Application Note 285

Highly sensitive determination of trace-level chlorophenols and common odorants in drinking and environmental waters

Water is a heavily regulated substance and understanding its volatile organic compound (VOC) content is crucial to ensuring consumer and environmental health. With multiple methods needing to be used to detect all analytes of concern in a sample, this makes analysis labour-intensive.

A single method is presented for the combined analysis of two important classes of contaminants in drinking and environmental waters – chlorophenols and common odorants – using immersive HiSorb high-capacity sorptive extraction and GC—MS. This method is highly sensitive, with limits of detection approximately 5 ng/L for chlorophenols and <1 ng/L for common odorants. Laboratory tests confirm excellent linearity and reproducibility, while analyses of real-world samples have confirmed the method's performance on a range of water matrices. It can also be fully automated, enabling unattended, high sample throughput of approximately 32 samples per system per day.

Introduction

Local authorities need to invest heavily to ensure drinking water is safe and palatable, that open waters such as lakes and rivers are environmentally sound, and that there is no contamination in ground waters that might leech into the drinking water supply. Water quality is determined in part by the VOC content, which can negatively affect human health, harm aquatic wildlife and/or impart tastes or odours that residents find unpleasant.

Used in industry as intermediates, chlorophenols are also used as disinfectants and pesticides. For outdoor fixtures such as telegraph poles, fence posts and garden furniture, pentachlorophenol (5CP) is primarily used as a wood preservative[1]. 2,3,4,5-tetrachlorophenol is also used but to a lesser extent. 5CP can also break down in the environment, especially in the presence of micro-organisms, losing chlorine atoms to give tetra- and trichlorophenols[2]. Chlorophenols are harmful to human health at high concentrations and can give an unpleasant antiseptic-like odour to water. They are regulated by various bodies including the US Environmental Protection Agency (EPA), the Korean Ministry of Environment and the European Commission, with the EPA stipulating a limit of 1 µg/L for 5CP in drinking water[3]. Standard methods for detecting chlorophenols including ISO 8615-2:1999 and US EPA Method 604 specify liquid-liquid extraction with derivatisation. These methods are manual, generate a lot of solvent waste and use harmful derivatising agents.

In addition to chlorophenols, many common odorants affect drinking and environmental waters. These odorants include methoxypyrazines, halogenated anisoles and some terpenes, and are naturally produced by microorganisms in the water. They have extremely low human odour threshold limits - in the region of 1 to 10 ng/L[4]. In particular, the terpenes geosmin and 2-methylisoborneol (MIB) are pervasive causes of customer complaints to water companies. Analytical methods must therefore be robust and of high-throughput for fast routine screening of large numbers of samples, and sensitive enough to detect rising odorant concentrations before they become noticeable to consumers. Most existing methods sacrifice at least one of these qualities in their testing and additionally, are not compatible with chlorophenols analysis. Laboratories attempting to analyse both must perform multiple tests on the same sample.

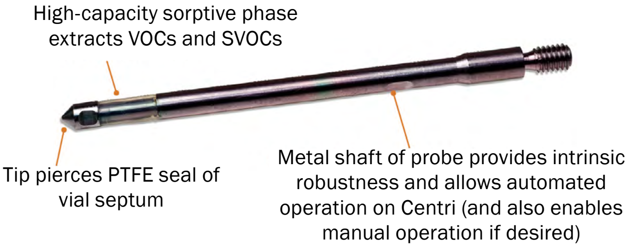


Figure 1: HiSorb probe.

HiSorb probe is inserted into sample vial

Vials are incubated and agitated for analyte extraction

After extraction, probes washed and dried to remove residual matrix before desorption

HiSorb probes fit into industry standard-size TD tubes for analysis by thermal desorption

Figure 2: HiSorb sampling and analysis workflow.

Figure 3: Markes' systems for GC–MS. Left: TD100-xr. Right: Centri 360.

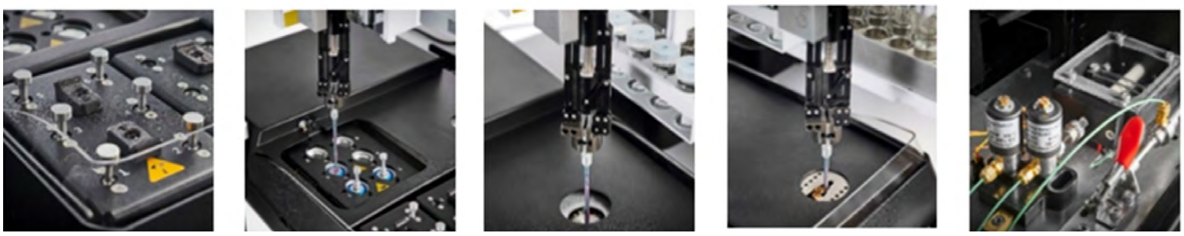
Simultaneous analysis

A simultaneous analysis of chlorophenols and other common odorants uses HiSorb™ high-capacity sorptive extraction probes in conjunction with gas chromatography—mass spectrometry (GC—MS). HiSorb (Figure 1) are robust, stainless-steel probes bearing a sorptive phase. Available HiSorb phases include pure poly(dimethylsiloxane) (PDMS), PDMS with carbon wide range (CWR), PDMS with divinylbenzene (DVB), and a combination of all three – DVB/CWR/PDMS. For sampling, the probe can either be suspended in the headspace above a sample or, as in this study, immersed directly in it. Analytes are absorbed from the sample matrix (water) onto the sorptive phase for 60 mins for this application, after which the probe is removed, washed and dried to remove residual sample matrix. It is then desorbed for analysis by thermal desorption (TD) (Figure 2). HiSorb probes are compatible with industry-standard TD tubes and can be analysed on any suitable thermal desorber such as Markes' TD100-xr™ (Figure 3).

HiSorb probes can also be paired with the Centri® 360 autosampler platform (Figure 3) which automates all aspects of HiSorb operation, from initial introduction of the probe to the sample vial through to probe desorption (Figure 4).

Markes' systems for GC–MS feature an electronically cooled focusing trap which, with its back-flush operation, retains a wide volatility range of analytes while allowing interferences such as residual water to be purged to vent. The trap is rapidly heated (> 100°C/sec) so analytes are transferred to the head of the GC column in a narrow band of vapour, enhancing peak shape.

Water testing laboratories often have large numbers of samples to process over short timeframes, so high throughput is important. Sample overlap on the TD system is critical to reducing overall run time to increase throughput per day. It works by desorbing the next sample while the previous sample is being analysed on the GC. On Centri 360, the multiple vial slots on the agitator allow up to six samples to undergo automated simultaneous extraction. This enables the next sample to be injected to the column immediately following the analysis of the previous sample (known as "prep-ahead" mode, Figure 5). Throughput becomes limited by GC run time only.



Probe storage on Centri platform

Sample extraction, incubation and agitation

Automation probe wash/dry to remove residual matrix

Analyte desorption to focusing trap

Rapid injection from trap to GC-MS

Figure 4: Automated HiSorb workflow on Centri 360.

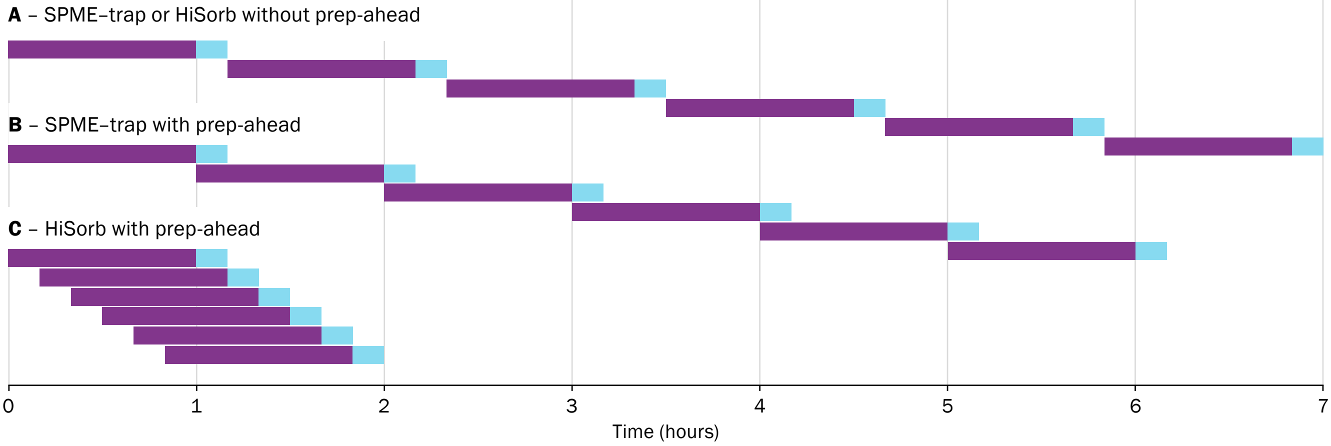


Figure 5: HiSorb prep-ahead operation on Centri 360. (🟦GC run time, 🟪Sample extraction time).

Experimental

Target compounds:

Target compounds for this study are described in Table 1.

Compound	Abbreviation	Quant ion	Confirming ion
2-Chlorophenol	1CP	128	64
2,4-Dichlorophenol	2CP	162	98
2,4,6-Trichlorophenol	3CP	196	132
2,3,4,6-Tetrachlorophenol	4CP	232	131
Pentachlorophenol	5CP	266	165
2-Isopropyl-3-methoxypyrazine	IPMP	137	152
2-Isobutyl-3-methoxypyrazine	IBMP	124	151
2-Chloroanisole	2-CA	142	99
3-Chloroanisole	3-CA	142	112
4-Chloroanisole	4-CA	142	127
2,3,4-Trichloroanisole	234-TCA	195	210
2,4,6-Trichloroanisole	246-TCA	210	197
2,4,6-Tribromoanisole	TBA	344	331
2-Methylisoborneol	MIB	95	107
Geosmin	-	112	55

Table 1: Target compounds.

Samples and Standards:

Calibration standards:

A primary stock (S1) was prepared in methanol to a concentration of 10 mg/L (chlorophenols) or 1 mg/L (other odorants) and stored at 4°C. Ahead of each analytical batch, a secondary stock (S2) was freshly prepared from S1 by 1,000-fold dilution in HPLC-grade water, and this secondary stock used to generate calibration standards as shown in Table 2. The concentration levels of the compounds used for the calibration range reach below the minimum odour threshold. The low concentrations enable the detection of compounds below the level where they become problematic and cause consumer complaints, in addition to higher concentrations that are often detected in real sample scenarios. Standards were dispensed into 20 mL vials each containing 1.5 g NaCl, and toluene-D8 was added at 20 ng/L as an internal standard.

Water samples:

Samples are described in Table 3. All were collected from the UK. These were prepared in the same way as the calibration standards except that sample water was used in place of HPLC-grade water, and no target analytes were added.

Name	Description	Chlorophenols concentration (ng/L)	Chlorophenols concentration (ng/L)
S1	Stock solution 1 in methanol	$1\text{x}10^7$	$1\text{x}10^6$
S2	Stock solution 2 in water	10,000	1,000
C1	Calibration 1	5	0.5
C2	Calibration 2	10	1
C3	Calibration 3	20	2
C4	Calibration 4	50	5
C5	Calibration 5	100	10
C6	Calibration 6	200	20
C7	Calibration 7	500	50
C8	Calibration 8	1,000	100
C9	Calibration 9	1,500	150

Table 2: Stock and calibration standards prepared.

Sample	Description	Colour	Odour	Particulates
A	Soft tap water from site A	Colourless	None	None
B	Hard Tap water from site B	Colourless	None	None
C	Soft Tap water from site C	Colourless	None	None
D	Raw spring water from site C’s source	Faintly yellow	None	None
E	Branded bottled spring water	Colourless	None	None
F	Water from a garden pond	Strongly yellow-green	Strong musty	Many, large
G	Water from lake G	Faintly yellow	None	Few, small
H	Water from lake H	Faintly yellow	None	Few, small
I	Freshly collected rainwater	Colourless	None	Few, small

Table 3: Water samples analysed in this study.

Autosampler:

Sample extraction and concentration platform: Centri 360 (Markes International)

Immersive HiSorb extraction:

Probe:	Standard-length, inert-coated stainless-steel, PDMS (part no. H1-AXAAC), PDMS/DVB (H3-AXAAC) or PDMS/CWR/DVB (H4-AXAAC).
Incubation:	45°C at 600 rpm, 60 min
Probe desorption:	260°C, 15 min

Preconcentration:

Flow path:	180°C
Focusing trap:	Tenax/Carbon multi-sorbent trap (part no. U-T12ME-2S)
Trap low:	30°C
Pre-desorption purge:	1 min at 50 mL/min
Trap desorption:	Max heating rate to 320°C, held for 5 min
Outlet split ratio:	3:1

GC–MS:

Column:	5-MS, 30 m x 0.25 mm x 0.25 µm
Constant flow:	Helium, 2 mL/min
Oven:	50°C (5 min hold), then 10°C/min to 280°C (5 min hold)
Transfer line:	230°C
Ion source:	150°C
Acquisition:	Selected ion monitoring (SIM) mode using quant and confirming ions from Table 1.

Results and discussion

Method development

Extraction phase selection

The method was optimised by sequentially testing various parameters to maximise response across target analytes. The most influential parameter was the sorptive phase combination on the HiSorb probe. While peak areas for other odorants were only weakly influenced by this, there was a striking effect on the chlorophenols with the DVB/CWR/PDMS probes substantially outperforming the other two combinations trialled (Figure 6). The strength of this effect depended on the number of chlorine atoms present, with an approximately 10-fold improvement over the PDMS-only probe observed for 1CP, and a 73-fold improvement observed for 5CP. Hence, we used DVB/CWR/PDMS probes for all further work.

Chromatography

Figure 7A shows SIM analysis of calibration standard C3. All compounds are readily detected, with excellent peak shapes. 2CP (#6) and IBMP (#7) partially co-elute, but as they have different quant and confirming ions, they are easily resolved. Figure 7B shows peaks from 7A (blue) overlaid with the analysis of a standard 4-times lower, at 5 ng/L for chlorophenols and 0.5 ng/L for other odorants (C1), demonstrating easy detection and excellent peak shapes even at extremely trace levels.

Method performance

Linearity

A calibration series comprising eight levels between 5 and 1,500 ng/L (chlorophenols) or 0.5 – 150 ng/L (other odorants) was analysed. R^2 values ranged from 0.9981 to 0.9997 (Table 4), indicating excellent linearity across all analytes.

Intra-batch reproducibility

Eight replicates of mid-level laboratory standard C4 were analysed, and the relative standard deviation (RSD) was calculated for each compound. RSDs ranged from 3.92% to 7.94%, except for 1CP and 5CP, which had values of 10.39% and 22.32% respectively (Table 4). Reproducibility is excellent for most target analytes and is good even for the chlorophenols. Further method development, such as increasing desorption temperature and flow rate, should improve the desorption of these compounds from the probe and provide enhanced reproducibility.

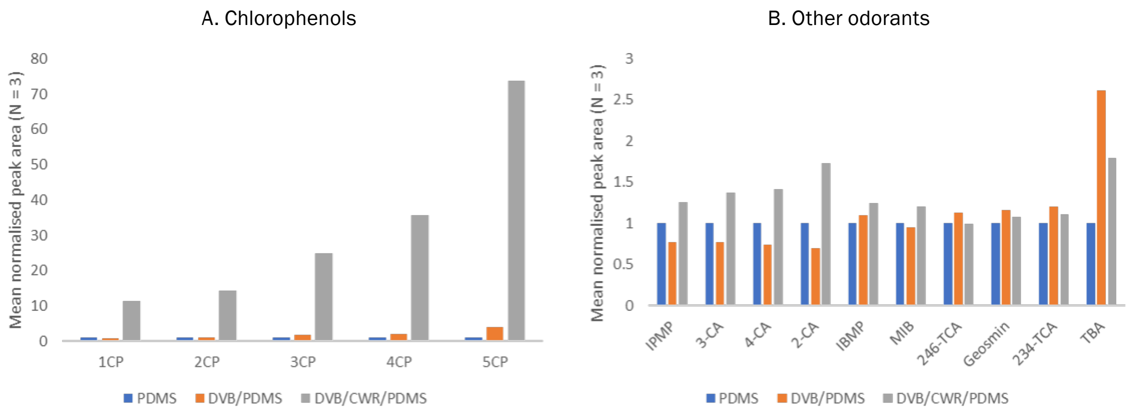
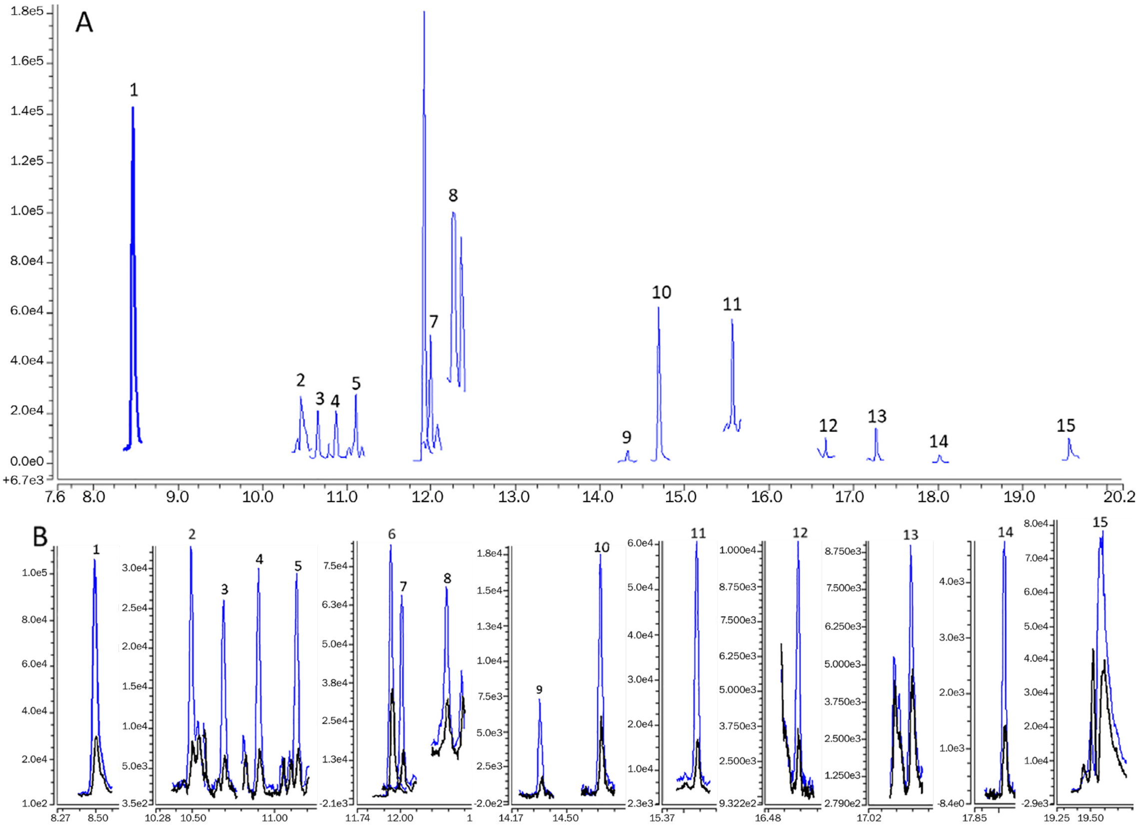


Figure 6: Comparison of three HiSorb sorptive phase combinations for the immersive extraction of compounds using calibration standard C8. Peak areas are normalised to the peak areas produced by the PDMS-only probe.



1	1CP	2	IPMP	3	3-CA	4	4-CA	5	2-CA
6	2CP	7	IBMP	8	MIB	9	246-TCA	10	3CP
11	Geosmin	12	234-TCA	13	4CP	14	TBA	15	5CP

Figure 7: SIM chromatograms for chlorophenols and other odorants using calibration standards C3 (blue) and C1 (black). A) All compounds with responses shown to scale. B) Isolated peaks for each compound.

#	Compound	R^2	Cal range (ng/L)	RSD(%)	Stability (%)	LOD (ng/L)	LOQ (ng/L)
1	2-Chlorophenol	0.9991	5 – 1,500	10.39	8.41	3.17	10.57
2	2-Isopropyl-3-methoxypyrazine	0.9997	0.5 – 150	5.49	11.68	0.26	0.78
3	3-Chloroanisole	0.9997	0.5 – 150	5.24	10.62	0.29	0.96
4	4-Chloroanisole	0.9996	0.5 – 150	4.96	2.12	0.34	1.14
5	2-Chloroanisole	0.9995	0.5 – 150	4.77	8.77	0.15	0.5
6	2,4-Dichlorophenol	0.9996	5 – 1,500	4.51	4.99	5.96	19.87
7	2-Isobutyl-3-methoxypyrazine	0.9991	0.5 – 150	5.73	10.27	0.27	0.89
8	2-Methylisoborneol	0.9995	0.5 – 150	5.05	2.37	0.53	1.78
9	2,4,6-Trichloroanisole	0.9994	0.5 – 150	5.31	8.15	0.4	1.34
10	2,4,6-Trichlorophenol	0.9981	5 – 1,500	7.58	5.74	3.91	13.05
11	Geosmin	0.9993	0.5 – 150	5.86	11.27	0.28	0.93
12	2,3,4-Trichloroanisole	0.9994	0.5 – 150	3.92	0.29	0.37	1.23
13	2,3,4,6-Tetrachlorophenol	0.998	5 – 1,500	7.94	4.24	8.76	29.21
14	2,4,6-Tribromoanisole	0.9992	0.5 – 150	5.47	7.70	0.24	0.79
15	Pentachlorophenol	0.9981	5 – 1,500	22.32	0.97	5.38	17.95

Table 4: Summary of method performance metrics. Stability refers to system stability, calculated over five days.

System stability over time

Laboratory standard C4 was run on different days, five days apart, with constant use of the analytical system for this method to assess repeatability. Results should agree within 20% of the previous multi-level calibration performed. Peak areas were normalised to the internal standard and then compared across the two runs finding that depending on target analyte, they diverged in the range of 0.29% to 11.68% (Table 4). The system was found to be stable across multiple days with continuous routine use.

Limits of detection and quantitation

Ten replicates of the lowest laboratory standard, C1, were analysed, and the standard deviation of the peak areas calculated. The deviation was compared against a calibration curve to determine an equivalent concentration. The limit of detection (LOD) was taken as this equivalent concentration multiplied by 3, while for the limit of quantitation (LOQ), it was multiplied by 10.

LODs for non-chlorophenol odorants were universally extremely low, with the highest being 0.53 ng/L for MIB. Crucially, these values are all well below the odour thresholds for these compounds in water, meaning the method can detect rising odorant concentrations before they become noticeable to consumers. Similarly, LODs for chlorophenols were well below levels required by regulatory bodies, with the highest being 8.76 ng/L for 4CP. As expected from these LODs, LOQs were also low, ranging from 0.5–1.78 ng/L for non-chlorophenols, and being below 30 ng/L for chlorophenols.

Real water samples

Having confirmed the performance of the method on laboratory standards, it was applied to real water samples (Table 5). Water samples were analysed in triplicate, and each sample batch contained a calibration curve comprised of laboratory standards.

The concentration of each detectable analyte in each sample was calculated from the mean peak area. Calculated concentrations were considered reliable if they surpassed the LOQs calculated previously. Analytes with calculated concentrations that were higher than the LOD but lower than the LOQ were present but at unquantifiable trace levels.

Results are summarised in Table 5.

RSDs were calculated for each quantifiable compound from each sample. 17 of the 19 RSD values were below 12% with a mean value of 7.35%. The exceptions were 4CP in sample F, pond water (20.51%), and 5CP in sample I, rainwater (13.54%).

Tap and spring water

As the tap water and bottled spring water were intended for human consumption, high levels of contaminants were not expected to be found. No target analytes were detected in the bottled spring water (sample E), and while geosmin and MIB were detected in all tap waters (samples A – C) the levels of these were well below odour thresholds and would not be expected to affect water quality. Geosmin and MIB are extremely common naturally occurring odorants that are difficult to fully eliminate, so their presence at low levels is not

Compound	Sample mean concentration (ng/L, N=3)								
	A	B	C	D	E	F	G	H	I
2-Chlorophenol	ND	ND	ND	ND	ND	ND	ND	ND	ND
2-Isopropyl-3-methoxypyrazine	ND	ND	ND	ND	ND	4.23	ND	ND	ND
3-Chloroanisole	ND	ND	ND	ND	ND	4.19	ND	ND	ND
4-Chloroanisole	ND	ND	ND	ND	ND	2.54	ND	ND	ND
2-Chloroanisole	ND	ND	ND	ND	ND	10.85	ND	ND	ND
2,4-Dichlorophenol	ND	ND	ND	ND	ND	ND	ND	ND	ND
2-Isobutyl-3-methoxypyrazine	ND	ND	ND	ND	ND	1.85	ND	ND	ND
2-Methylisoborneol	Trace	Trace	Trace	ND	ND	2.19	2.75	ND	ND
2,4,6-Trichloroanisole	Trace	ND	ND	ND	ND	1.87	Trace	ND	ND
2,4,6-Trichlorophenol	ND	ND	ND	ND	ND	Trace	ND	ND	ND
Geosmin	1.74	1.19	1.75	1.75	ND	2.50	1.52	2.1	ND
2,3,4-Trichloroanisole	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,3,4,6-Tetrachlorophenol	ND	ND	Trace	ND	ND	113.5	ND	Trace	ND
2,4,6-Tribromoanisole	ND	ND	ND	ND	ND	ND	ND	ND	ND
Pentachlorophenol	ND	ND	Trace	ND	ND	2,536	ND	43.74	204

Table 5: Summary of results for real water samples. “ND” indicates analyte not detected; “trace” indicates analyte detected below LOQ.

concerning or surprising. Similarly, the trace levels of 2,4,6-TCA in sample A likely originate from microorganisms in the water supply. In addition to geosmin and MIB, we also detected trace 4CP and 5CP in one tap water sample (sample C). Interestingly, neither these compounds nor MIB were detected in the raw spring water source of this sample (sample D), suggesting that they may have entered the water supply during processing.

Rainwater

Rainwater (Sample I) was collected in a clean glass jug. Any analytes in it were expected to have already been present when the rain fell, rather than having originated from the container. Therefore, it was surprising to find fairly high concentrations of 5CP (Figure 8). 5CP is a wood preservative and the collection jug was placed beside a wooden garden table. One possible source for the 5CP detected therefore, is run-off from the table that subsequently dripped into the jug.

Lake water

Water was collected from two natural lakes that are managed and maintained by the local water company. Higher levels of odorants might have been expected due to the lack of intensive filtering. Levels were in fact similar to tap and spring water samples, with sample G containing low (below odour threshold) levels of MIB, 246-TCA and geosmin and sample H containing low levels of geosmin, in addition to low levels of 4CP and 5CP. The chlorophenols in this case may have originated from telephone poles surrounding the lake, which may have been treated with wood preservatives.

Pond water

The pond water sample (sample F) came from a small (approx. 1m x 1m), stagnant garden pond in a semi-rural area. The pond was inhabited by algae, pondweed and animals such as arthropods and amphibians. Unlike all other samples, this one had a strong musty odour, hence we were not surprised to detect a high number of target analytes (Figure 9). This was the only sample to contain the monochloroanisoles and the methoxypyrazines, all of which have extremely low odour thresholds with distinctive musty/mouldy or green odour characteristics respectively. Geosmin, 246-TCA and MIB were also present.

All odorants likely originated from naturally occurring organisms living in the pond and, despite the strong smell, were present at fairly low concentrations (the highest being 10.85 ng/L for 2-CA).

Comparatively very high levels (~2.5 µg/L) of 5CP were detected in the pond water, exceeding the EPA maximum contaminant level for drinking water of 1 µg/L. 4CP and 3CP were also present at lower concentrations. The pond water was not intended for human consumption, however the presence of 5CP at such a high level was unexpected. This most likely resulted from run-off from preservative-treated fenceposts and telegraph poles accumulating in the pond due to it having no outflow. 4CP and 3CP can be produced via breakdown of 5CP, especially in the presence of microorganisms and this may explain the appearance of these compounds in the pond water. It is noted that the calculated concentration for 5CP exceeds the calibration range, and therefore further work with an extended calibration range would be required to formally report 5CP.

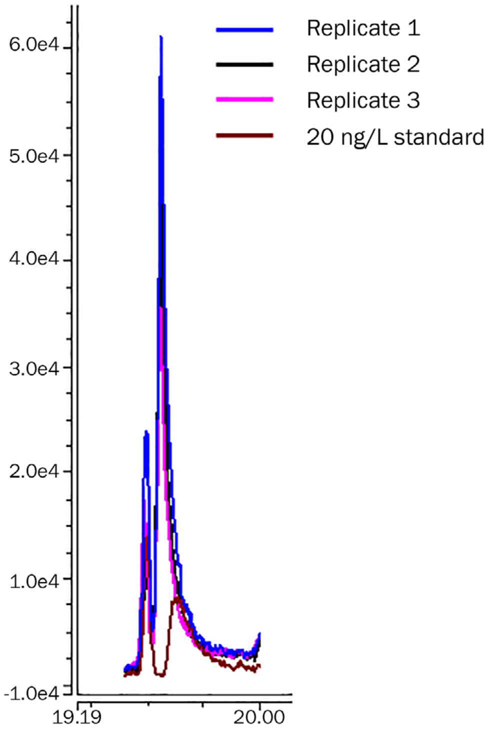
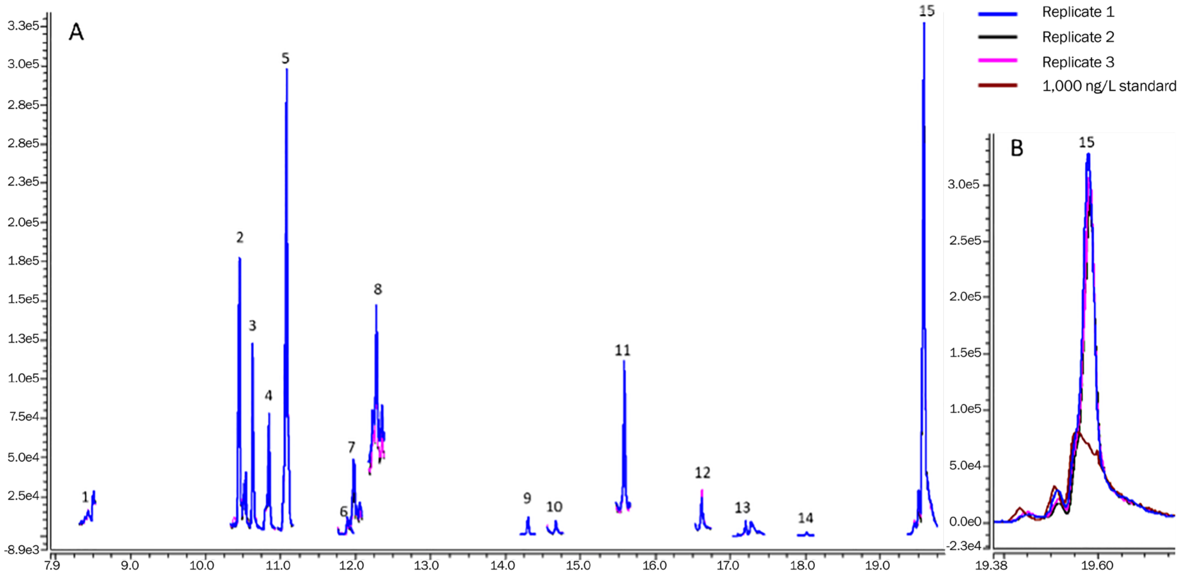


Figure 8: Overlaid peaks for pentachlorophenol in three rainwater replicates and a 20 ng/L laboratory standard.

Results summary

Findings were broadly in line with expectations. Most water samples, especially those intended for human consumption contained few target analytes, and when present these analytes were generally at low concentrations. Geosmin and MIB were both pervasive, with seven of the nine samples containing at least one of them, but in all cases their levels were below odour thresholds and so their presence would not affect perceived water quality. The next most common odorant was 246-TCA, being present in three samples. Due to its extremely low odour threshold (~1 ng/L), it's likely that its presence contributed to the strong odour of the pond water sample. Other odorants were present only in the pond water sample, while four targets (1CP, 2CP, 234-TCA and TBA) were not detected in any sample. Only one sample – a bottled spring water, contained no detectable targets.

Chlorophenols were found in four samples: one tap water, one lake water, the pond water and the rainwater. In all cases, 5CP was the most abundant chlorophenol. 4CP and 3CP, if present, were detected at increasingly lower levels. This suggests that 5CP is potentially entering these waters, most likely *via* the run-off from preservative treated wood, and subsequently being converted into 4CP and 3CP *via* microbial breakdown.



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6	2CP	7	IBMP	8	MIB	9	246-TCA	10	3CP
11	Geosmin	12	234-TCA	13	4CP	14	TBA	15	5CP

Figure 9: Results for target analytes detected in pond water:
A) All targets. B) Pentachlorophenol only with 1,000 ng/L laboratory standard for comparison.

In one sample, taken from a small pond, the concentration of 5CP was so high it exceeded our calibration range, suggesting that this analyte can accumulate in long-standing stagnant waters with no outflow.

Conclusion

A highly sensitive method for the simultaneous detection of chlorophenols and common water odorants using HiSorb high-capacity sorptive extraction probes and GC—MS has been developed. Unlike current methods used for the analysis of these compounds, HiSorb enables extraction and analysis of all target compounds in one run without the use of harmful solvents or derivatising agents, therefore simplifying and expediting the sample preparation process. Multiple samples can be prepared simultaneously, either offline with subsequent desorption in a dedicated TD instrument such as TD100-xr, or online with full automation on the Centri 360 platform.

Method performance was confirmed on laboratory standards with excellent linearity, reproducibility and long-term stability. LODs and LOQs were found to be well within requirements, with sub-ppt LODs for all non-chlorophenol odorants and LODs below 10 ppt for the chromatographically more challenging chlorophenols. The method performed well when subsequently applied to real water samples, with results being largely as expected, except for surprisingly high – but consistent among replicates – levels of 5CP in certain samples.

It is concluded that the method is ideal for water quality screening across a wide range of water matrices, combining high sensitivity with high throughput, convenience and low running costs.

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