

Determination of ppm Levels of ProClin® 300 Preservatives in Water-Dilutable Samples by HPLC

ProClin preservatives are highly effective biocides for the control of microorganisms in reagents and products intended for in vitro diagnostic use. At unusually low concentrations, ProClin preservatives eradicate bacteria, fungi, and reagents for prolonged periods, thereby increasing a product's shelf life. Further, ProClin 300 does not affect the functionality of most enzyme- or antibody-linked reactions, and will not interfere with assay indicators. This bulletin describes the analysis of water-dilutable samples for ppm levels of ProClin 150 and ProClin 300 preservatives. The active ingredients are: 5-chloro-2-methyl-4-isothiazolin-3-one (RH-651) and 2-methyl-4-isothiazolin-3-one (RH-573). Samples are analyzed by reverse-phase HPLC using UV detection at 275 nanometers.

Key Words:

● assay ● HPLC ● preservative ● standard

Safety

Isothiazolones are skin sensitizers and are corrosive. Handle with extreme care. Wear safety glasses, goggles, gloves, and a laboratory coat when handling standards and samples containing over 50ppm active ingredient.

Good laboratory practices should be used to reduce exposure to the extent feasible. Repeated or continual dermal contact with solutions containing >15ppm a.i. could result in allergic skin reactions in susceptible individuals.

Methanol is flammable and toxic. Consult MSDS of methanol and ProClin 150 and ProClin 300 preservative.

Apparatus

Liquid Chromatography (HPLC): Varian 5500 HPLC or equivalent with an autosampler and a 25µL sample loop.

Detector: Kratos Spectroflow 783 variable wavelength UV detector or equivalent.

Analytical Column: Spherisorb® ODS-1, 5µm, 25cm x 4.6mm ID. (Supelco equivalent: SUPELCOSIL™ LC-18, Cat. No. 5-8298.)

Guard Column: Spheri-5, RP18, 30mm x 4.6mm, 5µm. (Supelco equivalent: Supelguard™ guard column LC-18, Cat. No. 5-9564, pk. of 2)

Sample Filters: Millipore® 0.45µm Millex® HV filter units. (Supelco Cat. No. Z22,736-6.)

Repipet: Labindustries repipet capable of delivering up to 20mL of solution.

Syringes: Becton Dickinson 3cc disposable syringes. Fisher Cat. No. 14-823-38.

Reagents

Methanol: HPLC grade.

Water: Deionized HPLC grade.

Working Standard: Characterized lot of ProClin 150 preservative. The working standard is stable for at least 1 year if stored under refrigeration. The current working standard can be obtained from Supelco (Cat. No. 3-3360).

Chromatography Parameters

Column: Ambient

Temperature: Ambient

Flow Rate: 1.3mL/min

Column Back Pressure: Approximately 3500psi

Solvents: A: HPLC-grade water
B: HPLC-grade methanol

Solvent Program — I[■] (Isocratic):

| Time (min) | %A (water) | %B (methanol) |
|------------|------------|---------------|
| 0 | 50 | 50 |
| 8 | 50 | 50 |

■ If RH-573 or RH-651 cannot be resolved from other components present in sample by the above solvent program, then the following program, Solvent Program — II, may be used.

Solvent Program — II (Gradient):

| Time (min) | %A (water) | %B (methanol) |
|------------|------------|---------------|
| 0 | 75 | 25 |
| 20 | 75 | 25 |
| 22 | 0 | 100 |
| 28 | 0 | 100 |
| 29 | 75 | 25 |
| 35 | 75 | 25 |

Wavelength: 275nm
 Injection Volume: 25µL
 Retention Times: For program I:
 RH-573 — 3.2min
 RH-651 — 4.8min
 For program II:
 RH-573 — 5.8min
 RH-651 — 14.7min

Procedure

Prep Solvent

HPLC-grade water. Fill a 1-quart reservoir equipped with a 20mL repipet with water. Inject 25µL of this water to check for interfering components.

Preparation of Standards

Stock Solution

Weight 330mg ± 0.01mg of ProClin 150 preservative standard (1.5% Al) into a 1-ounce screw cap vial. Pipet 10mL of HPLC grade water into the vial and shake. This will yield approximately a 500ppm standard solution. Calculate actual ppm ProClin 150 preservative in water.

Working Standard

Pipet 1mL of 500ppm standard solution into a 1-ounce screw cap vial. Pipet 9mL of HPLC-grade water into the vial and shake. This will yield a 50ppm solution. Make serial dilutions to prepare 20, 10, 5, and 1ppm aqueous standards.

Use these standards to calibrate the instrument. Refrigerate standards; they are stable for at least 8 weeks.

Preparation of Sample

Usually samples treated with ProClin preservatives are chromatographically clean and do not require further dilution. Transfer sample to 3cc disposable syringe to which a 0.45µm filter unit has been affixed. Filter solution into an autosampler vial and cap with a Teflon®-faced septum.

If sample is viscous and contains high amounts of organic impurities, it should be diluted as follows: Weigh 0.8 - 1.2g ± 0.005g of sample into a 1oz screw cap vial. Dilute with 9mL of HPLC-grade water and shake. Follow the same filtering procedure as above.

Note: Always analyze control sample (sample without ProClin preservative) to determine presence of co-eluting interference.

Calibration

Depending on the expected concentration, inject in duplicate a 1, 5 or 10ppm standard. For long automated runs, inject standard after every five samples.

Calibrate the instrument with these standards using a single point or linear calibration mode using peak height or area as appropriate.

Calculation

Concentration (ppm) of active ingredient in sample = $A \times B \times Y / C$

A = Peak height of component in sample

B = ppm component in standard

C = Peak height of component in standard

Y = Dilution factor (y=1, if samples are injected neat without dilution)

Limit of Detection

The limit of detection for RH-573 is 0.2ppm, and for RH-651 it is 0.8ppm in samples. Report both results to 1 decimal place.

Precision

Precision of this HPLC procedure is ±0.5ppm at 25ppm active ingredient.

Figure A. 3ppm ProClin 150 Standard

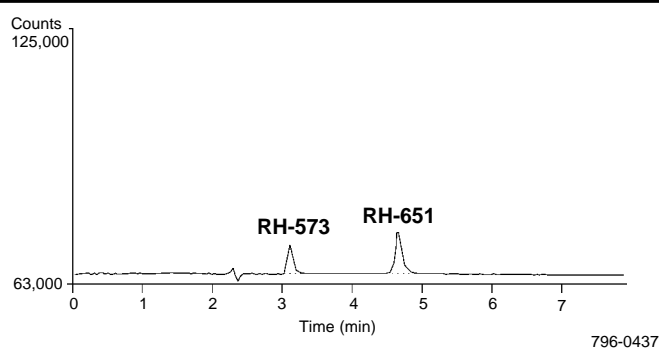


Figure B. 10ppm ProClin 150 Standard

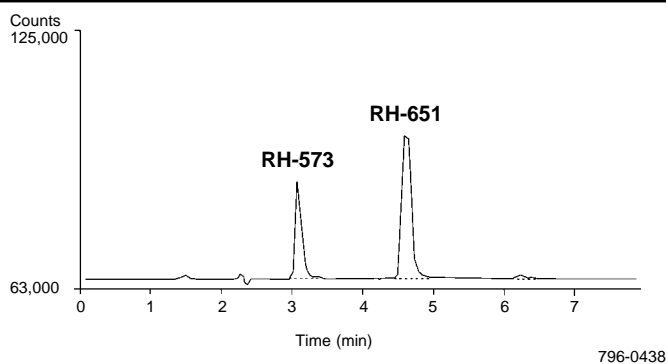


Figure C. Horseradish Peroxidase (HRP) Untreated

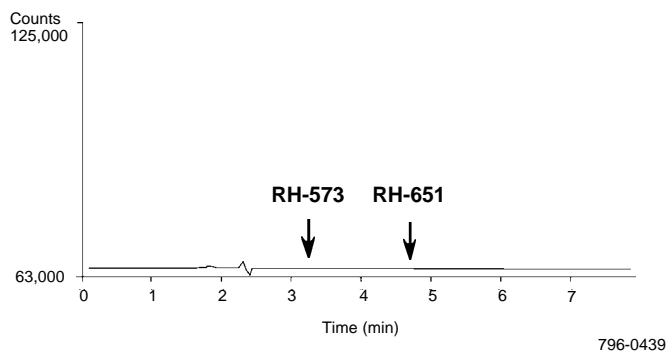


Figure D. 100 Units/mL HRP + 17.2ppm ProClin 300

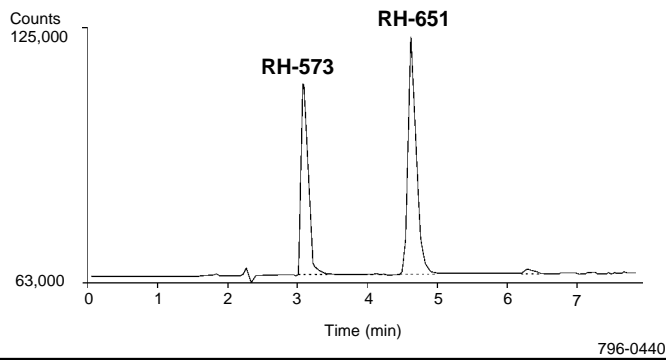


Figure H. 50mM Hepes (pH 7.2) + 18.0ppm ProClin 300

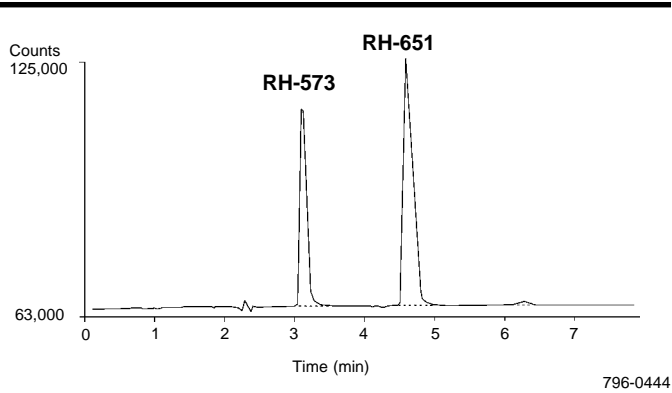


Figure E. Alkaline Phosphatase (AP) Untreated

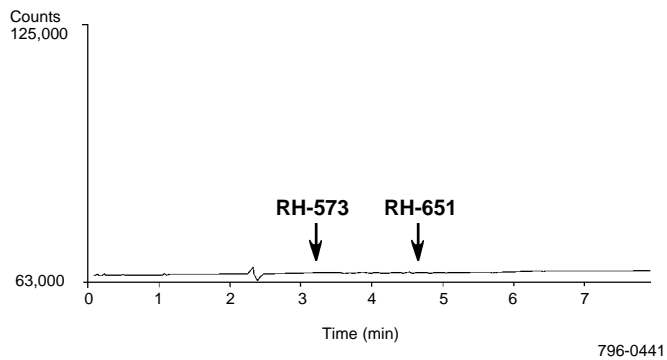


Figure I. Tris Untreated

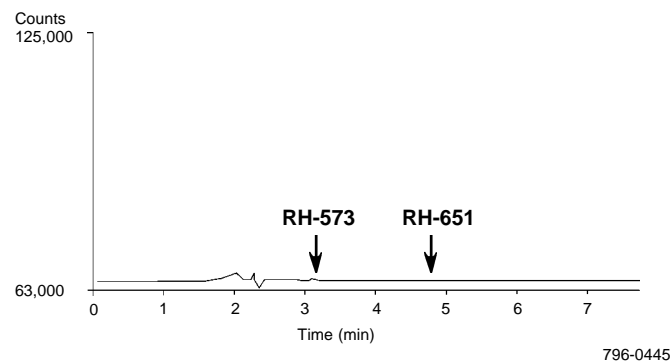


Figure F. 20 Units/mL AP + 16.2ppm ProClin 300

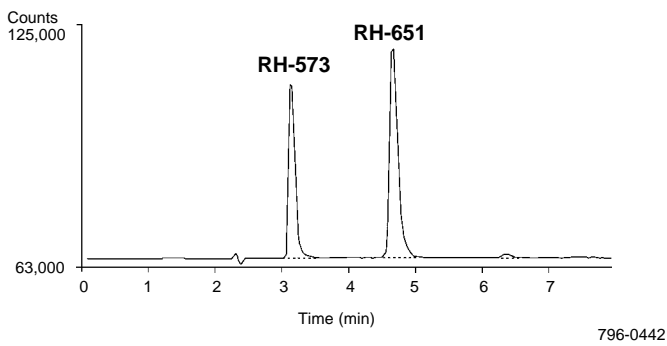


Figure J. 50mM Tris (pH 7.2) + 18.0ppm ProClin 300

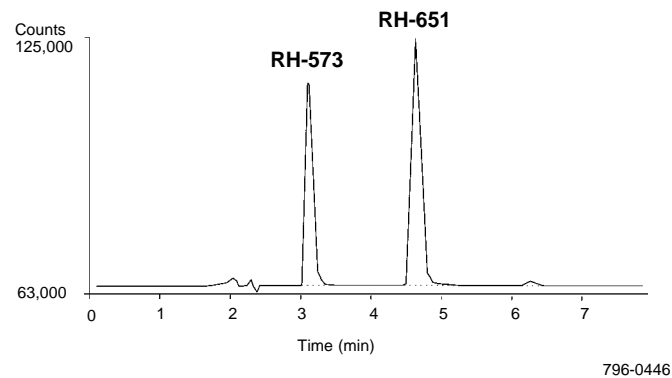
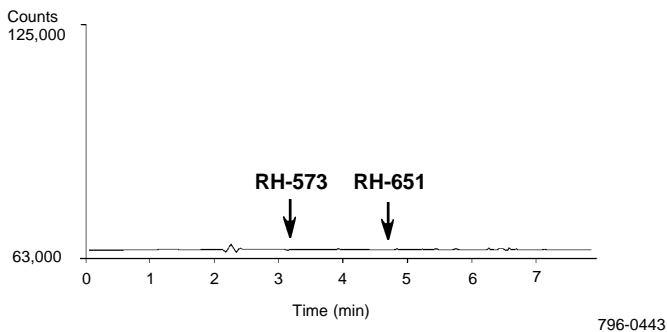


Figure G. Hepes Untreated



Safety and Handling

Handle Carefully: ProClin preservatives are corrosive to the eyes and skin and can cause skin burns and irreversible eye damage. May cause allergic skin reactions. Harmful if inhaled. ProClin preservatives are harmful if absorbed through the skin; large exposures may be fatal. May be fatal if swallowed.

Supelco maintains material safety data sheets (MSDS) on ProClin preservatives. These sheets contain important information that you may need to protect your employees and customers from any known health or safety hazards associated with the products.

We recommend that you obtain copies of MSDS from us before using the ProClin preservatives in your facilities. We also suggest that you contact suppliers of materials recommended for use with our products for appropriate health and safety information.

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