# MINISTRY OF THE ENVIRONMENT AND CLIMATE CHANGE
## LABORATORY SERVICES BRANCH

**METHOD TITLE:THE DETERMINATION OF 1,4-DIOXANE IN WATER BY AUTOMATED SPE GAS CHROMATOGRAPHY-HIGH RESOLUTION MASS SPECTROMETRY (GC-HRMS)**

**DATE OF LAST REVISION:  NEW METHOD**

**SECTION:     TOXIC ORGANICS, MASS SPECTROMETRY**

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**APPLICABLE REGULATIONS/PROGRAMS:**

   **SAFE DRINKING WATER ACT (SDWA), 2002**

   **DRINKING WATER SURVEILLANCE PROGRAM**

**MANAGER APPROVAL/CONTACT:  _____KAREN MACPHERSON_____**

**\* This method is deemed fit for purpose as of the date shown on this cover page by the data provided in Section 1.6 (Analytical Performance Summary)**

**\* The approval of this document is valid for two years at which time it will be subject to review to determine if any updates or modifications are warranted.\***

**NOTE:  Equivalent suppliers to that stated in the method are acceptable.  Reference to a particular brand does not constitute an endorsement by the Ontario Ministry of the Environment.**

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## THE DETERMINATION OF 1,4-DIOXANE IN WATER BY GAS CHROMATOGRAPHY-HIGH RESOLUTION MASS SPECTROMETRY (GC-HRMS)

### 1.0    SUMMARY

1,4-Dioxane or 1,4-dioxacyclohexane (CAS Registry Number 123-91-1), often called dioxane because the 1,2- and 1,3- isomers of dioxane are rare, is a heterocyclic organic compound. It is a colourless liquid with a faint sweet odour similar to that of diethyl ether. 1,4-Dioxane is extensively used in industrial applications involving paint strippers, dyes, greases and waxes as well as being found as impurities in many consumer products such as antifreeze/deicing fluids, polyethylene terephthalate plastics (PET), laundry detergents, soaps, shampoos and bubble baths (1.7.1). However, dioxane is used mainly as a stabilizer for various chlorinated solvents. 1,1,1-trichloroethane (TCA)-based solvents may contain as much as 8% 1,4-Dioxane. In 1996, nearly one million pounds of 1,4-Dioxane were released into the environment in the USA (1.7.2).

1,4-Dioxane is miscible with water and adsorbs only weakly to soils/sediments. Because it does not readily undergo biodegradation and is resistant to other natural attenuation processes it can move rapidly through hydrological regimes contaminating large volumes of surface and groundwater (1.7.3). The hydrophilic nature of 1,4-Dioxane makes it relatively immune to conventional treatment technologies commonly used for chlorinated solvents and makes it the fastest moving contaminant found at solvent release sites arriving first at municipal or domestic supply wells. Due to relative boiling point differences between stabilizers and solvents, degreasing operation industrial wastes have been known to have 1,4-Dioxane at levels more than 3x higher than the original products. Concentrations as high as 200,000 µg/L have been reported in contaminated groundwaters (1.7.4). As a by-product of ethoxylated detergents and surfactants dioxanes can enter surface waters from consumer products via municipal waste water effluent streams (1.7.5).

1,4-Dioxane is acutely toxic at high concentrations as well as being identified as a probable human carcinogen and an emerging contaminant in potable waters (1.7.6). 1,4-Dioxane is listed as a Group 2B compound by the World Health Organization's International Agency for Research on Cancer (IARC). WHO established a drinking water threshold of 50 µg/L in 2005 (1.7.9). In its Drinking Water Regulation (2001), the German Federal Environmental Agency suggested a precautionary limit for weak or non-genotoxic compounds such as 1,4-Dioxane at 0.1 µg/L (1.7.10). The USEPA recently revised the drinking water notification level from 3 µg/L to 0.35 µg/L (1 x 10$^{-6}$ lifetime cancer risk) with an associated minimum reporting level (MRL) of 0.07 µg/L (1.7.11). Most US State drinking water notification levels vary from 1-3 µg/L. Massachusetts ORSG drinking water guidelines for 1,4-Dioxane have been set at 0.30 µg/L (1.7.12).

The Colorado Department of Public Health and Environment (2012) has established an interim ground water quality clean-up standard of 0.35 µg/L.  In 2008, an interim Ontario Provincial Water Quality Guideline (PWQG) for the protection of aquatic life of 20 µg/L was developed (1.7.7). Ontario Soil, Water and Sediment Standards for Use Under Part XV.1 of the Environmental Protection Act state potable groundwater sites should not have 1,4-Dioxane background levels exceeding 50 µg/L (1.7.8). No Canadian or Ontario drinking water guidelines currently exist.

## 1.1     Principle of the Method

This method is designed to identify and quantify 1,4-Dioxane in water by solid phase extraction (SPE) with coconut charcoal using an in-line automated sample preparation station. Content is eluted with an organic solvent and analysed by Large-volume splitless injection with Concurrent Solvent Recondensation (LV-CRS) gas chromatography-high resolution mass spectrometry (GC-HRMS). The 1,4-Dioxane method was developed and validated in-house by MOECC-LaSB.

The internal standard $d_8$-1,4-Dioxane is added to 20 mL of sample aliquot.  The automated sample prep station conditions the coconut charcoal, loads 10 mL of the sample, dries the cartridge under low nitrogen flow, elutes analytes into 2 mL autosampler vial with 500 µL of dichloromethane and immediately after extraction injects 10 µL of organic extract into GC-HRMS.  1,4-Dioxane is quantified by isotope dilution using $d_8$-1,4-Dioxane.

1.1.1   Relationship to Other Methods.

Several extraction and concentration techniques have been used to analyse for 1,4-Dioxane. Heated Purge & Trap and SPME methods have been used but suffer from relatively high method detection limits - µg/L (1.7.13). Liquid/Liquid extraction (LLE) methods are effective but are labour intensive and require large amounts of hazardous solvents. Carbonaceous-Resin solid phase extraction (SPE) has been the most effective and efficient extraction technique to achieve low ng/L (ppt) detection limits. USEPA Method 522 uses coconut charcoal SPE cartridges – either Waters AC-2 Sep-Pak or Supelco ENVI-Carb Plus (1.7.14).

Separation, identification and quantitation of dioxanes are normally achieved with thick phase capillary column gas chromatography – mass spectrometry (GC-MS).  Low resolution full scan and single ion monitoring (SIM) mass spectrometry detection is almost exclusively used due to dioxanes' low molecular weight and volatility (1.7.13). Traditional

GC detectors such as flame ionization (FID) and photoionization (PID) are used but lack sufficient specificity and sensitivity.

There is no previous routine MOE LaSB method for the analysis of 1,4-Dioxane.

## 1.2     Parameters Measured

This method analyses 1,4-dioxane in water.

|              | Chemical Formula | CAS Number |
|--------------|------------------|------------|
| 1,4-Dioxane  | $C_4H_8O_2$      | 123-91-1   |

The **LIMS** (Laboratory Information Management System) (Reference 1.7.16) Product Code is **DIOXANE3534**.

Results are reported in μg/L.

## 1.3     Sample Matrices

The **LIMS** Matrix Codes used for this method are drinking water (**WD**), ground water (**WG**) and surface water (**WS**).

## 1.4     Sample Requirements

1.4.1   Specifications.

Samples are collected in 250 mL amber glass bottles with Teflon-lined caps with no headspace. If non-amber bottles are used, the samples must be protected from light by placement in an aluminium foil wrapper or in a light-tight box. Samples analysed by this method are unpreserved (see in-house storage study).

Samples are stored at 5±3°C.

Time Required for Analysis:

1) Sample preparation:          18 samples (limited by the sample tray size of the automated prep station), 1 replicate, 1

|  | Method Blank, 9 Calibration Curve Samples, 1 Calibration Control and 1 Matrix spike per day. |
|---|---|
| 2) Instrument set-up time: | warm-up, tuning, calibration (1 to 2 hours) |
| 3) Instrumental analysis: | 30 minutes/run |
| 4) Data processing: | 2 to 3 hours per batch of samples |

The holding time (before sample preparation) for unpreserved samples of 1,4-Dioxane is 28 days.

Extracts stored at -5°C are stable for 28 days (1.7.13).

The maximum turnaround time (TAT) in **LIMS** is 30 days.

1.4.2　Contingencies.

If an unsuitable sample is submitted, the client is notified and requested to submit a new sample. The analysis is withdrawn and the sample is discarded.

Improperly submitted samples may be analyzed under extenuating circumstances and at the discretion of the laboratory, i.e. emergency response or when a re-sample is not practical or available.

Drinking water samples exceeding their holding times are not analysed but flagged with the LIMS code NDHT: No Data Holding Time Exceeded.

The following **LIMS** remark codes may be used:

| | |
|---|---|
| NDAW | No data: analysis withdrawn |
| NDBT | No data: sample broken in transit |
| NDCC | No data: court case results reported elsewhere |
| NDEF | No data: laboratory equipment failure |
| NDFB | No data: container broken due to freezing |
| NDHT | No data: holding time exceeded |
| NDIC | No data: improper container |
| NDLA | No data: sample spoiled in laboratory accident |
| NDNR | No data: sample not received at laboratory |
| NDSM | No data: sample missing |
| UAL | Unreliable: sample age exceeds normal limit |
| UIC | Unreliable: improper container |
| UQC | Unreliable: possible lab QC problem(s) |
| USP | Unreliable: submitted in plastic for organics |

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## 1.5    Shortcomings

1.5.1    Interferences.

HRMS is used instead of LRMS because the effects of chemical interferences can be minimised and the selectivity of the method can be maximised.  The primary instrument, the Waters Micromass AutoSpec Ultima, and the back-up instrument, the Waters Micromass AutoSpec Premier, are operated at a mass resolution [i.e. resolving power (RP)] of 7,000 to 10,000 RP.

No chemical interferences have been detected for 1,4-Dioxane at 7,000 RP.

The isotopic purity of the $d_8$-1,4-Dioxane is 99.0%. Results are not corrected for isotopic purity.

The use of high purity reagents and solvents including Pure Water is essential in minimizing 1,4-Dioxane backgrounds and interferences.

1.5.2    Biases.

For 1,4-Dioxane, the isotope dilution method of analysis is used.  A known amount of the isotopically-labelled analogue $d_8$-1,4-Dioxane is added to the matrix prior to sample preparation.  The $d_8$-1,4-Dioxane has physical and chemical properties that are almost identical to those of the 1,4-Dioxane in the sample.  Results obtained by measuring the peak area ratios of 1,4-Dioxane to $d_8$-1,4-Dioxane are thus corrected for recovery and response factor.  Isotope dilution should produce highly accurate results.

Absolute recoveries are not monitored because the use of isotope dilution and internal standard methods of quantitation compensate for any losses of the native compounds during the sample preparation.

1,4-Dioxane can be present in Pure Water.

1,4-Dioxane may break down in samples during storage if samples contain significant amounts of bacteria. These samples would be biased low.

1.5.3    Limitations.

The sample preparation method is restricted to water samples.

Samples containing high amounts of settled particulates/solids are not filtered but the supernatant is decanted to a separate bottle for extraction. The particulates are not analysed separately for 1,4-Dioxane.

There is no sample clean-up. Organics that are adsorbed onto the coconut charcoal and desorbed into dichloromethane will be injected into the GC. Therefore, HRMS is necessary to differentiate the analytes from chemical interferences having similar retention times.

The multi-point calibration curve can handle widely varying concentrations of 1,4-Dioxane. However, when high µg/L concentrations of 1,4-Dioxane are found, the concentration is determined either by extrapolation and the value is reported with LIMS value qualifier AR> (Approximate result: Exceeded normal range limit) or smaller aliquots of the sample are taken and re-analysed.

Mechanical limitations of the current automated prep station do not allow for capping of GC vials containing the SPE extracts. Hence, only single injection is possible and the extracts cannot be stored. GC vials can be capped manually.

## 1.6 Analytical Performance Summary

Tables containing performance data are in Appendix I.

1.6.1 Within-run Method Performance (March 2016).

The within-run method performance was determined by analysing eight composite drinking water samples spiked at a concentration of 0.1 µg/L. The average concentration, standard deviation, RSD and method detection limits are listed in Table 1.6.1. The MDL was calculated according to the procedure described in LSBSOP.026.

1.6.2 Between-run Method Performance (March 2016).

The between-run method performance was determined from composite drinking water samples spiked at a concentration of 0.10 µg/L and analysed on three different days (eight replicates each day). The average concentration, average % recovery, standard deviation and RSD are listed in Table 1.6.2.

1.6.3    Method Working Range (March 2016).

The method working range was determined by analysing composite drinking water matrix spiked with 1,4-Dioxane at nine concentration levels ranging from 0.02 µg/L to 3 µg/L.  Three samples were prepared at each calibration level.  The average 1,4-Dioxane results and $R^2$ values are summarised in Table 1.6.3.

On a day-to-day basis, the working range is 0.02 to 0.75 µg/L.  If samples are suspected to have higher concentrations, then additional calibration levels, up to 3.0 µg/L, are processed.  Quantitation is done by quadratic curve-fitting.  Samples having concentrations greater than 3.0 µg/L are reanalysed by processing an appropriate aliquot (less than 10 mL) of the sample remaining in the original bottle through the entire analytical method.

## 1.7    Bibliography

**NOTE: Unless otherwise noted only current versions of LaSB methods and SOPs are used.**

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1.7.3    USEPA, Technical Fact Sheet – 1,4-Dioxane. USEPA Office of Solid Waste and Emergency Response, EPA 505-F-13-001 January 2013, 5 p.

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1.7.14  Munch, J. and Grimmett, P., Method 522 (v1.0): Determination of 1,4-Dioxane in drinking water by SPE and GC/MS with SIM. USEPA Document EPA/600/R-08/101, September 2008, 41p.

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1.7.17   Ministry of the Environment, MSSOP02 - Standard Operating Procedure for Operating the Glove Box for the Purpose of drying Ambersorb 572, current desktop version.

1.7.18   Ministry of the Environment Laboratory Services Branch, The Estimation of Uncertainty for Method E3534: The Determination of 1,4-Dioxane in Water by GC-HRMS, current version.

1.7.19   Ministry of the Environment and Climate Change, LaSB Procedures Manual, LSBSOP.026  - Determination of W, T and MDL, current desktop version.

1.7.20   Ministry of the Environment and Climate Change, LaSB Procedures Manual, LSBSOP.041 - Laboratory Services Branch Operating Procedure for Control Charting, current desktop version.

1.7.21   Ministry of the Environment , LaSB Procedures Manual, LSBSOP.039 – LaSB Procedure for Processing and Reporting Drinking Water Samples, current desktop version.

1.7.22   Instrument Operating / Reference Manuals.
Operating Manuals for 6890 Gas Chromatograph, Agilent Technologies, 1997.
Operating Guide for 7890A Gas Chromatograph, Agilent Technologies, 2007.
AutoSpec Operator Manual, Issue 3, Code No. 6666395, Micromass, 1996.
AutoSpec Instrument & Maintenance Manual, Issue 2, Code No. 6666419, Micromass, 1996.
Instrument Spares & Parts List, Issue 3, Code No. 6666437, Micromass, 1997.
GC Interface Assembly & Disassembly Manual, Issue 2, Code No. 6666438, Micromass, 1997.
AutoSpec Premier Operator's Guide, Revision B, Waters, 2005.
Operation Manual for Prep Station MPS Dual Head, Gerstel, 2014.
ITSP Set-up Guide, ITSP Solutions, 2013.

1.7.23   Supporting Forms and Logbooks:
Mass Spectrometry Group - Sample Reception Log Book
FRM_3534_001 E3534 - 1,4-Dioxane Sample Tracking Form
Sample/Standard Preparation Logbook – Dioxane
AutoSpec Ultima Maintenance Logbook - NDMA3291, NAMINE3388, TOCPDS3310, FORM3428, DIOXANE3534

AutoSpec Premier Maintenance Logbook - NAMINE3388, TOCPDS3310
AutoSpec Ultima Tuning/Calibration Binder – NAMINE3388, TOCPDS3310, FORM3428, DIOXANE3534
AutoSpec Premier Tuning/Calibration Binder – TOCPDS3310, NAMINE3388

## 1.8    History of Changes

January 2017 - New method to analyse 1,4-dioxane in water

## 1.9    Safety

Because of the toxicity and suspected carcinogenicity of 1,4-Dioxane, concentrated solutions and solvents must be handled with the utmost care.  The analyst must be provided with adequate protection. Safety glasses, gloves and lab coats must be worn.

Preparation of all standards must be done in fume hoods that provide adequate ventilation.

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## 2.0    SAMPLE PREPARATION

**NOTE:** Labware, reagents and equipment equivalent to those listed in this method are acceptable.

### 2.1    Labware

2.1.1    Clear headspace vial, 20 mL, with magnetic screw cap with PTFE/White silicone liner [for 2. 4.3, 2.4.4, 2.4.5 and 2.4.6].

2.1.2    Cylinders, graduated, glass, 25 mL [for 2. 4.3, 2.4.4, 2.4.5 and 2.4.6].

2.1.3    Syringe, glass, Hamilton, Gastight, 10 μL, 25 μL, 50 μL and 100 μL.

2.1.4    SPE cartridge, 20 mg coconut charcoal with prefilter (ITSP Solutions, Inc., PN: 20S-UCOCO30-T) or SPE cartridge, 20 mg coconut charcoal without prefilter. Evaluations of recoveries for cartridges with and without prefilter showed no significant differences. Cartridges with prefilter should be used for samples with high particulate matter. If the sample clogs the cartridge without prefilter, it will be re-run using the cartridge with prefilter.

2.1.5    Vial, autosampler, 2 mL.

2.1.6    Cap, Teflon/rubber lined.

2.1.7    Crimper.

### 2.2    Reagents

2.2.1    Dichloromethane, $CH_2Cl_2$, distilled-in-glass grade (DIG) or equivalent.

2.2.2    Methanol, $CH_3OH$, distilled-in-glass grade (DIG) or equivalent.

2.2.3    Water, $H_2O$, Pure: carbon-filtered/deionized/reverse osmosis (RO)/UV-treated.

2.2.4    Acetone, $CH_3O$, distilled-in-glass grade (DIG) or equivalent.

2.2.5    Nitrogen, $N_2$, low pressure from bulk cryo-tank.

2.2.6    Composite matrix. Samples that have been previously tested and where the concentration of 1,4-Dioxane was less than MDL (0.02 µg/L) are

collected in 4 L amber bottles and stored at 5±3°C. This matrix is used to prepared Method Blank, Calibration Curve, Calibration Control and Matrix spike. If clean blanks are not available (< 0.02 µg/L) samples will be run and the MDL will be appropriately adjusted to 3x the concentration of 1,4-Dioxane in the available method blank.

### 2.3 Equipment

2.3.1 MultiPurpose Sampler (MPS) Dual Head prep station (Gerstel) with:

2.3.1.1 Tray for 32 10 mL/20 mL vials, VT32-20 (Figure 1-A, Appendix IV).

2.3.1.2 Cartridge holder for 54 ITSP SPE cartridges, ITSPprep (Figure 1-B).

2.3.1.3 Tray for 54 2 mL vials with a custom aluminium cover, VT54ITSP (Figure1-C).

2.3.1.4 Tray for 98 2 mL vials, VT98 (Figure 1-D).

2.3.1.5 Left MPS arm with 2.5 mL syringe (Figure 1-E).

2.3.1.6 Right MPS arm with 10 µL syringe (Figure 1-F).

2.3.1.7 Three solvent reservoirs for SPE cartridge/2.5 mL syringe cleaning and conditioning (Figure 1-G).

2.3.1.8 Wash station for right MPS arm containing dichloromethane (Figure 1-H).

2.3.1.9 Wash station for left MPS arm containing methanol and RO water (Figure 1-I).

### 2.4 Operating Procedure

**Note:** Drinking water samples are not filtered.

2.4.1 When samples have been received, transfer them to the sample refrigerator set at 5±3°C.

2.4.2 Update the current **Sample Reception Logbook**.

2.4.3    Method Blank (MB)

For each batch of samples processed, prepare 1 Method Blank. Measure 20 mL of composite matrix (2.2.6) with graduated cylinder (2.1.2) and transfer to headspace vial (2.1.1).

2.4.4    Calibration Curve (MR1 to MR9)/ Calibration Control (MR6x)

For each batch of samples processed, prepare the following Calibration curve (MR1-MR9) / Calibration Control (MR6x) Samples consisting of 20 mL of composite matrix (2.2.6):

| Calibration Level | Concentration (µg/L water) | Solution C1 (5.2.11) (0.04 ng/µL) (µL) | Solution B1 (5.2.10) (0.2 ng/µL) (µL) | Solution A1 (5.2.6) (2 ng/µL) (µL) | Solution B2 (5.2.12) (0.2 ng/µL) (µL) |
|---|---|---|---|---|---|
| MR1 | 0.020 | 10 | | | |
| MR2 | 0.040 | 20 | | | |
| MR3 | 0.080 | 40 | 8 | | |
| MR4 | 0.10 | 50 | (10)[a] | | |
| MR5 | 0.25 | | 25 | | |
| MR6 | 0.50 | | 50 | | |
| MR7 | 0.75 | | 75 | (7.5)[a] | |
| MR8 | 1.00 | | | 10 | |
| MR9 | 3.00 | | | 30 | |
| MR6x | 0.50 | | | | 50 |

[a]    Calibration Level is prepared either by adding solution C1 or B1 (for MR4), B1 or A1 (for MR7) but not both.
On a day-to-day basis, the working range is 0.020 to 0.75 µg/L.  If samples are suspected to have higher concentrations, then additional calibration levels, up to 3.0 µg/L, are processed.

**NOTE:** Allow Working Spiking and Internal Standard Solutions to warm to room temperature before opening the vial.

**NOTE:** Calibration Curve and Calibration Control samples are spiked using standards that have been prepared either from two different sources or have separate lot numbers. Calibration Curve samples are spiked using the Primary working spiking solutions. Calibration Control sample is spiked with the Secondary working spiking solution.

2.4.5    Matrix Spike (MS6)

For each batch of samples processed, prepare Matrix spike Sample consisting of 20 mL of composite matrix (2.2.6) spiked with 50 µL of 1,4-Dioxane Working Spiking Solution B1 (5.2.10) to give (0.50 µg/L) of 1,4-Dioxane.

2.4.6   Samples

Allow the sample bottles to warm to room temperature.
Label pre-cleaned 20mL headspace vials (2.1.1) with LIMS sample numbers.  For replicates attach the suffix **R** after the LIMS number. Shake the sample bottles to homogenize the sample and transfer 20 mL of the sample into the headspace vial. Retain the remaining sample in the original bottle for re-analysis, if necessary.

For samples expected to contain high "µg/L" concentrations of 1,4-Dioxane, take a smaller volume (1 mL minimum).  Add composite matrix to make up to 20 mL.

Record the sample numbers, sample descriptions, sampling date and extraction volume on the Sample Tracking Form (FRM_3534_001).

**NOTE**:One sample per batch is randomly selected to be analysed in replicate.

2.4.7   Add 10 µL of the $d_8$-1,4-dioxane Working Internal Standard Solution (5.2.9, 0.4 ng/µL) to Method Blank, Calibration Curve / Calibration Control Samples, Matrix spike and samples.

2.4.8   Load headspace vials containing QC and samples onto tray VT32-20 (2.3.1.1).

2.4.9   Load ITSP cartridges (2.1.4) onto tray ITSPprep (2.3.1.2).

2.4.10  Load empty 2 mL autosampler vials (2.1.5) onto tray VT54ITSP (2.3.1.3). Cover the tray with aluminium block. Ensure that the cover sits snuggly over the vials to allow correct alignment of the ITSP cartridge with the autosampler vials during the elution step.

2.4.11  Fill up the Wash Station for right MPS arm (2.3.1.8) with dichloromethane.

2.4.12  Fill up the Wash Station for left MPS arm (2.3.1.9) with methanol and RO water.

2.4.13  Fill up the solvent reservoirs (2.3.1.7) with dichloromethane and acetone.

2.4.14  Change the status of the samples in the **LIMS** department **3211** from **NEED** to **FINI** (see Reference 1.7.16).

2.4.15  Prepare a file folder for the submissions to be processed.

2.4.16  Insert the Sample Tracking form FRM_E3534_001 for the submissions in the corresponding file folder.

**NOTE:** Update the **Sample Tracking Form** as each stage of analysis is completed.

### 3.0 ANALYTICAL PROCESSING

No further processing of the samples is required before submitting them to the Detection System.

### 4.0 DETECTION SYSTEM

**NOTE:** Labwares, reagents and equipment equivalent to that used in this method are acceptable.

#### 4.1 Labwares

4.1.1 Syringe, glass, Gastight, Hamilton, 5 μL or 10 μL.

#### 4.2 Reagents

4.2.1 Dichloromethane, $CH_2Cl_2$, distilled-in-glass grade (DIG) or equivalent.

4.2.2 Helium, He, ultrahigh purity (UHP).

4.2.3 Perfluorotributylamine (PFTBA, **FC43**), Reference Compound: SCM PCR Incorporated, Product No. 18201-4, (5 g).

4.2.4 1,4-Dioxane Working Instrument Check (WICS) Solution at 0.2 pg/μ/L (5.2.13) in dichloromethane.

#### 4.3 Equipment

4.3.1 MultiPurpose Sampler (MPS) Dual Head prep station (Gerstel) with:

4.3.1.1 Tray for 32 10 mL/20 mL vials, VT32-20 (Figure 1-A, Appendix IV).

4.3.1.2 Cartridge holder for 54 ITSP SPE cartridges, ITSPprep (Figure 1-B).

4.3.1.3 Tray for 54 2 mL vials with a custom aluminium cover, VT54ITSP (Figure1-C).

4.3.1.4 Tray for 98 2 mL vials, VT98 (Figure 1-D).

4.3.1.5 Left MPS arm with 2.5 mL syringe (Figure 1-E).

4.3.1.6  Right MPS arm with 10 µL syringe (Figure 1-F).

4.3.1.7  Three solvent reservoirs for SPE cartridge/syringe cleaning and conditioning (Figure 1-G).

4.3.1.8  Wash station for right MPS arm containing dichloromethane (Figure 1-H).

4.3.1.9  Wash station for left MPS arm containing methanol and RO water (Figure 1-I).

4.3.2  Gas Chromatograph: Agilent 6890N GC System or equivalent with split /splitless injectors and 4 mm i.d. single-tapered Gooseneck liner with glass wool.

4.3.3  Column:  J&W Scientific DB-1701, 30 m, 0.25 mm i.d., 1.0 µm film thickness connected to a guard column 5m x 0.53 mm i.d. with glass press-fit connector.

4.3.4  Mass Spectrometer:  Waters Micromass AutoSpec Ultima, [high resolution mass spectrometer (EBE geometry)].

4.3.5  Interface:  Waters EPC interface.

4.3.6  Data System (Instrument Control/ Data Acquisition):
ThinkStation Lenovo, Intel® Core™2 Duo CPU E6750 @ 2.66 GHz, 1.96 GB of RAM, 12x/8x/32x CD ReWritable Drive, HP L2245wg Monitor, HP 5550dtn colour Printer, Windows XP Service Pack 2, MassLynx v4.1 (Waters) software for GC and HR MS control, and Maestro 1 v1.4.25.8 software (Gerstel) for MultiPurpose Sampler Dual Head prep station control.

4.3.7  Data System (Data Processing):
ThinkStation Lenovo, Intel® Core™2 Duo CPU E6750 @ 2.66 GHz, 1.96 GB of RAM, 12x/8x/32x CD ReWritable Drive, Acer P246HL Monitor, HP 5550dtn colour Printer, Windows XP Service Pack 2 and MassLynx v4.1 Software, NIST and Wiley Libraries.

4.3.8  Recirculating Chiller: Neslab HX-500

Set the supply temperature to $15 \pm 2^{\circ}C$ to obtain a return temperature of $21^{\circ}C \pm 2^{\circ}C$.

4.3.9   Back-up Instrument:   Waters Micromass AutoSpec Premier [high resolution tandem mass spectrometer (EBE geometry)] with Agilent 7890A GC System with split / splitless injectors, Agilent 7683B Series Injector Towers and a 7683B Series Autosampler (100 sample tray).
ThinkCentre Lenovo, Intel® Core™2 Duo CPU E6750 @ 2.66 GHz, 1.96 GB of RAM, 12x/8x/32x CD ReWritable Drive, ThinkVision Lenovo Monitor, C8800 Series OKI Printer, Windows XP Service Pack 2 and MassLynx v4.1 Software, NIST and Wiley Libraries.

**4.4     Instrument Set-Up Procedures**
(References 1.7.22)

4.4.1   Data System Set-Up.

Double click on the **MassLynx** icon.
This will automatically search for a connection between the data system and the GC Inlet and Instrument (MS). The messages "**Connecting to Inlet**", "**Connecting to Instrument**" and "**Instrument Present**" should appear.

Double click on the **Maestro** icon.
This will open a connection between the data system and the MPS prep station.

4.4.2   MPS prep station Set-Up (see Appendix V).

Method **DX3534.mth** is used for solid-phase extraction of 10 mL sample on the ITSP cartridge and large-volume injection (10 µL) of the organic extract.

Method **LVI_10uL.mth** is used only for large-volume injection (10µL) without sample preparation i.e. WICS, solvent and continuous calibration extract.

4.4.3    Gas Chromatograph Set-Up.

In **MassLynx** under the **Instrument** tab select the **Inlet Method** icon,
then select **File**. Open inlet method **dx3534.h68** in
C:\MassLynx\Default.pro\Acqudb.
Ensure that the following parameters are loaded:

Under "**Inlet**" settings menu check control tabs parameters.
**Column 1** :
       **Enable Column**  = checked
       **Column dimensions**  = [30 m x 250 μm x 1.0 μm]
       **Carrier Gas** = Helium
       **Injection Pressure Mode** = Constant Flow
       **Injection Port** = Front
**Column 1 Pneumatics:**
       **Initial Flow** = 1.00 mL/min
       **Initial Time** = 1.00 min.
**Oven:**
       Max Oven Temperature = 255 °C
       Equilibrium Time = 0.20 min
       Cryo Cooling = Enabled [operating with Cryo = $CO_2$]
       Ambient Temp = 20 °C.
**Oven Temperature Ramps:**
       Initial Temperature = 38°C, Initial Time = 2.00 min
       Ramp Rate 1 = 10.0 °C/min, Final Temp = 250 °C, Hold Time =
       1.0 min.
       Run Time = 24.20 minutes
**Front Injector:**
Injection Port Type = Split/Splitless [operating in Splitless mode]
       Injector On = Enabled
       Temperature = 200°C
       Purge Flow = 20.0 mL/min
       Purge Time = 1.00 min

To **Configure** the GC, in **Inlet Method** menu, select **Tools**, **Instrument
Configuration.**
       In **Inlet Configuration** select **Configure**.
       In **Inlet Configuration Wizard** select **Next**.
       Select **HP 6890** as the pumping device and select **Next**.
       Select **None** as the autosampler and select **Next**.
       Select **None** as the detector and select **Next.**
       Select **Finish**.
       Select **Finish** in **Inlet Configuration**.

Exit the **Inlet Method** window.

In **MassLynx** select the **MS Tune**. Load **10k100_dioxane.ipr** file and set the GC transfer line temperatures on the **Inlets** tab of the **AutoSpec Tune Page**:

> Set **Capillary Line 1** to 230°C.
> Set **Capillary Line 2** to 230°C.
> Set **Re-entrant** to 230°C.
> Set **Septum** to 80°C.

4.4.3   Mass Spectrometer Set-Up.

In **MassLynx** under the **Instrument** tab, select **MS Method**.
Select **File**, **Open** and load **dx3534.exp** file stored in
C:\MassLynx\Default.pro\Acqudb. The **Experiment Setup** window with the following parameters will be displayed:

| No. | Type | Information | Time |
|-----|------|-------------|------|
| 1 | Voltage SIR | Time 8.0 to 9.80, Mass 88.05 to 99.99, EI+ | ▬▬▬ |

**NOTE:** The window start and end times are typical for a new 30 m column with 5 m guard column. The window is adjusted as required as the column is shortened to ensure that all of the analytes within the function continue to be monitored.

Select **Function 1**.  Function 1 must have the following parameters:
AutoSpec Parameter File is 10k100_dioxane.ipr

SIR Ions monitored:

| Mass(Da) | Channel Time (ms) | Delay Time (ms) | Lock Mass |
|----------|-------------------|-----------------|-----------|
| 88.0524 (1,4-dioxane) | 80.0 | 5.0 | No |
| 96.1026 ($d_8$-1,4-dioxane) | 80.0 | 5.0 | No |
| 99.9936 (PFTBA) | 30.0 | 5.0 | Yes |

| | |
|---|---|
| Start Time (min): | **8.0** |
| End Time (min): | **9.80** |
| Resolution: | **10,000** |
| Ionization Mode: | **EI+** |
| Primary Lock Span (pw): | **2** |
| Secondary Lock Span (pw): | **1** |
| Lock Mass Threshold (mV): | **0** |

|                        |          |
|------------------------|----------|
| Max Step Size (pw):    | **0.2**     |
| Use Fast Lock On       | **Enabled** |
| Cycle Magnet to Zero   | **Enabled** |

Exit **Function 1**.

Exit the **Experiment Setup** window.

In the **AutoSpec Tune** page open **10k100_dioxane.ipr** file and set the mass spectrometer to the following conditions:

|                        |                                               |
|------------------------|-----------------------------------------------|
| Ionization mode:       | electron ionization (positive ions) [EI$^+$]  |
| Resolving power:       | 10,000 RP (100 ppm) (AutoSpec Ultima)         |
|                        | (minimum 7,000 RP, 143 ppm) (see 6.2.9)       |
| Source temperature:    | 250°C                                         |
| Electron energy:       | 70 eV                                         |
| Trap current:          | 750 µA                                        |
| Alpha Slit:            | 100.00                                        |
| Detector:              | 350 V                                         |
| Accelerating voltage:  | 8 kV (nominal)                                |

## 4.5     Daily Tuning and Calibration Procedure for the Mass Spectrometer

4.5.1   Check the mass spectrometer vacuum.

Check the **Main Panel** on the instrument.
When the system is in the standby mode, the **Operate** light should be **red**. (If the switch is not in the Standby position but rather in Operate but the AutoSpec Tune Page indicates the instrument to be in Standby, the colour will be **orange**.)
Check the **AutoSpec Tune Page** which will also indicate **Standby** with a **red** light.
If there has been a high vacuum failure in the source and/or analyzer and at least one ion gauge is **OFF** both lights on the instrument will be **orange**. Typical pressure readings on the AutoSpec Ultima with two columns installed and at a total flow of 1.7 mL/min are:

The **Source Ion Gauge** should read approximately **4.0 x 10$^{-6}$ mbar.**
The **Source Pirani Gauge** should read approximately **1.8 x 10$^{-2}$ mbar.**
The **Analyser [1] Ion Gauge** should read approximately **4.3 x 10$^{-8}$ mbar.**
The **Analyser Pirani Gauge** should read approximately **2.0 x 10$^{-2}$ mbar**.
The **Inlets Pirani Gauge** should read approximately **2.4 x 10$^{-2}$ mbar**.

On the **AutoSpec Tune Page** under **Vacuum**, **Control** menu item 6 boxes should be illuminated with green lights to indicate that they are working:

> **Source Pumps** (**Fore=Rotary** and **Fine=Diffusion**)
> **Analyser Pumps** (**Fore=Rotary** and **Fine=Diffusion**)
> **Inlet Pumps** (**Inlets1 = Rotary**)
> **GC Solenoid**

Ensure that the **Trip Setting** for the **Source Pirani** and **Inlets1 Pirani** is 90.
Ensure that the **Trip Setting** for the **Source Ion Gauge** is set to 135 (approximately **9 x 10$^{-6}$ mbar**).

This completes the checks for major air leaks in the vacuum system.

4.5.2   Open the isolation valve between the source and the analyzer by toggling the Analyser Isolation Valve box on the **Vacuum** tab on the **AutoSpec Tune Page**.

4.5.3   Turn the system **ON** by switching the mass spectrometer main panel **Status** switch from **STANDBY** to **OPERATE**. Then press **Press for Operate** on the **AutoSpec Tune Page**.

4.5.4   Set the accelerating voltage (**V**) to **7950** kV.
Ensure that the Magnet is on by checking under **Options**, **Instrument Setup, Magnet** tab and click on the power to the magnet. The mode is **current**.
Turn **ON** the source by clicking the left mouse button on the **Tune** icon (♪).

4.5.5   Pump out the Septum Inlet by opening the valve to the **Inlet 1** rotary pump.
Wait 30 seconds and then close the valve.
Inject 0.2 μL of **PFTBA** (4.2.3) into the septum inlet (reference compound) reservoir.
Open the valve to the **Reference Compound Reservoir** to allow the PFTBA to bleed into the ion source.

4.5.6   Under **Control** 1 tab centre the **m/z 99.9936** peak on the tune page by setting the **Magnet Mass** to 100.0 and double clicking on the peak with the left button of the mouse.

**To ensure that there are no hysteresis effects, set the Magnet Current to 0 (i.e. m/z = 0), then back up for m/z = 100.0. Repeat if necessary to obtain a stable peak shape.**

4.5.7   Allow the system to equilibrate until peak shapes (and resolution) are stable.

4.5.8   Adjust the window span to 200 ppm on the tune page to obtain a peak width of 1-2 division(s). Repeat step 4.5.6 if necessary.

4.5.9   If a minor air leak is suspected, check the system as follows:

Pump out the PFTBA by opening the valves to the **Inlet 1** rotary pump. When the **Inlet 1** Pirani gauge drops to $1 \times 10^{-2}$ mbar, close the valve to the **Inlet 1** rotary pump.

In **Control 2** tab turn the **Detector** voltage down to 250 V.
Centre the helium (**m/z 4**) peak on the tune page window by selecting the **Magnet Mass** in the **Control 1** tab.
In the **Lenses** 2 tab adjust the **Y-focus** to optimize the peak shape and intensity.
If peak saturation is observed, reduce the detector voltage.
Check the height (volts) of the helium peak.
Centre the nitrogen (**m/z 28**) peak on the tune page window.
Adjust the **Y-focus** to optimize the peak shape and intensity.
Check the height (volts) of the nitrogen peak.
The ratio of **He:N$_2$** must be **>300:1** (purge A = on) or **> 30:1** (purge A = off). When this ratio is < 30:1, monitor **m/z 40** and use a stream of argon to check for leaks (see Section 6.2.8 #7). Once the source of the leak has been determined and corrective action taken, proceed.

Inject 0.2 μL of the reference compound **PFTBA** into the septum inlet reservoir.
Open the valve to the **Reference Compound Reservoir** to allow the PFTBA to bleed into the ion source.

Re-centre the **m/z 99.9936** peak on the tune page window by setting the **Magnet Mass** on the **Control 1** tab.
Turn the detector voltage up to its operational value of 350 V.
Adjust the **Y-focus** to optimize the peak shape and intensity or call up the tune parameters file **10k100_dioxane.ipr**.

4.5.10   Tune the mass spectrometer to **10,000 RP** on **m/z 99.9936** by adjusting the
           following controls iteratively (see Appendix II for a detailed tuning
           procedure for the AutoSpec Ultima with MassLynx software and
           Appendix III for typical tuning parameters):

- **Ion Repeller**
- **Focus 1**
- **Beam Centre**
- **Focus 2**
- **Y Deflect 1**
- **Z Deflect 1**
- **Z Deflect 2**
- **Z Focus 2** [set to approximately 2000 initially]
- **Z Focus 3** [set to zero when tuning to 10,000 RP]
- **Z Deflect 3**
- **Y Focus**
- **Rotate 2**
- **Curve 2**
- **Curve 3**
- **Rotate 3**
- **Rotate 4**

Save the tune parameters under the file **10k100_dioxane.ipr.**

4.5.11   Set the **Magnet Options** (Mass Range) as follows:

On the **AutoSpec Tune Page** select **Options** then **Instrument Setup**.
In the **Magnet** tab, ensure that the power to the magnet is **on** and the
magnet is in **current** mode.
In the **Limits** tab, ensure the voltage limit is **8000** and the magnet limit is
**2290.4** (this value does not need to be changed).
In the **Y Focus** tab**,** the **Setup Parameter** file is
C:\MassLynx\Default.pro\Acqudb\**BLANK.ypr**.
In the **Slits** tab, ensure the **Source Slit Setup** and **Collector Slit Setup** left
side and right side limits are maximum =100.00 and minimum = 0.00. The
AutoSpec Ultima utilizes hot wire slits. The Source Slit and Collector Slit
**Settings** are adjusted to obtain 10,000 RP.

4.5.12   Perform the voltage calibration.

Before performing a calibration, the instrument must not be in **tune** mode
(to check this ensure that the tune icon, ♪, is greyed out).

In the **AutoSpec Tune Page** menu select **Options**, **Experiment Calibration**. The **Experiment Calibration** window is displayed. In **Experiment file** select **dioxane_voltage SIR.exp**. In **Reference File** select **hepta.ref**. Set the **Rate (ms)** to **500** and the **Span** to **200** ppm. Select **Start**.

4.5.13 The peak profiles for **Fn 1** of the PFTBA reference masses **68.9952** and **99.9935** should appear during the calibration.
**Centroid** the peaks.
**Print** the peak profiles.
Select **Continue**.

Insert the Calibration Peak Profile printout in the appropriate **AutoSpec Instrument Tuning/Calibration** binder.

## 4.6     Analysis Procedure

4.6.1   Set up the sequence in **MassLynx**.

In **MassLynx**, select **File, New…**. An empty **Sample List** is loaded. Fill in the appropriate information under the [Filename], [File Text], [MS File], [Inlet File], [Bottle], [Inject Volume] column headings. (e.g. [DX150626_1] (for Dioxane yymmdd_sequence number), [WICS], [dx3534.exp], [dx3534.h68], [1], [20]).
Save the Sample List as **DXyymmdd.spl** in the C:\MassLynx\Default.pro\Sampledb folder.

A resolution checking macro program can be invoked at the completion of any sample acquisition in a sequence. To ensure mass peak shape and resolution has been maintained throughout the analytical sequence (4.6.14), trigger the resolution check after the first and last standards in the analytical sequence by filling in the macro information in the [Parameter File] C:\MassLynx\Default.pro\DX_res.dat, [Process Options] DX_res.dat and [Process] Resolution Check columns of the first and last standards.

4.6.2   Check the instrument sensitivity.

Set up sample #1 in the **Mass Lynx** sample list to run the Working Instrument Check Standard (WICS) (5.2.13) to check for instrument sensitivity (Section 6.2.1). For the WICS, place a 200 μL glass insert into an autosampler vial. Transfer approximately 60 μL of the WICS into an insert using a Pasteur pipette. Cap the vial with a Teflon-lined cap and crimp to seal.

4.6.3  Place the vial into the appropriate position in the autosampler tray VT98 (Figure 1-D).

4.6.4  Place the solvent vials (tray VT98), Method Blank, Method Calibration Curve/Calibration Control, Matrix spike and samples in the tray VT32-20 (Figure 1-A) in the appropriate positions.

Include solvent injections after samples expected to have high concentrations of 1,4-Dioxane.

4.6.5  Ensure that the solvent rinse vials (Figure 1-H) are filled with dichloromethane and that the waste vials are empty.

4.6.6  In **Maestro**, select **Method, Load**, **LVI_10uL.mth** (Appendix V).

4.6.7  Start the **Acquisition** of WICS first in **MassLynx** and then in **Maestro** by selecting **Run** button. A **Start Single Run** panel will appear:

Get sample from: **Tray1, VT98**
Inject to: **Front**
Vial number: **1**

Press **Start Run** button.

The following messages should flash up on the GC display:
**Downloading Method. Please Wait.**

The magnet current will drop to zero and go back up to the setting for Function 1.
Following the solvent delay, the lock mass should be acquired and displayed on the tune page window.

The solvent trip (set at **9 x 10$^{-6}$ mbar** on the source ion gauge) will turn **OFF** the high tension (**HT**) and **filament** but will leave the mass spectrometer in **OPERATE**.  The source ion gauge will increase to **1.8 x 10$^{-4}$** mbar when 10 μL are injected into a 4 mm i.d. single-tapered injector liner with glass wool.  The source Pirani gauge will increase to **6.5 x 10$^{-2}$** mbar.
Ensure that the **Source Ion Gauge** does **NOT** trip the system into **STANDBY** otherwise the run will be lost.
Ensure that the **Source Pirani Gauge** does **NOT** trip the source diffusion pump.

When the source pressure falls below **9 x 10$^{-6}$ mbar**, the **HT** and **filament** will be turned **ON**.

When the solvent delay is over, the data system should begin the acquisition and acquire the lock mass.  The lock mass signal should be visible on the tune page window.  If the lock mass signal is not acquired, then terminate the acquisition with **Stop**, wait until the GC cycles back to the starting temperature and start the acquisition again (start at Step 4.6.7) by overwriting the file.

4.6.8    Determine the **S/N AVG** for 1,4-Dioxane.

For the Working Instrument Check Solution (WICS) (5.2.13), display the ion chromatogram for 1,4-Dioxane (m/z 88.0524) and run the signal-to-noise algorithm.

In **MassLynx**, select **File**, **Open Data File**.
Select the filename from **Directory**.
Once selected the **Chromatogram** window will automatically appear.
In **Chromatogram**, select **Display**, **Mass**, select **Ch2 88.0524** for 1,4-Dioxane and **OK**.
In **Chromatogram** select **Process** and **Signal to Noise...**
In **Signal to Noise**, enter the signal window span and the noise window span and then **OK**.
If the **S/N AVG** is **≥ 10:1**, continue.
If this ratio is **< 10:1**, **Stop** the acquisition and start over at Step 4.5.6 but by-pass Step 4.5.7.
**Print** the Signal to Noise display.
File the hardcopy in the appropriate **AutoSpec Instrument Tuning/Calibration** binder.

4.6.9    Set up the sequence in **Maestro**.

In **Maestro**, select **Sequence**, **New…**. An empty sequence table is loaded containing [Vial], [Method], [Volume], [Tray], [Injector], [Sample Name] and [Sample Info] columns.

From **MassLynx** copy the content of the [File Name] column into **Maestro** [Sample Name] column and the content of the [File Text] column into [Sample Info] column.
For solvent and Continuous Calibration samples, select **LVI_10uL.mth** from the [Method] drop-down list. This will automatically populate [Volume], [Tray] and [Injector] columns with 10.0, Tray1, VT98, and Front, respectively.

For all other samples select method **DX3534.mth** from the [Method] drop-down list. This will automatically populate [Volume], [Tray] and [Injector] columns with 10.0, MT1-Rear, HW-54P and Front, respectively.

Click **OK** button and save the Sequence Table by selecting **Sequence, Save as…** DXyymmdd.seq.

4.6.10   Start the Sequence first in **MassLynx** and then in **Maestro** by selecting **Sequence, Run Interactive Mode**.

4.6.11   Check the **MassLynx** window for the following messages:

If the message reads **Sample # Acquiring**, then the system will have acquired data only up to and including that sample and may still be acquiring data. Do **NOT Stop** the acquisition.

If the message reads **Queue is Empty**, this confirms that all runs were completed.

4.6.12   Check one mass chromatogram in each sample to ensure that the sample extract was injected properly (analyte or labelled analyte peaks are acquired) and that the lock mass was acquired (see lock mass check). See Step 4.6.8 to display a mass chromatogram.

4.6.13   If no analyte or labelled analyte peaks were acquired, the sample must be re-extracted and re-injected, preferably without recalibrating the instrument. If the instrument was re-calibrated (Step 4.5.12), the Calibration Curve samples (Step 2.4.4) used to generate the standard curve must be re-run.

4.6.14   If the **Acquisition** was **Completed**, assess the Resolution Check macro printouts or place the instrument in the tuning mode so that the resolution can be assessed on the oscilloscope:

Select the tune icon (♪) and set the magnet mass to 100.0 with a window span of 200 ppm.

**To ensure that there are no hysteresis effects, set the Magnet Current to 0 (i.e. m/z = 0), then back up for m/z = 100.0. Repeat if necessary to obtain a stable peak shape.**

**Print** the Resolution check display.

File the hardcopy in the appropriate **AutoSpec Instrument Tuning/Calibration** binder. The resolution should be within 10% of the initial RP.

4.6.15   Change the status of the samples in the **LIMS** department **3222** from **NEED** to **FINI** (see Reference 1.7.16).

4.6.16   After all runs are completed, check the vacuum system (see Step 4.5.1):

The **Source Ion Gauge** should read approximately **4.0 x 10$^{-6}$** mbar.
The **Analyser [1] Ion Gauge** should read approximately **4.3 x 10$^{-8}$** mbar.

4.6.17   If no other samples are scheduled to be analyzed, place the instrument in the Standby mode:

Close the **Analyser Isolation Valve** (by clicking on the toggle switch in the vacuum menu of the tune page.
Set the **AutoSpec Tune Page** to **Standby**.
Switch the instrument Main Panel "Status" control to **Standby**.
Close the **Reference Compound Reservoir Valve** and pump out the reservoir.
Set the GC column temperature to **110°C**.

4.6.18   Bake out the flight tube (optional).

Monitor the magnet status by selecting **Options**, then **Instrument Setup** and **Magnet Setup**.  The magnet power should be **ON**.
Select **Vacuum**, then **Bake**.
In the **Baking Menu**, use **Manual Control**.
Set the baking time to a minimum of **12.0 hours** and turn **ON**.
The magnet power will be turned **OFF** by a solenoid (audible).

4.6.19   Process the data according to Sections 5.3 and 6.3.

## 5.0    CALIBRATION

### 5.1    Labwares

**NOTE:** Pipettes and flasks are cleaned using detergent and water, rinsed with Pure Water (2.2.3) and then rinsed with solvent (methanol, then dichloromethane).

5.1.1    Flasks, volumetric, glass, Class A, 10 mL, 50 mL, 100 mL.

5.1.2    Vials, amber glass with Teflon-lined caps, 2 mL, 10 mL, 20 mL, 40 mL.

5.1.3    Pipettes, volumetric, glass, Class A, 0.5 mL, 1 mL, 2 mL, 5 mL.

5.1.4    Syringes, glass, 10 µL, 25 µL, 100 µL, 250 µL.

5.1.5    Pipette Bulb or No. 2 (blue) Glasfirn pi pump.

### 5.2    Reagents, Standards and Reference Materials

5.2.1    Methanol, $CH_3OH$, distilled-in-glass grade (DIG) or equivalent.

5.2.2    Dichloromethane, $CH_2Cl_2$, distilled-in-glass grade (DIG) or equivalent.

5.2.3    1,4-Dioxane, $C_4H_8O_2$, CAS# 123-91-1, Ultra Scientific US NV 1501 (1mL), 100 µg/mL in methanol, 99.8%.

5.2.4    1,4-Dioxane, $C_4H_8O_2$, CAS# 123-91-1, AccuStandard #AAPP-9-096 (1mL), 100 µg/mL in methanol, $> 99\%$.

5.2.5    $d_8$-1,4-Dioxane, $C_4D_8O_2$, CAS# 17647-74-4, Cambridge Isotope Labs #DLM-28-SM-1.2 (1.2 mL), 1 000µg/mL in methanol, 99% D.

**NOTE:** Stock Solutions have a shelf life of 5 years and are labelled with an expiry date of 5 years from the date the solution was first prepared.  Intermediate Solutions and Working Solutions have a shelf life of 2 years and are labelled with an expiry date of 2 years from the date the solution was first prepared.

**NOTE:** Allow standard solutions to warm to room temperature before opening vials or ampules and working with solutions.

**NOTE:** Preparation of all standard solutions is recorded in the **Sample/Standard Preparation Logbook – Dioxane** (Reference 1.7.23).

5.2.6    Primary 1,4-Dioxane Stock Solution **A1** (2 ng/µL).

Transfer 1.0 mL of 100 µg/mL 1,4-Dioxane solution (5.2.3) to a 50 mL volumetric flask containing 10 mL of methanol and swirl to mix. Dilute to the mark with methanol. Cap the flask and invert 3 times to mix. The concentration of this solution is 2 ng/µL.  Store the solution at 5±3°C in the dark in amber vials (5.1.2).

5.2.7    Secondary 1,4-Dioxane Stock Solution **A2** (2 ng/µL).

Transfer 1.0 mL of 100 µg/mL 1,4-Dioxane solution (5.2.4) to a 50 mL volumetric flask containing 10 mL of methanol and swirl to mix. Dilute to the mark with methanol.  Cap the flask and invert 3 times to mix. The concentration of this solution is 2 ng/µL.  Store the solution at 5±3°C in the dark in amber vials (5.1.2).

5.2.8    $d_8$-1,4-Dioxane Stock Solution **A3** (20 ng/µL).

Transfer 200 µL of 1 000 µg/mL $d_8$-1,4-Dioxane solution (5.2.5) to a 10 mL volumetric flask containing 5 mL of methanol and swirl to mix. Dilute to the mark with methanol. Cap the flask and invert 3 times to mix. The concentration of this solution is 20 ng/µL.  Store the solution at 5±3°C in the dark in amber vial (5.1.2).

5.2.9    $d_8$-1,4-Dioxane Working Internal Standard (**IS**) Solution (0.4 ng/µL).

Transfer 1.0 mL of $d_8$-1,4-Dioxane Stock Solution A3 (5.2.8) to a 50 mL volumetric flask containing approximately 10 mL of methanol and swirl to mix. Dilute to the mark with methanol. Cap the flask and invert 3 times to mix.  The concentration of this solution is 0.4 ng/µL.  Store the solution at 5±3°C in the dark in amber vials (5.1.2).

5.2.10   1,4-Dioxane Primary Working Spiking Solution **B1** (0.2 ng/µL).

Transfer 5 mL of the Primary 1,4-Dioxane Stock Solution A1 (5.2.6) to a 50 mL volumetric flask and dilute to the mark with methanol. Cap the flask and invert 3 times to mix. The concentration of 1,4-Dioxane is 0.2 ng/µL.  Store the solution at 5±3°C in the dark in amber vials (5.1.2).

5.2.11  1,4-Dioxane Primary Working Spiking Solution **C1** (0.04 ng/μL).

Transfer 1 mL of the Primary 1,4-Dioxane Stock Solution A1 (5.2.6) to a 50 mL volumetric flask and dilute to the mark with methanol. Cap the flask and invert 3 times to mix. The concentration of 1,4-Dioxane is 0.04 ng/μL.  When 10 μL of this solution is added to 20 mL of water, the concentration of 1,4-Dioxane is 0.02 µg/L (2.4.4).  Store the solution at 5±3°C in the dark in amber vials (5.1.2).

5.2.12  1,4-Dioxane Secondary Working Spiking Solution **B2** (0.2 ng/μL).

Transfer 1 mL of the Secondary 1,4-Dioxane Stock Solution A2 (5.2.7) to a 10 mL volumetric flask and dilute to the mark with methanol. Cap the flask and invert 3 times to mix. The concentration of 1,4-Dioxane in this solution is 0.2 ng/μL.  When 50 μL of this solution is added to 20 mL of water, the concentration of 1,4-Dioxane is 0.50 µg/L (2.4.4). Store the solution at 5±3°C in the dark in amber vials (5.1.2).

5.2.13  Working Instrument Check (**WICS**) Solution (1,4-Dioxane and $d_8$-1,4-Dioxane at 0.2 pg/μL).

Transfer 0.5 mL of the Primary Working Spiking Solution C1 (5.2.11) and 50 μL of the $d_8$-1,4-Dioxane Working Internal Standard (**IS**) Solution (5.2.9) to a 100 mL volumetric flask containing 50mL of dichloromethane, swirl to mix and dilute to 100 mL with dichloromethane. Cap the flask and invert 3 times to mix. The concentration of each component in this solution is 0.2 pg/μL. Store the solution at 5±3°C in the dark in amber vials (5.1.2).

**NOTE:**  All new working solutions are verified against the old working solutions by analysing eight replicates of both solutions.  The new solution is accepted if the average response does not differ by more than 15% from the average response of the old solution.  If the difference is greater than 15%, a new solution is prepared for testing and the validation/verification redone.

**5.3**    **Creating a TargetLynx Quantitation Method**

The acquired data are integrated, calibrated and quantified using **TargetLynx** software. On the data processing computer in **MassLynx**, select **TargetLynx** tab and **Edit Method** icon. A **Method Editor** window is displayed. In **Method Editor** select **File**, **New**.
Fill in the information for each of the compounds of interest.

For d$_8$-**1,4-Dioxane**:

Under **Compound Properties** tab:

| | |
|---|---|
| Compound Name | d$_8$-**1,4-Dioxane** |
| Quantification Trace | **96.1026** |
| Include Primary Trace in Response? | **Yes** |
| Use Absolute Mass Window? | **Yes** |
| Chromatogram mass window (Da) | **0.020** |
| Response Type | **External (absolute)** |
| Response Uses | **Area** |
| Acquisition Function Number | **One** |
| Concentration of Standard: Level | **Fixed** |
| Concentration of Standard | **1.0** |

*View Retention Time Parameters*

| | |
|---|---|
| Locate Peak Using | **Retention Time** |
| Predicted Retention Time | **8.37** (determined from WICS chromatogram) |
| Relative Retention Time Reference | **None** |
| Retention Time Window (mins) ± | **0.0400** |
| Locate Peak Selection | **Nearest** |

*View Internal Standards*

| | |
|---|---|
| Internal Standard: 1 | **None** |

Under **Integration Properties** tab:

| | |
|---|---|
| Smoothing Enabled? | **Yes** |

*View Smooth Parameters*

| | |
|---|---|
| Smoothing Method | **Savitzky-Golay** |
| Smoothing Iterations | **1** |
| Smoothing Width | **3** |
| Apex Track Enabled? | **No** |

*Standard Peak Detection Parameters:*

| | |
|---|---|
| Peak-to-peak noise amplitude | **0** |
| Automatic Noise Measurement | **Yes** |
| Balance | **10** |
| Splitting | **90** |
| Detect Shoulder Peaks? | **No** |
| Reduce Tail | **30** |
| Reduce Height | **5** |

*View Threshold Parameters:*

| | |
|---|---|
| Threshold Relative Height | **1.5** |
| Threshold Relative Area | **2.0** |
| Integration Window Extent | **2.0** |
| Minimum Signal/Noise Ratio | **2.0** |

Under **Calibration Properties** tab:

| | |
|---|---|
| Polynomial Type | **Average RF** |
| Origin | **Exclude** |

Weighting Function                    **1/x**
Axis Transformation                   **None**
Concentration Units                   **µg/L**


Select **Compound, Add** menu item.

For **1,4-Dioxane**:

Under **Compound Properties** tab:

Compound Name                         **1,4-Dioxane**
Quantification Trace                   **88.0524**
Include Primary Trace in Response?     **Yes**
Use Absolute Mass Window?              **Yes**
Chromatogram mass window (Da)          **0.020**
Response Type                         **Internal (relative)**
Response Uses                         **Area**
Acquisition Function Number           **One**
Concentration of Standard: Level       **Conc A**
Concentration of Standard              **0**
*View Retention Time Parameters*
Locate Peak Using                     **Retention Time**
Predicted Retention Time              **8.41** (determined from WICS chromatogram)
Relative Retention Time Reference     **None**
Retention Time Window (mins) ±         **0.0400**
Locate Peak Selection                 **Nearest**
*View Internal Standards*
Internal Standard: 1                  **d$_8$-1,4-Dioxane**

Under **Integration Properties** tab:
Smoothing Enabled?                    **Yes**
*View Smooth Parameters*
Smoothing Method                      **Savitzky-Golay**
Smoothing Iterations                  **1**
Smoothing Width                       **3**
Apex Track Enabled?                   **No**
*Standard Peak Detection Parameters:*
Peak-to-peak noise amplitude          **100**
Automatic Noise Measurement           **Yes**
Balance                               **10**
Splitting                             **90**
Detect Shoulder Peaks?                **No**
Reduce Tail                           **30**
Reduce Height                         **5**
*View Threshold Parameters:*
Threshold Relative Height             **1.5**

| | |
|---|---|
| Threshold Relative Area | **2.0** |
| Integration Window Extent | **2.0** |
| Minimum Signal/Noise Ratio | **2.0** |

Under **Calibration Properties** tab:

| | |
|---|---|
| Polynomial Type | **Quadratic** |
| Origin | **Exclude** |
| Weighting Function | **1/x** |
| Axis Transformation | **None** |
| Concentration Units | **µg/L** |

Select **File**, **Save As** and type the file name in the format DX_3534_yymmdd.mdb. Select **OK**.

## 5.4    Calibration Procedure

Calibration curves are plots of native/deuterated target compound peak area ratios in the Calibration Curve Sample extracts versus the concentration (µg/L) of native target compound in the original Calibration Curve Samples (2.4.4).  The concentration of the deuterated compound in the Calibration Curve Samples remains fixed ($d_8$-1,4-Dioxane at 0.20 µg/L) while the concentration of the native compound or analyte added to the Calibration Curve sample is varied (1,4-Dioxane at 0.020 µg/L to 3.0 µg/L).

Create a calibration curve using all calibration standards in the batch with a 1/x weighting, origin excluded and quadratic regression. The coefficient of determination ($R^2$) for the calibration curve generated must be $\geq$0.995. If a point on the curve is an outlier (i.e. it deviates by >25% from the curve of best fit), this point may be excluded from the calibration curve equation calculation.

The absolute responses (peak areas) and native/deuterated target compound peak area ratios for a particular Calibration Curve Sample (2.4.4) extract will vary from day to day.  The signal-to-noise (S/N) of the Working Instrument Check (WICS) Solution will vary from day to day.  The minimum S/N for 2 pg injected [10 µL of Working Instrument Check Solution (WICS) (5.2.13)] is $\geq$10:1 for 1,4-Dioxane (see Step 6.2.1).

**6.0     RUN PROCESSING AND QUALITY ASSURANCE**

**6.1     Run Format**

6.1.1   The daily run format includes analyzing:

1)   Solvent
2)   Working Instrument Check Solution [WICS] (5.2.13)
3)   1 Method Blank (2.4.3)
4)   Calibration Curve Samples MR1 – MR6 [0.020 – 0.50 µg/L] (2.4.4)
5)   Calibration Control Sample MR6x  [0.50 µg/L] (2.4.4)
6)   Calibration Curve Samples MR7 – MR9  [0.750 µg/L, 1.0 and 3.0 µg/L if processed] (2.4.4)
7)   Matrix spike MS6 [0.5 µg/L] (2.4.5)
8)   Solvent
9)   Samples (2.4.6)
10)  Sample replicates [one per batch] (2.4.6)
11)  Instrument Drift Check MR6 [0.50 µg/L] (6.1.1 #4, re-injection of the extract)

**NOTE:** Samples are run in the order of Emergency (EM), Repeat Analysis (WD first), Priority (PR), Regulatory (RE), Drinking Water (WD) - Normal (N) priority, Court Case (CC), Other matrices - Normal (N)priority.

**NOTE:** Solvent injection(s) are included in the run after samples expected to have high concentrations of 1,4-Dioxane.

**6.2     Run Control Operations/Limits**

6.2.1   Instrument Sensitivity

Expectation:   The S/N for 1,4-Dioxane in the **Working Instrument Check Solution (WICS)** (5.2.13) (2 pg 1,4-Dioxane injected) (Step 4.6.7) must be **≥ 10:1**.
Action:         If it is < 10:1, then proceed as follows:
1)   Check the chromatography (Step 6.2.5).
2)   Replace the injector septum.
3)   Replace the injector liner.
1)   Replace the injector liner O-ring.
2)   Replace the injector liner seat.
3)   Retune and recalibrate the mass spectrometer (Section 4.5).

4)  Check for air leaks at the injector, the connector between the column and the interface and the mass spectrometer itself including the septum inlet. Set up the system to monitor argon at m/z 40 and use a stream of argon to check for the source(s) of the leak(s).

5)  Clean or replace the inner ion source.

6)  Clean or replace the outer ion source.

7)  Clean or replace the first lens stack.

8)  Replace the source slit.

9)  Check the photomultiplier and the phosphor. The gain of the photomultiplier is determined by a statistical calculation of the noise. Check the manual for the procedure. A gain of 100,000 should be achieved with a photomultiplier voltage of 350 to 400 V. The gain should be constant for the life of the photomultiplier tube. A gain of 50,000 to 100,000 is necessary for this method. Replace the photomultiplier and/or phosphor if necessary.

10) If sufficient mass resolution (10,000 RP) and sensitivity cannot be attained, detune the instrument to 7,000 RP.

6.2.2   Peak Width

Expectation:   Good chromatography is exemplified by a peak width of ≤7 seconds at the baseline.

Action:   If the chromatography is poor (exemplified by severe peak tailing-peak, peak width > 14 seconds), then proceed as follows:

1)  Replace the injector liner.

2)  Replace the guard column.

3)  Shorten the analytical column by 0.5-2.0 m at the injector end. Update the column dimensions in Gas Chromatograph Set-Up Section 4.4.2 in order to maintain comparable retention times under constant flow conditions.

4)  Replace the column (if necessary). Update the column dimensions in Gas Chromatograph Set-Up Section 4.4.2.

6.2.3   Method Blank

The 1,4-Dioxane concentration in the **Method Blank** (2.4.3) is monitored but the concentrations of the 1,4-Dioxane is not corrected for the method blanks.

Expectation:   The concentration of 1,4-Dioxane in the Method Blank should be less than 0.020 µg/L.

Action:   When clean composite matrix is not available (< 0.020 µg/L) samples will be run and the MDL will be appropriately adjusted to 3x the concentration of 1,4-Dioxane in the available method blank.

6.2.4   Calibration Control (MR6x)

The 1,4-Dioxane recovery from the **Calibration Control** Sample (2.4.4) spiked at 0.50 µg/L is plotted in a control chart. The control charts are reviewed for trends on a per run basis prior to APP1 approval in LIMS according to the LSBSOP041 (1.7.20).

Expectation:   Maximum acceptable difference between the calculated and target concentration is ± 25%.

Action:   If the Calibration Control Sample is out of control the source of the problem is investigated. Sample results are reported if the source of the problem is traced back to the calibration control sample itself (i.e. spiking standard or processing). If other quality control indicators (i.e. calibration curve, internal standard peak areas, etc.) point to a larger problem, sample results are flagged with a remark code UQC and corrective actions are taken. Possible corrective actions include re-analysing the sample set, injector/column maintenance and inner or outer source replacement.

6.2.5   Replicates

The relative percent difference (RPD) of the replicates is monitored.  RPD of the replicate results is calculated as [(replicate 1 – replicate 2)/(average of replicate 1 & replicate 2)]*100 and must be equal to or less than 30%.

Expectation:   When replicate sample RPD is ≤ 30% the higher value of
               the replicate results is reported in LIMS.

Action:        If the replicate sample RPD is > 30%, the higher of the
               two values will be reported with the LIMS remark code
               UDD (Unreliable: sample duplicates differ).

6.2.6   Instrument Drift (MR6)

The **Instrument Drift Check** is a Calibration Curve MR6 extract at 0.5
µg/L, that was prepared at the start of the sequence and the vial was re-
capped. This extract is run again at the end of the sequence to monitor
instrument drift.

Expectation:   Maximum acceptable difference between the initial and the
               final concentration is ± 30%.

Action:        If the final calculated concentration is 30% higher than the
               target then all positive results are flagged with a remark
               code ABH (Approximate Result: May be biased high).
               If the final calculated concentration is 30% lower than the
               target then all results are flagged with a remark code ABL
               (Approximate Result: May be biased low).

## 6.3   Identifications and Calculations

6.3.1   The criteria for positive identification of 1,4-Dioxane are:

6.3.1.1   The lock mass signal must be picked up on the tune page (Step
          4.6.7) and its intensity must be constant across the peaks. This
          can be checked by monitoring the lock mass trace (4.6.12).

6.3.1.2   The retention times of the 1,4-Dioxane and $d_8$-1,4-Dioxane are
          within 2 seconds of the expected retention times determined for
          the Working Instrument Check (WICS) Solution (4.6.8).

6.3.1.3   The retention time of the $d_8$-1,4-Dioxane is approximately 2.5
          seconds less than 1,4-Dioxane.

6.3.1.4   If the retention times of the 1,4-Dioxane or $d_8$-1,4-Dioxane are
          shifted because of "matrix effects", then criterion 6.3.1.3 must be
          met.

6.3.2   To process the data, copy the data files from the data acquisition to the data processing computer in the directory **C:\MassLynx\Dioxane.pro\Data** and the Sample List to the directory **C:\MassLynx\Dioxane.pro\SampleDB**.

In **MassLynx**, select the **TargetLynx** tab.  Then select **File**, **Open Project,** Dioxine.pro and **OK**.
Load the **Sample List** table by selecting **File, Open…..** and select the filename (with extension .spl) from the **SampleDB** directory and **OK**. The **Sample List** table should include the headings **File Name**, **File Text**, **MS File**, **Inlet File**, **Sample Type** and **Conc. A**.  If it does not contain these headings, right click the mouse on the table and select **Customize Options**.  Select the above headings from the list and select **OK**.  For each of the Calibration Curve samples to be used as **Standards**, select the corresponding **Sample Type** cell with the left button of the mouse and select **Standard**.  For each of these **Standards**, fill in the appropriate concentrations in the **Conc. A** column by clicking on the table cell with the left button of the mouse and typing in the concentration.  For the Method Blank, Matrix spike and Calibration Control samples select **Blank**, **QC** and **QC**, respectively in the **Sample Type** cell and type the appropriate concentrations in the **Con. A** column.

Select **Process Samples** icon. A **Create TargetLynx Dataset** window is displayed. Select **Integrate Samples, Calibrate Standards, Quantify Samples**.  Check the **Quantify** box to confirm that the correct **Method** is selected.  If it is not, select **Browse** and select the quantify method from **MethodDB** directory.

Select **OK**.  This will begin the processing of the entire data set and the results will be automatically displayed in the **TargetLynx** Browser window.
To view the chromatograms of the next sample in the list, select **Display**, **Next Sample**.
To view the integration results for the next compound, select **Display**, **Next Compound**.

If manual integration is required, highlight the sample from the sample list to call up the chromatograms for the compound selected and left-click on the peak of interest.  This will indicate the outline width of the peak that was integrated.  To change the area, hold down the left button of the mouse on the side of interest and drag it to the position of interest and release the button.  On the now shaded peak, hold down the right button of the mouse and select **Save Peak Modifications** and **NO** (for note of

change).  The new area will be recorded in the **Summary Table**.
Integration of the peak should be from peak start to peak end along the
baseline.  The baseline is defined by its position in the absence of a peak.

Print the **Compound Summary** and **Samples Report** by selecting these
reports under **File**, **Report Format** and then **File**, **Print** in the
**TargetLynx** window.  Save the **TargetLynx** file as a **DXyymmdd.qld**
file.

Save a text file of the sets of tables on a removable hard drive by selecting
**File**, **Export**, **All Groups Summary** in the **TargetLynx** Browser
window.  A prompt window will open. Type in the name of the file as
DXyymmdd and save as a .txt file.  The .txt file can now be opened in
eLab.

6.3.3    Update all control charts and check for trends as described in the
         LSBSOP.041.

6.3.4    **Update** the status of the samples in Instrument Workgroup in **LIMS**
         department **3222** to **FINI.**  Create the Data Reporting Workgroup and add
         the samples in **LIMS** department **3241.** Change the status from **NEED** to
         **WIP**.

         Sequence the Workgroup in **Modify wGroup**.

         The results can be transferred to LIMS manually or through **DCI** the
         Workgroup using **eLab**.

6.3.5    Open **eLab** and press the **Workgroup List** button.

         Select the Workgroup number and click on **Import File** tab. Load the text
         file DXyymmdd.txt saved on the removable hard drive (6.3.2). Then select
         **Submit** tab followed by **OK**. A message will appear to indicate "# records
         applied".

         Import the Workgroup to **LIMS** by depressing the **Update LIMS** tab and
         then **OK**.  A message will appear to indicate how many records have been
         applied and saved (this number is related to the number of samples in the
         Workgroup). Close **eLab**.

6.3.6    In **LIMS** go to **Results**, **Results Entry** and select the Workgroup. Depress
         **Page up** and check the data for transcription errors. Then exit.

6.3.7    Go to **Results**, **Move Data**. Select the workgroup and then select **Page down**. Type **Y** to update and select **OK**. A Pre-APP1 report is emailed to the submitter. Print a Pre-APP1 report by going to **Reports**, **Results** and **Pre-App1 Approval Report** and select the workgroup number. Select **Run Report**.

6.3.8    Check the data/calculations for transcription errors on the Pre-APP1 report.

6.3.9    If the data and calculations are acceptable, update the status of the Workgroup to **APP1**.  The data in the Workgroup must be checked by another analyst and the status of the Workgroup changed to **APP2** in **LQAS2** and to **FINI** in **LIMS** department **3201**.  The folder containing all chromatograms, reports and forms is filed in the DIOXANE filing cabinet in room WL03A.  The manager or designate (e.g. Senior Scientist, Group Leader) updates the LIMS department **3001.**

## 6.4    Reporting

6.4.1    Results are reported in LIMS using the MDL format.  Results are reported in µg/L to 2 "significant figures".

6.4.2    The **LIMS MDL** is the Reporting Detection Limit (**RDL**).  The RDL is **0.020 µg/L** (20 ng/L) for **1,4-Dioxane**. Results below the MDL are reported with the numerical MDL value and the value qualifier **<MDL**.

## 6.5    Proficiency Testing

6.5.1    Analyst proficiency is determined as follows:

1)    Sample preparation proficiency is determined by preparing a series of eight composite drinking water samples spiked at a level of 0.10 µg/L with primary working spiking solution.  The analyst is proficient if the MDL calculated from this set of data according to the LSBSOP.026 is smaller than the LIMS Reporting MDL (6.4.2).

2)    GC-HRMS proficiency is determined by following Section 4.5, Daily Tuning and Calibration Procedure for the Mass Spectrometer, and meeting the criteria.

3)    Tuning proficiency is determined by following the procedure in Appendix II, to tune the instrument to10,000 resolution, running WICS (4.6.7) and meeting the criteria in Section 6.2.1.

4)   Data processing proficiency is determined by following Section 6.3, Identifications and Calculations.

5)   Additional proficiency will be determined by Performance Evaluation (PE) Samples from the Quality Management Unit (QMU) and from Interlaboratory Studies (ILS).

6)   Final proficiency will be determined by the "manager" or designee.

6.5.2   The proficiency records are kept in the filing cabinet in WL03A.

**Appendix I Analytical Performance Data**

**Table 1.6.1**    Within-run Method Performance (repeatability) and Method Detection Limits (MDL). Results for 1,4-Dioxane spiked at a concentration of 0.10 µg/L in composite drinking water matrix, n=8. (March, 2016)

| Parameter | Determined Concentration µg/L | Average Recovery % | Standard Deviation µg/L | RSD % | Calculated MDL µg/L | LIMS MDL µg/L |
|---|---|---|---|---|---|---|
| **1,4-Dioxane** | 0.097 | 97 | 0.0031 | 3.2 | 0.0093 | 0.020 |

**Table 1.6.2**    Between-run Method Performance (reproducibility).  Results for 1,4-Dioxane spiked at a concentration of 0.10 µg/L in composite drinking water matrix.  (March 2016)

| Parameter | Number of measurements | Average Determined Concentration (µg/L) | Average Recovery (%) | Standard Deviation (µg/L) | RSD (%) |
|---|---|---|---|---|---|
| **1,4-Dioxane** | 24 | 0.097 | 97 | 0.0045 | 4.7 |

**Table 1.6.3**    Method Working Range.  Average concentration of 1,4-Dioxane determined in composite drinking water matrix spiked with the primary working spiking solutions in the concentration range 0.02 to 3.0 µg/L. (n=3, Quadratic fit, origin excluded, 1/x weighting. (March 2016)

| Parameter | 1,4-Dioxane (µg/L) |
|---|---|
| **Level 1 – 0.02 µg/L** | 0.021 |
| **Level 2 – 0.04 µg/L** | 0.036 |
| **Level 3 – 0.08 µg/L** | 0.073 |
| **Level 4 – 0.10 µg/L** | 0.093 |
| **Level 5 – 0.20 µg/L** | 0.233 |
| **Level 6 – 0.50 µg/L** | 0.489 |
| **Level 7 – 0.75 µg/L** | 0.759 |
| **Level 8 – 1.00 µg/L** | 1.080 |
| **Level 9 – 3.00 µg/L** | 3.006 |
| **$R^2$** | 0.9973 |

**Appendix II  Tuning Procedure (AutoSpec Ultima and AutoSpec Premier)**

The procedure is outlined as follows:

**Section 1**       Vacuum System Checks
**Section 2**       Beam ON
**Section 3**       Tune to Low Resolution [approximately 1,000 RP]
**Section 4**       Tune to High Resolution from Low Resolution
**Section 5**       Retune in High Resolution [Daily for High Resolution Operation]

**Section 1 - Vacuum System Checks**

      See Section 4.5.1.

**Section 2 - Beam ON**

      See Steps 4.5.2 to 4.5.9.

**Section 3 - Tuning at Low Resolution**

      Centre the **m/z 68.9952** peak on the tune page window by selecting a magnet mass of **68.9** in **Magnet Mass** under the **Control 1** panel in the **AutoSpec Tune** page.

      **To ensure that there are no hysteresis effects, set the Magnet Current to 0 (i.e. m/z = 0), then back up for m/z = 68.9.  Repeat if necessary to obtain a stable peak shape.**

      Set the detector (photomultiplier) voltage to **250 V**.

      Set the **Source Slit** and **Collector Slit** (**Slit Tune**) to their approximate positions for 1,000 RP (1,000 ppm). Open the **Collector Slit** to flat topping and then close to approximately 90% to obtain a triangular peak.

      Set the **Source Control** parameters (**Source Tune**) to their midpoints except as noted:

      - **Ion Repeller** [set to approximately -4 V]
      - **Focus 1**
      - **Beam Centre**
      - **Focus 2**

      Set the **Beam Control** parameters (**Lens Tune**) to their midpoints except as noted:

      - **Y deflect 1**
      - **Ion Energy** (decrease to drop the peak height to 50%, then increase through the maximum to drop the peak height to 50%, then set to the midpoint)
      - **Z Deflect 1**
      - **Z Deflect 2**
      - **Z focus 2** [set to approximately 2000 initially]

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- **Z Focus 3** [set to 0]
- **Y focus**
- **Rotate 2**
- **Curve 2**
- **Curve 3**
- **Rotate 3**
- **Rotate 4**

Iterate through the **Source Control** parameters, **Beam Control** parameters, **Source Slit** and **Collector Slit** to obtain symmetrical triangular peaks with optimum resolution (resolving power) and sensitivity (signal strength).

Open the **Collector Slit** to flat topping and then close to approximately 90% to obtain a triangular peak. Note the resolution. This should be ≥1,000 RP (≤1,000 ppm).

If the instrument is under-resolved, close the **Source Slit** incrementally and repeat the iterative tuning procedure. If the instrument is over-resolved, open the **Source Slit** incrementally and repeat the iterative tuning procedure.

NOTE: The **source slit** is the **resolution-determining** slit. The **collector slit** should be set to **90% transmission** for an optimum tune.

## Section 4 - Tuning from Low Resolution to High Resolution

Tune the instrument to 1,000 RP (1,000 ppm).

Open the **Collector Slit** to flat topping and note the voltage.
Close the **Source Slit** to 10% of the voltage above.
Close the **Collector Slit** to 5% of the voltage above.
Adjust the **Y-focus** to obtain a symmetrical peak.
Open the **Collector Slit** to flat topping and then close to approximately 90% to obtain a triangular peak.

NOTE: There should be a significant improvement in resolution, i.e. to >5,000 RP. If this does not occur, then go back to 1,000 RP and retune.

Increase the **Detector Voltage** to **350 V**.

Iterate through the **Source Control** parameters, **Beam Control** parameters, **Source Slit** and **Collector Slit** to obtain optimum resolution (resolving power) and sensitivity (signal strength) with symmetrical triangular peaks.

NOTE: If no improvement in resolution can be seen, close the **Collector Slit** to 50% and iterate through the **Source Control** and **Beam Control** parameters.

Open the **Collector Slit** to flat topping and then close to approximately 90% to obtain a triangular peak.

Measure the resolution in ppm.

If the resolution is still less than 10,000 (under-resolved), close the **Source Slit** incrementally and repeat the iterative tuning procedure.

If the resolution is still less than 10,000, carefully observe its shape. If the peak has wide shallow tails, which cause the 5% height points (10% valley) to be wide, proceed as follows:

1) Adjust the **α-Slit**.
2) Adjust the **Z4 Restrictor**.

**NOTE:** The objective is to reduce the peak tails with a minimal loss in peak height.

**Section 5 - High Resolution Re-tuning**

Follow the procedure in Section 4.5.

Typical tuning parameter values for the AutoSpec Ultima and AutoSpec Premier instruments are listed in Appendix III.

**Appendix III Typical Tuning Parameters (AutoSpec Ultima and AutoSpec Premier)**

|  | AutoSpec Premier | AutoSpec Ultima |
|---|---|---|
| Source | EI + | EI+ |
| Ion Repeller (V) | -1.4 | -3.9 |
| Focus 1 | 806 | 1061 |
| Beam Centre | 4.4 | -26.1 |
| Focus 2 | 4206 | 4066 |
| Temperature | 250 | 250 |
| Electron Energy (eV) | 70 | 70 |
| Trap Current (μA) | 750 | 750 |
| Y Deflect 1 | 18.7 | 1 |
| Z Deflect 1 | 18.5 | -61.2 |
| Z Deflect 2 | 7.3 | -24.1 |
| Z Focus 2 | 2350 | 2117 |
| Z Focus 3 | 0 | 0 |
| Z Deflect 3 | -16.8 | 2 |
| Y Focus | 3403 | 3510 |
| Rotate 2 | -8.1 | 0.9 |
| Curve 2 | -0.4 | 2.3 |
| Curve 3 | -2.9 | 9.7 |
| Rotate 3 | 18.5 | 7.8 |
| Rotate 4 | -11.2 | -3.4 |
| V Acc (V) | 7959 | 7907.1 |
| Magnet Mass | 130.9 | 130.9 |
| Source Slit | 21.2 | 48.3 |
| Collector Slit | 13.32 | 43.4 |
| Alpha | 100 | 100 |
| Detector Voltage | 350 | 350 |
| Ion Energy | -5.5 | -0.1 |
| Z4 Restrictor | 0 | 0 |
| Voltage Limit | 8000 | 8000 |

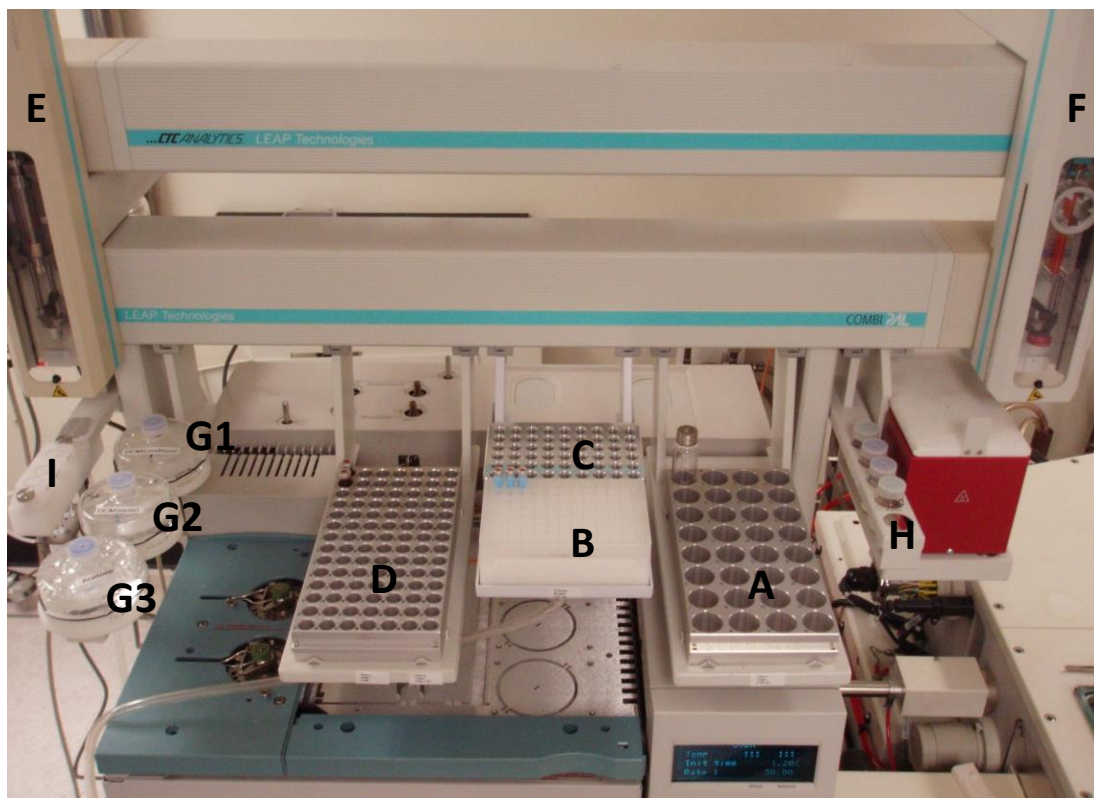**Appendix IV Multi Purpose Sampler (MPS) Dual Head Prep Station**



**Figure 1.** MultiPurpose Sampler (MPS) Dual Head Prep Station.
A – Tray for 32 10 mL/20 mL vials, VT32-20
B – Cartridge holder for 54 ITSP SPE cartridges, ITSP Prep
C – Tray for 54 2 mL vials with a custom aluminium cover, VT54ITSP
D – Tray for 98 2 mL vials, VT98
E – Left MPS arm with 2.5 mL syringe
F – Right MPS arm with 10 µL syringe
G – Three solvent reservoirs for SPE cartridge/syringe cleaning and conditioning
H – Wash station for right MPS arm containing dichloromethane
I – Wash station for left MPS arm containing methanol and RO water

### Appendix V GERSTEL MAESTRO Method Parameters


## Method - DX3534.mth

**NOTE:** This method includes sample extraction on the ITSP cartridge and large-volume injection (10uL) of the organic extract.

**MPS SAMPLE PREP ACTION LIST**

| ACTION | METHOD / VALUE | SOURCE | DESTINATION |
|---|---|---|---|
| ADD | Condition ITSP - 1000ul DCM | SolvRes1 | TSPprep,ITSP96 |
| ADD | Condition ITSP - 500ul MeOH | Wash1 | TSPprep,ITSP96 |
| ADD | Condition ITSP - 500ul RO Water | Wash2 | ITSPprep,ITSP96 |
| ADD | Load Sample (50uL/s) to ITSP | Tray2,VT32-20 | ITSPprep,ITSP96 |
| ADD | Rinse Syringe - 2500uL Acetone | SolvRes3 | Waste |
| FLUSH | 0.10 min | Waste | |
| ADD | Air flush cartridge @ 32 | Tray2,VT32-20 | ITSPprep,ITSP96 |
| FLUSH | 6.00 min | ITSPprep,ITSP96 | |
| ADD | Air flush cartridge @ 32 | Tray2,VT32-20 | ITSPprep,ITSP96 |
| FLUSH | 6.00 min | ITSPprep,ITSP96 | |
| ADD | Air flush cartridge @ 32 | Tray2,VT32-20 | ITSPprep,ITSP96 |
| ADD | Aspirate 500 uL DCM | SolvRes2 | Syringe |
| MOVE | | ITSPprep,ITSP96 | ITSPelut,VT54ITSP |
| ADD | Elute ITSP - 500 uL DCM | Syringe | ITSPelut,VT54ITSP |
| MOVE | | ITSPelut,VT54 | ITSPITSPprep,ITSP96 |


### SAMPLE PREP ACTION PARAMETERS

**ADD Method "Condition ITSP - 1000ul DCM"**

Syringe: 2.5mlALX
Fill Volume: 2500.0 µL
Fill Strokes: 0
Fill Speed: 50.00 µL/s
Add Volume: 1000.0 µL
Add Speed: 10.00 µL/s
Viscosity Delay: 0 s
Eject Speed: 500.00 µL/s
Source Vial Penetration: 47.00 mm
Dest. Vial Penetration: 18.00 mm
Post Add Delay: 0 s
Pressurize: No
Preclean with Sample: 0

Rinse 1: Wash1
Preclean with Solvent 1: 0
Postclean with Solvent 1: 0
Fill Speed Solvent 1: 500.00 µL/s
Viscosity Delay Solvent 1: 0 s
Eject Speed Solvent 1: 500.00 µL/s

Rinse 2: Wash2
Preclean with Solvent 2: 0
Postclean with Solvent 2: 0
Fill Speed Solvent 2: 500.00 µL/s
Viscosity Delay Solvent 2: 0 s

Eject Speed Solvent 2: 500.00 µL/s


**ADD Method "Condition ITSP - 500ul MeOH"**

Syringe: 2.5mlALX

Rinse 1: Wash1
Preclean with Solvent 1: 0

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Fill Volume: 2500.0 µL
Fill Strokes: 0
Fill Speed: 50.00 µL/s
Add Volume: 500.0 µL
Add Speed: 10.00 µL/s
Viscosity Delay: 0 s
Eject Speed: 500.00 µL/s
Source Vial Penetration: 32.00 mm
Dest. Vial Penetration: 18.00 mm
Post Add Delay: 0 s
Pressurize: No
Preclean with Sample: 0

Postclean with Solvent 1: 0
Fill Speed Solvent 1: 500.00 µL/s
Viscosity Delay Solvent 1: 0 s
Eject Speed Solvent 1: 500.00 µL/s

Rinse 2: Wash2
Preclean with Solvent 2: 0
Postclean with Solvent 2: 0
Fill Speed Solvent 2: 500.00 µL/s
Viscosity Delay Solvent 2: 0 s
Eject Speed Solvent 2: 500.00 µL/s

**ADD Method "Condition ITSP - 500ul RO Water"**

Syringe: 2.5mlALX
Fill Volume: 2500.0 µL
Fill Strokes: 0
Fill Speed: 50.00 µL/s
Add Volume: 500.0 µL
Add Speed: 10.00 µL/s
Viscosity Delay: 0 s
Eject Speed: 500.00 µL/s
Source Vial Penetration: 32.00 mm
Dest. Vial Penetration: 18.00 mm
Post Add Delay: 0 s
Pressurize: No
Preclean with Sample: 0

Rinse 1: Wash1
Preclean with Solvent 1: 0
Postclean with Solvent 1: 0
Fill Speed Solvent 1: 500.00 µL/s
Viscosity Delay Solvent 1: 0 s
Eject Speed Solvent 1: 500.00 µL/s

Rinse 2: Wash2
Preclean with Solvent 2: 0
Postclean with Solvent 2: 0
Fill Speed Solvent 2: 500.00 µL/s
Viscosity Delay Solvent 2: 0 s
Eject Speed Solvent 2: 500.00 µL/s

**ADD Method "Load Sample (50uL/s) to ITSP"**

Syringe: 2.5mlALX
Fill Volume: 2500.0 µL
Fill Strokes: 0
Fill Speed: 50.00 µL/s
Add Volume: 10000.0 µL
Add Speed: 50.00 µL/s
Viscosity Delay: 0 s
Eject Speed: 50.00 µL/s
Source Vial Penetration: 51.00 mm
Dest. Vial Penetration: 18.00 mm
Post Add Delay: 0 s
Preclean with Sample: 0

Rinse 1: Wash1
Preclean with Solvent 1: 0
Postclean with Solvent 1: 0
Fill Speed Solvent 1: 500.00 µL/s
Viscosity Delay Solvent 1: 0 s
Eject Speed Solvent 1: 500.00 µL/s

Rinse 2: Wash2
Preclean with Solvent 2: 0
Postclean with Solvent 2: 0
Fill Speed Solvent 2: 500.00 µL/s
Viscosity Delay Solvent 2: 0 s
Eject Speed Solvent 2: 500.00 µL/s

**ADD Method "Rinse Syringe - 2500uL Acetone"**

Syringe: 2.5mlALX
Fill Volume: 2500.0 µL

Rinse 1: Wash1
Preclean with Solvent 1: 0

Fill Strokes: 0
Fill Speed: 200.00 µL/s
Add Volume: 2500.0 µL
Add Speed: 500.00 µL/s
Viscosity Delay: 0 s
Eject Speed: 500.00 µL/s
Source Vial Penetration: 47.00 mm
Dest. Vial Penetration: 18.00 mm
Post Add Delay: 0 s
Pressurize: No
Preclean with Sample: 0

Postclean with Solvent 1: 0
Fill Speed Solvent 1: 500.00 µL/s
Viscosity Delay Solvent 1: 0 s
Eject Speed Solvent 1: 500.00 µL/s

Rinse 2: Wash2
Preclean with Solvent 2: 0
Postclean with Solvent 2: 0
Fill Speed Solvent 2: 500.00 µL/s
Viscosity Delay Solvent 2: 0 s
Eject Speed Solvent 2: 500.00 µL/s

**ADD Method "Air Flush Cartridge"**

Syringe: 2.5mlALX
Fill Volume: 2500.0 µL
Fill Strokes: 0
Fill Speed: 700.00 µL/s
Add Volume: 2500.0 µL
Add Speed: 700.00 µL/s
Viscosity Delay: 0 s
Eject Speed: 500.00 µL/s
Source Vial Penetration: 47.00 mm
Dest. Vial Penetration: 10.00 mm
Post Add Delay: 0 s
Pressurize: No
Preclean with Sample: 0

Rinse 1: Wash1
Preclean with Solvent 1: 0
Postclean with Solvent 1: 0
Fill Speed Solvent 1: 500.00 µL/s
Viscosity Delay Solvent 1: 0 s
Eject Speed Solvent 1: 500.00 µL/s

Rinse 2: Wash2
Preclean with Solvent 2: 0
Postclean with Solvent 2: 0
Fill Speed Solvent 2: 500.00 µL/s
Viscosity Delay Solvent 2: 0 s
Eject Speed Solvent 2: 500.00 µL/s

**ADD Method "Aspirate 500 uL DCM"**

Syringe: 2.5mlALX
Fill Volume: 2500.0 µL
Fill Strokes: 0
Fill Speed: 50.00 µL/s
Add Volume: 500.0 µL
Add Speed: 10.00 µL/s
Viscosity Delay: 3 s
Eject Speed: 500.00 µL/s
Source Vial Penetration: 47.00 mm
Dest. Vial Penetration: 32.00 mm
Post Add Delay: 0 s
Pressurize: No
Preclean with Sample: 0

Rinse 1: Wash1
Preclean with Solvent 1: 0
Postclean with Solvent 1: 0
Fill Speed Solvent 1: 500.00 µL/s
Viscosity Delay Solvent 1: 0 s
Eject Speed Solvent 1: 500.00 µL/s

Rinse 2: Wash2
Preclean with Solvent 2: 0
Postclean with Solvent 2: 0
Fill Speed Solvent 2: 500.00 µL/s
Viscosity Delay Solvent 2: 0 s
Eject Speed Solvent 2: 500.00 µL/s

**ADD Method "Elute ITSP - 500 uL DCM"**

Syringe: 2.5mlALX
Fill Volume: 2500.0 µL
Fill Strokes: 0

Rinse 1: Wash1
Preclean with Solvent 1: 0
Postclean with Solvent 1: 0

Fill Speed: 50.00 µL/s
Add Volume: 500.0 µL
Add Speed: 10.00 µL/s
Viscosity Delay: 0 s
Eject Speed: 500.00 µL/s
Source Vial Penetration: 23.00 mm
Dest. Vial Penetration: 18.00 mm
Post Add Delay: 0 s
Pressurize: No
Preclean with Sample: 0

Fill Speed Solvent 1: 500.00 µL/s
Viscosity Delay Solvent 1: 0 s
Eject Speed Solvent 1: 500.00 µL/s

Rinse 2: Wash2
Preclean with Solvent 2: 0
Postclean with Solvent 2: 0
Fill Speed Solvent 2: 500.00 µL/s
Viscosity Delay Solvent 2: 0 s
Eject Speed Solvent 2: 500.00 µL/s
Left MPS Syringe: 2.5mlALX

## AUTOSAMPLER INJECTOR PARAMETERS

### MPS Liquid Injection into GC

Sample Parameters
    Syringe: 10ul
    Sandwich: No Sandwich
    Inj. Volume: 10.0 µL
    Air Volume below: 0.0 µL
    Inj. Speed: 100.00 µL/s
    Fill Volume: 10.0 µL
    Fill Strokes for Sample: 1
    Fill Speed: 2.50 µL/s
    Viscosity Delay: 10 s
    Eject Speed: 20.00 µL/s
    Pre Inj. Delay: 0 s
    Post Inj. Delay: 0 s Inj.
    Penetration: 40.00 mm
    Sample Tray Type: HW-54P
    Vial Penetration: 43.00 mm

Rinse Parameters
    Preclean with Sample: 0

    Rinse 1: Wash1
    Preclean with Solvent 1: 1
    Postclean with Solvent 1: 2
    Fill Speed Solvent 1: 5.00 µL/s
    Viscosity Delay Solvent 1: 0 s
    Eject Speed Solvent 1: 50.00 µL/s

    Rinse 2: Wash2
    Preclean with Solvent 2: 1
    Postclean with Solvent 2: 2
    Fill Speed Solvent 2: 5.00 µL/s
    Viscosity Delay Solvent 2: 2 s
    Eject Speed Solvent 2: 50.00 µL/s

Runtime: 24.00 min

    Cool Down Time: 3.00 min

## GERSTEL MAESTRO Method – LVI_10uL.mth

**NOTE:** This method is only for large-volume injection (10µL) of WICS, solvent and continuous calibration extract.

Sample Parameters
    Syringe: 10µl
    Sandwich: No Sandwich
    Inj. Volume: 10.0 µL
    Air Volume below: 0.0 µL
    Inj. Speed: 100.00 µL/s
    Fill Volume: 10.0 µL
    Fill Strokes for Sample: 1
    Fill Speed: 2.50 µL/s

Rinse Parameters
    Preclean with Sample: 0

    Rinse 1: Wash1
    Preclean with Solvent 1: 1
    Postclean with Solvent 1: 2
    Fill Speed Solvent 1: 5.00 µL/s
    Viscosity Delay Solvent 1: 0 s
    Eject Speed Solvent 1: 50.00 µL/s

Viscosity Delay: 7 s
Eject Speed: 20.00 µL/s                      Rinse 2: Wash2
Pre Inj. Delay: 0 s                           Preclean with Solvent 2: 1
Post Inj. Delay: 0 s                          Postclean with Solvent 2: 2
Inj. Penetration: 40.00 mm                    Fill Speed Solvent 2: 5.00 µL/s
Sample Tray Type: VT98                        Viscosity Delay Solvent 2: 2 s
Vial Penetration: 32.00 mm                    Eject Speed Solvent 2: 50.00 µL/s

Runtime: 24.00 min
Cool Down Time: 3.00 min