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Trace Level estimation of Glyphosate and Glufosinate in Red Chilli Pepper using FMOC-derivatization

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Introduction

Abstract

Glyphosate and Glufosinate are common herbicides used among crops for weed control but have adverse health effects oh human. Derivatization with FMOC-Cl is useful to overcome the analytical challenges such as poor chromatography, poor ionization and sample preparation issues. Red Chili is considered among difficult plant-based matrix for pesticide analysis.

Introduction

The tolerance limits are up to 0.1ppm as per FDA[1], up to 0.03 mg/kg as per EU-MRL[2] and up to 0.01mg/kg as per FSSAI[3]. Current methodology targets Glyphosate and Glufosinate at 0.001ppm ie 0.001 mg/kg, in red chili powder and is suitable to guidelines. The data was acquired in positive as well as in negative mode, ensuring robustness. Pre-spike linearity ensured good recovery and wide calibration range indicates suitability of method various food guidelines.

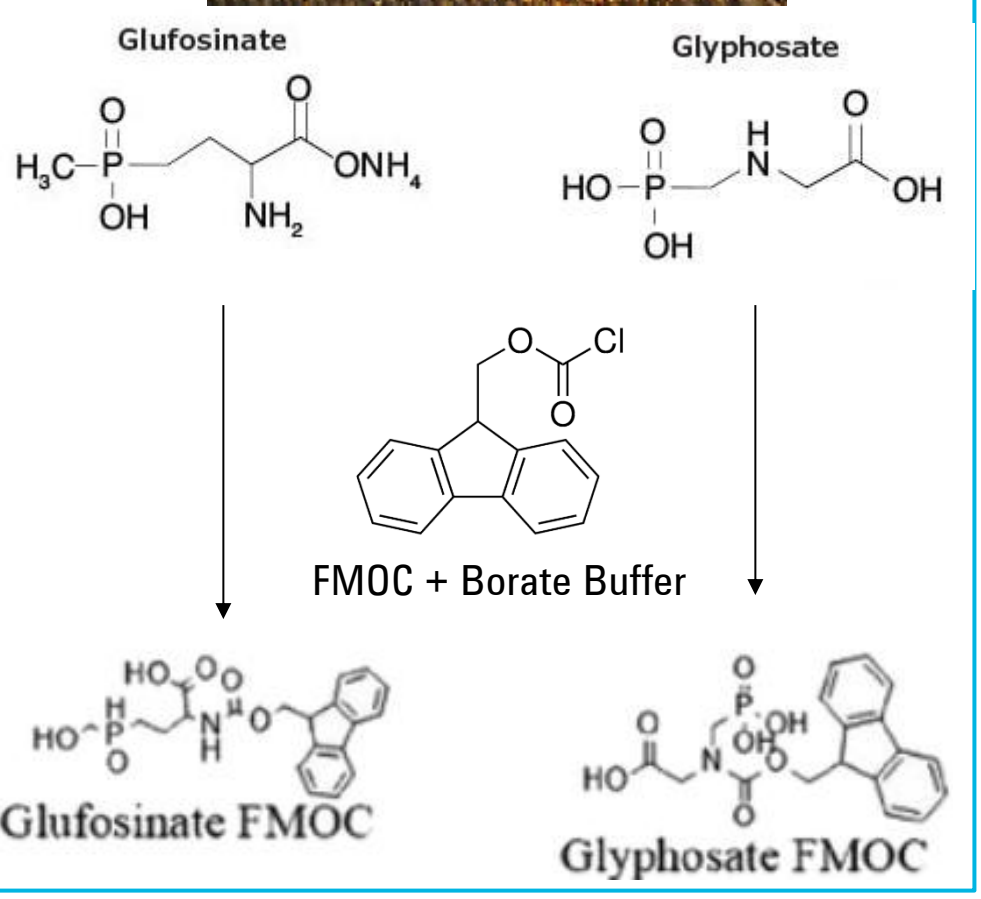


Figure 1: Red Chili Pepper, Derivatization reaction

Experimental

Sample Preparation and LCMS Parameters

Soak 2.5g sample in 10mL 1% Formic acid and extract with 10mL dichloromethane. After centrifugation, add 0.7mL 5% borate buffer and 0.3mL of 40mM FMOC-Cl in 1mL supernatant. Derivatization to be done at 40 °C for 45min in dark shaking water bath. Add 0.05mL formic acid in cooled sample, followed by centrifugation at 5000rpm for 5min. The matrix based (pre-spike) samples were prepared and analyzed using the LC/TQ parameters as seen in table 1, below.

Parameter	Value
Multi sampler	5 °C
Injection Volume	20 µL
Column	40 °C, Agilent P/ N 693975-302
Drying gas	9 L/min at 300 °C
Nebulizer gas	35 psi
Sheath Gas	10 L/min at 300 °C
Capillary Voltage	2700 V (+ve) and 3500 V (-ve)
Nozzle voltage	500 (+/-)
Ionization	ESI-AJS Positive and Negative

Time (min)	Flow Rate	Mob Phase A (5mM Amm Acetate)	Mob Phase B (MeOH)
0.0	0.4 ml/min	99	1
2.0	0.4 ml/min	99	1
7.0	0.4 ml/min	1	99
9.0	0.4 ml/min	1	99
9.1.0	0.4 ml/min	99	1
13.0	0.4 ml/min	99	1

Table 1: LC/TQ method parameters

Figure 2: Agilent 1290 UHPLC, 6475 LC/TQ

Results and Discussion

Data in ESI Negative Mode:

The data obtained for Glufosinate is seen in figure 3 and data for Glyphosate is seen in figure 4. Well distinguished and sensitive peaks are seen for the polar herbicides.

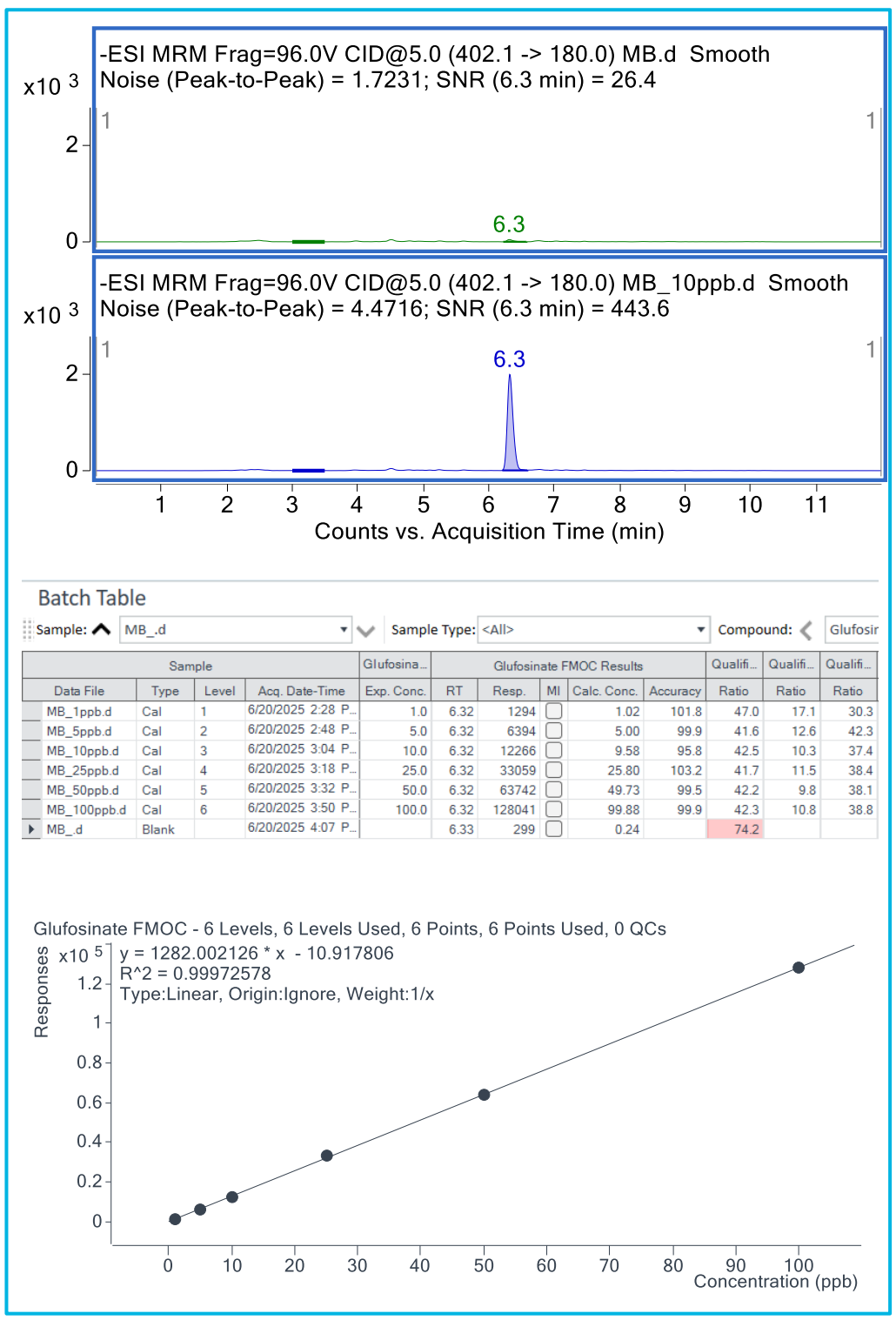
The peak seen in matrix blank (MB) is considerably lower than targeted level of 0.001 mg/kg (MB_10ppb). A linearity plot from 0.001-0.1 mg/kg (1-100 ppb) with r2 value >0.9996 and >0.9997 indicates robust and dynamic method in ESI negative mode for the derivatized analytes.

Further the method complies with the norms as per SANTE/2026, including ion ratio criteria for both the challenging herbicides.

Data ESI Positive Mode:

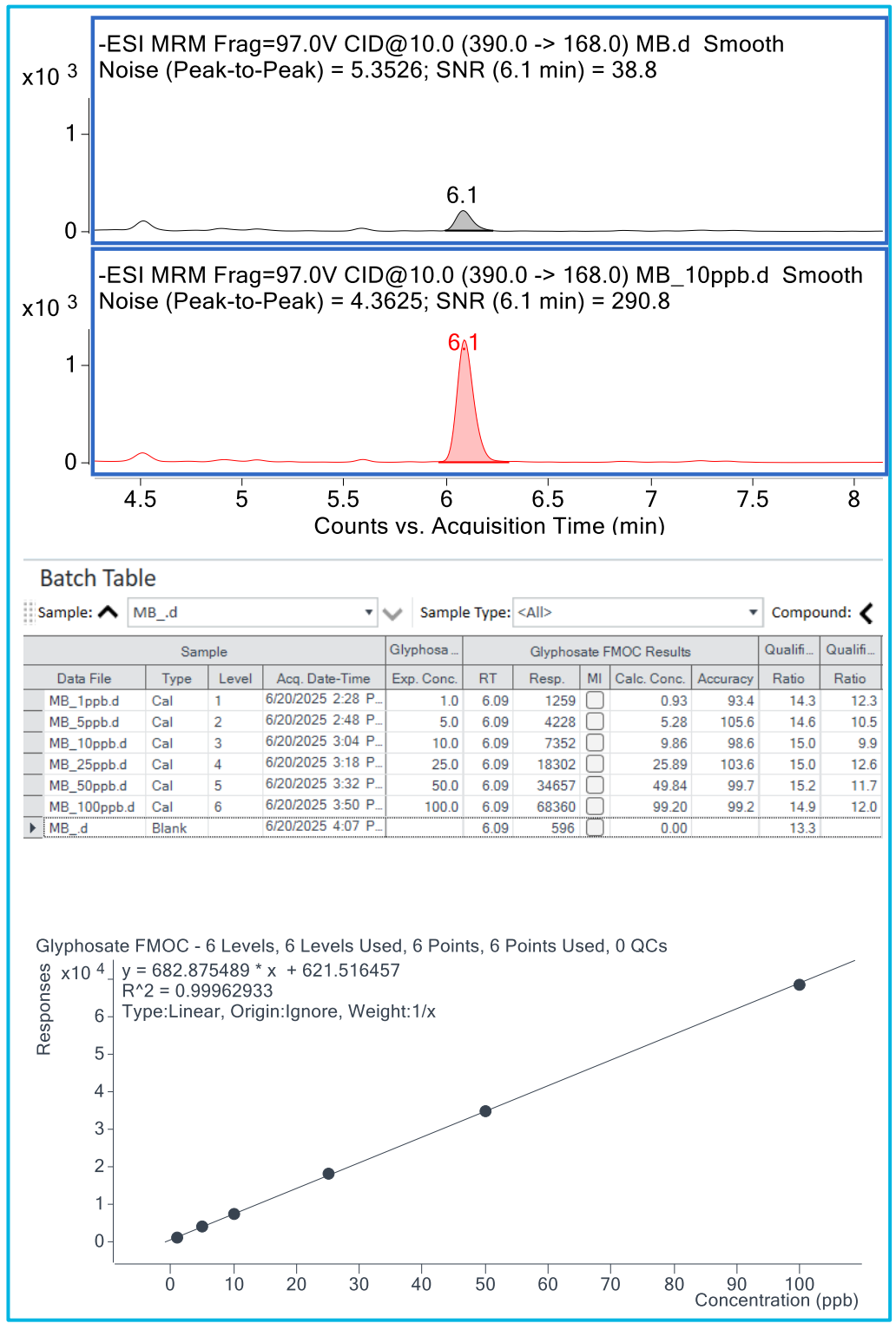
The data obtained for Glufosinate is seen in figure 5 and data for Glyphosate is seen in figure 6. Well distinguished and sensitive peaks are seen for the polar herbicides. Though the baseline has more disturbance in positive, than negative mode, indicating considerable matrix effect. Another observation is that area counts and signal to noise values are better in negative than positive mode.

The peak seen in matrix blank (MB) is considerably lower than targeted LOQ. A wide linearity plot and r2 value above 0.995 for both analytes indicates that method is well suitable to ESI positive based LC/TQ experiments as well.



Sample				Glufosina...	Glufosinate FMOC Results					Qualifi...	Qualifi...	Qualifi...
Data File	Type	Level	Acq. Date-Time	Exp. Conc.	RT	Resp.	MI	Calc. Conc.	Accuracy	Ratio	Ratio	Ratio
MB_1ppb.d	Cal	1	6/20/2025 2:28 P...	1.0	6.32	1294		1.02	101.8	47.0	17.1	30.3
MB_5ppb.d	Cal	2	6/20/2025 2:48 P...	5.0	6.32	6394		5.00	99.9	41.6	12.6	42.3
MB_10ppb.d	Cal	3	6/20/2025 3:04 P...	10.0	6.32	12266		9.58	95.8	42.5	10.3	37.4
MB_25ppb.d	Cal	4	6/20/2025 3:18 P...	25.0	6.32	33059		25.80	103.2	41.7	11.5	38.4
MB_50ppb.d	Cal	5	6/20/2025 3:32 P...	50.0	6.32	63742		49.73	99.5	42.2	9.8	38.1
MB_100ppb.d	Cal	6	6/20/2025 3:50 P...	100.0	6.32	128041		99.88	99.9	42.3	10.8	38.8
MB_.d	Blank		6/20/2025 4:07 P...		6.33	299		0.24		74.2		

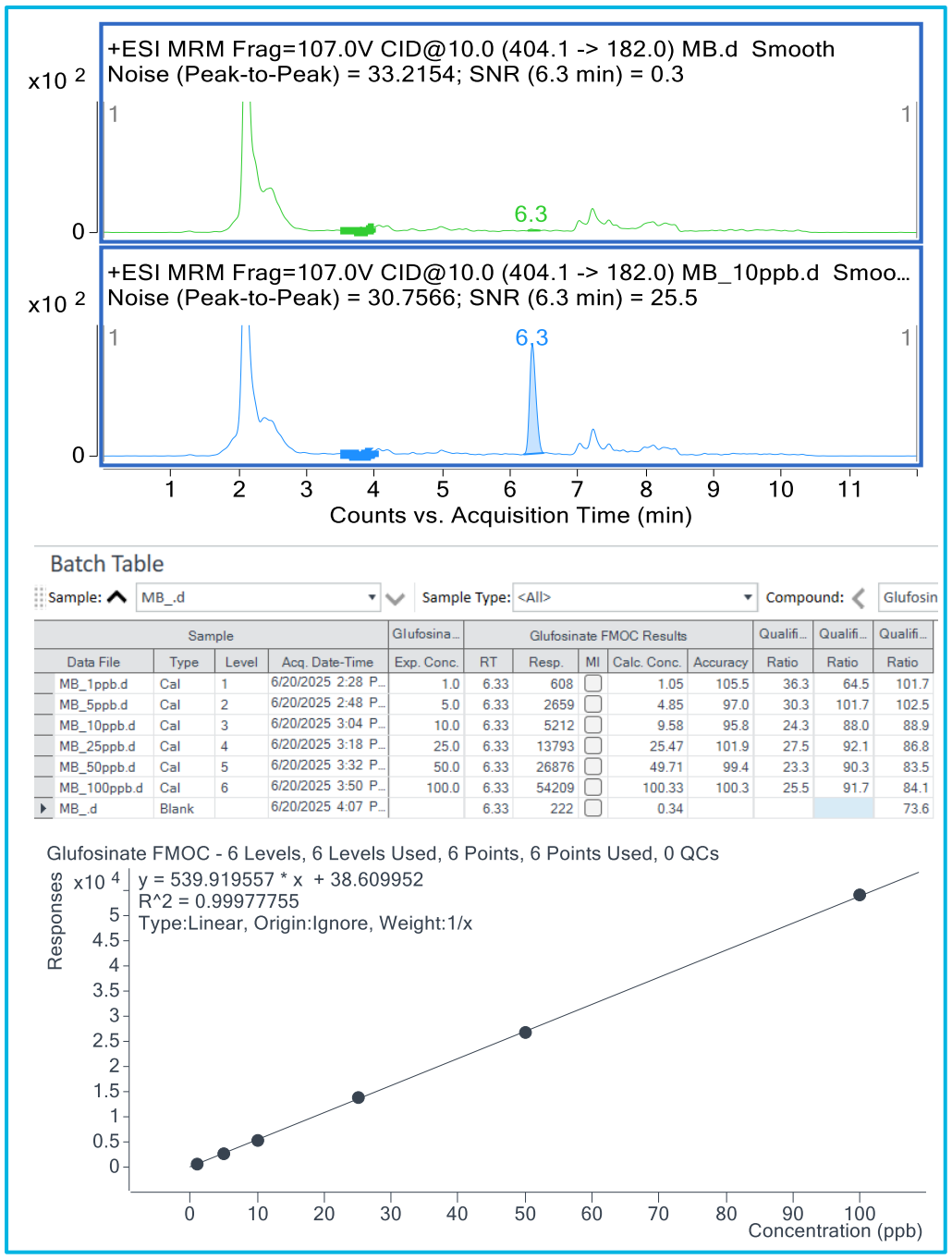
Figure 3: Sensitivity and Calibration having ion ratio, r2 values for Glufosinate in ESI negative mode



Sample				Glyphosa...	Glyphosate FMOC Results					Qualifi...	Qualifi...
Data File	Type	Level	Acq. Date-Time	Exp. Conc.	RT	Resp.	MI	Calc. Conc.	Accuracy	Ratio	Ratio
MB_1ppb.d	Cal	1	6/20/2025 2:28 P...	1.0	6.09	1259		0.93	93.4	14.3	12.3
MB_5ppb.d	Cal	2	6/20/2025 2:48 P...	5.0	6.09	4228		5.28	105.6	14.6	10.5
MB_10ppb.d	Cal	3	6/20/2025 3:04 P...	10.0	6.09	7352		9.86	98.6	15.0	9.9
MB_25ppb.d	Cal	4	6/20/2025 3:18 P...	25.0	6.09	18302		25.89	103.6	15.0	12.6
MB_50ppb.d	Cal	5	6/20/2025 3:32 P...	50.0	6.09	34657		49.84	99.7	15.2	11.7
MB_100ppb.d	Cal	6	6/20/2025 3:50 P...	100.0	6.09	68360		99.20	99.2	14.9	12.0
MB_.d	Blank		6/20/2025 4:07 P...		6.09	596		0.00		13.3	

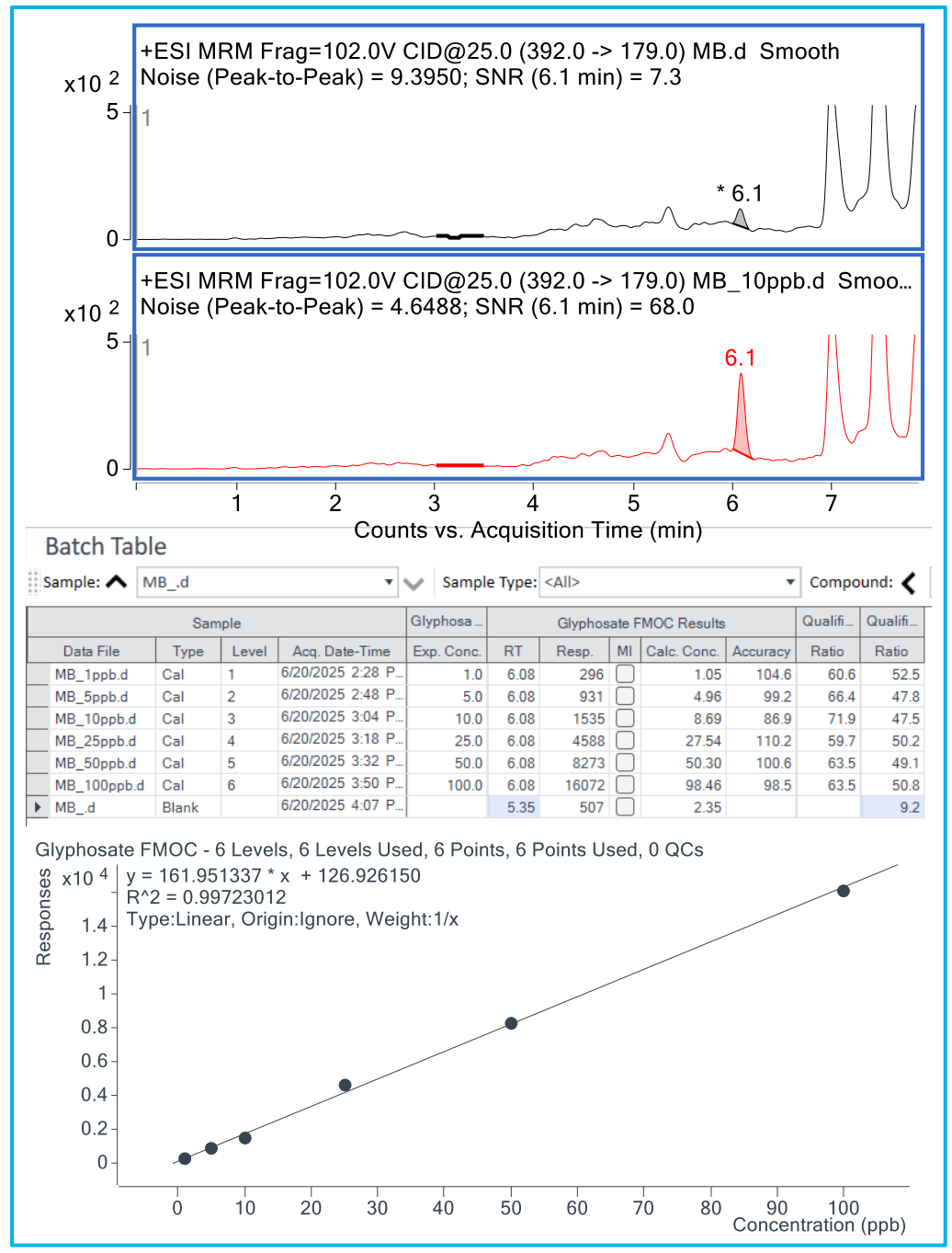
Figure 4: Sensitivity and Calibration having ion ratio, r2 values for Glyphosate in ESI negative mode

Results and Discussion



Sample				Glufosina…	Glufosinate FMOC Results					Qualifi…	Qualifi…	Qualifi…
Data File	Type	Level	Acq. Date-Time	Exp. Conc.	RT	Resp.	MI	Calc. Conc.	Accuracy	Ratio	Ratio	Ratio
MB_1ppb.d	Cal	1	6/20/2025 2:28 P…	1.0	6.33	608		1.05	105.5	36.3	64.5	101.7
MB_5ppb.d	Cal	2	6/20/2025 2:48 P…	5.0	6.33	2659		4.85	97.0	30.3	101.7	102.5
MB_10ppb.d	Cal	3	6/20/2025 3:04 P…	10.0	6.33	5212		9.58	95.8	24.3	88.0	88.9
MB_25ppb.d	Cal	4	6/20/2025 3:18 P…	25.0	6.33	13793		25.47	101.9	27.5	92.1	86.8
MB_50ppb.d	Cal	5	6/20/2025 3:32 P…	50.0	6.33	26876		49.71	99.4	23.3	90.3	83.5
MB_100ppb.d	Cal	6	6/20/2025 3:50 P…	100.0	6.33	54209		100.33	100.3	25.5	91.7	84.1
MB_.d	Blank		6/20/2025 4:07 P…		6.33	222		0.34				73.6

Figure 5: Sensitivity and Calibration having ion ratio, r2 values for Glufosinate in ESI positive mode



Sample				Glyphosa…	Glyphosate FMOC Results					Qualifi…	Qualifi…
Data File	Type	Level	Acq. Date-Time	Exp. Conc.	RT	Resp.	MI	Calc. Conc.	Accuracy	Ratio	Ratio
MB_1ppb.d	Cal	1	6/20/2025 2:28 P…	1.0	6.08	296		1.05	104.6	60.6	52.5
MB_5ppb.d	Cal	2	6/20/2025 2:48 P…	5.0	6.08	931		4.96	99.2	66.4	47.8
MB_10ppb.d	Cal	3	6/20/2025 3:04 P…	10.0	6.08	1535		8.69	86.9	71.9	47.5
MB_25ppb.d	Cal	4	6/20/2025 3:18 P…	25.0	6.08	4588		27.54	110.2	59.7	50.2
MB_50ppb.d	Cal	5	6/20/2025 3:32 P…	50.0	6.08	8273		50.30	100.6	63.5	49.1
MB_100ppb.d	Cal	6	6/20/2025 3:50 P…	100.0	6.08	16072		98.46	98.5	63.5	50.8
MB_.d	Blank		6/20/2025 4:07 P…		5.35	507		2.35			9.2

Figure 6: Sensitivity and Calibration having ion ratio, r2 values for Glyphosate in ESI positive mode

Conclusions

1. This FMOC derivatization methodology meet FDA, EU and FSSAI sensitivity method requirements.
2. The method uses pre-spike sample preparation demonstrating good recovery values, in difficult matrix.
3. The methodology is particularly valuable for commercial laboratories, as if provides flexibility between traditional ESI positive-mode methods and matrix systems better suited for ESI negative-mode analysis.

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