Observations concerning…

☑ ***a compound*** ☑ ***a matrix*** ☐ ***a method*** ☐ ***other***

Matrix-induced Signal Enhancement of Propamocarb in LC-MS/MS

Reported by: EURL-SRM

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Brief description of problem/observation/solution:

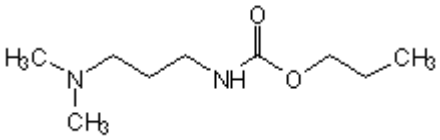
Very strong LC-MS/MS signal enhancement effects of propamocarb in QuEChERS extracts of cucumber and lettuce were observed when applying a quick method with poor chromatographic retention of propamocarb. Virtually no signal enhancement/suppression effects were observed using a different LC separation approach where propamocarb was better retained.

Compound profile:

Propamocarb is a systemic fungicide with protective action which is commonly used on leafy and fruiting vegetables as well as on potatoes. In formulations propamocarb is mostly contained as the hydrochloride salt. It is applied by watering or spraying but it also used for seed treatment. Propamocarb is absorbed by the roots and leaves, and transported acropetally.

Parameter	Value	Notes
Pka	9.6	at 20°C[1] (basic compound)
LogP	-1.2	at pH7
Water solubility	1000 g/L	for the hydrochloride
Hydrolytic behavior	stable	<10% losses at pH 4, 7 & 9 at 50ºC over 5 days (in water)
Residue definition EU	Propamocarb (Sum of propamocarb and its salt expressed as propamocarb)	
Authorized in…[2]	AT, BE, BG, CY, CZ, DE, DK, EE, EL, ES, FI, FR, HU, IE, IT, LT, LU, LV, MT, NL, PL, PT, RO, SE, SI, SK, UK	

Propamocarb



[1] *EFSA Scientific Report* (2006) 78, 1-80, Conclusion on the peer review of propamocarb, http://www.efsa.europa.eu/en/efsajournal/doc/78r.pdf

[2] EU-Pesticides-Database by DG_SANCO (http://ec.europa.eu/sanco_pesticides)

Experiments conducted and observations:

Strongly deviating propamocarb results were obtained from the same extracts using different LC-MS/MS conditions and calibration standards on pure solvent in both cases. To explore this phenomenon the following solutions were prepared and analyzed by both LC-MS/MS methods.
The results of this experiment are summarized in the following table.

#	Solutions prepared	Spiked Propamocarb Level	1st method	2nd method
			Signals in %	
1	**QuEChERS extract of blank cucumber**	-	< 0,1%	< 0,1%
2	**QuEChERS extract of blank lettuce**	-	< 0,1%	< 0,1%
3	**Std in QuEChERS extract of blank cucumber**	0.1 µg/mL	**486%**	107%
4	**Std in QuEChERS extract of blank lettuce**	0.1 µg/mL	**492%**	92%
5	**Std in neat acetonitrile**	0.1 µg/mL	100%*	100%*

*Set at 100%

Materials:

Propamocarb (purity 98%), purchased from Dr. Ehrenstorfer (Cat #: 16390000)

Instrumentation details:

1st LC-MS/MS method:	
LC	WATERS Acquity UPLC
MS/MS	ABSCIEX API 4000 Q-Trap, run in ESI positive mode
MRMs	189/102; 189/144; 189/74
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 / 90 / 10 5 / 10 / 90 5.1 / 10 / 90 11 / 90 / 10
Flow	0.4 mL min^{-1}
Injection volume	2 µL, partial loop with needle overfill
Column temperature	40°C

2nd LC-MS/MS method:	
LC	Shimadzu HPLC Prominence
MS/MS	ABSCIEX API 3200 Q-Trap, run in ESI positive mode
MRMs	189/102; 189/144
Column	Phenomenex Synergi 2.5µ Fusion RP100A, 2x100 mm
Pre-column	Fusion-RP, C18 Polar Embedded, 4x2 mm
Mobile Phase	A: 5 mmol NH_4formate in purified water B: 5 mmol NH_4formate in Methanol
Gradient	

Time min	Mobile Phase A %	Mobile Phase B %
0	100	0
1	100	0
2	60	40
12	10	90
17	10	90
17.1	100	0
22	100	0

Flow	0.3 mL min^{-1}
Injection volume	5 µL
Column temperature	40°C

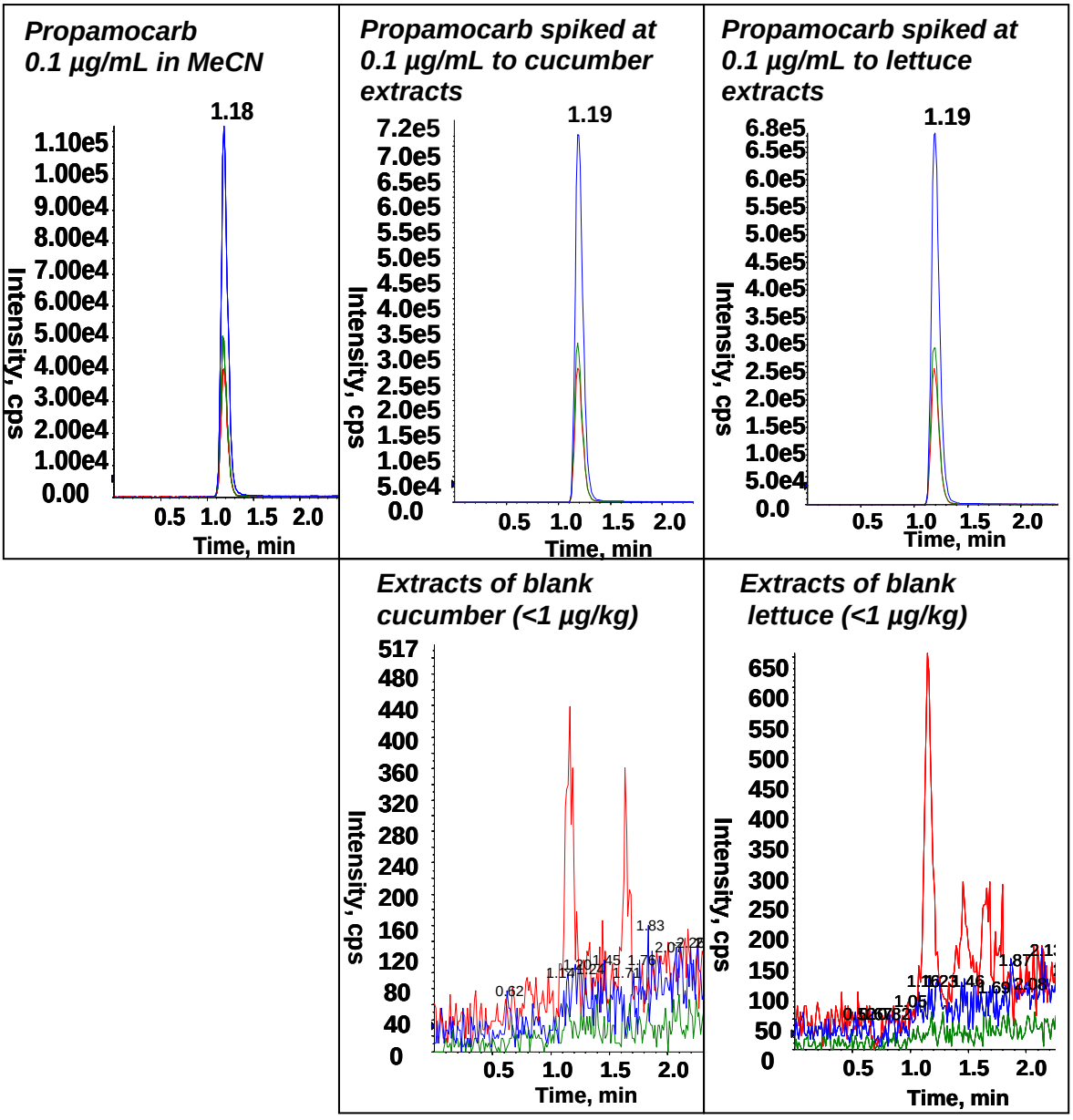
Discussion and Conclusions:

In the **1st method**, separation was conducted on a relatively non-polar stationary LC-phase with the gradient composition starting at 10% methanol (90% water). Propamocarb eluted very early under these conditions (RT at 1.2 min) obviously co-eluting with certain matrix components inducing strong signal enhancement.
In the **2nd method**, a polar embedded C18 phase was employed which allowed the gradient to start at 100% aqueous conditions thus allowing a good retention of propamocarb via lipophilic interactions (RT at 5.7 min) and obviously also a good separation from the interfering matrix components. No enhancement or suppression could be detected in the second approach.

Matrix-induced signal enhancement in LC-MS/MS (ESI-mode):
A possible explanation for the matrix-induced signal enhancement phenomenon is the co-elution of the analyte with compounds that facilitate the release of analyte ions within the ion-source. Such compounds can be bipolar molecules that, having a surfactant activity, can reduce the surface tension of the ion-spray droplets especially at highly aqueous compositions thus facilitating the release of analyte ions from the ion-spray micro-droplets (either via direct release from the droplet surface or indirectly through the facilitation of coulomb explosions). This results in a higher yield of free ions released into space within the ion-source.

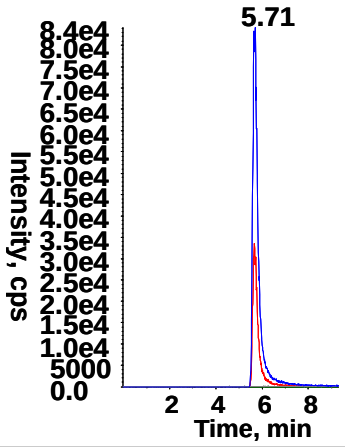
Annex:

LC-MS/MS chromatograms for 1st Methos:

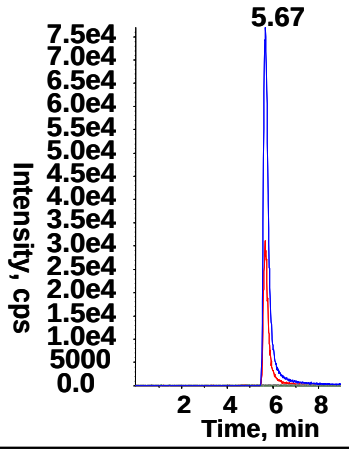


LC-MS/MS chromatograms for 2nd approach:

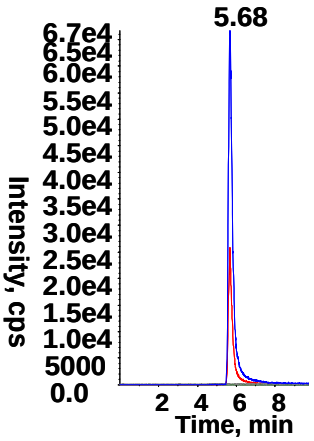
Propamocarb 0.1 µg/mL in MeCN



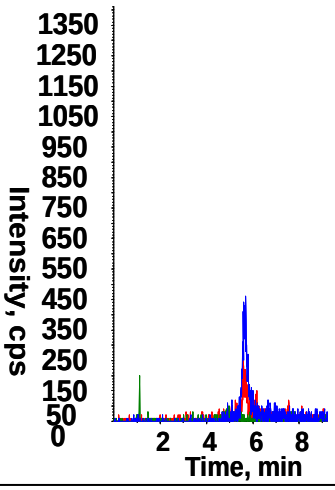
Propamocarb spiked at 0.1 µg/mL to cucumber extracts



Propamocarb spiked at 0.1 µg/mL to lettuce extracts



Extracts of blank cucumber (<1 µg/kg)



Extracts of blank lettuce (<1 µg/kg)