UPLC EPA Methods Book

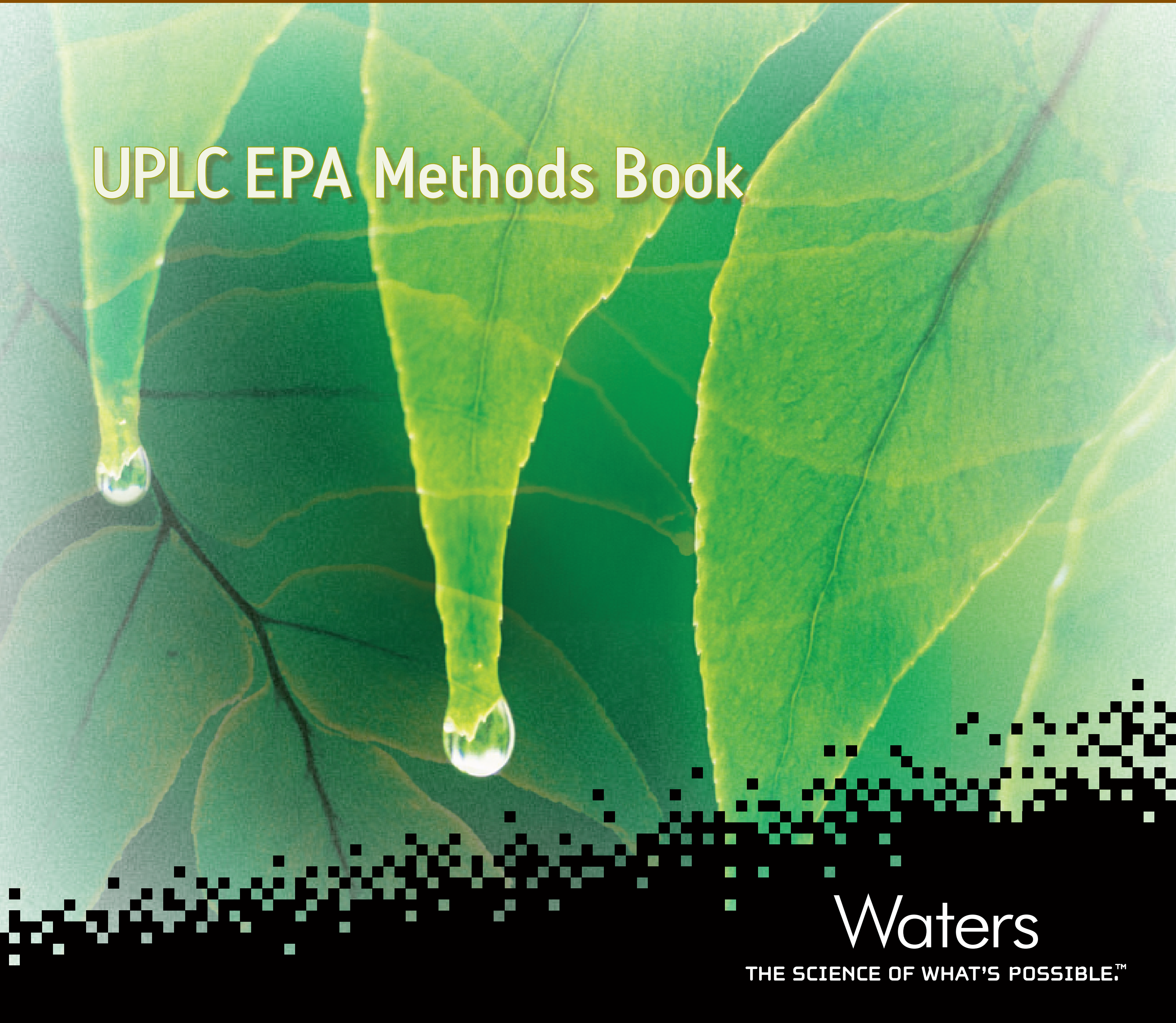

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TABLE OF CONTENTS

EPA METHOD 532

Phenylureas 6

EPA METHOD 535

Chloroacetanilide and Acetamide Herbidice Degradates in Drinking Water 8

EPA METHOD 549.2

Diquat-Paraquat 12

EPA METHODS 550.1, 610, 8330

Polynuclear Aromatic Hydrocarbons (PAHs) 14

EPA METHOD 555

Chlorinated Acids 18

EPA METHODS TO11, 554, 8315

Options 1 and 2, Aldehydes and Ketones as DNPH Derivatives 20

EPA METHOD 8330B

Explosives 22

532

PHENYLUREAS

ACQUITY UPLC System with TUV Detector

EPA Method 532 describes the analysis of phenylurea pesticides using HPLC with UV detection.

Compounds that may be analyzed using this method include:

- Tebuthiuron; Siduron A
- Thidiazuron; Siduron B
- Monuron*; Linuron
- Fluometuron; Carbazole*
- Diuron; Diflubenzuron
- Propanil

** surrogate compounds*

Conditions

LC system:	ACQUITY UPLC
UV detection:	ACQUITY UPLC TUV, dual wavelength, 245 nm
Eluent:	Binary gradient *(see below)*
Column:	Waters ACQUITY® BEH C_{18} 1.7 µm, 2.1 x 100 mm
Column temp.:	43 °C
Injection volume:	5.0 µL (full loop)
Weak wash:	5% aqueous acetonitrile (800 µL)
Strong wash:	50% aqueous acetonitrile (500 µL)
Flow rate:	0.7 mL/min

Standard Preparation

Prepare AccuStandard Mix M- 532 diluted 1:100 water/acetonitrile, and AccuStandard Mix M- 532SS, diluted 1:250 in 1:1 water/acetonitrile.

Time	Flow (mL/min)	%A	%B	Curve
Initial	0.7	73	27	-
3.0	0.7	47	53	6
3.5	0.7	47	53	6
4.0	0.7	73	27	11

Table 1. Eluent gradient for EPA Method 532.

Sample Preparation

EPA Method	Sample Matrix	Sample Preparation
532	Ground water and surface water, low concentration	Solid-Phase Extraction (SPE) – Use an SPE 500-mL water sample with an Oasis® HLB Cartridge; rinse cartridge; then elute with 5 mL 1:1 methanol/acetonitrile, evaporate and reconstitute in 1 mL initial mobile phase (500:1) sample enrichment.

Table 2. EPA Method 532 sample matrix and preparation.

Data Acquisition And Processing

The data were acquired using Empower™ 2 Software, Waters' flagship chromatography data software (CDS) package for advanced data acquisition, management, processing, reporting, and distribution.

Eluent Preparation

A: 25-mM phosphate, dissolve 1.7 g KH_2PO_4 and 850-µL H_3PO_4 in HPLC grade water, and dilute to one liter.

B: Acetonitrile
Filter and degas through a 0.45-µm PES filter.

Comments

- Detection limits of less than 1.0 ppb can be achieved using UV detection.
- Diuron is also known as Karmex.
- Siduron is a mixture of two isomers, A and B.
- At column temperatures greater than 60 °C, tebuthiuron and thidiazuron reverse elution order.
- EPA 532 may be found at the EPA website, www.EPA.gov

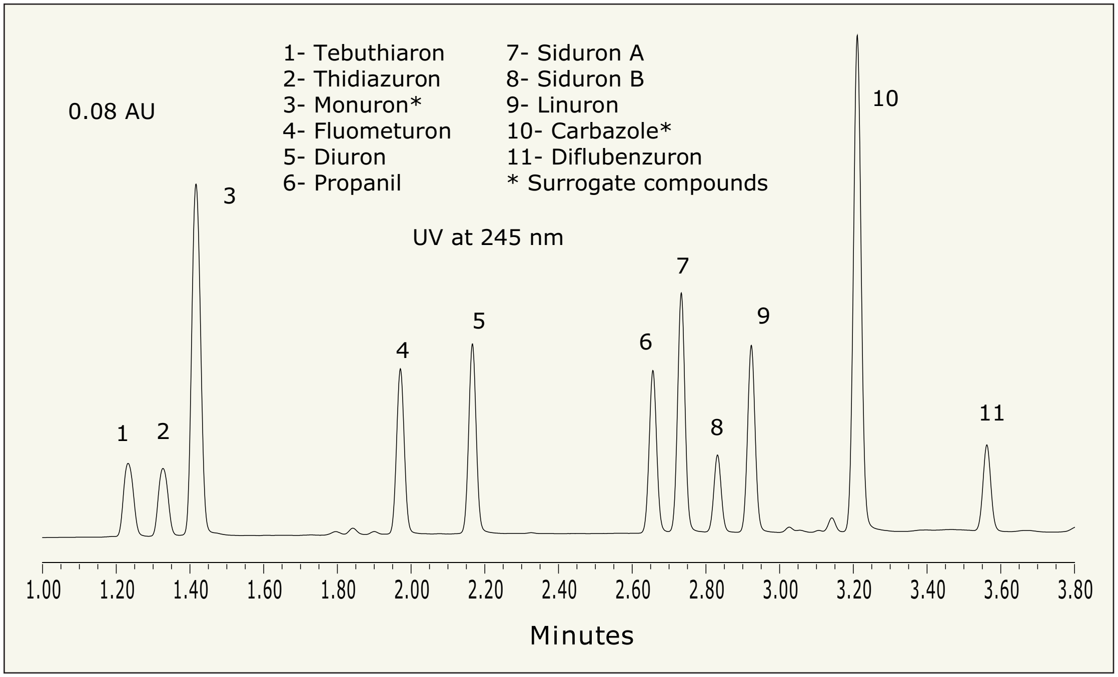

Figure 1. EPA 532 analytes, 1 ppm, 2 ppm surrogate compounds.

535

CHLOROACETANILIDE AND ACETAMIDE HERBICIDE DEGRADATES IN DRINKING WATER

ACQUITY UPLC System with TQ Detector or Quattro micro API Mass Spectrometer

EPA Method 535 describes the use of high performance liquid chromatography (HPLC) with tandem quadrupole mass spectrometry (LC/MS/MS) for the analysis of the metabolites of chloroacetanilide and acetamide herbicide degradates in drinking water matrices.

Compounds that may be analyzed using this method include:

- Alachlor oxanilic acid (OA)
- Alachlor ethanesulfonic acid (ESA)
- Acetochlor OA
- Acetochlor ESA
- Metolachlor OA
- Metolachlor ESA
- Propachlor OA
- Propachlor ESA
- Flufenacet OA
- Flufenacet ESA
- Dimetheamid OA
- Dimetheamid ESA

LC Conditions

LC system:	ACQUITY UPLC
Eluent:	Binary gradient (see below)
Column:	ACQUITY UPLC BEH C_{18} 2.1 x 150 mm, 1.7 µm
Column temp.:	60 °C
Injection volume:	50.0 µL
Flow rate:	0.45 mL/min

MS Conditions

Instrument:	ACQUITY TQD or Quattro Micro API
Ion mode:	Electrospray negative (ESI-)
Mode:	Multiple reaction monitoring (MRM)

The MRM transitions, cone voltages (CV), and collision energies (CE) are listed in Table 2.

	Compound	MRM Transition	CV	CE
1	Propachlor OA	206.3 > 134.2	13	11
2	Flufenacet OA	224.3 > 152.3	13	11
3	Propachlor ESA	256.3 > 80.0	35	25
4	Flufenacet ESA	274.3 > 80.0	35	25
5	Dimethenamid OA	270.3 > 198.3	15	11
6	Dimethenamid ESA	320.3 > 80.0	40	30
7	Acetachlor OA	264.1 > 146.2	20	11
8	Alachlor OA	264.1 > 160.1	20	11
9	Metolachlor OA	278.4 > 206.3	20	11
10	Alachlor ESA	314.1 > 80.0	40	25
11	Acetachlor ESA	314.1 > 80.0	40	25
12	Metolachlor ESA	328.2 > 80.0	37	25
13	Butachlor ESA*	356.2 > 80.0	35	25

Table 2. Optimized MRM transition parameters for EPA Method 535 in ES-.

Data Acquisition and Processing

Data were acquired using Waters MassLynx™ Software v1.1. MassLynx intelligently controls any Waters mass spectrometry system, from sample and solvent management components to mass spectrometer and auxiliary detectors.

Eluent Preparation

A: 5-mM aqueous ammonium acetate
B: Methanol

Time	Flow (mL/min)	%A	%B
Initial	0.45	90	10
5.0	0.45	75	25
7.5	0.45	66	34
10.5	0.45	65	35
11.0	0.45	10	90
13.0	0.45	90	10

Table 1. Eluent gradient.

Sample Preparation

A 250-mL water sample was extracted using a graphitized carbon SPE cartridge, dried and made up to 1 mL with 5-mM ammonium acetate.

Comments

- The use of UPLC/MS/MS for the analysis of the herbicide metabolites in EPA method 535 has many benefits over traditional HPLC/MS/MS. The advantages include a 2.5 to 4 times reduction in total runtime, improved resolution for the structural isomers acetochlor ESA, and alachlor ESA and a 2 to 4 times increase in sensitivity.
- The HPLC tandem quadrupole MS method described in EPA method 535 has a 35 min runtime and the HPLC – Ion Trap MS method has a 60 min runtime. Figure 1 illustrates the improved chromatography with respect to runtime when the analysis is carried out by UPLC/MS/MS. The total run time was shortened to 14 min.
- Two of the analytes in this method, alachlor ESA and acetochlor ESA, are structural isomers and are monitored using the same MRM transition (314 > 80). Therefore, chromatographic separation is required. The method states that these analytes must be separated chromatographically with a resolution (Rs) of 1.0 or greater. The resolution obtained on the UPLC/MS/MS System is superior to that obtained with HPLC/MS/MS and surpasses the method requirement of Rs > 1 for these compounds, as shown in Figure 3 on page 10.
- The 5-mM aqueous ammonium acetate mobile phase should be replaced at least every 48 hours, preferably every 24 hours.
- EPA method 535 may be found on the EPA website, www.EPA.gov

535

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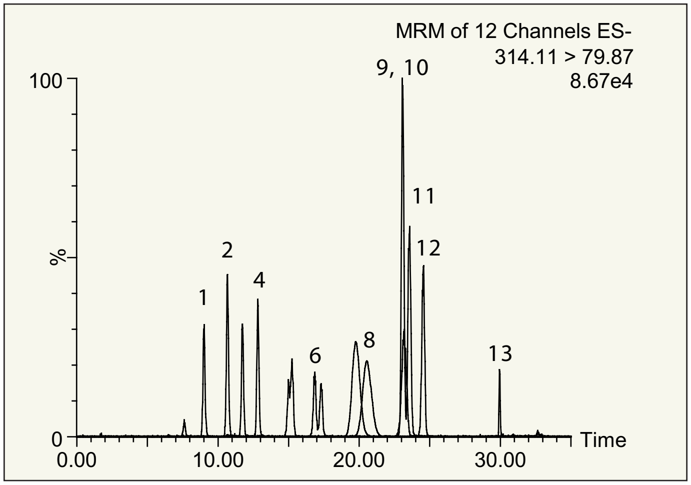

Figure 1. HPLC chromatogram for EPA Method 535 analytes.

Figure 1 shows the chromatogram obtained using a traditional HPLC/MS/MS system and the gradient specified in the EPA 535 method. The first eluting compound is propachlor OA with a retention time of 9 min. The internal standard, butachlor ESA elutes last at 30 min. The total runtime for the HPLC method is 35 min and runtimes of up to 60 min using the ion trap MS instrument are reported in the EPA method.

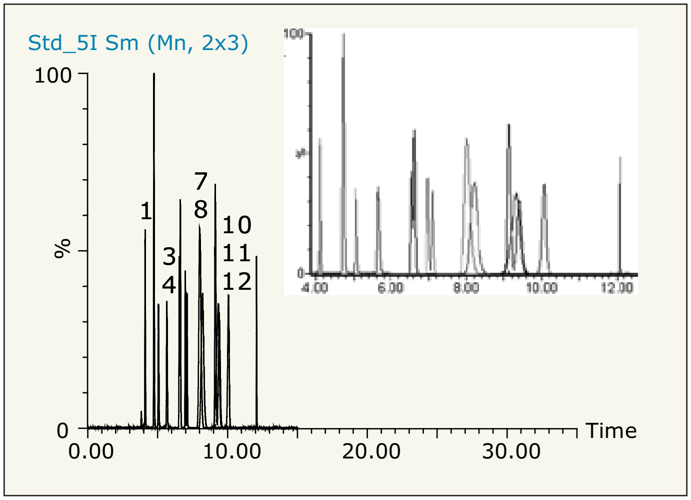

Figure 2. UPLC/MS/MS chromatogram for EPA Method 535 analytes.

Figure 2 illustrates the improved chromatography with respect to runtime when the analysis is carried out by UPLC/MS/MS. The total runtime is shortened to 14 min.

The resolution obtained on the UPLC/MS/MS System is superior to that obtained with HPLC/MS/MS and surpasses the method requirement of Rs > 1 for these compounds, as shown in Figure 3.

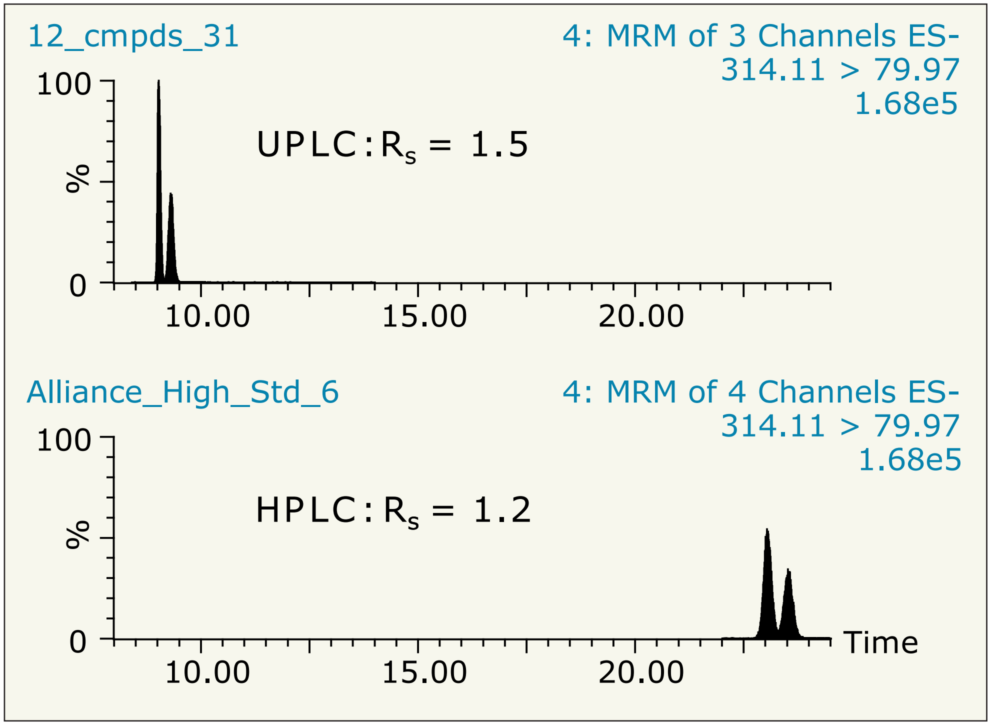

Figure 3. Comparison of resolution of Alachlor ESA and Acetochlor ESA obtained on UPLC (top) and HPLC (bottom).

549.2

DIQUAT-PARAQUAT

ACQUITY UPLC System with PDA Detector

EPA Method 549.2 describes the use of HPLC with photodiode array detector (PDA) for the determination of diquat (1,1'-ethylene-2,2'-bipyridilium dibromide salt) and paraquat (1,1'-dimethyl-4,4'- bipyridilium dichloride salt) in drinking water sources and finished drinking water.

Conditions

LC system:	ACQUITY UPLC
Eluent:	Isocratic *(see below)*
Column:	ACQUITY BEH HILIC 2.1 x 100 mm, 1.7 µm
Column temp.:	30 °C
Injection:	5.0 µL of AccuStandard Mix M- 549.1 diluted 1:1000 in eluent (full loop)
Weak wash:	5% aqueous acetonitrile (800 µL)
Strong wash:	50% aqueous acetonitrile (500 µL)
Flow rate:	0.3 mL/min
Detection:	PDA (210 to 350 nm) Diquat at 308 nm Paraquat at 257 nm

Data Acquisition and Processing

Data were acquired using Waters Empower 2 Software, which has more enhanced detection capabilities when using Waters' mass detectors or the PDA Detector.

Sample Preparation

EPA Method	Sample Matrix	Sample Preparation
549.2	Ground water and surface water, low concentration	Solid-Phase Extraction – Use a 25.0-mL sample using Oasis Weak Cation Exchange (WCX) sorbents. Elute with 1.5-mL ACN/water/TFA, 84:14:2, evaporate and reconstitute in 0.5 mL eluent (50:1 sample enrichment).

Table 1. EPA Method 549.2 sample matrix and preparation.

Comments

- Detection limits of <1.0 ppb can be achieved using PDA detection.
- LC/MS may allow for detection at parts per trillion (ppt) levels.
- Use silanized vials to prevent quaternary compounds from adhering to glass.
- For more information on sample prep see Young et. al., Waters publication (WAT41855).
- EPA method 549.2 may be found at the EPA website, www.EPA.gov

Eluent Preparation

A: 100-mM ammonium formate, dissolve 3.16 g NH_4HCO_2 in HPLC-water and make to 500 mL pH to 3.5 with formic acid.

B: Acetonitrile

Mix well: 75 mL of A, and 425 mL of B, and filter through a 0.45-µm PES filter.

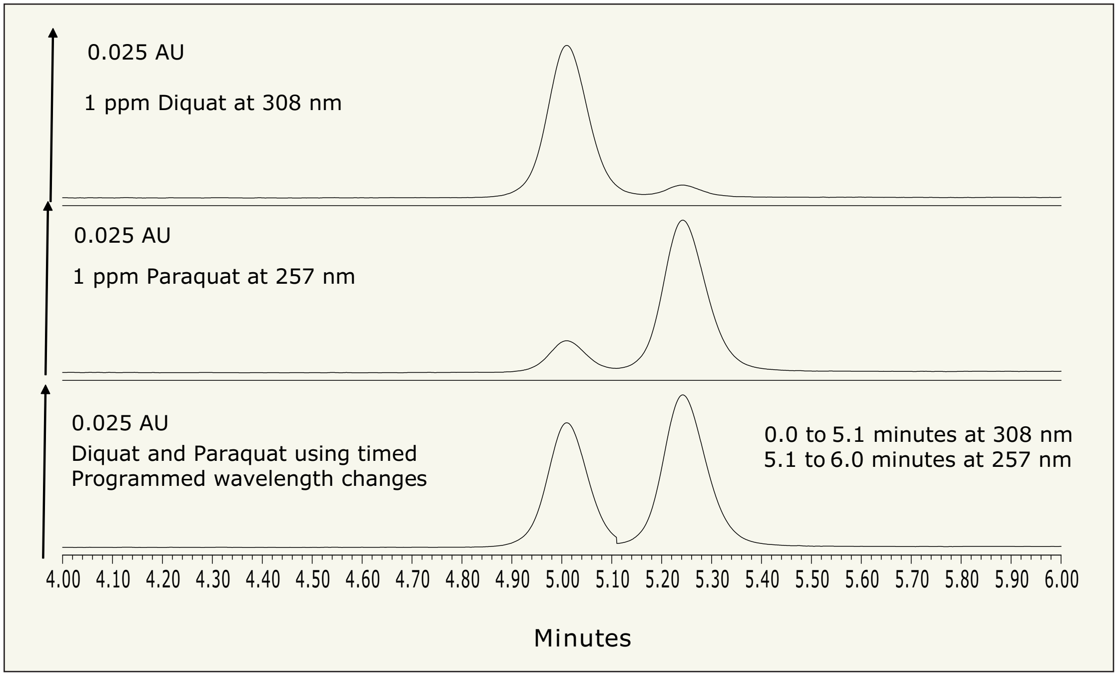

Figure 1. EPA Method 549.2 chromatograms of diquat and paraquat.

550.1, 610, 8330

POLYNUCLEAR AROMATIC HYDROCARBONS (PAHs)

ACQUITY UPLC System with PDA and FLR Detectors

EPA methods 550.1, 610, and 8310 describe the use of HPLC with ultra violet (UV) and fluorescence detection for the identification of polynuclear aromatic hydrocarbons (PAHs) in drinking water (550.1), ground and waste water (8310), and municipal and industrial waste water.

Compounds that may be identified with these methods include:

- Acenaphthene; Chrysene
- Acenaphthylene; Dibenzo(a,h)anthracene
- Anthracene; Fluoranthene
- Benzo(a)anthracene; Fluorene
- Benzo(a)pyrene; Indeno(1,2,3-cd)pyrene
- Benzo(b)fluoranthene; Naphthalene
- Benzo(ghi)perylene; Phenanthrene
- Benzo(k)fluoranthene; Pyrene

Conditions (UV)

Instrument:	UPLC System with PDA Detector
Eluent:	Water/methanol *(See Table 1)*
Column:	PAH 4.6 x 50 mm (part no. 186001260)
Column temp.:	35 °C
Injection:	5 µL AccuStandard Mix EPA 610 (#M-610) diluted 1:100 in methanol
Flow rate:	2.0 mL/min
Detection:	UV at 254 nm
Data:	Empower 2 Software

Sample Preparation

EPA Method	Sample Matrix	Sample Preparation
550.1	Drinking water	Extract with C_{18} cartridge, elute with $MeCl_2$
610	Waste water	Liquid/liquid extraction with $MeCl_2$
8310	Solid waste leachates	$MeCl_2$ extraction

Table 3. EPA method sample matrix and preparation.

Comments

- Stable column temperature control is crucial for retention time stability.
- Analyte 2, acenaphthylene, is non-fluorescent.
- For specific sample preparation protocols, see the appropriate EPA method, which can be found at www.EPA.gov.
- Using UV detection, 254 nm is a compromise wavelength, Max for the individual analytes range from 220 to 307 nm. For optimum UV sensitivity, use the optimum wavelength listed in Table 4. Another option is to use the UV Maxplot feature, however, any impurities absorbing in this range will also be enhanced.
- Fluorescence detection will yield the optimum detection limits for all compounds except acenaphthylene. See Table 4 below for excitation and emission wavelengths.

Eluent Preparation

Filter and degas through a 0.45-µm PES filter.

A: Water

B: Methanol

Time	Flow	%A	%B	Curve
Initial	2.0	50	50	-
4.0	2.0	0	100	4
4.1	2.0	50	50	6
4.0	0.7	73	27	11

Table 1. Eluent gradient (UV).

Conditions (Fluorescence)

Instrument:	UPLC System with FLD Detector
Eluent:	Water/Acetonitrile *(see Table 2)*
Column:	ACQUITY BEH Shield RP18, 2.1 X 150 mm (Part no. 186003376)
Column temp.:	45 °C
Injection:	5 µL AccuStandard Mix EPA 610QC (M-610QC) diluted 1:100 in acetonitrile
Injection volume:	2 µL
Flow rate:	0.6 mL/min
Detection:	Fluorescence using timed programmed wavelengths
Data:	Empower 2 Software

Eluent Preparation

Filter and degas through a 0.45-µm filter.

A: Water

B: Acetonitrile

Time	Flow	%A	%B	Curve
Initial	0.6	50	50	-
4.5	0.6	50	50	6
5.5	0.6	33	67	6
7.5	0.6	33	67	6
9.5	0.6	23	77	6
12.0	0.6	50	50	11

Table 2. Eluent gradient (fluorescence).

	Analyte	UV max (nm)	EX (nm)	EM (nm)	Detection Limit (ppb)[1]
1	Naphthalene	220	270	326	0.14
2	Acenaphthylene	229	NA	NA	NA
3	Acenaphthene	227	294	326	0.01
4	Fluorene	261	293	306	0.03
5	Phenanthrene	251	242	358	0.02
6	Anthracene	252	243	397	0.01
7	Fluoranthene	236	243	354	0.02
8	Pyrene	240	332	378	0.01
9	Benzo(a)anthracene	287	279	385	0.01
10	Chrysene	267	304	375	0.04
11	Benzo(b)fluoranthene	256	294	435	0.09
12	Benzo(k)fluoranthene	307	297	408	0.01
13	Benzo(a)pyrene[2]	296	292	402	0.03
14	Dibenzo(a,h)anthracene	297	292	393	0.01
15	Benzo(g,h,I)perylene	299	294	410	0.03
16	Indeno(1,2,3-cd)pyrene	250	303	495	0.49

Table 4. PAH target analytes.

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[1] Fluorescence mode used for detection limit determination, no pre-concentration. Seven replicates per 40 CFR pt. 136, App. B.

[2] Regulated compound; action level 0.17 ppb.

550.1, 610, 8330

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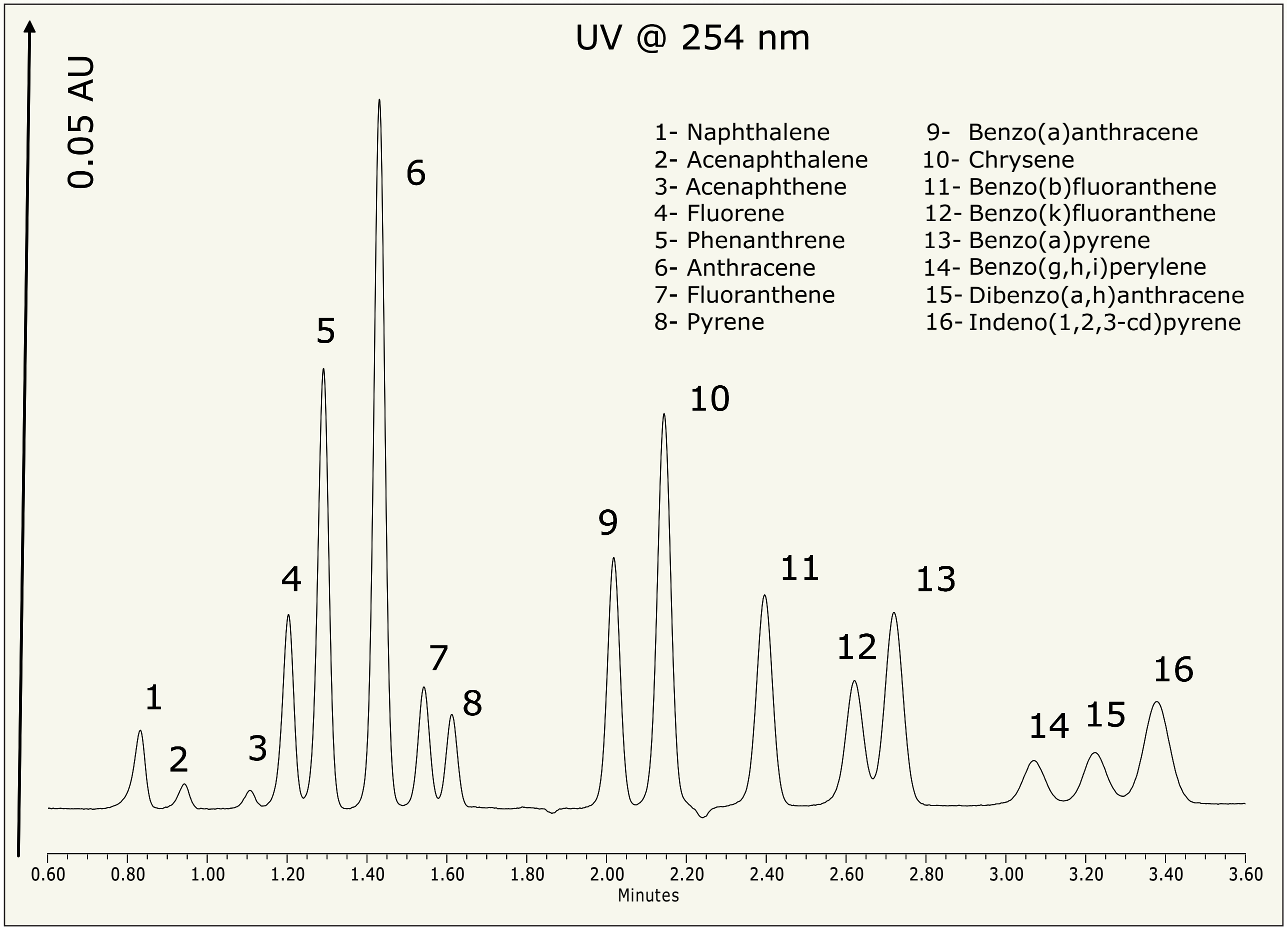


Figure 1. 1 ppm PAH analytes, UV at 254 nm.

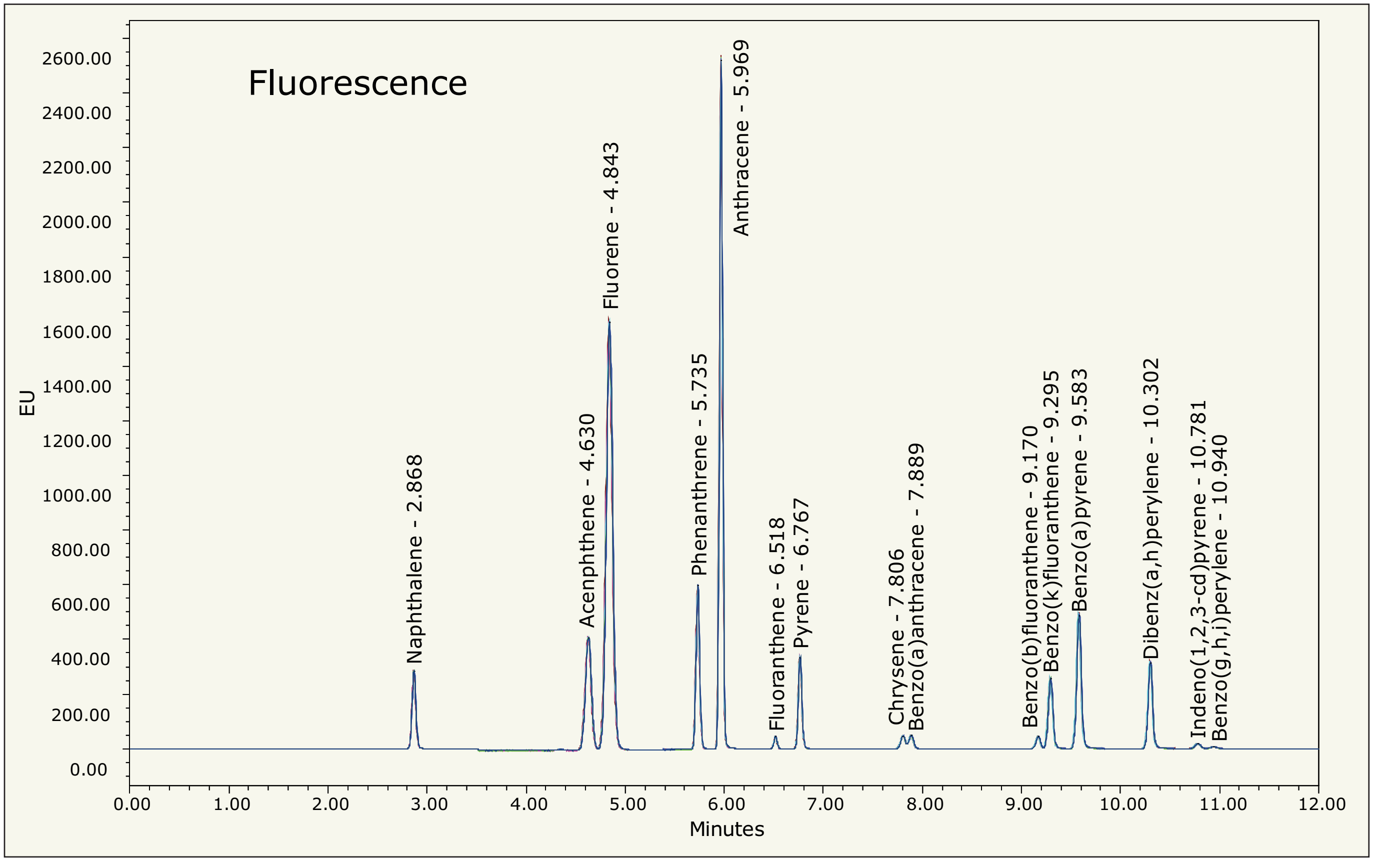


Figure 2. PAH analytes, fluorescence, time-programmed wavelength changes.

555

CHLORINATED ACIDS

ACQUITY UPLC System with Photodiode Array (PDA) Detector

EPA Method 555 describes the analysis of chlorinated acid pesticides using HPLC with UV detection. With the PDA Detector, you can utilize multiple UPLC/MS strategies for the identification of compounds that are difficult to resolve by conventional HPLC-based methods, detect and quantify lower concentrations of sample analytes, and compare spectra across wavelengths and broad concentration ranges.

Compounds that may be analyzed using this method include:

Group A

- Picloram
- Chloramben
- Dicamba
- Bentazon
- 2,4-D
- Dichlorprop
- 2,4,5-TP
- Acifluorfen

Group B

- 4-Nitrophenol
- MCPA
- 3,5- Dichlorobenzoic acid
- MCPP
- 2,4,5-T
- 2,4-DB
- Dinoseb
- Pentachlorophenol

Conditions

LC system:	ACQUITY UPLC
Eluent:	Binary gradient *(see below)*
Column:	ACQUITY BEH C_{18}, 2.1 x 100 mm, 1.7 µm
Column temp.:	40.7 °C
Injection:	5.0 µL each of AccuStandard Mix M- 555 (Mix A and B) diluted 1:1000 in 1:1 water/acetonitrile
Weak wash:	5% Aqueous acetonitrile (800 µL)
Strong wash:	50% Aqueous acetonitrile (500 µL)
Flow rate:	0.6 mL/min
Detection:	UV-PDA Scan 210 to 350 nm

Sample Preparation

EPA Method	Sample Matrix	Sample Preparation
555	Ground and finished drinking water	Solid Phase Extraction – Adjust a 75.0-mL water sample to pH 12 with 6 N NaOH. Allow to set for 1 hr, then acidify to pH 2 with H_3PO_4 and filter. Condition an Oasis Cartridge with 3.0 mL 10:90 methanol/ MTBE, rinse with 2.0 mL MeOH, 2.0 mL water. Load sample, wash with 1.0 mL water, elute with 2.0 mL 10:90 MeOH/MTBE, evaporate to 0.2 mL then reconstitute to 0.5 mL with water.

Table 2. EPA Method 555 sample matrix and preparation.

Comments

- Detection limits of <1.0 ppb can be achieved using UV detection.
- 2,4,5-TP is also known as Silvex.
- 5-Hydroxydicamba, listed in method, is no longer available as a standard.
- Use retention time, confirmation wavelengths, and area response ratios to confirm analytes (see Table 3). A PDA spectral library can also be created.
- Analytes are grouped into two sets A and B and run separately.
- EPA Method 555 may be found at the EPA website, www.EPA.gov

Data Acquisition and Processing

Data were acquired using Waters Empower 2 Software. This system provides simultaneous 2D and 3D operation in either Empower or MassLynx Software.

Eluent Preparation

A: 25-mM phosphoric acid, pipette 2 mL of 85% H_3PO_4 into HPLC grade water, and dilute to 1 L.

B: Acetonitrile

Filter and degas through a 0.45-µm PES filter.

Time	Flow (mL/min)	%A	%B	Curve
Initial	0.6	72	28	-
3.7	0.6	37	63	6
4.5	0.6	37	63	6
4.6	0.6	72	28	6

Table 1. Eluent gradient for EPA Method 555.

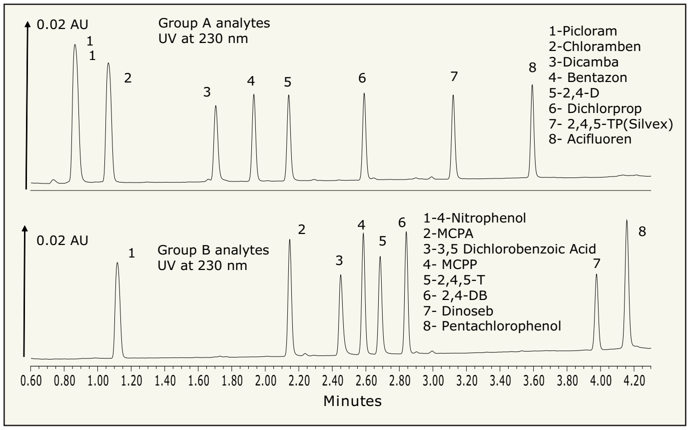


Figure 1. EPA Method 555 analytes, groups A and B (1 ppm).

Analyte and Group	Confirmation Wavelength (nm)	Area Response Ratio
Acifluorfen (A)	293	1.72
Bentazon (A)	240	1.08
Chloramben (A)	214	0.61
2,4-D (A)	285	4.02
2,4-DB (B)	285	5.93
Dicamba (A)	220	0.66
3,5-Dichlorobenzoic acid (B)	285	5.15
Dichlorprop (A)	285	4.07
Dinoseb (B)	268	0.48
MCPA (B)	285	6.66
MCPP (B)	285	6.49
4-Nitrophenol (B)	310	0.56
Pentachlorophenol (B)	290	5.65
Picloram (A)	223	0.82
2,4,5-T (B)	290	4.00
2,4,5-TP (A)	293	3.84

Table 3. Confirmation wavelengths and area response ratios (ARR) for method analytes. ARA= peak area at 230 nm/peak area at confirmation wavelength.

TO11, 554, 8315

OPTIONS 1 AND 2, ALDEHYDES AND KETONES AS DNPH DERIVATIVES

ACQUITY UPLC System with UV Detector

EPA Methods TO11, 554, and 8315 (Options 1 and 2) describe the use of HPLC with ultra violet (UV) for the identification of aldehydes and ketones as Dinitrophenylhydrazine (DNPH) derivatives in drinking water (554), soil, air, water, waste, or stacks collected by Method 0011 (8315 Option 1) ambient air (TO11), and samples collected from indoor air by Method 0100 (8315 Option 2). Methods 554 and 8315 Option 1 target the same 12 compounds. Likewise, Methods TO11 and 8315 Option 2 target the same 15 compounds. Several analytes are common to all four methods.

Compounds that may be identified with these methods include:

- Acetaldehyde[1,2]
- Acetone[2]
- Acrolein[2]
- Benzaldehyde[2]
- Butanal (Butyraldehyde)[1,2]
- Crotonaldehyde[1,2]
- Cyclohexanone[1]
- Decanal[1]
- 2,5- Dimethylbenzaldehyde[1,2]
- Formaldehyde[1,2]
- Heptanal[1]
- Hexanal (Hexaldehyde)[1,2]
- Isovaleraldehyde[2]
- Nonanal[1]
- Octanal[1]
- Pentanal (Valeraldehyde)[1,2]
- Propanal (Propionaldehyde)[1,2]
- m-Tolualdehyde[2]
- o-Tolualdehyde[2]
- p-Tolualdehyde[2]

Sample Preparation

EPA Method	Sample Matrix	Sample Preparation
554, 8315 Option 1	Drinking water, solid waste leachate	DNPH reagent added to 100-mL sample, extract with Oasis HLB or use methylene chloride extraction option.
TO11, 8315 Option 2	Indoor or ambient air	Use Sep-Pak® DNPH Silica Cartridge, backflush cartridge with acetonitrile.

Table 3. EPA Method TO11, 554, and 8315 sample matrix and preparation.

Comment

- California State Method 1004 can also be done using a modified gradient, contact Waters at 508-482-2000 x8360.

[1] *Analyte per Methods 554 and 8315, Option 1*

[2] *Analyte per Methods TO11 and 8315, Option 2*

Conditions

LC system:	ACQUITY UPLC
Eluent:	Binary gradient *(see below)*
Column:	ACQUITY BEH Phenyl 2.1 x 100 mm, 1.7 µm
Column temp.:	35 °C
Injection volume:	5.0 µL each of AccuStandard Mix M- 8315-R1-DNPH and M- 8315-R2- DNPH diluted 1:5 in 40:60 water/acetonitrile (full loop)
Weak wash:	5% aqueous acetonitrile (800 µL)
Strong wash:	50% aqueous acetonitrile (500 µL)
Flow rate:	0.5 mL/min
Detection:	Tunable UV (TUV) at 360 nm
Data:	Empower 2 Software

Eluent Preparation

A:	90% water, 10% THF (mix 900 mL water and 100-mL stabilized tetrahydrofuran (THF))
B:	Acetonitrile Filter and degas through a 0.45-µm PES filter.

Time	Flow (mL/min)	%A	%B	Curve
Initial	0.5	70	30	-
9.0	0.5	36	64	6
9.5	0.5	70	30	11

Table 1. Eluent gradient for methods 554 and 8315 Option 1.

Time	Flow (mL/min)	%A	%B	Curve
Initial	0.5	70	30	-
6.5	0.5	53	47	6
9.5	0.5	53	47	11

Table 2. Eluent gradient for methods TO11 and 8315 Option 2.

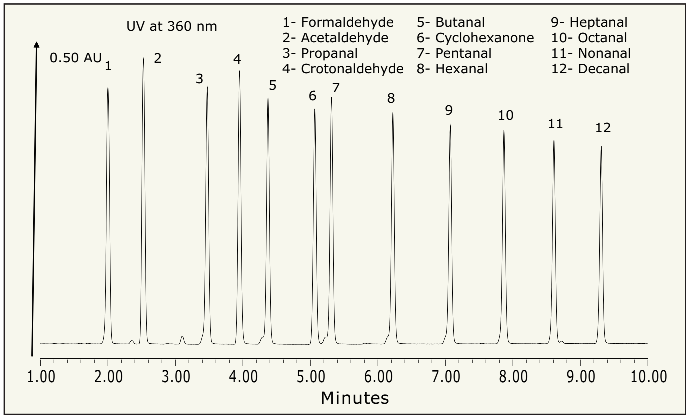
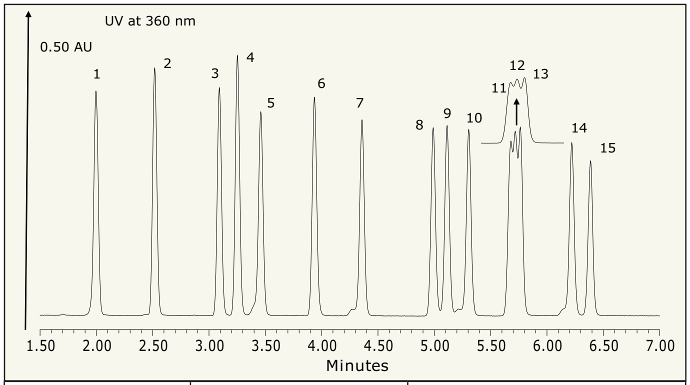


Figure 1. EPA Methods 554 and 8315-01, analytes as DNPH derivatives (20 ppm).



1- Formaldehyde	6- Crotonaldehyde	11- o-Tolualdehyde
2- Acetaldehyde	7- Butanal	12- p-Tolualdehyde
3- Acetone	8- Benzaldehyde	13- m-Tolualdehyde
4- Acrolein	9- Isovaleraldehyde	14- Hexanal
5- Propanal	10- Pentanal	15- 2,5- Dimethylbenzaldehyde

Figure 2. EPA Methods TO11 and 8315-02, analytes as DNPH derivatives (20 ppm).

8330B

EXPLOSIVES

ACQUITY UPLC System with TUV Detector

EPA Method 8330B describes the analysis of nitroaromatics, nitramines, and nitrate esters using HPLC with TUV detection.

Compounds included in this method are:

- HMX; 2-amino-4,6-DNT
- RDX; 4-amino-2,6-DNT
- TNB; 3,4-DNT**
- 1,2-DNB*; 2,4-DNT
- 1,3-DNB; 2,6-DNT
- 3,5-DNA; 2-NT
- NB; 4-NT
- Tetryl; 3-NT
- Nitroglycerine; PETN
- TNT

** System Surrogate*

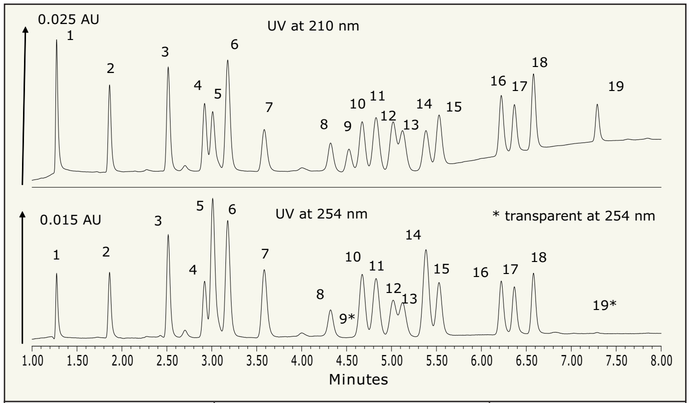
*** Internal Standard*

Conditions

LC system:	ACQUITY UPLC
Eluent:	Binary Gradient *(see below)*
Column:	ACQUITY BEH C_{18} 2.1 x 100 mm, 1.7 µm
Column temp.:	55.4 °C
Injection volume:	5.0 µL each of AccuStandard Mix M- 8330R, IS, SS, adds 1, 2, and 3, diluted 1:100 in mobile phase
Weak wash:	5% aqueous acetonitrile (800 µL)
Strong wash:	50% aqueous acetonitrile (500 µL)
Flow rate:	0.5 mL/min
Detection:	ACQUITY UPLC TUV at 210 and 254 nm
Data:	Empower 2 Software

Comments

- Detection limits of less than 1.0 ppb can be achieved using UV detection.
- The separation is very dependent on accurate column temperature.
- Nitroglycerin and PETN are transparent at 254 nm.
- EPA Method 8330B may be found at the EPA website, www.EPA.gov



1- HMX	8- Tetryl	14- 2,4-DNT
2- RDX	9- Nitroglycerin	15- 2,6-DNT
3- TNB	10- TNT	16- 2-NT
4- 1,2-DNB	11- 2-Amino-4,6DNT	17- 4-NT
5- 1,3-DNB	12- 4-Amino-2,6DNT	18- 3-NT
6- 3,5-DNA	13- 3,4-DNT	19- PETN
7- NB		

Figure 1. EPA Method 8330B analytes (1 ppb level).

Eluent Preparation

A: Water (HPLC grade)

B: Methanol

Filter and degas through a 0.45-µm PES filter.

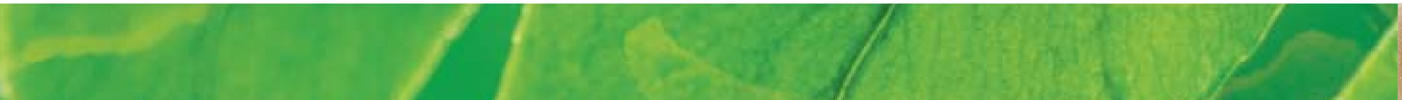
Time	Flow (mL/min)	%A	%B	Curve
Initial	0.5	90	10	-
3.5	0.5	70	30	2
4.5	0.5	70	30	6
7.5	0.5	50	50	5
8	0.5	90	10	6

Table 1. Eluent gradient for EPA Method 8330B.

Sample Preparation

EPA Method	Sample Matrix	Sample Preparation
8330B	Ground water and surface water, low concentration	Solid-Phase Extraction – Use a 500-mL water sample with an Oasis HLB Cartridge, wash cartridge then elute with 2 x 1 mL of 15:85 water: acetonitrile. Bring to volume with 1% formic acid.

Table 2. EPA Method 8330B sample matrix and preparation.



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