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Application News

No. AD-0125

Water Analysis / LCMS-8050

A High Sensitivity Method for Quantitative Determination of Ten Phenols in Surface Water on LC/MS/MS with APCI Interface

❑ Introduction

There are a variety of phenolic compounds such as alkylphenols (AP), chlorophenols (CP), nitrophenols (NP), bisphenol A (BPA) and triclosan, etc. Phenols are widely used as chemical precursors in industries and for other purposes in agriculture, medical and domestic processes. Many phenolic compounds are toxic and carcinogenic to human and they are classified as the priority pollutants in surface and drinking waters [1, 2]. The US Environmental Protection Agency (EPA) and the European Community (EC) have set a legal tolerance level of 0.5 µg/L for total phenols and 0.1 µg/L for individual phenolic compound in drinking water [3,4]. Japan's MHLW (Ministry of Health, Labor and Welfare) has designated six phenols as the index of water quality standard requirements. Various analytical methods such as GC, GCMS, HPLC and LC/MS/MS have been used for detection and quantitation of phenols in drinking waters [5, 6]. These methods require sample pre-treatments including derivatization and/or pre-concentration by SPE, etc. In this Application News, a MRM based method is described, which was developed for detection and quantitation of phenol and nine substituted phenols (see Table 2) in treated water and reservoir water on triple quadrupole LC/MS/MS with an APCI interface.

❑ Experimental

Analytical conditions

A LCMS-8050 triple quadrupole system coupled with Nexera UHPLC system was employed in this work. A pentafluorophenyl (PFP) column from Phenomenex was used with an optimized gradient elution program for ten phenols. Details of the HPLC conditions and MS/MS conditions are shown in Table 1.

Preparation of standards and water samples

Phenol and nine substituted phenols including three chlorophenols (CP), four nitrophenols (NP) and two alkylphenols (AP) are listed in Table 2. Standard stock solutions were prepared in MeOH, which were diluted in series with MilliQ water to obtain calibrants of 50, 100, 250, 500, 1000, 2500, 5000 and 10,000 ng/L. The testing samples were obtained from a third party laboratory, including treated water, local reservoir water and a few spiked samples as controls. All the water samples were injected to LC/MS/MS without any pre-treatment or enrichment.

Table 1: Analytical conditions of phenols on LCMS-8050

Column	Kinetex 2.6u PFP 100A (100 mm L. x 2.10mm I.D.)
Mobile Phase	A: Water B: Methanol
Elution Program	Gradient elution, 5%B (0.00-0.01 min), 95%B (5.00-6.40 min), 5%B (6.41-8.00 min)
Flow Rate	0.5 mL/min
Oven Temp.	40 ºC
Injection	10 µL
Interface	APCI
MS Mode	MRM, Negative mode
Block Temp.	200 ºC
DL Temp.	200 ºC
Interface Temp.	500 ºC
Nebulizing gas	N_2, 4.0 L/min
Drying gas	N_2, 5.0 L/min

❑ Results and Discussion

A. Establishment of MRM method for ten phenols

The MRM optimization was carried out with 1ppm mixed standards by direct injection or on-column method. Two MRM transitions were selected for each compound, with one as quantifying ion and the other for confirmation. Details of

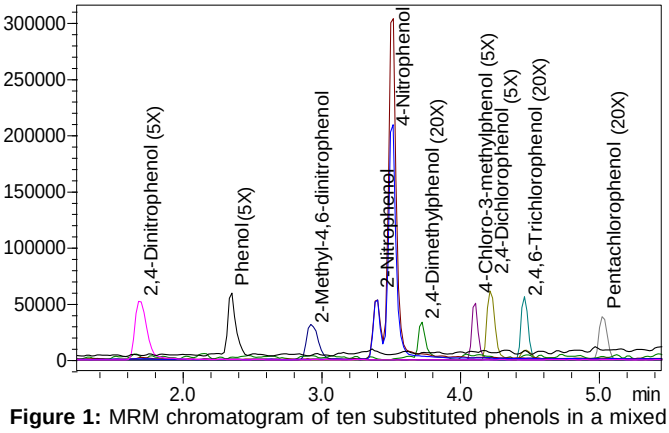

Figure 1: MRM chromatogram of ten substituted phenols in a mixed standard solution with each 5 µg/L.

Table 2: Summary of calibration range, linearity and detection sensitivity of MRM for substituted phenols on LCMS-8050

Compound	Abbr.	MRM Parameter				RT and Calibration Curve					
		Precursor	Product	CE (V)	Relative Int.	RT (min)	Range (µg/L)	R^2	LOD (µg/L)	LOQ (µg/L)	%RSD (n=3)*
2,4-dintrophenol	2,4-NP	183.0	109.1	26	100	1.70	0.05 – 10	0.9997	0.05	0.15	5.4%
			123.0	21	33						
Phenol	P	93.1	65.0	24	100	2.36	0.5 – 10	0.9978	1.0	3.0	3.2%
2-methyl-4,6-dinitrophenol	2-M-4,6-NP	197.0	180.1	20	100	2.94	0.1 – 10	0.9991	0.05	0.15	12.1%
			137.1	20	74						
2-nitrophenol	2-NP	138.0	108.0	18	100	3.40	0.1 – 10	0.9956	0.1	0.3	8.9%
			46.1	28	55						
4-nitrophenol	4-NP	138.1	108.1	22	100	3.51	0.05 – 10	0.9995	0.02	0.06	5.4%
			92.1	24	17						
2,4-dimethylphenol	2,4-MP	121.1	91.1	21	100	3.73	1.0 – 5	0.9995	1.0	3.0	8.8%
			106.1	23	65						
4-chloro-3-methylphenol	4-C-3-MP	141.0	35.0	21	100	4.11	0.1 – 10	0.9981	0.1	0.3	12.4%
			105.0	18	30						
2,4-dichlorophenol	2,4-CP	160.9	125.0	20	100	4.23	0.1 – 10	0.9971	0.05	0.015	8.1%
			35.1	25	43						
2,4,6-trichlorophenol	2,4,6-CP	194.9	35.0	29	100	4.47	0.1 – 10	0.9989	0.1	0.3	8.0%
		196.8	35.1	27	94						
Pentachloro-phenol	PCP	262.9	35.0	25	100	5.03	0.1 – 10	0.9994	0.1	0.3	1.4%
		264.9	35.0	29	128						

***** *Data obtained with the concentration levels closest to LOQ of the compound*

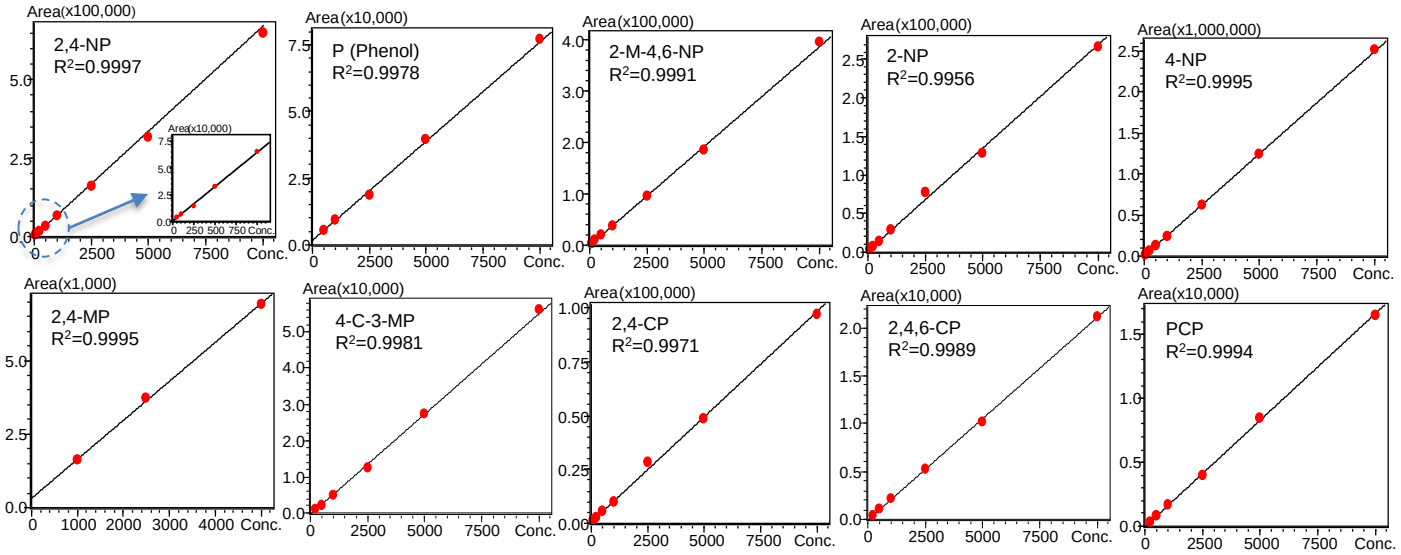

Figure 2: Calibration curves of ten phenol standards in MRM mode with an injection volume of 10 µL. Constructed with weighing method 1/C.

the MRM parameters are compiled into Table 2. The current method has adopted an APCI interface due to the relatively low polarity of some phenolic compounds including phenol and 2,4-MP. Figure 1 shows the MRM chromatograms of the ten phenols in a mixed standard solution. It is worth to note that the isomer pair of 2-NP and 4-NP can be separated and detected under the conditions at retentions of 3.40 minutes and 3.51 minutes, respectively. The MRM transitions of 2-NP and 4-NP are similar including 138>108, 138>46 and 138>92, but the intensity ratios of quantifying ion (138>108) to reference ions are different (see Table 2).

The calibration curves for quantitation of the compounds are shown in Figure 2. Good linearity of $R^2 > 0.995$ was obtained for the calibration range from 0.05 µg/L to 10 µg/L (except for 2,4-MP). The LODs of the method estimated from the lowest calibration points are at 0.02 ~ 0.25 µg/L except for Phenol and 2,4-MP (LOD = 1.0 µg/L).

B. Analysis of Treated Water and Reservoir Water

The MRM quantitation method established was applied to actual samples, a treated water S1 and a reservoir water S2, for detection of the targeted ten phenols. The water samples and two control samples (C/H and C/L) were obtained from a third party laboratory. These samples were injected into the LC/MS/MS without further pre-treatment or pre-concentration. The analysis results are summarized in Table 3. All the ten phenols were detected and quantified in the control sample C/H and in C/L (L=low) except 2,4-MP. However, the levels of phenol and 2,4-MP are below the LODs as remarked in the table. In the actual surface water samples S1 and S2, only 2-NP and 4-NP were detected and quantified. A suspected peak of 2,4-P was observed in S2, but its level (0.03 µg/L) is below the LOD of the method (0.05 µg/L). The individual MRM peaks of 2-NP, 4-NP and 2,4-NP of samples S1 and S2 are shown in Figure 3.

Table 3: Quantitation results of ten phenols in water samples

Compd.	RT (min)	Phenols in Water Samples (µg/L)			
		C/L	C/H	S1	S2
2,4-NP	1.70	0.30	0.79	N.D.	~0.03*
P	2.36	~0.32*	~0.66*	N.D	N.D.
2-M-4,6-NP	2.94	0.17	0.75	N.D.	N.D.
2-NP	3.40	0.23	0.67	0.28	0.17
4-NP	3.51	0.22	0.45	0.08	0.08
2,4-MP	3.73	N.D.	~0.62*	N.D.	N.D.
4-C-3-MP	4.1	018	0.63	N.D.	N.D.
2,4-CP	4.2	0.14	0.64	N.D.	N.D.
2,4,6-CP	4.5	0.15	0.71	N.D.	N.D.
PCP	5.0	0.29	0.74	N.D.	N.D.

** Lower than LODs of the method; N.D. = Not Detected*

In summary, this study focuses on evaluation of a MRM based method and its applicability in detection and quantitation of trace levels of phenol and substituted phenols in surface water samples. The results indicate the high sensitivity of the method, which offers a possibility to determine these substituted phenols directly to achieve the required LOD of 0.1 µg/L [3,4] without the need of pre-concentration. However, the sensitivity for phenol and 2,4-MP are not sufficient, which is likely related to their poorer ionization due to low polarity of the molecules. Sample concentration of ten times or more before analysis is needed.

❑ Conclusions

A MRM-based LC-APCI-MS/MS method with fast gradient elution of 8 minutes was established and evaluated for detection and quantitation of ten phenols in surface waters. The limits of detection (LOD) of the method achieved are better than 0.1 µg/L for each individual compound except phenol and 2,4-MP, which LODs are at 1.0 µg/L. The high sensitivity of the method for the eight substituted phenols offers the possibility to determine them quantitatively and directly without the need of pre-concentration. For phenol and 2,4-MP, it requires at least ten times of pre-concentration before analysis.

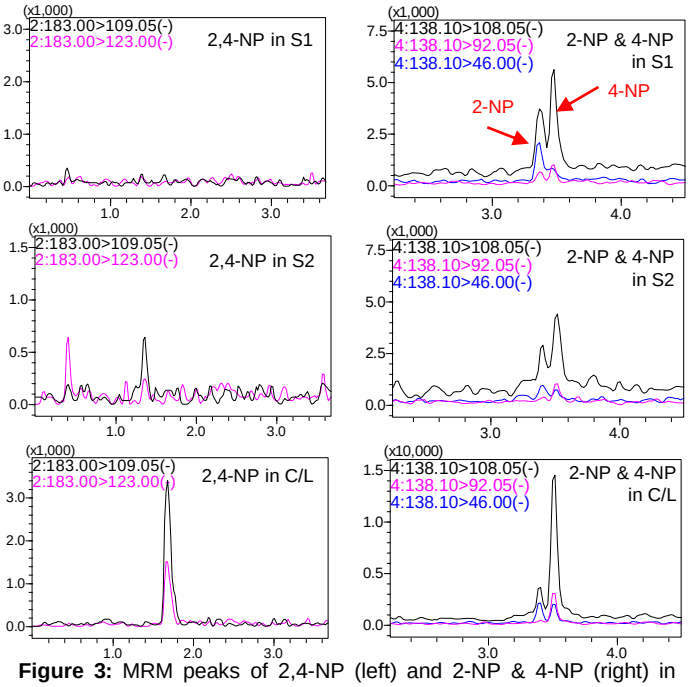

Figure 3: MRM peaks of 2,4-NP (left) and 2-NP & 4-NP (right) in samples S1, S2 and C/L with injection volume of 10 µL on LCMS-8050 with APCI interface.

❑ References

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