

## Environmental testing

# Trace analysis of chlorophenolics using triple quadrupole GC-MS

## Authors

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## Keywords

TSQ 9610 mass spectrometer, TRACE 1610 GC, TriPlus RSH autosampler, water, 2,4,6-trichlorophenol, catechol, chlorocatechols, guaiacol, chloroguaiacols, syringol, gas chromatography-mass spectrometry, GC-MS, triple quadrupole, Advanced Ionization Ion source (AEI)

## Goal

To develop and assess a reliable, robust, and future-proof method for the analysis of chlorophenols at trace and ultra-trace levels in water.

## Introduction

Chlorophenolic compounds are widely used in the industrial manufacturing of pharmaceuticals, agricultural chemicals, and dyes. They are ubiquitous in the environment, having been released via pesticides, industrial waste, or the breakdown of chlorinated compounds.<sup>1</sup> Chlorophenolics are also produced as disinfection by-products in drinking water due to the chlorination of phenols.<sup>2</sup> Chlorophenol derivatives such as catechol, chlorocatechols, guaiacol, chloroguaiacols, and syringol exhibit toxic properties including cytotoxic, mutagenic, and cancerogenic activity. Subsequently, substitution of these compounds with chlorine atoms may increase their toxicity and prolong the period of bioaccumulation in living organisms.

Due to the toxicity of these chemicals, there are a number of regulations implemented throughout the world to limit the public's exposure to them. These regulations include the United States Environmental Protection Agency (U.S. EPA) Methods 604, 525, and 8270. However, the EPA Methods only cover a subset of chlorophenolic compounds and do not assess the risk of the entire class of compounds. Limited information is present regarding the toxicity of the wide range of chlorophenolic compounds. Currently, guidelines are given only for 2,4,6-trichlorophenol (2,4,6-TCP) at 2 µg/L in water.

With increasing attention being given to these compounds, intensified research into the toxic effects of compounds is underway. It is therefore important to develop consistent, reliable, and future-proof methods to detect these compounds at trace and ultra-trace levels to meet the limits of the ever-evolving regulations.

In this work, an expanded list of chlorophenolic compounds were analyzed in water using GC-MS/MS. Linearity, method detection limits, precision, and accuracy were assessed to evaluate the method performance.

## Experimental

### Reagents and standards

To prepare the samples, an 800 mL aliquot of carbon filtered lab water was placed into a 1 L amber bottle for extraction. Eight samples were spiked with 100  $\mu\text{L}$  of labeled chlorophenolic (10  $\mu\text{g}/\text{mL}$ ) and chlorovanillin solutions (10  $\mu\text{g}/\text{mL}$ ). Samples were then spiked with 20  $\mu\text{L}$  of the chlorophenolic at 2.5, 5, and 10  $\mu\text{g}/\text{mL}$  and unlabeled chlorovanillin at 5 and 10  $\mu\text{g}/\text{mL}$ . 25 mL of  $\text{K}_2\text{CO}_3$  buffer, 25 mL of acetic anhydride, and 100 mL of hexane were added to each bottle before placing it on a mechanical roller overnight (at least 16 hours) for the initial extraction. The aqueous and organic layers were allowed to separate (around 5 minutes) and the organic top layer was collected. The extraction was repeated with an additional 50 mL of hexane added to the aqueous layer and placed back onto the roller for another 1 hour. After the layers were allowed to separate, the top organic layer was collected and concentrated using the Thermo Scientific™ Rocket™ Evaporator down to 5 mL. The recovery standards were then added. Figure 1 shows a summary schematic of the sample preparation workflow.

All standards were purchased from Cambridge Isotope Laboratories. The solutions were prepared as follows:

- Chlorophenolics  $^{13}\text{C}$  surrogate solution (10  $\mu\text{g}/\text{mL}$ ) was prepared by diluting 1,000  $\mu\text{L}$  of EM-4173-1  $^{13}\text{C}$  Chlorophenolics (250  $\mu\text{g}/\text{mL}$ ) to 25 mL methanol in a 25 mL volumetric flask.

- Chlorovanillin  $^{13}\text{C}$  surrogate solution (10  $\mu\text{g}/\text{mL}$ ) was prepared by diluting 1,000  $\mu\text{L}$  of EM-4173-2  $^{13}\text{C}$  Chlorovanillin (250  $\mu\text{g}/\text{mL}$ ) to 25 mL acetone in a 25 mL volumetric flask.
- Chlorophenolics unlabelled solutions (25/50/100  $\mu\text{g}/\text{mL}$ ) were prepared by diluting 1 mL of EM-4181 and 1 mL of EM-4183 (250/500/1,000  $\mu\text{g}/\text{mL}$ ) to 10 mL methanol in a 10 mL volumetric flask.
- Chlorovanillin, chlorosyringaldehyde, chlorosyringol unlabelled solutions (50/100  $\mu\text{g}/\text{mL}$ ) were prepared by diluting 1 mL of EM-4182 and 1 mL of EM-4184 Unlabelled Chlorophenolic Standards (500/1,000  $\mu\text{g}/\text{mL}$ ) to 10 mL acetone in a 10 mL volumetric flask.
- For recovery standard, a semi-volatile internal standards mix containing 2-methylnaphthalene- $\text{d}_{10}$ , *p*-terphenyl- $\text{d}_{14}$  and benzo(*a*)pyrene- $\text{d}_{12}$  at 250  $\mu\text{g}/\text{mL}$  in DCM was used. A 10  $\mu\text{g}/\text{mL}$  solution of recovery standard was prepared by diluting 1 mL at 250  $\mu\text{g}/\text{mL}$  in 25.0 mL hexane.

To assess the linearity of the system, a calibration curve was prepared. Five separatory funnels containing 1 L of carbon filtered lab water were prepared. Each separatory funnel was spiked with 0.5 mL of labeled chlorophenolic solution (10  $\mu\text{g}/\text{mL}$ ) and 0.5 mL of labelled chlorovanillin solution (10  $\mu\text{g}/\text{mL}$ ). Then each separate funnel was spiked with the unlabeled solutions at volumes of 10  $\mu\text{L}$ , 30  $\mu\text{L}$ , 100  $\mu\text{L}$ , 300  $\mu\text{L}$ , and 1,000  $\mu\text{L}$ . 25 mL of  $\text{K}_2\text{CO}_3$  buffer and 25 mL of acetic anhydride were added to each funnel. The solution was allowed to sit for 1 hour. 100 mL of hexane was added, and the mixture shaken for 2 minutes. The organic layer was left to separate and then collected. The process was repeated two additional times. The organic layer was concentrated via the Rocket Evaporator and made up to 25 mL. Then 250  $\mu\text{L}$  of recovery standard (10  $\mu\text{g}/\text{mL}$ ) was added. Each calibration mix was then transferred to a 20 mL amber glass vial with Teflon lined silicone septa closure and stored at 4  $^\circ\text{C}$ . Table 1 gives the details of the volumes added to each calibration standard. Figure 2 shows a summary schematic of the calibration preparation workflow for the analysis.



Figure 1. Sample preparation workflow

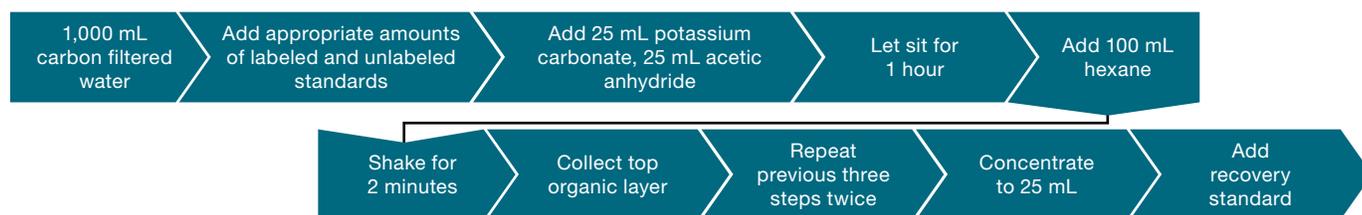


Figure 2. Schematic for the preparation of the calibration curve

Table 1. Calibration curve preparation

Standard name	Calibration level	Volume of unlabeled chlorophenolic and chlorovanillin solution (10 ppm)	Volume of labelled chlorophenolic and chlorovanillin solution (10 ppm)	Volume of recovery STD mix (10 ppm)
	Concentration ng/mL	Volume added (µL)	Volume added (µL)	Volume added (µL)
CS1	10	10	500	250
CS2	30	30	500	250
CS3	100	100	500	250
CS4	300	300	500	250
CS5	1,000	1,000	500	250

## GC-MS/MS analysis

For the analysis, the Thermo Scientific™ TSQ™ 9610 triple quadrupole mass spectrometer coupled to a Thermo Scientific™ TRACE™ 1610 gas chromatograph (GC) and a Thermo Scientific™ TriPlus™ RSH autosampler were used. The TSQ 9610 mass spectrometer was equipped with the Thermo Scientific™ Advanced Electron Ionization™ (AEI) ion source, which provided ultra-trace level sensitivity and robustness. Chromatographic separation of the target analytes was achieved using a Thermo Scientific™ TraceGOLD™ TG-Dioxin GC column (60 m x 0.25 mm x 0.25 µm) (P/N 26066-1540). Full GC conditions are shown in Table 2. Appendix 1 gives the SRM transitions used for the TSQ 9610 mass spectrometer.

## Results and discussion

### Chromatography

The optimized GC conditions allowed for the chlorophenolic method run time to be 25 minutes. The superior performance and the high thermal stability of the TraceGOLD TG-Dioxin GC column allowed for Gaussian peak shapes and baseline resolution of the target analytes, thus ensuring precise and reliable quantitation of chlorophenols at trace and ultra-trace concentrations. Figure 3 shows an example of the chromatographic separation for all target compounds in a mid-level solvent standard at 100–200 ng/mL. Figure 4 shows an example chromatogram for the separation of the trichlorophenols at 5.0 ng/L with 2,4,6-TCP highlighted.

Table 2. Settings for the TRACE 1610 gas chromatograph

Inlet settings	
Injector	SSL
Temperature (°C)	220
Inlet parameters	
Operating mode	Splitless with surge
Split flow (mL/min)	40
Column flow	1.2 mL/min
Split ratio	33 to 1
Splitless time (min)	0.4
Purge flow (mL/min)	2.0
Stop purge for (min)	0.5
Surge pressure (psi)	37.71
Surge duration (min)	0.5
Vacuum comp.	On
Gas saver mode	On
Gas save flow (mL/min)	20.0
Gas save time (min)	5.0
Oven settings	
Initial temp. (°C)	60
Initial time (min)	3
Temperature rate #1 (°C/min)	30
Final temp. (°C)	140
Hold time (min)	0
Temperature rate #2 (°C/min)	5
Final temp. (°C)	240
Hold time (min)	0
Temperature rate #1 (°C/min)	30
Final temp. (°C)	330
Hold time (min)	5

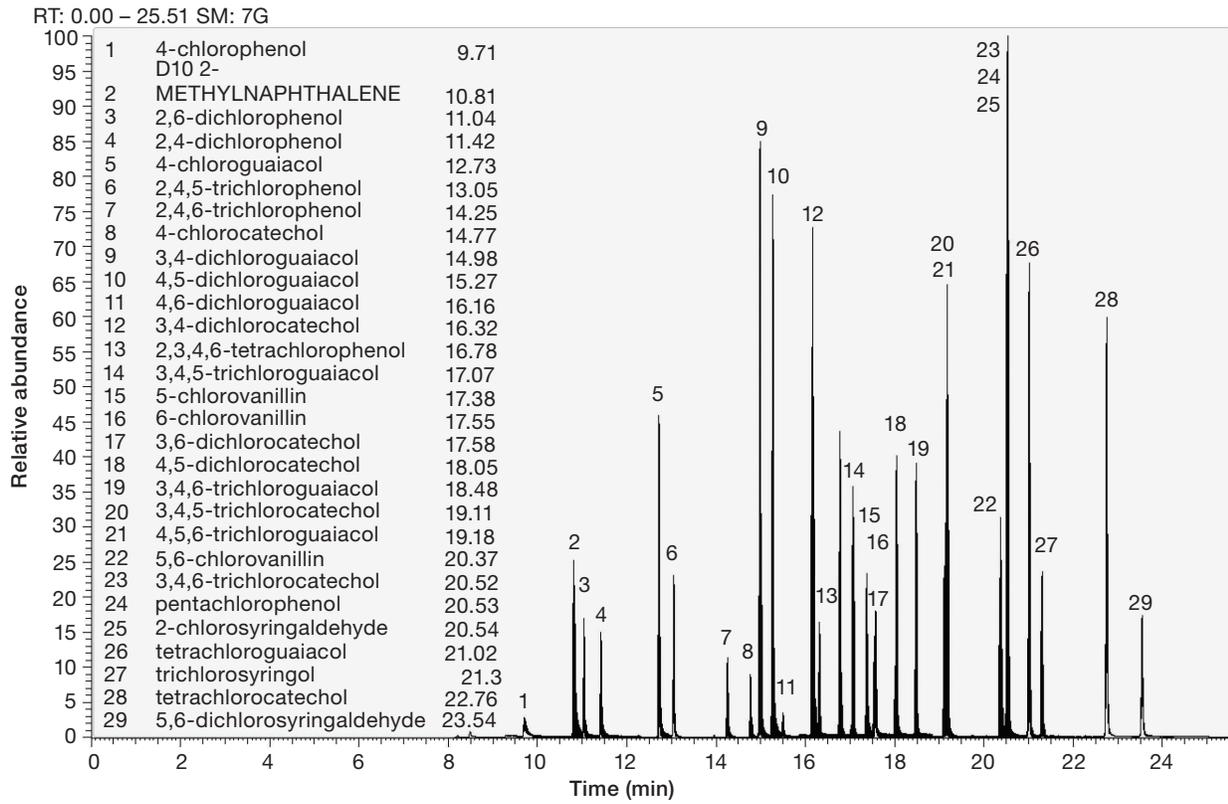


Figure 3. Example chromatogram showing the separation of all 29 analytes in a standard at 100–200 ng/mL

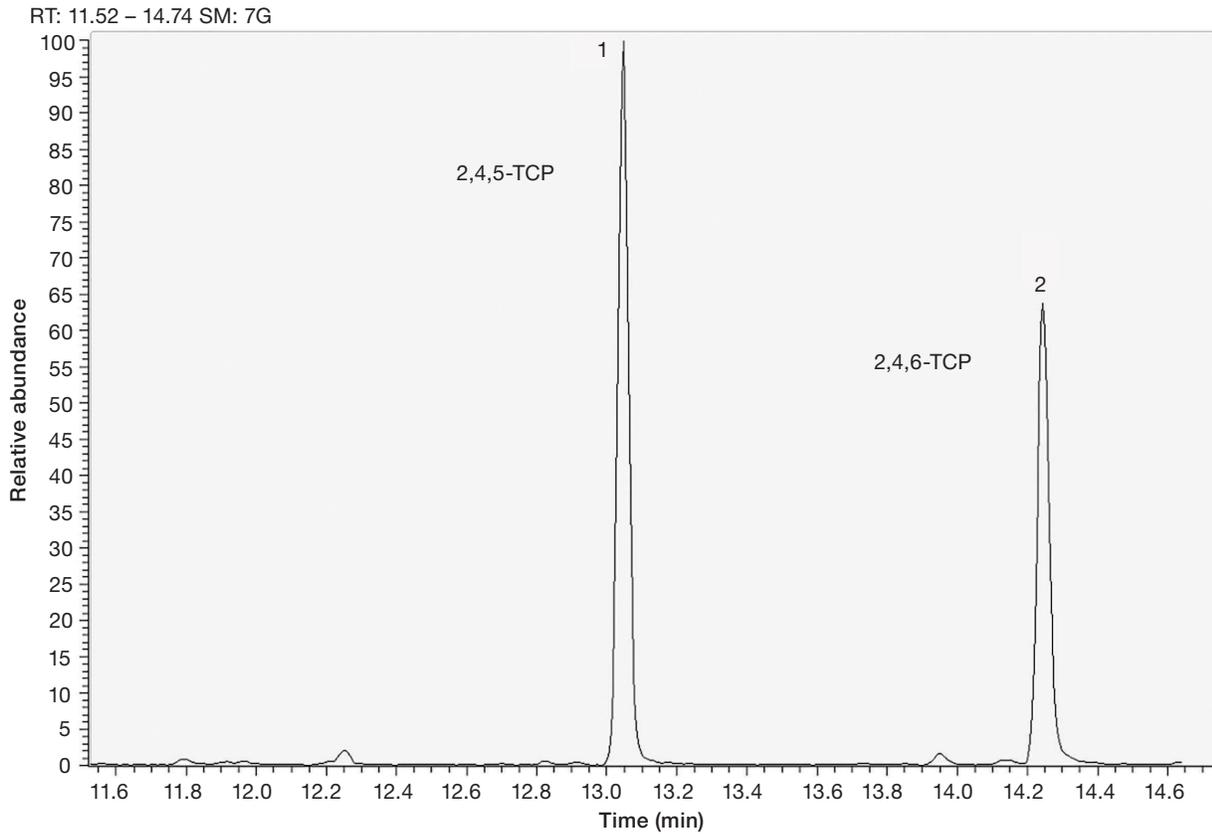


Figure 4. Separation of trichlorophenols in the sample at 5.0 ng/L

## Linearity and sensitivity

The extended dynamic range of the Thermo Scientific™ XLXR™ detector was assessed using six solvent calibration standards with levels ranging from 2.5 to 10,000 ng/mL depending on the analyte. Good linearity was obtained with an average coefficient of determination ( $R^2$ ) of >0.99 and an average response factor RSD of 3.6% (compliant with AvCF %RSD <20), confirming the linear trend across the specified concentration range and ensuring confidence for quantitative analysis. Figure 5 shows an example of the calibration curve obtained for 2,4,6-TCP. Calculated  $R^2$  values, as well as AvCF %RSD and calibration ranges for the individual chlorophenols, are reported in Appendix 2.

The MDLs were assessed by using n=9 replicated injections of a sample spiked at 2.5–10 ng/L. The MDL was calculated by taking three times the standard deviation ( $\sigma$ ) of the results. The MDLs are shown in Appendix 2. The calculated MDL was below 0.001  $\mu\text{g/L}$  (1 ng/L) for all investigated compounds, which is well below the current guideline value for 2,4,6-TCP of 2  $\mu\text{g/L}$ . The precision of this method is well defined by the low RSD for all compounds with values <10%. Figure 6 shows a summary of the MDLs and the %RSDs for all compounds. Calculated recovery was between 75% to 125%, except for the catechols due to their poor extraction efficiency for which it was <35%. Figure 7 shows an example of a low-level spike for 4-chlorophenol (2.5 ng) in water.

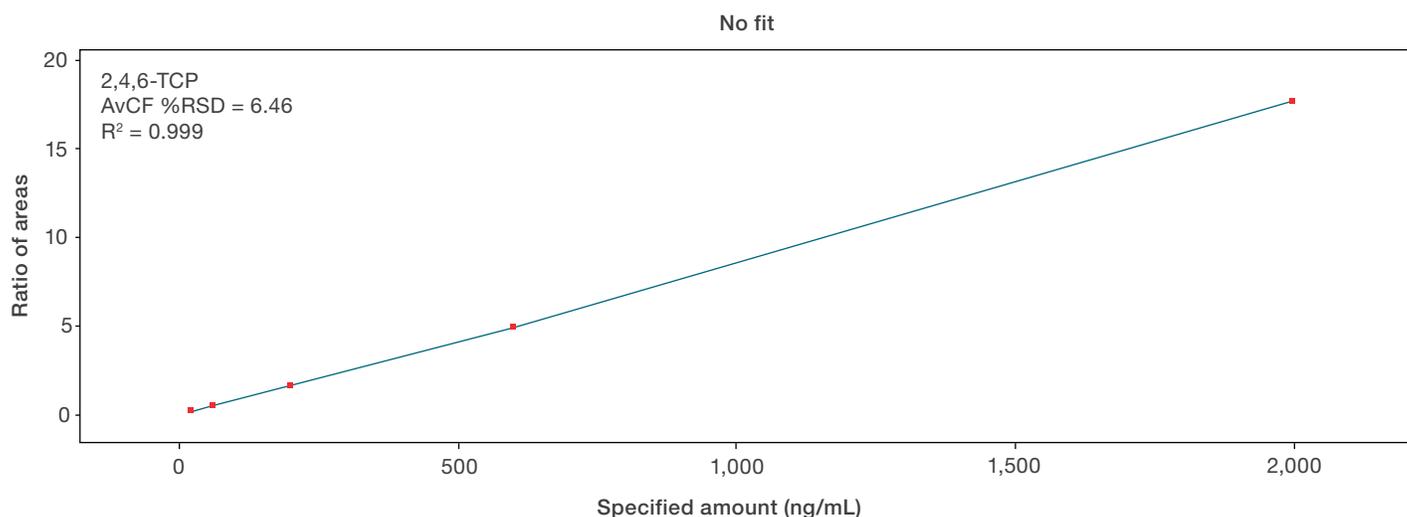


Figure 5. Calibration curve showing 2,4,6-trichlorophenol between 2.5 and 2,000 ng/mL

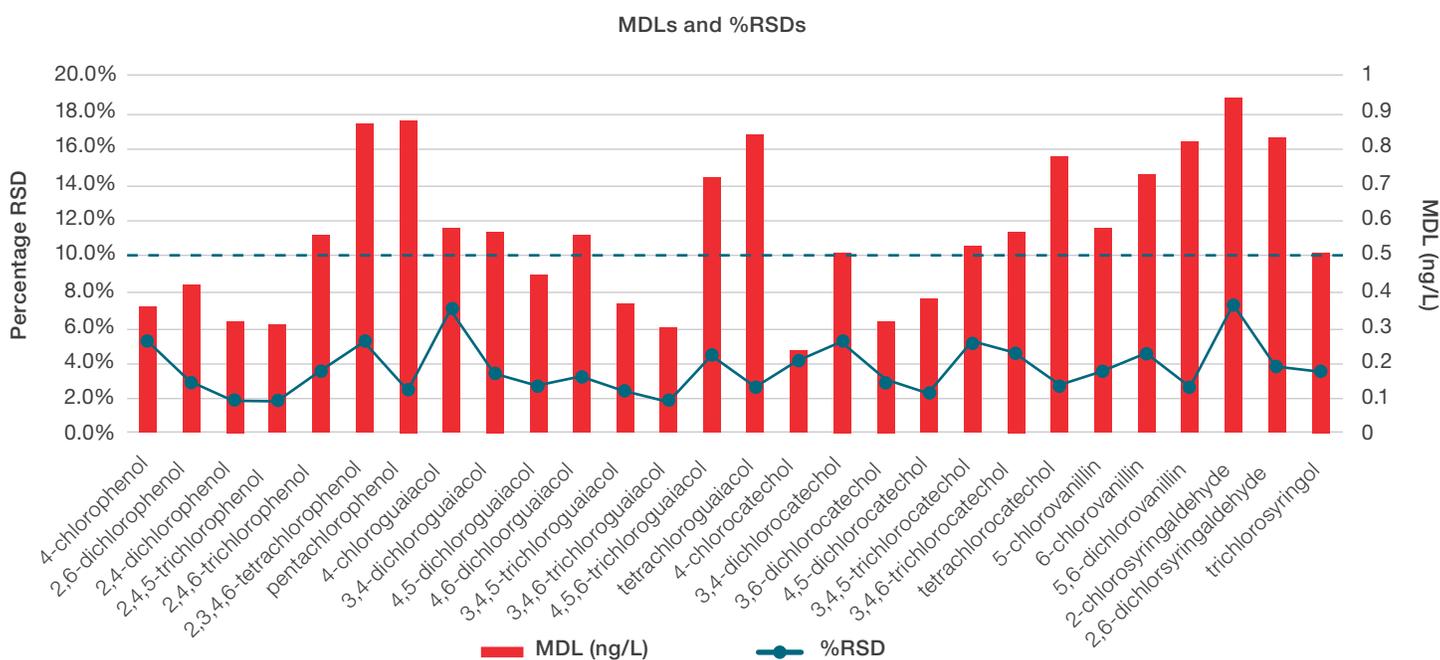


Figure 6. Calculated MDLs and %RSDs for all chlorophenolic compounds. The blue dotted line indicates the 10% RSD.

RT: 8.60 – 11.52 SM: 7G

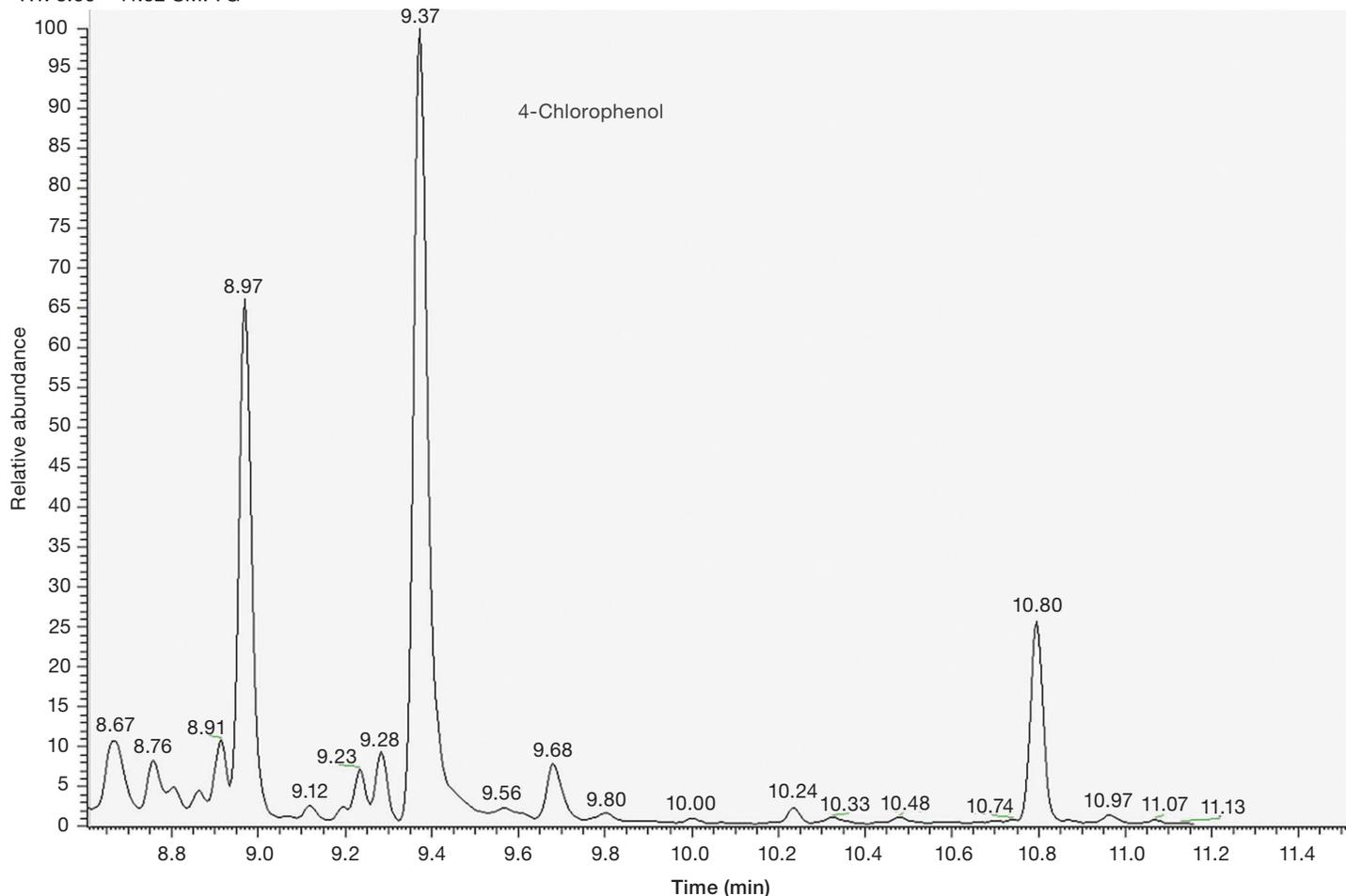


Figure 7. Low-level water spike showing 4-chlorophenol at 2.5 ng/L (at 9.37 minutes)

## Conclusion

The results obtained in this study demonstrate that the TSQ 9610 triple quadrupole GC-MS/MS system in combination with the TRACE 1610 GC and the TriPlus RSH autosampler represent a reliable, robust, and future-proof tool for the analysis of chlorophenols at trace and ultra-trace levels in water:

- Extended linearity was achieved thanks to the XLXR detector, which allowed for average coefficients of determination ( $R^2$ ) of  $>0.99$  in the range 2.5–10,000 ng/mL and AvCF %RSDs below 10%.
- The optimized design and the high sensitivity of the NeverVent™ AEI ion source allowed for MDLs below 1 ng/L for all the target compounds, which is significantly below the current guideline values for 2,4,6-TCP of 2 µg/L.
- The inertness of the flow path and the precise control of the TriPlus RSH autosampler ensure excellent precision for all compounds with peak area %RSD  $<10\%$  in a sample spiked at 1 ng/L.

## References

1. Igbinsosa, E.; Odjajare, E.; Chigor, V.; Igbinsosa, I.; Emoghene, A.; Ekhaise, F.; Igiehon, N.; Idemudia, O. Toxicological Profile of Chlorophenols and Their Derivatives in the Environment: The Public Health Perspective. *The Scientific World Journal*, **2013**, pp.1-11.
2. World Health Organization, 1996. *Guidelines for drinking-water quality*. Geneva: World Health Organization.

**Appendix 1. SRM transitions for chlorophenol compounds**

Analyte	RT	Mass	Product mass	Collision energy (eV)	Labeled standards	RT	Mass	Product mass	Collision energy (eV)
4-chlorophenol	9.7	128	100	12	2,4-DICHLOROPHENOL	11.41	168	132	13
4-chlorophenol	9.7	130	102	12	2,4-DICHLOROPHENOL	11.41	170	134	13
2,6-dichlorophenol	11.04	162	98	13	4-CHLOROGUAIACOL	12.71	164	149	10
2,6-dichlorophenol	11.04	164	100	13	4-CHLOROGUAIACOL	12.71	166	151	10
2,4 dichlorophenol	11.42	162	126	13	5-CHLOROVANILLIN	17.38	192	192	8
2,4 dichlorophenol	11.42	164	128	13	5-CHLOROVANILLIN	17.38	194	194	8
4-chloroguaiacol	12.72	158	143	6	4,5-DICHLOROCATECHOL	18.04	226	184	6
4-chloroguaiacol	12.72	160	145	6	4,5-DICHLOROCATECHOL	18.04	228	186	6
2,4,5-trichlorophenol	13.04	196	132	10	4,5,6-TRICHLOROGUAIACOL	19.17	232	217	8
2,4,5-trichlorophenol	13.04	198	134	10	4,5,6-TRICHLOROGUAIACOL	19.17	234	219	8
2,4,6-trichlorophenol	14.26	196	132	13	PENTACHLOROPHENOL	20.53	272	272	8
2,4,6-trichlorophenol	14.26	198	134	13	PENTACHLOROPHENOL	20.53	274	274	8
4-chlorocatechol	14.78	186	144	6	TETRACHLOROGUAIACOL	21.01	268	253	10
4-chlorocatechol	14.78	188	146	6	TETRACHLOROGUAIACOL	21.01	270	255	10
3,4-dichloroguaiacol	14.99	192	177	13	TETRACHLOROCATECHOL	22.75	254	254	6
3,4-dichloroguaiacol	14.99	194	179	13	TETRACHLOROCATECHOL	22.75	256	256	6
4,5-dichloroguaiacol	15.27	192	177	8					
4,5-dichloroguaiacol	15.27	194	179	8	Recovery standard				
4,6-dichloroguaiacol	16.17	192	177	11	D10 2METHNAPHTHALENE	10.81	152	152	8
4,6-dichloroguaiacol	16.17	194	179	11					
3,4-dichlorocatechol	16.32	220	178	11					
3,4-dichlorocatechol	16.32	222	180	11					
2,3,4,6-tetrachlorophenol	16.78	232	232	8					
2,3,4,6-tetrachlorophenol	16.78	234	234	8					
3,4,5-trichloroguaiacol	17.07	226	211	8					
3,4,5-trichloroguaiacol	17.07	228	213	8					
5-chlorovanillin	17.39	186	186	8					
5-chlorovanillin	17.39	188	188	8					
6-chlorovanillin	17.56	186	186	8					
6-chlorovanillin	17.56	188	188	8					
3,6-dichlorocatechol	17.59	220	178	20					
3,6-dichlorocatechol	17.59	222	180	20					
4,5-dichlorocatechol	18.05	220	178	8					
4,5-dichlorocatechol	18.05	222	180	8					
3,4,6-trichloroguaiacol	18.48	226	211	6					
3,4,6-trichloroguaiacol	18.48	228	213	6					
3,4,5-trichlorocatechol	19.11	254	212	6					
3,4,5-trichlorocatechol	19.11	256	214	6					
4,5,6-trichloroguaiacol	19.18	226	211	12					
4,5,6-trichloroguaiacol	19.18	228	213	12					
5,6-dichlorovanillin	20.38	220	220	8					
5,6-dichlorovanillin	20.38	222	222	8					
3,4,6-trichlorocatechol	20.53	254	212	12					
3,4,6-trichlorocatechol	20.53	256	214	12					
pentachlorophenol	20.54	266	266	8					
pentachlorophenol	20.54	268	268	8					
2 chlorosyringaldehyde	20.6	216	216	8					

**Appendix 1 continued. SRM transitions for chlorophenol compounds**

Analyte	RT	Mass	Product mass	Collision energy (eV)
tetrachloroguaiacol	21.02	262	247	10
tetrachloroguaiacol	21.02	264	249	10
trichlorosyringol	21.29	256	241	6
trichlorosyringol	21.29	258	243	6

Analyte	RT	Mass	Product mass	Collision energy (eV)
tetrachlorocatechol	22.76	248	248	6
tetrachlorocatechol	22.76	250	250	6
5,6 dichorosyringaldehyde	23.55	250	250	6
5,6 dichorosyringaldehyde	23.55	252	252	6

**Appendix 2. Linearity and MDLs for chlorophenol compounds**

Analyte	Level of fortification (ng)	Average calculated amount (ng)	Average recovery (%)	Standard deviation ( $\sigma$ )	%RSD	MDL (ng/L)	R <sup>2</sup>
4-chlorophenol	2.5	2.394	96	0.123	5.10	0.35	0.998
2,6-dichlorophenol	5	5.085	102	0.142	2.80	0.41	0.998
2,4-dichlorophenol	5	6.189	124	0.108	1.80	0.31	0.999
2,4,5-trichlorophenol	5	5.637	113	0.103	1.80	0.3	0.999
2,4,6-trichlorophenol	5	5.635	113	0.19	3.40	0.55	1.000
2,3,4,6-tetrachlorophenol	5	5.79	116	0.296	5.10	0.86	0.995
pentachlorophenol	10	12.766	128	0.301	2.40	0.87	0.998
4-chloroguaiacol	2.5	2.864	115	0.198	6.90	0.57	0.999
3,4-dichloroguaiacol	5	5.974	119	0.195	3.30	0.56	1.000
4,5-dichloroguaiacol	5	5.917	118	0.151	2.60	0.44	0.999
4,6-dichloroguaiacol	5	6.112	122	0.191	3.10	0.55	1.000
3,4,5-trichloroguaiacol	5	5.398	108	0.125	2.30	0.36	0.999
3,4,6-trichloroguaiacol	5	5.466	109	0.099	1.80	0.29	0.999
4,5,6-trichloroguaiacol	5	5.685	114	0.246	4.30	0.71	0.998
tetrachloroguaiacol	10	11.626	116	0.286	2.50	0.83	0.999
4-chlorocatechol	2.5	1.95	78	0.078	4.00	0.23	0.997
3,4-dichlorocatechol	5	3.367	67	0.172	5.10	0.5	0.999
3,6-dichlorocatechol	5	3.806	76	0.108	2.80	0.31	1.000
4,5-dichlorocatechol	5	5.786	116	0.129	2.20	0.37	0.999
3,4,5-trichlorocatechol	10	3.526	35	0.178	5.00	0.52	0.996
3,4,6-trichlorocatechol	10	4.378	44	0.193	4.40	0.56	0.998
tetrachlorocatechol	10	10.122	101	0.267	2.60	0.77	0.999
5-chlorovanillin	5	5.857	117	0.197	3.40	0.57	0.998
6-chlorovanillin	5	5.607	112	0.248	4.40	0.72	0.999
5,6-dichlorovanillin	10	10.969	110	0.279	2.50	0.81	1.000
2-chlorosyringaldehyde	5	4.556	91	0.323	7.10	0.93	0.999
2,6-dichlorosyringaldehyde	10	7.601	76	0.283	3.70	0.82	0.993
trichlorosyringol	5	5.014	100	0.172	3.40	0.5	0.995

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