ROHM AND HAAS BIOCIDES

TECHNICAL NOTE

KATHON™ Microbicide

High Performance Liquid Chromatography Test Method for the Analysis of Percent Level Concentrations of KATHON™ Microbicide Formulations

1.5% Formulation

Scope:

This method is intended for the determination of percent level concentrations of methylisothiazolone (MI or RH-573) and methylchloroisothiazolone (MCI or RH-651) in KATHON microbicide. Samples are analysed by reverse phase High Performance Liquid Chromatography (HPLC) using ultraviolet (UV) detection at 254 nanometers. Concentrations are estimated by an internal standard, single point calibration method.

Safety:

KATHON microbicide as supplied is corrosive and a skin sensitizer and must be handled with extreme care. Safety glasses, chemical impervious gloves and a lab coat must be worn when handling KATHON microbicide.

Methanol is a toxic, flammable solvent.

Please consult the appropriate Material Safety Data Sheets for more detailed safety information.

References:

1. CIS Dept. Test Method # 89-03-03
2. CIS Dept. Report # GLP-2001-006, "GLP Report on Validation of CIS Test Method # 89-03-03 for the Analysis of KATHON Microbicide Formulations for Active Ingredients Under Protocol # GLP 24P-2000-026", D. Doshi, February 15, 2001.

Apparatus

Liquid Chromatograph	Spectra SystemP4000 HPLC HPLC Quaternary Pump or equivalent
Detector	UV 2000 UV/Vis Detector or equivalent
Autosampler	Spectra System AS3000 autosampler or equivalent
Analytical Column:	Sphericlone ODS-1 150mm X 4.6 mm i.d., 5 micron, Phenomenex Inc. Catalog # 00F-4143-E0 or equivalent
Guard Column:	NewGuard RP-18, 7 micron,15mm X 3.2m Rainin Part No. G18-013 or equivalent (Note: Use of guard column is optional)
Repipet	Brinkman Dispensette with 20 ml capacity or equivalent

Reagents

Methanol	HPLC Grade, J. T. Baker Co. or equivalent
Water	Milli-Q™ Deionized HPLC grade Water, Millipore Corporation, or equivalent.
Analytical Standard	Characterized Lot of KATHON Microbicide, Rohm and Haas Company
Internal Standard	Dimethyl Phthalate (DMP) 99% + purity, Sigma Aldrich or equivalent.

HPLC Chromatographic Parameters:

Column Temperature	Ambient
Flow Rate	1.7 milliliter/minute
Mobile Phase	50% HPLC Water/50% Methanol, Isocratic
Initial back pressure	2900 psi (207 bars)
Detection Wavelength	254 nanometers
Detector Sensitivity	1.0 AUFS (Absorbance Unit Full Scale)
Injection Volume	5 microliters
Retention Times:	MI approximately 1.5 minutes MCI approximately 2.5 minutes DMP approximately 3 minutes

Note: The retention times will vary depending on the instrument, type and age of column used.

Procedure:

I. Preparation of Internal Standard Prep Solution

Prepare 500 parts per million (ppm) w/v dimethyl phthalate (DMP) internal standard (ISTD) solution in 50/50 v/v water/methanol. Transfer the solution to an appropriate reservoir equipped with a repipet. Inject the ISTD solution to look for interfering components. If interfering components are present then a different batch of DMP or solvent should be used.

II. Preparation of Working Standards:

Analytical standards are refrigerated when not in use. Remove the required working standard and allow it to come to room temperature before use. Shake to mix the standard prior to use.

Weigh 200 milligrams (± 1.0 mg) of KATHON microbicide analytical standard into a one oz. vial. Using a volumetric pipet, add 20 mls DMP internal standard solution into the vial. This results in a 0.015 percent a.i. weight/volume stock standard. This standard is stable at least one week when refrigerated at 4°

III. Control sample

Prepare duplicate stock standard (0.015% a.i.) solutions following the instructions mentioned previously. This standard is then prepared and analyzed as a sample with every analysis to check on sample preparation and quantitation. If the results of the control samples do not agree with the label values, then a thorough investigation of the analytical procedure should be conducted. This may involve preparation and reanalysis of standards and samples.

IV. Sample Analysis

Weigh 200 milligrams (± 1.0 mg) of KATHON microbicide analytical standard into a one oz. vial. Using a volumetric pipet, add 20 mls DMP internal standard solution into the vial. This results in a 0.015 percent a.i. weight/volume stock standard. This standard is stable at least one week when refrigerated at 4°

Instrument Calibration

Once linearity has been established a single point, internal standard calibration is adequate for the analysis The standard should be injected at least twice, and over a long run the standard should be injected every six to eight samples. Use peak height or area as appropriate

Calculation:

Calculations can be performed by chromoatographic software using a single point internal standard calibration method. For manual calculations, the following formula may be used:

1. Response Factor (RF) = A1 xW1 / W2 x A2

   Where, A1= Area of component peak in the standard.
   W1= Weight of the ISTD in 20 ml of ISTD solution (nominally applied as 1 for simplicity as the weight of the ISTD is constant, provided the same ISTD preparation is used throughout the run.).
   A2= Area of ISTD peak
   W2= Weight of component in the standard in milligrams.

2. Weight % Component = A3 x W3 x 100 / A4 x RF x W4

   Where, A3= Area of component peak in the sample.
   W3= Weight of ISTD in ISTD solution (see above).
   A4= Area of ISTD peak in the sample.
   W4= weight of the sample in milligrams.
   RF= Response Factor of component (see above).

Method Validation:

The method validation was conducted following US EPA GLP (Good Laboratory Practice) protocols (Ref. 1). Details are given below.

1) Linearity Detector response is linear for both MI and MCI for the concentration ranges of interest. (Figures 1 and 2). Correlation coefficients for both components are better than 0.999 with negligible intercept.

2) Precision Samples were analysed by duplicate analysts on different days in different laboratories. Each analyst prepared each sample four times and each preparation was injected four times resulting in sixteen determinations for each sample. Precision of the test method is 0.8% and 0.6% relative standard deviation for MI and MCI, respectively.

Chromatogram of Internal Standard:

500 ppm Internal Standard (ISTD) Dimethyl Phthalate in 50/50 water/methanol

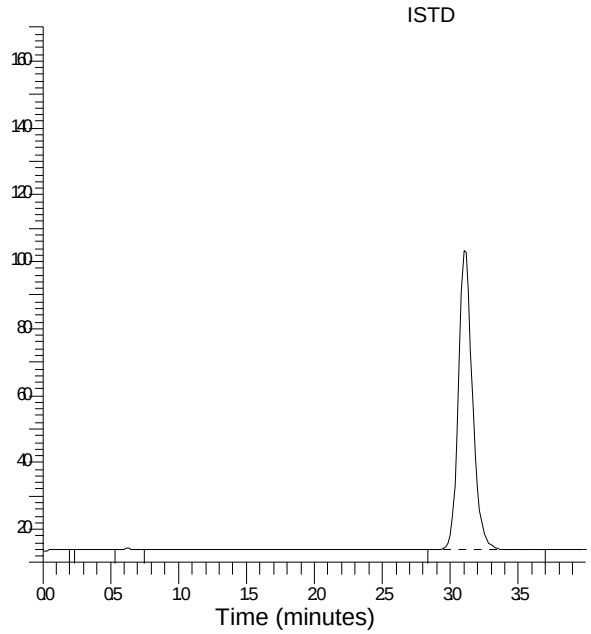


Figure 1: 500 ppm Internal Standard (ISTD) Dimethyl Phthalate in 50/50 water/methanol. (ISTD Blank)

Chromatogram of Calibration Standard:

206.35 mgs KATHON™ 1.5% formulation in 20 ml Internal Standard Solution

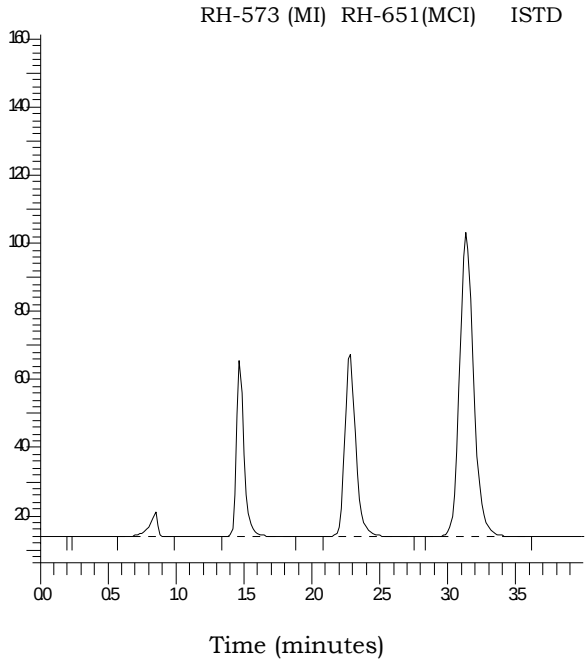
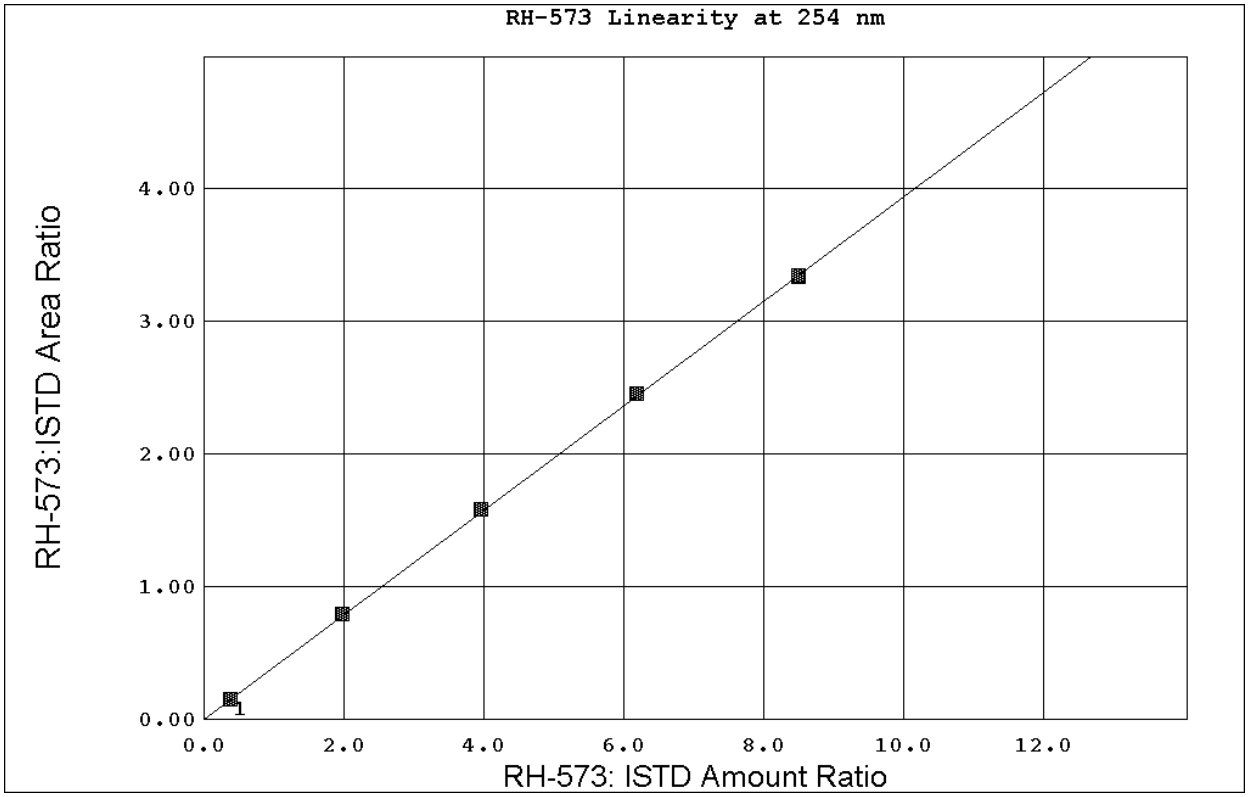
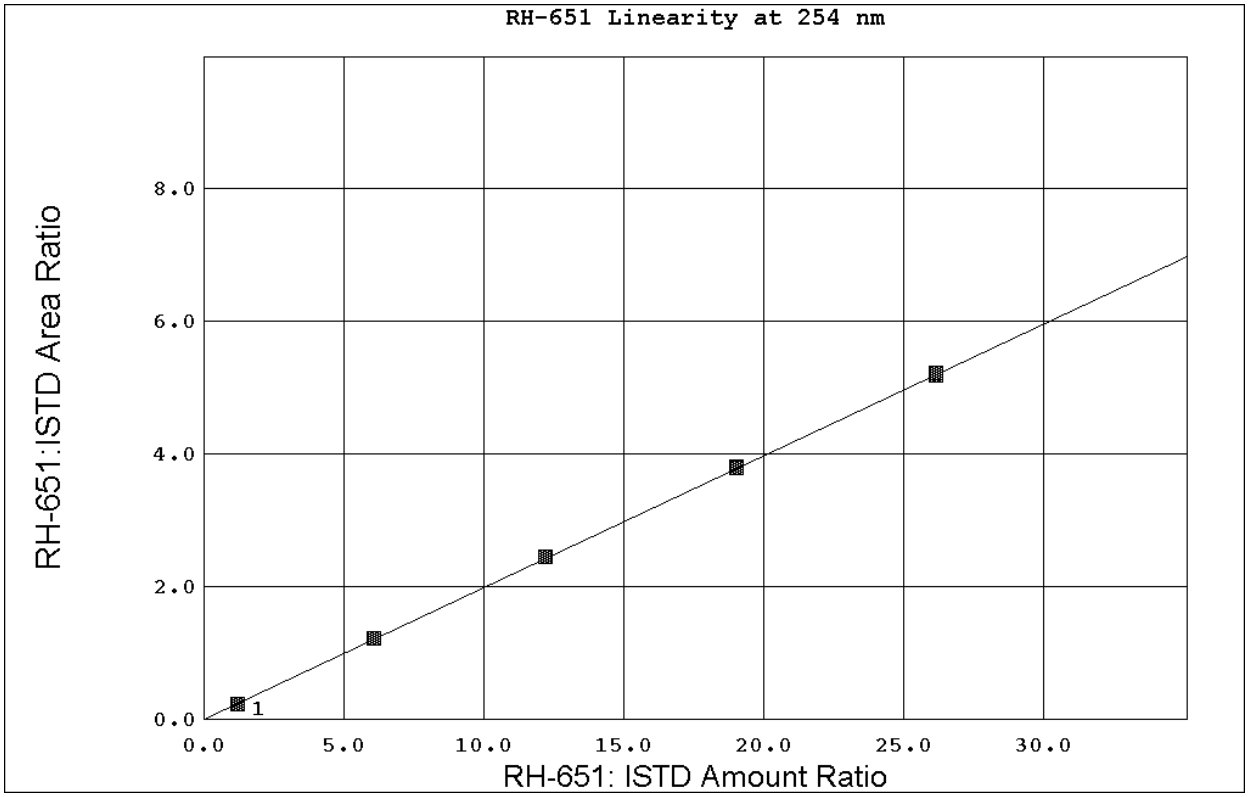


Fig 1: Linearity of detector response for MI (RH-573) at 254 nanometers.



Level	573:ISTD Amount Ratio	573:ISTD Area Ratio
1	8.4898	3.351574
2	6.1806	2.46245
3	3.9645	1.593987
4	1.9822	0.8012825
5	0.3964	0.162039
Linearity Parameters: Ist Order, R Squared = 0.999910 Calibration Curve: Y = (0.017671) + (0.394174) x		

Fig 2: Linearity of detector response for MCI (RH-651) at 254 nanometers.



Level	651:ISTD Amount Ratio	651:ISTD Area Ratio
1	26.1148	5.21269
2	19.0117	3.81855
3	12.1948	2.47078
4	6.0974	1.24166
5	1.2195	0.25076
Linearity Parameters: Ist Order, R Squared = 0.999930 Calibration Curve: Y = (0.022912) + (0.199261) x		