1.0810
4	4.0	0.829±0.0075	0.9035
5	5.0	0.936±0.0126	1.3435

S.D: Standard deviation, %RSD: Relative standard deviation

Table 2: Optical Characteristics and Regression Equation of Fluindione (FLU)

Parameters	*Fluindione*
Apparent molar absorptivity (l mol^{-1} cm^{-1})	$4.79x10^4$
Linearity range (µg/ml)	1-5
Correlation coefficient	0.9999
Regression equation (Y)*	0.097
Slope (a)	0.1277
Intercept (b)	0.850(2.305)
Calculated F-value (critical F-value)[b]	
Specificity - *t*Cal (*t*Crit)	0.01 (2.35)

Y* = ax+b, where 'x' is concentration in µg/ml and Y is absorbance
b Theoretical value of *F* (4, 45) based on one-way ANOVA test at *P* = 0.05 level of significance.

c *t*Cal is calculated value and *t*Crit is theoretical value (at 8 d.f.) based on paired *t*-test at *P* = 0.05 level of significance.

Table 3: Accuracy data of the developed method

S.No.	*Concentration (mcg/ml)*	*Mean Absorbance*	*S.D*	*%RSD*	*%Recovery*
1	1.0	0.311	0.0045	1.2400	100.74
2	2.0	0.525	0.0026	0.4945	100.65
3	3.0	0.703	0.0010	0.1381	100.25
4	4.0	0.839	0.0016	0.1896	100.41
5	5.0	0.924	0.0007	0.0708	100.06

Table 4: Precision data of the developed method

S.No.	*Concentration (mcg/ml)*	*Intra-day repeatability % R.S.D(N = 9)*			*Inter-day repeatability % R.S.D (N = 27)*
		Day 1	*Day 2*	*Day 3*	
1	1.0	1.437	1.562	1.475	1.4188
2	3.0	1.761	1.758	1.749	1.7582
3	5.0	1.668	1.713	1.713	1.7135

S.No.	*Concentration (mcg/ml)*	*Mean Absorbance*	*Inter-instrument repeatability % RSD (N=6)*
1	1.0	0.3148±0.00519	1.649
2	3.0	0.7117±0.01329	1.867
3	5.0	0.926±0.01413	1.526

Table 5: Detection limit (DL) and Quantitation limit (QL) data of the developed method

S.No.		*Mean of calibration curve (N=9)*	*SD*	*DL*	*QL*
1	Slope	0.15811	0.00446	0.09302	0.28186
2	Intercept	0.20911			
3	R^2	0.998			

Table-6: Robustness data of the developed method

S.No.	*Concentration (mcg/ml)*	*Mean Absorbance (N=9)*	*S.D*	*%Recovery*
1	1.0	0.315	0.0045	100.38
2	3.0	0.731	0.0079	99.31
3	5.0	0.936	0.0126	99.43

Analytical validation

Specificity and selectivity

The UV-spectrum of Fluindione (FLU) was not changed in the presence of common excipients in both the selected media. The calculated *t*-values were found to be less than that of the critical *t*-value, indicating that statistically there was no significant difference between mean absorbance of solutions prepared from pure drug sample (Table-2). Therefore proposed methods are specific and selective for the drug.

Accuracy

The excellent % recovery values (nearly 100%) and their low standard deviation values (S.D. < 1.5) represent accuracy. In 25% methanol the mean percentage recoveries (% R.S.D.) for lower, intermediate and higher concentrations were found to be 99.93(0.301), 100.02 (0.110) and 100.11 (0.271), respectively and 10% methanol in 0.1N hydrochloric acid the mean percentage recoveries (% R.S.D.) for lower, intermediate and higher concentrations were found to be 100.74 (1.24), 100.25 (0.138) and 100.06 (0.070), respectively. This result revealed that any small change in the drug concentration in the solution can be accurately determined by the proposed method. (Table-3)

Precision

Precision determined by studying repeatability and intermediate precision. Repeatability (% R.S.D.) in 10%methanol in 0.1N hydrochloric acid medium, at all three levels of concentrations (Table-4). Repeatability results indicate the precision under the same Operating conditions over a short interval of time and inter-assay precision. Intermediate precision expresses within-laboratory variations in different days and in different instruments. In

intermediate precision study, % R.S.D. values were not more than 2% in all the cases (Table 4). R.S.D. values were within the acceptable range indicating that these methods have excellent repeatability and intermediate precision.

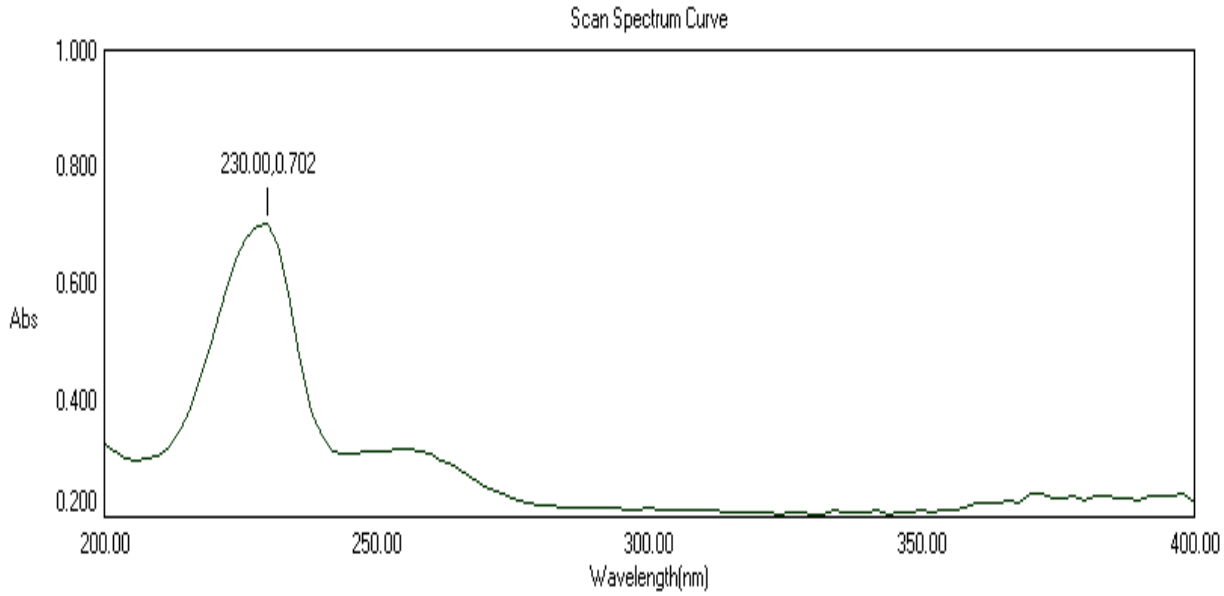

Fig- 2. Absorption spectrum of Fluindione (FLU)

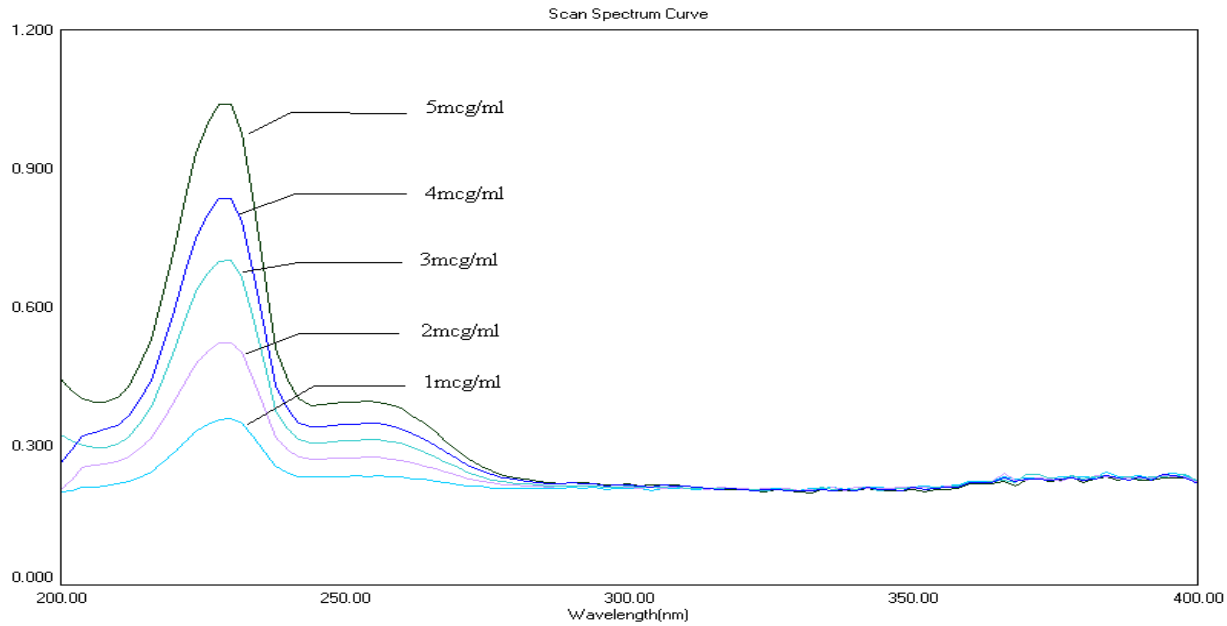

Fig- 3: Overlaid Spectra of Fluindione (FLU)

Linearity

In and 10% methanol in hydrochloric acid medium the linearity range was found to be 1-5µg ml–1 at 230 nm. Lower values of parameters like standard error of slope and intercept (Table 2) indicated high precision of the proposed methods. The mean slope and intercept values are within the 95% confidence interval. Goodness of fit of regression equations was supported by high regression coefficient values and less calculated *F*-values (Table 2).

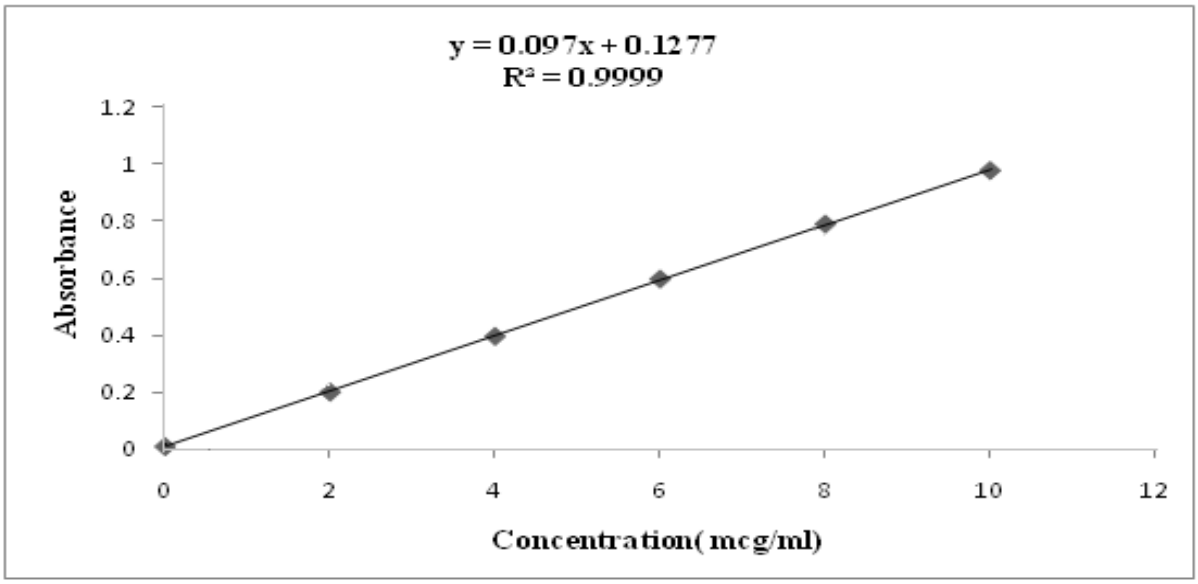

Fig 4: Calibration curve of Fluindione (FLU)

DL and QL

In 10% methanol in 0.1N hydrochloric acid DL and QL were found to be 0.093 and 0.281µg/ml, respectively (Table-5)

Robustness

Variation of strength in the selected media by ± 2% did not have any significant effect on absorbance. The mean % recovery (± S.D.) was found to be 99.7(± 0.586) in 10% methanol in 0.1N hydrochloric acid media, respectively (Table -6).

CONCLUSION

In summary, the proposed method was simple, rapid, accurate, precise and inexpensive and can be used for routine analysis of Fluindione (FLU) in bulk, pharmaceutical formulations and for dissolution studies. In general, 100 mM phosphate buffer and 100 mM hydrochloric acid are used as media for dissolution studies and therefore, developed analytical methods will be useful for normal dissolution studies.

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