EURL-SRM - Analytical Observations Report

concerning the following…

- **Compound(s)**: AMTT (metabolite of tritosulfuron)
- **Commodities**: Fruit and vegetables, cereals
- **Extraction Method(s)**: QuEChERS
- **Instrumental analysis**: LC-MS/MS

Analysis of the Tritosulfuron Metabolite AMTT by QuEChERS Method using LC-MS/MS

Version 1 (last update: 30.03.2017)

Background information / Initial Observations:

AMTT results from the use of tritosulfuron. It is both a metabolite of tritosulfuron as well as an impurity in formulations (specification max. 0.2 g/kg)[1]. Tritosulfuron is intended to be used in cereals (autumn and spring sown) and in maize. It is a post-emergence herbicide with systemic action. Due to the high toxicity of AMTT, EFSA in its reasoned opinion on Tritosulfuron in 2015[2], proposed to distinctly regulate AMTT proposing very low MRLs at 0.001 mg/kg for cereals and various products of animal origin. Based on the metabolism study it was concluded that the residues in milk were expected to be so low, that no exceedances of the toxicological thresholds are expected. The development of a method for milk that is validated at 0.0005 mg/kg was advised.
With regulation 2016/2016/EC, applicable from 14 January 2017 onwards, the maximum residue level of AMTT for milk and cereals was set at 0.001 mg/kg and for all other products at 0.01 mg/kg. In the preamble of this regulation it is indicated that "*Analytical methods to achieve the lowest possible LOD need to be developed for AMTT. Once those methods are available, the levels set by this Regulation may be reviewed at any stage*". Initial tests have shown that using unmodified QuEChERS and routine instrument settings of an older generation LC-MS/MS instrument (ABI-Sciex 4000), LOQs below 0.01 mg/kg are difficult to achieve, especially for dry commodities, where the sample weight is reduced to 5 g.

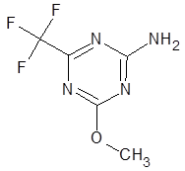
[1] http://www.bvl.bund.de/SharedDocs/Downloads/04_Pflanzenschutzmittel/02_eu_berichte/Tritosulfuron-DAR.pdf?__blob=publicationFile

[2] Reasoned opinion on the review of the existing maximum residue levels (MRLs) for tritosulfuron according to Article 12 of Regulation (EC) No 396/2005 EFSA Journal 2015;13(1):3964

Compound details

AMTT (CAS: 5311-05-7), IUPAC: 4-Methoxy-6-(trifluoromethyl)-1,3,5-triazin-2-amine
2-amino-4-methoxy-6-(trifluormethyl)-1,3,5-triazine

Parameter	Value
Molecular Mass	194.117 g/mol
Exact mass	194.04154 Da
Pka	not ionized when pH < 13 (Chemicalize.org)[3]
LogD	1.65 (calculated by Chemicalize.org) 0.62 (20°C/pH7) according to BASF-report[4]
Residue definition EU	2-amino-4-methoxy-6-(trifluormethyl)-1,3,5-triazine (AMTT), resulting from the use of tritosulfuron
Tritosulfuron is approved in…	AT, BE, BG, CY, CZ, DE, EE, EL, ES, FI, FR, HR, HU, IE, IT, LT, LU, LV, NL, PL, PT, RO, SI, SK
ADI / ARfD	0.0001 mg/kg bw per day / 0.0001 mg/kg bw (EC2008)



Materials[5]:

Substance	Purity	CAS	Source
Tritosulfuron	98.5%	142469-14-5	LGC (Dr. Ehrenstorfer)
AMTT	99.9%	5311-05-7	BASF (friendly donation)
Chlorpyrifos D10	97.0%	285138-81-0	LGC (Dr. Ehrenstorfer)

- All other materials and chemicals used as listed in EN 15662

Measurement conditions

Measurement was conducted by LC-MS/MS instrument (ESI-positive mode). Details are given in Table 2 and Table 3

Table 2: Instrumentation details

LC	WATERS Acquity UPLC		
MS/MS	SCIEX API 5500 Q-Trap, run in ESI positive mode		
Column	Acquity BEH C18, 2.1x100 mm, 1.7 µm		
Pre-column	Acquity BEH C18, 2.1x5 mm, 1.7 µm		
Mobile Phase	A: 5 mmol NH_4formate in purified water + 5% methanol B: 5 mmol NH_4formate in methanol		
Gradient	**Time** (min)	**Mobile Phase A** (%)	**Mobile Phase B** (%)
	0	60	40
	10	10	90
	13	10	90
	13.1	60	40
	19	60	40
Flow	0.4 mL min^{-1}		
Injection volume	2 µL, partial loop with needle overfill		
Column temperature	40°C		

[3] Chemicalize.org
[4] https://www.agrar.basf.de/agroportal/de/media/migrated/de/productfiles/sdb/sdb-arrat.pdf
[5] **Disclaimer**: Names of companies are given for the convenience of the reader and do not indicate any preference by the EURL-SRM towards these companies and their products

Table 3: MRM details for AMTT (ESI-pos. mode using Sciex API 4000 QTrap):

Compound	Intensity ranking	Q 1	Q 3	DP	CE	CXP
AMTT	3	**195**	**57**	66	37	4
	2	**195**	**69**	66	69	2
	4	**195**	**110**	66	35	6
	1	**195**	**175**	66	23	10
	5	**195**	**138**	66	27	8
Chlorpyrifos D10	**-**	**360**	**199**	**66**	**23**	**12**

Note: If you measure on an **API 5500 instrument** you may also use the settings above. **DP values could be increased by 20.**

Experiments conducted and observations:

The validation experiments were based on common QuEChERS procedure (EN-15662[6]). The main focus of validation experiments was on cereals. Various cleanup approaches tested (PSA, C18 or freeze out) had no negative effect on recovery.

A summary of the validation experiments is given in Table 4. The results were evaluated with Chlorpyriphos-D10 as internal standard. For mean recovery and RSD only the target MRM of m/z 195/175 was used. All samples, except the 0.02 ppm level of flour, were measured with the more sensitive API5500 instrument. The 0.02 ppm validation level of flour was run on an API 4000. Comparison of API4000 and the more sensitive API5500 is shown in Figure 1.

Some exemplary chromatograms showing the various MRM transition when analyzing by API5500 are shown in Figure 2.

Table 4: Recovery data for AMTT from various commodities:

Matrix Type	Matrix	Sample Weight + water addition	Spiking Level	QuEChERS w/o cleanup			QuEChERS with PSA/C18			QuEChERS with freeze out		
				n	Mean Rec.%	RSD %	n	Mean Rec.%	RSD %	n	Mean Rec.%	RSD %
High water	Cucumber	10 g	0.001	5	98	3.4						
			0.01	5	100	1.6						
High water + low pH	Orange Juice	10 g	0.001**	5	108	7.0						
			0.01	5	96	1.6						
Dry	Flour	5 g + 10 mL water	0.001	5	113	7.6						
			0.002				5	94	6.1			
			0.005				5	94	3.8			
			0.01	5	105	3.5						
			0.02*	5	109	7.8	5	102	11.9			
Animal	Milk	5 g	0.001							5	109	19.0

*measured with API4000
** matrix interference in qualifier

[6] Detailed instructions on the QuEChERS method are given in the CEN method EN 15662 (citrate buffered), see also brief description under www.quechers.de .

Figure 1: Comparison of sensitivity between API 4000 and API 5500 instrument, exemplary with a recovery sample of 0.02 ppm AMTT on flour

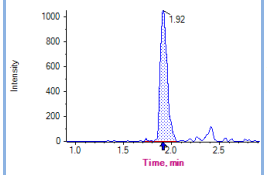
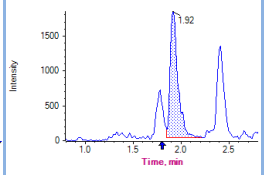
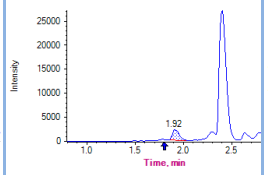
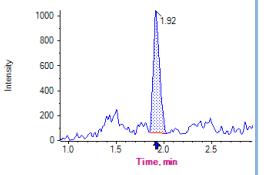
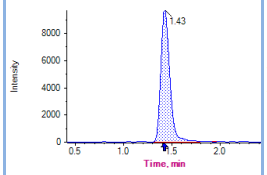
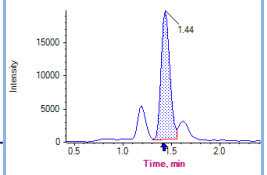
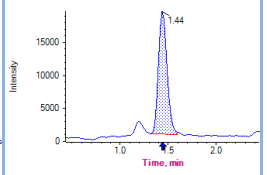
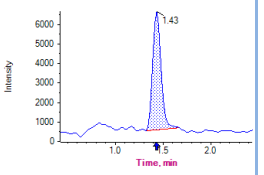
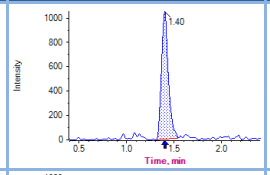
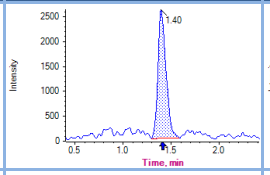
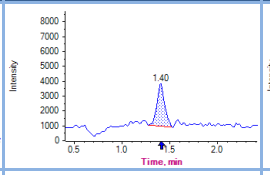
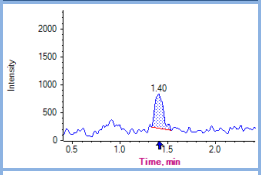
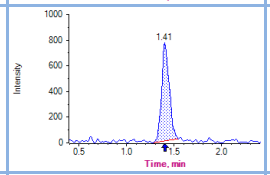
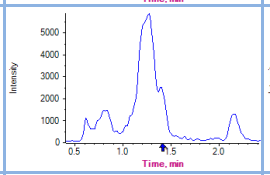
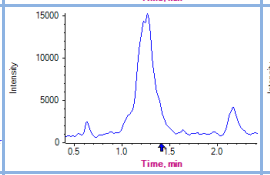
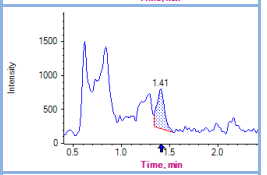
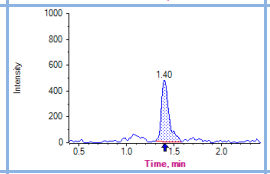
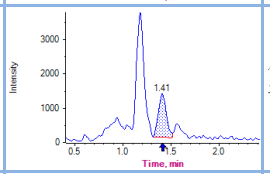
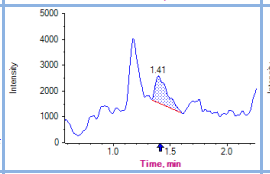
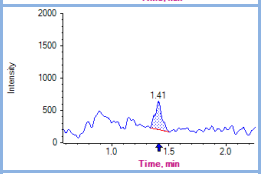
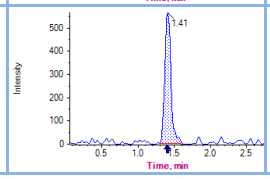
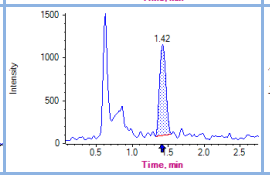
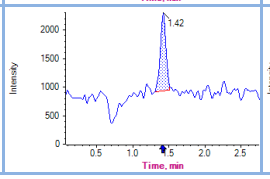
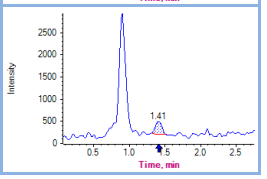
Instrument	m/z 195/175 (target)	m/z 195/69	m/z 195/57	m/z 195/110
API4000				
API5500				

Figure 2: Exemplary chromatograms of 0.001ppm in different matrices measured with API 5500

Matrix	m/z 195/175 (target)	m/z 195/69	m/z 195/57	m/z 195/110
Cucumber				
Orange juice *(matrix interference on qualifier!)*				
Flour				
Milk				

Discussion and conclusions:

It was shown that AMTT gives good recoveries using the citrate buffered QuEChERS method and that the very low MRLs in cereals and milk (at 0.001 mg/kg) can be routinely enforced but only if high end instrumentation is used. Cleanup with various approaches (PSA, C18 or freeze out) had no negative effect on recovery.
Tritosulfuron (parent) is also QuEChERS amenable and may be measured via LC-MS/MS in ESI neg. or ESI pos. mode, but sensitivity in negative mode is much higher (f=40).

History

Action	When	Document Version
Initial Experiments	Oct - Dec 2015	
Further Experiments	Jan – August 2016	
Observation document placed on-line	March 2017	V1