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

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Julia Sander[1], Anja Grüning[1], Robert Ludwig[1], Philipp Jochems[1]
1 Shimadzu Europa, Albert-Hahn-Str. 6-10, 47269 Duisburg, Germany

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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.

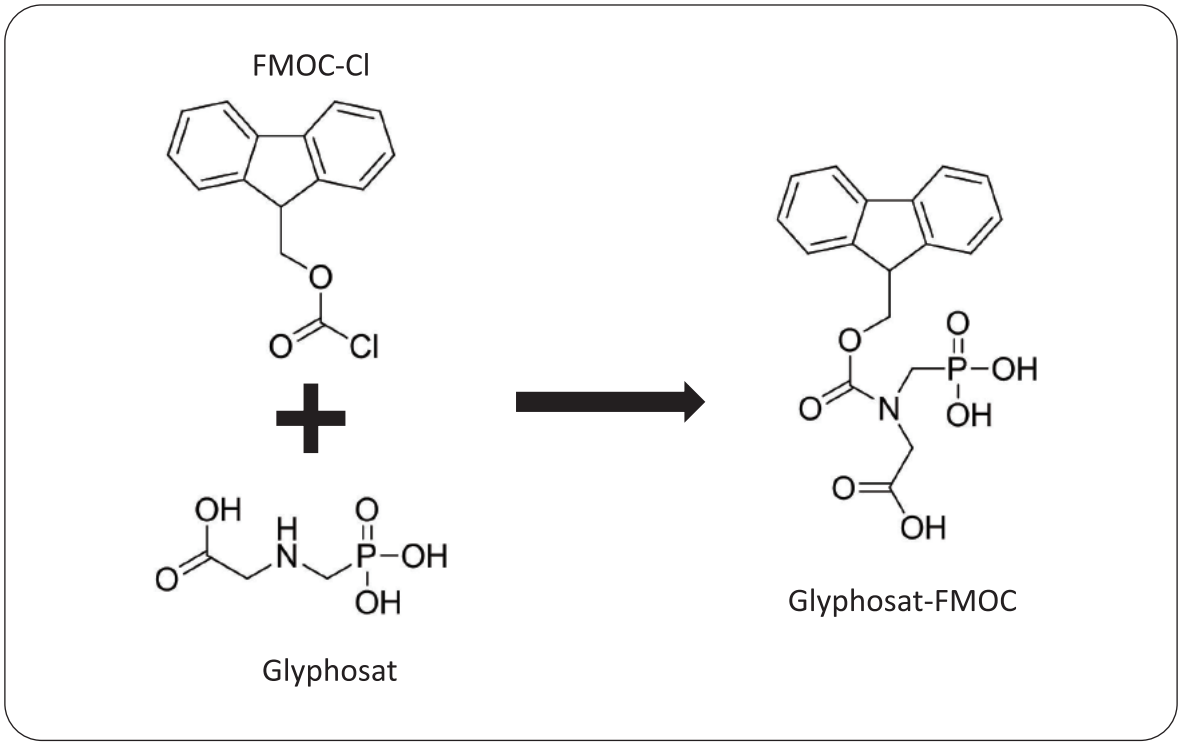

Figure 1 Derivatization of Glyphosat with FMOC

Methods and Materials

Sample Preparation

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

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UHPLC method

Instrument	: Nexera UHPLC, Shimadzu
Column	: Gemini 5 µm C18, 150 x 2 mm
Mobile phase A	: 2 mm NH_4HCO_3, pH 9.5
B	: acetonitrile
Flow rate	: 0.4 mL/min
Time program	: B conc. 5%(0 min) -50%(7 min) - 95%(7.01-12min) – 5% (12.01 min – 15 min)
Injection vol.	: 50 µL
Column temperature	: 35 °C

MS conditions

Instrument	: LCMS-8060, Shimadzu
Ionization	: pos/neg ESI
Nebulizing gas	: 3 L/min
Heating gas	: 15 L/min
Drying gas	: 5 L/min
Interface temperature	: 325 °C
DL temperature	: 150 °C
Heat block temperature	: 400 °C
CID gas	: 270 kPa
Interface voltage	: 4 kV/ -3 kV

Results

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.

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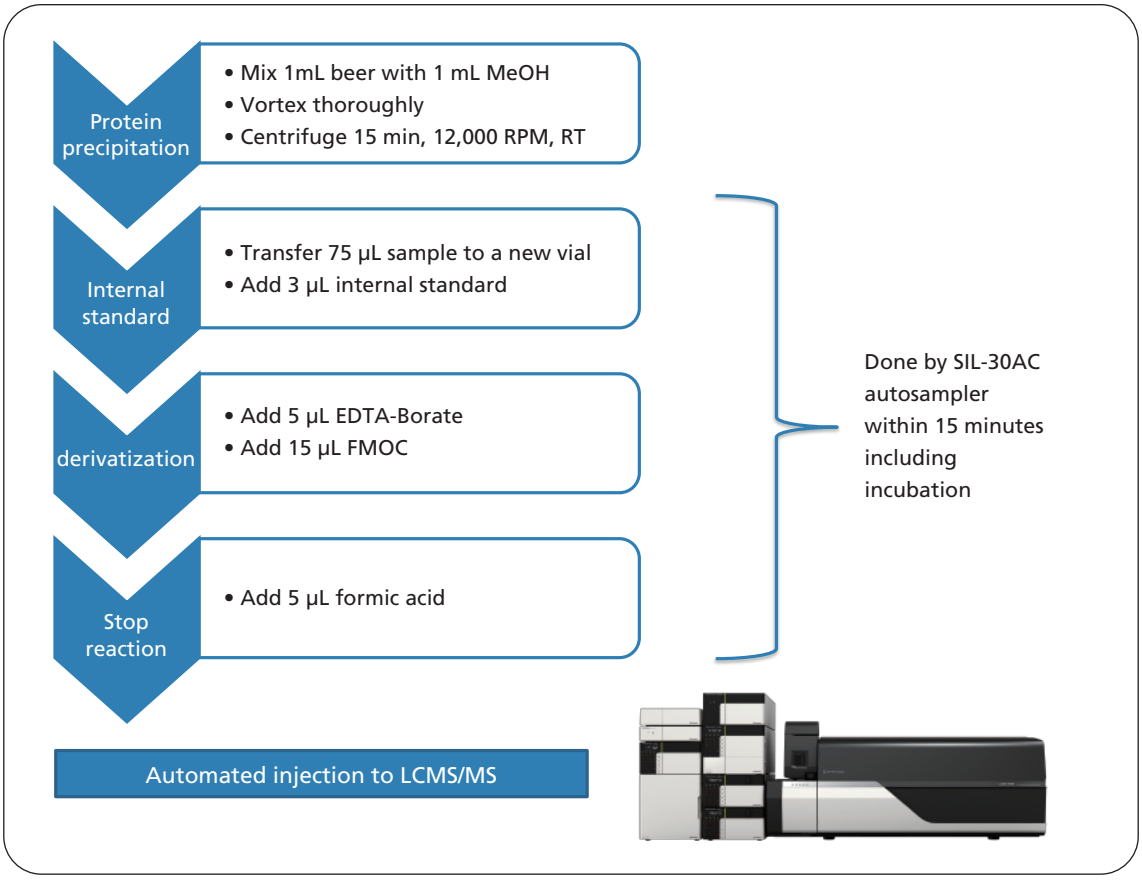

Figure 2: Workflow of sample pretreatment. Addition of internal standard as well as derivatization is done by the autosampler.

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.

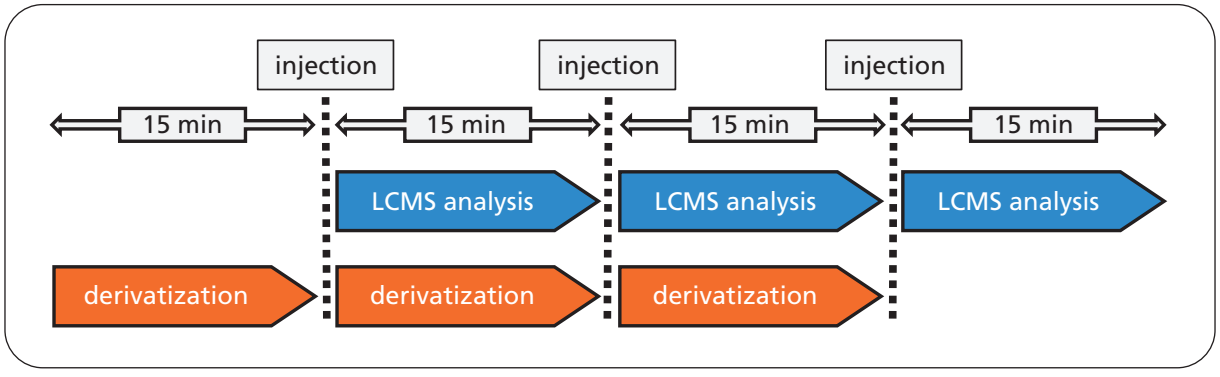

Figure 3: Overlapping sample pretreatment and analysis done by SIL-30AC. Total time per sample is reduced to 15 minutes.

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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**	
								extrapolated				

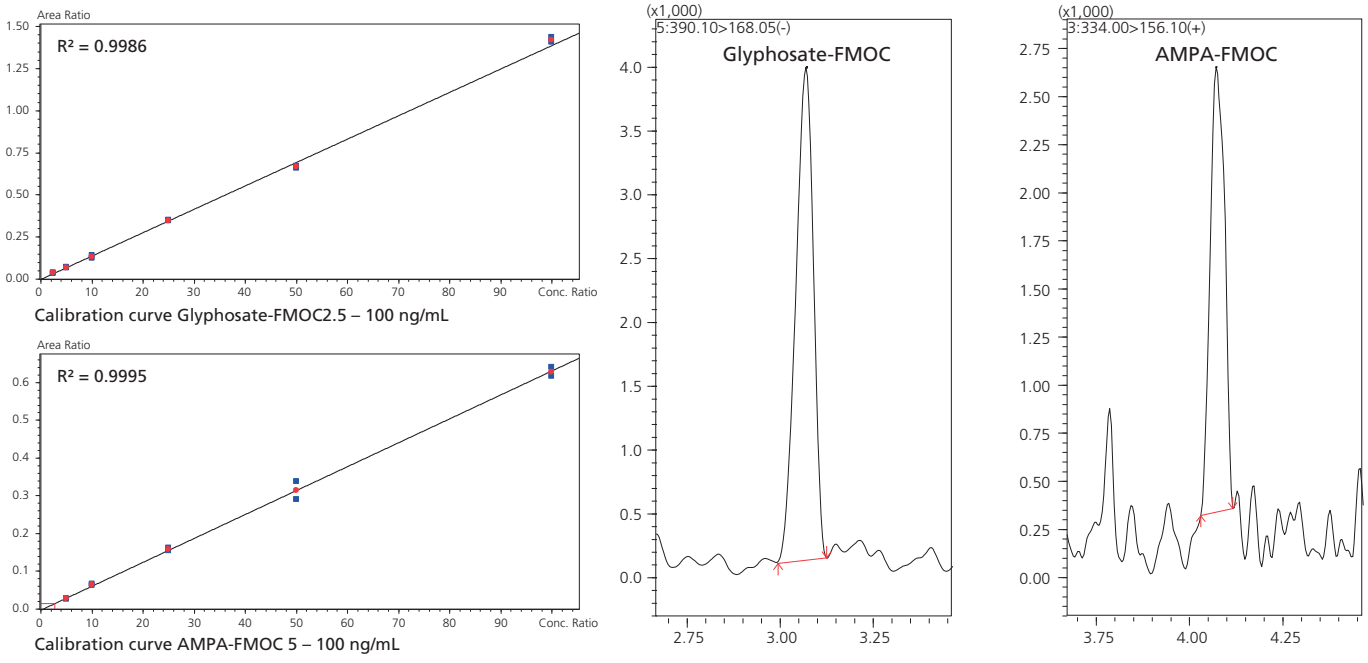

Figure 4: Chromatogram of Glyphosate-FMOC (2.5 ng/mL) and AMPA-FMOC (5 ng/mL) at their respective LOQs and calibration curves.

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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

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Conclusions

- 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

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