APPLICATION NOTE 73755

Comprehensive analysis of components and degradation products in coolants

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Keywords: Coolants, IC, HPLC, UV, DAD, CAD, automated sample preparation, water quality, inhibitors, degradation products, contaminants

Application benefits

- Straightforward automated sample loading and sample preparation capabilities, such as simultaneous injection, concentration, matrix elimination, and separation of ionic substances by ion chromatography.
- Complementary detectors (ultraviolet detection and charged aerosol detection) enable the elimination of false positive or false negative results.

Goal

Separation and quantification of ingredients and degradation products in coolants using complementary techniques (IC-CD, HPLC-UV, HPLC-UV-CAD)

Introduction

Coolants are required when operating engines or other machines to ensure that such elements do not overheat and are durable. In addition, the coolant must serve as frost and corrosion protection. The largest fraction of commonly used coolants consists of deionized water, glycol for frost protection, inhibitors to protect against corrosion, cavitation, and foaming, and additives for pH stabilization.

A variety of analytical methods are used for comprehensive analysis of in-service coolants. The refractive index (RI) is often a first parameter for determining the correct mixing ratio, while inductively coupled plasma optical emission spectrometry (ICP-OES) provides important information about water hardness and quality. In addition, ion chromatography (IC) and high-performance liquid chromatography (HPLC) deliver detailed information about

the chemical composition of the coolant. As both, the glycol and the additives will degrade during the period of use; they must be carefully monitored to define the appropriate time when the coolant needs to be replaced.

High temperature and air contact accelerate degradation and generate oxidation products. The corrosion protection is not only achieved by adding a single inhibitor, but by adding combinations of inhibitors, typically two or three compounds, which are intended to protect different metals and alloys and to maximize engine reliability. A number of different inhibitor formulations are available since different engines and materials require different inhibition strategies. These formulations are usually not miscible, and the appropriate one must be used. Coolants based on organic acid technologies (OAT) are widely used, because they offer a long-term stability, and multimetal protection, with organic acids being added, as they are able to stabilize the pH and are readily oxidizable thus fulfilling a dual purpose.[1] OAT-coolants contain a wide range of ionic, polar, and nonpolar compounds such as monocarboxylic acids, dicarboxylic acids, azoles, and aromatic acids. Small ionic analytes as inorganic anions and low molecular weight organic acids as part of the inhibitor package or derived from coolant degradation can be separated and trace level detected in various matrices using IC equipped with conductivity detection (CD). Reversed phase (RP)-HPLC, on the other hand, is suitable for the separation of less polar molecules such as azoles and weak organic acids with aromatic or long-chain saturated substitutes, in particular the latter showing poor ultraviolet (UV)-absorption. However, coupling charged aerosol detection (CAD) in series to UV greatly improves sensitivity and provides additional selectivity.

This application note demonstrates a workflow for automated sample preparation and analysis of representative coolant samples with IC-CD and direct sample injection in RP-HPLC using UV detection only as well as serially coupled to CAD. Measurements performed on IC-CD and HPLC-UV only were carried out by OELCHECK.

The owner-managed family business OELCHECK, founded in 1991, is currently the leading laboratory for lubricant and operating fluid analyses in Europe. The laboratory of the company is located in Brannenburg, Germany.

Experimental IC

The analysis of the coolant samples using IC was carried out in the OELCHECK laboratory.

Chemicals

- Deionized water, 18.2 MΩ·cm resistivity or higher
- VWR potassium hydroxide EMSURE™ ACS (P/N 1.05029.1000)
- Fisher Scientific™ Alfa Aesar™ adipic acid (P/N AAA137050I)
- LGC standards Glycolate Standard, 1000 µg/mL in water (P/N VHG-IGLY-100)
- LGC standards Acetate Standard, 1000 µg/mL in water (P/N VHG-IACET-100)
- LGC standards Nitrite Standard, 1000 µg/mL in water (P/N VHG-INO2-100)
- LGC standards Nitrate Standard, 1000 µg/mL in water (P/N VHG-INO3-100)
- LGC standards Formate Standard, 1000 µg/mL in water (P/N VHG-IFORM-100)
- LGC standards Oxalate Standard, 1000 µg/mL in water (P/N VHG-IOXAL-100)
- LGC standards Multi-Anion Standard, 100 µg/mL in water (P/N VHG-ICMI-100)

Equipment

- VWR 0.2 µm PP Syringe Filter (P/N 514-0064)
- PP Microvial Crimp/Snap, Fisher Scientific (P/N C04011-14)
- Blue Snap-It™ Seal T/SSLT, Fisher Scientific (P/N C04011-55B)

Instrumentation

A Thermo Scientific™ Dionex™ Integrion™ HPIC™ system with RFIC-EG™ was used for the analysis.

- Thermo Scientific™ Dionex™ Integrion™ RFIC™ system with Thermostatted Detector Compartment (P/N 22153-60305)
- Thermo Scientific™ Dionex™ AXP pump (P/N 063973)
- Thermo Scientific™ Dionex™ AS-AP Autosampler (P/N 074921)

- Thermo Scientific™ Dionex™ InGuard™ Na/HRP cartridge (P/N 074035)
- Thermo Scientific™ Dionex™ CR-ATC 600 Continuously Regenerated Anion Trap Column (P/N 088662)
- Thermo Scientific™ Dionex™ IonPac™ UTAC LP2 Trap Column (P/N 079917)
- Thermo Scientific™ Dionex™ Suppressor AERS 500 (P/N 082541)

Table 1. Chromatographic conditions for IC-CD

Parameter	Value
Column	Dionex IonPac AS 15 2 × 250 mm (P/N 053941)
Eluent	Aqueous potassium hydroxide (KOH)
Eluent source	Thermo Scientific™ Dionex™ EGC 500 KOH potassium hydroxide eluent generator cartridge (P/N 075778)
AXP pump (transfer pump)	Time [min] / Flow rate [mL/min] 0.0 / 1.0 2.0 / 1.0 2.1 / 0.2 50.0 / 0.2
Flow rate analytical pump	0.3 mL/min
Analytical gradient	Time [min] / Concentration KOH [mM] -0.5 / 7.0 0.0 / 7.0 11.0 / 7.0 22.0 / 43.5 29.0 / 50.0 32.0 / 60.0 35.0 / 60.0 36.0 / 65.0 40.0 / 65.0 42.0 / 7.0 50.0 / 7.0
Injection volume	2.5 µL
Column temperature	30 °C
Detection	Suppressed conductivity
Suppressor	Dionex AERS 500
Detection compartment temperature	15 °C
Cell temperature	35 °C
Background conductance	<0.5 µS
System backpressure	~2000 psi (~138 bar)
Noise	<0.025 µS

Ion chromatography with automated sample preparation was performed to determine small ionic analytes for water quality, degradation products, contaminants, and inhibitors of the coolant samples. A four-stage flow diagram is shown in Figure 1. The initial flow path through two valves is shown in A. To load the sample loop, the injection valve is switched as shown in B. After completing sample loading, the trap and the injection valve switch to the position in which the sample loop is emptied onto the trap column passing through the Dionex InGuard cartridge. In this step, the matrix is separated from the target analytes (C). After switching the trap valve, the flow of the analytical pump is introduced and pumped through the trap column to the pre-column and analytical column, where the separation takes place (D). Subsequently, the analytes are detected using a suppressed conductivity detector.

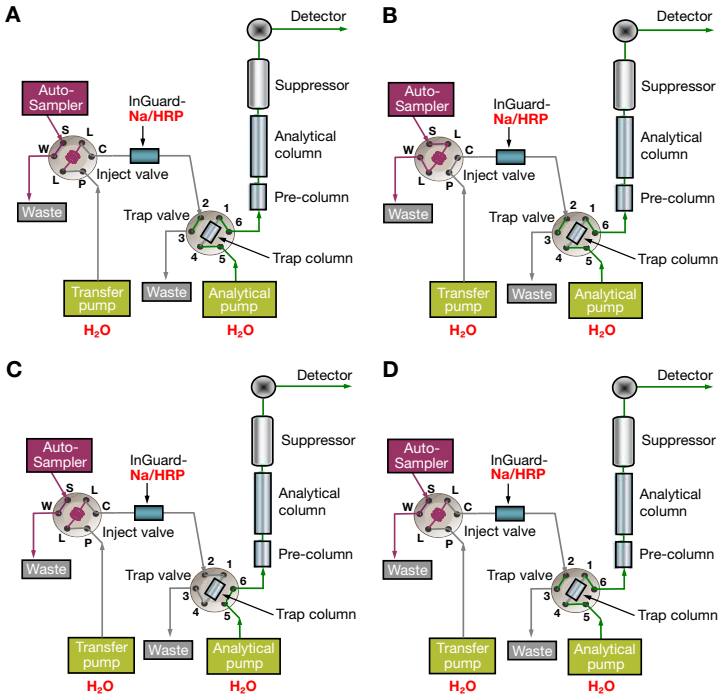

Figure 1. Fluidic scheme of IC workflow with automated sample preparation with A) initial position, B) loading sample loop, C) flushing sample loop through Dionex InGuard Na/HRP cartridge to trap column, and D) flushing analytes from trap column onto pre-column and analytical column followed by suppressed conductivity detection

Preparation of standards and samples

A multi-element stock solution was prepared from commercial standards gravimetrically as 100 mg/L each, containing chloride, sulfate, glycolate, acetate, formate, oxalate, fluoride, bromide, nitrite, and nitrate, in water. A stock solution of adipinate with 1000 mg/L was prepared in water.

Calibration standards were prepared with concentrations of 0.5, 1, 5, 10, 25, 50, and 100 mg/L by volumetric dilution with water. Additionally, adipinate single standards were prepared of 200, 300, 400, and 500 mg/L by volumetric dilution with water.

The coolant samples were filtered and diluted 1:10 or 1:20 with ultrapure water prior to injection.

Experimental HPLC-UV and HPLC-UV-CAD

The analysis of the coolant samples using HPLC-UV only was carried out in the OELCHECK laboratory. Analysis using HPLC-UV-CAD was performed in the Thermo Fisher Scientific laboratory.

Chemicals

OELCHECK laboratory

- Deionized water, 18.2 MΩ·cm resistivity or higher
- VWR acetonitrile HiPerSolv CHROMANORM™ (P/N 83639.320)
- VWR ortho-phosphoric acid (≥85%) HiPerSolv CHROMANORM (P/N 153154DP)
- VWR potassium hydroxide EMSURE™ ACS (P/N 1.05029.1000)
- VWR benzoic acid AnalaR NORMAPUR™ (P/N 20172.180)
- Sigma-Aldrich decanedioic acid (P/N 283258)
- Fisher Scientific™ Alfa Aesar™ 2-ethylhexanoic acid (P/N AAA12644AE)
- Fisher Scientific™ Alfa Aesar™ iso-nonanoic acid (P/N AAL13360AE)
- Sigma-Aldrich octanoic acid (P/N C2875)
- Supelco *p*-toluic acid (P/N 41768)
- Fisher Scientific™ Alfa Aesar™ hexanedioic acid (P/N AAA137050I)
- Sigma-Aldrich 2-mercaptobenzothiazole (P/N M3302)
- Fisher Scientific™ Alfa Aesar™ 1H-benzotriazole (P/N AAA1542318)
- Supelco 5-tolyltriazole (P/N 14949)
- Fisher Scientific™ Alfa Aesar™ nonanoic acid (P/N AAB21568AK)
- Fisher Scientific™ Alfa Aesar™ decanoic acid (P/N AAA1478830)
- Fisher Scientific™ Alfa Aesar™ dodecanoic acid (P/N AA4203818)
- Fisher Scientific™ Alfa Aesar™ heptanedioic acid (P/N AAA1849514)
- Fisher Scientific™ Alfa Aesar™ octanedioic acid (P/N AAA1396322)
- Fisher Scientific™ Alfa Aesar™ dodecandioic acid (P/N AAA1038730)

Thermo Fisher Scientific laboratory

- Deionized water, 18.2 MΩ·cm resistivity or higher
- Fisher Scientific™ acetonitrile Optima™ LC/MS grade (P/N A955-212)
- Thermo Scientific™ Pierce™ LC-MS grade trifluoroacetic acid (TFA) (P/N PI85183)

Equipment

OELCHECK laboratory

- Fisherbrand™ 11 mm crimp neck vial, amber glass (2 mL) (P/N 11545884)
- Thermo Scientific™ 11 mm autosampler vial crimp caps (Chlorobutyl, PTFE) (P/N 11568150)
- VWR 0.2 µm PP Syringe Filter (P/N 514-0064)

Thermo Fisher Scientific laboratory

- Fisherbrand™ 11 mm crimp neck vial, amber glass (2 mL) (P/N 11545884)
- Thermo Scientific™ 11 mm autosampler vial crimp caps (Chlorobutyl, PTFE) (P/N 11568150)
- Thermo Scientific™ Target2™ regenerated cellulose syringe filters (P/N F2513-8)

Instrumentation

OELCHECK laboratory

A Thermo Scientific™ UltiMate™ 3000 SD HPLC system was used for the analysis.

- Thermo Scientific™ UltiMate™ 3000 Solvent Rack with 4 degasser channels (SRD-3400) (P/N 5035.9245)
- Thermo Scientific™ UltiMate™ 3000 HPG-3400SD (P/N 5040.0041)
- Thermo Scientific™ UltiMate™ 3000 WPS-3000TSL Analytical Sampler (P/N 5822.0020)
- Thermo Scientific™ UltiMate™ 3000 TCC-3000SD Thermostatted Column Compartment (P/N 5730.0010)
- Thermo Scientific™ UltiMate™ 3000 DAD-3000 Diode Array Detector (P/N 5082.0010) with analytical flow cell, 13 µL (P/N 6082.0400)

Thermo Fisher Scientific laboratory

A Thermo Scientific™ Vanquish™ Flex Quaternary UHPLC system was used for the analysis.

- Thermo Scientific™ Vanquish™ System Base Vanquish Flex (P/N VF-S01-A)
- Thermo Scientific™ Vanquish™ Dual Gradient Pump F (P/N VF-P32-A)
- Thermo Scientific™ Vanquish™ Sampler FT (P/N VF-A10-A)
- Thermo Scientific™ Vanquish™ Column Compartment H (P/N VH-C10-A-02)
- Thermo Scientific™ Vanquish™ Diode Array Detector FG (P/N VF-D11-A) with flow cell, 2.5 µL (P/N 6083.0550)
- Thermo Scientific™ Vanquish™ Charged Aerosol Detector H (P/N VH-D20-A)

Table 2. Chromatographic conditions for HPLC-UV (OELCHECK laboratory)

Parameter	Value	
Column	Thermo Scientific™ Acclaim™ RSLC 120 C18 150 × 2.1 mm; 2.2 µm, (P/N 071399)	
Mobile phase	A: 90/10 water/acetonitrile (v/v) with 0.05% phosphoric acid B: 10/90 water/acetonitrile (v/v) with 0.05% phosphoric acid	
Flow rate	0.3 mL/min	
Injection volume	2 µL	
Gradient	Time [min]	%B
	0.0	0
	15.4	70
	20.0	100
	22.0	100
	24.0	0
	37.0	0
Column temperature	30 °C	
Autosampler temperature	10 °C	
UV wavelength	214 nm	
UV data collection rate	10 Hz	
UV response time	0.5 s	
Spectral scan	190–400 nm	

Table 3. Chromatographic conditions for HPLC-UV-CAD (Thermo Fisher Scientific laboratory)

Parameter	Value
Column	Acclaim RSLC 120 C18 150 × 2.1 mm; 2.2 µm, (P/N 071399)
Mobile phase	A: 90/10 water/acetonitrile (v/v) with 0.05% TFA B: 10/90 water/acetonitrile (v/v) with 0.05% TFA
Flow rate	0.3 mL/min
Injection volume	1 µL
Gradient	Time [min] %B 0.0 0 15.4 70 20.0 100 22.0 100 24.0 0 34.0 0
Column temperature	30 °C with active pre-heater at 30 °C (forced air mode with fan speed 5)
Autosampler temperature	10 °C
DAD FG detector settings	UV wavelength: 214 nm UV data collection rate: 10 Hz UV response time: 0.5 s Spectral scan: 190–400 nm
CAD detector settings	Evaporation temperature: 35 °C Filter: 3.6 s Power function value: 1.00 Data collection rate: 5 Hz

Table 4. Stock solutions as standard mixtures used for the preparation of calibration standards in HPLC-UV and HPLC-UV-CAD measurements. Concentration of KOH solvent is 0.15 mol/L.

Compound	Concentration [mg/L]	Solvent	Standard mixture
Monocarboxylic acids			
2-Ethylhexanoic acid	2500	KOH	IV
Octanoic acid	2500	KOH	IV
Nonanoic acid	2500	KOH	IV
Iso-nonanoic acid	2500	KOH	IV
Decanoic acid	2500	KOH	IV
Dodecanoic acid	2500	KOH	IV
Dicarboxylic acids			
Heptanedioic acid	2500	KOH	III
Hexanedioic acid	2500	KOH	III
Octanedioic acid	2500	KOH	I
Decanedioic acid	2500	KOH	II
Dodecanedioic acid	2500	KOH	II
Aromatic acids			
Benzoic acid	500	KOH	I
Toluic acid	500	KOH	I
Azoles			
Benzotriazole	500	KOH	I
Tolyltriazole	500	KOH	I
Mercaptobenzothiazole	500	KOH	I

Preparation of standards and samples

The stock solutions of the standard mixtures I-IV and three representative coolant samples were provided by the OELCHECK laboratory.

The exact composition of the standard mixtures, shown in Table 4, is important to ensure solubility of all compounds. The standard solutions produced in this way are stable for up to 4 weeks at room temperature.

Tolyltriazole shows two isomeric peaks in coolant samples, which is why a standard of technical quality was used to prepare the calibration standards. Since this quality also contains the isomeric tolyltriazole, both peaks were quantified together.

HPLC-UV: External calibration was performed in the range of 2 to 500 mg/L for aromatic acids and azoles, and of 32 to 2500 mg/L for monocarboxylic and dicarboxylic acids by diluting the stock solutions with the appropriate amount of ultrapure water.

HPLC-UV-CAD: External calibration was performed in the range of 0.1 to 500 mg/L for aromatic acids and azoles, and of 10 to 2500 mg/L for monocarboxylic and dicarboxylic acids by diluting the stock solutions with the appropriate amount of ultrapure water.

The coolant samples were filtered and diluted 1:10 or 1:20 with ultrapure water prior to injection.

Data processing and software

OELCHECK laboratory

The Thermo Scientific™ Chromeleon™ 7.2 SR4 Chromatography Data System (CDS) was used for data acquisition and analysis for IC-CD and HPLC-UV.

Thermo Fisher Scientific laboratory

The Thermo Scientific™ Chromeleon™ 7.3 Chromatography Data System (CDS) was used for data acquisition and analysis for HPLC-UV-CAD.

Results and discussion

Ion chromatography

Coolants are available as concentrates and are diluted with water before use. Chloride and sulfate ions contained in the water can be used as a marker for water quality. The diluted coolant sample is flushed onto the analytical column after a concentration step (refer to Figure 1 for more details) and retained due to its ionic interaction with the stationary phase. Separation takes place when the ionic strength in the mobile phase is increased. Figure 2 shows the chromatogram of a 50 mg/L standard, containing anions of degradation products, water quality indicators, inhibitors, and contaminants.

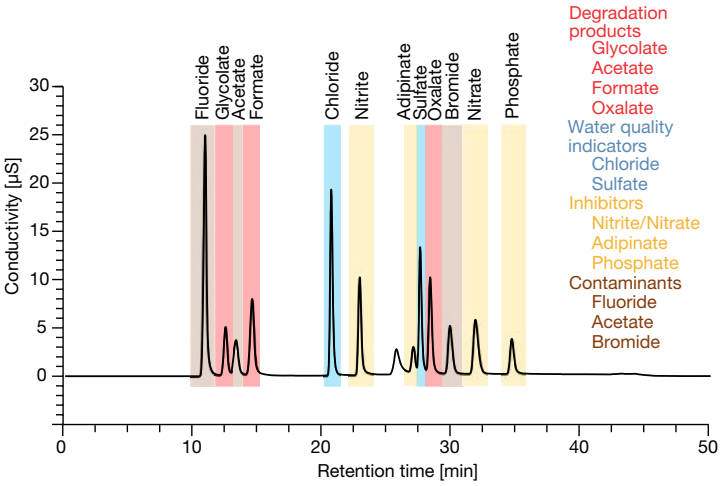

Figure 2. Representative IC chromatogram on the separation of degradation products, water quality indicators, inhibitors, and contaminants. Standard mixture concentration was 50 mg/L each.

The obtained calibration results are listed in Table 5. Excellent correlation coefficients with ≥0.99974 are found, while applying a quadratic curve fit type. To estimate the limit of detection (LOD) and the limit of quantification (LOQ), the signal-to-noise ratio (S/N) of each analyte at the lowest calibration level (0.5 mg/L) was determined using a fixed retention time interval of 0.5 min before or after each peak. Lowest LOD (S/N 3) and LOQ (S/N 10) values were obtained for fluoride with 0.001 mg/L and 0.002 mg/L, respectively, while the highest values were achieved for adipinate with 1.0 mg/L for LOD and 3.33 mg/L for LOQ.

Table 5. Concentration range used for calibration and obtained correlation coefficients in IC-CD measurement. The compounds are sorted according to their elution order.

Compound	Concentration range [mg/L]	Correlation coefficient (R^2)
Fluoride	0.5–100	0.99992
Glycolate	0.5–100	0.99994
Acetate	0.5–100	0.99974
Formate	0.5–100	0.99988
Chloride	0.5–100	0.99998
Nitrite	0.5–100	0.99997
Adipinate	0.5–500	0.99985
Sulfate	0.5–100	0.99998
Oxalate	0.5–100	0.99996
Bromide	0.5–100	0.99998
Nitrate	0.5–100	0.99998
Phosphate	0.5–100	0.99991

Three coolant samples were analyzed after a 1:10 or 1:20 dilution with ultrapure water and the online sample pre-treatment as described in experimental IC section. Figure 3 shows the IC chromatograms and Table 6 summarizes the quantitative results.

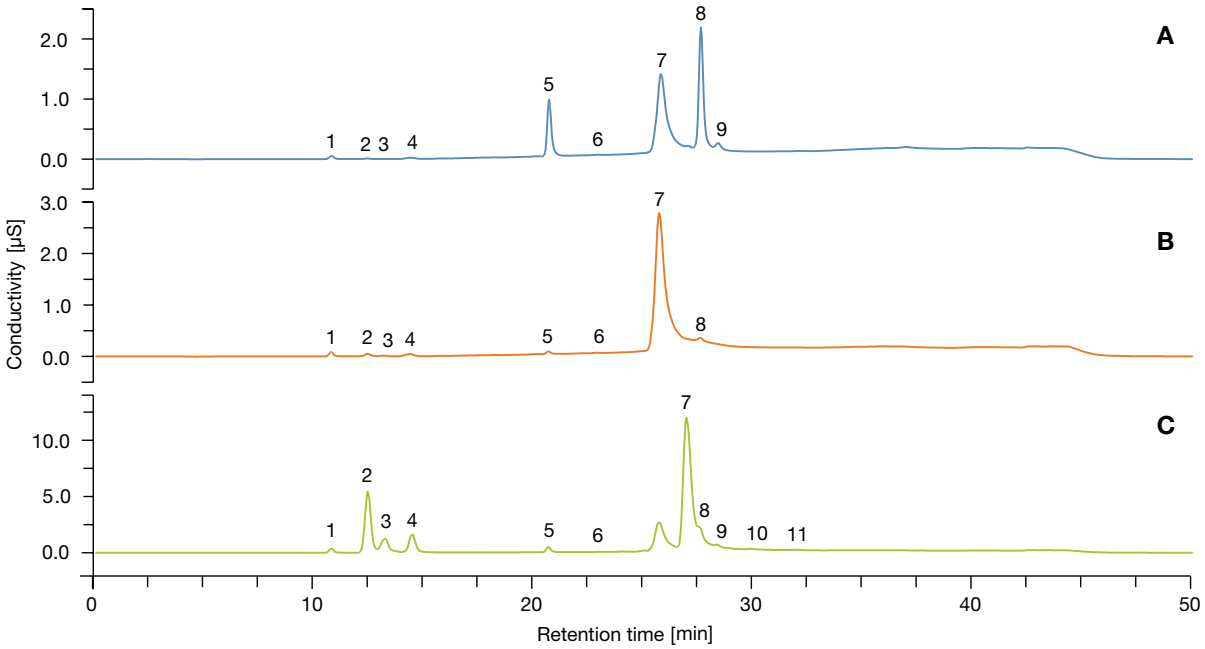

Figure 3. IC chromatograms of three representative coolant samples; (A) sample 1, (B) sample 2, (C) sample 3. Refer to Table 6 for peak assignments.

Table 6. Quantitative IC results for three representative coolant samples. Samples 1 and 3 have been diluted 1:10 and sample 2 1:20 with water and filtered prior to analysis; the amount [mg/L] given in the table has been corrected with the appropriate dilution factor.

Peak	Compound	Sample 1		Sample 2		Sample 3	
		RT [min]	Amount [mg/L]	RT [min]	Amount [mg/L]	RT [min]	Amount [mg/L]
1	Fluoride	10.75	0.9	10.73	2.9	10.74	6.4
2	Glycolate	12.38	5.7	12.39	20.5	12.41	555.1
3	Acetate	13.19	5.2	13.12	12.5	13.18	155.6
4	Formate	14.36	3.0	14.34	9.4	14.42	106.3
5	Chloride	20.66	28.3	20.62	5.7	20.63	15.8
6	Nitrite	22.87	3.2	22.84	6.5	22.84	3.3
7	Adipinate	26.98	5.1	< LOD		26.92	4378.4
8	Sulfate	27.58	84.7	27.53	9.4	27.54	13.9
9	Oxalate	28.36	9.1	< LOD		28.32	11.6
10	Bromide	< LOD		< LOD		29.88	7.1
11	Nitrate	32.12	3.6	< LOQ		31.92	6.7
12	Phosphate	< LOD		< LOQ		< LOD	

During engine operation, particularly from excessive heat, glycol can be degraded into glycolate, acetate, formate, and oxalate. Sample 3 shows high amounts of these acids, which leads to the assumption that the coolant was already heavily used.

Chloride and sulfate ions are a measure of water quality from the source water used for coolant preparation. Sample 1 shows higher amounts of both ions, indicating a poorer water quality. High levels of chloride in the sample can increase the risk of metal corrosion. In addition, in an acidic environment, sulfate can form sulfuric acid, which promotes cavitation and pitting of cast iron surfaces as well iron corrosion or red rust, while calcium sulfate can form in an alkaline milieu and cause undesired deposits on hot metal surfaces.

Adipinate, nitrite, and nitrate are inhibitors that are used against corrosion for various metals. Sample 3 shows a high amount of adipinate, while nitrite and nitrate are also detected. From this perspective, the coolant sample still contains inhibitors to protect the engine from corrosion. Samples 1 and 2 do not contain adipinate, but nitrite as corrosion inhibitor.

HPLC-UV and HPLC-UV-CAD

The RP-HPLC-UV method was originally developed on an UltiMate 3000 SD system to separate and detect commonly contained aromatic acids, azoles, and organic acid inhibitors, such as mono- and dicarboxylic acids, which are used as corrosion protection for various metals. To neutralize the acidic analytes to achieve adequate separation, the pH of the mobile phase must be low.

Phosphoric acid was used as an additive for the mobile phase at a level of 0.05%, which resulted in a pH of about 2. However, this pH is already at the lower end of the specification limit of the Acclaim RSLC C18 column, which can lead to a certain column bleeding with the effect of a higher baseline noise and a higher LOD and LOQ as a consequence.

Since the monocarboxylic and dicarboxylic acids absorb poorly in the UV, a more suitable detection technique was sought. Therefore, the method was transferred to a Vanquish Flex Quaternary UHPLC system equipped with a UV detector and a CAD. Only the additive in the mobile phase was changed to 0.05% TFA, while keeping the column, column temperature, and gradient profile the same. The additive TFA was chosen to replace phosphoric acid because only volatile additives can be used with CAD to obtain minimal background interferences and best performance. At the same time, the pH was increased from about 2 to 2.3 to not operate the column at its minimum pH limit, but still below the pK_a values of the analytes to keep them neutral, which led to symmetric peaks.

Figure 4 shows two UV chromatograms of the six monocarboxylic acids contained in standard mixture IV with (A) 0.05% phosphoric acid and (B) 0.05% TFA in the mobile phase. The differences in signal response is due to different injection volumes (2 µL was used for the phosphoric acid method and 1 µL used for the TFA method). Table 7 summarizes resolution and peak parameters (peak width at 50% height and asymmetry) obtained for the two methods.

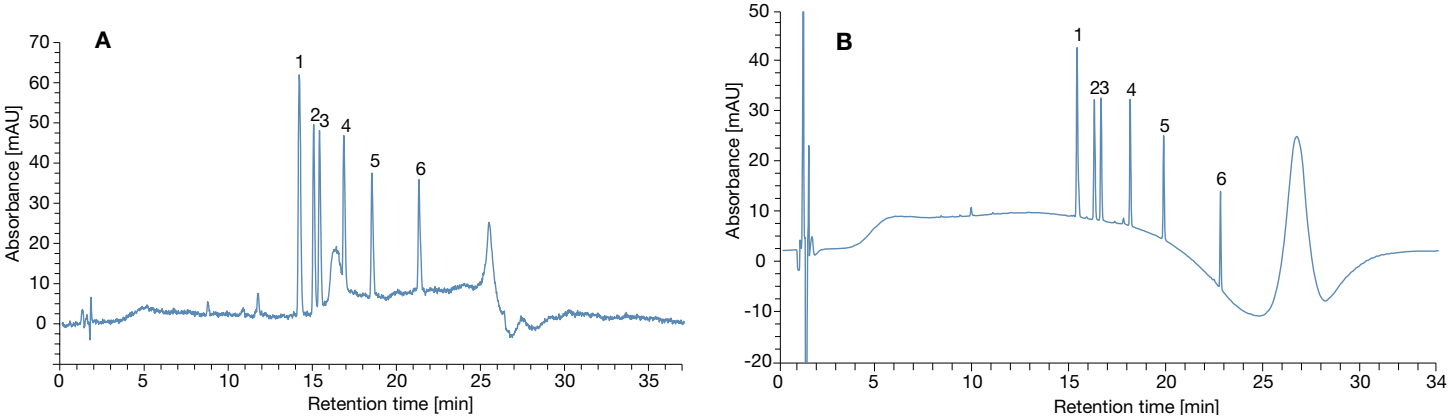

Figure 4. UV chromatograms of monocarboxylic acids with (A) 0.05% phosphoric acid and (B) 0.05% TFA in mobile phase; sample: standard mixture IV at a concentration of 2500 mg/L. Differences in signal response are due to different injection volumes: 2 µL for the phosphoric acid method and 1 µL for the TFA method. Refer to Table 7 for peak assignments.

Table 7. Resolution (Rs), peak width (50%), and asymmetry values obtained for phosphoric acid and TFA method; sample: standard mixture IV at a concentration of 2500 mg/L

Peak	Compound	Phosphoric acid method			TFA method		
		R_s	Peak width (50%) [min]	Asymmetry	R_s	Peak width (50%) [min]	Asymmetry
1	2-Ethylhexanoic acid	3.68	0.156	1.34	7.02	0.076	1.42
2	Octanoic acid	1.68	0.119	1.28	2.84	0.074	1.22
3	Iso-nonanoic acid	7.05	0.123	1.30	13.41	0.069	1.18
4	Nonanoic acid	7.87	0.119	1.43	16.22	0.063	1.12
5	Decanoic acid	12.80	0.131	1.43	29.05	0.062	1.12
6	Dodecanoic acid	n.a.	0.128	1.49	n.a.	0.057	1.08

The decision to use a Vanquish Flex Quaternary UHPLC system for the method transfer was based on the fact that the Thermo Scientific™ Vanquish™ UHPLC Platform is currently state-of-the-art with new innovative technologies for improved performance. The new generation instrument enables less peak dispersion due to lower extra column volume. The Vanquish Flex diode array detector (DAD) in particular allows for a higher detector sensitivity compared to the UltiMate 3000 DAD. As obvious from Table 7, the peak width (50%) and peak asymmetry decreases, while resolution increases, when applying the TFA method on the Vanquish Flex instrument. The differences in the baseline noise, seen in Figure 4, can be attributed to the use of the technically improved Vanquish Flex DAD, and the column bleeding effect, when operating the column at their lower pH limit, as previously mentioned. This assumption was not further verified during the study.

The signal response in the CAD for the dicarboxylic acids is higher than in the UV, expressed as the S/N in Figure 5A, while no increased sensitivity for the monocarboxylic acids could be achieved, as they were too volatile. The azoles and aromatic acids show no or only low signal response in CAD, but a high UV activity (Figure 5B).

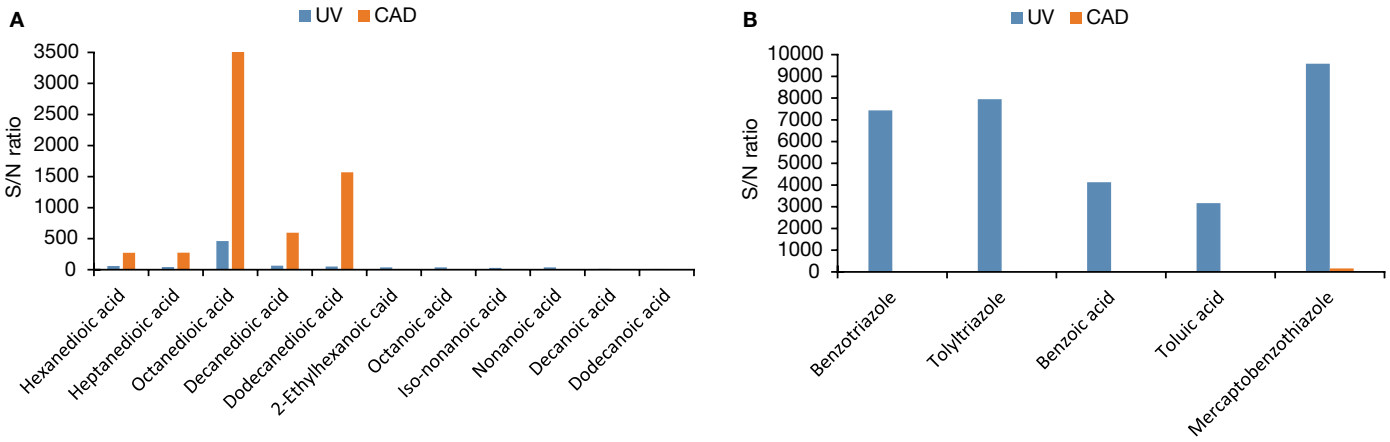

Figure 5. Comparison on S/N ratio obtained with UV and CAD for 100 mg/L standards (n=5); (A) monocarboxylic and dicarboxylic acids, (B) aromatic acids and azoles

Five consecutive injections of each standard mixture with 100 mg/L were performed using the TFA method and method performance data evaluated. Table 8 summarizes the results on relative standard deviation of retention time (%RSD RT) and area (%RSD area), as well as on peak asymmetry. Excellent %RSD RT values were obtained with ≤0.05%. Acceptable area reproducibility (%RSD area) was achieved between 0.1 and 2.8%. Asymmetry values were obtained with 0.9–1.2, which indicates very symmetric peak shapes in the entire chromatogram.

Table 8. Method performance data for %RSD RT, %RSD area, and peak asymmetry obtained for standard mixtures at 100 mg/L from five consecutive injections. The * indicates that the value could not be determined because the peak (tolyltriazole) is an isomeric unresolved double peak. Obtained %RSD RT values <0.05, %RSD area between 0.1 and 2.8, and peak asymmetries between 0.9 and 1.2.

Compound	Detector	RT [min]	%RSD RT	%RSD area	Asymmetry
Hexanedioic acid	CAD	3.4	0.05	1.8	1.2
Heptanedioic acid	CAD	6.2	0.03	2.1	1.2
Benzotriazole	UV	6.8	0.03	0.5	1.1
Octanedioic acid	CAD	8.3	0.02	0.7	1.1
Tolyltriazole	UV	9.1	0.01	0.5	n.a.*
Benzoic acid	UV	10.0	0.01	1.1	1.1
Decanedioic acid	CAD	11.4	0.02	1.6	1.2
Toluic acid	UV	12.1	0.01	0.1	1.1
Mercaptobenzothiazole	UV	12.6	0.01	0.5	1.1
Dodecanedioic acid	CAD	14.2	0.01	0.3	1.1
2-ethylhexanoic acid	UV	15.4	0.00	1.1	1.0
Octanoic acid	UV	16.2	0.01	1.8	1.1
Iso-nonanoic acid	UV	16.6	0.01	2.4	1.1
Nonanoic acid	UV	18.1	0.01	1.7	1.1
Decanoic acid	UV	19.8	0.01	2.8	0.9
Dodecanoic acid	UV	22.7	0.01	2.7	1.2

Comprehensive analysis of azoles, aromatic acids, and carboxylic acids in coolants can require both UV and CAD detection, depending on the composition of the coolant sample and expected target analytes. Performing only UV analysis can be sufficient, if the concentration of dicarboxylic acids in the real samples is above the LOQ of the UV method (Table 9).

The S/N ratio was determined by using a solvent blank run (100% water) for noise calculation. The S/N was then calculated based on the peak to peak method within Chromeleon CDS software with a multiple time span factor of 10 at half peak height (peak width (50%)). The LOD and LOQ values were determined by diluting the standard mixture until a S/N ratio between 3 and 20 was observed. The exact concentrations corresponding to S/N 3 for LOD and 10 for LOQ were then calculated based on extrapolation from the measured values. Table 9 summarizes the results for calibration and LOD, LOQ determination for the chromatographic method. LOD and LOQ for coolant samples are 10 times higher since those are diluted 1:10 prior to analysis.

Table 9. Calibration parameters, LOD and LOQ values of the HPLC-UV-CAD method. The values in brackets for LOD and LOQ show the obtained values with UV detection. The compounds are sorted according to their elution order.

Compound	Detector	Concentration range [mg/L]	Calibration type	Correlation coefficient (R^2)	LOD [mg/L]	LOQ [mg/L]
Hexanedioic acid	CAD (UV)	10–2500	Quadratic	0.99899	1.8 (3.0)	5.9 (10.0)
Heptanedioic acid	CAD (UV)	10–2500	Quadratic	0.99914	1.3 (7.1)	4.2 (23.8)
Benzotriazole	UV	0.5–500	Linear	0.99914	0.09	0.3
Octanedioic acid	CAD (UV)	2.5–1250	Quadratic	0.99993	0.6 (3.0)	1.9 (9.9)
Tolyltriazole	UV	0.1–500	Linear	0.99984	0.04	0.1
Benzoic acid	UV	0.5–500	Linear	0.99999	0.05	0.2
Decanedioic acid	CAD (UV)	1–1000	Quadratic	0.99792	0.3 (4.6)	0.9 (15.2)
Toluic acid	UV	0.5–500	Linear	0.99993	0.1	0.4
Mercaptobenzothiazole	UV	0.5–500	Linear	0.99999	0.1	0.4
Dodecanedioic acid	CAD (UV)	1–1000	Quadratic	0.99934	0.5 (3.7)	1.7 (12.4)
2-ethylhexanoic acid	UV	25–2500	Linear	0.99985	7.6	25.3
Octanoic acid	UV	25–2500	Linear	0.99988	5.7	19.0
Iso-nonanoic acid	UV	50–2500	Linear	0.99993	13.9	46.3
Nonanoic acid	UV	25–2500	Linear	0.99992	10.2	34.1
Decanoic acid	UV	50–2500	Linear	0.99947	22.2	73.9
Dodecanoic acid	UV	50–2500	Linear	0.99984	26.4	88.0

The same coolant samples analyzed by IC-CD were further analyzed by HPLC-UV-CAD. Aromatic acids, azoles, and monocarboxylic acids have been quantified based on the UV signal, while dicarboxylic acids were quantified on the CAD response. Figure 6 shows chromatograms of the coolant samples and the quantitative results are summarized in Table 10.

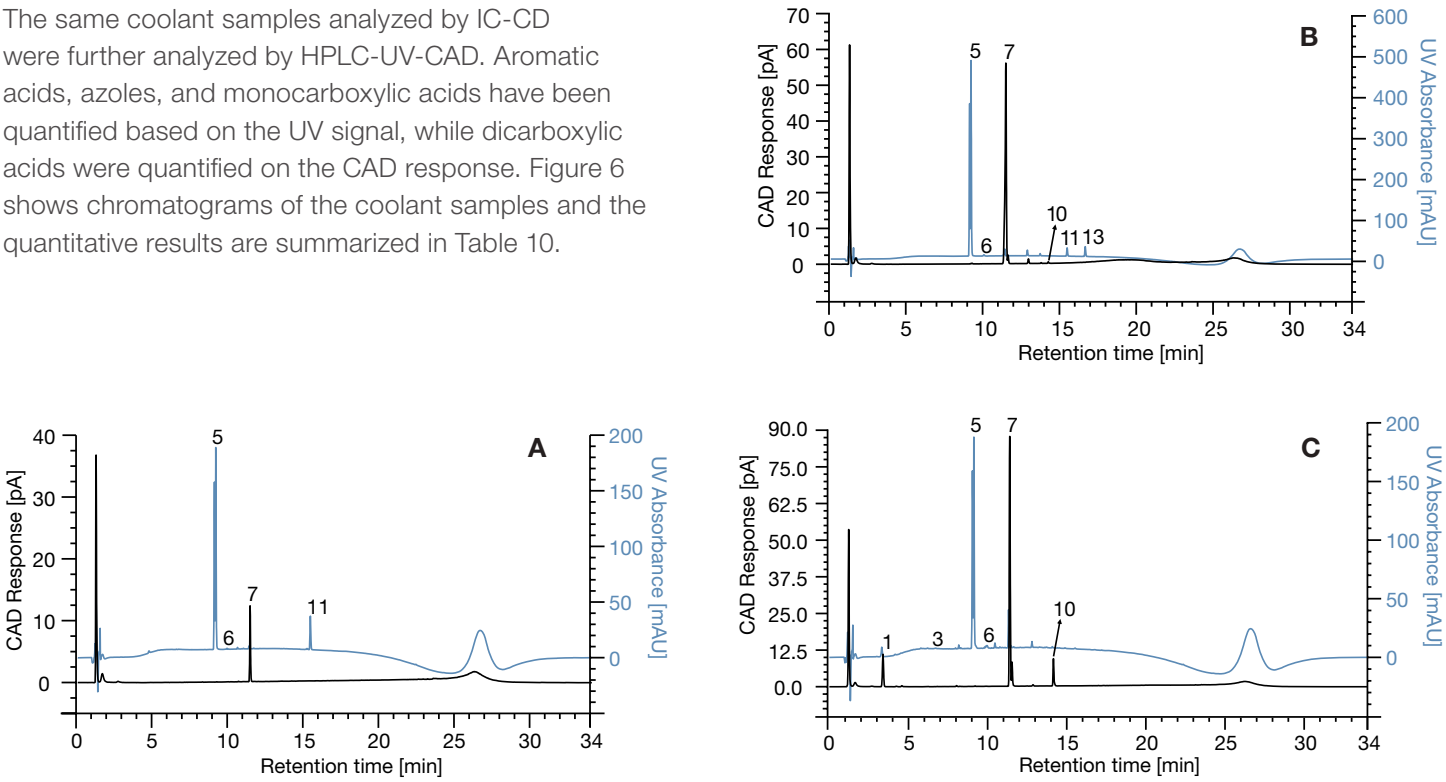

Figure 6. Chromatograms of three coolant samples with overlaid traces of UV detection (blue) and CAD (black); (A) sample 1; (B) sample 2; (C) sample 3. Refer to Table 10 for peak assignments.

Table 10. Quantitation results for three coolant samples. Samples have been diluted 1:10 with ultrapure water and filtered prior to analysis; aromatic acids, azoles and monocarboxylic acids have been quantified based on the UV signal, dicarboxylic acids based on CAD response.

Peak	Compound	Sample 1		Sample 2		Sample 3	
		RT [min]	Amount [mg/L]	RT [min]	Amount [mg/L]	RT [min]	Amount [mg/L]
1	Hexanedioic acid	< LOD		< LOD		3.39	4662.5
2	Heptanedioic acid	< LOD		< LOD		< LOQ	
3	Benzotriazole	< LOD		< LOQ		6.73	3.76
4	Octanedioic acid	< LOD		< LOD		< LOD	
5	Tolyltriazole	9.13	845.3	9.13	2176.7	9.13	853.5
6	Benzoic acid	9.89	5.86	9.98	32.34	9.96	26.03
7	Decanedioic acid	11.40	1339.8	11.40	11865.0	11.40	13608.8
8	Toluic acid	< LOQ		< LOQ		< LOQ	
9	Mercaptobenzothiazole	< LOD		< LOD		< LOD	
10	Dodecanedioic acid	< LOQ		14.16	41.17	14.16	804.9
11	2-Ethylhexanoic acid	15.39	14736.4	15.39	9897.0	< LOD	
12	Octanoic acid	< LOD		< LOQ		< LOD	
13	Iso-nonanoic acid	< LOD		16.57	20829.3	< LOD	
14	Nonanoic acid	< LOD		< LOD		< LOD	
15	Decanoic acid	< LOD		< LOD		< LOD	
16	Dodecanoic acid	< LOD		< LOD		< LOD	

From the quantitative results, it can be concluded that all three coolant samples still contain high amounts of several inhibitors, such as tolyltriazole, decandioic acid, and/or 2-ethylhexanoic acid, giving evidence to continuous protection for various metals from corrosion. In comparison to the fresh coolants, however, especially sample 3 shows a remarkable depletion of inhibitor concentrations. This might be due to the wrong dilution ratio if the coolant was prepared from a concentrate, mixture with a different coolant, or deterioration of the coolant.

For sample 1, the slight depletion of all inhibitor components points to a wrong mixing ratio. This is also supported by a low coolant concentration as calculated from the refractive index. The IC results showing elevated concentrations of chloride (28.3 mg/L) and sulfate (84.7 mg/L) finally indicate the use of low-quality water for the preparation of the coolant mixture. Deterioration of the coolant can be excluded by the absence of the typical oxidation indicators, mainly glycolate and formate, in the IC results. Sample 2 is showing slight depletion in inhibitor concentrations only. Glycolate (20.5 mg/L) and formate (9.4 mg/L) concentrations as derived from IC analyses, however, indicate the onset of coolant degradation with glycolate being the first degradation product of ethylene glycol, the most common antifreeze in coolants.

Sample 3 finally shows, beside the more significant depletion in inhibitor concentrations, severe amounts of degradation products (glycolate: 555.1 mg/L, formate 106.3 mg/L). These results strongly support a severe degradation/oxidation of the coolant, mainly due to improper operation conditions or long-term use. The poor condition of sample 3 is reflected in more results than the inhibitor and degradation concentrations derived from IC and HPLC measurements. The pH of 7.0 indicates acidification of the coolant, and an iron concentration of 5.3 mg/L (ICP-OES) finally confirms the lack of inhibitors; corrosion has started in this equipment.

The use of two complementary detectors helps to eliminate false positive or false negative results. As illustrated in Figure 7A and B, the UV trace shows a peak at 8.2 min with a S/N of 107. Based on the UV signal only, the peak would be assigned as octanedioic acid. From the results given in Figure 5, however, it can be seen that the S/N ratio in CAD is approximately 40 times higher than in UV for this molecule. Therefore, the presence of the analyte can be excluded since the peak at 8.3 min in the CAD chromatogram shows a 10 times lower S/N ratio. On the other hand, the use of the CAD helped to reduce false negative results (Figure 7C and D). The small peak observed for dodecanedioic acid in the UV chromatogram

at 14.1 min is determined with a S/N ratio of 3 and cannot be quantified with UV detection, while the signal in the CAD is well above the LOQ of the method showing a S/N ratio of 53. Additional peaks can be observed in each chromatogram, which did not match any of the retention times for the target analytes. The time delay of 0.1 min between UV and CAD is caused by the longer flow path with the additional capillary after the UV outlet.

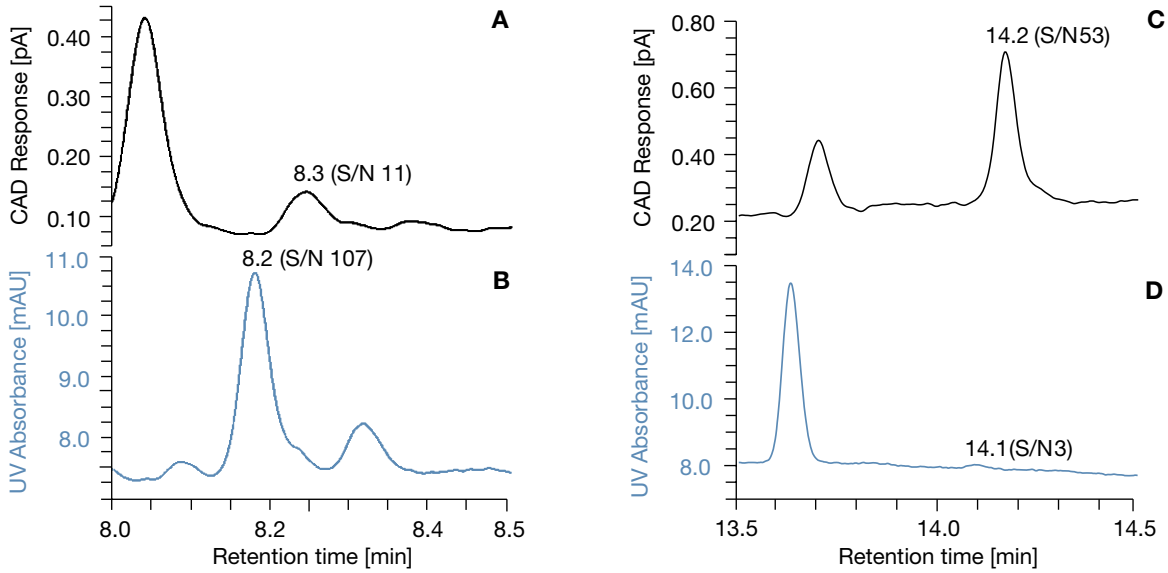

Figure 7. Left: chromatograms of sample 1 of (A) CAD response and (B) UV absorbance showing the retention time window for octanedioic acid; right: chromatograms of sample 2 of (C) CAD response and (D) UV absorbance showing the retention time window for dodecanedioic acid. A false positive peak is assigned in UV for octanedioic acid; a false negative result is obtained for UV for dodecanedioic acid. The time delay of 0.1 min between UV and CAD signal is caused by the longer flow path with the additional capillary after the UV outlet.

Conclusion

- IC and RP-HPLC with UV and CAD allow for a comprehensive monitoring of coolant samples.
- While UV is commonly used for this analysis, the CAD offers a higher sensitivity for dicarboxylic acids, which are barely detectable with UV.
- Serial coupling of UV and CAD enables the elimination of false positive and negative results by complementing each other.

Reference

1. Gershun, A.V. and Woyciesjes, P.M, Engine Coolants and Coolant System Corrosion, ASM Handbook, Volume 13C, *Corrosion: Environments and Industries,* page 531–537, **2006**, https://doi.org/10.31399/asm.hb.v13c.a0004164

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