EURL-SRM - Analytical Observations Report

concerning the following…

- **Compound(s)**: Pymetrozine
- **Commodities**: Various commodities of plant origin
- **Method(s)**: QuEChERS, adjustment of pH
- **Instrumentation**: LC-MS/MS

Analysis of Pymetrozine by the QuEChERS Method - Impact of pH on Recovery Rate

Reported by: EURL-SRM
Version 1 (last update: 18.04.2016)

Background information / Initial Observations:

When using the citrate buffered QuEChERS method (EN15662), recovery rates of pymetrozine are often < 70 %, mostly in the range between 45 to 65 %. Some examples are shown in Table 1:

Table 1: Recovery rates for pymetrozine using citrate buffered QuEChERS (EN15662)

Commodity	n	Level [mg/kg]	Recovery rates by QuEChERS EN 15662, (Spiking level 0.1 mg/kg)	CV
Cucumber	5	0.01	62 %	5 %
Avocado	5	0.01	59 %	10 %
Wheat	5	0.1	58 %	6 %
Grapes	5	0.1	55 %	4 %
Lemon	4	0.1	46 %	6 %

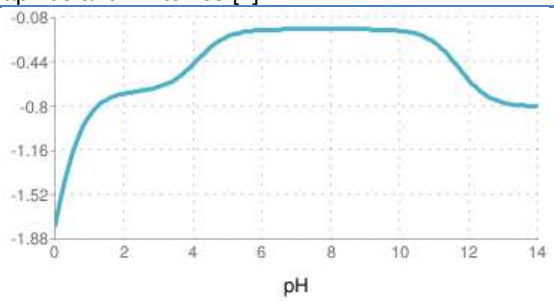
Without buffering, recovery rates are more strongly influenced by the natural pH of the extracted commodities, with recoveries from neutral commodities like lettuce being higher than those from acidic ones.
Pymetrozine, being weakly basic at the pyridine group (pK_a of the corresponding acid ~4.4) and weakly acidic at the triazinone group (pK_a ~11.4), exhibits an ambiphilic character[1]. At pH values < 4.4 the protonated form predominates. At pH values <2 even a double protonated form comes into play (pK_a ~0.7 at the triazinon group).
As the pH decreases the logD value (a measure of lipophilicity) drops from ~-0.25 at pH 5 to ~-0.5 at pH 4 and further to ~-0.7 at pH 3. This explains the lower recovery rates at lower pH.

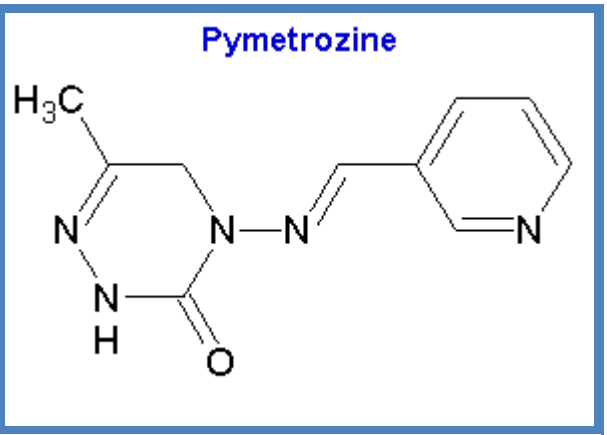
[1] pKa values calculated by www.chemicalize.com; in the EFSA peer review on pymetrozine (**http://www.efsa.europa.eu/sites/default/files/scientific_output/files/main_documents/3817.pdf**) the pka is given as 4.06

Between pH 6 and 10 pymetrozine is almost exclusively present in its - still quite polar - neutral form (logP ~ -0.2), and has a more favorable transfer into the acetonitrile phase.

Using the citrate buffering salts of EN 15662 the pH value during extraction is around 4. Although the citrate salt mixture in itself buffers in the pH range of 5-5.5, the presence of $MgSO_4$ causes the pH to drop as Mg^{2+} ions form a complex with citrate, thus releasing protons. As a result the recovery of pymetrozine remains well below 70%.

Facts at a glance:

Pymetrozine	
Mode of action	Insecticide selective against Homoptera like aphids and whiteflies [1]
Log D	 www.chemicalize.org
Partition coefficient	log Po/w = -0.19 (pH 5, 25 °C) log Po/w = -0.19 (pH 7, 25 °C) log Po/w = -0.20 (pH 9, 25 °C) [3]
Water solubility	320 mg/L (25 °C; pH 5) 270 mg/L (25 °C; pH 7) 270 mg/L (25 °C; pH 9), all 99.7% [3]
Dissociation constant	pKa,1 = 4.06 (basic, protonation at nitrogen atom of the pyridine ring) [1,3] pKa,2 < 1 (basic, protonation at nitrogen atom of triazinone ring) [1,3] pka,3 = 11.4 (acidic, deprotonation at nitrogen of triazinone ring) [chemicalize.org]
Residue definition EU	Pymetrozine For milk: pymetrozine, 6-hydroxymethylpymetrozine and its phosphate conjugate, expressed as pymetrozine [2]
Registration Status	Approved within the EU: AT, BE, CY, CZ, DE, DK, EE, EL, ES, FI, FR, HR, HU, IE, IT, LT, LU, LV, NL, PL, PT, RO, SE, SI, UK
ADI	0.03 mg/kg bw/day [3]
ARfD	0.1 mg/kg bw [3]



Observations and experiments conducted:

Materials and instrumentation (exemplary[2]):

Pymetrozine (purity 99.9%), purchased from Riedel-de-Haen (stock solution at 1 mg/ml was prepared in methanol)
Magnesium acetate tetrahydrate, min. 99% from Sigma-Aldrich
Sodium acetate p.a. anhydrous, min. 99% from Merck

Table 2: Instrumentation details

LC	Agilent 1290			
MS/MS	ABSCIEX 4000, run in ESI positive mode			
MRMs	218.2 / 105.0 (target); 218.2 / 78.0 (qualifier)			
Column	Phenomenex Synergi 4 µ Hydro-RP; 150x2 mm			
Pre-column	Aqua C18 125A 4 mm x 2 mm			
Mobile Phase	A: 5 mmol NH_4-formiate in purified H2O without addition of methanol (use brown bottles to avoid formation of algae) B: 5 mmol NH_4formate in methanol			
Gradient	**Time** (min)	**Mobile Phase A** (%)	**Mobile Phase B** (%)	**Flow** (mL/min)
	0	100	0	0.1
	3	30	70	0.1
	6	15	85	0.3
	9	10	90	0.3
	18.5	10	0	0.3
	19	100	0	0.3
	28	100	0	0.3
Column temperature	40 °C			
Injection volume	3 µL, automatically diluted with 10 µL of mobile phase A during injection procedure Note: Should the possibility for an automated dilution of the solutions in the instrument injector not exist, the sample extracts should be manually diluted with mobile phase A (e.g. 1+1 to 1+4)			
Internal Standard	Chlorpyrifos-ethyl D10			

Direct injection of QuEChERS extracts onto traditional C-18 columns often results in peak distortion or even splitting. Diluting the extracts with the aqueous mobile phase A prior or during injection, improves the situation. The use of gradients starting with highly aqueous eluent composition facilitates reversed phase interactions of highly polar compounds such as pymetrozine with the stationary phase. Polar end-capped RP columns are helpful in this respect as they can tolerate purely aqueous eluents without the hydrocarbon-chains collapsing.

[2] 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

Impact of extraction pH on pymetrozine recoveries

To study the influence of various salt mixtures on the pH various combinations of salts (used instead the citrate buffering salts described in EN15662) were tested using cucumber as neutral and orange juice as acidic commodity:

The resulting pH values in the extracts are shown in Table 3.

Table 3: pH values of QuEChERS extracts using different buffering salt combinations

Salt mixture	pH-values of QuEChERS extracts (measured at ~ 23 °C)		Recovery rates of Pymetrozine (n=2) at 0.1 mg/kg	
	Orange juice	Cucumber	Orange juice	Cucumber
Citrate buffering salts EN15662	3.88	4.20	55 %	65 %
4 g $MgSO_4$. 1 g NaCl	2.55	4.84	22 %	80 %
4 g $MgSO_4$. 1 g NaCl. 0.5 g Na-Acetate	5.21	6.55	94 %	86 %
4 g $MgSO_4$. 0.5 g Mg-Acetate	5.33	6.63	Not analyzed	Not analyzed
4 g $MgSO_4$. 1 g Mg-Acetate	5.54	6.63	89 %	97 %

The resulting pH values indicate that magnesium or sodium acetate is necessary to elevate the pH value of acidic commodities above pH=5 in order to ensure that pymetrozine lays almost entirely in its less polar neutral form. The recoveries achieved with the different variations are shown below:

Table 4: Recovery figures for pymetrozine using different QuEChERS salt compositions (Chlorpyrifos D10 was used as internal standard)

Salt mixture	Orange juice		Cucumber	
Fortification level 0.1 mg/kg (n=5)	Recovery %	CV %	Recovery %	CV %
4 g $MgSO_4$, 1 g Mg-Acetate	86	3	86	2
4 g $MgSO_4$, 0.5 g Na-Acetate, 1 g NaCl	86	2	89	3

Using 10 mL water and the salt mixtures listed above, the volume of the organic phase was measured. In the case of 4 g $MgSO_4$ + 1 g Mg-Acetate the volume of the organic phase was 10.8 mL and in the case of 4 g $MgSO_4$ + 0.5 g Na-Acetate + 1 g NaCl it was 10.0 mL. In principle, such volume deviations can be compensated by internal standards, however if no internal standard is used the second option is favored.

To prove that the poor recoveries of pymetrozine are not due to degradation orange juice was spiked and extracted a) immediately and b) after 1 hour delay. The mean recoveries did not decrease indicating that pymetrozine is not particularly sensitive to degradation at low pH.

Overall Conclusions:

Pymetrozine shows pH-dependent recovery rates using the QuEChERS method. pH should optimally exceed 5 for the pymetrozine recovery rates to be well above 70%. pH can be increased by replacing the citrate salts with acetate salts. The following mixtures were found to work well for 10 g sample: 4 g $MgSO_4$ +1 g NaCl + 0.5 g sodium acetate and 4 g $MgSO_4$ +1 g Mg-Acetate.

[1] The Pesticide Manual 14th Edition. C D S Tomlin. BCPC 2006
[2] Regulation (EU) 2015/401
[3] EFSA Journal 2014;12(9):3817. Conclusion on the peer review of the pesticide risk assessment of the active substance pymetrozine

History

Action	When	Version
Conduction of experiments	June 2015 – February 2016	
Method placed on-line	April 2016	V1