

Instructions for G1969-85001 ES-TOF Reference Mass Solution Kit

Contents

This kit contains two ampoules each (2.2 mL/ampoule) of the following solutions:

- 100 mM (millimolar) ammonium trifluoroacetate in 90:10 acetonitrile:water
This solution is abbreviated 100 mM TFANH₄.
- 5 mM (millimolar) purine in 90:10 acetonitrile:water
- 2.5 mM (millimolar) hexakis(1H, 1H, 3H-tetrafluoropropoxy)phosphazine in 90:10 acetonitrile:water. This solution is abbreviated 2.5 mM HP-0921.

One kit of ES-TOF Reference Mass Solution is expected to prepare several liters of ES-Reference Mass Solution. This amount of ES reference mass solution would be expected to provide over two months of continuous operation. Consumption is based upon a solution prepared in 95:5 acetonitrile:water delivered through the calibrant delivery system (CDS) reference channel "A" at an average flow rate of 45 ± 10 μ L/min.

Use of ES-TOF Reference Mass Solution

Use of this internal reference mass solution allows users to obtain accurate mass time-of-flight data. The actual concentrations of the mass reference compounds in the solution prepared by the user will depend upon several instrument operating parameters:

- LC gradient or isocratic operation
- LC flow rate, mobile phases and modifiers
- MS source settings including fragmentor and octopole RF voltages

All of the above may affect the reference mass signal response, however, some guidelines have been established for normal operation. If samples contain compounds that are relatively easy to ionize under electrospray conditions (either positive or negative mode), then no LC modifiers are required. The best sensitivity for reference masses will generally occur when LC mobile phase modifiers are not present or are at low levels. Table 1. below provides some suggested concentrations for several LC mobile phase selections. The solutions provide internal reference mass correction for both positive and negative operation.

The concentrations listed (aliquots in parentheses) are based upon a one liter preparation of ES-TOF Reference Mass Solution *in 95:5 acetonitrile:water*.

Table 1. Typical Concentrations of ES Reference Compounds

LC Mobile Phase / Modifiers	TFANH ₄	Purine	HP-0921
A: Water B: Acetonitrile	25 μ M (0.25 mL)	1.0 μ M (0.2 mL)	0.25 μ M (0.1 mL)
A: Water w/ 0.1% formic acid B: Acetonitrile w/ 0.1% formic acid	100 μ M (1.0 mL)	10 μ M (2.0 mL)	2.0 μ M (0.8 mL)
A: Water w/ 5 mM ammonium formate B: Methanol w/ 5 mM ammonium formate	100 μ M (1.0 mL)	10 μ M (2.0 mL)	2.0 μ M (0.8 mL)

Preparation Guidelines

Protective gloves should be worn when preparing the ES-TOF Reference Mass Solution. Prior to breaking open an ampoule, invert the ampoule several times to mix. Inspect the ampoule's contents to ensure that all the solution is contained in the lower cylindrical base. Shake the ampoule, if necessary, to dislodge any air pocket which prevents solution from settling in the lower portion of the ampoule.

- "Acetonitrile UV" B&J Brand High Purity Solvent for HPLC, Gas Chromatography, Pesticide Residue Analysis and Spectrophotometry. (Material number: 10071618, Cat. number 015-4).
- "Nanopure" D.I. Water (18 megaohm-cm, organic-free)

Add aliquots of the individual calibrants (listed in Table 1.) to a one-liter Nalgene volumetric flask according to one of the LC mobile phase selections, Fill flask to the calibration mark with 95:5 acetonitrile:water. Cap and invert flask several times to mix. Transfer 100 mL of this solution to CDS Reference Bottle "A."

Table 2. ES-TOF Reference Masses

Species	Positive Ion (m/z)	Negative Ion (m/z)	Formula Wt.	Molecular Formula
CF3 (TFA fragment)		68.995758		C F3
TFA anion		112.985587	131.06	C2 O2 F3 (N H4)
Purine	121.050873	119.036320	120.11	C5 H4 N4
HP-0921	922.009798	1033.988109	921.24	C18 H18 O6 N3 P3 F24
HP-0921 (+ formate)		966.000725		
HP-0921 (+ acetate)		980.016375		

Note: Reference mass responses will vary depending upon LC mobile phases, use of modifiers, LC gradient conditions and MS source settings including fragmentor & octopole RF voltage. A minimum reference mass signal abundance of several thousand counts and maximum abundance of several hundred thousand counts will provide accurate reference mass corrections.

If LC mobile phase modifiers are present (e.g. Na⁺, K⁺, acetate, formate), competition may result in multiple molecular species attenuating the reference mass response. Principal ions are in shaded cells above. ES-TOF fragmentor settings > 250V will result in extensive fragmentation of reference mass ions.

The data acquisition mass range should be set wide enough to include all of the reference masses. For small molecule analysis, this range is typically m/z 50 - 1000 for positive mode and m/z 50 - 1100 for negative mode (note m/z 1034 is the TFA adduct of HP-0921).