

Fully automated derivatization and quantification of Glyphosate and AMPA in beer using a standard UHPLC-MS/MS system

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1. Introduction

Glyphosate is currently one of the most common pesticides used worldwide. In spite of its approval by regulatory bodies all over the world, the concern about its harm to humans and the environment persists. Therefore, the strict control of Glyphosate and its metabolite Aminomethylphosphonic acid (AMPA) in food and environment is mandatory.

The chromatography of glyphosate is challenging due to its high polarity. In order to overcome this, there exists a well-established method including a derivatization step with 9-fluorenylmethyl chloroformate (FMOC) followed by LCMS analysis.

Here we report a fully automated derivatization followed by LC-MS/MS analysis of beer samples. The instrumental set-up does not require any additional hardware for sample pretreatment but uses the built-in pretreatment function of the autosampler.

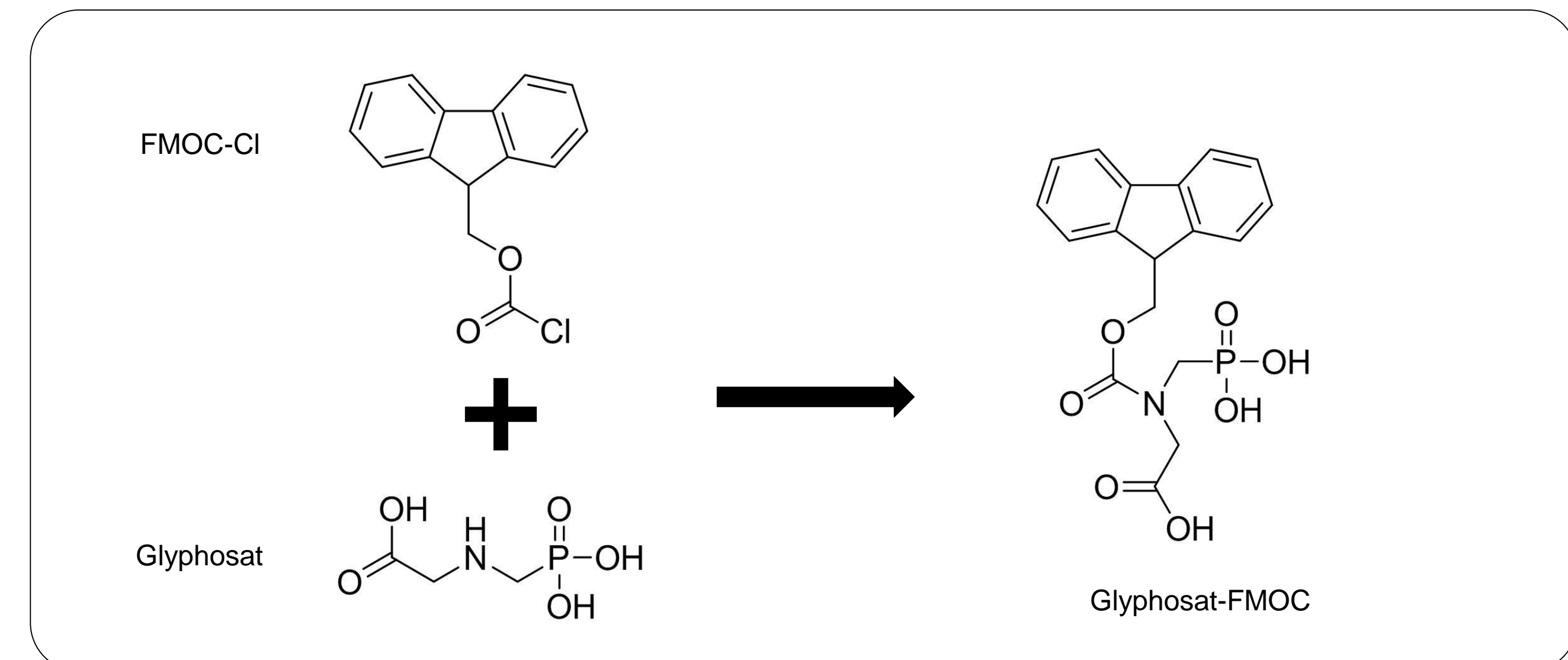


Figure 1 Derivatization of Glyphosat with FMOC

2. Materials and Methods

2.1 Sample Preparation

After precipitation with methanol (50:50) and centrifugation the beer samples were set into the autosampler.

2-1. Analytical Conditions

LC system	
LC system:	Nexera X2 (Shimadzu, Japan)
Analytical Column:	Gemini 5µm C18, 150 mm x 2 mm
Mobile Phase A:	2 mM NH ₄ HCO ₃ , pH 9.5
Mobile Phase B:	Acetonitrile
Time program:	B conc. 5%(0 min) -50%(7min) -95%(7.01-12 min) -5% (12.01-15min)
Injection volume:	50 µL
Column Temperature:	35 °C

MS	
MS system:	LCMS-8060 (Shimadzu, Japan)
Ionization:	HESI (positive/negative)
Nebulizing Gas Flow:	3.00 L/min (N ₂)
Drying Gas Flow:	5.0 L/min (N ₂)
Heating Gas Flow:	15.0 L/min (Air)
CID gas:	270 kPa
DL Temperature :	150 °C
Block Temperature :	400 °C
Interface Temperature :	325 °C

3. Results

3.1 Method development for automatization of derivatization

The addition of internal standards as well as the derivatization of Glyphosate and AMPA with FMOC was done fully automated by the autosampler SIL-30AC within 15 minutes. After derivatization the sample was injected directly to the LC-MS/MS and analyzed accordingly.

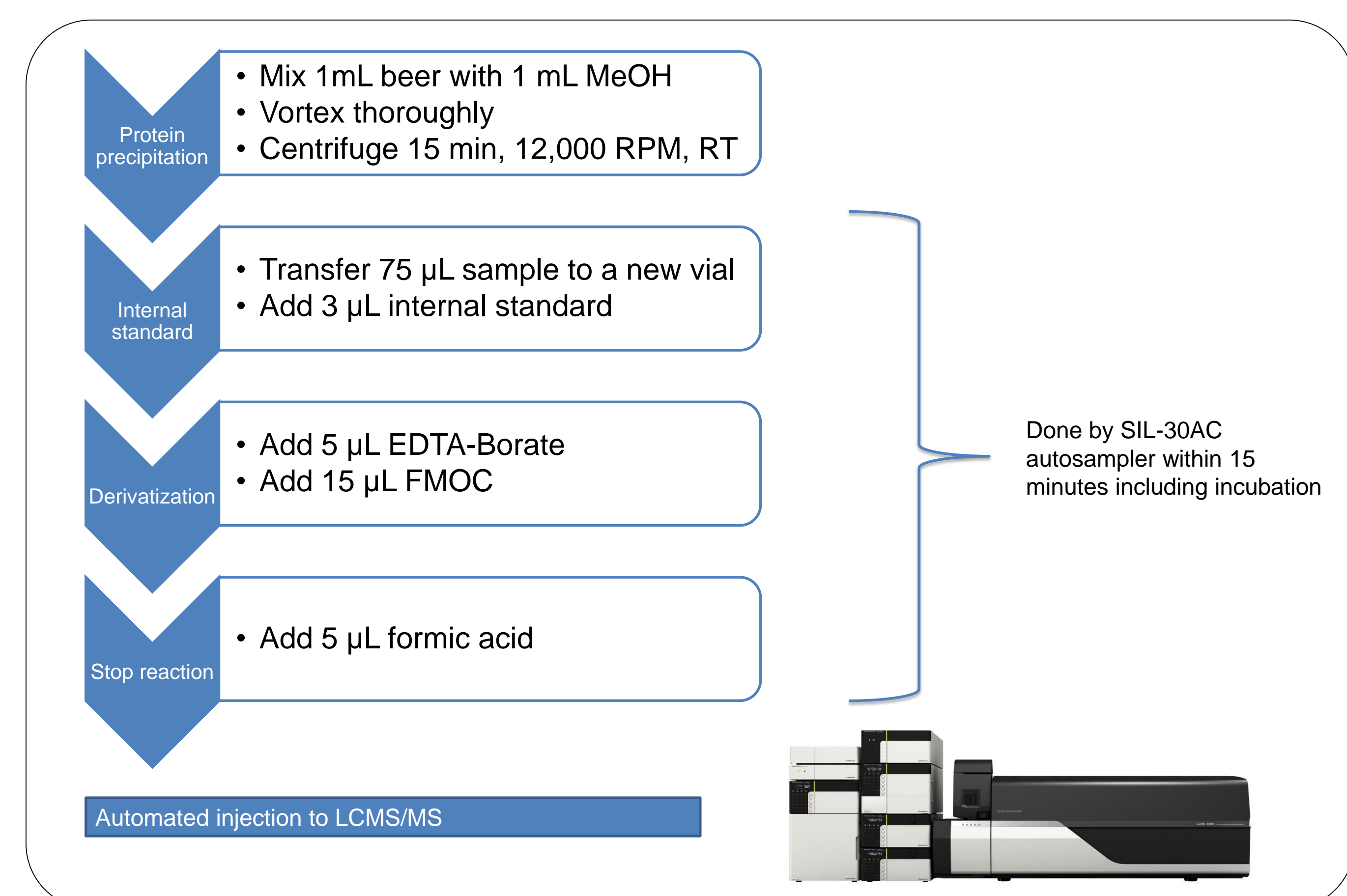


Figure 2: Workflow of sample pretreatment. Addition of internal standard as well as derivatization is done by the autosampler.

Due to overlapping sample pretreatment functionality, the next sample was already pretreated during the on-going analysis in order to maximize sample throughput. Except for the first and the last sample, the total time per sample for automated pretreatment and analysis can be reduced to 15 minutes.

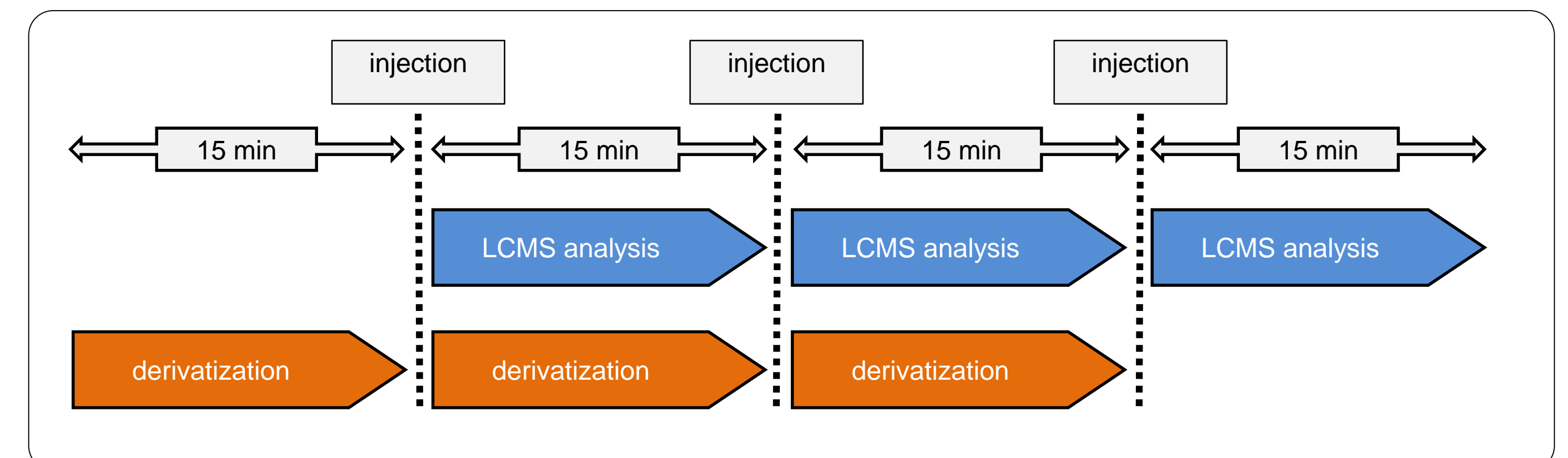


Figure 3: Overlapping sample pretreatment and analysis done by SIL-30AC. Total time per sample is reduced to 15 minutes.

Table 1: QC sample results

Batch	Glyphosate-FMOC						AMPA-FMOC					
	QC 3 ng/mL		QC 15 ng/mL		QC 75 ng/mL		QC 3 ng/mL		QC 15 ng/mL		QC 75 ng/mL	
	Conc.	Acc. %	Conc.	Acc. %	Conc.	Acc. %	Conc.	Acc. %	Conc.	Acc. %	Conc.	Acc. %
A	2.60	86.5	14.89	99.3	74.14	98.9	4.76	158.5	15.66	104.4	80.80	107.7
A	2.87	95.7	14.96	99.7	81.22	108.3	2.71	90.3	16.16	107.7	85.65	114.2
A	3.41	113.5	15.14	100.9	77.94	103.9	3.15	105.0	15.99	106.6	81.38	108.5
B	2.81	93.7	16.00	106.7	79.18	105.6	4.11	137.0	15.33	102.2	78.40	104.5
B	3.20	106.7	16.08	107.2	76.19	101.6	3.49	116.2	15.20	101.3	82.23	109.6
B	3.46	115.3	15.42	102.8	83.74	111.6	3.02	100.8	15.66	104.4	84.15	112.2
C	2.82	93.9	14.94	99.6	67.88	90.5	3.48	115.9	15.48	103.2	83.97	112.0
C	2.73	91.1	15.67	104.5	76.89	102.5	3.25	108.3	16.55	110.3	79.72	106.3
C	3.27	109.0	15.87	105.8	84.87	113.2	3.38	112.6	16.87	112.5	82.65	110.2
D	3.19	106.2	16.42	109.5	82.82	110.4	2.73	90.9	16.85	112.3	75.46	100.6
D	3.33	110.9	16.00	106.7	85.29	113.7	3.31	110.4	14.35	95.7	72.06	96.1
D	3.23	107.6	17.14	114.3	84.74	113.0	3.55	118.3	15.50	103.3	75.97	101.3
Mean	3.08		15.71		79.57		3.41		15.80		80.20	
SD	0.2915		0.6816		5.2735		0.5676		0.7306		4.0615	
RSD (%)	9.5		4.3		6.6		16.6		4.6		5.1	

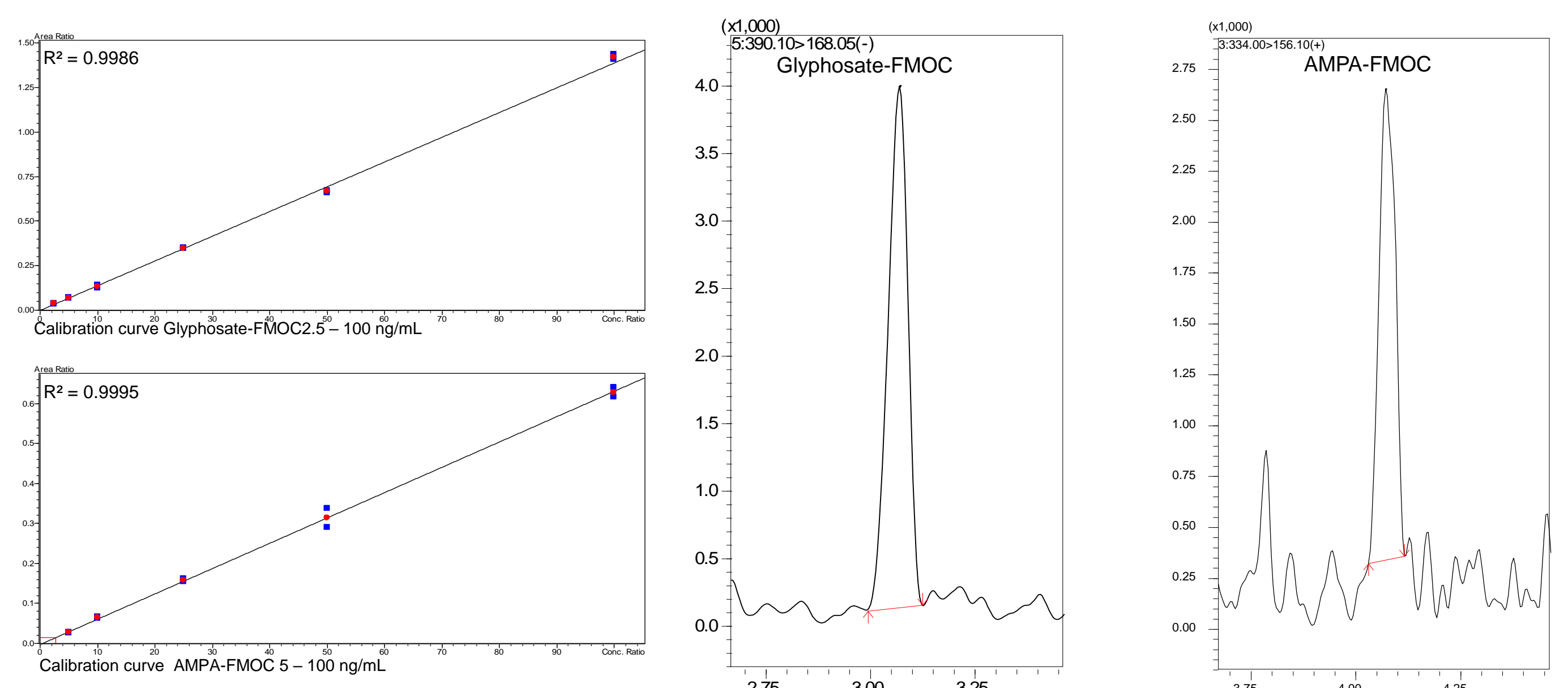


Figure 4: Chromatogram of Glyphosate-FMOC (2.5 ng/mL) and AMPA-FMOC (5 ng/mL) at their respective LOQs and calibration curves.

3.2 Quantitative analysis of 40 beer samples

A total of 40 commercially available beer samples were analysed. Among these samples there were 21 samples of beer brewed according to Pilsener style, 3 samples of organic beer, 10 samples of other types of beer and 6 samples of alcohol-free beers or non alcoholic beer mix drinks. All samples were analysed in duplicate in two consecutive runs. While Glyphosate was detected in 60 % of all samples its metabolite AMPA was below LOQ in all samples.

Table 2: Analysis of beer samples

	Glyphosat-FMOC						AMPA-FMOC	
	Conc. ng/mL	Conc. ng/mL	Mean	SD	% RSD	Conc. ng/mL	Conc. ng/mL	
Pils								
Sample 1	<LOQ	<LOQ				<LOQ	<LOQ	
Sample 2	8.37	8.95	8.7	0.4087	4.7	<LOQ	<LOQ	
Sample 3	20.85	20.28	20.6	0.4038	2.0	<LOQ	<LOQ	
Sample 4	<LOQ	<LOQ				<LOQ	<LOQ	
Sample 5	6.78	6.57	6.7	0.1549	2.3	<LOQ	<LOQ	
Sample 6	11.34	12.08	11.7	0.5240	4.5	<LOQ	<LOQ	
Sample 7	<LOQ	<LOQ				<LOQ	<LOQ	
Sample 8	8.61	9.41	9.0	0.5706	6.3	<LOQ	<LOQ	
Sample 9	4.74	4.63	4.7	0.0834	1.8	<LOQ	<LOQ	
Sample 10	<LOQ	<LOQ				<LOQ	<LOQ	
Sample 11	10.81	12.03	11.4	0.8627	7.6	<LOQ	<LOQ	
Sample 12	13.95	14.65	14.3	0.4943	3.5	<LOQ	<LOQ	
Sample 13	33.06	27.61	30.3	3.8509	12.7	<LOQ	<LOQ	
Sample 14	20.29	18.68	19.5	1.1377	5.8	<LOQ	<LOQ	
Sample 15	25.28	22.09	23.7	2.2578	9.5	<LOQ	<LOQ	
Sample 16	3.23	2.93	3.1	0.2171	7.1	<LOQ	<LOQ	
Sample 17	3.66	3.48	3.6	0.1308	3.7	<LOQ	<LOQ	
Sample 18	5.25	5.65	5.4	0.2807	5.2	<LOQ	<LOQ	
Sample 19	2.67	2.93	2.8	0.1881	6.7	<LOQ	<LOQ	
Sample 20	3.87	4.39	4.1	0.3698	9.0	<LOQ	<LOQ	
Sample 21	<LOQ	<LOQ				<LOQ	<LOQ	
Organic Beer								
Sample 22	<LOQ	<LOQ				<LOQ	<LOQ	
Sample 23	<LOQ	<LOQ				<LOQ	<LOQ	
Sample 24	<LOQ	<LOQ				<LOQ	<LOQ	
Others								
Sample 25	2.79	3.26	3.0	0.3323	11.0	<LOQ	<LOQ	
Sample 26	4.61	4.15	4.4	0.3260	7.4	<LOQ	<LOQ	
Sample 27	<LOQ	<LOQ				<LOQ	<LOQ	
Sample 28	<LOQ	<LOQ				<LOQ	<LOQ	
Sample 29	2.52	<LOQ				<LOQ	<LOQ	
Sample 30	<LOQ	<LOQ				<LOQ	<LOQ	
Sample 31	<LOQ	<LOQ				<LOQ	<LOQ	
Sample 32	8.06	7.27	7.7	0.5621	7.3	<LOQ	<LOQ	
Sample 33	11.19	11.57	11.4	0.2737	2.4	<LOQ	<LOQ	
Sample 34	<LOQ	<LOQ				<LOQ	<LOQ	
Non alcoholic								
Sample 35	4.75	4.47	4.6	0.1952	4.2	<LOQ	<LOQ	
Sample 36	16.05	15.71	15.9	0.2454	1.5	<LOQ	<LOQ	
Sample 37	<LOQ	<LOQ				<LOQ	<LOQ	
Sample 38	<LOQ	<LOQ				<LOQ	<LOQ	
Sample 39	<LOQ	<LOQ				<LOQ	<LOQ	
Sample 40	2.50	2.85	2.7	0.2482	9.3	<LOQ	<LOQ	

4. Conclusion

- Fully automated FMOC-derivatization of Glyphosate and AMPA within 15 minutes
- No additional hardware required
- Sample derivatization and internal standard addition done by autosampler SIL-30AC
- Maximized sample throughput due to overlapping sample pretreatment functionality
- Robust and reliable method for Glyphosat and AMPA even in a complex matrix like beer