e-ISSN 2320 –7876 www.ijfans.org
Vol.11,S Iss.1, 2022

DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR DETERMINATION OF FLUINDIONE AND ITS THREE INHERENT IMPURITIES IN PHARMACEUTICAL DOSAGE FORMS

Birendra Shrivastava[1], Tattari Praveenkumar[1]*, Alavala Manikanta Kumar[2]

[1]School of Pharmaceutical Sciences, Jaipur National University, Jaipur, Rajasthan, India.

[2]Department of Quality Control, M/s Aurobindo Pharma Limited, Visakhapatnam.

***Email for correspondence:** praveenpark8@gmail.com**

Abstract

A reversed-phase high-performance liquid chromatography (RP-HPLC) method was developed and validated for the identification and quantification of Fluindione and its three inherent related substances in finished dosage forms. Chromatographic separation was achieved on an Ascentis Column (150 mm x 4.6mm, 2.7µm) with the mobile phase (0.1% Ortho Phosphoric acid as buffer and Acetonitrile in gradient mode. The HPLC flow rate was 1.0 ml/ min and peaks were monitored at 226 nm using a UV detector. The column temperature was kept constant at 30°C, and the injection volume was 10 µl. The run time of the method was 20 min. The method was validated as per the International Conference on Harmonization (ICH) guidelines. Linearity was recorded at various concentrations ranging from 0.25 to 1.5 µg/ml for all the Fluindione impurities. Linearity, regression value, recovery, % relative standard deviation (RSD) of method precision values were found within the acceptance limits. The method for related substances in Fluindione was found to be specific, linear, accurate, precise, rugged, and robust. The validated method was suitable for the quantification of the Related Substances in Fluindione drug products. The method can be applied for routine analysis of Fluindione drug substance and drug products in labs.

Keywords: Fluindione, Ascentis, Inherent, parafluorobenzaldehyde, Desflourobenzaldehyde, phthalide

Introduction

Anticoagulants are medicines that help prevent blood clots. They're given to people at a high risk of getting clots, to reduce their chances of developing serious conditions such as strokes and heart attacks[1–3]. A blood clot is a seal created by the blood to stop bleeding from wounds. In general Vitamin K antagonists (VKA), direct oral anticoagulants (DOACs), and low-molecular weight heparins (LMWH) are used to treat blot clot. VKA are a group of

substances that reduce blood clotting by reducing the action of Vitamin K. The term "vitamin K antagonist" is technically a misnomer, as the drugs do not directly antagonise the action of vitamin K in the pharmacological sense, but rather the recycling of vitamin K and these were categorised as Coumarins and Indandiones drugs[4,5].

Indandiones are superseded by second-generation anticoagulants because warfarin-resistant rodents have become more common. Anisindione, fluindione and phenindione are oral anticoagulant medicines having similar actions of that as warfarin. Fluindione is a member of indanones and a cyclic ketone and fluindione is under investigation for the treatment of Venous Thrombosis, Pulmonary Embolism, Permanent Atrial Fibrillation, and Anticoagulating Treatment[6,7].

A detailed literature survey shows that few analytical methods for determination of fluindione and its impurities were available by UV, high-performance liquid chromatography (HPLC), LC-MS/MS Method[8–19], but there is no method available with three impurities (Fig. 1). The present study describes a novel method of determination of inherent impurities in fluindione[3] which is accurate, simple, reproducible and cost saving method which can be adopted for routine analysis at quality control labs in pharma industries, which is in line with ICH-Q2B Guidelines. The structure of Fluindione along with its inherent impurities are depicted in Fig. 1.

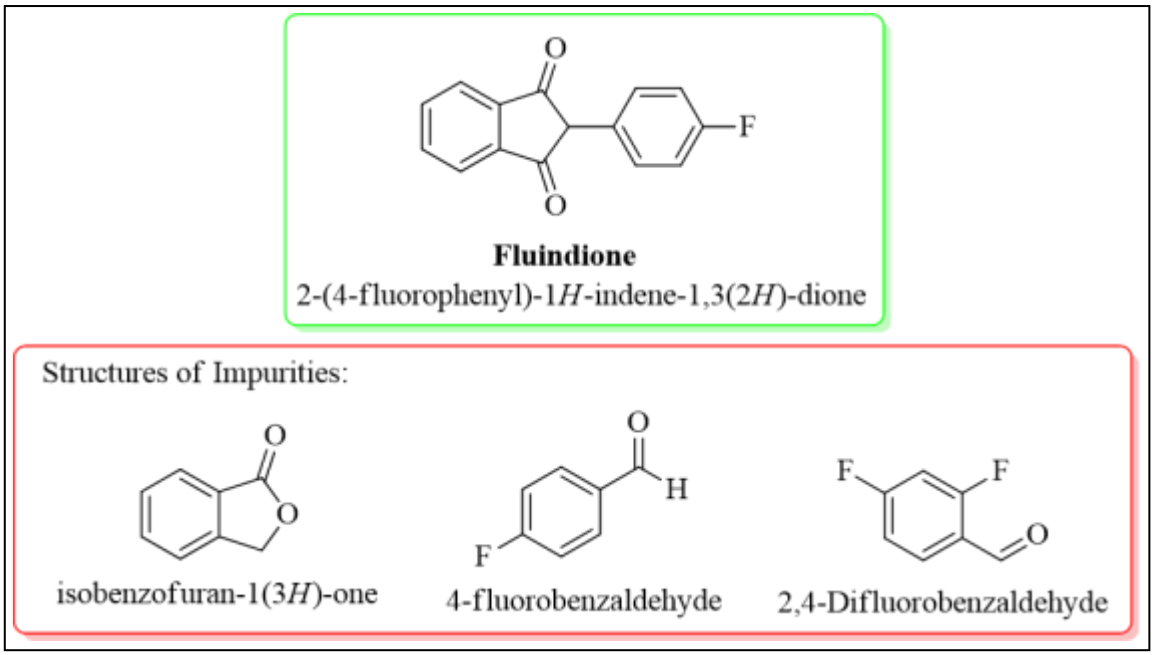


Fig. 1. The structure of Fluindione along with its inherent impurities

Methods and Materials

Instruments used

Electronics balance of Denver, Ultrasonicator of Labman, and Vacuum pump of Crompton and High performance liquid chromatography 2695 system with PDA detector with Empower 2 software was used for Method validation.

Chemicals and Reagents

Acetonitrile, Methanol(HPLC Grade) were purchased from M/s Merck Chemicals division and that of Potassium dihydrogen Ortho phosphate, Triethyl Amine, Ortho-Phosphoric acid and diSodium hydrogen Ortho phosphate was procured from M/s Rankem avantor. Standards and API of Fluindione were gifted by M/s Shodhana labs, Hyderabad and Fluindione tablets were arranged by M/s Spectrum labs, Hyderabad.

Preparation of Mobile phase

Preparation of 3.5%w/v of Disodium hydrogen ortho phosphate

Transferred 3.5gms of Disodium hydrogen ortho phosphate to 100ml volumetric flask and diluted to 100ml with milli-Q water.

Preparation of 0.01 Potassium dihydrogen Phosphate buffer

Dissolved 1.369gms of potassium dihydrogen phosphate to 100ml volumetric flask and made up to 100ml with milli-Q water and then adjusted the pH with 3.5%w/v disodium hydrogen ortho phosphate. Further taken above solution of 100ml and diluted to 1000ml with milli-Q water and degas.

Mobile phase preparation

Used 0.01M Potassium di hydrogen phosphate as mobile phase solution A. Used Acetonitrile as Mobile phase B

Diluent

Water: Acetonitrile (50:50 %v/v)

Standard Preparation

Transfer 10mg of Fluindione into 50ml volumetric flask, dissolve and dilute volume with diluents (Concentration=200ppm). Further 2.5ml of above stock transfer into a 50ml volumetric flask, dilute to volume with diluent. (Concentration=10ppm). Again, further dilute above solution 5.0ml into 50ml volumetric flask, dilute to volume with diluent. (Concentration=1ppm).

Sample preparation

Crush not less than 10 tablets into fine powder, weigh equivalent to 20mg of Fluindione and transfer into a 100ml volumetric flask. Add about 35 mL of diluent, sonicate for 20 minutes with intermittent shaking. Attain to room temperature. Dilute up to the volume with diluent and mix well.

Tablet average Weight: 190mg

Placebo solution

Weight and transfer placebo powder equivalent to 25 mg of fluindione into a 50 mL of volumetric flask, add about 35 mL of diluent, sonicate for 20minutes with intermittent shanking. Attain to room temperature. Dilute up to the volume with diluent and mix well.

Chromatographic conditions

Mobile phase	**:**	0.01N kH2PO4: Acetonitrile (65:35 v\v ratio)
Flow rate	**:**	1 ml/min
Column	**:**	Ascentis 150 mm x 4.6mm, 2.7µm
Detector wave length	**:**	226 nm
Column temperature	**:**	30°C
Injection volume	**:**	10µL
Run time	**:**	20min
Diluent	**:**	Water: Acetonitrile 50:50

Method Development study

The aim of the research work study was to develop a simple, robust, accurate and sensitive HPLC method for the determination of Fluindione and its inherent impurities .Initially various mobile phases and stationary phases were tested to obtain the separation. In development trail 01, Impurity peak desfluorobenzaldheyde and Fluindione peak were merged and not resolved properly, so stopped the run after 18mins and made new trail with changing the mobile phase ratio to Acetonitrile: 0.1% OPA (45:55). In development trail 02, all the impurities along with Fluindione peak was resolved properly but all the peak shapes were not good, so stopped the run after 10min and made new trail by changing the mobile phase ratio to Acetonitrile: 0.1% OPA (40:60). In development trail 03, all the impurities peaks along with Fluindione peaks were not resolved and hump like peaks were found, hence stopped the run after 08mins and made new trail by changing column to Discovery 250x4.6mm, 5micros and mobile phase ratio to Acetonitrile: 0.1% OPA (50:50)[20,21].

In development trail 04, all Impurities and fluindione peak were resolved properly with good peak shape expect phthalide impurity as the peak got spilited, hence stopped the

run after 20mins and made new trail by changing column to Interstil 250x4.6mm, 5micros and mobile phase ratio to Acetonitrile: 0.1% OPA (40:60). In development trail 05, phthalide Impurity got merged with an unknown impurity and desfluorobenzaldeheyde was merged with fluindione peak, hence again new trail was made by changing column to Ascentis 150x4.6mm, 2.7micros and mobile phase ratio to 0.01M Potassium dihydrogen ortho Phosphate: Acetonitrile (65:35)[22–24].

Basing on development trail 05 we had optimised the conditions as follows Mobile phase ratio to 0.01M Potassium dihydrogen ortho Phosphate: Acetonitrile (65:35), Column Ascentis 150x4.6mm, 2.7µ, flow rate 1.0ml/min detection at 226nm and run time as 20mins and found that all the impurities were resolved , peak shape and base line found satisfactory. Hence this finalised method was used for method validation of Fluindione. The optimized chromatogram of Fluindione and impurities is depicted in Fig. 2.

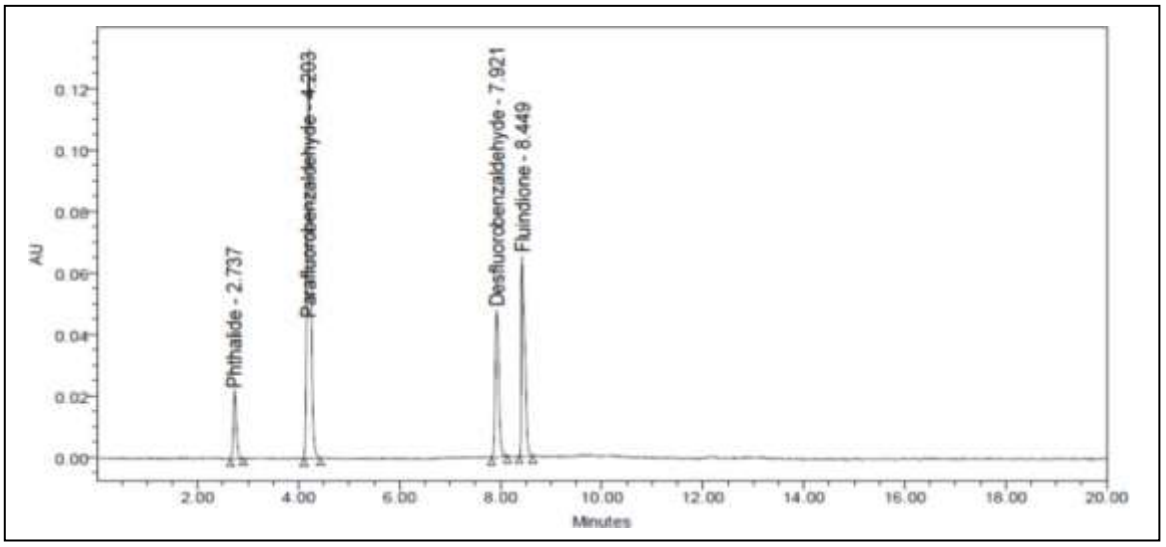


Fig. 2. The optimized chromatogram of Fluindione and impurities

Results and Discussion

Method Validation

The method was validated in accordance with recognized ICH guidelines[25–27].

System suitability

Prepared the standard solution as per methodology and injected six times into the chromatographic system and obtained % RSD from six replicate injections was 0.6. The observed tailing factor for Fluindione peak from the first injection of standard solution was 1.11, suitability results are given in Table 1.

Table 1. System suitability results

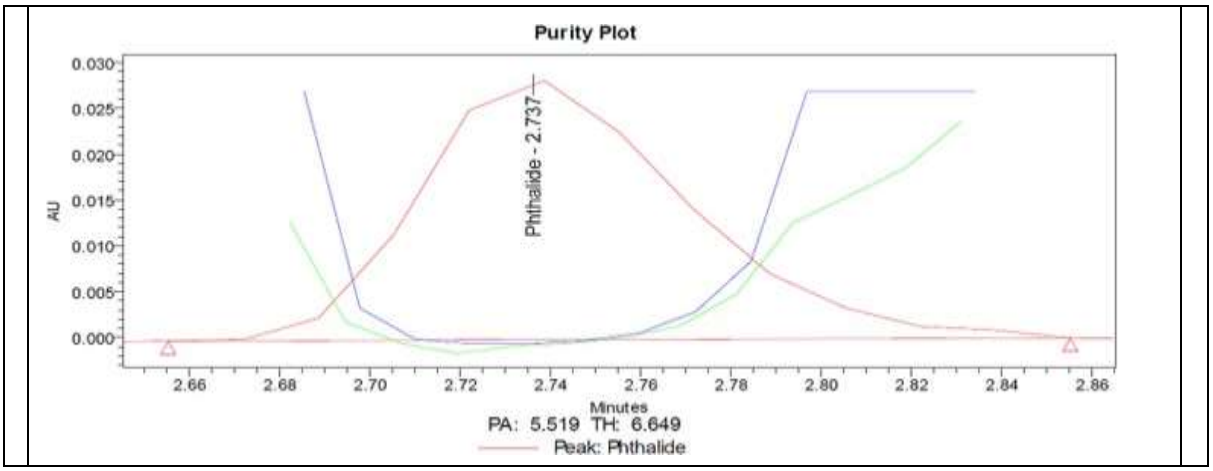
Injections	Peak area of Fluindione
1	220393
2	222738
3	223443
4	223740
5	222203
6	221086
Mean	222267
SD	1318.0
%RSD	0.6

Specificity

Specificity is demonstrated by checking the blank, placebo, known and degradant impurities interference with the analyte peak. Prepared blank, placebo, standard solution, sample solution, Impurities spiked sample solution and individual impurities solutions as per method and injected into HPLC system to evaluate the peak purity and interference of any peak with Fluindione and known impurities. All blank and placebo peaks were well separated from known impurities and Fluindione peak. All known peaks were separated with each other and Fluindione peak. Specificity results are addressed in table 2 and specificity chromatograms and peak purity plots are shown from Fig. 3 to 5. Blank, placebo and impurities have not shown any interference with Fluindione and Known degradant impurities. Hence the above method is specific.

Table 2. Specificity results

Name of the active/impurity	Retention time (min)	Peak purity	
		Purity angle	Purity threshold
Phthalide	2.737	5.519	6.649
Parafluorobenzaldehyde	4.179	0.583	0.557
Desfluorobenzaldehyde	7.835	2.731	3.147
Control sample (Fluindione)	8.362	3.560	4.171



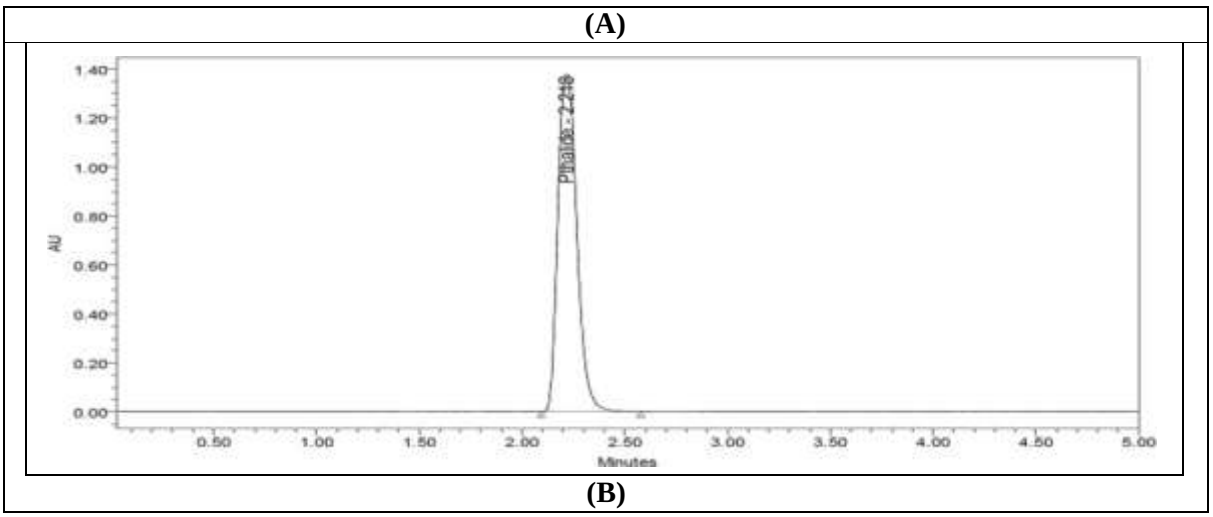


Fig. 3. (A) The peak purity of Phthalide impurity and (B) The chromatogram of Phthalide impurity

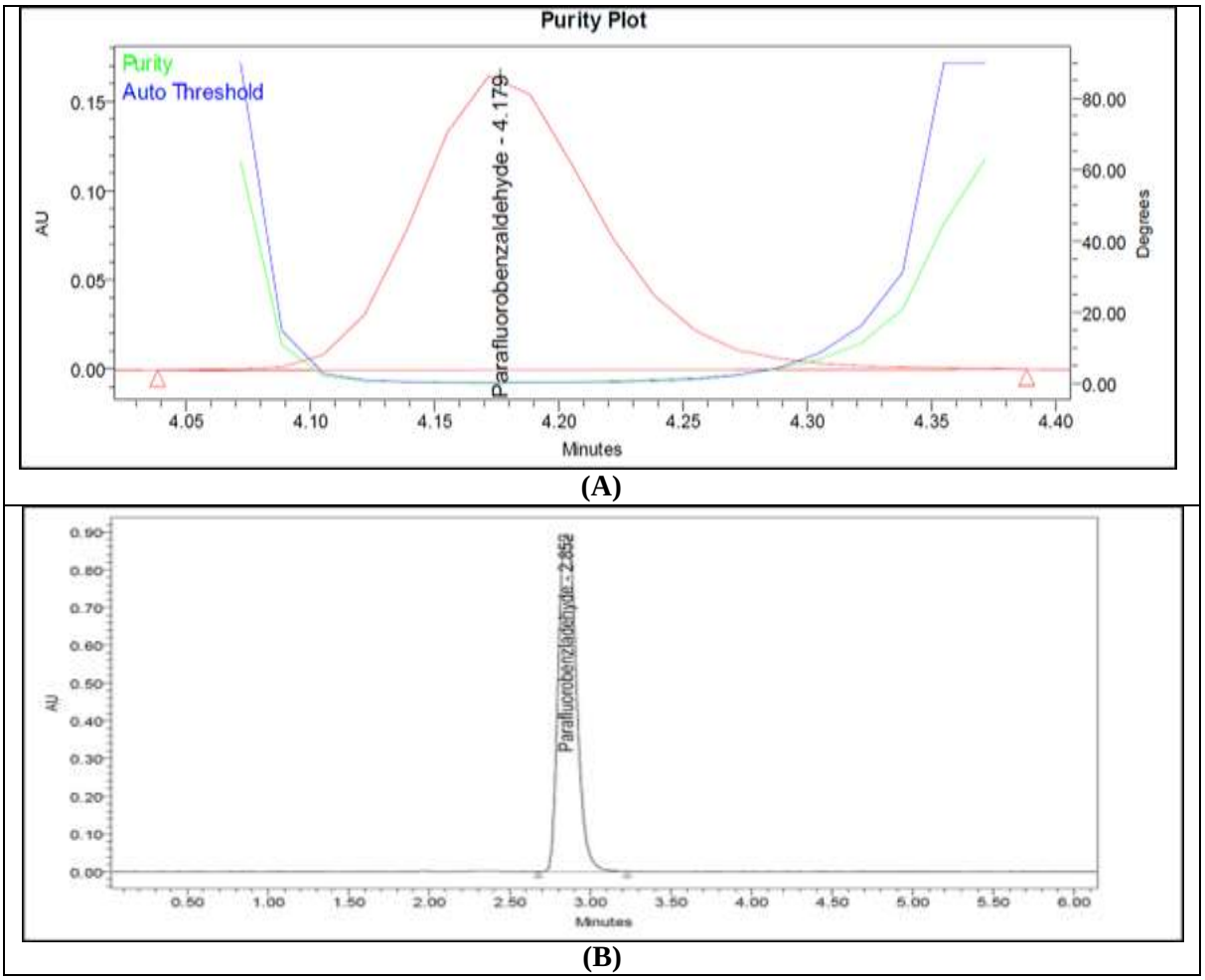


Fig. 4. (A) The peak purity of Parafluorobenzaldehyde impurity and (B) The chromatogram of Parafluorobenzaldehyde impurity

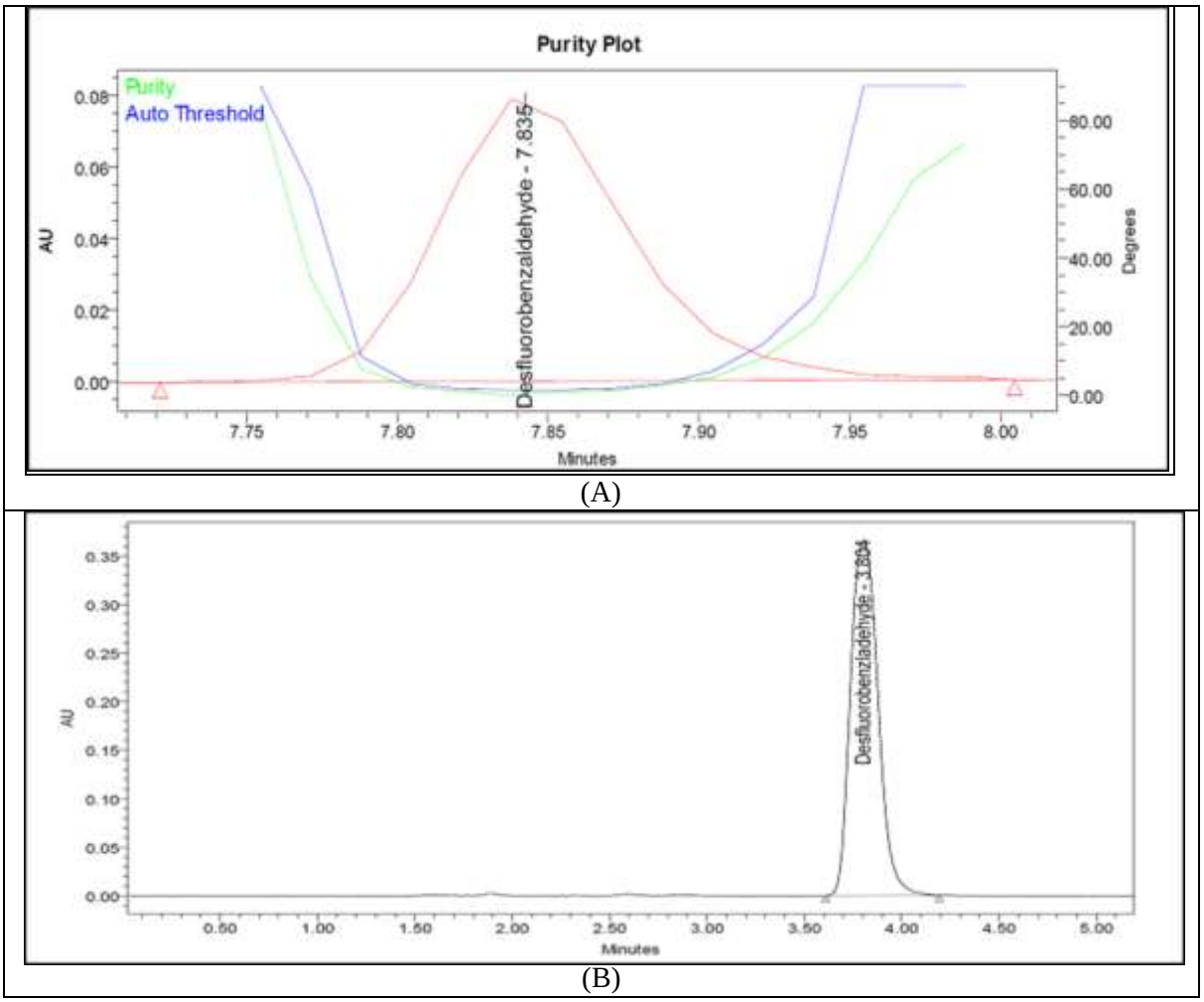


Fig. 5. (A) The peak purity of Desfluorobenzaldehyde impurity and (B) The chromatogram of Desfluorobenzaldehyde impurity

Forced degradation

Forced degradation of Fluindione in Fluindione tablets was carried out, to confirm that, during the stability study or throughout the shelf life, any degradation product if found will not interfere with Fluindione and known impurities peaks and also the forced degradation study will help to identify the type of degradation pathway (whether oxidative, alkali hydrolysis, acid hydrolysis, photolytic, dry heat and humidity) for each of the degradants.

Fluindione tablets was forcefully stressed by exposure to acid hydrolysis, base hydrolysis, peroxide, Hydrolysis, UV and thermal. Control and stressed samples were injected into the HPLC system and evaluate the Peak purity, interference of degradants and mass balance. In force degradation studies fortunately all generated impurities have not interfered with the Fluindione peak, known impurities peaks and also with each other. The purity angle of fluindione and its known impurities is less than the purity threshold. Forced

degradation chromatograms shows peak purity, peak threshold, assay and degradations and the results for forced degradation studies were addressed in the table 3.

Table 3. Forced degradation Studies

Sample Name	Pthalide (%w/w)	Parafluorobenzaldehyde (%w/w)	Desfluorobenzaldehyde (%w/w)	Single max. Unknown (% area)	Purity Angle	Purity Threshold	% assay
Control sample	ND	ND	ND	ND	2.315	2.946	100.0
0.01N HCl / 24hrs at Bench top.	N.D	ND	ND	6.33	4.789	6.394	93.67
0.01N NaOH / 24hrs at Bench top	N.D	ND	ND	5.91	2.712	2.935	94.09
3.0% H_2O_2 for 24hrs on Bench top	ND	ND	ND	3.08	2.890	3.339	94.68
Water /40°C For 24Hrs	ND	ND	ND	NA	3.625	3.438	100.0
UV for 24hrs	ND	ND	ND	0.11	2.654	2.997	100.0
Thermal 105 ^{0}C for 6 Hours	ND	ND	ND	0.18	2.488	3.031	100.0

Linearity and RRF establishment

A series of known impurity and Fluindione from LOQ to 150% of specification level and injected into HPLC system as per method. Linearity was conducted by preparing the five levels of linearity solutions and Plot a graph of concentration versus response for impurity solutions and standard solutions. Relative response factors for all individual impurities established based on slope method and calculate the RRF values from the linearity data. Calculate the relative response factor for all the known impurities using following formula.

$$\textbf{Factor (RRF) of impurity} = \frac{\textbf{Slope of impurity solution}}{\textbf{Slope of standard solution}}$$

The obtained all known impurities and Fluindione correlation coefficient were not less than 0.999. All the linearity data and RRF values are addressed from table 4 and table 5 and the linearity graphs are shown from Fig. 6 to 9.

Table 4. Linearity results of Pthalide and Parafluorobenzaldehyde

Linearity levels	Pthalide		Parafluorobenzaldehyde	
	Concentration (ppm)	Area response	Concentration (ppm)	Area response
25%	0.25	28256	0.25	205120
50 %	0.50	56561	0.50	415848
75%	0.75	84789	0.75	613442
100 %	1.00	116114	1.00	827290
125%	1.25	143018	1.25	1026411

150 %	1.50	168673	1.50	1226614
Correlation Coefficient (r)	**0.99**		**0.99**	
Square Correlation Coefficient (r^2)	0.999		0.999	
Slope	113461		817487	
Y-Intercept	290.24		3820	
Relative response factor	0.51		3.65	

Table 5. Linearity results of Desfluorobenzaldehyde and Fluindione

Linearity levels	**Desfluorobenzaldehyde**		**Fluindione**	
	Concentration (ppm)	**Area response**	**Concentration (ppm)**	**Area response**
25%	0.25	83651	0.25	55688
50 %	0.50	167694	0.50	116324
75%	0.75	255329	0.75	166520
100 %	1.00	335040	1.00	226820
125%	1.25	412694	1.25	283198
150 %	1.50	503819	1.50	335583
Correlation Coefficient (r)	**0.99**		**0.99**	
Square Correlation Coefficient (r^2)	0.999		0.999	
Slope	333206		224045	
Y-Intercept	1482.6		1315.8	
Relative response factor	1.49		NA	

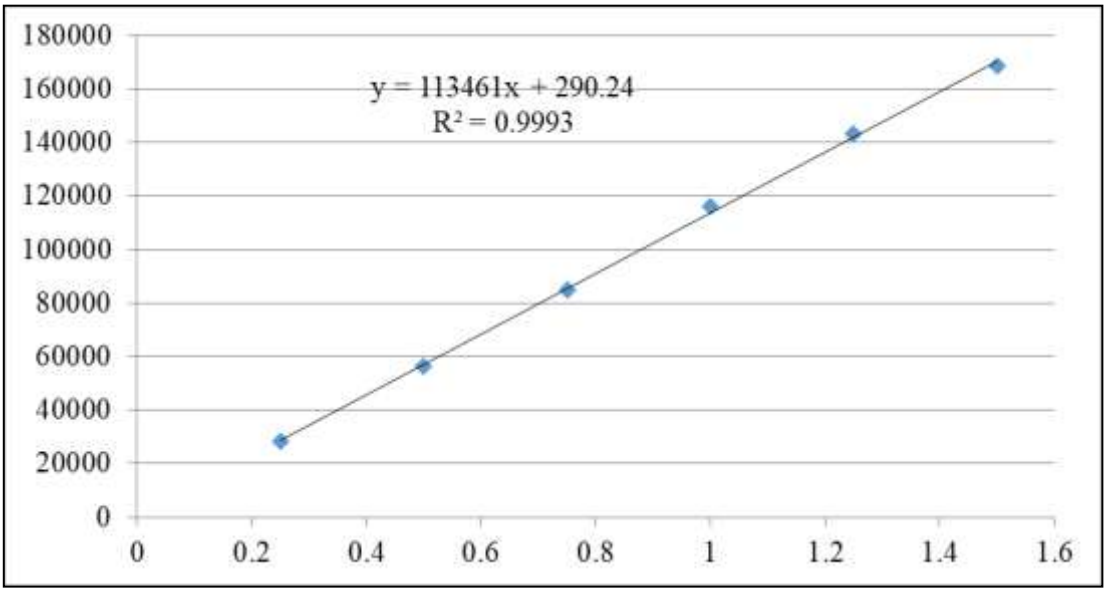


Fig. 6. The linearity graph of Phthalide impurity

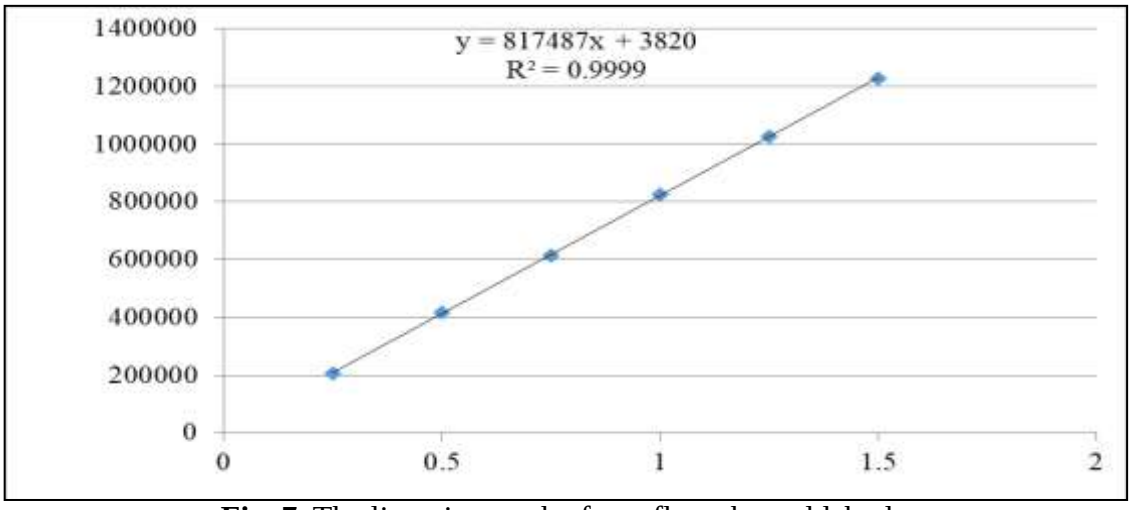


Fig. 7. The linearity graph of parafluorobenzaldehyde

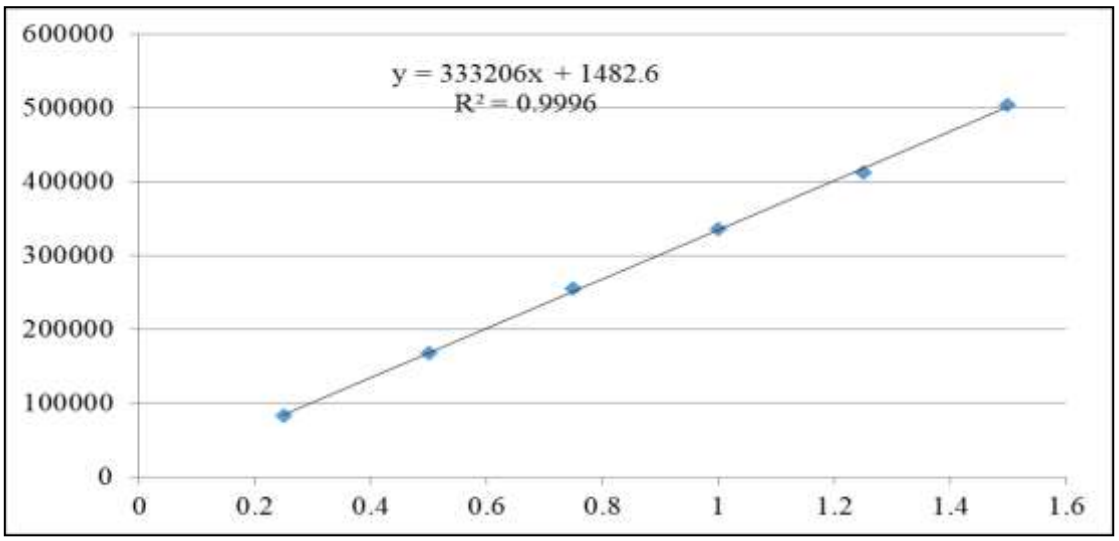


Fig. 8. The linearity graph of Desflourobenzaldehyde

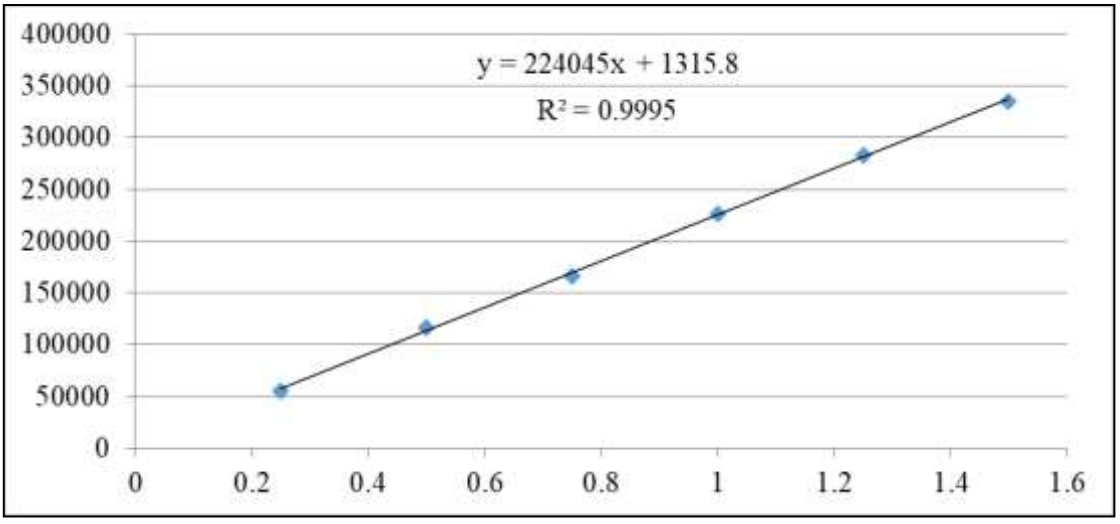


Fig. 9. The linearity graph of Fluindione

Establishment of LOD and LOQ

The LOD and LOQ values of all known impurities and fluindione were determined by using Standard deviation of the response and Slope. From the above linearity curve, standard deviation on response and slope of known impurities and main peak were calculated at different concentrations. LOD and LOQ values are expressed as a known concentration of fluindione and its known impurities at a low concentration for LOQ 10:1, for LOD 3:1 were quantitated or detected by HPLC method but LOD and LOQ is determined here by signal to noise ratio method. The LOD and LOQ values are addressed in table 6.

Table 6. Signal to noise ratio of LOD and LOQ

Name of the Active/Impurity	LOD (S/N)	LOQ (S/N)
Phthalide	4.9	23.9
Parafluorobenzaldehyde	8.4	37.6
Desfluorobenzaldheyde	10.8	34.6
Fluindione	9.1	30.8

Precision

System precision

It is demonstrated by calculating %RSD for retention time and peak areas of fluindione peak from six replicate injections of standard solution preparation. The system precision results are addressed in table 7.

Table 7. System precision results

Injection	Fluindione Retention time	Peak area of Fluindione
1	7.965	220393
2	8.015	222738
3	8.043	223443
4	8.210	223740
5	8.222	222203
6	8.291	221086
Mean	8.12	222267
SD	0.133	1318.0
%RSD	1.6	0.6

Method precision

Method precision was evaluated by injecting spiked known impurities on drug product at specification level. The results of Precision are reported from table 11 to table 13. % RSD values for Retention time and peak area responses of individual impurities should not be more than 10%. %RSD of Phthalide Impurity found 0.5 and 0.7, Parafluorobenzaldehyde 1.5 and 0.4 and Desfluorobenzaldehyde found 0.9 and 1.0. The data demonstrated that the values are met the acceptance criteria. Hence the method was found Precise and the results are addressed from table 8 and table 9.

Table 8. Method precision for Phthalide and Parafluorobenzaldehyde

Injection	Phthalide Retention time	Peak area of Phthalide	Parafluorobenzaldehyde Retention time	Peak area of Parafluorobenzaldehyde
1	2.742	112448	7.809	335054
2	2.748	112160	7.824	333113
3	2.753	113953	7.868	333650
4	2.764	112447	7.996	335512
5	2.772	114041	8.063	334526
6	2.779	112714	8.073	331730
Mean	2.760	112961	7.939	333931
SD	0.014	822.3	0.12	1393.36
%RSD	0.52	0.73	1.51	0.42

Table 9. Method precision for Desfluorobenzaldheyde

Injection	Desfluorobenzaldheyde Retention time	Peak area of Desfluorobenzaldheyde
1	4.233	815731
2	4.262	814603
3	4.274	809443
4	4.292	826370
5	4.3	828842
6	4.344	826763
Mean	4.284	820292
SD	0.038	8034.76
%RSD	0.88	0.98

Accuracy

The accuracy was evaluated by measurement (n=3) applying the method to the sample spiked with known amounts of known impurities corresponding to 50 %, 100 % and 150 % of specification. The recovery data of all known impurities obtained from a study of formulation from 50% level to 150 %. The test sample were prepared at each % level and tested against standard according to the description of the methodology. The total average recovery for Phthalide Impurity is 100.8% with 1.0 % RSD, Parafluorobenzaldehyde Impurity is 101.0 % with 0.8 % RSD and Desfluorobenzaldehyde Impurity is 101.0 % with 0.4 % RSD.The accuracy results are addressed in table 10. Based on the impurities recovery results, it is concluded that there was no interference from excipients present in the formulation and the method is accurate.

Table 10. Accuracy results (% Recovery)

Level	Phthalide	Parafluorobenzaldehyde	Desfluorobenzaldheyde
50 % Mean % Recovery	101.38	101.35	101.17

50 % % RSD	0.4	0.3	1.0
100 % Mean % Recovery	101.44	101.63	101.33
100 % % RSD	1.3	0.3	0.3
150 % Mean % Recovery	99.56	100.04	100.50
150 % % RSD	1.6	0.2	0.4
Overall Mean % Recovery	100.78	101.01	101.00
RSD of overall % Recovery	1.0	0.8	0.4

Solution stability

Spiked sample solution was found stable up to 24 hours at room temperature with the difference in 10% individual known impurity from initial to time intervals. Solution stability results are addressed in table 11.

Table 11. Spiked Sample solution stability at Room temperature

	At Room temperature (25°C) %Difference			
Hours	Phthalide	Parafluorobenzaldehyde	Desfluorobenzaldheyde	Fluindione
Initial	NA	NA	NA	NA
24 Hours	5.50	0.61	6.60	0.15

Mobile phase stability

The mobile phase was found stable for 6 days at bench top condition, no haziness of mobile phase was observed.

Robustness

Robustness of the method was assessed by varying the instrumental conditions such as column temperature (±5 ^{0}C), flow rate (±0.1 mL) and Organic variation of mobile phase (±5%). The deliberate changes in the method have no significant changes in retention time, relative retention time and no distorted chromatography was observed for fluindione and its known impurities. This indicates that the method is robust. Results for robustness studies are addressed in the table 12 and table 13.

Table 12. Robustness studies for spiked sample

RRT of Impurities in spiked sample				
parameter	Variation	Pthalide	Parafluorobenzaldehyde	Desfluorobenzaldehyde
Original conditions	None	0.33	0.52	0.94
Mobile phase variation	Organic 5% minus	0.34	0.54	0.95
	Organic 5% plus	0.32	0.51	0.94
Flow Rate mL/min	0.9mL/min	0.33	0.52	0.94
	1.1mL/min	0.33	0.50	0.92
Column oven temperature	25°C	0.32	0.52	0.95
	35°C	0.32	0.50	0.92

Conclusion

A validated reverse phase HPLC method concluded that the method is suitable, specific, linear, accurate, precise and robust. The range of the analytical method is from 50% to 150% of its specification limit and it can be used for intended purpose. This method is suitable for routine analysis for determination of five inherent impurities in fluindione drug substances and Pharmaceutical dosage forms.

Acknowledgments

This research article is made possible through the help and support from M/s Shodhana labs, Hyderabad & M/s spectrum labs, Hyderabad.

Conflict of Interest

The authors declare no conflict of interest.

References

(1) Tripathi, K. D. *Essentials of Medical Pharmacology Essentials of Medical Pharmacology, Sixth Edition*; 2008.

(2) Vonkeman, H. E.; van de Laar, M. A. F. J. Nonsteroidal Anti-Inflammatory Drugs: Adverse Effects and Their Prevention. *Semin. Arthritis Rheum.* **2010**, *39* (4), 294–312. https://doi.org/10.1016/j.semarthrit.2008.08.001.

(3) Bansal, Y.; Silakari, O. The Therapeutic Journey of Benzimidazoles: A Review. *Bioorganic Med. Chem.* **2012**, *20* (21), 6208–6236. https://doi.org/10.1016/j.bmc.2012.09.013.

(4) Husain, A. A.; Husain, A. S.; Gawhale, N. D.; Khan, S. L.; Murlidhar, D. V. An Overview of Natural and Synthetic Coumarin Derivatives as Potential Antidiabetic

Agents. *Journal of Pharmaceutical Negative Results* **2022**, *13* (5), 739–744. https://doi.org/10.47750/pnr.2022.13.

(5) Bossavy, J. P.; Sakariassen, K. S.; Thalamas, C.; Boneu, B.; Cadroy, Y. Antithrombotic Efficacy of the Vitamin K Antagonist Fluindione in a Human Ex Vivo Model of Arterial Thrombosis: Effect of Anticoagulation Level and Combination Therapy with Aspirin. *Arterioscler. Thromb. Vasc. Biol.* **1999**, *19* (9), 2269–2275. https://doi.org/10.1161/01.ATV.19.9.2269.

(6) Darban, M.; Bagheri, B. Drug Reaction with Eosinophilia and Systemic Symptoms Induced by Valproic Acid: A Case Report. *Iran. Red Crescent Med. J.* **2016**, *18* (9). https://doi.org/10.5812/ircmj.36825.

(7) Chen, X.; Jin, D. Y.; Stafford, D. W.; Tie, J. K. Evaluation of Oral Anticoagulants with Vitamin K Epoxide Reductase in Its Native Milieu. *Blood* **2018**, *132* (18), 1974–1984. https://doi.org/10.1182/blood-2018-05-846592.

(8) Crepin, T.; Bamoulid, J.; Courivaud, C.; Dahmani, O.; Felix, S.; Ducloux, D. Reversible Fluindione-Induced Chronic Interstitial Nephritis. *Case Reports Nephrol.* **2016**, *2016*, 1–3. https://doi.org/10.1155/2016/9818195.

(9) Comets, E.; Diquet, B.; Legrain, S.; Huisse, M. G.; Godon, A.; Bruhat, C.; Chauveheid, M. P.; Delpierre, S.; Duval, X.; Berrut, G.; Verstuyft, C.; Aumont, M. C.; Mentré, F. Pharmacokinetic and Pharmacodynamic Variability of Fluindione in Octogenarians. *Clin. Pharmacol. Ther.* **2012**, *91* (5), 777–786. https://doi.org/10.1038/clpt.2011.309.

(10) Verstuyft, C.; Delavenne, X.; Rousseau, A.; Robert, A.; Tod, M.; Diquet, B.; Lebot, M.; Jaillon, P.; Becquemont, L. A Pharmacokinetic-Pharmacodynamic Model for Predicting the Impact of CYP2C9 and VKORC1 Polymorphisms on Fluindione and Acenocoumarol during Induction Therapy. *Clin. Pharmacokinet.* **2012**, *51* (1), 41–53. https://doi.org/10.2165/11595560-000000000-00000.

(11) Lacut, K.; Ayme-Dietrich, E.; Gourhant, L.; Poulhazan, E.; Andro, M.; Becquemont, L.; Mottier, D.; Le Gal, G.; Verstuyft, C. Impact of Genetic Factors (VKORC1, CYP2C9, CYP4F2 and EPHX1) on the Anticoagulation Response to Fluindione. *Br. J. Clin. Pharmacol.* **2012**, *73* (3), 428–436. https://doi.org/10.1111/j.1365-2125.2011.04095.x.

(12) Cam, G.; Kwetcheu, A. T.; Vigneau, C.; Siohan, P.; Queffeulou, G.; Gatault, P.; Laruelle, E.; Crémault, A.; Le Cacheux, P.; Rioux-Leclercq, N.; Renaudineau, E.

Acute and Chronic Nephropathy Induced by Fluindione Must Be Addressed. *Nephrol. Dial. Transplant.* **2012**, *27* (4), 1554–1558. https://doi.org/10.1093/ndt/gfr500.

(13) Imbert, P.; Pernod, G.; Jacquet, J. P.; Bailly, C.; Laporte, S.; Lievre, M. Evaluation of a Mobile Electronic Assistant to Aid in Fluindione Prescription: The INRPlus Cluster Randomized Trial. *Thromb. Res.* **2014**, *133* (5), 756–761. https://doi.org/10.1016/j.thromres.2014.02.003.

(14) Pautas, E.; Despres, J.; Peyron, I.; Golmard, J. L.; Grange, J.; Koenig, N.; Gouronnec, A.; Mitha, N.; Siguret, V.; Gouin-Thibault, I. Sécabilité Comparée Des Comprimés de Warfarine et de Fluindione Pour Les Patients Âgés et Leur Entourage. *Geriatr. Psychol. Neuropsychiatr. Vieil.* **2011**, *9* (2), 171–177. https://doi.org/10.1684/pnv.2011.0269.

(15) Manckoundia, P.; Rosay, C.; Menu, D.; Nuss, V.; Mihai, A. M.; Vovelle, J.; Nuémi, G.; D'athis, P.; Putot, A.; Barben, J. The Prescription of Vitamin K Antagonists in a Very Old Population: A Cross-Sectional Study of 8696 Ambulatory Subjects Aged over 85 Years. *Int. J. Environ. Res. Public Health* **2020**, *17* (18), 1–12. https://doi.org/10.3390/ijerph17186685.

(16) Daveluy, A.; Milpied, B.; Barbaud, A.; Lebrun-Vignes, B.; Gouraud, A.; Laroche, M. L.; Ciobanu, E.; Bégaud, B.; Moore, N.; Miremont-Salamé, G.; Haramburu, F. Fluindione and Drug Reaction with Eosinophilia and Systemic Symptoms: An Unrecognised Adverse Effect? *Eur. J. Clin. Pharmacol.* **2012**, *68* (1), 101–105. https://doi.org/10.1007/s00228-011-1101-9.

(17) Moreau, C.; Pautas, E.; Duverlie, C.; Berndt, C.; Andro, M.; Mahé, I.; Emmerich, J.; Lacut, K.; Le Gal, G.; Peyron, I.; Gouin-Thibault, I.; Golmard, J. L.; Loriot, M. A.; Siguret, V. A Model Predicting Fluindione Dose Requirement in Elderly Inpatients Including Genotypes, Body Weight, and Amiodarone. *Thromb. Haemost.* **2013**, *111* (4), 705–712. https://doi.org/10.1160/TH13-07-0555.

(18) Mazumder, O.; Sundararajan, R. Development and Validation of UV Spectrophotometric Method for determination of Acalabrutinib. *Ymer* **2022**, *21* (6), 723–731. https://doi.org/10.37896/YMER21.06/73.

(19) Giron, A.; Lambert, C.; Berland, M.; Schmidt, J.; Poinas, L.; Charuel, E.; Ménini, T.; Pereira, B.; Vorilhon, P. Comparative Study in Primary Care of the Stability of INR in Patients Treated with Warfarin or Fluindione for Atrial Fibrillation. The FLOWER Study (FLuindione Or WarfarinE – Result INR). *Ann. Cardiol. Angeiol. (Paris).* **2022**,

71 (3), 123–129. https://doi.org/10.1016/j.ancard.2021.09.013.

(20) Baokar, S.; Mulgund, S.; Ranpise, N. Development and Validation of RP-HPLC Method for Simultaneous Estimation of Vildagliptin and Metformin. *Res. J. Pharm. Dos. Forms Technol.* **2013**, *5* (2), 95–98.

(21) Kavathia, A.; Misra, M. Development and Validation of RP-HPLC and UV-Spectrophotometric Methods for Rapid Simultaneous Estimation of Amlodipine and Benazepril in Pure and Fixed Dose Combination. *Arab. J. Chem.* **2017**, *10*, S3021–S3028. https://doi.org/10.1016/j.arabjc.2013.11.043.

(22) The United States Pharmacopeial Convention. United State Pharmacopoeia. *United States Pharmacopoeia* **2011**, *USP 35* (c), 867–868.

(23) IP. Indian Pharmacopoeia. *Appendix 9.4*. 1996, pp 110–117.

(24) British Pharmacopoeia. British Pharmacopoeia 2016. *Append. XIII Part. Contam. Sub-visible Part.* **2016**, *I*, 1027–1028.

(25) Chordiya, M.; Gangurde, H.; Sancheti, V. N. Quality by Design: A Roadmap for Quality Pharmaceutical Products. *Journal of Reports in Pharmaceutical Sciences*. 2019, pp 289–294. https://doi.org/10.4103/jrptps.JRPTPS_2_18.

(26) Kenett, R. S.; Kenett, D. A. Quality by Design Applications in Biosimilar Pharmaceutical Products. *Accreditation and Quality Assurance*. 2008, pp 681–690. https://doi.org/10.1007/s00769-008-0459-6.

(27) El-Fatatry, H. M.; Mabrouk, M. M.; Hewala, I. I.; Emam, E. H. Stability-Indicating HPLC-DAD Methods for Determination of Two Binary Mixtures: Rabeprazole Sodium-Mosapride Citrate and Rabeprazole Sodium-Itopride Hydrochloride. *J. Pharm. Anal.* **2014**, *4* (4), 258–269. https://doi.org/10.1016/j.jpha.2013.09.009.