Development and validation of a high-throughput method for biomonitoring exposure of invertebrates to contaminants in aquatic systems

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1. Introduction

The aim of this work was to find a faster and more effective extraction method to help measure pharmaceuticals in aquatic systems

- Drugs enter the environment through human use
- Aquatic organisms are exposed but effects are not well understood
- Extracting compounds can pose many analytical challenges

2. Experimental

Testing micro-elution solvents (50 µL):

- Methanol
- Acetonitrile
- 50:50 Methanol:Acetonitrile
- 50:50 Ethyl acetate: acetone

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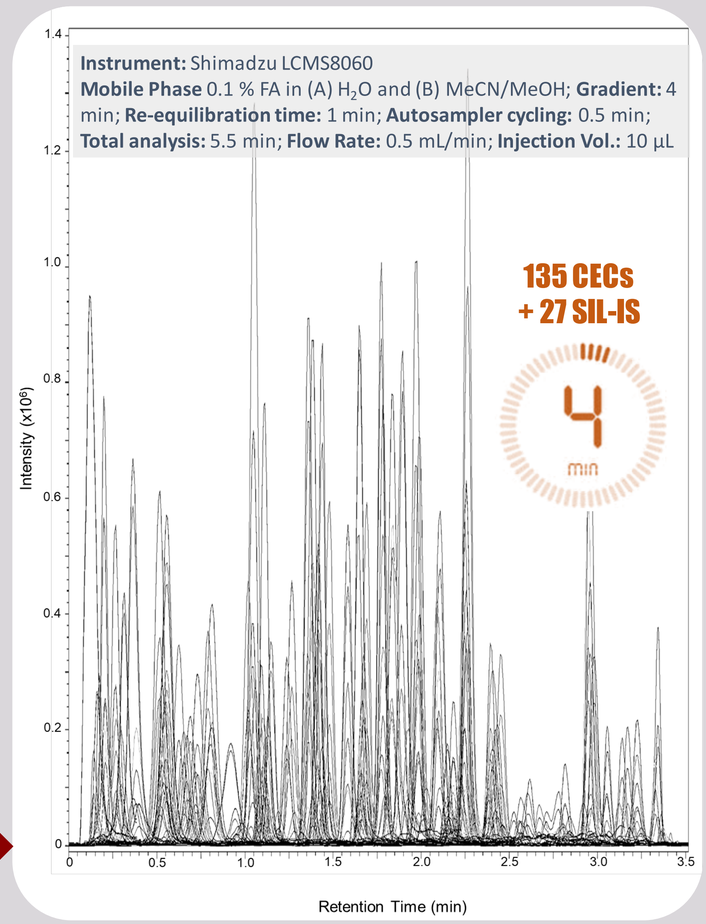

Fig. 1. Chromatogram showing all 135 compounds + 27 internal standards (for more info, see **3.14.1** *)*

3. Results & Discussion

Though clear chromatograms were obtained for many compounds (Fig 3.), unusually high and irreproducible recovery were observed using micro-elution (Table 1). The cause was found to be evaporation of eluate from the wells (Fig. 2).

Table 1. Summary statistics of elution in ultra-pure water to prevent evaporation

	Intra-day precision (RSD %)	Recovery (%)
Min	3	8
Max	141	12130
Mean	25	238
Standard deviation	±19	±468

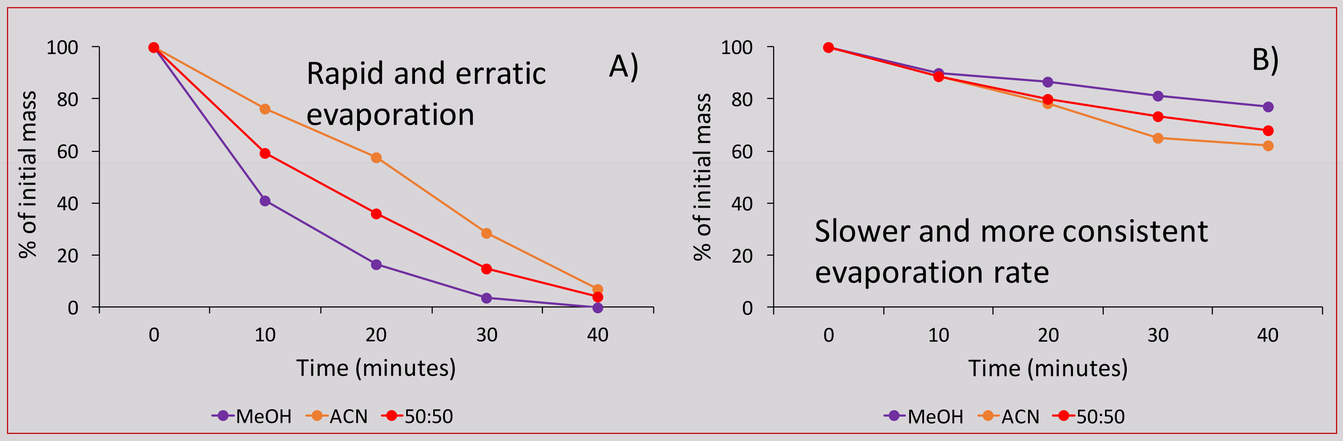

Figure 2. *Evaporation of A) 30 uL of organic solvent alone, and B) same solvents but with 30 uL of water in the well*

Table 2. Summary of analytical performance characteristics for 67 contaminants of emerging concern in Gammarus pulex using PuLE, 2xSPE, and LC-MS/MS

	Linearity	Precision			Matrix Effect	Inaccuracy		Sensitivity	
	n≥5 (max n=10)	RSD% n=3	RSD% n=3	Inter Day n=3	Recovery n=5	n=5	CV%, n=3		LLOD n=6	LLOQ n=6
	R^2	at 25 ng g^{-1}	at 100 ng L^{-1}	100 ng L^{-1}	50 ng g^{-1}	50 ng g^{-1}	25 ng g^{-1}	100 ng g^{-1}	ng g^{-1}	ng g^{-1}
Median	0.9955	9	8	15	76	54	9	1	1	2
Mean	0.9950	9	8	14	74	51	9	4	1	3
(± SD)	(±0.0033)	(±5)	(±4)	(±4)	(±13)	(±20)	(±14)	(±14)	(±1)	(±4)

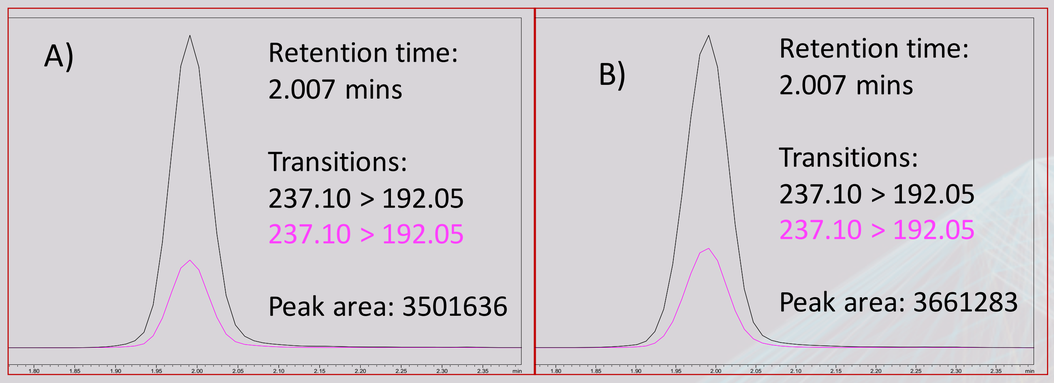

Figure 3. *Chromatograms showing A) Standard of 250 ng/L carbamazepine in 10mM ammonium acetate and B) 250 ng/L carbamazepine subjected to µ-elution SPE and eluted in methanol*

5. Conclusions

- ✓ Evaporation was a major limitation of micro-elution SPE
- ✓ Evaporation led to inconsistent recovery values
- ✓ Methanol was performed better as an elution solvent for pharmaceuticals
- ✓ Water may slow evaporation of small solvent volumes

6. Future

- ✓ Investigate other methods to overcome evaporation
- ✓ Understand uptake of pharmaceuticals into biota using multi-platform analysis

Options to reduce result of evaporation:

- Let solvent evaporate completely then reconstitute
- Have water in wells before elution.

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References: [1] https://www.youtube.com/watch?v=UMzjCOWjs_8