

Analysis of Cyanide Ions (CN^-) and Cyanogen Chloride (CNCl) by Ion Chromatography with Post-column Derivatization and UV-Visible Spectroscopic Detection

Introduction

Cyanide is rarely present naturally in water; however, cyanide ions (CN^-) can enter water supplies through contamination from wastewater and other sources. Cyanogen chloride (CNCl) can also form during water chlorination. Because of their potential impact on drinking water safety, these substances are designated as regulated water quality parameters under Japan's Water Supply Act (Ministry of Health, Labour and Welfare Ordinance No. 101).

Ion chromatography with post-column derivatization and UV-Visible spectroscopic detection is the analytical method established for determining CN^- and CNCl under the Ministerial Ordinance on Water Quality Standards (Ministry of Health, Labour and Welfare Notification No. 261, Final Revision; Ministry of the Environment Notification No. 25, March 26, 2025). The 2025 revision introduced ethanol as a less toxic alternative to N,N-dimethylformamide (DMF) for the color-developing reagent, improving laboratory safety.

Following chromatographic separation, CN^- reacts with chloramine-T to form CNCl. Both the resulting CNCl and CNCl that was originally present in the sample subsequently react with a 4-pyridinecarboxylic acid and 1-Phenyl-3-methyl-5-pyrazolone solution to produce a blue-colored product detected at 636 nm. This approach enables the separate quantification of CN^- and CNCl. For water quality testing, measurement variability must be $\leq 10\%$ at concentrations approximately one-tenth of the water quality standard value.

In this application note, CN^- and CNCl were analyzed using an RSpak KC-811 6E column for cyanide analysis. Calibration curves for both analytes were evaluated in accordance with the "Validity Assessment Guidelines" (Attachment to Health and Water Notice No. 0906-1 September 6, 2012, Final Revision; Pharmaceutical and Food Safety Bureau Notice No. 1018-1 October 18, 2017).

Keywords

Cyanide, cyanide ions, CN^- , Cyanogen chloride, CNCl, chloramine-T, 4-pyridinecarboxylic acid, 1-Phenyl-3-methyl-5-pyrazolone, ethanol, EtOH, ethyl alcohol, N,N-dimethylformamide, DMF, RSpak KC-811 6E, RSpak KC-G 6B, ion chromatography, water quality standards, water quality testing, post-column derivatization, validation guidelines, UV-Visible spectrophotometer, spectrophotometric detection, UV-Visible detection

Experimental

Instruments

Eluent pump: PU-4180*
 Reaction pump 1: PU-4185
 Reaction pump 2: PU-4185
 Autosampler: AS-4050*
 Column oven: CO-4060
 Reaction oven: RO-4068
 Detector: UV-4070

* with option units

LC Conditions

Column: RSpak KC-811 6E
 (6.0 mm I.D. x 250 mm L, 6 μ m)
 Guard column: RSpak KC-G 6B
 (6.0 mm I.D. x 50 mm L)
 Eluent: 1 mM sulfuric acid aqueous solution
 Reagent 1: 0.1 M phosphate buffer solution
 containing 0.1 % (w/v) chloramine-T
 Reagent 2: 23 mM 1-phenyl-3-methyl-5-pyrazolone,
 77 mM sodium 4-pyridinecarboxylate,
 aqueous solution containing 30% ethanol
 Eluent flow rate: 1.0 mL/min
 Reagent 1 flow rate: 0.5 mL/min
 Reagent 2 flow rate: 0.4 mL/min
 Column temperature: 40 $^{\circ}$ C
 Reaction temperature: 100 $^{\circ}$ C
 Wavelength: 636 nm
 Injection volume: 100 μ L
 Standard sample: Mixed standard solution of CN^- and CNCl

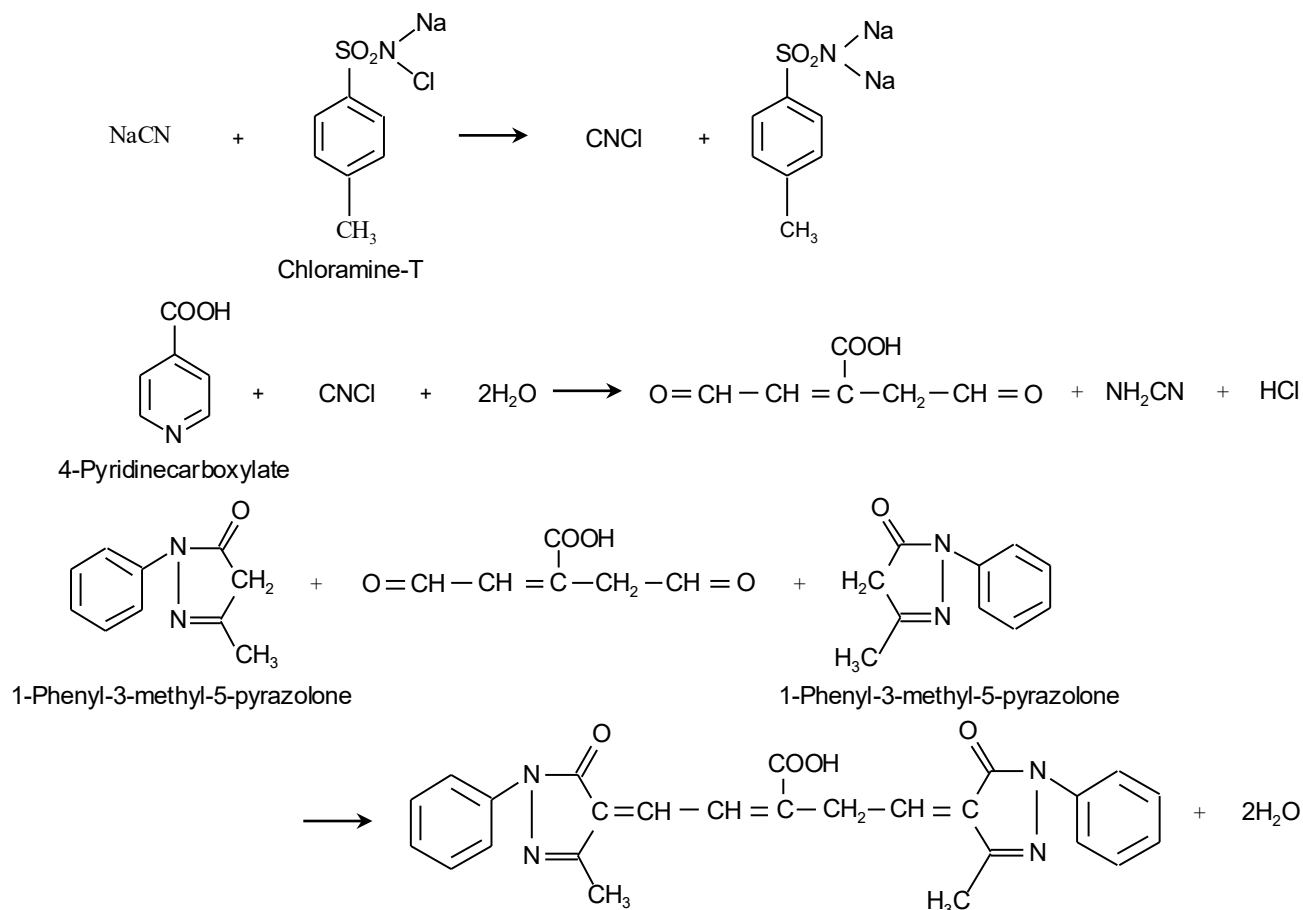


Fig. 1 Post-column derivatization reaction scheme for cyanide detection using chloramine-T and 4-pyridinecarboxylic acid

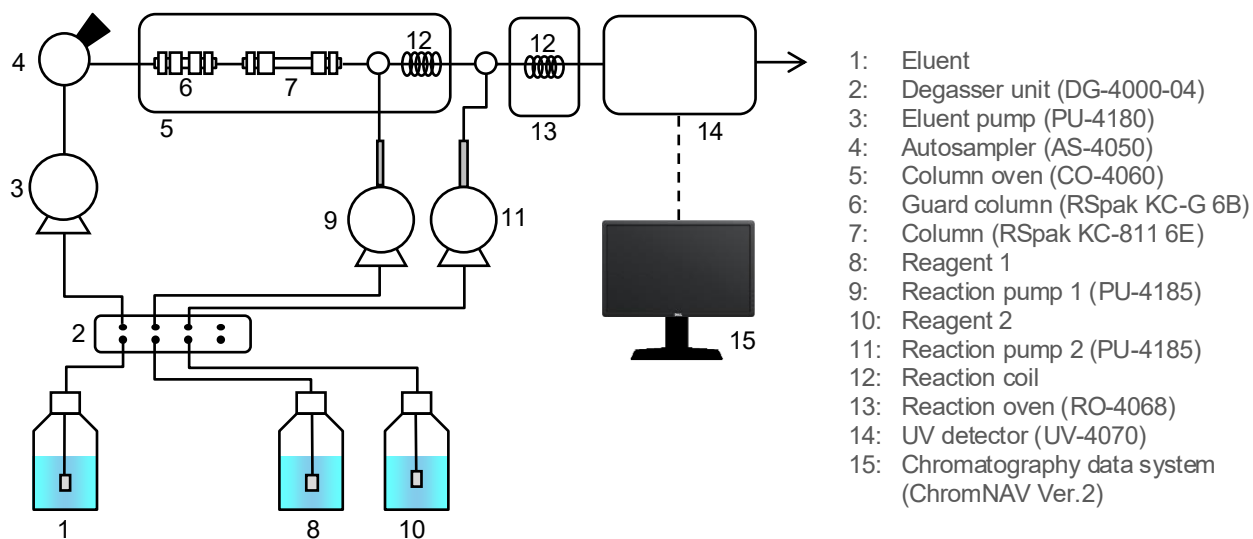


Fig. 2 System flow diagram

Results

According to the Waterworks Act, the measurement accuracy for CN^- and CNCl is generally required to be one-tenth of the target concentration, which is set at $\leq 10 \mu\text{g/L}$, and the quantification limit is $1.0 \mu\text{g/L}$. A calibration curve was prepared by measuring the peak areas for mixed standard solutions at five different concentrations (0.5, 1.0, 2.0, 5.0, and $10 \mu\text{g/L}$), with each solution measured three times.

Figure 3 shows the chromatograms of the CN^- and CNCl mixed standard solutions. The calibration curves shown in Figure 4 exhibited good linearity for both analytes, with correlation coefficients (r) of ≥ 0.9992 .

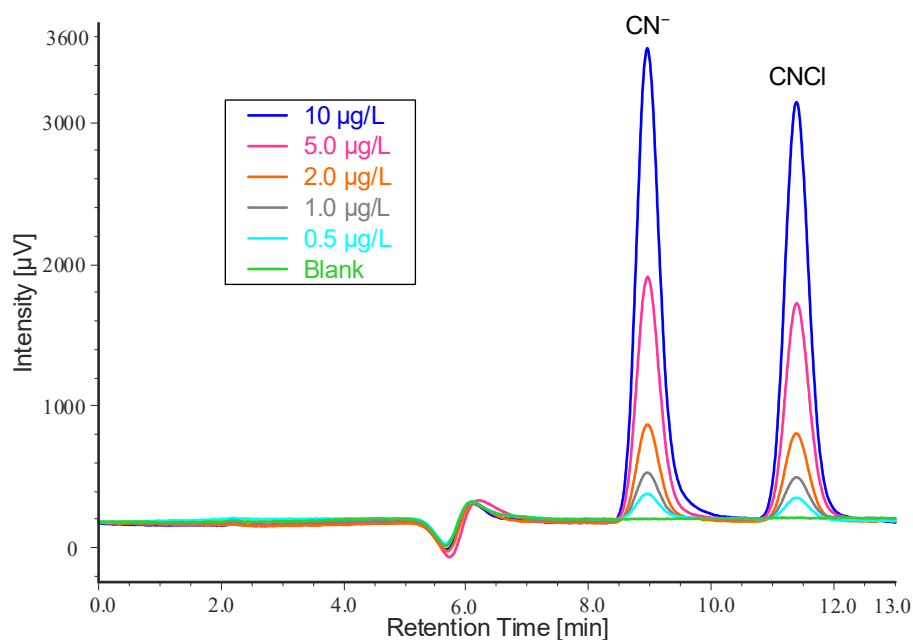


Fig. 3 Chromatograms of the CN^- and CNCl mixed standard solutions (0.5, 1.0, 2.0, 5.0, and $10 \mu\text{g/L}$)

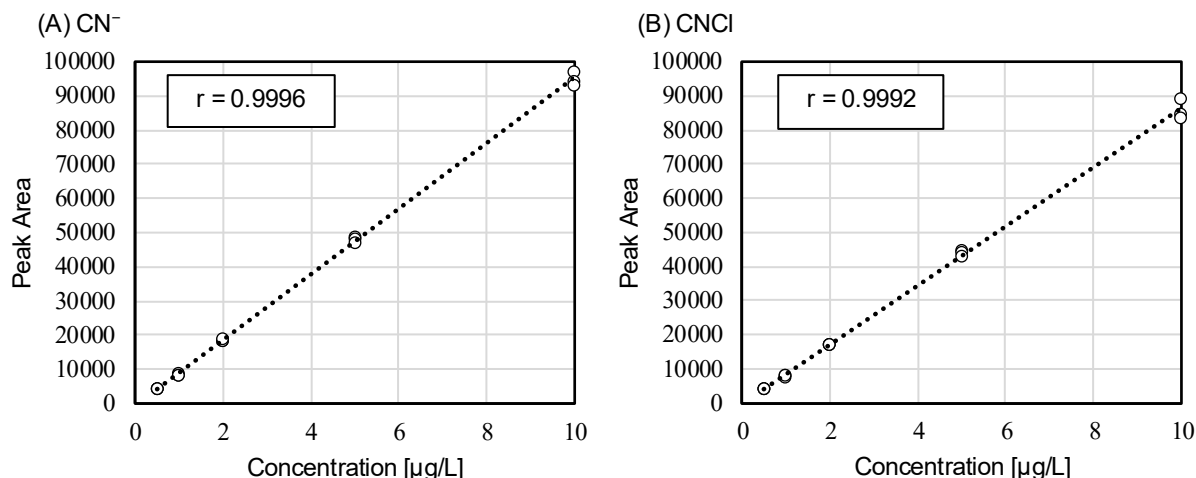


Fig. 4 Calibration curves of the mixed standard solutions
(A) CN⁻ (B) CNCl

Tables 1 and 2 summarize the calibration curve validation results. No peaks corresponding to CN⁻ or CNCl were detected in the carryover blanks. Across all standard solution calibration levels, measured concentrations ranged from 94% to 104% of the nominal concentrations, with relative standard deviations (RSDs) of ≤4.2%.

Table 1. Calibration curve validation results for CN⁻

Concentration [μg/L]	Peak Area						Quantitative Value [μg/L]	Accuracy [%]
	#1	#2	#3	Average	SD	RSD [%]		
0.5	4,696	4,487	4,375	4,519	163	3.61	0.52	103.7
1.0	9,041	8,354	8,502	8,632	362	4.19	0.95	94.8
2.0	18,382	18,242	19,086	18,570	452	2.44	1.99	99.3
5.0	49,146	48,066	47,421	48,211	872	1.81	5.08	101.6
10.0	97,352	94,181	93,444	94,992	2,076	2.19	9.97	99.7
Blank ^{*1}	N.D. ^{*2}	N.D. ^{*2}	N.D. ^{*2}					

^{*1} Blank (ultrapure water) analyzed immediately after the highest-concentration mixed standard solution

^{*2} N.D.: not detected

Table 2. Calibration curve validation results for CNCl

Concentration [μg/L]	Peak Area						Quantitative Value [μg/L]	Accuracy [%]
	#1	#2	#3	Average	SD	RSD [%]		
0.5	4,149	4,080	4,068	4,099	44	1.07	0.49	98.6
1.0	8,061	7,891	7,972	7,975	85	1.07	0.94	94.1
2.0	17,183	17,110	17,133	17,142	37	0.22	2.00	100.0
5.0	44,903	44,267	43,341	44,170	785	1.78	5.12	102.4
10.0	89,196	84,825	83,779	85,933	2,874	3.34	9.95	99.5
Blank ^{*1}	N.D. ^{*2}	N.D. ^{*2}	N.D. ^{*2}					

^{*1} Blank (ultrapure water) analyzed immediately after the highest-concentration mixed standard solution

^{*2} N.D.: not detected

Table 3 summarizes the repeatability and accuracy results for replicate measurements of the 0.5 µg/L mixed standard solution of CN⁻ and CNCl. Both peak areas and measured concentrations exhibited good repeatability, with RSDs of ≤4.6% and the of the measured concentrations ranged from 98% to 112% of the nominal value.

Table 3. Repeatability and accuracy the CN⁻ and CNCl mixed standard solutions (0.5 µg/L each, n = 6)

Injection Number	Peak Area		Quantitative Value [µg/L]		Accuracy [%]	
	CN ⁻	CNCl	CN ⁻	CNCl	CN ⁻	CNCl
1	4,537	4,112	0.52	0.49	104.1	98.9
2	4,496	4,266	0.52	0.51	103.2	102.4
3	4,709	4,651	0.54	0.56	107.7	111.3
4	4,298	4,220	0.50	0.51	99.1	101.4
5	4,423	4,175	0.51	0.50	101.7	100.3
6	4,606	4,208	0.53	0.51	105.5	101.1
Average	4,512	4,272	0.52	0.51	103.5	102.6
SD	143.0	192.6	0.01	0.02	2.99	4.45
RSD [%]	3.17	4.51	2.88	4.34	2.88	4.34

Conclusion

Cyanide ions (CN⁻) and cyanogen chloride (CNCl) were analyzed by ion chromatography with post-column derivatization and UV-Visible spectroscopic detection, using ethanol in place of N,N-dimethylformamide (DMF) as the solvent for the color developing reagent. The method provided sufficient sensitivity to detect both analytes at 1.0 µg/L (0.001 mg/L), equivalent to one-tenth of the water quality standard value required by the Water Supply Act.

Calibration curves generated over the range of 0.5 to 10 µg/L exhibited good linearity, and evaluation of the calibration standards demonstrated good accuracy and precision, with no detectable carryover. Replicate measurements of the 0.5 µg/L mixed standard solution also demonstrated good repeatability and accuracy. These results support the use of ethanol as an alternative to DMF for the color developing reagent in the analysis of CN⁻ and CNCl.

Author: LC System Group
N. Suzuki