

**Analytical and Bioanalytical Chemistry**

**Electronic Supplementary Material**

**Development of an LC-MS method for determination of nitrogen-containing heterocycles using mixed-mode liquid chromatography**

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**Table S1** Chromatographic parameters used for investigation of the separation performance

Parameter	Symbol used	Unit	Measurement
Void time	$t_0$	min	By injecting NaBr
Retention	$t_R$	min	
Retention factor	$k'$	-	$k' = \frac{t_R - t_0}{t_0}$
Peak width	$W_{50}$	min	At 50% height
Peak asymmetry	$A_s$	-	At 10% height
Selectivity	$\alpha$	-	Ratio of the retention factor to the retention factor of the previous peak
Efficiency	$N$	Plates/Column	$5,54 \times \left(\frac{t_R}{W_{50}}\right)^2$
Resolution	$R_s$	-	$\frac{1}{4} \times \sqrt{N} \times \frac{k'}{(k' + 1)} \times \frac{(\alpha - 1)}{\alpha}$

**Table S2** Empirical constants calculated to describe retention factor ( $\log(k')$ ) in isocratic elution of NCHs at different ACN volume fractions according to Equation 4 in the main text.. Eluent A: Water + 0.1% FA, Eluent B: ACN Flowrate 300  $\mu\text{L}/\text{min}$ , 5  $\mu\text{L}$  injection volume. Separation column: Primesep 200

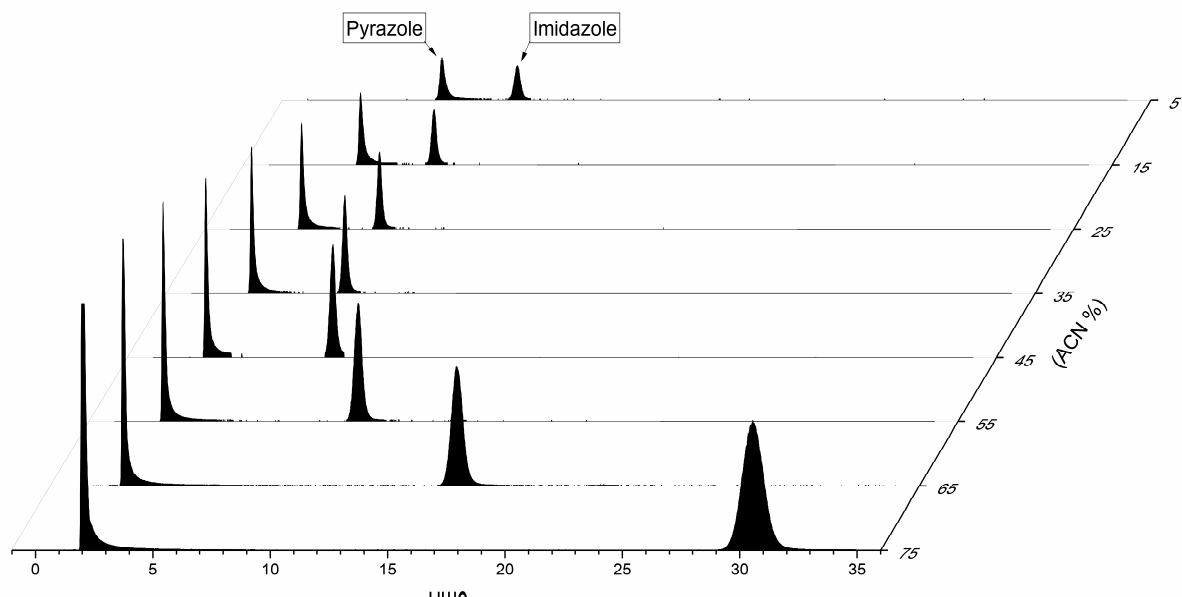
Compound	$m_1$	$m_2$	C	SSE	$R^2$
Imidazole	$2.63 \pm 0.37$	$4.31 \pm 0.45$	$-1.83 \pm 0.34$	0.00997	0.979
Pyrazole	$2.48 \pm 0.24$	$1.75 \pm 0.31$	$-1.85 \pm 0.23$	0.00457	0.991
Pyridine	$2.76 \pm 0.66$	$4.16 \pm 0.81$	$-1.72 \pm 0.6$	0.03187	0.901
Pyridazine	$3 \pm 0.41$	$2.81 \pm 0.51$	$-2.44 \pm 0.38$	0.01254	0.962
Piperidine	$3.18 \pm 0.61$	$4.76 \pm 0.75$	$2.09 \pm 0.56$	0.02788	0.932

**Table S3** Gradient of the separation of NCHs in method optimized for surface water measurement

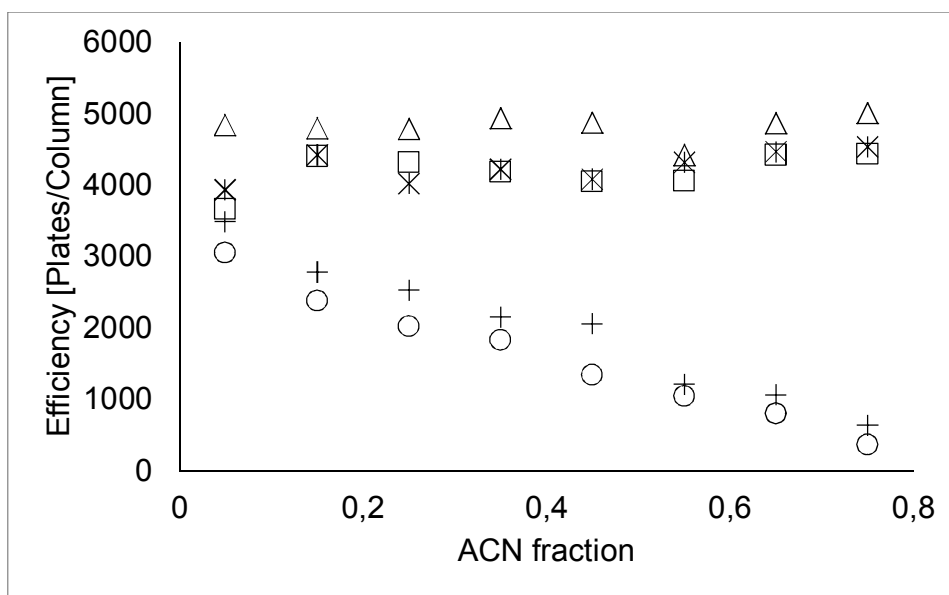
<b>Time</b>	<b>Eluent A Water + 0.1% FA [%]</b>	<b>Eluent B ACN + 0.2% FA [%]</b>
0	95	5
5	95	5
8	50	50
15	50	50
15.01	95	5
25	95	5

**Table S4** MS settings for measurement of NCHs

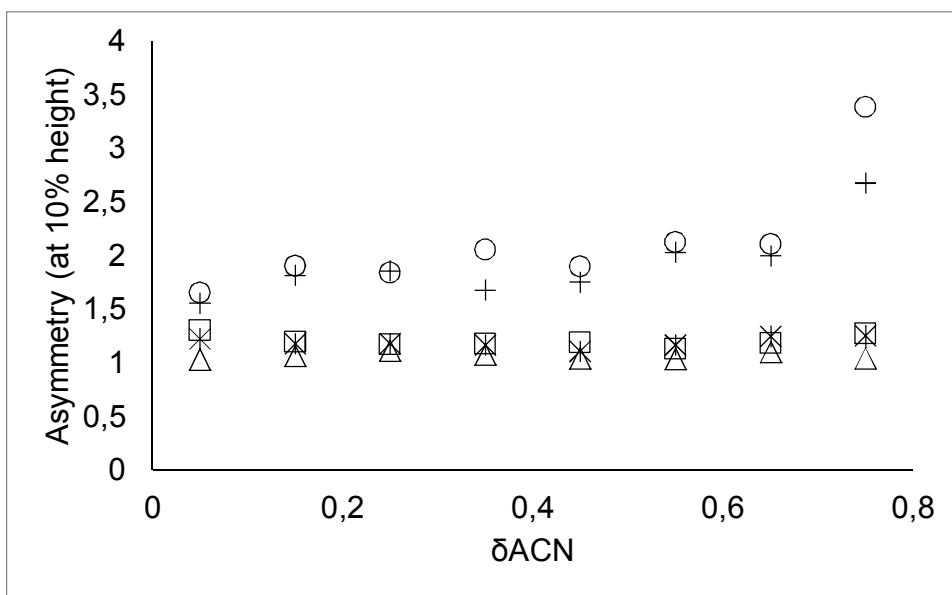
	<b>Signal 1 TIC</b>	<b>Signal 2 SIM</b>		<b>Signal 3 SIM</b>	
Cycle percent	<b>20</b>	<b>40</b>		<b>40</b>	
Mass range	65-90	81	80	69	86.10
Time span	0-25	0-8	8-25	0-10.5	10.5-25



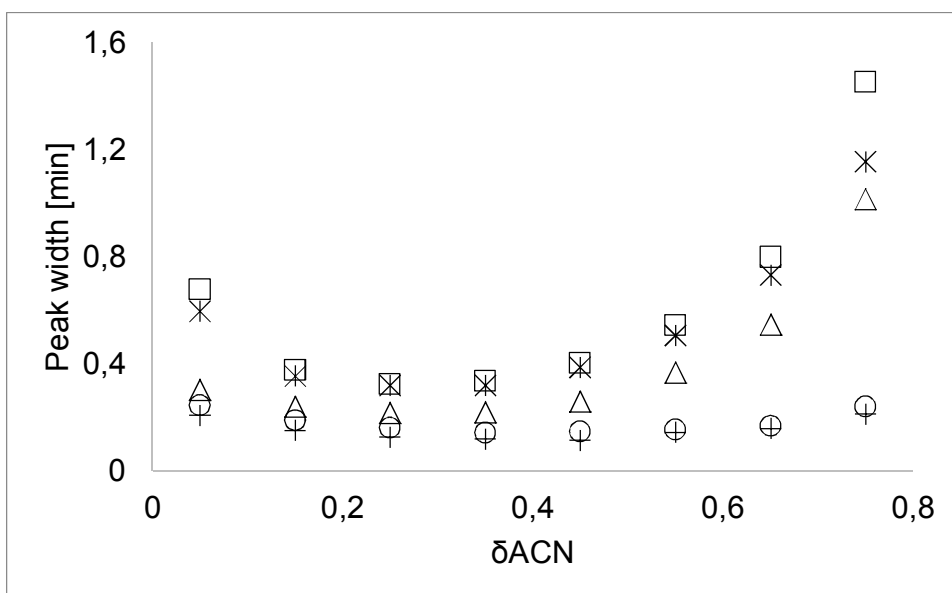
**Fig. S1** EIC ( $m/z = 69$ ), of pyrazole and imidazole at different  $\delta_{ACN}$  (isocratic) showing different modes of interaction. NHCs concentration  $1000 \mu\text{g/L}$ , Eluent A: Water +  $0.1 \text{ FA}$ , Eluent B: ACN Flowrate  $300 \mu\text{L/min}$ ,  $5 \mu\text{L}$  injection volume. Primesep 200



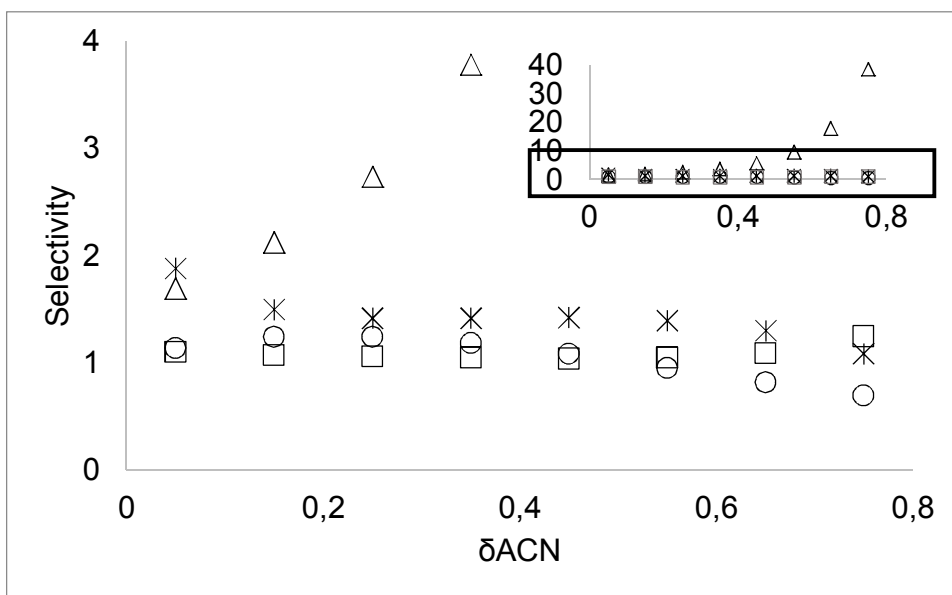
**Fig. S2** Efficiency in isocratic elution of NCHs at different ACN volume fractions. Imidazole (triangles) pyrazole (circles) pyridine (stars) pyridazine (crosses), and piperidine (squares) Eluent A: Water +  $0.1\% \text{ FA}$ , Eluent B: ACN Flowrate  $300 \mu\text{L/min}$ ,  $5 \mu\text{L}$  injection volume. Separation column: Primesep 200



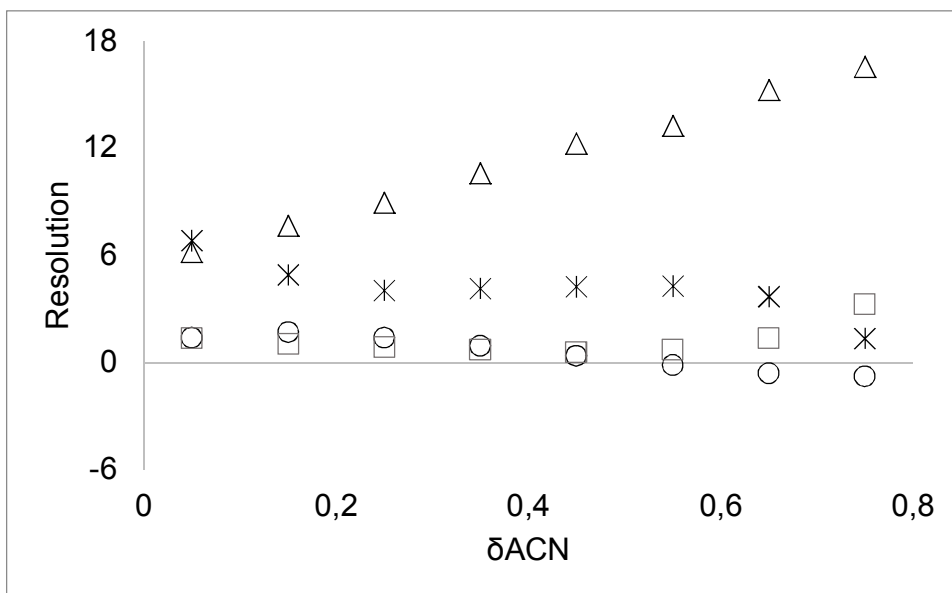
**Fig. S3** Peak asymmetry in isocratic elution of NHCs at different ACN volume fractions. Imidazole (triangles) Pyrazole (circles) Pyridine (stars) Pyridazine (crosses) and piperidine (squares) Eluent A: Water + 0.1 Formic acid, Eluent B: ACN Flowrate 300  $\mu\text{L}/\text{min}$ , 5  $\mu\text{L}$  injection volume. Separation column: Primesep 200



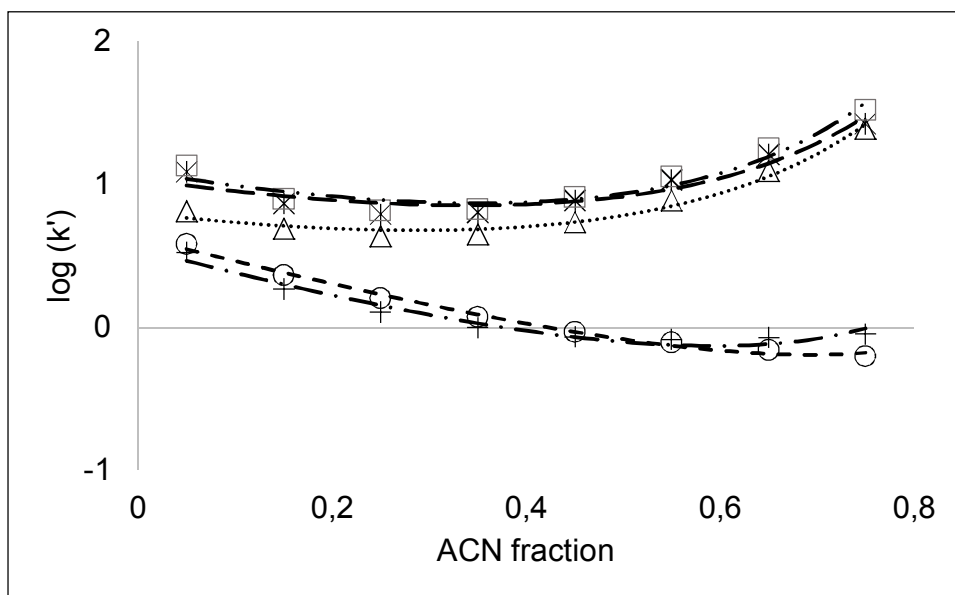
**Fig. S4** Peak width in isocratic elution of NHCs at different ACN volume fractions. Imidazole (triangles) Pyrazole (circles) Pyridine (stars) Pyridazine (crosses) and piperidine (squares) Eluent A: Water + 0.1 Formic acid, Eluent B: ACN Flowrate 300  $\mu\text{L}/\text{min}$ , 5  $\mu\text{L}$  injection volume. Separation column: Primesep 200



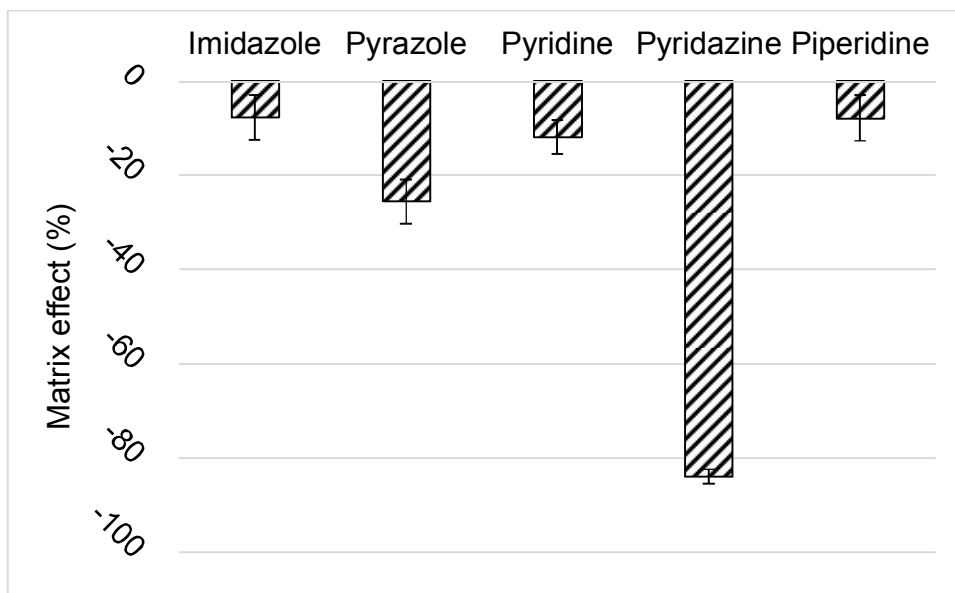
**Fig. S5** Selectivity in isocratic elution of NHCs at different ACN volume fractions. (main figure represents an enlarged view of the black-bordered box of the insert) Imidazole (triangles) Pyrazole (circles) Pyridine (stars) Pyridazine (crosses) and piperidine (squares) Eluent A: Water + 0.1 Formic acid, Eluent B: ACN Flowrate 300  $\mu$ L/min, 5  $\mu$ L injection volume. Separation column: Primesep 200



**Fig. S6** Resolution in isocratic elution of NHCs at different ACN volume fractions. Imidazole (triangles) Pyrazole (circles) Pyridine (stars) Pyridazine (crosses) and piperidine (squares) Eluent A: Water + 0.1 Formic acid, Eluent B: ACN Flowrate 300  $\mu$ L/min, 5  $\mu$ L injection volume. Separation column: Primesep 200



**Fig. S7** Experimental and calculated logarithm of retention factor ( $\log(k')$ ) in isocratic elution of NCHs at different ACN volume fractions. Imidazole (triangles, dotted line) pyrazole (circles, dashed line) pyridine (stars, long dashed line) pyridazine (crosses, long dash-dotted line), and piperidine (squares, long dash double dotted line). Eluent A: Water + 0.1% FA, Eluent B: ACN Flowrate 300  $\mu\text{L}/\text{min}$ , 5  $\mu\text{L}$  injection volume. Separation column: Primesep 200



**Fig. S8** Matrix effect observed in the measurement of NCHs in spiked surface water showing ion suppression for all NCHs. Flowrate 300  $\mu\text{L}/\text{min}$ , 5  $\mu\text{L}$  injection volume. Separation column: Primesep 200, Gradient according to Table S2. MS condition according to Table S3