

Trace Organic and Inorganic Materials Detection and Analysis in Pipette Tips

A number of studies have indicated that inclusion of contaminating trace metals and trace organic compounds can have a negative impact on the expected final results of an experiment. To confirm that tips manufactured by Rainin do not provide sufficient quantities of contaminating inhibitors, a number of experiments were performed by independent external analysts the results of which are shown below.

Materials and methods

Trace metal analysis: A random selection of newly manufactured and packaged tips was removed from the stock inventory for testing, these included 10, 250, 300 and 1000 μL volumes. All experiments were performed under a HEPA filtered clean hood.

All other vessels used in the experiment were cleaned with concentrated nitric acid to ensure no cross contamination occurred. For the washing of tips, Optima grade nitric acid (Fisher Scientific) with trace metal amounts less than 100 ppt, was used.

Test tips were cycled with concentrated nitric acid as follows: maximum volume for each tip was aspirated with the appropriate pipette and held in position in the tip for 5 minutes. The acid was dispensed into a holding vessel and a new tip placed on the pipette. The same acid was now aspirated and held for 5 minutes for each new tip tested and the final product, the leachate, was used to quantitatively determine the amounts of trace metals washed from the tips. A total of 10 different tips was used for each volume tested. Linearity standards, diluted from commercial High Purity Standards were prepared for the ICP-MS calibration and the instrument was calibrated using a five-point curve (0, 2, 5, 10 and 20 ppb).

The leachate from the tips was diluted with ultrapure deionized water to an acidity of 3% before injection into the VG Axiom high resolution ICP-MS system.

Trace organics determination: A random selection of newly manufactured and packaged 1000 μL volume tips was removed from the stock inventory for testing by two methods:

1) GC-MS: Samples were prepared by rinsing 15 pipet tips/sample with 1.5 mL of a 3:1 solution of methanol and toluene, respectively. Multiple sample tips were rinsed with the same small volume of solvent (i.e., 0.75 mL) in order to concentrate the quantity of oleamide in solution.

GC-MS experimental conditions: An aliquot of the sample rinse was analyzed on an Agilent 6890 GC-MS in selective ion monitoring (SIM) mode using a 5973 mass selective detector (MSD). SIM was performed using ions 59, 72 and 281. A ZB-5 MS with 5 m guardian column (30 m x 0.25 mm x 0.25 μm film) was used to obtain the necessary chromatographic separation.

2) LC-MS: Samples were prepared in the same manner as for GC-MS by rinsing 15 pipet tips/sample, but with 1.5 mL of a 50% HPLC grade water/50% acetonitrile (0.2% formic acid) solution as the extraction solvent.

LC-MS Experimental Conditions: The samples and blank were injected into an Agilent 1100 Series HPLC system which was equipped with a LC/MSD Trap mass spectrometer (ion trap). The mass spectrometer utilized electrospray ionization (ESI) as the ionization source for sample injection. Separations were achieved using a 30 x 4.6 mm Cadenza CD-C18 column with 3- μm particles using water and acetonitrile as the eluents. The analyses were conducted with the positive mode of ionization and a 50 μL injection.

Results

Trace metal analysis

For the tips tested, the typical results in ng/μL were as follows:

Element	Al	Cr	Mn	Fe	Ni	Cu	Zn	Cd	Hg	Pb
Typical values (ng/μL)	<0.003	<0.00005	<0.00005	<0.0007	<0.0002	<0.00015	<0.001	<0.00002	<0.00004	<0.000025

These results indicate that even under the harsh conditions of the experiment, the amount of trace metal that can leach from the tip should not inhibit or affect any experimental outcome.

Trace organics analysis

Results from the GC-MS experiment to detect potential contamination by the releasing agent oleamide are seen in Figure 1, comparing a standard with the washate from Rainin tips.

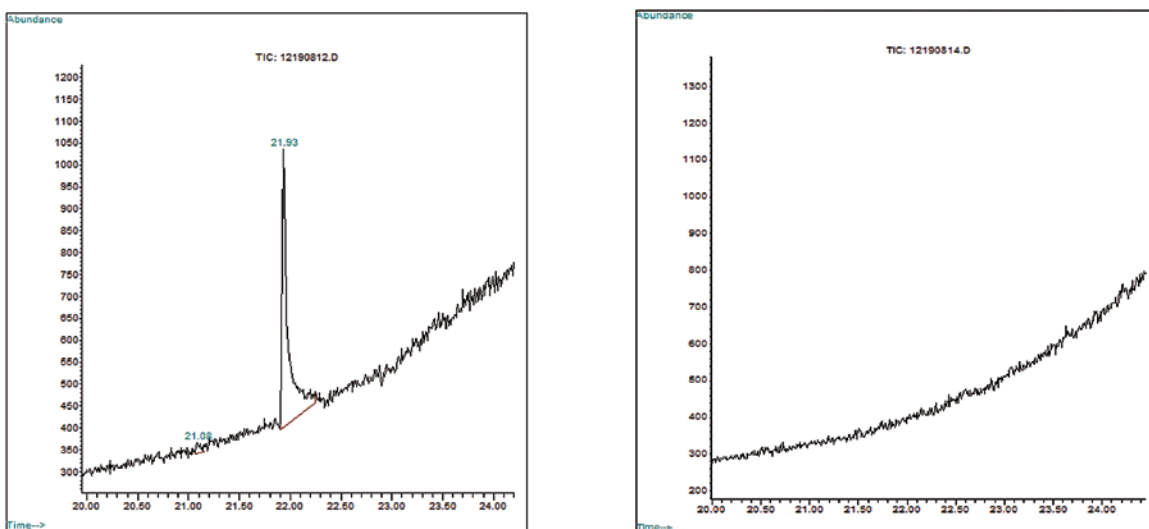


Figure 1. Limit of detection (LOD) calculation using a 10.8 μg/mL oleamide standard (GC-MS, SIM mode), prepared in 3:1 methanol/toluene mixture – left. At right, GC-MS chromatogram (SIM mode) of sample RT-L1000LR pipet tips indicating zero level of contaminating Oleamide releasing reagent. Results from the LC-MS experiment were similar to those for the GC-MS, indicating zero levels of contamination from the detergent DiHEMDA.

Conclusions

The experiments conducted by independent external analysts indicate that tips manufactured by Rainin do not include trace organics such as oleamide and DiHEMDA in sufficient quantities to act as contaminating inhibitors. Separately, trace metal analysis indicates that potentially inhibiting elements are in sufficiently low quantities so as not to influence experimental results.