

Sensitive and Fast Determination of Endothall in Water Samples by IC-MS/MS

INTRODUCTION

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. Human exposure to endothall in excess of the maximum contamination level (MCL) may cause gastrointestinal problems. Endothall is regulated by the United States Environmental Protection Agency (EPA) with an MCL at 0.1 mg/L or 100 ppb for drinking water;¹ and the California EPA developed the Public Health Goal (PHG) of 0.58 mg/L or 580 ppb for this compound.²

Current analytical methods for quantitation of endothall in water samples are described in EPA method 548.1³ as gas chromatography with mass spectrometry or flame ionization detection (GC-MS or GC-FID). These methods involve ion-exchange solid phase extraction, sample enrichment, and dimethyl ester derivatization, followed by a 20 min GC separation and MS or FID detection.

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 min, thus significantly improving method throughput. An MS/MS instrument was operated in selected reaction monitoring (SRM) mode requiring minimal sample cleanup and ensuring highly sensitive (low ppb) and selective quantitation. Isotope labeled glutaric acid (glutaric acid- d_6) was used as an internal standard to ensure quantitation accuracy. This method has been successfully used for quantification of endothall in various water matrices including fresh creek water, lake water, and high salt content lake water. Method performance parameters such as linearity, calibration range, precision, accuracy, detection limits, and recovery were evaluated and will be presented in this application note.

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EQUIPMENT

Dionex ICS-2000 or ICS-2100 Reagent-Free™ Ion Chromatography (RFIC™) system
TSQ Quantum Access™ triple quadrupole mass spectrometer
XCalibur® 2.1 with integrated Dionex Chromatography Mass Spectrometry Link (DCMS^{Link}™) 2.8 for instrument control, data acquisition and processing, and report generation
Two Dionex AXP-MS auxiliary pumps

CHROMATOGRAPHIC CONDITIONS

Column: IonPac® AS16 and AG16 hydroxide selective anion-exchange columns (2 mm)
Column Temp.: 30 °C
Flow Rate: 400 µL/min
Eluent Source: EGC II KOH with Continuously-Regenerated Anion Trap Column (CR-ATC), 2 mm
Eluent: Hydroxide gradient

| Time/min | Conc./mM |
|----------|----------|
| -4.0 | 15 |
| 0.0 | 15 |
| 5.0 | 15 |
| 6.0 | 80 |
| 9.0 | 80 |
| 9.5 | 15 |
| 10.0 | 5 |

Solvent: 200 µL/min acetonitrile delivered by an AXP-MS pump
Detection: 1st detector: Suppressed Conductivity with Anion Self-Regenerating Suppressor® (ASRS®), 2 mm (external water at 0.5 mL/min delivered by an AXP-MS pump)
2nd detector: TSQ Quantum Access Mass Spectrometer

MASS SPECTROMETRIC CONDITIONS

Interface: Negative Electrospray Ionization (ESI)
Spray Voltage: 3500 V
Sheath Gas: 50 Arbitrary units
Auxiliary Gas: 30 Arbitrary units
Capillary Temp.: 350 °C
Collision Gas: Argon at 1.5 mTorr
SRM Acquisition: 4.2 to 6 min
Operating Mode: Selected Reaction Monitoring (SRM)

| Analyte | Parent Ion (m/z) | Product Ion (m/z) | Collision Energy (V) |
|------------------------------|------------------|-------------------|----------------------|
| Endothall-1 | 185 | 141 | 17 |
| Endothall-2 | 185 | 123 | 19 |
| Glutaric Acid-d ₆ | 137 | 74 | 21 |

REAGENTS AND STANDARDS

Endothall standard solution, 1 mg/mL in methanol (AccuStandard P/N P-183S-10XT)
Isotope labeled internal standard (IStd) glutaric acid-d₆ (C/D/N Isotopes P/N D-5227)
Seven anion standard solution with various concentrations from 20 ppm (fluoride) to 150 ppm (sulfate and phosphate) (Dionex P/N 056933)
Acetonitrile (HPLC grade, Burdick & Jackson)
Deionized (DI) water with 18.2 MΩ-cm resistance (Millipore Corporation)

The chemical structures of endothall and isotope labeled internal standard glutaric acid-d₆ are shown in Figure 1.

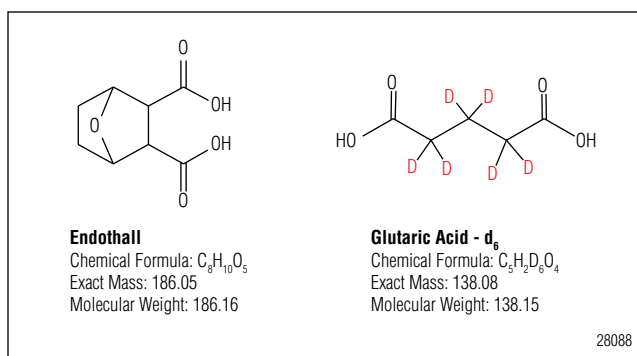


Figure 1. Chemical structures of studied compounds.

STANDARD PREPARATION

Dilute endothall standard in DI water to 10 ppm and 1 ppm as the two working standards to prepare calibration standards. Dilute the internal standard stock solution (1000 ppm) to 10 ppm for calibration standard preparation and for spiking unknown samples.

Prepare calibration standards by serial dilution from the working standards at 10 concentrations: 1 ppb, 2 ppb, 5 ppb, 10 ppb, 20 ppb, 50 ppb, 100 ppb, 200 ppb, 500 ppb, and 1000 ppb with the IStd spiked at 100 ppb for each concentration with the presence of diluted (100× dilution) seven common anions (fluoride 0.2 ppm, chloride 0.3 ppm, nitrite 1 ppm, bromide 1 ppm, nitrate 1 ppm, phosphate 1.5 ppm, and sulfate 1.5 ppm).

SAMPLE PREPARATION

Ground water samples were collected from a local creek and Lake Tahoe west shore. A lake water sample with a high salt content was kindly provided by a customer. These samples were spiked with IStd at 100 ppb and injected directly for ion chromatography tandem mass spectrometry (IC-MS/MS) quantification. (For samples with observed particulates, filtration is required prior to injection.) The salty water sample was diluted with DI water at a 1 to 10 ratio (v/v) due to the observed high concentration of ionic species.

RESULTS AND DISCUSSION

Chromatography

As shown in Figure 1, endothall is a dicarboxylic acid. IC is the preferred separation technique for ionic or ionizable compounds; therefore, it was selected in this study for the separation of endothall from interferences. For IC-MS analysis, the major interferences are inorganic matrix ions such as chloride and sulfate, and other ionizable organics at high concentration. The goals of chromatographic separation were to achieve sufficient chromatographic resolution for endothall from common anions, and to have a short run time to improve method throughput.

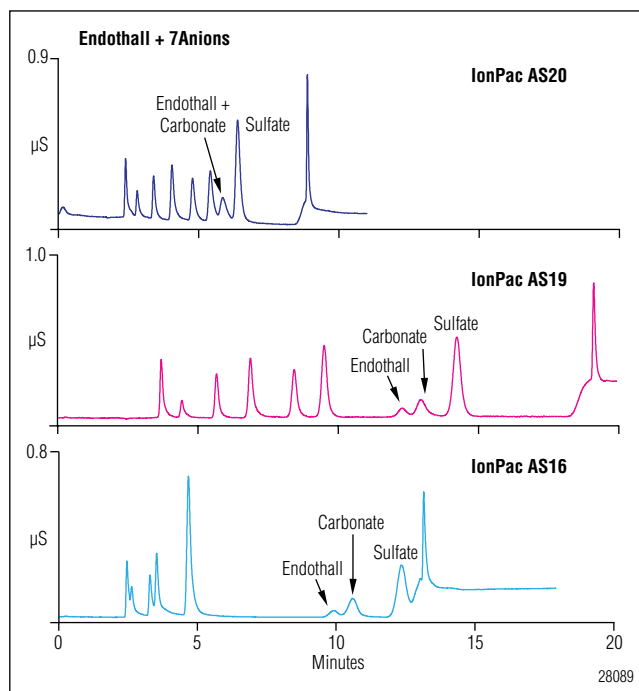


Figure 2. Column selection for endothall separation.

In this study, several high-capacity anion-exchange columns were evaluated for their selectivity; namely, the IonPac AS20, AS19, and AS16 columns. The IonPac AS16 column exhibited the best performance by: 1) providing a wide elution window for endothall between chloride and sulfate, and 2) requiring substantially less chromatographic run time than the IonPac AS19 column. Note that the IonPac AS19 column would be a better choice for simultaneous quantification of endothall and seven anion profiling, as it provided complete chromatographic resolution for all analytes (Figure 2).

CHROMATOGRAPHIC CONDITIONS

System: ICS-2000 or ICS-2100 RFIC system

Column: IonPac AS16 and AG16 (2 mm)

Column Temp.: 30 °C

Flow Rate: 400 µL/min

Eluent Source: EGC II KOH with CR-ATC (2 mm)

Eluent: Hydroxide gradient

| Time/min | Conc./mM |
|----------|----------|
| -4.0 | 15 |
| 0.0 | 15 |
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| 9.0 | 80 |
| 9.5 | 15 |
| 10.0 | 5 |

Solvent: 200 µL/min acetonitrile delivered

by an AXP-MS pump

Detection: 1st detector: Suppressed Conductivity using ASRS 300 (2 mm)

(external water at 0.5 mL/min delivered by an AXP-MS pump)

2nd detector: TSQ Quantum Access Mass Spectrometer

MASS SPECTROMETRIC CONDITIONS

System: TSQ Quantum Access triple quadrupole mass spectrometer

Interface: Negative Electrospray Ionization (ESI)

Spray Voltage: 3500 V

Sheath Gas: 50 Arbitrary units

Auxiliary Gas: 30 Arbitrary units

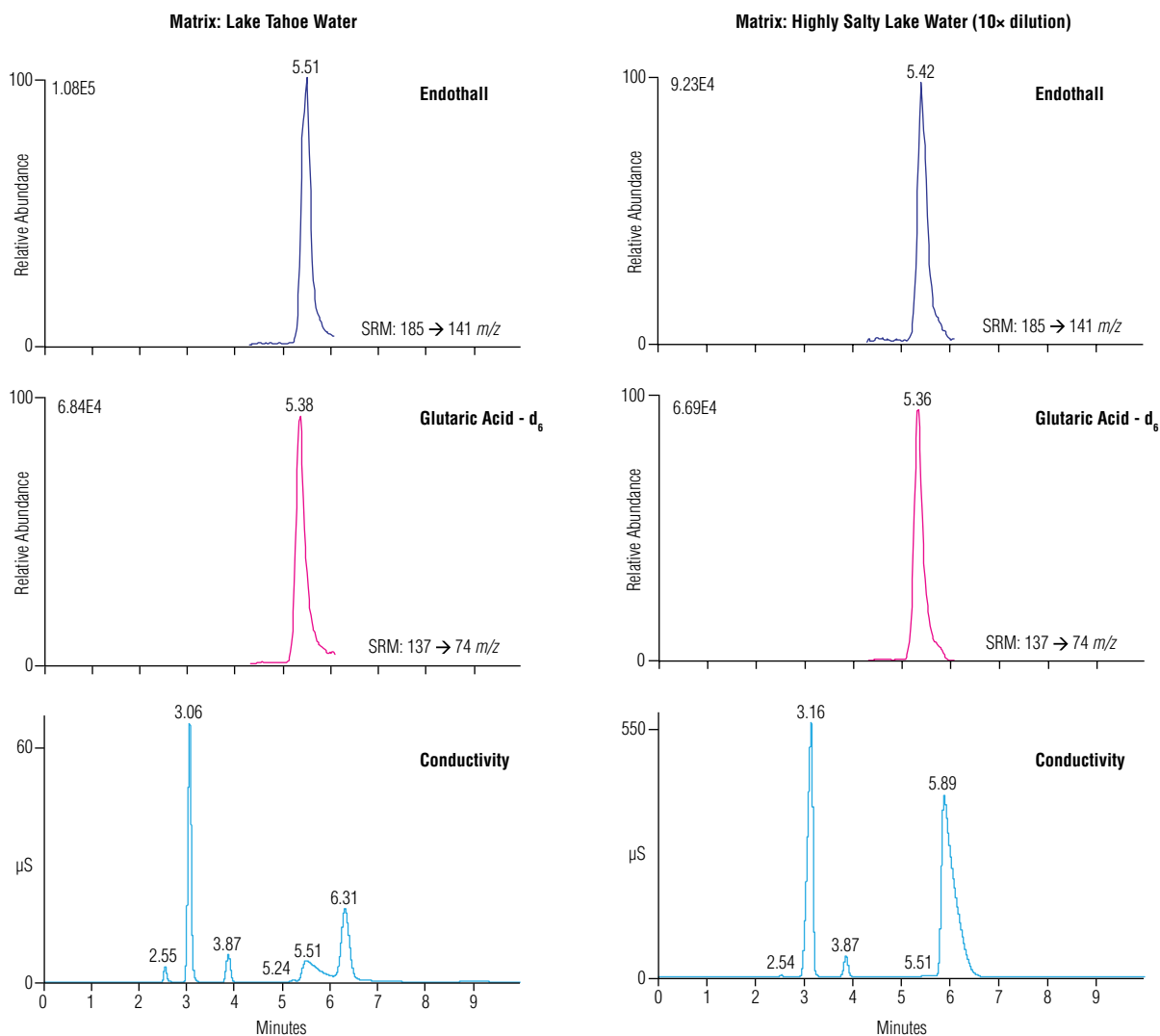
Capillary Temp.: 350 °C

Collision Gas: Argon at 1.5 mTorr

SRM Acquisition: 4.2 to 6 min

Operating Mode: Selected Reaction Monitoring (SRM)

| Analyte | Parent Ion (m/z) | Product Ion (m/z) | Collision Energy (V) |
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Figure 3. Conductivity and SRM chromatograms of endothall spiked in different samples. Left, 5 ppb endothall spiked in Lake Tahoe water; right, 50 ppb endothall in salty lake water with 10-fold dilution.

Mass Spectrometry

A TSQ Quantum Access triple quadrupole mass spectrometer was selected in this study for sensitivity and selectivity, which allowed minimum sample preparation such as cleanup and enrichment. A matrix diversion valve was used to divert high concentrations of inorganic anions to waste to prevent MS entrance fouling and to maintain long-term system stability. It is important to adjust the diversion window for specific matrices to ensure the analyte signal is collected by the MS and inorganic salts are diverted to waste.

As seen in Figure 3, two different matrices, Lake Tahoe water (LTW) and highly salty lake water (HSW), were spiked with endothall at 5 ppb and analyzed by this method. The HSW is a highly salty water sample (~1000 ppm sulfate, estimated by conductivity peak area), and the retention time of endothall in this 10× diluted matrix was slightly shifted (0.09 min earlier than in the LTW matrix). In addition, the retention time for sulfate shifted significantly earlier by 0.42 min; thus, the diversion window had to be adjusted accordingly to avoid MS entrance contamination. In rare situations where dilution of the original sample is not applicable, such as when endothall is present in very low concentration in a high salt matrix, the gradient program will have to be adjusted, i.e. decrease the gradient slope from 5 to 9 min, in order to increase the resolution of endothall from sulfate (as seen in Figure 2).

The quantitation of endothall was carried out in SRM mode, the precursor ion was observed as the deprotonated molecular ion $[M-H]^-$ at 185 m/z , and the predominant product ion was observed as $[M-H-CO_2]^-$ at 141 m/z and used as the quantitative SRM transition; another characteristic product ion was also observed as $[M-H-CO_2-H_2O]^-$ at 123 m/z with less intensity and was used as a confirmative SRM transition. Isotope labeled glutaric acid- d_6 was used as the internal standard due to its similarity in chemical structure and chromatographic retention to endothall.

Because the IC eluent is 100% aqueous post-suppression, acetonitrile was introduced as the desolvation solvent to assist desolvation/ionization and was delivered by an auxiliary pump at 0.2 mL/min.

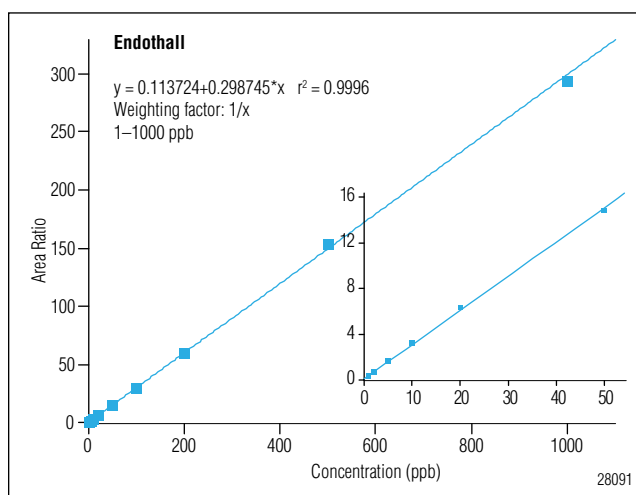


Figure 4. Calibration curve of endothall using glutaric acid- d_6 as an internal standard.

Method Performance

Typical conductivity and SRM chromatograms are shown in Figure 3. Sulfate, chloride, and nitrate were observed as the main anionic species in tested ground water, and were chromatographically separated from the target analyte, endothall, and the internal standard, glutaric acid- d_6 . The MS/MS instrument operated in SRM mode provided sensitive and selective detection: each target analyte was observed as a single peak in each SRM channel with excellent intensity.

This method was used to determine endothall in various water samples mentioned in the Sample Preparation section, and no quantifiable level of target analyte was observed in any of the tested samples. Thus, these three matrices were used to evaluate the recovery from matrices.

Method performance was evaluated against quality parameters such as linearity, reproducibility, precision, accuracy, detection limits, and recovery from matrix. Calibration was performed by analyzing calibration standards at 10 concentrations from 1 to 1000 ppb using internal calibration with IStd at 100 ppb in each standard. Excellent linearity was achieved through three orders of magnitude with the coefficient of determination (r^2) at 0.9996. The calibration curve is shown in Figure 4, and the insert shows the calibration curve at lower concentrations.

Table 1. Recovery of Endothall from Three Matrices

| Sample | 5 ppb | | | 500 ppb | | |
|-------------|-------|--------------------|------------|---------|--------------------|------------|
| | Mean | Standard Deviation | % Recovery | Mean | Standard Deviation | % Recovery |
| Creek Water | 5.00 | 0.15 | 100 | 551 | 31.6 | 110 |
| LTW | 5.20 | 0.08 | 104 | 540 | 7.32 | 108 |
| HSW | 4.76 | 0.06 | 95.1 | 535 | 50.3 | 107 |

Precision and accuracy were evaluated by seven replicate assays of a standard at 5 ppb, with the calculated mean at 4.89 ppb (97.8% accuracy) with standard deviation at 0.18 (3.63% RSD). The method detection limit (MDL) was calculated following the equation $MDL = s \times t$, where s is the standard deviation and t is the Student's t at 99% confidence interval. The calculated MDL is 0.56 ppb in DI water ($n = 7$).

Recovery was evaluated by spiking three samples (Creek Water, LTW, and a HSW [10-fold dilution]) at two levels: 5 ppb and 500 ppb with IStd spiked at 100 ppb. The results are summarized in Table 1.

CONCLUSION

A fast and sensitive IC-MS/MS method was presented for the quantitative determination of trace level endothall in environmental water samples. The detection limit was estimated at 0.56 ppb, and linear response was observed from 1 ppb to 1000 ppb. The significantly improved sensitivity enables direct analysis of water samples without labor intensive sample enrichment and derivatization. The total chromatographic run time was halved from the 20 min GC run in EPA Method 548.1.

REFERENCES

1. United States Environmental Protection Agency, *Basic Information about Endothall in Drinking Water*. <http://water.epa.gov/drink/contaminants/basicinformation/endothall.cfm> (accessed September, 2010).
2. Pesticide and Environmental Toxicology Section, Office of Environmental Health Hazard Assessment, California Environmental Protection Agency, *Public Health Goal for ENDOTHALL in Drinking Water*, December 1997.
3. United States Environmental Protection Agency, *Method 548.1: Determination of Endothall in Drinking Water by Ion-Exchange Extraction, Acidic Methanol Methylation and Gas Chromatography/Mass Spectrometry*. Available from http://www.epa.gov/waterscience/methods/method/files/548_1.pdf (accessed January 31, 2011).

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