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Simultaneous determination of 40 weedicide residues in grapes using liquid chromatography, tandem mass spectrometry (LC-MS/MS)

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Abstract

Most of the herbicides are characteristically toxic to humans and carcinogenic in nature, it is necessary to evaluate the safety aspects of table grapes with regards to their residues to ensure consumer safety. Aggregate 40 weedicides were selected for the study, which contain some of CIB registered and included in "List of agrochemicals to be monitored for the grape season 2021-2022(Annexure-9, APEDA)". Grape variety Thompson Seedless was selected as study matrix. Single laboratory method was selected for the validation of analytical method and SANTE/11312/2021 was the guideline followed for the same. A liquid Chromatography (Agilent 1200 series) tandem mass spectrometry (Agilent triple quadruple 6460) was used for the residue analysis in grape samples. Observed results from the validation of test parameters are within acceptable criteria specified in SANTE/11312/2021 guideline. It is concluded that this method is fit for the purpose of residues analysis of enlisted 40 weedicide molecules in grapes.

Keywords: Grapes; Weedicides; Residues analysis; Method validation; Thompson seedless

1. Introduction

Grape is an important horticultural crop in India, the commercial cultivation of which receives frequent application of a massive number of weedicides throughout the cropping season to control a variety of weeds. Monitoring of weedicide residues in table grapes is essential since diversified kinds of herbicides are frequently applied in viticulture, though only 3 weedicides are approved by CIB-RC for usage in grape vineyards [1]. Injudicious usage of weedicides may become a source to withstand the residue till and after harvesting [2]. Since most of the herbicides are characteristically toxic to humans and carcinogenic in nature, it is necessary to evaluate the safety aspects of table grapes with regards to their residues to ensure consumer safety.

Judicial limits have become stricter than ever due to the concerns of food safety and the demands of trade barriers of different countries, motivating the demand for new sensitive and reliable analytical methods for pesticide residues [3]. 60 herbicide molecules registered by CIB-RC in India for use in various crops. So it became necessity to develop a multi residue analytical method to analyze maximum weedicides in a single attempt.

Grape has a complex matrix nature, therefore to reduce influences from the interferences, we preferred ethyl acetate based sample preparation method instead of Acetonitrile based QuEChERS, because Ethyl acetate is an economically cheaper and toxicologically safer solvent than acetonitrile and thus found more appropriate for extraction [4]. Moreover

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Selection of ethyl acetate offers precise advantages over acetonitrile in minimizing the matrix components in the final extract and reducing the cost of analysis of matrix-like grape, which contains high sugar and less fat [6].

2. Material and methods

2.1. Selection of weedicides

Aggregate 40 weedicides which are amenable for LC-MS/MS analysis were selected for the study, which contain some of CIB registered and included in "List of agrochemicals to be monitored for the grape season 2021-2022(Annexure-9, APEDA)" [6]. Grape variety Thompson Seedless was selected as study matrix because it is a major constitute among all grape varieties being exported from India. Organically grown mature fruits were collected and screened to confirm an absence of any weedicide residues before using for method development and validation.

2.2. Reagents and materials

Certified reference materials/ standards of the weedicides (listed in table.1) were purchased with minimum purity of 98% from Dr. Ehrenstorfer GmbH (Augsburg, Germany). Residue analysis grade (dried) ethyl acetate, anhydrous sodium sulfate, methanol, sodium acetate and diethylene glycol and acetic acid (all of AR Grade) were purchased form Sigma-Aldrich Chemicals Private Limited (Bangalore, India). Primary secondary amine (PSA, 40µm, Bondesil) bought from Agilent Technologies (California, USA). Before use of anhydrous Sodium sulfate, it was activated at 450ºC for 6 hours and kept in desiccator [7].

2.3. Preparation of Standard Solutions

The stock solutions of the individual weedicide standard were prepared by weighing accurately 10 (±0.1) mg of each CRM in volumetric flasks (certified 'A' class) and dissolving in 10 ml of Methanol. These were stored in dark vials at -20 ºC and brought at room temperature before use. A working standard mixture of 5 mg L-1 was prepared by appropriate dilution of the stock solution, from which the calibration standards (10, 25, 50,100 and 200 µg L-1) were prepared by serial dilution with Methanol [8] [9].

2.4. Sample preparation method

Accurately10 (±0.1)g of finely crushed and homogenized grape sample was taken in a 50 ml PP centrifuge tube, 10 (±0.1)ml ethyl acetate was added, further 10 (±0.1)g of anhydrous sodium sulfate and 1.5 gm sodium acetate was added and vortexed for 2 min [10].

The solution was centrifuged at 4000 rpm at 20 ºC. 5ml of aliquot was taken in a 15 ml polypropylene tube containing 25mg. 2ml aliquot was taken in a test tube which already contain 200µl of 10% DEG Solution (in methanol). Extract was dried with the help of low volume concentrator, under a gentle stream of nitrogen gas at 35 ºC until dryness. Finally the residue in test tube was reconstituted with 1ml methanol and later with 1ml of 0.1% acetic acid solution in water. Test tube containing dissolved residue was exposed to ultrasonic bath for 15-20 seconds and then vortexed for 30 seconds, followed by centrifuge at 10000 rpm. Further the reconstituted solution was filtered through 0.22µm nylon filter and filtrate was collected in an auto sampler vial for LC-MS/MS analysis [10] [11].

2.5. Method Vallidation

The single laboratory method was selected for the validation of analytical method [12] and SANTE/11312/2021 was the guideline followed for the same. The quantification was done with the help of five point matrix match calibration curve, which was drawn against the area of the daughter ion of the individual target compounds against concentrations of the calibration standards**.** The limits of quantification (LOQs) were determined by considering a signal to noise ratio of 10 [13].

2.5.1. Linearity

Five level matrix match linearity of 10, 25, 50,100 and 200 μgL^{-1} was prepared from the working standard of $5mgL^{-1}$. Blank matrix extract was used as diluent for each level. The variation of back calculated concentrations of the related region should be within ±20%. The linearity started from the LOQ level [14].

2.5.2. Evaluation of Matrix effect

Mean responses of 5 replicates of solvent and 5 replicates of matrix match standards having concentration 10μgL^{-1} were compared to understand the matrix effect (ME).

$$\text{ME \%} = \frac{\text{Peak area of matrix matched standard}}{\text{Peak area of Solvent standard}} \times 100 \quad \text{..... [15]}$$

Values of ME % smaller than 90 signify matrix induced signal suppressions, whereas values above 110 signifies improvement in the signal. The matrix effect for different parts of the fruit was also evaluated in a similar approach. [15].

2.5.3. LOQ

The theoretical LOQs were observed different for each compound, so it was better to bring LOQ at a common level which would be ≥RL or MRL. There for the limit of quantification was set to the lowest spike level meeting the requirements for identification and method performance criteria for recovery and precision [SANTE/11312/2021] and that was 10 μgL^{-1}.

$$\text{LOQ} = 10 \times \frac{\text{Signal}}{\text{Noise}} \quad \text{..... [11]}$$

2.5.4. Specificity

Responses of analytes were examined in reagent and matrix blank. The blank material should be free from any analyte and if it is not then the spiking value should be ≥3 time the level present or it may be considered that the blank values should not be higher than 30% of the residue level corresponding the reporting limit (RL).

2.5.5. Recovery

Sample was spiked with two different concentrations that is 10 and 100μgL^{-1}. Five replicates of each concentration were spiked and injected separately to the LC-MS/MS. Further the percent recovery was calculated by formula

$$\text{\% Recovery} = \frac{\text{Observed concentration}}{\text{Spiked concentration}} \times 100 \quad \text{……[17]}$$

The present recoveries should lie between 70 to 120% but in exceptional cases, mean recovery outside the range of 70-120% can be accepted if they are consistent (RSD ≤20%) the basis for this is well established, but the mean recovery must not be lower than 30% or above 140%.

2.5.6. Precision (RSD_R)

Relative standard deviation of six spiked replicates was calculated.

2.5.7. Precision/ Robustness (RSD_{WR})

Within laboratory reproducibility of results were examined by calculating relative standard deviation between recoveries of spiked samples at different time intervals but with the same concentration level.

2.5.8. Robustness

Average recovery and RSD_{WR}, derived from on-going method validation were observed at different time intervals that are extended validation.

Afterward the individual recovery results were compared with the mean recovery results and RSDs taken from the initial validation.

2.5.9. Ion ratio

Ion ratio from spiked sample extracts were monitored against average of matrix match calibration standards from the same sequence.

2.5.10. Retention time

Retention time from spiked sample extracts were monitored against average of matrix match calibration standards.

2.6. Instrumental analysis by LC-MS/MS

A liquid Chromatography (Agilent 1200 series) tandem mass spectrometry (Agilent triple quadruple 6460) was used for the residue analysis of the grape samples. Mass spectrometer with ion source ESI jet stream was operated in positive mode. Continual elucidation of each compound was carried out in positive ionization mode by an ESI source. To begin with Full scan mass spectra were recorded in order to select the most ample mass fragments. The relative intensity for the most abundant m/z was used to evaluate the performance of each ionization mode. The signal intensities obtained in the positive mode were high. Full-scan daughter mass spectra were obtained with continuous infusion of each analyte in product–ion scan mode. The most abundant product ion for each compound chosen for quantification and the fragment with next relative abundance for confirmation, the values of the voltages applied to the ion source (ESI), collision cell and quadruples were optimized in the MRM mode by continuous exclusion in order to achieve the highest sensitivity as possible[18]. The optimization of the nebulizing gas, auxiliary gas and curtain gas pressure further improved the sensitivity.

The HPLC separation was performed by injecting 5 µL via auto-sampler on a Zorbax Eclipse C-18 (50mm×4.6mm×5µm) column (Agilent Technologies), maintained at ambient temperature and the flow rate of 0.8 mL/min. The mobile phase was composed of Phase A= 5mM ammonium formate along 0.1% formic acid in water and Phase B= 5mM ammonium formate with 0.1% formic acid in water; gradient 0–1.0 min/80% A, 1–7 min 80%–50%A, 7–12 min 50-20%A, 12–15 min 20–0%A, 15–18 min 0-0%A, 18.1-20min 80-80% A.

The detector was configured with Agilent Jet Stream- Electro Spray Ionization (AJS-ESI) source. The MS parameters included capillary voltage of 3500V; nebulizer gas 55 psi; gas flow 8L/min; gas temperature 250 ºC; sheath gas flow 10 L/min; sheath gas heater 350 ºC. The mass transitions and their parameters of MS/MS analysis are presented in Table 1.

Table 1 MRM Transitions for LC-MS/MS

SN	Compound Name	RT	RT window	Precurser Ion	Fragmen - tor (V)	Product Ion-1	CE (V)	Product Ion-2	CE (V)
1	Metamitron	6.530	0.1	203.1	100	175.1	12	145	12
2	Imazethapyr	9.452	0.1	290.2	60	177	28	86.1	28
3	Metribuzin	10.110	0.1	215	90	187.1	13	84	20
4	Triasulfuron	10.121	0.1	6.62	257	220.9	8	109	16
5	Simazine	10.217	0.1	202.2	121	132	17	68	17
6	Hexazinone	10.392	0.1	253.3	80	171.1	15	71.1	30
7	Sulfentrazone	10.414	0.1	387.1	170	308.1	17	146	49
8	Triafemone	10.702	0.1	407	110	245.1	15	160	30
9	Tri-allate	10.725	0.1	304	100	141.1	20	85.9	20
10	Diclosulam	10.741	0.1	405.9	120	377.9	12	160.9	15
11	Penoxulam	10.753	0.1	484	130	195	25	164.1	25
12	Methabenzthiazuron	11.192	0.1	222.1	100	165	24	149.1	24
13	Pyroxasulfone	11.415	0.1	392.1	135	229	15	178.9	15
14	Atrazine	11.483	0.1	216	85	174	13	1039	33
15	Isoproturon	11.751	0.1	207.6	25	165.5	6	72.2	6
16	Diuron	12.112	0.1	233	102	72.1	16	46.1	16
17	Azimsulfuron	12.348	0.1	425.2	60	182	10	156	35
18	Orthosulfamuron	12.601	0.1	425.1	110	199.1	5	120	41
19	Ametryn	12.782	0.1	228.1	120	186.1	20	96.1	25

20	Iodosulfuron methyl sodium	12.796	0.1	508	135	167.1	2	141	5
21	Clomazone	12.802	0.1	240.1	120	125	20	89	30
22	Linuron	13.083	0.1	249	120	182	15	160	20
23	Propanil	13.372	0.1	218	106	161.9	12	127	24
24	Fluazifop P	13.425	0.1	328.2	100	282.2	15	253.9	15
25	Bensulfuron Methyl	13.667	0.1	411.1	27	182	20	149	22
26	Fluthiacet methyl	13.708	0.1	404.1	100	344	20	274	30
27	Halosulfuron Methyl	13.909	0.1	434.9	100	182	10	83.4	32
28	Flufenacet	14.072	0.1	364	100	152	4	124	4
29	Pyrazosulfuron ethyl	14.182	0.1	415	60	182	20	82.9	45
30	Indaziflam	14.236	0.1	302.1	135	158	35	145	30
31	Metalachlor	15.002	0.1	284	92	252.1	8	91	64
32	Pinoxaden	15.079	0.1	401.2	120	317.1	15	289	42
33	Anilofos	15.136	0.1	368	100	199	5	171	20
34	Thiobencarb	15.147	0.1	258.3	119	125.1	13	89.1	61
35	Butachlor	15.374	0.1	312.2	100	238.1	5	147.1	28
36	Fluazifop butyl	15.397	0.1	384.1	38	328.1	15	282.1	22
37	Metamifop	15.621	0.1	441.1	100	288	15	123	30
38	Pretilachlor	15.722	0.1	312.2	100	252	15	176	5
39	Oxadiazon	16.002	0.1	345	141	303	8	85	80
40	Cinmethylene	16.098	0.1	275.1	90	105	5	77.3	44

Where, RT: Retention time, CE: Collission energy

Residues were estimated by dynamic multiple reaction monitoring (DMRM) with two mass transitions for each weedicide molecule with cell acceleration voltage 7V; one with higher response is for quantification and another for confirmation. The ion ratio for these two mass transitions was used for definite identification of each pesticide as per the European Commission (EC) guidelines [19].

3. Results and discussion

3.1. Selection of weedicides and method performance

Total 62 CIB registered weedicides were selected for this experiment, from which 22 weedicides (including glyphosate, paraquat dichloride and 2,4-D etc.) were infirm for the analysis with same method because of the limitations about their solubility, volatility, ionization problem, stability, etc. MS parameters for all 40 compounds were optimized on LC–MS/MS and then their performances and responses were tested in different scan modes. For the 40 weedicides belonging to different chemical classes, LC–MS/MS multiple reaction monitoring (MRM) gave excellent responses in terms of peak shape, linearity, sensitivity, etc. The detail MS/MS parameters are presented in Table 1.

All the 40 optimized molecules could be analyzed by single chromatographic run of 20 min (Fig. 1). Pesticides could be detectable at 5 μgL^{-1} or even at lower level. The LOQ for the test pesticides are presented in Table 3.

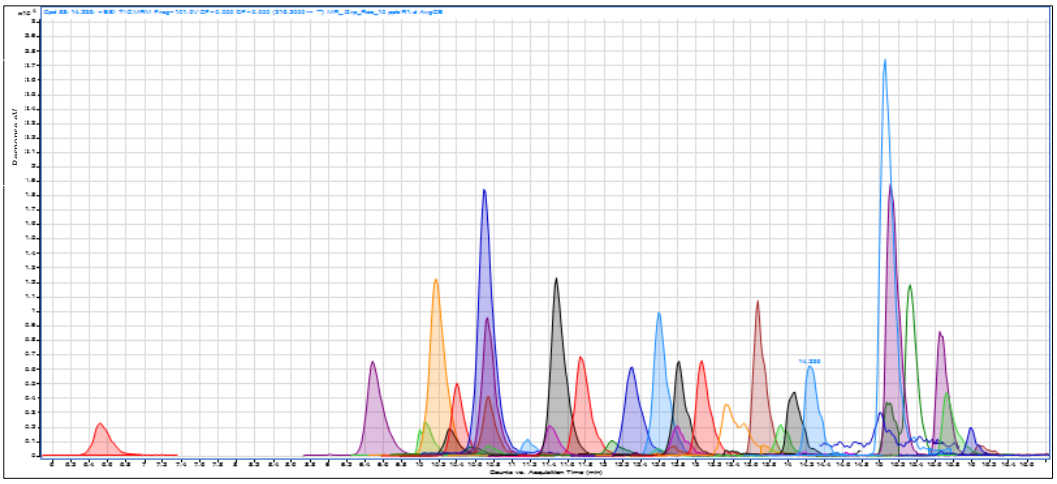

Figure 1 Total Ion Chromatogram of MRM detections of compounds

3.2. Method validation

The analytical method was validated as per the SANTE/11312/2021 guideline. The performance of the method was evaluated considering different validation parameters that include the following points.

3.2.1. Linearity

Linearity of the calibration curve was established for all the weedicides. The correlation coefficient (*R*2) of the calibration curve drawn for matrix-matched were >0.99 for all the compounds (Table 2). The calibration curve for all the compounds were obtained by plotting the graph for peak area against respective concentration and the standards, at five different levels 10, 25, 50, 100 and 200 μgL^{-1}. The concentrations of each point were back calculated and they were observed between ± 20 % of actual spiked concentration.

$$X = Y- C/M$$

Where; X is unknown Concentration,
Y= Peak Area,
C= Intercept,
M = Slope

Table 2 Linearity of compounds with back calculated concentrations

Sr. No.	Name	Back calculated Concentrations in μgL^{-1}					
		10	**25**	**50**	**100**	**200**	**R^2**
1	Metamitron	8.72	27.14	49.49	99.46	200.19	0.9997
2	Imazethapyr	8.92	23.35	49.76	105.31	197.67	0.9984
3	Metribuzin	8.82	24.27	50.81	102.31	198.79	0.9996
4	Triasulfuron	11.82	28.10	48.28	93.69	203.10	0.9972
5	Simazine	9.43	23.89	49.65	103.57	198.47	0.9993
6	Hexazinone	8.07	25.48	48.78	104.67	198.01	0.9987
7	Triafemone	10.14	24.30	49.76	101.33	199.48	0.9999
8	Sulfentrazone 1	8.46	23.69	47.53	108.93	196.39	0.9956
9	Tri-allate	9.50	22.68	50.63	104.07	198.12	0.9989
10	Penoxulam	9.62	24.46	48.72	103.60	198.61	0.9993
11	Diclosulam	9.80	25.97	48.16	101.44	199.63	0.9997

12	Methabenzthiazuron	10.14	23.97	49.26	102.63	198.99	0.9996
13	Pyroxasulfone	8.53	23.26	49.56	106.49	197.16	0.9976
14	Atrazine	9.36	24.00	49.60	103.58	198.47	0.9993
15	Azimsulfuron	9.18	24.42	48.86	104.29	198.25	0.9990
16	Isoproturon	10.26	24.09	48.66	103.11	198.88	0.9994
17	Diuron	8.83	23.55	50.35	104.24	198.03	0.9989
18	Orthosulfamuron	9.01	23.49	49.72	104.94	197.84	0.9986
19	Ametryn	10.54	24.26	49.36	101.25	199.60	0.9999
20	Iodosulfuron methyl sodium	11.35	25.10	47.65	100.79	200.11	0.9997
21	Bensulfuron Methyl	11.87	26.88	49.20	94.36	202.69	0.9980
22	Clomazone	10.20	24.42	49.50	101.38	199.50	0.9999
23	Linuron	8.40	23.21	50.13	105.98	197.28	0.9979
24	Fluthiacet methyl	8.54	23.78	48.85	106.64	197.19	0.9976
25	Propanil	8.11	22.96	51.16	105.41	197.35	0.9981
26	Fluazifop P	11.82	27.15	48.27	95.36	202.39	0.9984
27	Halosulfuron Methyl	8.84	23.22	49.55	105.99	197.40	0.9980
28	Pyrazosulfuron ethyl	9.98	25.91	46.94	103.03	199.14	0.9991
29	Flufenacet	8.40	23.93	50.58	104.02	198.05	0.9990
30	Indaziflam	8.78	23.48	50.22	104.67	197.86	0.9987
31	Metalachlor	8.11	23.60	51.11	104.37	197.81	0.9987
32	Anilofos	8.58	23.39	50.98	104.03	198.01	0.9989
33	Pinoxaden	9.25	22.22	49.77	106.64	197.12	0.9974
34	Thiobencarb	9.92	20.04	49.98	108.87	196.19	0.9950
35	Pretilachlor	8.32	22.88	51.08	105.26	197.45	0.9982
36	Metamifop	11.85	28.68	48.56	92.20	203.71	0.9960
37	Fluazifop butyl	8.54	24.22	50.31	103.68	198.25	0.9992
38	Oxadiazon	8.08	24.23	50.85	103.70	198.13	0.9991
39	Butachlor	8.91	23.35	49.91	105.09	197.74	0.9985
40	Cinmethylene	8.58	21.72	49.12	109.75	195.83	0.9947

Where, R^2 is correlation coefficient

3.2.2. Matrix effect

Whereas Most of the weedicides showed noticeable matrix effect (Table 3). Since the variable matrix influences for different compounds in mixture, the matrix-matched calibrations were used for the quantification purposes to elude any over or under-estimation of residues. Since only under estimation of the signal was observed related to the selected molecules.

3.2.3. Limit of quantitation

The limits of quantification (LOQ) were determined by considering a signal-to-noise ratio of 10 (Table 3). As the calculated LOQs were observed with different concentrations, practically they could not show reproducible recoveries. So we have decided to bring them to 10 μgL^{-1} as a common reporting level (RL) ≤ MRL and essentially gave considerable reproducibility.

Table 3 Matrix effect, LOQ, Recovery and Ion ratio of different molecules

Sr. No.	Name	% ME	LOQ	Recovery % (mean ± RSD_R)		Recovery % (mean ± RSD_{WR})	Ion ratio T1		Ion ratio T2	
				Recovery-I	Recovery-II		Avg	RSD	Avg	RSD
1	Metamitron	73.36	2.28	94.39 ± 8.41	116.74±6.86	93.34±9.72	68.5±6.5		11.2±11	
2	Imazethapyr	67.85	2.45	97.81±7.57	93.47±8.93	96.09±7.14	6.8±8.1		4.6±11.1	
3	Metribuzin	66.79	3.45	98.14±10.37	101.14±9.09	96.34±10.95	92.6±3.7		56.9±6.8	
4	Triasulfuron	79.28	2.14	88.45±13.52	107.37±11.45	88.04±11.69	67±12.7		23.1±13.3	
5	Simazine	67.70	2.17	96.15±8.69	95.31±8.32	96.03±9.95	72.8±4.6		52.9±5.6	
6	Hexazinone	69.03	4.24	95.32±10.39	95.07±7.12	93.34±9.59	99±5.7		7.2±4.7	
7	Triafemone	69.05	3.22	96.36±8.58	94.08±7.53	95.51±9.21	9.7±10.1		5.2±18.3	
8	Sulfentrazone 1	66.97	8.40	96.72 ± 13.30	109.06± 10.41	95.32±12.01	87.7 ±3		14.1±1.7	
9	Tri-allate	93.26	4.81	52.39±13.29	62.93±12.25	51.32±13.21	60.2±4.1		48.6±6.2	
10	Penoxulam	69.32	7.20	99.16±6.37	83.02±8.85	99.22±6.52	25.9±7.2		25.9±7.8	
11	Diclosulam	62.96	6.57	103.94±9.14	99.89±11.09	102.18±10.32	21.6±5.6		13.4±6.8	
12	Methabenzthiazuron	71.35	6.40	93.54±10.51	91.48±8.13	96.02±9.66	27.6±5.6		97.6±3.6	
13	Pyroxasulfone	80.93	4.50	93.76±10.74	97.68±8.55	91.90±11.91	90.6±4.2		37.3±5.8	
14	Atrazine	70.82	1.58	97.09±9.50	101.01±8.82	96.53±8.88	99.8±3.5		7.6±17.1	
15	Azimsulfuron	68.87	3.02	93.71±12.33	97.09±9.97	93.41±10.85	62.8±6.2		62.8±8.5	
16	Isoproturon	70.34	1.10	91.98±9.92	90.28±7.63	92.38±9.71	93±4		41.1±4.1	
17	Diuron	73.20	4.01	98.70±8.78	96.30±6.92	97.75±8.83	60.8±2.4		17.4±2	
18	Orthosulfamuron	59.94	1.67	91.63±14.49	102.63±11.27	91.47±12.70	86.4±3.7		46.5±6.3	
19	Ametryn	69.14	7.60	93.72±9.96	101.03±8.01	92.27±10.11	42.9±3.7		23.6±5.7	
20	Iodosulfuron methyl sodium	58.92	2.32	106.08±8.78	109.57±7.90	101.72±10.04	86.9±11.3		30.9±18.9	
21	Bensulfuron Methyl	66.69	2.30	90.81±10.02	95.56±8.90	93.36 ±9.91	61.6±1.7		13.2±4.2	
22	Clomazone	67.83	3.58	99.06±8.26	100.44±11.84	96.67±8.44	99.2±3.2		52.6±7.4	
23	Linuron	68.73	1.91	92.17±11.85	95.31±7.69	91.49±11.01	25.2±19.8		20.1±17.9	

24	Fluthiacet methyl	65.09	7.20	88.60±13.63	90.14±9.25	89.71±12.26	17.4±9.8	21±16.4
25	Propanil	64.43	1.34	96.61±9.21	104.56±8.14	93.34±11.48	63.17±3.7	15.3±9.8
26	Fluazifop P	70.66	6.77	85.71±10.02	97.74±7.96	89.74±10.08	35.5±5.2	27.1±11.2
27	Halosulfuron Methyl	66.94	3.70	91.60±10.10	90.55±7.99	94.15±9.15	27.5±12.2	23.4±15
28	Pyrazosulfuron ethyl	68.15	1.60	92.97±9.14	95.86±8.76	94.79±9.38	52±10.4	33±3.2
29	Flufenacet	68.68	6.94	98.43±9.02	93.51±7.92	97.34±8.20	30.7±9.7	19.6±7
30	Indaziflam	68.53	7.62	94.10±10.14	106.19±12.27	94.58±11.62	52.8±2.6	15.4±6.5
31	Metalachlor	68.36	2.45	99.86±11.77	102.43±10.12	98.52±10.32	25.4±7.6	4.9±16.7
32	Anilofos	70.40	7.23	97.52±10.18	103.61±9.73	95.75±11.23	17.7±8.4	15.54±7.1
33	Pinoxaden	71.79	3.11	87.13±12.24	98.64±9.64	90.86±11.76	23±6.9	17.9±18.6
34	Thiobencarb	70.09	1.25	91.47±11.27	94.38±7.46	93.48 ±10.64	44.2±12.8	24.1±7.9
35	Pretilachlor	67.20	5.27	95.91±9.06	98.01±8.78	97.48±8.81	94.9±2.8	6.9±10.1
36	Metamifop	68.52	5.33	90.74±9.96	103.18±11.35	91.61±9.25	65.2±10.2	60.8±6.6
37	Fluazifop butyl	66.92	1.91	102.27±10.39	100.20±8.80	101.35±10.61	58.5±4.4	51.9±4.5
38	Oxadiazon	65.08	4.43	95.57±14.23	102.64±11.32	90.64±13.68	31.8±3.4	29.6±6.4
39	Butachlor	69.95	1.46	95.34±9.82	94.25±9.53	93.75±10.98	64.20±11.70	33.1±7.99
40	Cinmethylene	64.63	3.24	90.81±10.66	96.62±8.14	92.72±12.30	55.2±10.1	38.1±13.7

Where, ME : matrix effect, LOQ : Limit of Quantitaiton, RSDR : Relative Standard Deviation for precision, RSDWR: Relative Standard Deviation for within laboratory reproducibilty

3.2.4. Specificity

Both Reagent blank and control blank complying the criterion for specificity by showing no any target peak throughout the run.

3.2.5. Recovery

As far as the matrix effect is considered, the spiked samples for recovery studies were evaluated against the linearity of five point matrix match standards. It was seen that all of the molecules are showing recoveries (Table 3) in-between 70 to 120% except 'Triallet', whose mean recovery was revealed 52% at 10µgL^{-1} and 63% at 100µgL^{-1}, still it's RSD was observed ±13.29 and ±12.25 respectively. After all these recoveries could be acceptable according to the guideline but the recovery corrections would be applicable for the real time or commercial samples.

3.2.6. Precision (RSD_R)

All the compounds represented % recoveries with acceptable values. We also examined the precision (RSD_R) in six replicates of spiked samples at 10µgL^{-1} and 100µgL^{-1}, those were also observed below 20% for every analyte. The overall precision in terms of the relative standard deviations was satisfactory (Table 3).

3.2.7. Precision/ Reproducibility (RSD_{WR})

The experiment of within laboratory reproducibility was resulted percent recoveries with RSDs ≤ 20% two sets of recovery studies were carried out at 10µgL-1 with six replicates at day-1 and six at day-2. The extent of within laboratory reproducibility (RSD_{WR}) was quite agreeable with relative standard deviation of values of two sets of six replicates was less than 20% for every compound analyzed in over 2 different time intervals (Table 3).

3.2.8. Robustness

The method was executed for the study of reproducibility and found recovery of each individual replicate of day-2 was amid ±2X RSD of mean recovery at day-1 (Table 4).

Table 4 % Recoveries with RSD for robustness study

SN.	Compound Name	% Reovery at spike level 10 µgL-1									
		Day 1		± 2 x RSD		day-2					
		Avg	RSD	Lower Limit	Upper Limit	R1	R2	R3	R4	R5	R6
1	Metamitron	94.39	8.41	77.57	111.20	100.50	105.99	78.10	97.51	82.00	89.63
2	Imazethapyr	97.81	7.57	82.66	112.95	98.21	102.91	91.35	91.97	96.97	84.83
3	Metribuzin	98.14	10.37	77.40	118.89	97.66	105.89	81.51	108.43	81.57	92.19
4	Triasulfuron	88.45	13.52	61.42	115.48	89.55	96.52	89.59	96.56	78.95	74.56
5	Simazine	96.15	8.69	78.77	113.52	110.52	107.16	96.15	87.17	79.85	94.56
6	Hexazinone	95.32	10.39	74.54	116.11	103.96	89.56	80.94	86.05	96.52	91.17
7	Triafemone	96.36	8.58	79.19	113.52	88.47	107.52	92.47	104.82	94.06	80.62
8	Sulfentrazone 1	96.72	13.30	70.13	123.32	84.23	102.59	94.75	105.20	98.36	78.40
9	Tri-allate	52.39	13.29	25.82	78.96	49.60	50.25	51.65	45.92	62.53	41.56
10	Penoxulam	99.16	6.37	86.43	111.90	103.53	103.63	88.63	108.56	96.20	95.16
11	Diclosulam	103.94	9.14	85.65	122.23	106.30	95.16	87.27	110.93	115.65	87.15
12	Methabenzthiazuron	93.54	10.51	72.52	114.57	109.60	99.81	106.52	96.52	86.52	92.00
13	Pyroxasulfone	93.76	10.74	72.28	115.24	101.53	93.27	80.56	107.15	76.23	81.56
14	Atrazine	97.09	9.50	78.09	116.10	106.22	95.26	96.15	85.62	105.07	87.52

Sr. *No.	Compound Name	% Reovery at spike level 10 µgL-1									
		Day 1		± 2 x RSD		day-2					
		RSD		Lower Limit	Upper Limit	R1	R2	R3	R4	R5	R6
15	Azimsulfuron	93.71	12.33	69.05	118.36	98.04	80.25	87.65	106.75	89.51	96.52
16	Isoproturon	91.98	9.92	72.14	111.82	89.60	80.56	91.52	103.26	86.52	105.17
17	Diuron	98.70	8.78	81.14	116.26	105.06	89.56	83.56	104.13	93.27	105.21
18	Orthosulfamuron	91.63	14.49	62.64	120.62	98.25	73.26	83.43	98.16	96.21	98.55
19	Ametryn	93.72	9.96	73.81	113.63	83.77	89.28	95.24	107.00	78.05	91.56
20	Iodosulfuron methyl sodium	106.08	8.78	88.51	123.64	92.65	89.20	99.27	116.07	89.60	97.45
21	Bensulfuron Methyl	90.81	10.02	70.77	110.86	99.27	109.17	88.80	81.64	97.83	98.72
22	Clomazone	99.06	8.26	82.53	115.58	105.89	86.53	95.17	83.55	97.26	97.30
23	Linuron	92.17	11.85	68.47	115.86	83.77	89.28	95.24	107.00	78.05	91.56
24	Fluthiacet methyl	88.60	13.63	61.35	115.85	75.29	87.18	94.93	107.25	93.27	86.96
25	Propanil	96.61	9.21	78.19	115.04	84.65	78.90	87.91	112.13	96.25	80.57
26	Flufenacet	98.43	9.02	80.39	116.47	101.83	102.97	93.70	83.02	100.63	95.40
27	Fluazifop P	85.71	10.02	65.67	105.74	95.36	99.56	91.37	103.21	93.96	79.18
28	Halosulfuron Methyl	91.60	10.10	71.40	111.81	94.97	88.42	100.21	109.23	89.21	98.21
29	Pyrazosulfuron ethyl	92.97	9.14	74.69	111.26	98.54	101.68	110.19	95.60	80.96	92.71
30	Indaziflam	94.10	10.14	73.81	114.39	79.24	103.39	79.63	113.26	98.05	96.86
31	Metalachlor	99.86	11.77	76.31	123.41	87.01	99.60	86.65	106.35	106.41	97.07
32	Anilofos	97.52	10.18	77.15	117.89	102.65	109.82	80.65	95.61	78.39	96.77
33	Pinoxaden	87.13	12.24	62.66	111.61	86.91	102.65	95.29	109.41	81.68	91.54
34	Thiobencarb	91.47	11.27	68.93	114.01	106.51	89.23	80.93	104.67	100.55	91.10
35	Pretilachlor	95.91	9.06	77.79	114.02	105.06	108.06	91.56	107.43	95.15	87.09
36	Metamifop	90.74	9.96	70.82	110.66	103.02	99.03	90.53	85.66	96.21	80.51
37	Fluazifop butyl	102.27	10.39	81.49	123.05	115.42	107.91	92.33	106.80	83.03	97.08
38	Oxadiazon	95.57	14.23	67.10	124.04	78.15	101.26	91.71	85.89	76.83	80.40
39	Butachlor	95.34	9.82	75.69	114.99	78.40	81.56	86.13	107.16	103.96	95.78
40	Cinmethylene	90.81	10.66	69.50	112.12	111.60	106.96	78.32	95.44	78.95	96.55

Where, Avg : Average, R1, R2,Rn: Replicates

3.2.9. Ion ratio

Ion ratio for each compound was seen specific with ≤20% RSD (Table 3). Each of the target compound showing ion ratio within ±30% with respect to the calibration standards, which is satisfying the requirements of SANTE/11312/2021.

3.2.10. Retention time (RT)

It was observed that the RT of the every single compound was differing with ≤0.1 minute, confirming the analyte occurrence according to SANTE/11312/2021 (Table 1).

4. Conclusion

Observed results from the validation of test parameters are within acceptable criteria specified in SANTE/11312/2021 guideline. So it is concluded that this method is fit for the purpose of residues analysis of enlisted 40 weedicide molecules in grapes.

Compliance with ethical standards

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Disclosure of conflict of interest

We hereby declare that the disclosed research article is correct and there is no other situation of real, potential or apparent conflict of interest.

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