



Quantitative Analysis of Flubendiamide and its Related Impurities in Bulk Batch by RP-HPLC Method

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An RP-HPLC method is validated to separate and quantify flubendiamide and its relevant eight related impurities. The ideal chromatographic separation of flubendiamide and its eight allied impurities was performed on Ace-5 C-18 column (5 μm , 25 cm \times 4.6 mm) with formic acid (0.1%) in Milli-Q water plus acetonitrile in a gradient elution at a flowing rate of 1.2 mL/min as mobile phase. Photodiode array based detection at wavelength of 240 nm was deployed to monitor flubendiamide and its eight related impurities. The robustness, accuracy, linearity, precision and detection and quantitation limitations of the new RP-HPLC technique for flubendiamide and its eight related impurities were all validated. Both FDE and eight process allied impurities had quantitation limits in the range of 0.07 to 0.12 $\mu\text{g/mL}$. This new HPLC technique was successfully applied to ascertain the purity of flubendiamide analyte and levels of eight related impurities in five bulk lot products of flubendiamide synthesized in the laboratory. The present study also deals with the bulk batch synthesis of flubendiamide and characterization of flubendiamide and its relevant eight impurities by ^1H NMR, ESI-MS and IR spectroscopy.

Keywords: Flubendiamide, RP-HPLC, Impurities.

INTRODUCTION

Flubendiamide (FDE) is an insecticide belongs to phthalic acid diamide class. Japanese Pesticide Corporation Limited developed FDE and initially enrolled in Philippines in 2007 and in India, was introduced by Bayer Crop Science [1-4]. Flubendiamide is a water dispersible granule that has been employed in plant safeguard as a novel agricultural ingredient in Japan for increased production and high resilient insect pest management. Flubendiamide is primarily effective towards lepidopteron pests, along with resistant strains, in fruits, vegetables, tobacco, corn, cotton and rice [5]. It is a new family of insecticides with extraordinarily high effectiveness against a wide spectrum of lepidopteron insect pests [6].

Flubendiamide, a novel phthalic acid diamide pesticide, operates as a ryanodine receptor agonist, activating the intracellular calcium releasing channel mechanism, which is a main source of calcium releasing which is unregulated [7]. Due to the unique mechanistic route, there's been no cross-resistance to the traditional insecticides discovered and it's also proven reported to be harmless for mammals as well as arthropods [8-13].

In general, regulatory authorities not only focus on main compound's potency but also the presence of other analytes like process related impurities and degradation products which may arise in the course of manufacturing procedure and active molecule's storage. Impurities should not exceed 0.15%, according to the regulatory agencies and any amount over that should be recognized and evaluated using proper methodological approaches [14]. The in-house method of flubendiamide synthesis has a total of eight process related impurities. Literature survey of flubendiamide insecticide risk assessment revealed that the associated impurities may possess more toxicity than its parent drug [15]. Hence, it is necessary to measure all these impurities level by using appropriate analytical procedures. Only a limited analytical techniques for determining flubendiamide [16-24] and desiodo flubendiamide are also accessible [17,25], so far no competent RP-HPLC approach for the simultaneous removal and quantification of flubendiamide and its probable process related impurities is available.

However, none of the reported procedures used any sophisticated analytical approach to quantify the separation and detection of impurities together with flubendiamide active

component in bulk batch manufacturing. Eight related impurities of flubendiamide (Fig. 1) were derived from the multi-stage synthetic processes. The profiles of impurities are affected by routine common factors such as the choice of synthetic route, reaction conditions, purification and the involved process equipment design in the manufacturing process. Both the manufacturers and regulatory authorities paid close attention to the impurity profile owing to the safety and effectiveness concerns [16,26].

In this work, the authors had carried out the the synthetic procedure involved in the bulk batch synthesis of flubendiamide. Moreover, the separation and quantification of flubendiamide and its relevant eight process related impurities were also performed by RP-HPLC method.

EXPERIMENTAL

The HPLC grade methanol, acetonitrile and the analytical grade of highest purity chemicals and solvents *viz.* dimethyl sulfoxide, KBr and formic acid were procured from Merck, India. Diluent used was 1:1 v/v mixture of formic acid (0.01%) in water of highly pure and acetonitrile. Standard flubendiamide samples and its eight related impurities (Imp 1-8) were received as gift samples from Nagarjuna Agrochemicals Ltd., India. A highly pure water was acquired from the Millipore, Bangalore, India.

Preparation of stock solutions of flubendiamide and its eight related impurities: The stock solutions of flubendiamide (2.0 mg/mL) and its eight related impurities (0.5 mg/mL) were

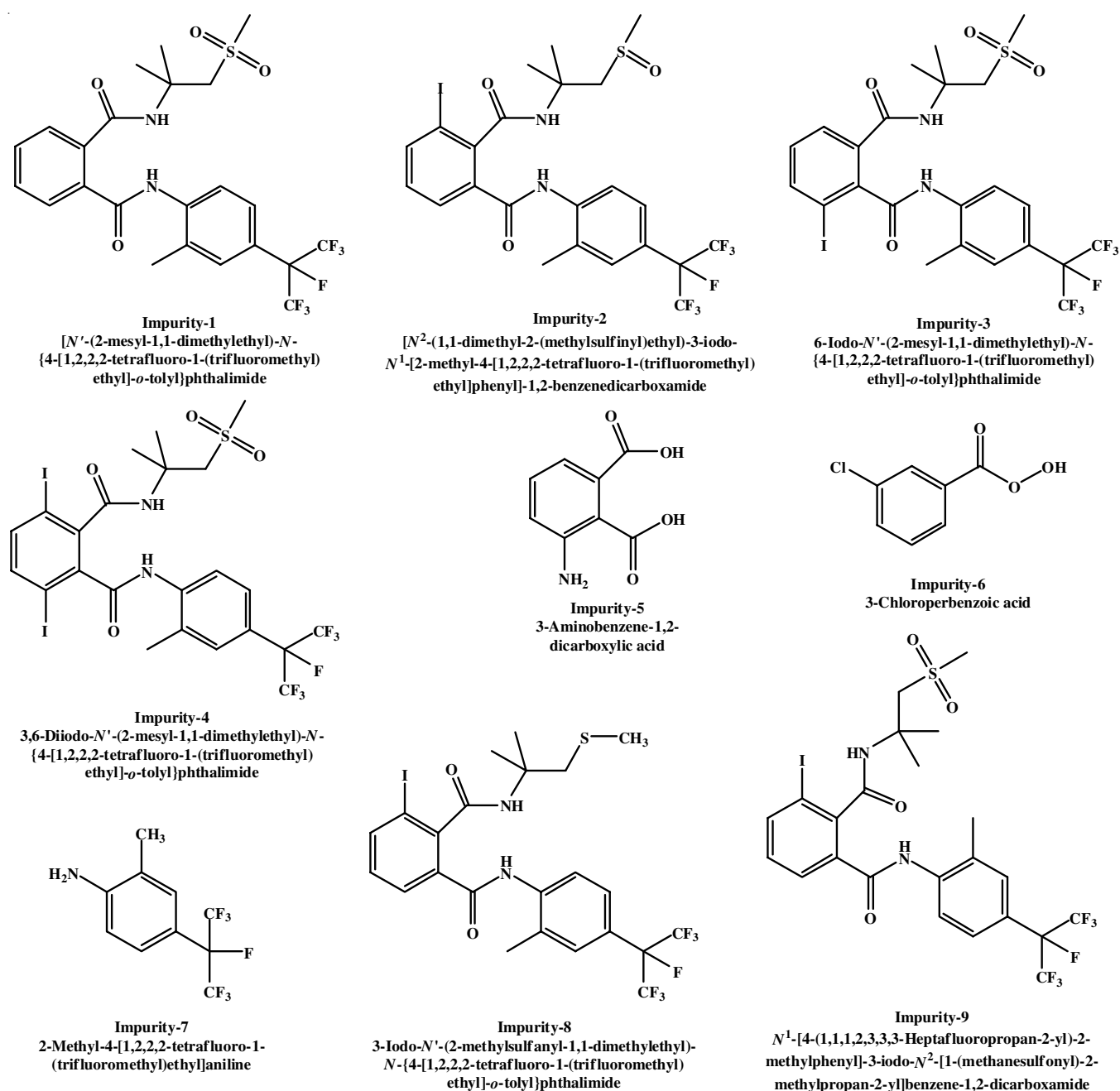


Fig. 1. Structures and chemical terms of FDE and its probable process allied impurities

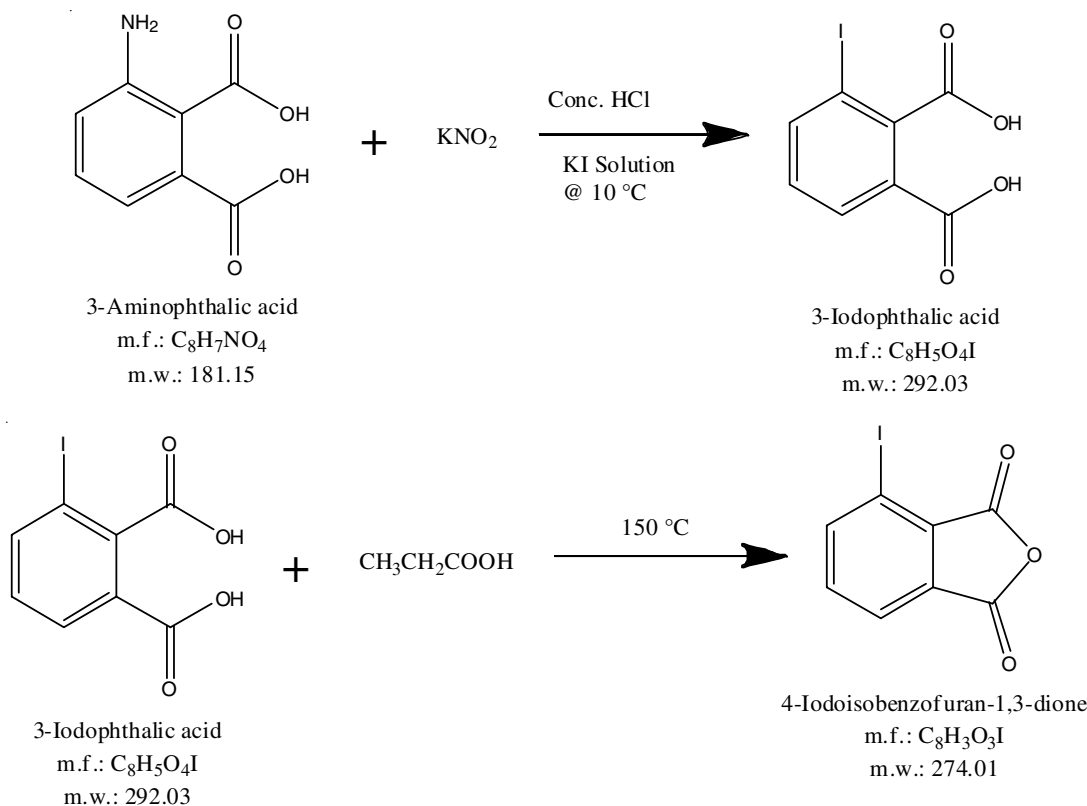
separately prepared by dissolving them in a small quantity of acetonitrile, sonicating for 2 min and added diluent to make up the desired volume. The specification quantities of flubendiamide were 1000 and 100 $\mu\text{g/mL}$ for the eight related impurities and assay determinations, respectively. The specification quantity (100%) of the eight impurities is 0.15% (*i.e.* 1.5 $\mu\text{g/mL}$) with regard to flubendiamide quantity of 1000 $\mu\text{g/mL}$. For the assessments of the eight related impurities as well as assay analysis, the working solutions of flubendiamide at 1000 $\mu\text{g/mL}$ supplemented with 0.15% of impurities and 100 $\mu\text{g/mL}$ (unspiked flubendiamide) were made from the aforesaid stock solutions. The stock solutions of flubendiamide and its eight related impurities were adequately blended and then diluted from the intermediate stock solutions to study the validation parameters *viz.* accuracy, precision, linearity, robustness, detection limits and quantification limits. Sample solutions for bulk batch manufactured were also prepared same as to the standard solution preparations.

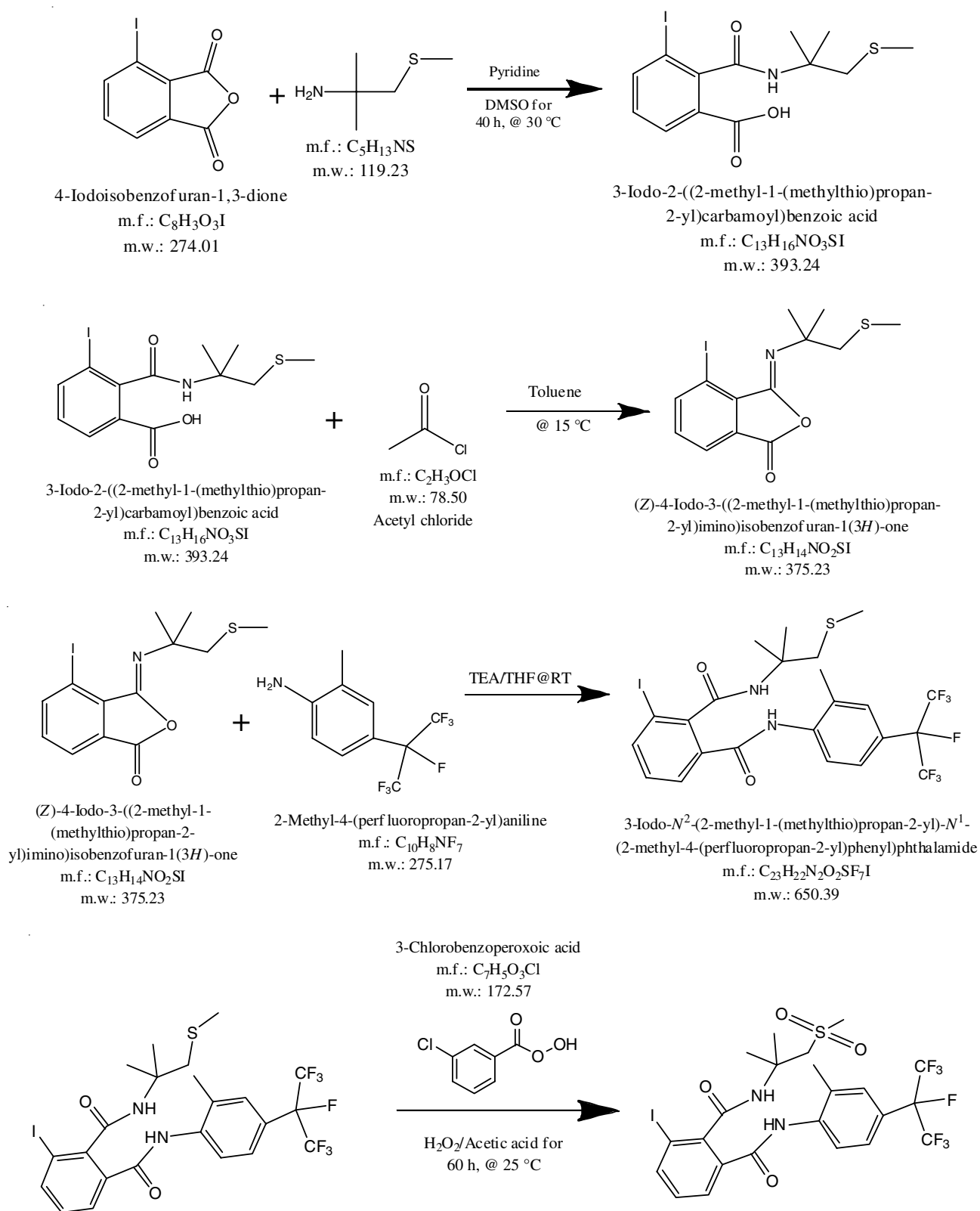
Instrumentation: The chromatographic experiments were carried out using Waters instrument 2695 model having quaternary pump 2995 module and detector of photodiode array mode and Shimadzu 2010 HPLC system having quaternary pumping module possessing excessive pressure gradient with low delay volumes and detector of photodiode array mode. Mass spectrometric studies were carried out on Agilent Technologies 6120 mass quadrupole spectrometer having an ESI supply. Both in positive and negative modes, data was captured and Typical ESI supply settings were as follows: capillary voltage 3.0 kV for negative polarity and 2.5 kV for positive polarity, 2 kV was charging voltage for positive and negative polarities, nebulizer pressure: 50 psi, vaporizer temperature: 200 $^{\circ}\text{C}$, dry

gas temperature: 250 $^{\circ}\text{C}$ and flow rate of drying gas: 15 L/min. In full scan operation, the ions gathered from the mass detector were studied in the mass range of 100-1200 m/z . The FT-IR spectral investigative studies of flubendiamide and its eight related impurities were carried out on Jasco FT-IR spectrometer-4100 instrument in the range of 4000-450 cm^{-1} . The ^1H NMR spectral studies were carried on Bruker Avance 300 (Palo Alto, USA) with BBO 5 mm probe in the $\text{DMSO-}d_6$ solvent.

Chromatographic conditions: The analysis of flubendiamide and its eight related impurities was performed with Ace-5 C-18 column [5 μm , 25 cm \times 4.6 mm] using formic acid (0.1% and pH 2.7) in Milli-Q water (solvent A) and pure acetonitrile (solvent B) as mobile phase combination solvents. To get good separation of flubendiamide and its eight related impurities, a precised linear gradient course program was developed (0.0 min/20% acetonitrile/80% formic acid, 10 min/40% acetonitrile/60% formic acid, 40 min/55% acetonitrile/45% formic acid, 53 min/90% acetonitrile/10% formic acid, 55 min/20% acetonitrile/80% formic acid, 65 min/20% acetonitrile/80% formic acid) at a rate of 1.2 mL/min. Prior to analyze the samples, the column was conditioned with an initial gradient mobile phase flow. At room temperature (25-27 $^{\circ}\text{C}$), aliquot samples of 20 μL were injected onto Ace-5 C-18 column [5 μm , 25 cm \times 4.6 mm] column. At a wavelength detection of 240 nm, the chromatograms of the flubendiamide and its related impurities were retrieved from PDA.

Synthetic procedure for flubendiamide and its process related impurities: Flubendiamide drug was synthesized in this research in six stages, as illustrated in **Scheme-I**. **Stage-I** involves a Sand-Meyer chemical reaction, where amino group from 3-aminophthalic acid (Imp-5) was substituted by iodine





Scheme-I: Synthetic pathway of flubendiamide

through a diazonium salt intermediate formation. The impurities of 3-aminophthalic acid were usually unreacted phthalic acid and diamino phthalic acid. **Stage II** involved the formation of anhydride through dehydration, whereas **Stage III** involved in the coupling of anhydride with amino compound, which resulted

in the formation of amine analogue. **Stage IV** involves the cyclization of formed amine analogue when treated with acetyl chloride. In **stage V**, the cyclized compound was coupled with 2-methyl-4-(perfluoropropan-2-yl)aniline and formed diamide compound. Finally, the oxidation of diamide compound with

3-chlorobezoperoxoic acid resulted in formation of flubendiamide in **stage VI**.

Small amounts of other compounds (impurities 1-4) were also obtained in final stage. Impurities 1 and 4 were formed from phthalic acid and 3,6-diiodophthalic acid present in the starting material 3-iodophthalic acid. Impurity-3 was found to be iodine positional isomer of flubendiamide, which might be resulted from the amination stage, where amination occurred on another carbonyl group resulted in isomer. Isomeric nature of impurity-3 was also confirmed by 2D NMR NOE studies. Impurity-4 was supposed to be monooxidized sulphur compound. All these four impurities were separated and characterized by NMR, IR and mass spectral techniques. In order to confirm the structures of impurities 1 and 4, same synthetic methodology of flubendiamide was applied to synthesize impurity-1 from phthalic acid, impurity-4 from 3,6-diaminophthalic acid.

Flubendiamide: ESI-MS: m/z 680.9 M^- . 1H NMR (DMSO- d_6): δ 1.45 (s, 6H), 2.94 (s, 3H), 2.45 (s, 3H), 3.73 (s, 2H), 7.24-7.27 (1H), 7.53 (s, 2H), 7.62-7.65 (d, 1H), 7.92-7.95 (t, 1H), 8.00-8.03 (d, 1H), 8.21 (s, 1H), 10.01 (s, 1H). IR (KBr, ν_{max} , cm^{-1}): 3230, 3015, 1542, 1653, 1542, 1443, 1311, 1235, 822.

Imp-1: ESI-MS: m/z 555.0 M^- . 1H NMR (DMSO- d_6): δ 1.48 (s, 6H), 2.39 (s, 3H), 2.96 (s, 3H), 3.75 (s, 2H), 7.52-7.54 (t, 1H), 7.53 (s, 2H), 7.62-7.65 (d, 1H), 7.92-7.95 (t, 1H), 8.00-8.03 (d, 1H), 8.31 (s, 1H), 9.44 (s, 1H). IR (KBr, ν_{max} , cm^{-1}): 3288, 2978, 1661, 1523, 1442, 1312, 1219, 829.

Imp-2: ESI-MS: m/z 664.9 M^- . 1H NMR (DMSO- d_6): δ 1.45 (s, 6H), 2.28 (s, 3H), 2.30 (s, 3H), 3.73 (s, 2H), 7.24-7.27 (t, 1H), 7.53 (s, 2H), 7.62-7.65 (d, 1H), 7.92-7.95 (t, 1H), 8.00-8.03 (d, 1H), 8.21 (s, 1H), 10.01 (s, 1H). IR (KBr, ν_{max} , cm^{-1}): 3298, 3090, 1668, 1520, 1443, 1306, 1233, 821.

Imp-3: ESI-MS: m/z 680.9 M^- . 1H NMR (DMSO- d_6): δ 1.45 (s, 6H), 2.45 (s, 3H), 2.94 (s, 3H), 3.73 (s, 2H), 7.24-7.29 (t, 1H), 7.53-7.55 (d, 2H), 7.62-7.63 (d, 1H), 7.92-7.95 (d, 1H), 8.00-8.03 (d, 1H), 8.21 (s, 1H), 10.01 (s, 1H). IR (KBr, ν_{max} , cm^{-1}): 3283, 3018, 1639, 1523, 1444, 1310, 1220, 821.

Imp-4: ESI-MS: m/z 806.8 M^- . 1H NMR (DMSO- d_6): δ 1.46 (s, 6H), 2.43 (s, 3H), 2.93 (s, 3H), 3.34 (s, 2H), 7.54 (s, 2H), 7.67 (s, 2H), 7.98-8.01 (d, 1H), 8.38 (s, 1H), 9.98 (s, 1H). IR (KBr, ν_{max} , cm^{-1}): 3283, 3017, 1644, 1525, 1448, 1308, 1219, 823.

Imp-5: ESI-MS: m/z 180.0 M^- . 1H NMR (DMSO- d_6): δ 6.55 (s, 2H), 6.75-6.78 (d, 1H), 7.00-7.05 (t, 1H), 7.12-7.14 (d, 1H). IR (KBr, ν_{max} , cm^{-1}): 3285, 3068, 1689, 1537, 1469, 1316, 1158, 832.

Imp-6: ESI-MS: m/z 171.0 M^- . 1H NMR (DMSO- d_6): δ 7.52-7.69 (m, 1H), 7.70-7.72 (d, 1H), 7.88-7.90 (t, 2H), 13.33 (s, 1H). IR (KBr, ν_{max} , cm^{-1}): 3147, 3024, 1720, 1549, 1456, 1324, 1155, 847.

Imp-7: ESI-MS: m/z 276.0 M^+ . 1H NMR (DMSO- d_6): δ 2.09 (s, 3H), 5.51 (s, 2H), 6.71-6.74 (d, 1H), 7.11-7.13 (d, 2H). IR (KBr, ν_{max} , cm^{-1}): 3213, 3008, 1727, 1539, 1466, 1315, 1173, 855.

Imp-8: ESI-MS: m/z 648.9 M^- . 1H NMR (DMSO- d_6): δ 1.30 (s, 6H), 1.93 (s, 3H), 2.36 (s, 3H), 2.84 (s, 2H), 7.23-7.28 (t, 1H), 7.54 (s, 2H), 7.65-7.68 (d, 1H), 7.96-8.02 (t, 2H), 8.15

(s, 1H), 9.54 (s, 1H). IR (KBr, ν_{max} , cm^{-1}): 3243, 3066, 1655, 1537, 1441, 1312, 1228, 830.

RESULTS AND DISCUSSION

The isomeric nature of impurity-3 was confirmed by NMR studies. Nuclear overhauser effect (NOE) and 1H NMR spectral studies confirmed the presence of hydrogen in impurity-3 at a position where iodine is present in flubendiamide, presence of iodine at the *para*-position. In general, amine group protons produce broader signal in NMR spectroscopy. Impurity-3 shows two broader signals at chemical shift values δ 8.21 and δ 10.01 ppm. These two amino group proton signals were differentiated by chemical moiety attached to it. Higher chemical shift value of amine proton is due to deshielding effect of phenyl ring and electronegative $-C_3F_7$ attached to phenyl ring, therefore chemical shift values at δ 10.01 ppm corresponds to NH at 6th position, while δ 8.21 ppm corresponds to another NH at 5th position.

In order to explain the NOESY spectrum, numbering is given to impurity-3 as shown in Fig. 2a and the NOESY spectrum of impurity-3 is shown in Fig. 2b. For NOE studies, the NH proton at 5th position was chosen for the double irradiation. The cross-peak A represents the NOESY interaction between the protons at C-1 (δ 7.8 ppm) and NH-9 proton (δ 8.2 ppm), which suggests the presence of proton and absence of iodine at C-1 position. 1H NMR spectrum of impurity-3 shows all the aromatic protons with splitting patterns in iodine attached phenyl ring suggests that couplings were happened between the aromatic protons, which is only possible when there is no element other than hydrogen present at the *meta*-positions 2 and 3. The presence of iodine at *para*-position (4th) was also confirmed from the chemical shift values. If iodine was present at 2nd or 3rd position, two adjacent protons chemical shift values get increased due to the deshielding effect of iodine, but spectrum shows only one proton with shielding effect. From these NMR results, it was clear that iodine is present at *para* position in impurity-3.

RP-HPLC method: At initial stages of development process, all the compounds including both flubendiamide and its eight related impurities were run through photodiode type detector and recorded the maximum absorption wavelength (λ_{max}) by scanning them between 190 to 400 nm individually and chosen suitable maximum wavelength for development at 240 nm. Preliminary trials for separation of analytes were done to develop a chromatographic system competent of eluting and resolving flubendiamide and its eight related impurities from one another. As most of the process related impurities were completely different structures from one another and have different pK_a values, it was found difficult to choose suitable mobile phase buffers to run on HPLC, so that trials were done with several mobile phase combinations with buffers including ammonium acetate, ammonium bicarbonate, ammonium formate, formic acid and organic solvents modifiers include methanol and also acetonitrile. After several attempts, it was observed that ammonium bicarbonate and formic acid buffer solutions show better peak constraints compared to formate buffer and acetate buffer. Therefore, ammonium bicarbonate and formic acid were chosen for further development.

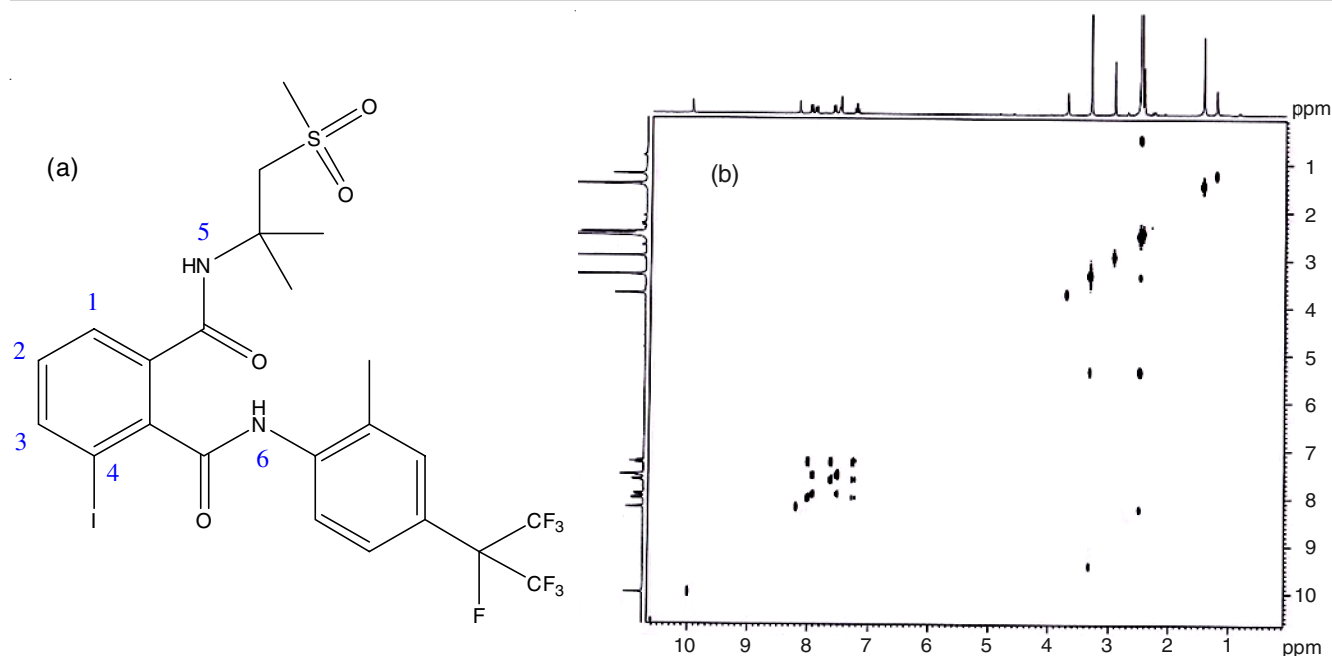


Fig. 2. (a) FDE related impurity 3 structure, (b) NOE spectrum of impurity-3

Thorough mobile phase combinational and column screening experiments observed that the isocratic elution programs need longer run times to elute all analytes because of the large polarity difference between analytes. To avoid this, runtime constraint gradient programs have been evaluated. A desirable separation with resolution > 2 was observed between flubendiamide and its eight related impurities when used mobile phase combination of ammonium bicarbonate (10 mM) and methanol/acetonitrile organic solvent modifier in a gradient elution manner with Gemini C-18 [5 μm , 25 cm \times 4.6 mm] but with lack of peak symmetry for impurities. Replacement of Gemini C-18 with X-Bridge C-18 column mobile phase consisting of 10 mM ammonium bicarbonate and acetonitrile resulted a symmetrical peak shape with poor resolution between flubendiamide and impurities 3 and 4.

Trials with Inertsil ODS column using ammonium acetate (10 mM) buffer and acetonitrile combinations in gradient manner elution showed co-elution of flubendiamide, impurities 3 and 7 and resulted only six impurities in the chromatogram. Replacement carbonate buffers with formic acid showed better separations with good peak symmetries. After several attempts with different C-18 columns and mobile phase combinations, better separations with acceptable analytical parameters for all analytes was accomplished on a Ace C-18, [5 μm , 25 cm \times 4.6 mm] column with formic acid (0.1% and pH 2.7) in Milli-Q water (solvent A) and pure acetonitrile (solvent B) as mobile phase combination solvents. Fig. 3 depicts a chromatogram

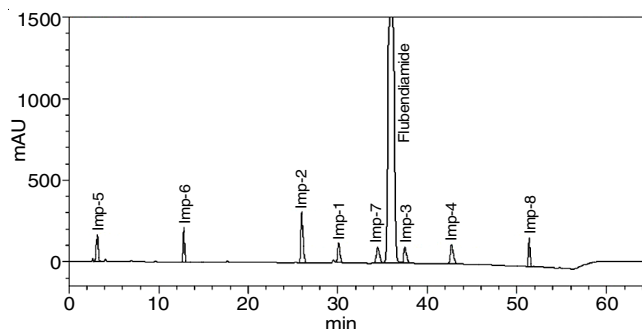


Fig. 3. A typical chromatogram of FDE and its eight process allied impurities under optimized conditions of chromatography

illustrating the separation of flubendiamide its eight related impurities.

Method validation: The current method was validated by the ensuing guidelines from SANCO/3030/99/Rev. 4 and Office of Chemical Safety and Pollution Prevention [27,28].

System suitability: Since the tailing factor for flubendiamide, eight related impurities were below 1.4 value and resolution among any two adjacent eluting analytes was better than 1.5 value, the system was judged appropriate for use. Table-1 shows information on system appropriateness.

Specificity: For this test, flubendiamide and its eight related impurities (1-8) were injected separately followed then by blank and standard mixture sample of flubendiamide with eight process related impurities to check the purity of flubendiamide. Relying

TABLE-1
INFORMATION ON SYSTEM APPROPRIATENESS FOR FDE AND ITS PROCESS ALLIED IMPURITIES

Parameter	FDE process allied impurities								FDE
	-1	-2	-3	-4	-5	-6	-7	-8	
Retention time (min)	30.15	25.98	37.51	42.75	3.21	12.82	34.51	51.42	35.98
Resolution	7.05	26.5	2.43	9.52	2.80	18.56	7.92	17.84	2.25
Plate count	10240	15025	9850	9975	10014	12012	11030	10260	16238
Tailing factor	1.35	1.08	1.07	0.92	1.10	1.15	0.98	1.04	0.95

on the retention times and elution characteristics, the active analyte flubendiamide and eight related impurities were identified. Peaks of eight process allied impurities were observed as well separated from flubendiamide active ingredient and met the resolution of better than 1.5. The active analyte flubendiamide assay was revealed to be 99.82% with a percent RSD of 0.26% for three determinations and 99.68% with a percent RSD of 0.49 in the vicinity of eight related impurities (0.15% w/w). Since there was no interference between peaks of active analyte flubendiamide and its eight related impurities, this strategy was regarded to be specific. Peak purity data obtained from the PDA detector also showed that no peak is interfered and hence further confirms good separation. Both flubendiamide and its related impurities were also found well separated from one another with resolution greater than 1.5, the method was also considered to be possessing a good selectivity.

Linearity: Linearity was verified by ascertaining linearity test solutions generated at six concentration levels of flubendiamide impurity concentration, spanning from the LOQ amount level to 2.25 µg/mL (equivalent to 150% of the specification amount level). These were made through spiking defined quantities of flubendiamide related impurities into flubendiamide solution (1000 µg/mL).

Similarly, the linearity of flubendiamide assay technique was investigated by infusing flubendiamide solutions at five varied concentration levels *viz.* 50%, 75%, 100%, 125% and 150% of the flubendiamide concentration (100 µg/mL). The peak area *versus* concentration of both flubendiamide and its related impurities were studied with least square methodology (Table-2). The computed correlation coefficient (r^2) values (≥ 0.998) from the regression analysis specified that this developed approach is linear.

Sensitivity: By evaluating a range of dilute solutions having known concentrations, the limit of detection (LOD) and limit of quantitation (LOQ) for flubendiamide and its related substances (impurities 1-8) were obtained at signal:noise ratios of 3:1 (LOD) and 10:1 (LOQ). The LOD and LOQ values (Table-3) of flubendiamide and its related impurities were obtained between range 0.03-0.10 µg/mL (flubendiamide) and 0.02-0.12 µg/mL (Impurities 1-8) indicating the higher detection sensitivity.

Accuracy: The test method's accuracy was ascertained for all substances (impurities 1-8) along with flubendiamide by using standard addition and recovery method which was carried out by fortifying known quantities (µg/mL) of all impurities into the test flubendiamide sample. The recovery assessments were conducted in triplicate for flubendiamide impurities at four content levels (LOQ, 50, 100 and 150%) at the specification content level (0.15%) of impurities (*i.e.* LOQ, 0.75, 1.5 and 2.25 µg/mL) with regard to flubendiamide quantity (1000 µg/mL). The percentage recoveries of flubendiamide and its eight impurities were obtained between range 96.50-103.71% and 99.41-100.01%, respectively (Table-4) validating the higher accuracy.

Precision: Six repeats of standard analyte solutions for both the assay (flubendiamide 100 µg/mL) and related impurity (1000 µg/mL added to 0.15% of each of eight impurities) were analyzed independently to ensure system and also method precision. For system and also method precision investigations, the percent RSD measures of each impurity chromatography response and flubendiamide chromatography response were computed. Ruggedness was evaluated to establish the test method's quality of repeatability. The intermediate precisions were assessed on a separate day by a separate analyst, using a separate batch column from the same vendor and an equipment in the similar laboratory, with the similar sample solutions in use for method/system precision evaluation. The intermediate precision test for flubendiamide added with the related impurities was carried out at 100% specification amount level for impurities. The relative standard deviations of peak areas were calculated for six replicates injections. The %RSD values obtained for all analytes in precision study were well within the acceptable limits (Table-5) and confirms the precision of the method.

Robustness: The system aptness limitations and assay of flubendiamide and its eight impurities were examined under each altered circumstances and found no significant changes suggests the robustness of the method. The resolution of closely eluted analytes impurity-7, flubendiamide and impurity-3 are given in Table-6.

Stabilities of solution and mobile phase: Freshly prepared mobile phase stability investigations were conducted for 48 h at 8 h intervals by infusing freshly made sample analyte

TABLE-2
INFORMATION ON LINEARITY FOR FDE AND ITS PROCESS ALLIED IMPURITIES

Parameter	FDE process allied impurities								FDE
	-1	-2	-3	-4	-5	-6	-7	-8	
Range (µg/mL)	0.12-2.25	0.07-2.25	0.10-2.25	0.11-2.25	0.09-2.25	0.08-2.25	0.12-2.25	0.09-2.25	50-150
r^2	0.9984	0.9995	0.9987	0.9982	0.9991	0.9989	0.9996	0.9995	0.9998
Slope	23731	42722	28236	28829	35312	34743	24727	34823	823017
Intercept	-263.79	-124.13	208.67	1242.7	-489.66	-705.86	-622.67	267.48	73291

TABLE-3
INFORMATION ON LINEARITY FOR FDE AND ITS PROCESS ALLIED IMPURITIES

Parameter	FDE process allied impurities (µg/mL)								FDE
	-1	-2	-3	-4	-5	-6	-7	-8	
LOD	0.04	0.02	0.03	0.04	0.03	0.03	0.04	0.03	0.03
LOQ	0.12	0.07	0.10	0.11	0.09	0.08	0.12	0.09	0.10

TABLE-4
INFORMATION ON ACCURACY FOR FDE AND ITS PROCESS ALLIED IMPURITIES

Values	FDE process allied impurities								FDE
	-1	-2	-3	-4	-5	-6	-7	-8	
At 50% added level									
Add up ($\mu\text{g/mL}$)	0.75								50
Quantity recovered ($\mu\text{g/mL}$)	0.724	0.778	0.779	0.736	0.770	0.775	0.773	0.748	49.85
Recovery (%)	96.50	103.71	103.83	98.19	102.69	103.33	103.02	99.73	99.71
RSD (%)	3.01	1.28	1.77	1.09	3.06	2.58	2.13	0.82	0.57
At 100% added level									
Add up ($\mu\text{g/mL}$)	1.5								100
Quantity recovered ($\mu\text{g/mL}$)	1.469	1.519	1.557	1.515	1.530	1.546	1.473	1.495	100.01
Recovery (%)	97.91	101.29	103.79	101.00	101.98	103.05	98.23	99.69	100.01
RSD (%)	1.76	0.85	1.15	1.09	1.03	1.07	1.40	1.00	0.08
At 150% added level									
Add up ($\mu\text{g/mL}$)	2.25								150
Quantity recovered ($\mu\text{g/mL}$)	2.196	2.243	2.311	2.238	2.281	2.327	2.210	2.304	149.12
Recovery (%)	97.61	99.69	102.71	99.47	101.36	103.41	98.21	102.39	99.41
RSD (%)	0.78	1.42	0.45	0.63	1.09	0.93	0.58	0.51	0.14
At LOQ added level									
Add up ($\mu\text{g/mL}$)	0.12	0.07	0.1	0.11	0.09	0.08	0.12	0.09	–
Quantity recovered ($\mu\text{g/mL}$)	0.1202	0.0724	0.0979	0.1104	0.0911	0.0783	0.1243	0.0910	–
Recovery (%)	100.14	103.42	97.93	100.37	101.20	97.90	103.57	101.15	–
RSD (%)	3.18	3.38	2.27	0.86	1.45	1.22	1.67	2.49	–

TABLE-5
INFORMATION ON PRECISION FOR FDE AND ITS PROCESS ALLIED IMPURITIES

Precision	RSD values of FDE process allied impurities peak area								FDE
	-1	-2	-3	-4	-5	-6	-7	-8	
System	1.42	0.93	1.42	1.2	1.15	0.99	1.31	0.95	0.17
Method	1.37	1.64	2.15	2.24	1.20	0.96	1.60	1.17	0.43
Intermediate	1.03	2.11	0.97	1.13	1.02	0.56	0.87	1.45	0.57

TABLE-6
INFORMATION ON ROBUSTNESS FOR FDE AND ITS PROCESS ALLIED IMPURITIES

Parameter assessed	Flow streaming rate (mL/min)			Buffer strength (%)			Temperature ($^{\circ}\text{C}$)		
Parameter values	1.15	1.20	1.25	0.09	0.1	0.11	23	25	27
Resolution between FDE and impurity-7	2.28	2.25	2.18	2.24	2.25	2.25	2.18	2.25	2.29
Resolution between FIDE and impurity-7	2.47	2.43	2.35	2.44	2.43	2.43	2.32	2.43	2.48

solution. The peak areas of FDE and its eight process allied impurities were recorded and their %RSD measurements were computed. The %RSD values obtained (0.65-2.66) were well within the acceptable limits suggest the good stability of sample solution and mobile phases used for method.

Application of method to bulk batch analysis: Five bulk batches of synthesized flubendiamide were analyzed by using this RP-HPLC method. Flubendiamide related impurities 1-4 were detected (Table-7), whereas the other impurities 5-8 were not detected in all five batches.

Conclusion

Flubendiamide and its eight related impurities were determined using a simple and selective gradient RP-HPLC validated method. The statistical outcome of validation criteria falls well within the limits mentioned in guidelines of the SANCO/3030/99/Rev. 4 and Office of Chemical Safety and Pollution Prevention specified limits. The research also includes the bulk batch synthesis of flubendiamide and its eight related impurities. All the synthetic process related impurities were characterized

TABLE-7
DATA OF DETECTION OF SELF-MADE BULK DRUG

FDE impurity	Impurity content ($\mu\text{g/mL}$) in batch				
	1	2	3	4	5
-1	0.015	0.036	0.041	0.038	0.046
-2	0.243	0.217	0.284	0.350	0.400
-3	0.012	0.005	0.019	0.033	0.028
-4	0.241	0.198	0.268	0.324	0.388
-5	ND	ND	ND	ND	ND
-6	ND	ND	ND	ND	ND
-7	ND	ND	ND	ND	ND
-8	ND	ND	ND	ND	ND

ND = Not detected

by FT-IR, ^1H NMR and ESI-MS techniques. The new approach has also been proven to be useful for bulk batch analysis of flubendiamide and its eight related impurities, which is a need for registering agrochemicals in the agricultural business. As a result, a newly designed RP-HPLC approach may be more suited for the continuous process control assessment in bulk production as well as impurity profiling in quantitative research.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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