

Direct Determination of Endothall by Ion Chromatography with Mass Spectrometric Detection

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ABSTRACT

Endothall is a widely used herbicide for both terrestrial and aquatic weeds. Major uses of endothall include defoliation of cotton, the control of aquatic weeds and algae, and as a desiccating agent for lucerne and potatoes. It has been used for sugar beets, turf, hops, sucker suppression; alfalfa, clover desiccants, and potato vine killers.¹ Human exposure to endothall in excess of the maximum contamination level (MCL) may cause gastrointestinal problems.⁷ Endothall is regulated by the U.S. EPA with an MCL at 0.1 mg/L or 100 ppb for drinking water;⁸ and by the California EPA at 0.58 mg/L or 580 ppb as the Public Health Goal (PHG).¹

Current analytical methods for quantitation of endothall in water samples are described in U.S.EPA method 548.1 as gas chromatography with mass spectrometry or flame ionization detection (GC-MS or GC-FID). These methods involve ion exchange solid phase extraction and employ sample enrichment, with dimethyl ester derivatization, followed by a 20 min GC separation and MS or FID detection.²

This study describes the direct analysis of trace-level endothall in water samples by ion chromatography-mass spectrometry (IC-MS). Water samples were directly injected without labor intensive sample preparation and chromatographic separation was achieved in 10 minutes, significantly improving method throughput. An MS/MS instrument was operated in SRM (selected reaction monitoring) mode, requiring minimal sample cleanup and ensuring highly sensitive (low ppb) and selective quantitation. Isotope-labeled glutaric acid (Glutaric Acid-d6) was used as the internal standard to ensure quantitation accuracy. This method has been successfully used for quantification of endothall in various water matrices including creek water, lake water and high-salt content lake water. Method performance parameters such as linearity, calibration range, precision and accuracy, detection limits, and recovery are also shown.

INTRODUCTION

Endothall (7-Oxabicyclo (2.2.1)heptane-2,3 dicarboxylic acid) is a specific inhibitor of protein phosphatase 2A. It is highly mobile in soil and has a reported half life of 4-9 days. Endothall is not expected to oxidize, chemically hydrolyze, photolyze, volatilize, bioaccumulate, or adsorb to suspended solids or sediments in water.³ If released to the atmosphere, endothall is expected to exist predominantly on particles and should either settle out or wash out in precipitation. It is not expected to chemically react or photolyze in the atmosphere.⁴ The most probable routes of human exposure to endothall are inhalation and dermal contact of workers involved in the manufacture, handling or application of endothall. The general public can potentially be exposed through use for lawn weed control.⁵⁻⁷

Endothall is currently analyzed by GC/MS or GC/FID with reported MDLs of 1.79 and 0.7, respectively.⁶ Considerable sample preparation is required prior to the analysis step. This includes:

SAMPLE CARTRIDGE CONDITIONING FOR CURRENT GC METHOD

- 10 mL methanol
- 10 mL reagent water
- 10 mL 10% sulfuric acid in methanol
- 10 mL reagent water
- 20 mL 1 N sodium hydroxide
- 20 mL reagent water

Loading and Extraction Steps Using GC Method

- Trap a 100 aliquot of sampler on an SPE column
- Wash with methanol
- Elute endothall with 10% sulfuric acid in methanol
- Add 6 mL methylene chloride
- Heat to 500 °C for 60 minutes
- Extract with methylene chloride 3 times
- Inject

In addition to the above, this method is also subject to matrix interferences that must be removed using EDTA. The goal of this work was to develop a direct-injection technique using IC-MS to eliminate the preparation steps shown above.

MATERIALS AND METHODS

ICS-5000 Chromatography System

DP—Dual Pump
EG—Eluent Generator
DC—Detector Chromatography Compartment
CD—Conductivity Detector
AS—Autosampler

Columns

IonPac® AG16 (2.1 × 50 mm) and IonPac AS16 (2.1 × 250 mm);

Accessories

EGC-KOH Potassium Hydroxide Eluent Generator cartridge to electrolytically produce hydroxide gradients for separation
ASRS® 3000 (2 mm) removes cations from eluent and samples that can cause signal suppression
AXP-MS pump for postcolumn addition of acetonitrile

Mass Spectrometer

TSQ Plus™ Triple Quadrupole Mass Spectrometer

Software

Chromleon® 6.8 SR6 Chromatography Data System

Chromatographic Conditions

Temperature: 30 °C
Mobile Phase: Hydroxide from EGC II KOH cartridge

Time /min	OH Conc.
-4.0	15 mM
5.0	15 mM
6.0	80 mM
9.0	80 mM
9.5	15 mM
10.0	15 mM

Flow Rate: 0.40 mL/min
Injection: 100 µL
Detection: Conductivity; Quantum TSQ Access MS

Mass Spectrometric Conditions

Interface: ESI positive
Desolvation Solvent: 0.2 mL/min acetonitrile
Scan Mode: MRM
Spray Voltage: 3500 V
Sheath Gas: 50 arbitrary units
Auxiliary Gas: 30 arbitrary units
Capillary Temp.: 300 °C

EXPERIMENTAL

Eluent generation technology allows automatic in-situ production of high-purity IC eluent (Figure 1). The pump delivers water to an eluent generator cartridge (EGC) which converts the water into a selected concentration of potassium hydroxide eluent using electrolysis. After separation on the column, the eluent enters the ASRS suppressor, which produces hydronium ions to exchange with potassium in the eluent and neutralize the hydroxide, making the mobile phase compatible with a mass spectrometer liquid inlet system. In addition, the ASRS removes cations present in the sample matrix prior to entry into the MS detector. Figure 1 shows the configuration used for this experiment. The ICS-5000 IC system is directly coupled to the MS/MS detector along with the AXP-MS pump. Desolvation of water requires elevated temperatures, which may cause breakdown of analytes in the ESI, thus the AXP-MS pump is used to add acetonitrile to assist with the desolvation in the ESI source. Electrolytic generation of eluent, suppression of eluent and trapping of eluent contaminants is known as Reagent Free Ion Chromatography (RFIC®).

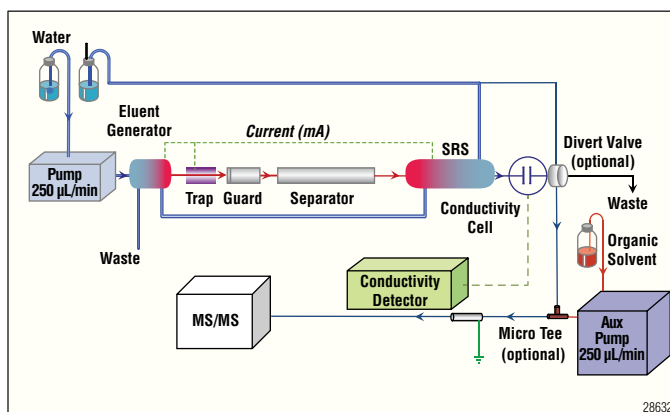


Figure 1. Schematic of the IC-MS configuration used showing the IC with eluent generation, columns, and suppression prior to the MS injection. A conductivity detector was used inline to check standards and calibrate the IC. The auxiliary pump is used to assist with desolvation in the ESI source.

Endothall is not available as an internal standard. Glutaric acid was chosen because its dicarboxylic acid components impart similar chemical and physical properties and its retention time is the same. Two MRM transitions were used for each analyte. Real samples were directly injected after filtration or dilution, if necessary. Carryover was evaluated by injecting a DI water blank followed by a 1000 ppb injection. Calibration curves were generated from 1 to 1000 ppb. The internal standard weighted calibration with 1/x for better accuracy at lower level quantitation.

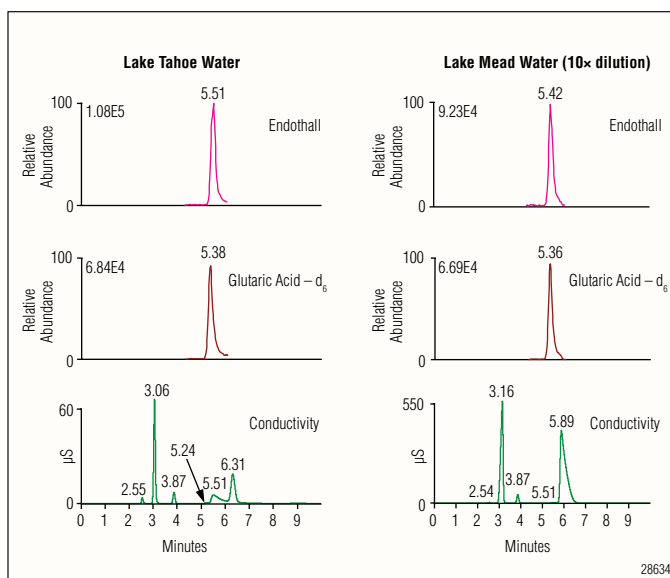
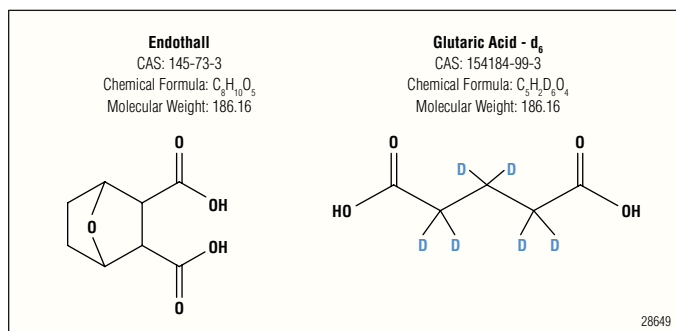


Figure 3. Detection of endothall in 5 ppb spiked water samples. Note the conductivity trace does not overlap significantly with the endothall and ISTD, allowing for optimal detection.

RESULTS

Figure 2 shows the conductivity trace for the separation of spiked endothall in an anion matrix. The IonPac AS16 column is a medium-capacity column which chromatographically resolves endothall from nitrate and sulfate anions which cause signal suppression in the MS that can interfere with detection. Figure 3 shows the results of endothall and glutaric acid in spiked surface water samples. The parent and daughter transition states (MRM's), conductivity trace, and retention times for endothall and glutaric acid are shown. Note that the large conductivity traces resulting from Cl⁻ and SO₄⁻ are at different retention times than either endothall and glutaric acid.

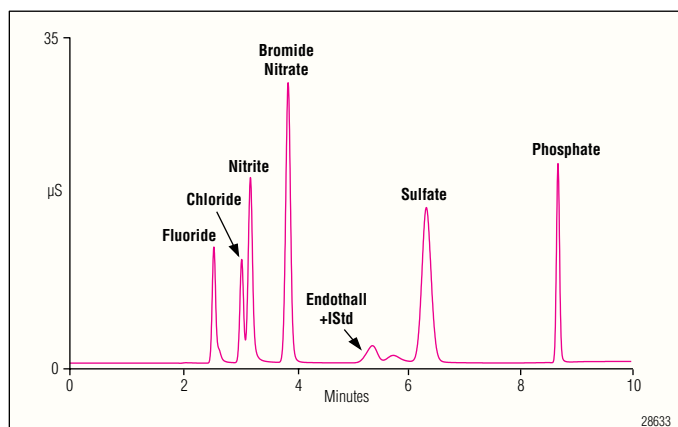


Figure 2. Chromatographic separation of anions and endothall, including the glutaric acid ISTD. Note the chromatographic separation of endothall from nitrate and sulfate, and the short run time.

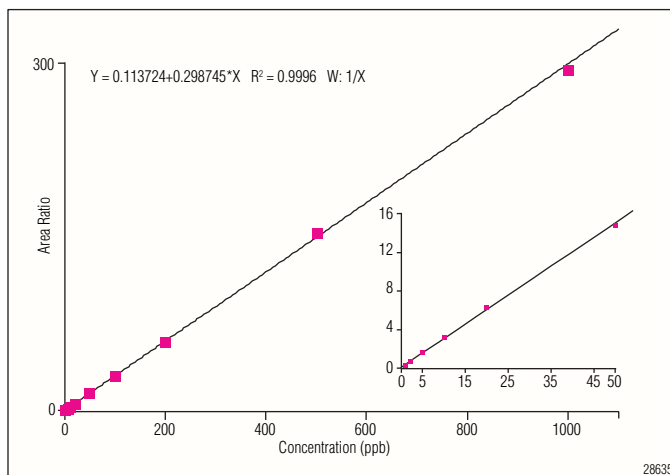


Figure 4. Linear calibration of endothall in DI water. Range: 1 to 1000 ppb.

Calibration curves were generated using concentrations from low ppb to 1 ppm, and method detection limits (MDL) were calculated by: $MDL = S \cdot t_{99\%}$, $n=7$, where S is the standard deviation and t is the Student's t at 99% confidence interval. Seven replicate injections of a calibration standard at 5 ppb were performed to calculate the MDL. Calibration range, coefficient of determination, and MDL values are summarized under Table 1. Figure 4 shows the calibration curve for endothall from 5 ppb to 500 ppb and the graphic insert shows the linear curve at the lower levels.

Table 1 shows the precision, accuracy, and recovery for low- and high-end spikes from three different samples. For the 0.5 ppb spikes, excellent standard deviation, % RSD, and recoveries between 95 and 105% were achieved. The 500 ppb spike also showed excellent recoveries, however, one of the samples showed a greater standard deviation than the others.

Table 1. Precision and Accuracy for Three Water Samples Using Low- and High-Level Spikes

Matrix	5 ppb				500 ppb			
	Mean (n=3)	Std. Deviation	Precision (%RSD)	% Recovery	Mean (n=3)	Std. Deviation	Precision (%RSD)	% Recovery
Local Creek	5.00	0.15	2.98	100	551	31.6	5.74	110
Lake Tahoe	5.20	0.08	1.44	104	540	7.32	1.36	108
Lake Mead	4.76	0.06	1.28	95.1	535	50.3	9.41	107

The MDL was determined using seven replicate injections of a 5 ppb standard, which resulted in a standard deviation of ± 0.18 with a calculated MDL of 0.566 ppb.

DISCUSSION

Current U.S. EPA guidelines found in Method 548.1 specify MDLs for endothall at 1.76 ppb using GC/MS and 0.7 using GC/FID. The method shown here demonstrates that RFIC coupled to MS/MS detection meets the requirements for endothall detection in matrix waters. More importantly, it eliminates many steps.

The use of multi-dimensional chromatography or a high-capacity single-dimension with matrix diversion significantly reduces the introduction of matrix ions to the mass spectrometer, improving the method robustness with challenging sample matrices. The relative standard deviations for both separation methods were 5% or less for both compounds, without internal standard correction.

In the two-dimensional analysis, the first chromatographic dimension separated the AMPA and glyphosate from the majority of the matrix ions using an IonPac AS19 column. However, poor peak shape was observed once the samples were introduced into the mass spectrometer. Trapping a heart-cut region of AMPA and glyphosate onto the anion concentrator column and eluting the analytes of interest onto a lower-capacity, higher-efficiency second-dimension column yielded improved peak shape and the added benefit of detecting the analytes in higher concentrations of matrix. Careful attention was given to the time allotted for the heart-cut regions onto the concentrator column. When diverting to the concentrator column for extended periods, recovery yields for glyphosate were poor. Low glyphosate yields are likely due to excess salts from the sample prematurely eluting the glyphosate off the trap column.

CONCLUSION

IC-MS is a valuable tool for any laboratory interested in the analysis of environmental contaminants. Here, the authors successfully demonstrated the use of IC-MS for direct injection analysis for endothall in natural waters. This method eliminates over 10 steps and two hours of manual sample preparation compared to the current SPE and GC/MS-based detection method, and meets the U.S. EPA requirements for detection of endothall in drinking waters.

REFERENCES

1. Technical factsheet on: ENDOTHALL
2. ...National Primary Drinking Water Regulations
3. Atochem North America, Inc. October 22, 1991a. Hydrolysis of ¹⁴C-endothall dipotassium salt in water at pH 5, 7 and 9. Xenobiotics Laboratory, Inc. Princeton, N.J.
4. Atochem North America, Inc. October 31, 1991b. Photogradation of ¹⁴C endothall in a buffered aqueous solution under artificial sunlight. Battelle. Columbus, Ohio.
5. Langeland, K.A. and J.P Warner. 1986. Persistence of diquat, endothall, and fluridone in ponds. *J. Aquat. Plant Manage.* 24:43-6.
6. Phipps, G.L. *et al.* 1984. *J. Water Pollution Control Fed.* 56(6):725-58.
7. U.S. EPA (U.S. Environmental Protection Agency). 1988. Endothall Health Advisory. Office of Drinking Water. Washington, D.C.
8. U.S. EPA (U.S. Environmental Protection Agency). January 1992a. Final Drinking Water Criteria Document for Endothall. Health and Ecological Criteria Division. Office of Science and Technology. Office of Water. Washington, D.C.

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