EURL-SRM Analytical Observations Report

concerning the following:

- **Compound(s)**: Internal Standard BNPU (a component of Nicarbazin)
- **Commodities**: Various commodities of plant origin
- **Method(s)**: QuEChERS, QuOil, dSPE-cleanup with PSA, dSPE-cleanup with GCB
- **Instrumentation**: LC-MS/MS, negative mode

Errors due to losses of the Internal Standard BNPU (Nicarbazin) during dSPE cleanup of QuEChERS extracts with GCB or of QuOil extracts with PSA

Reported by: EURL-SRM
Version 1 (last update: 14.04.2016)

Background information / Initial thoughts and Observations:

BNPU shows nice peaks and good signals in LC-MS/MS in the ESI (neg.) mode and is thus often used as Internal Standard (IS) for acidic pesticides. As BNPU recoveries are nearly quantitative with QuEChERS and QuOil methods BNPU appears to be a good IS choice for volumetric corrections provided that there is no losses during cleanup (if this is conducted).

Acidic pesticides tend to experience losses during dSPE cleanup with PSA as sorbent, thus QuEChERS and QuPPe methods foresee the direct analysis of acidic pesticides from the raw extracts.

There is however other pesticides which are also typically measured by LC-MS/MS in the ESI (neg.) mode (such as benzoylureas, propyzamide, oryzalin, flurtamone and fipronil), that do not show notable losses during dSPE cleanup of QuEChERS extracts with PSA. Fortunately BNPH losses by interactions with PSA were also negligible making it principally suitable as IS in these cases.

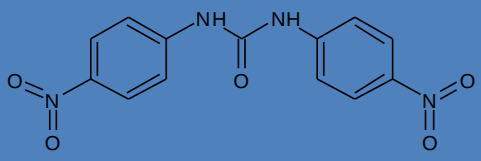
In contrast to QuEChERS, the use of PSA in dSPE cleanup of QuOil extracts results in extensive BNPU-losses. This leads to overestimated results for pesticides experiencing significantly less or no PSA-induced losses.

For the cleanup of extracts of commodities with high content of carotenoids or chlorophyll QuEChERS foresees dSPE cleanup with Graphitized Carbon Black (GCB) sorbent. During preliminary validation experiments it was observed that BNPU experiences considerable losses upon contact with GCB.

Experiments demonstrating these aspects are shown within this observation report.

Compound profile

1,3-bis(4-nitrophenyl)urea (BNPU)	
Mode of action	A component of **nicarbazine** (an equimolar complex between BNPU and HDP (4,6-dimethyl-1*H*-pyrimidin-2-one (= 2-hydroxy - 4,6 dimethylpirimidine). HDP is reported to improve the bio-assimilation of the active component BNPH). It is is used as a coccidiostat for chickens and pigeons.
Synonym	4,4'- dinitrocarbanilide (DNC)
LogP	3.0 (calculated by chemicalize.org) at pH 0-9
Water solubility	46 ppb (pH 7) (Nicarbazin)
pKa	10.8 (calculated by chemicalize.org), very weakly acidic at the urea group
Registration Status	Not approved within the EU
Formula	$C_{19}H_{18}N_6O_6$



Experiments conducted and observations:

In order to study BNPU-losses more closely various QuEChERS and QuOil extracts were cleaned-up by dSPE with PSA and GCB as sorbents (see Table 1). The herbicide propyzamide, which was previously shown not to be affected by PSA or GCB was used as Internal Standard in these experiments.

Table 1: Recovery rates for BNPU, n=2, spiking: 0.1 µg/mL raw QuEChERS extract or raw QuOil extract or acetonitrile (measured by LC-MS/MS in ESI negative mode)

Method used to produce raw extract to be spiked	Commodity extracted by this method	dSPE cleanup approach tested	Recovery rate BNPU %
QuEChERS	Spinach	GCB-1 (2.5 mg GCB / 150 mg $MgSO_4$ per mL)	26
	Spinach	GCB-2 (7.5 mg GCB / 150 mg $MgSO_4$ per mL)	9
	None (10 mL water used)	GCB-1 (2.5 mg GCB / 150 mg $MgSO_4$ per mL)	1
	Cucumber	PSA (25 mg PSA / 150 mg $MgSO_4$ per mL)	103
	None (10 mL water used)	PSA (25 mg PSA / 150 mg $MgSO_4$ per mL)	103
QuOil	Olive Oil	PSA (25 mg PSA / 150 mg $MgSO_4$ per mL)	22
	Hazelnut	PSA (25 mg PSA / 150 mg $MgSO_4$ per mL)	43
	Hazelnut	PSA+W (25 mg PSA / 150 mg $MgSO_4$ + 50µL H_2O*per mL)	98
None	Pure acetonitrile	PSA (25 mg PSA and 150 mg MgSO4 per mL)	23

***** Water added after extraction but before cleanup (50µL to 1 mL)

Discussion and conclusions:

In absence of matrix components the BNPU losses during dSPE cleanup with GCB were nearly quantitative. In spinach extracts, and despite the strong competition by chlorophyll, the losses were more moderate but still dramatic indicating that the affinity of BNPU towards GCB, surpasses that of any other pesticide tested so far. Looking at the 3D structure of BNPU it becomes clear that the molecule can arrange into a largely planar configuration, which explains its strong affinity towards GCB.

When conducting cleanup with PSA as sorbent no BNPU losses were notable in the case of QuEChERS extracts, irrespective of the presence or absence of matrix components. However, when applying dSPE with PSA sorbent to a spiked QuOil extract of oil or Hazelnut or to spiked acetonitrile, BNPU losses were in the range of 60-80%. This can be explained by the absence of water in QuOil extracts and pure acetonitrile. In the case of QuEChERS extracts residual water (ca 7-8% in raw extracts and ca. 2-3% in cleaned-up extracts) interferes with the formation of hydrogen bonds and reduces interactions between BNPU and PSA. A solution to this problem Is the addition of water to QuOil extracts after extraction and before dSPE with PSA. Adding 5 % water (50 µL to 1 mL extract resulted in quantitative recoveries for BNPU (98 %).

Summary

BNPU (a component of nicarbazin) is often used as a volumetric Internal Standard (IS) for acidic pesticides. As IS-losses can lead to severe overestimation of residue values of pesticides it is important that there is no IS losses along the procedure. Extensive BNPU-losses are detected when conducting dSPE-cleanup of QuEChERS extracts with GCB (Graphitized carbon black). So, if nicarbazin (BNPU) is used as IS, dSPE with GCB sorbent should be avoided and vice versa. dSPE cleanup of QuEChERS extracts does not result in any notable losses but if QuOil extract are subjected to cleanup with PSA BNPU losses are dramatic. No notable losses of BNPU are noticed when 5% water are added to QuOil extracts prior to cleanup with PSA.

Document History

Date	Action	Changes
Apr. 2016	Publication of V1	