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RESEARCH ARTICLE

DEVELOPMENT AND INDEPENDENT LABORATORY VALIDATION OF AN ANALYTICAL METHOD FOR THE DETERMINATION OF OXYACETANILIDE RESIDUE AND ITS METABOLITES IN TRITICUM AESTIVUM (GRAIN & STRAW) BY LC-MS/MS TANDEM MASS SPECTROMETRY

Purushottam Trivedi, Alpesh H. Patel, Meet R. Patel and Padmaja S. Prabhu
Department of Chemistry, Jai Research Foundation, Vapi, 396105 Gujarat.

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Abstract

A novel and robust high-throughput liquid chromatography/tandem mass spectrometry (LC-MS/MS) method was developed for the determination of residue of oxyacetanilide as its metabolitesnamely the N-fluorophenyl-N-isopropyl in Triticum Aestivum (Grain & Straw). The performance characteristics included linearity, specificity (matrix interferences), LOQ, reproducibility (%RSD) and trueness (%recovery). The method is, therefore, compliant with the definition of the residue for oxyacetanilide containing the N fluorophenyl-N-isopropyl as oxyacetanilide equivalent. This design specifically evaluates the effects of sample extraction interferences. Two precursors/product ion transitions (154/112 and 154/95) were monitored for target compound to achieve true positive identification. 5 g of the sample was taken with water containing 1 N H_2SO_4 and $KMnO_4$ in RBF. Afterwards, 1 g of $NaHSO_3$- $NaHSO_4$ mixture was mixed. Conc. H_2SO_4 was added through the condenser and the mixture was heated under reflux for 20 h. The mixture was cool for approx. 30 minutes and added water. An amount of this extract was taken for liquid liquid extraction. CH_2Cl_2 was added for 1 minute and the organic phase was disposed of, and 50 % aq. NaOH was added into the aq. Phase. CH_2Cl_2 was again added and shaken for 1 min. The lower phase was filtered and repeated twice. 0.10 % HCOOH in water was added to the extract. The mixture was ultra-sonicated for about 30 seconds. This research highlights the satisfactory linearity, trueness, and reproducibility were achieved within the linear range 0.00008- 0.01 mg/L with a correlation co-efficient >0.99. %RSD and % recovery of the analytical method was determined from recovery experiment (n=5 replicates) at 0.01 mg kg-1 (grain) and 0.05 mgkg-1 (straw). The mean recovery of the targeted compound in wheat grain and straw obtained wasin the range of 72.30 – 80.50 % with associated % RSD values lower than or equal to 7% for quantification and confirmation.

Introduction:-

India is principally an agriculture-based country with more than 70% of its population reliant on agriculture and covers a maximum portion of its budget[1]. The Indian agriculture sector has a remarkable long-term record of taking the country out of

Corresponding Author:- Purushottam Trivedi
Address:- Department of Chemistry, Jai Research Foundation, Vapi, 396105 Gujarat.

serious food shortages despite a rapid population increase.As reported earlier that 45% of the annual food construction is depleted by pest infestations[2], effective pest organisation seems one of the main strategies to increase crop output for a rapidly growing population, that requires application of a wide variety of pesticides in agricultural fields to contest pests.

Pesticides have been broadly used all over the world since the20th century. These chemicals belong to various classes. Organochlorine, pyrethroid, herbicide, fungicide, nicotinoid and organophosphorus compounds, are the most vital groups. The Phys-chem characteristics of insecticides may differ significantly. Some chemicals contain halogens, others phosphorous, sulphur, chlorine or nitrogen. Herbicides play an important role in modern agriculture.Several compounds are very volatile and unstable, but several do not vanish at all. This assortment causes serious difficulties in the expansion of a worldwide residue analytical method, which should have the broadestpossibility possible. TheseMRM methods are instantlydesirable. Possibly, no other use of chemicals is regulated more extensively than that of pesticides. MRLrecognized for pesticides in crops and drinking water in utmost countries to avoid any antagonistic impact on public strength.Insecticide residues in surface water may cause adverse effects on marinecreatures. For these reasons,many laboratories are involved in the investigation of MRL or in the identification and quantification of these residues in ecologicalmedia. In this context, the use of several single-residue methods is usually too expensive. It must be noted that eachcorporation which applies for registering of a new insecticidemust provide residue analytical information. Contingent on the purpose, determination of pesticide residues may be target analysis or non-target analysis. These different chemicals act on a surprisingly low number of molecular targets in the plant.[3,4] An example of target analysis is the inspection of MRLs in food. The relevant analytes are fixed by the residue definition given in the MRL regulation. These residue definitions may include relevant metabolites or degradation products of the pesticides.

Wheat (Triticum aestivum) is leading food grain crop being a staple diet and prime importance in the realms of food crops in the world. It is foremost among cereals and as a main source of carbohydrates and protein for both human beings and animals; contains starch (60-90%), protein (11-16.5%), fat (1.5-2%), inorganic ions (1.2-2%) and vitamins (B-complex and vitamin E).[5,6] In the past decades, many hypotheses have been made concerning the primary target sites of these herbicides. In general, these herbicides are more active on monocotyledonous plants than thedicotyledonous plants. The accumulation of pesticides in food commodities may have serious cascading effects on human health and ecosystems throughfood chains.Therefore, it is of great importance to investigate the uptake and accumulation behaviours of pesticides. The total area of the world under wheat is around 212.99 million ha with a grain yield of 596.20 million tons.[7] The most important species of wheat is Triticum aestivum occupying 85% of the total area under wheat cultivation. Weeds infestation is one of the major threats to crop growth and yield. Weeds compete with crop plants for nutrients, solar radiation, water, carbon dioxide, space, and many other growth factors. The fast-growing population of the country makes it imperative to achieve matching increases in the rate of food production.

Oxyacetanilide {N-(4-fluorophenyl)-N-(1-methyl-ethyl)-2-[5-(trifluoromethyl)-1, 3, 4-thiadiazol 2-yl] oxy] acetamide}, a selective herbicide to control grassy weeds in a wide range of crops disinclining cereals.[8,9]The objectives of this study were to develop a robust and reliable analytical method for the extraction, separation and identification of N-fluorophenyl-N-isopropyl in grain and straw extracts; for the purpose of accurate quantification using oxyacetanilide. The work was to independently validate for the determination of residue of oxyacetanilide in Wheat is the principal staple food crop for rural societies in Gujarat. This research work provides the framework for efficient, reproducible, specific, linear, accurate, high throughput, and cost-effective analytical approach for the analysis of oxyacetanilide and its metabolite in Grain & Straw for residue analysis. Analytical method validation isapillar for standardising reference methods for its intended purpose. Laboratory studies provide reliable data forregulatory requirements, science, and quality control requirements.

Experimental:-

Chemicals and Herbicide Standard:-

Oxyacetanilide (99.5 %) and 4-Fluoro-N-isopropylaniline (95.75%) were procured from Sigma Aldrich and Arro-Biochem respectively. All solvents like methanol (HPLC) milli-Q water, sulphuric acid (AR), sodium sulphate (AR), sodium hydroxide (AR), dichloromethane (HPLC), formic acid (AR), potassium permanganate (AR), sodium bisulfite (AR) and sodium bisulfate (AR) were procured from Merck, Finar, SDFCL and Arro-Biochem respectively.

Grinder Cum Mixer, Magnetic Hot Plate Stirrer, Analytical Balance, Micro-Balance, Refrigerator, Rotary Vacuum Evaporator, Spinix Vortex Shaker, Sonicator Ultrasonic Cleaning bath, HPLC (Nexera X2_Shimadzu), LC-MS/MS (API4000_ AB Sciex).

Sample collection:-
Triticum Aestivum (Wheat grain and straw) specimens originating from the agricultural region of Saurashtra Gujarat, India and homogenized using a cutting mill for straw and a knife mill for grain samples, and stored in the deep freezer until extraction.

Standard Preparation:-
The validation procedure was performed based on SANCO 825 guidelines [31], evaluating several parameters: linearity, specificity (matrix interferences), sensitivity, trueness (% recovery), reproducibility (%RSD) and limits of quantification (LOQ).

The individual stock solutions of oxyacetanilide(?) and 4-Fluoro-N-Isopropylaniline at 1 mg/mL were prepared in methanol. These stock solutions were used to prepare working standard solutions at 0.00008, 0.0001, 0.0004, 0.002, 0.004, 0.008 and 0.01 mg/L. The working standard solution prepared in 0.1 % formic acid in milli Q water
(80%) and 0.1 % formic acid in methanol (20%). The stock, intermediate and working solutions were maintained at refrigerator condition (2-8°C).

Blank grain and straw samples were chosen for the preparation of matrix-interferences and recovery studies. Specificity was measured by analyzing blank wheat straw and grain sample evaluate potential interferences from endogenous components. Also, a comparison was made of the t_R of standard solutions of compounds. In the validation study, the concentration of oxyacetanilide standard spiked- wheat (straw and grain) sample were selected to be at around 0.05 and 0.01 mg/L respectively as LOQ level. However, for 10 x LOQ level,this concentration level wasselected to be at around 0.5 and 0.1 mg/L respectively. For each concentration, the standard solution mixture of oxyacetanilide was spiked into wheat (straw and grain) (n=5).

For stock solution stability the stock solutions of oxyacetanilide and 4-fluoro-N-isopropylaniline were prepared and stored in refrigerator condition (2-8 °C) and compared with the freshly stock solutions of 4-fluoro-N-isopropylaniline and oxyacetanilideafter 21 days and 26 days respectively. For linearity solution stability, a 4-fluoro-N-isopropylaniline solutionwas prepared and stored in refrigerator condition (2-8 °C) and subsequently compared with the freshly prepared 4-fluoro-N-isopropylanilinesolution after 31 days. For extract solution stability, wheat grain and straw at LOQ and 10 x LOQ level were extracted and analysed on the day 0 and stored in refrigerated condition (2-8 °C). After 27 days for wheat straw and 21 days for wheat grain, the stored extract solutions were analysed. (n = 3 for each).

Calculation:-
The below-mentionedequation was used to calculate the 4-fluoro-N-isopropylaniline residue R in samples for the transition 154 m/z -> 112/95 m/z:

$R = C_{End} \times DF \times [(V_{Ex} \times V_{End}) / (VR1 \times W)]$
$= C_{End} \times \text{Multiplier M} \times DF$
Where: R: Residue found, in mg/kg
C_{End}: Final concentration of the analyte in the extract, in ng/mL
V_{Ex}: Water extract volume: 250 mL
VR1: Aliquot of water extract: 10 mL.
V_{End}: Final volume: 5.0 mL (wheat grain), 25 mL (wheat straw)
W: Specimen weight used: 5.0 g
DF: Dilution factor

Results and Discussion:-

Optimisation of Extraction and Clean-up Procedure:-
An amount of 5.0 g sample was taken into a 500 mL round bottom flask (RBF) and add water (40 mL for grain and 80 mL for straw). RBF was placed on magnetic stirring and the mixture was stirred for 1 h at room temperature. Then 5 mL of aqueous 1 N H_2SO_4 was added and stirred for 2 minutes. An amount of 1 g $KMnO_4$ was added and the mixture was stirred for 5 min. The colour of the entire mixture appeared purple. Afterwards, 1 g of sodium bisulfite-sodium bisulfate-mixture was added to the flask and the mixture lost whole purple colour. A reflux condenser was attached on to the round bottom flask and 25 mL concentrated H_2SO_4 was added through the reflux condenser into the flask. The mixture in the flask was heated and boil under reflux for at least 20 h. The mixture was allowed to cool for approximately 30 minutes and 100 mL of water

would be added through the reflux condenser. The reflux condenser was removed and the mixture filtered through a folded filter into a 250 mL graduated measuring cylinder. The cylinder was filled up to 250 mL with water. 10 mL of the extract was taken into a separating funnel containing 90 mL of water, the pH value wasin the range of 0.7 to 1. A volume of 20 mL of dichloromethane was added to the separating funnel, the funnel was shaken for 1 minute vigorously by hand and the organic (lower) phase was disposed of. This rinsingstep was repeated once again. 5 mL of 50 % aqueous sodium hydroxide solution (in an ice bath during addition) was added into the aq. phase, the mixture was stirred with a glass rod and pH was checked and the value of the mixture in the separation funnel wasin the range of 12.2 to 12.4.

A volume of 20 mL of dichloromethane was added and the separation funnel, shaken for 1 min. The lower phase was filtered through a glass funnel with anhydrous sodium sulphate into a round bottom flask. The sodium sulphate was wetted with dichloromethane before filtration. This step was repeated twice. 0.10 % formic acid in water was added to the extract (20 mL for wheat straw and 4 mL for wheat grain) as the keeper. The filtrate in the round bottom flask was evaporated in a rotary vacuum evaporator to the volume of the keeper. Water was added gravimetrically to the target water content (20 mL for wheat straw and 4 mL for wheat grain) and methanol was added (5 mL for wheat straw and 1 mL for wheat grain) and mixed. The mixture was ultra-sonicated for about 30 seconds.

Chromatography Optimization:-

LC-MS/MS analysis was conducted on a Shimadzu NEXERA X2 equipped with an autosampler (SIL-30AC), quaternary pump (LC-30AD), UV/VIS detector (SPD-20A) and column oven (CTO-20AC). The separation was carried out on Phenomenex Kinetex C18 100 mm x 4.6 mm, particle size 2.6 µm.The mobile phase was gradient A) Water + 0.1% Formic Acid B) Methanol + 0.1% Formic Acid The analysis was carried out by injecting 20 µL sample into the chromatography system at a flow rate of 0.40 mL min^{-1} and maintaining the column temperature at 40 °C.

For 4-fluoro-N-isopropylaniline:

Gradient MS Parameters

Time (min)	% A	% B	Analyte	4-fluoro-N-isopropylaniline	
0.01	90	10	MRM Transitions	154.0 – 112.0	154.0 – 95.0
1.00	90	10	Dwell	250	250
4.00	5.0	95	DP	85	85
6.50	5.0	95	CE	17	39
6.60	90	10	CXP	20	8
9.00	90	10	EP	10	10

For Oxyacetanilide

Gradien MS Parameter

Time (min)	% A	% B	Analyte		Oxyacetanilide
0.01	90	10	MRM Transitions	364.0 -152.2	364.0 -194.0
1.00	90	10	Dwell	250	250
4.00	5.0	95	DP	55	76
7.50	5.0	95	CE	15	17
7.60	90	10	CXP	12	10
10.00	90	10	EP	15	15

Ion Spray Voltage : 5500
CUR : 20
GS 1 (Ion Source gas 1) : 55
GS 2 (Ion Source gas 2) : 60
Temp : 500 °C
CAD : 10.0
Polarity : Positive

The total analysis time was 9 min for the developed semi-automated method based on LC-MS/MS coupled to quadrupoles high-resolution mass spectrometry.4-fluoro-N-isopropylaniline was analysed with 154 m/z quantification along with two confirmation m/z ion 112 and m/z ion 95 and oxyacetanilide was analysed with 364 m/z quantification along with two confirmation m/z ion 152.2 and m/z ion 194. Moreover, the use of a semiautomated method minimizes sample handling,

which reduces interferences that may occur in the extraction stage.In this work, the influence of co-extracted compounds on analytical signals was assessed by injecting mobile phase, methanol, blank matrix extracts of wheat straw and wheat grain, a standard solution of 4-fluoro-N-isopropylaniline and oxyacetanilide. In separation selectivity testing, all blanks showed no interfering peaks at the t_R of the target analytes, as can be seen from the chromatograms in Figures 3 and 4. Also, with the aim of studying the specificity of the method, a comparison was made of the t_R of reference standard solutions sample as well as blank matrix extracts of wheat straw and wheat grain samples. As can be seen in Figures 3 and 4, the t_R values were not significantly influenced by other interferences from the matrix, the method was specific for the analyte. The matrix of wheat straw and wheat grain was residue-free.

The slopes achieved insolvent 0.1 % formic acid in milli Q water (80%) and 0.1 % formic acid in methanol (20%). Next, the linearity and correlation coefficients were obtained in the range of 0.994 - 0.999 from the calibration curves at concentrations ranging viz., 0.00008, 0.0001, 0.0004, 0.002, 0.004, 0.008 and 0.01 mg/L in Figures 1 and 2, from the blank levels and were found to be sufficient.

The LOQ for the analysis of oxyacetanilide established at 0.05 mg/kg for straw, 0.01 mg/kg for grain. The reproducibility of 4-fluoro-N-isopropylaniline residue in wheat (straw and grain) at LOQ level were 0.00, 4.05 and 1.43, 4.83 for quantification and confirmation, respectively. The reproducibility at 10 times LOQ level was 2.68, 2.07 and 6.63, 6.67 for quantification and confirmation, respectively. The mean trueness (% recovery) of 4-fluoro-N-isopropylaniline, expressed as oxyacetanilide, residue in wheat (straw and grain) at LOQ level were 75.00, 80.50 and 78.00, 78.50 for quantification and confirmation, respectively. The mean trueness (% recovery) at 10 x LOQ level was 75.00, 74.60 and 72.30, 74.15 for quantification and confirmation, respectively. (Table 1 and 2)

Recoveries ranged from 72.30 % to 80.50 %, whereas RSD values were lower than or equal to 10% for repeatability conditions and 24% for reproducibility. Because the obtained results are suitable, the use of the internal standard is not necessary, simplifying the proposed method, which agreed with SANCO (Document No. SANCO/825/00 rev.8.1 Guidance Document on Residue Analytical Methods. European Commission, Directorate General/ Health and Consumer Protection).[10]

Extract solutions of wheat straw and wheat grain were stable up to 27 days and 21 days in refrigerator condition (2-8 °C) (Table 3 and 4). 4-fluoro-N-isopropylaniline and oxyacetanilide stock solution were stable up to 21 days and 26 days and calibration solution was stable up to 31 days in refrigerator condition (2-8 °C) (Table 5, 6 and 7).As far as we know, this is the first study focused on the development of a semiautomated method based on LC-MS/MS quadrupole for the determination of 4-fluoro-N-isopropylaniline, expressed as oxyacetanilide in wheat (straw and grain). Finally, the proposed methodology can be implemented in analytical laboratories for the determination of target compounds due to the simplicity of the procedure.

Conclusion:-

In-house inter-laboratory validation studies, using wheat straw and wheat grain samples containing N-fluorophenyl-N-isopropyl residues of oxyacetanilide demonstrated that the method is quick, rugged, selective, and sensitive enough to determine residue. This method is highly robust and suitable for cost-effective routine analysis of these herbicides, achieving acceptable recoveries for all the spike concentration, good sensitivity (LOQ, 0.01 $mgkg^{-1}$ for wheat grain and 0.05 $mgkg^{-1}$ for wheat straw) and acceptable % RSDs. The proposed LC-MS/MS method is rapid, sensitive, and successfully applicable for the simultaneous analysis of oxyacetanilide in their metabolite form N-fluorophenyl-N-isopropyl, in wheat straw and wheat grain. An analytical gradient elution developed in this method improved the peak shape and retention of the analytes over gradient elution. Positive mode ion-spray with MS/MS dimension gives admirable sensitivity and selectivity that produce distinct chromatographic peaks with slight nosiness. The present method allows the simultaneous determination of the presence and quantification with high reliability.

Table 01:- Reproducibility (% RSD) and Trueness (% Recovery) for Residue of 4-fluoro-N-isopropylaniline in Triticum Aestivum (Wheat Grain).

Sample ID	4-Fluoro-N-Isopropylaniline *					
	Quantification			Confirmation		
	Q1/Q3 (154 m/z -> 112 m/z)			Q1/Q3 (154 m/z -> 95 m/z)		
	C_{End}	R	Trueness	C_{End}	R	Trueness
	mg/L	mg/kg	%	mg/L	mg/kg	%
QR1	0.00031	0.00775	77.50	0.00033	0.00825	82.50

QR2	0.00031	0.00775	77.50	0.00030	0.00750	75.00
QR3	0.00031	0.00775	77.50	0.00033	0.00825	82.50
QR4	0.00031	0.00775	77.50	0.00030	0.00750	75.00
QR5	0.00032	0.00800	80.00	0.00031	0.00775	77.50
Average (n=5)			**78.00**	**78.50**		
% RSD (n=5)			**1.43**	**4.83**		
10 QR1	0.00298	0.07450	74.50	0.00307	0.07675	76.75
10 QR2	0.00255	0.06375	63.75	0.00262	0.06550	65.50
10 QR3	0.00297	0.07425	74.25	0.00303	0.07575	75.75
10 QR4	0.00296	0.07400	74.00	0.00311	0.07775	77.75
10 QR5	0.00300	0.07500	75.00	0.00300	0.07500	75.00
Average (n = 5)			**72.30**	**74.15**		
% RSD (n = 5)			**6.63**	**6.67**		
Overall Average (n = 10)			**75.15**	**76.33**		
Overall % RSD (n = 10)			**4.03**	**5.75**		
Typical Calculation						
W	V_{EX}	V_{R1}	V_{END}	R = 0.00031 x 1 x 250 x 5		
g	mL	mL	mL	10 x 5		
5	250	10	5	= 0.00775 mg/kg		
Residue R	= C_{END} x DF x [(V_{EX} x V_{END}) / (V_{R1} x W)]			LOQ Level = 0.01 mg/kg	LOQ Level = 0.1 mg/kg	
* Fortified as Oxyacetanilide, determined as 4-Fluoro-N-Isopropylaniline				R = Residue	Dilution factor = 1	

Table 2:- Calculation of Reproducibility (% RSD) and Trueness (% Recovery) for Residue of 4-fluoro-N-isopropylaniline in Wheat Straw.

Sample ID	**4-Fluoro-N-Isopropylaniline ***					
	Quantification			**Confirmation**		
	Q1/Q3 (154 m/z -> 112 m/z)			**Q1/Q3 (154 m/z -> 95 m/z)**		
	C_{End}	**R**	**Trueness**	**C_{End}**	**R**	**Trueness**
	mg/L	mg/kg	%	mg/L	mg/kg	%
QR1	0.00030	0.0375	75.00	0.00030	0.03750	75.00
QR2	0.00030	0.0375	75.00	0.00033	0.04125	82.50
QR3	0.00030	0.0375	75.00	0.00033	0.04125	82.50
QR4	0.00030	0.0375	75.00	0.00032	0.04000	80.00
QR5	0.00030	0.0375	75.00	0.00033	0.04125	82.50
Average (n=5)			**75.00**	**80.50**		
% RSD (n=5)			**0.00**	**4.05**		
10 QR1	0.00305	0.38125	76.25	0.00305	0.38125	76.25
10 QR2	0.00308	0.38500	77.00	0.00304	0.38000	76.00
10 QR3	0.00303	0.37875	75.75	0.00296	0.37000	74.00
10 QR4	0.00296	0.37000	74.00	0.00297	0.37125	74.25
10 QR5	0.00288	0.36000	72.00	0.00290	0.36250	72.50
Average (n = 5)			**75.00**	**74.60**		
% RSD (n = 5)			**2.68**	**2.07**		
Overall Average (n = 10)			**75.00**	**77.55**		
Overall % RSD (n = 10)			**1.34**	**3.06**		
Typical Calculation						
W	V_{EX}	V_{R1}	V_{END}			
g	mL	mL	mL			
5	250	10	25			
Residue R	= C_{END} x DF x [(V_{EX} x V_{END}) / (V_{R1} x W)]			LOQ Level = 0.05 mg/kg	LOQ Level = 0.5 mg/kg	
* Fortified as Oxyacetanilide, determined as 4-Fluoro-N-Isopropylaniline				R = Residue	Dilution factor = 1	

Table 3:- Extract Stability in Wheat Straw (27 Day).

Sample ID	Fortification Concentration	4-Fluoro-N-Isopropylaniline *					
		Quantification			Confirmation		
		Q1/Q3 (154 m/z -> 112 m/z)			Q1/Q3 (154 m/z -> 95 m/z)		
		C_{End}	R	C_{End}	R	C_{End}	R
	(mg/kg)	mg/L	mg/kg	%	mg/L	mg/kg	%
Q (27) _I	0.05	0.00034	Mean of 2		0.00033	Mean of 2	
Q (27) _II		0.00035	0.0438	87.60	0.00035	0.0425	85.00
Q (0) _I		0.00031	Mean of 2		0.00031	Mean of 2	
Q (0) _II		0.00031	0.0388	77.50	0.00031	0.0388	77.50
10Q (27) _I	0.50	0.00332	Mean of 2		0.00322	Mean of 2	
10Q (27) _II		0.00326	0.4113	82.25	0.00321	0.4025	80.50
10Q (0) _I		0.00310	Mean of 2		0.00308	Mean of 2	
10Q (0) _II		0.00307	0.3863	77.26	0.00304	0.3825	80.50

Table 4:- Extract Stability in Wheat Grain (21 Day).

Sample ID	Fortification Concentration	4-Fluoro-N-Isopropylaniline *					
		Quantification			Confirmation		
		Q1/Q3 (154 m/z -> 112 m/z)			Q1/Q3 (154 m/z -> 95 m/z)		
		C_{End}	R	C_{End}	R	C_{End}	R
	(mg/kg)	mg/L	mg/kg	%	mg/L	mg/kg	%
Q (27) _I	0.01	0.00031	Mean of 2		0.00033	Mean of 2	
Q (27) _II		0.00030	0.0078	78.00	0.00030	0.0078	78.00
Q (0) _I		0.00031	Mean of 2		0.00029	Mean of 2	
Q (0) _II		0.00029	0.0075	75.00	0.00030	0.0075	75.00
10Q (27) _I	0.10	0.0029	Mean of 2		0.00293	Mean of 2	
10Q (27) _II		0.00287	0.0723	72.30	0.00286	0.0723	72.30
10Q (0) _I		0.00292	Mean of 2		0.00287	Mean of 2	
10Q (0) _II		0.00294	0.0733	73.25	0.00287	0.0733	73.25

Table 5:- Linearity Solution Stability of 4-Fluoro-N-Isopropylaniline at 31 days.

Sample ID	Conc. mg/L	4-Fluoro-N-Isopropylaniline	
		Quantification	Confirmation
		Q1/Q3 (154 m/z -> 112 m/z)	Q1/Q3 (154 m/z -> 95 m/z)
		Peak Area Counts	Peak Area Counts
LO_I	0.002	242401	25971
LO_II		261783	28276
LO_III		270067	29110
Average (n = 3)		**258083.67**	**27785.67**
% RSD (n = 3)		**5.50%**	**5.85%**
LN_I	0.002	262104	28453
LN_II		265490	31908
LN_III		252894	28351
Average (n = 3)		**260162.67**	**29570.67**
% RSD (n = 3)		**2.51%**	**6.85%**
LO = Linearity Old and LN = linearity New			

Table 6:- Stock Solution Stability of 4-Fluoro-N-Isopropylaniline at 21 days.

Sample ID	Conc. mg/L	4-Fluoro-N-Isopropylaniline	
		Quantification	Confirmation
		Q1/Q3 (154 m/z -> 112 m/z)	Q1/Q3 (154 m/z -> 95 m/z)
		Peak Area Counts	Peak Area Counts
SO_I	0.001	103781	11711

SO_II		112350	13367
LO_III		119896	13763
Average (n = 3)		112009.00	12947.00
% RSD (n = 3)		7.20%	8.41%
SN_I	0.001	122564	13089
SN_II		123599	13374
SN_III		120073	13316
Average (n = 3)		122078.67	13259.67
% RSD (n = 3)		1.48%	1.14%
SO = Stock Old and SN = Stock New			

Table 7:- Stock Solution Stability of Oxyacetanilide at 26 Days.

Sample ID	**Conc. mg/L**	**4-Fluoro-N-Isopropylaniline**	
		Quantification	**Confirmation**
		Q1/Q3 (364 m/z -> 194 m/z)	**Q1/Q3 (364 m/z -> 152 m/z)**
		Peak Area Counts	**Peak Area Counts**
SO_I	0.001	334073	103490
SO_II		340660	106666
LO_III		341685	105894
Average (n = 3)		338806.00	105350.00
% RSD (n = 3)		1.22%	1.57%
SN_I	0.001	334297	104319
SN_II		324170	102017
SN_III		331534	103388
Average (n = 3)		330000.33	103241.33
% RSD (n = 3)		1.59%	1.12%
SO = Stock Old and SN = Stock New			

Fig:1:- Linearity Curve of 4-fluoro-N-isopropylaniline (Quantification).

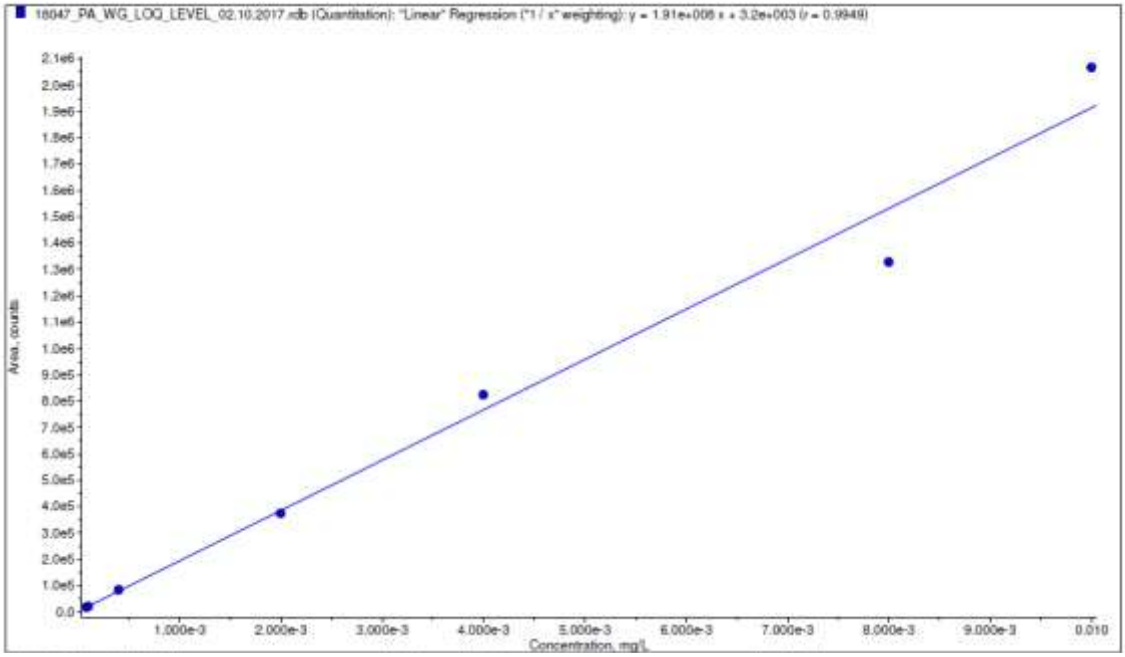

Fig. 2:- Linearity Curve of 4-fluoro-N-isopropylaniline (Confirmation).

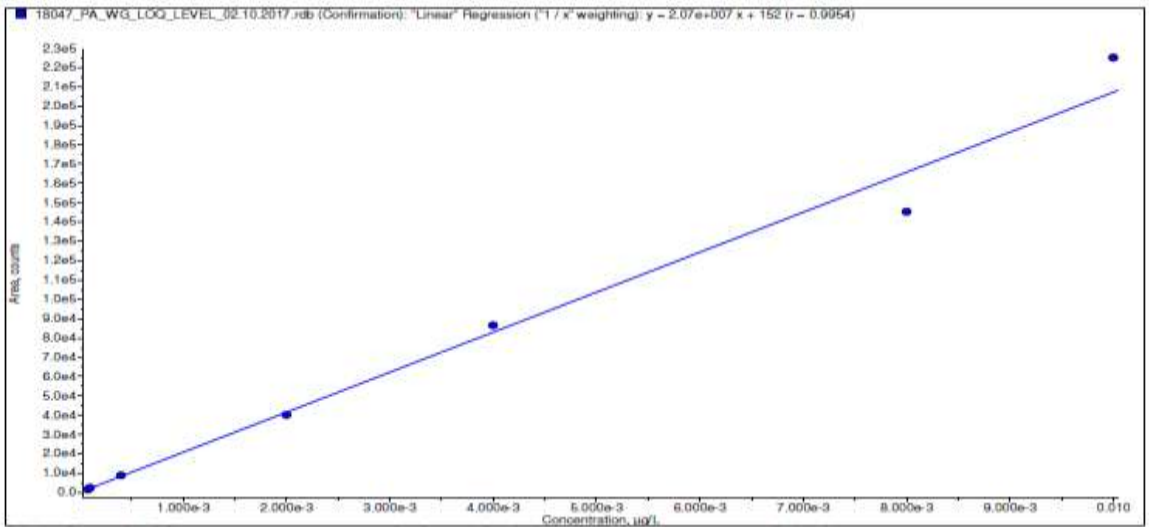

Fig. 3:- Chromatogram of Methanol, Mobile Phase, Blank Extract of Wheat Grain and Straw and Reference Standard Solution of 4-Fluoro-N Isopropyl aniline (0.002 mg/L) (Mass Transition 154 -> 112 m/z).

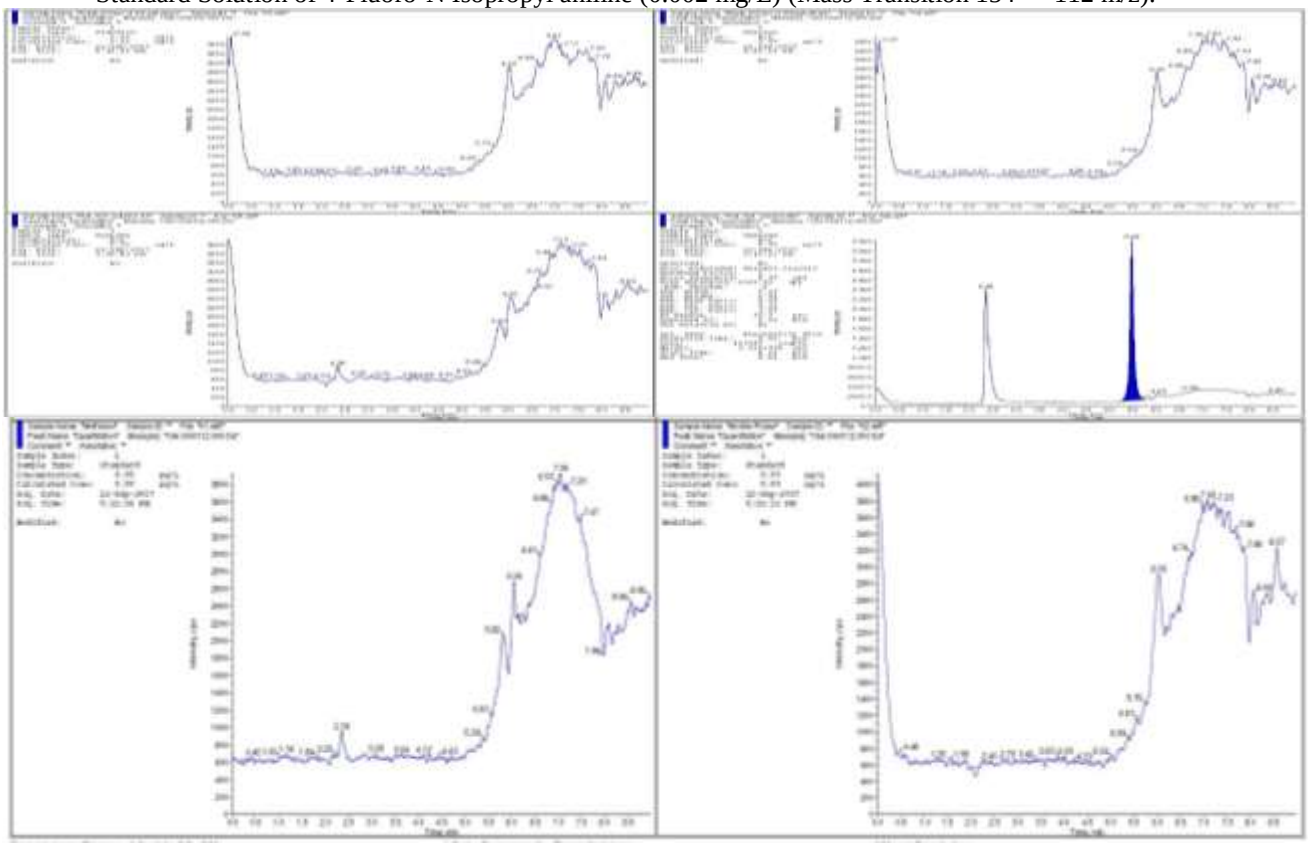

Fig. 4:- Chromatogram of Methanol, Mobile Phase, Blank Extract of Wheat Grain and Wheat Straw and Reference Standard Solution of 4-Fluoro-N Isopropyl aniline (0.002 mg/L) (Mass Transition 154 -> 95 m/z).

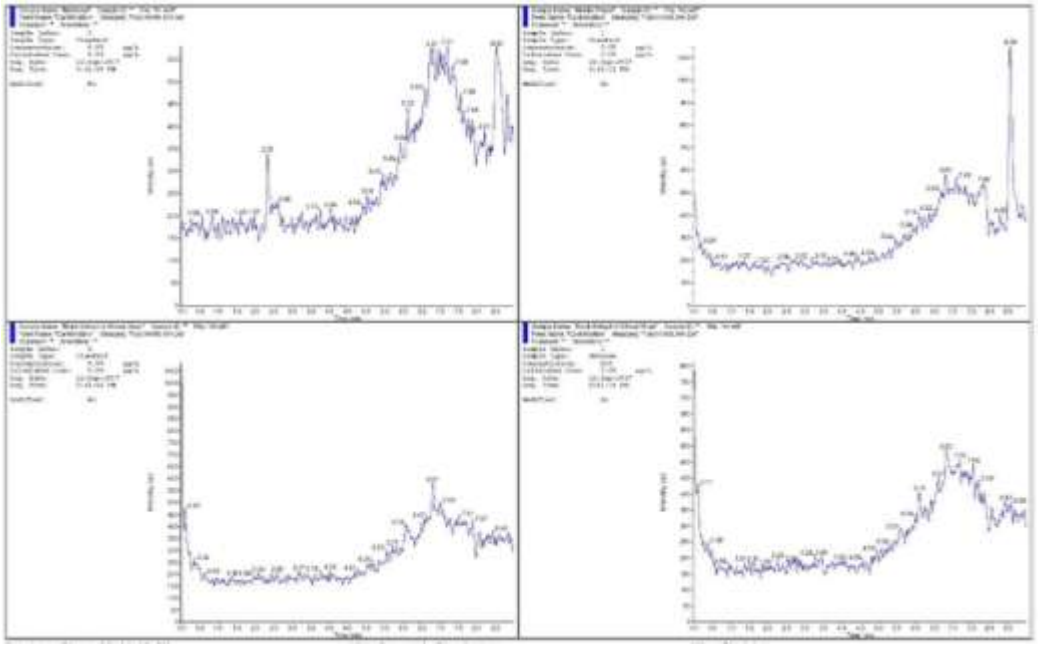

Fig: 5:- Chromatogram of Wheat Grain sample at LOQ Level _ (0.01 mg/kg) (Mass Transition 154 -> 112 m/z).

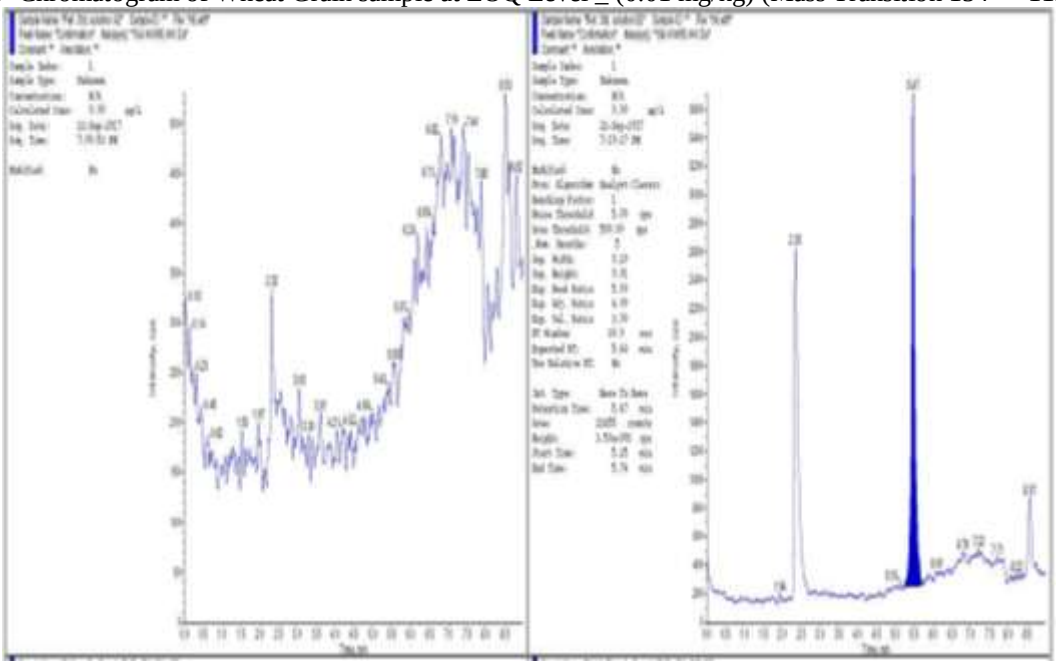

Fig. 6:- Chromatogram of Wheat Grain sample at LOQ Level _ (0.01 mg/kg) (Mass Transition 154 -> 95 m/z).

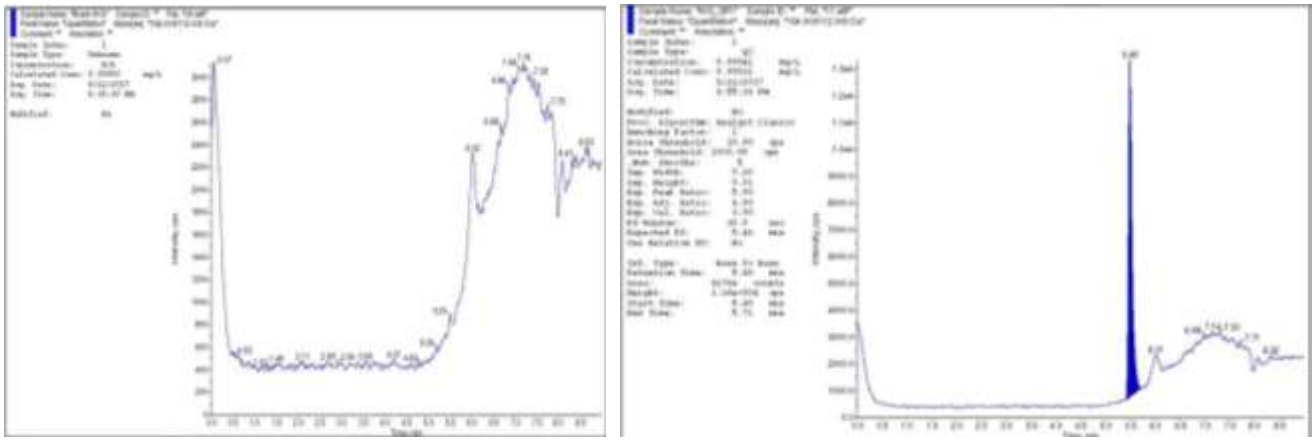

Fig. 7:- Chromatogram of Wheat Grain sample at 10 x LOQ Level _(0.1 mg/kg) (Mass Transition 154 -> 112 m/z).

Fig. 8:- Chromatogram of Wheat Grain sample at 10 x LOQ Level _(0.1 mg/kg) (Mass Transition 154 -> 95 m/z).

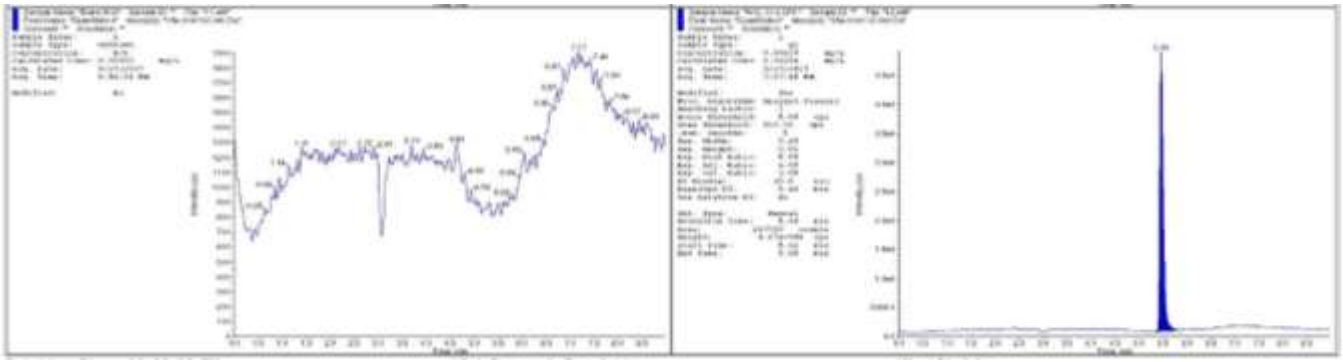

Fig. 9:- Chromatogram of Wheat Straw sample at LOQ Level _ (0.05 mg/kg) (Mass Transition 154 -> 112 m/z).

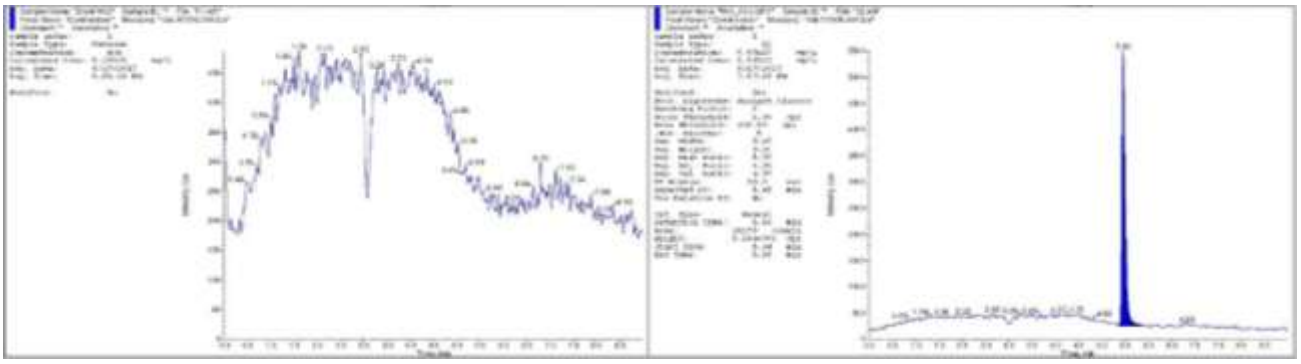

Fig.10:- Chromatogram of Wheat Straw sample at LOQ Level _ (0.05 mg/kg) (Mass Transition 154 -> 95 m/z).

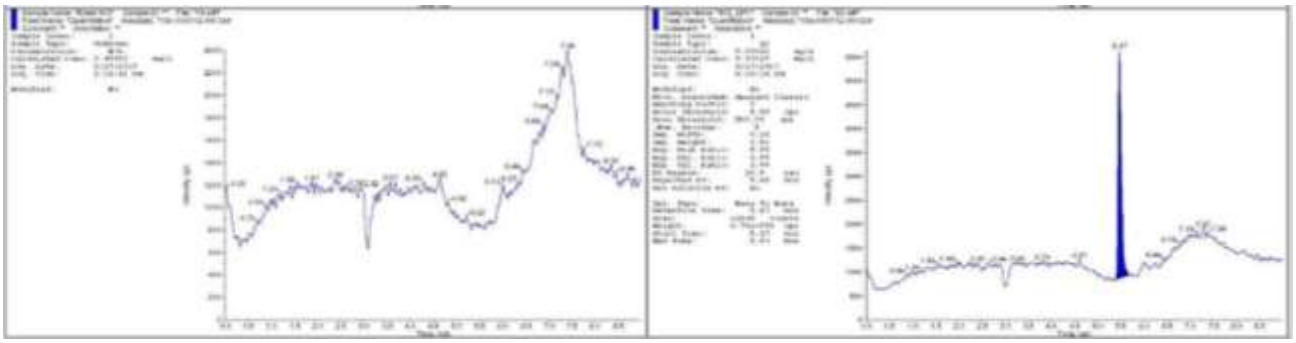

Fig. 11:- Chromatogram of Wheat Straw sample at 10 x LOQ Level _ (0.5 mg/kg) (Mass Transition 154 -> 112 m/z).

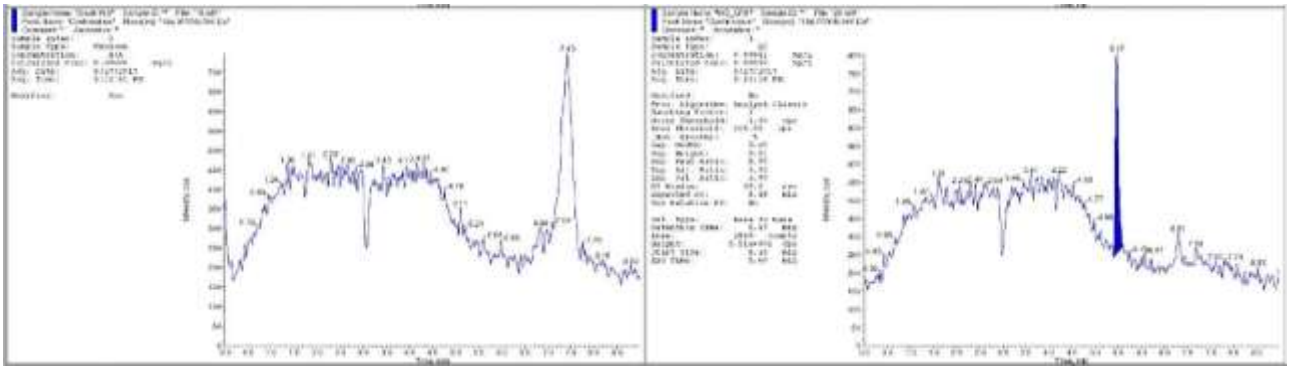

Fig. 12:- Chromatogram of Wheat Straw sample at 10 x LOQ Level _ (0.5 mg/kg) (Mass Transition 154 -> 95 m/z).

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