

TR 34-98-102  
INDEPENDENT LABORATORY METHOD VALIDATION TRIALS OF RH-2485 ANALYTICAL METHOD FOR  
WATER

**6. Method**

*6.1 Chemicals / Supplies / Solutions*

<b>CHEMICALS/SUPPLIES</b>	<b>SUPPLIER</b>
Acetonitrile (HPLC)	Fisher
Methanol (HPLC)	Fisher
H <sub>2</sub> O (nanopure)	Barnstead
n-Propanol	Fisher
RH-2485 Analytical Standard	Rohm & Haas
Untreated Well Water	Rohm & Haas

<b>SOLUTIONS</b>	<b>PREPARATIONS</b>
15% Acetonitrile/Water (v/v)	150mL Acetonitrile + 850mL Water
40% Acetonitrile/Water (v/v)	400mL Acetonitrile + 600mL Water
52% Acetonitrile/Water (v/v)	520mL Acetonitrile + 480mL Water
54% Acetonitrile/Water (v/v)	1080mL Acetonitrile + 920mL Water

### 6.2 Equipment

EQUIPMENT	SUPPLIER
Empore 47mm C-18 extraction disc	3M
Mobile Phase Filtration Apparatus	Supelco
Roundbottom Flask-500mL	Pyrex
Rotary Evaporator	Buchi
Standard Laboratory Equipment, Balances, Beakers, Test tubes	

### 6.3 Preparation of Standards

#### (1) Stock solution

Weigh a known amount of RH-2485 analytical standard between 1 and 10mg on an analytical balance and transfer into a 50mL volumetric flask. Dissolve the standard in 52% ACN/Water. Bring to volume with 52% ACN/Water. (5mg is weighed and brought to volume in a 50mL volumetric flask to yield a 100ug/mL stock solution). Store stock solution at -10 °C +/- 8 °C for up to six months.

#### (2) Intermediate Standard

A 10ug/mL intermediate standard is made by taking an accurate volume of stock solution (1) to a precise volume with the 52% ACN/Water used. (10mL of 100ug/mL stock solution is added to a 100mL volumetric flask and brought to volume with 52% ACN/Water to yield a 10ug/mL solution). Store intermediate standard at -10°C +/- 8 °C for up to six months.

#### (3) Intermediate Standard

A 1ug/mL intermediate standard is made by taking an accurate volume of stock solution (1) to a precise volume with the 52% ACN/Water used. ( 10mL of 10µg/mL stock solution is added to a 100mL volumetric flask and brought to volume with 52% ACN/Water to yield a 1µg/mL solution). Store intermediate standard frozen at -10°C +/- 8 °C for up to six months.

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Working Standards

	Concentration	Take	Final Volume
(4)	0.5µg/mL	1.25mL of 10µg/mL (2)	25mL
(5)	0.25µg/mL	0.625mL of 10µg/mL (2)	25mL
(6)	0.1µg/mL	2.50mL of 1.0µg/mL(3)	25mL
(7)	0.05µg/mL	1.25mL of 1.0µg/mL (3)	25mL
(8)	0.025µg/mL	0.625mL of 1.0µg/mL (3)	25mL
(9)	0.01µg/mL	0.250mL of 1.0µg/mL (3)	25mL

Dilute working standards to volume in 25mL volumetric flasks with 52%ACN/Water and store at 5°C for up to six months.

**6.4 Analytical Procedure**

**6.4.1 Sample processing**

Water samples are used as collected from untreated wells.

**6.4.2 Detailed Method**

**Extraction**

Set up a filtration apparatus with Empore 47mm Extraction Disk (C-18) and attach to the vacuum line. Condition the disk with 40mL of 40% ACN/Water, then 40mL Milli-Q water. Do not allow disk to go dry. Add 500mL of well water (with appropriate fortification if desired) and let drain through disk. Do not allow disk to go dry. Rinse disk with 30mL of 15% ACN/Water. Do not allow disk to go dry. Discard all filtrate to this point. Put a clean flask below the filtration apparatus. Add 40mL of 40% ACN/Water to the disk. Allow eluent to completely drain through till disk is dry. Transfer eluent to 500mL 24/40 ST round bottom flask. Rinse filtration flask with small amount of MeOH and add to round bottom flask. Add 100mL of n-Propanol to the round bottom flask and rotovap to dryness. Dissolve the residue in 2.5mL of 52%ACN/Water.

## 7. Test and Reference Material

<u>Identity</u>	<u>Lot #</u>	<u>Purity</u>	<u>Appearance</u>	<u>Expiration Date</u>
RH-2485	WCT3386A	99.7%	White Solid	Aug. 12, 1998

## 8. HPLC Quantitation

### 8.1 Standards

A minimum of four standard solutions of RH-2485 are prepared in the concentration range of 0.01 µg/mL to 1.0 µg/mL. Inject samples at the same volume (150 µL) as the RH-2485 standards. If necessary, the samples are diluted to an appropriate volume to give a response within the standard curve range. Standards and samples are quantitated by peak height. At least four standards, run in duplicate, are required to construct a linear standard curve.

### 8.2 Sample Analysis

Inject fortification samples at the same volume (between 50-150 µL) as RH-2485 standards. If necessary, the samples are diluted to an appropriate volume to give a final concentration within the standard curve range. The peak heights are measured and the concentration of each component is determined from the standard curves. The limit of quantitation was established at 0.10 ppb by analysis of fortifications at that level. The retention time for RH-2485 is found to be ≈ 4.9 minutes under normal HPLC conditions.

The residue concentration is determined as follows:

Equation 1:

$$\frac{\text{Concentration (ng/mL)} \times \text{Final Volume (mL)}}{\text{Initial Sample Volume (mL)}} = \text{ppb}$$

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### *8.3 Fortification Recovery*

Control samples are fortified with known amounts of RH-2485 prior to extraction.

Equation 2: Fortification Recovery

$$\frac{(\text{found (ng/mL)} \times \text{final vol. (mL)})}{\text{fortification amount (ng)}} \times 100 = \% \text{ Recovery}$$

### *8.4 HPLC System Set-Up and Conditions*

Pump: Perkin Elmer Series 200  
Flow Rate: 2.00 mL/min.

Mobile Phase: 54% ACN/Water

Autosampler: Perkin Elmer S200  
Stop Time: 11.8 minutes  
Injection Volume: 50-150  $\mu$ L

Column Heater: Waters 600E Temperature = 45°C

Analytical Column: Supelco C-18, 25cm x 4.6mm, 5 $\mu$ m

Detector: PE LC-95 UV Visible, 240nm

Data Acquisition: PE Turbochrome