Poster Reprint

ASMS 2022
Poster number MP156

Comparative Study of High-Resolution Q-TOF Fast Polarity Switching versus Single Polarity Data Acquisition on Mass Accuracy, Resolution and Analytical Sensitivity

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Introduction

Laboratories around the world face the challenge of an ever-increasing sample load demanding higher productivity and faster turnaround times, due to COVID-19 related restrictions on laboratory access. The use of Fast Polarity Switching (FPS) in MS only data acquisition mode is one way to increase sample through-put two-fold but at what impact to mass accuracy, mass resolution and analytical sensitivity?

A potential drawback of FPS, is the use of the same LC mobile phase buffers for both positive and negative ion modes of operation. It commonly known that even low concentrations of Formic acid (~0.1-0.2%) in Water, Methanol or Acetonitrile, can cause ion suppression effects in negative ion mode resulting in lower ion signals but higher response in positive ion mode.

To answer the above questions, a complex mixture of pesticides were analyzed using reverse phase (RP) LC separation with common organic buffers in FPS and Single Polarity (SP) modes using several different models of Agilent Q-TOF's. Here, we report the average and compound specific mass accuracy differences observed as a function of concentration and overall analytical sensitivity (10 ppb-1 ppm). The best performance was achieved using the 6546 LC/Q-TOF that acquires LCMS data with both high resolution and wide in-spectra dynamic range.

Figure 1: Agilent 6546 LC/Q-TOF

Mass Spectrometer Parameters

The source parameters were optimized from the single positive and negative ion polarity experiments and then used in the FPS experiments. The maximum mass range was set to *m/z* 3200 and data was collected between *m/z* 80 and 1100. For the positive or negative ion modes, the acquisition rate was varied from 4, 6, 8 and 10 spectra per second. For fast polarity switching mode, the acquisition rate was set at 1.5 spectra/sec using the optimized source parameters for positive and negative ion polarity (Nozzle Voltage).

Experimental

Experimental Samples and Method Preparation

The Agilent LCMS pesticide comprehensive mixture (PN: 5190-0551) that contains standards in eight individual vials at 100 mg/mL concentration. Six of the eight vials (for a total of 214 standards) were used to build a retention time locked database needed to separate the 28 isomeric compounds in the mixture. These standards were then mixed and serially diluted using both Methanol or Water to a final concentration of 10 ppb, 100 ppb and 1 ppm.

LC Separation Conditions

The mixtures of pesticides were analyzed in both FPS and SP MS only data acquisition modes using an Agilent 1290 Infinity II LC system interfaced with the high resolution 6546 LC/Q-TOF with the Dual Jet Stream (AJS) electrospray ionization source.

The reverse phase LC separation for the pesticide standards used an Agilent Poroshell Eclipse-Plus C18 2.1 x 150 mm diameter, 2.7 μm particle size column heated to 45°C and with flow rate of 350 μL/min that resulted in peaks of 6-8 second wide and a total separation time of 20 minutes. To reduce ion suppression caused by buffers in the mobile phase, 0.2% Acetic acid was used in both the MilliQ water and Methanol in place of the standard Formic acid (0.1%) or NH_4Formate/Acetate. The LC gradient for the separation is shown in Table 1. The resultant chromatogram from both positive and negative ions at 1 ppm is shown in Figure 2.

Time	%A	%B
0.0	98.0	2.0
0.5	98.0	2.0
1.0	50.0	50.0
4.0	35.0	65.0
17.0	0.0	100.0
20.0	0.0	100.0
20.1	98.0	2.0

Post Time: 3.0

Table 1: Pesticide Mixture Gradient

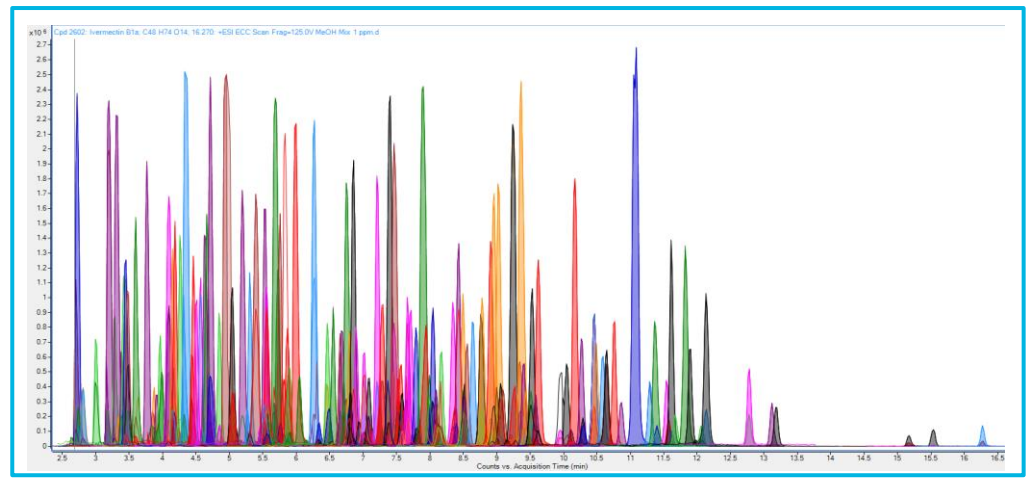
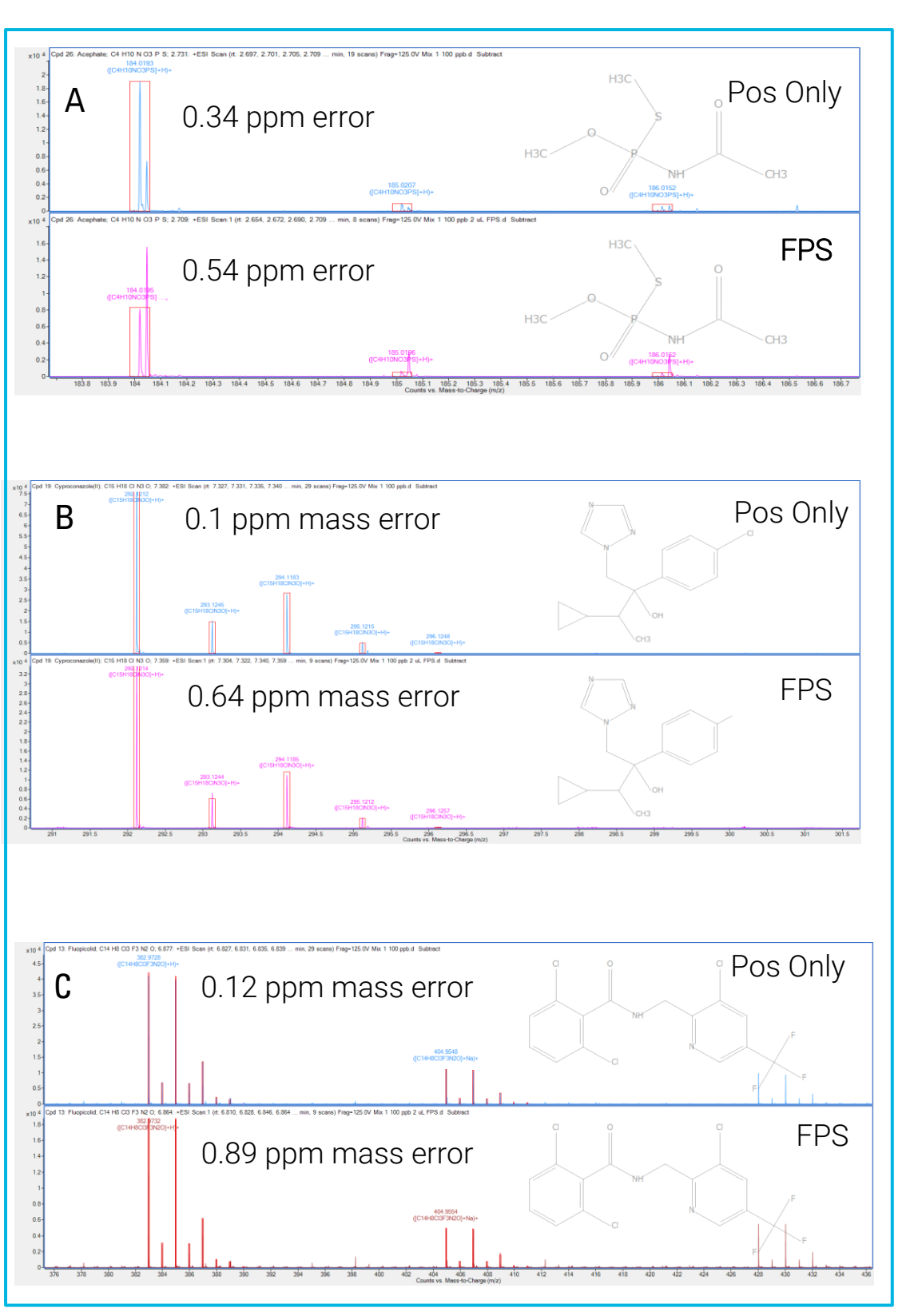


Figure 2: Overlay of the 192 Individual Compound chromatograms from the Pesticide Mixture 1 ppm

Single versus Fast Polarity Switching: Impact on Isotope Fidelity, Resolution and Mass Accuracy

The Agilent comprehensive pesticide standard mix was analyzed using the 6546 LC/Q-TOF in both SP and FPS modes of operation. The average mass spectra of Acephate (Figure 3A), Cyproconazole (II) (Figure 3B) and Fluopicolid (Figure 3C) all show no change in mass resolution and isotope fidelity (red boxes theoretical pattern) and only small changes in mass accuracy.



Figures 3A-3C: The average mass spectra of Acephate (A), Cyproconazole (B) and Fluopicolid (C) in positive ion and FPS modes

Impact on Mass Accuracy

To test the impact on mass by acquisition mode, a subset of pesticides, 20-40 standards per vial were diluted to a final concentration of 100 ppb in Methanol. Targeted data analysis using the retention locked database (Find-by-Formula) was compared with untargeted data analysis (compound discovery MFE) grouping adducts and isotopes together into a single feature that is separated by polarity. The custom database scored by mass accuracy and isotope fidelity were used to identify these untargeted compounds.

The summary of the results from Mixture 1 for **positive ion mode** (Table 2) shows that mass accuracy in SP mode varied from 0.0 to 0.83 ppm (red box) and in FPS from 0.16 to 2.18 ppm (green box). The mass error was slightly higher using FPS for many of the compounds can be lower for saturated compounds.

Positive Ion Only 4 Spectra/s | Fast Polarity Switching Pos Ions

Name	Hits	RT	Mass	Height	Diff (Tgt, ppm)	Name	Hits	RT	Mass	Height	Diff (Tgt, ppm)
Acephate	1	2.735	183.0119	70323	0.22	Acephate	1	2.741	183.0121	15270	0.84
Azaconazole	1	5.686	299.0228	262696	-0.11	Azaconazole	1	5.688	299.0228	44620	-0.09
Azinphos-ethyl (Guthion ethyl)	1	7.492	345.0368	92970	-0.64	Azinphos-ethyl (Guthion ethyl)	1	7.501	345.037	20247	-0.11
Azinphos-methyl (Guthion)	1	5.881	317.0057	61402	-0.37	Azinphos-methyl (Guthion)	1	5.89	317.0063	11619	1.7
Buprofezin	1	11.079	305.1563	626254	0.37	Buprofezin	1	11.071	305.1568	174148	1.93
Cyproconazole(I)	1	7.009	291.1139	112982	0.17	Cyproconazole(I)	1	7.007	291.1138	18588	-0.21
Cyproconazole(II)	1	7.386	291.1138	199063	-0.27	Cyproconazole(II)	1	7.391	291.1143	34225	1.5
Dimethachlor	1	5.839	255.1026	207007	-0.09	Dimethachlor	1	5.835	255.1029	56202	1.08
Dimoxystrobin	1	8.434	326.1632	252652	0.37	Dimoxystrobin	1	8.435	326.1633	62324	0.7
Fosthiazate	1	5.033	283.0465	197270	-0.3	Fosthiazate	1	5.029	283.0469	48977	1.07
Isoprothiolane	1	6.852	290.0646	408927	-0.14	Isoprothiolane	1	6.86	290.0649	101861	0.92
Lenacil	1	5.466	234.1369	46861	0.33	Lenacil	1	5.46	234.1372	4022	1.44
Methamidophos (Metamidophos)	1	2.65	141.0014	102028	0.59	Methamidophos (Metamidophos)	1	2.649	141.0011	16639	-1.33
Myclobutanil	1	7.085	288.1144	186561	0.83	Myclobutanil	1	7.089	288.1139	7232	-0.88
Prochloraz	1	9.036	375.0308	367667	-0.11	Prochloraz	1	9.039	375.0312	84013	0.94
Proquinazid	1	13.187	372.0334	40887	-0.3	Proquinazid	1	13.176	372.0335	11612	0.16
Spiroxamine	1	6.012	297.2668	271690	-0.01	Spiroxamine	1	6.018	297.2669	84468	0.4
Tifatol (Cymiazole)	1	3.197	218.0878	527700	0.23	Tifatol (Cymiazole)	1	3.198	218.0882	135807	2.18
Tralkoxydim	1	12.139	329.1991	151765	0.16	Tralkoxydim	1	12.133	329.1993	38702	0.52

Table 2: Mass Accuracy Comparison of Mixture 1 in Positive Ion Only Mode and FPS Mode

A summary of the results from Mixture 4 for **negative ion mode** shows that the mass error in SP mode varied from 0.06 to -1.38 ppm and in FPS mode varied from 0.04 to -1.44 ppm (Table 3). There are several cases where the mass error is lower in the FPS mode than in negative ion only mode of data collection.

Negative Ion Only 4 Spectra/s | Fast Polarity Switching Negative Ion

Name	RT	Mass	Height	Diff (DB, ppm)	Name	RT	Mass	Height	Diff (DB, ppm)
Boscalid (Nicobifen)	6.653	342.0323	76574	-1.04	Boscalid (Nicobifen)	6.674	342.0322	11140	-1.33
Chloridazon (PAC)	3.468	221.0354	81426	-0.68	Chloridazon (PAC)	3.47	221.0355	17703	-0.27
Cyazofamid	7.781	324.0447	41889	-0.27	Cyazofamid	7.812	324.0446	7044	-0.43
Diflubenzuron	8.069	310.0321	118871	-0.03	Diflubenzuron	8.099	310.0318	25269	-0.81
Ethirimol	3.929	209.1527	146036	-0.52	Ethirimol	3.923	209.1528	32817	-0.16
Fipronil	8.155	435.9386	348659	-0.3	Fipronil	8.187	435.9381	78592	-1.43
Flufenoxuron	12.733	488.0357	146885	-1.1	Flufenoxuron	12.777	488.0356	41175	-1.34
Isoxaben	6.738	332.1736	94547	0.06	Isoxaben	6.766	332.1731	10067	-1.44
Lufenuron	11.852	509.9781	146189	-0.7	Lufenuron	11.896	509.9781	37249	-0.54
Mandipropamid	6.668	411.1232	70796	-1.22	Mandipropamid	6.69	411.1233	13945	-1.07
Metaflumizone	11.572	506.1175	307589	-0.46	Metaflumizone	11.615	506.1185	85622	1.41
Metsulfuron-methyl	4.32	381.0744	83866	0.14	Metsulfuron-methyl	4.333	381.0745	24587	0.61
Novaluron	10.716	492.0119	182384	-0.8	Novaluron	10.76	492.0123	58105	0.04
Teflubenzuron	11.503	379.974	95489	-0.53	Teflubenzuron	11.545	379.9742	26676	-0.04
Thifensulfuron-methyl (DPX-M6316	4.178	387.0307	87682	-0.15	Thifensulfuron-methyl (DPX-M6316	4.186	387.0305	26110	-0.46
Triasulfuron (Logran)	4.144	401.0558	75951	-0.78	Triasulfuron (Logran)	4.154	401.0562	20778	0.31
Triflumuron	9.372	358.0327	148683	-1.38	Triflumuron	9.408	358.0332	36556	0.11

Table 3: Mass Accuracy Comparison Mixture 4 in Negative Ion Only Mode and Fast Polarity Switching Mode

Results and Discussion

Impact of Mixture Concentration of Detection

In LCMS, analytical sensitivity relates back to how well a compound ionizes in electrospray and the resultant signal produced. The tested pesticide mixture contained 214 standards, 28 of which were structural isomers that were not separated, thus a total of 200 pesticides. When using the Compound Discovery mode of data processing the total number of standards detected as a function of polarity and concentration is shown in Table 4A. When processing the data files using a targeted approach where only the best fit in terms of polarity is shown results in better coverage for the lower concentration mixture shown in Table 4B

Concentration Level	Total Detected	A: Compound Discovery Pos & Neg	Pos Only	Neg Only
1 ppm	192	74	108	10
100 ppb	176	45	124	7
10 ppb	90	1	89	0

Concentration Level	Total Number Compound	B: Targeted Analysis Primary Pos Ion	Primary Neg Ion
1 ppm	192	149	43
100 ppb	182	164	18
10 ppb	133	122	11

Table 4: Total compounds detected as a function of polarity and data processing method with A) Compound Discovery and B) Targeted Find-by-Formula

The impact of the reported mass error as a function of the concentration and data processing methods shows that lower errors were obtained using compound discovery where both polarities are reported and multiple adducts (Table 5).

Concentration Level	Average Mass Error MFE Pos	Average Mass Error FBF Pos	Average Mass Error MFE Neg	Average Mass Error FBF Neg
1 ppm	0.89 ppm	1.34 ppm	0.07 ppm	0.38 ppm
100 ppb	0.83 ppm	1.00 ppm	0.42 ppm	0.22 ppm
10 ppb	0.60 ppm	1.10 ppm		0.21 ppm

Table 5: Impact of Data Processing method on average reported mass accuracy by polarity and concentration

Comparison of FPS to Negative Ion Only

For pesticides contains basic groups the positive ion signals will be higher than the negative ion responses. For a small class of pesticides containing acid groups, negative ion will give higher response than the positive ion detection. Data collected using negative ion mode detection at 4 spectra/second was compared with data collected in FPS mode at 1 ppm.

Data Analysis used compound discovery mode mass filtering with custom database. Less than half of the standards (92 out of 214) could be detected in negative ion mode and only 81 in FPS mode. The mass errors were slightly higher in the FPS mode but in 18 standards the measured mass error was better in FPS mode many of which are saturated at the 1 ppm concentration level (Table 6).

Concentration Level	Number Neg Ion	Number FPS Neg Ion	Neg < FPS	Neg > FPS	# Saturated
1 ppm	92	81	63	18	39
100 ppb	72	43	32	11	

Table 6:Comparison of Negative Ion and FPS Negative Ion Detection for Pesticide Mixture at 1 ppm and 100 ppb

Conclusions

Fast Polarity Switching using 6546 LC/Q-TOF

- No Impact on Isotope Fidelity
- Minor Impact on Mass Accuracy
- Flexible Data Processing in either Targeted or Compound Discovery Modes
- Leverage Accurate Mass Databases to Enhance Detection
- Optimal for high-level suspect screening

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DE81390694

Published in USA, May 25, 2022

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