Long Term Stability Monitoring of Pesticide Stock Solutions by Quantitative NMR – Results

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Introduction

A significant source of error in pesticide residue analysis is the degradation of neat standards, standards in stock solutions, working solutions and sample extracts. QC protocols require laboratories to ensure that this source of error remains insignificant. Currently LC-MS/MS and GC-MS(/MS) are the most widely employed techniques for testing the stability of pesticides. These techniques are sensitive and selective enough for testing of mixtures, but measurement uncertainty requires multiple injections (typically ≥5) to achieve the accuracy required for reliable conclusions about compliance with the stipulated thresholds. An additional disadvantage is the unavailability of the instrument during measurements for routine pesticide analyses of samples and the need to prepare a new stock solution that is measured against the old one, which can be quite costly.

Quantitative NMR - an Alternative!?

Quantitative proton NMR (qNMR) has been gaining popularity e.g. in drug analysis and quality control applications, as it produces qualitative and quantitative information simultaneously. Compared with mass spectrometry, qNMR yields relatively low-sensitivity measurements, but a major advantage is that the reference standard does not need to be the identical material, but only one universal standard unrelated to the target analyte. This considerably reduces the costs associated with the purchase and preparation of "new" standard stock solutions. Additionally, qNMR has been reported to be highly precise and accurate, thus reducing the number of replicate measurements required. The non-destructive nature of this technique makes it possible for samples to be kept for measurements over the course of several years, e.g. in flame-sealed NRM tubes. A comparison of the main characteristics of qNMR and chromatographic techniques is shown here (based on [1]):

Quantitative NMR		LC-MS/MS GC-MS(/MS)
Weight/dilution Non-destructive analysis	**Sample preparation**	Weight/dilutions destructive analysis
Structural properties (restriction: e.g. ¹H)	**Detection**	Physical properties (restriction: e.g. ionization)
Certified reference material (one universal calibrant!)	**Calibrant**	Structurally identical reference needed
Internal/external standard (with/without calibration curve)	**Quantitation**	Internal/external standard (with/without calibration curve)
Low µM	**Sensitivity**	Low nM - pM
Resonance overlapping	**Selectivity & Specifity**	Chromatographic separation = better specifity
Instrument independent	**Reproducibility**	Instrument-dependent
Available to very few pesticide routine labs	**Availability of technique**	Available to most pesticide routine labs

These merits and positive results from pre-tests (e.g. high level of reproducibility and correct quantification) encouraged us to start exploring the suitability of qNMR for monitoring the stability of pesticides.

Experiments

Pesticide stock solutions were prepared at 1 mg/mL in acetonitrile, in formic acid-acidified (0.4 Vol.%) acetonitrile (started in 05/2015) and/or deuterated acetonitrile (started in 07/2017), filled in NMR-tubes, flame-sealed and stored at 4°C. 1H-NMR spectra were recorded at 400 MHz (Bruker Avance 400). As acetonitrile has a proton resonance, the acetonitrile-signal was reduced by solvent suppression techniques. The certified reference standards 3,5-dinitrobenzoic acid or prochloraz were used as universal calibration standards.

Reference: 1. Simmler Ch, Napolitano JG, McAlpine JB; Current Opinion in Biotechnology 2014, 25:51-59

Results

Purity Assessment of Reference Standards

The NMR-spectra of 130 compounds revealed that the reference standards of most pesticides were of high purity as no detectable impurities of organic compounds could be identified in the spectra, even when the recommended maximum storage time has expired. But for some pesticide standards, qNMR measurements revealed lower purities than the purities given by the supplier (Table 1). In case of a newly purchased benfuracarb standard, the observed purity was determined to be 10% lower than the given purity. Here, the degradation product carbofuran was identified.

Table 1 Purity observed by qNMR of some pesticide standards

Pesticide	Purchase Date (Y/M)	Given Expiry Date (Y/M)	qNMR Measur. Date (Y/M)	Expiration (qNMR Meas. Date - Given Exp. Date; Y/M)	Given Purity of Ref. Std. (%)	Purity observed by qNMR (%)
Benfuracarb	2017/04	2020/02	2017/07	(within expiry d.)	99.7	**90**
Dichlorvos	2014/05	2015/07	2017/07	2Y	98	**91.6**
Omethoate	2012/11	2014/08	2017/07	2Y/11M	98	**79**
Dioxacarb	2012/03	2014/01	2017/07	5Y/4M	96.5	**86.5**

Stability Monitoring of Stock Solutions

During the period covered by the study, no or very minor degradation could be observed for most of the compounds. Neither new NMR-signals nor decreasing integrals could be observed in the spectra indicating that no degradation occurred. Regarding the NMR measurements over a time course of more than one year, the individual NMR-signals revealed a good correlation to the concentration of the freshly prepared solution and very low relative standard deviations (data not shown). Both results indicate a high level of reproducibility and correct quantification. Exemplary results of this study are shown in Table 2 for some selected pesticide stock solutions.

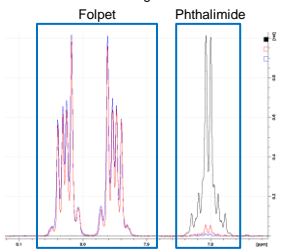**Stability Monitoring of Folpet Stock Solution**

Figure 1. Superimposed NMR-spectra of the freshly prepared folpet stock solution using acidified acetonitrile as solvent (blue), of the same solution after 2 years of storage (red) and of a phthalimide-stock solution (black). Phthalimide was identified in the 2-year-old folpet stock solution (red NMR-signals at 7.8 ppm).

Table 2 Stability monitoring of pesticide stock solutions

Pesticide	Solvent [*]	Concentration of Stock Solution [mM]		%-Difference from Observed Concentration by qNMR after … Months of Storage at 4°C			
		Nominal (Weighed [**])	Observed by qNMR	3	6	12	24
Folpet	A	3.4	3.6	-0.9	-2.9	-2.4	-4.2
	B		3.37	-0.7	0.4	-0.7	-7.9
	C		3.47	-1.4	-0.1	[/]	[/]
Captan	A	3.33	3.33	-0.4	-2.3	-2.6	-2.8
	B		3.33	0.2	2.0	-0.2	-2.9
	C		3.36	1.2	1.0	[/]	[/]
Difenoconazole	A	2.46	2.51	1.5	1.2	1.8	-2.3
	C		2.52	0.1	0.6	[/]	[/]
Carbofuran	B	4.52	4.51	-1.0	-0.2	-2.5	-3.4
	C		4.52	3.0	3.0	[/]	[/]
Dicofol	B	2.7	2.80	-2.0	-0.2	-1.5	-4.7
	C		2.70	0.8	1.1	[/]	[/]
Tolylfluanid	A	2.9	2.93	-0.4	-1.4	-3.2	-5.1
	B		2.92	-3.6	-3.1	-4.5	-6.7
	C		2.96	0.2	-0.8	[/]	[/]
Alanycarb	C	2.5	2.52	-3.1	-5.2	[/]	[/]
Amidosulfuron	C	2.63	2.76	-10.7	-17.2	[/]	[/]

[*] A: acetonitrile, B: formic acid-acidified (0.4 Vol.%) acetonitrile, C: deuterated Acetonitrile; [**] 1 mg/ml; [/] NMR measurements in 07/2018 and 07/2019

Summary

Although some drawbacks have to be considered (e.g. resonance overlapping, technique is available only to very few pesticide routine labs) the outcome of this study indicates that qNMR is a reliable technique to assess the purity of neat standards and the intrinsic stability of pesticide stock solutions at specified storage conditions. The results of this study will be imported into the compound stability database of the EURL DataPool-website.

EPRW 2018

Baden-Württemberg