

How clean are your vials and closures?

L. Pereira¹, T. Edge¹, L. Shick², M. Slade²

National Scientific Mass Spec Certified Vials

Industry's **first and only** pre-cleaned, low particle, low background chromatography vial

When your instrumentation, sample handling and methodology is pushing the limits, a chromatography vial that can keep up is essential.

- The only chromatography vials pre-cleaned to provide unmatched consistency
- The first low particle, low background chromatography vials
- Pre-cleaned vial packaging protects the product integrity
- High purity closures packed in air-tight re-closeable container
- Tested and certified for 13 critical physical characteristics affecting vial performance
- Tested and certified for low background by positive ESI LC/MS
- Tested and certified for low background by GC/MS

Low Particle Background

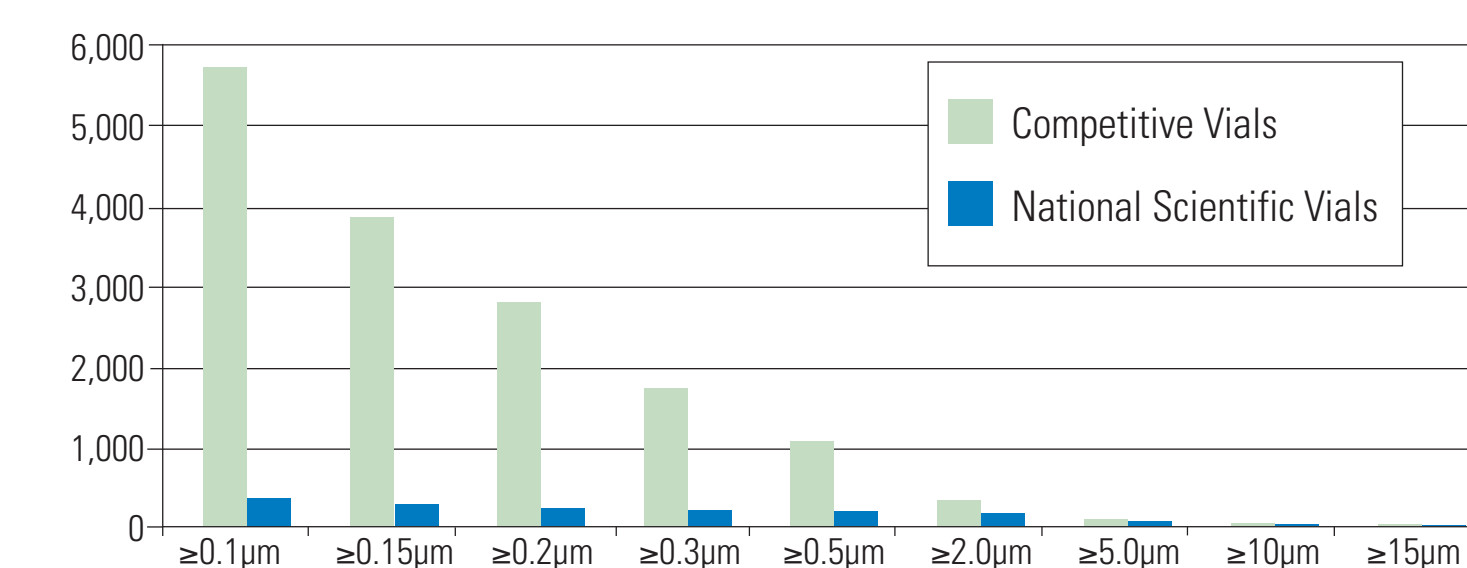
The presence of inorganic sub micron particles in all glass vials as a byproduct of the manufacturing process is a little known phenomenon that has not been extensively studied. Gas chromatographers depend on injection port liners to act as traps for particulates while the HPLC chromatographer takes extensive steps to eliminate them during sample preparation. This has been an effective strategy for routine analytical methods, but the need to work with ever lower concentrations of analytes creates the possibility of interactions with compounds of interest.

National Scientific Mass Spec Vials undergo a proprietary cleaning process that greatly reduces the background particulates and their potential affect on high sensitivity chromatography. The table below gives a comparison of the particle distribution obtained from an analysis of standard vials versus the National Scientific Mass Spec Vials. All National Scientific Mass Spec Vials are processed and tested for background particulates.

A typical vial that has not been processed can exhibit particle counts exceeding 5000 particles per mL with the highest counts occurring in the range below 0.5µm. This has traditionally been of little concern when GC inlet liners or HPLC guard columns are used. GC techniques employing on-column injection create the need for a sample vial with minimal background particulates to prevent an accumulation of foreign material at the head of the column than might adversely affect a separation. Similarly newer techniques employing finely packed HPLC columns, capillary columns and direct connection of the analytical column to the sample valve also require the elimination of as much particulate matter as possible from the sample stream.

The table below shows the results obtained from particulate analysis of a typical unprocessed vial compared to the National Scientific Certified Mass Spec vials. The processed vial shows a significant reduction in total particle counts.

Typical Cumulative Particle Counts



Abstract

Purpose: The evaluation of a new vial and closure for reducing interferences.

Methods: Vials and closures were exposed to acetonitrile for 2 hours and subsequently analysed by LC/UV, LC/MS and GC/MS to characterise the interferences. Comparisons were made between pre-cleaned MS Certified vials and closures, a control and a competitor vial and closure set.

Introduction

Improvements in chromatographic techniques, instrumentation and sample handling continue to push the limits of detection in trace analysis. As such, the cleanliness of the total workflow process becomes even more important to reduce the potential for interferences and contamination that can ultimately reduce the sensitivity of the assay. The selection of the correct auto-sampler vial and closure is thus an important consideration. Vials that are not effectively cleaned can introduce particulate matter that can cause blockages and accumulation of foreign material at the head of the separation column affecting chromatographic performance. Additionally, residual organic compounds that might survive the glass forming process or that leach from the closure when exposed to the sample solvent can reduce the analysis sensitivity.

The work presented in this poster evaluates the performance of the new MS certified vial and an ultra high pure bonded PTFE/silicone closure compared with a control and a competitor vial and closure set.

Materials & Methods

LC/MS and LC/UV

Instrument: Surveyor with LCQ Deca XP
 Column: Hypersil GOLD 3µm, 50x2.1mm
 Mobile phase: A – H₂O + 0.1% formic acid; B – MeOH + 0.1% formic acid
 Gradient:

Time (min)	%B
0	10
10	50
20	100
25	100
25.01	10
30	10

Flow rate: 0.3 mL/min
 Temperature: 40° C
 Injection vol.: 10 µL (2 µL for system suitability test)
 UV: 190 to 500nm
 MS: Full scan 50 to 1500 amu

GC/MS

Instrument: DSQII DurabriteXL GC/MS with Triplus autosampler
 Column: TRACE TR-5MS, 30m x 0.25mm x 0.25µm
 Carrier gas: Helium
 Flow rate: 1.2 mL/min
 Oven program: 40° C, hold for 0.5min; 15° C to 150° C, hold for 1min; 10° C to 290° C, hold for 5 min
 Inlet temperature: 250° C; Split flow: 50 mL/min
 Injection vol.: 1 µL splitless
 MS transfer line: 290° C
 MS ion source: 230° C
 MS detection: EI; Full scan 50 to 650 amu

Sample Preparation

- Vials were filled with 1.0mL of acetonitrile (LC/MS grade).
- The filled vials were capped and the vial+closure incubated at room temperature in the upright position and in the inverted position (in duplicate) for 2 hours.

System Suitability Tests (SST)

- LC/MS –5 repeated injections of reserpine solution at 10ng/µL in water.
- GC/MS –5 repeated injections of phenanthrene at 1ng/µL in acetonitrile.

SST's were injected before each set of injections for each vial+closure type.

Control

The control (or blank) was generated by rinsing a vial (with no cap) twice with injection solvent (acetonitrile). 1 mL of acetonitrile was measured into the vial and vial with no closure was placed in the autosampler tray for analysis.

Results

Figure 1 illustrates the UV traces for a control, a vial with PTFE/silicone closure and a vial + closure with pre-slit, incubated in the inverted position at room temperature. The three traces are identical, demonstrating that in LC/UV it is not feasible to see the interferences using this detector technology.

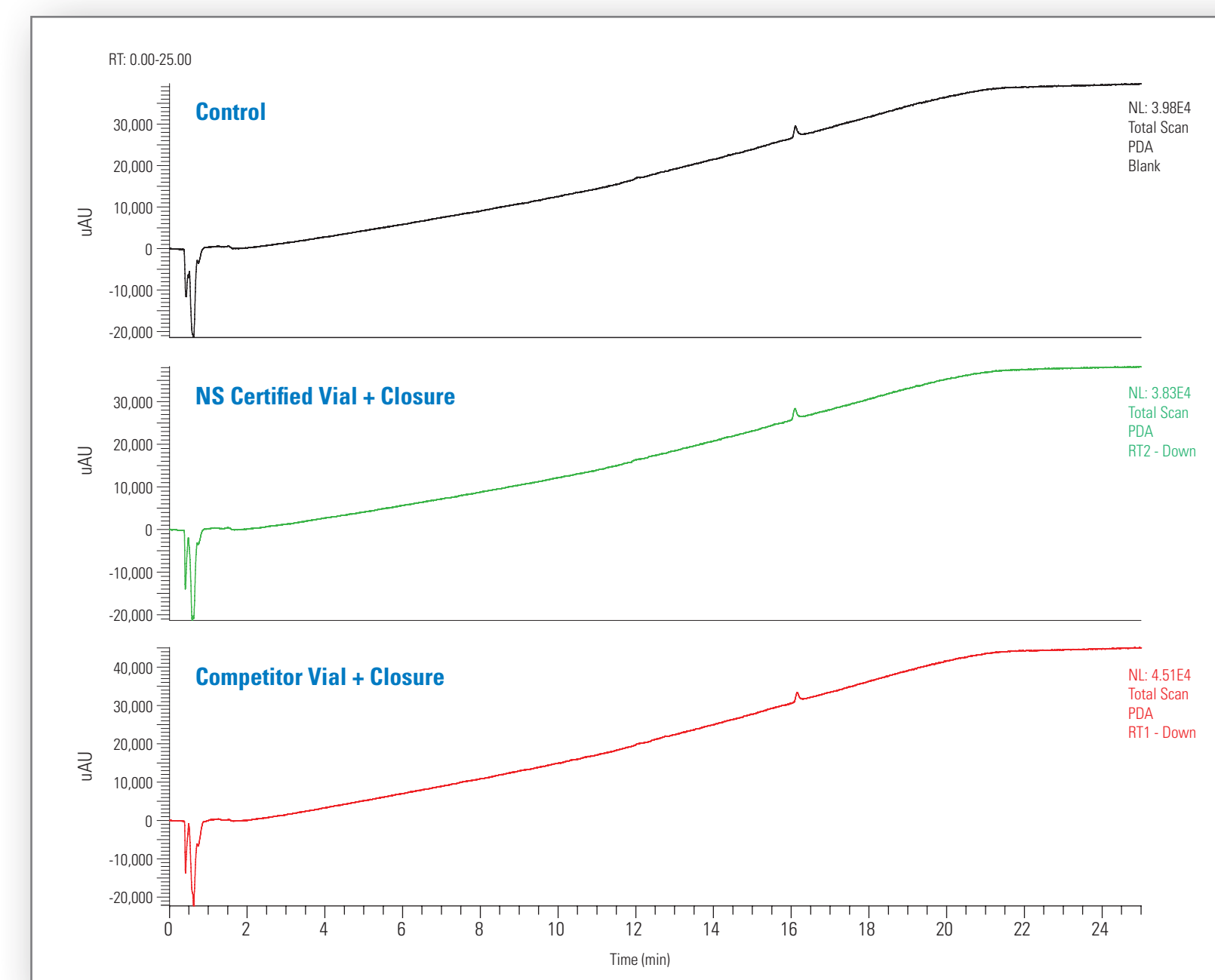


FIGURE 1. LC/UV chromatograms of a blank, a vial with PTFE/silicone closure and a vial with pre-slit closure, incubated at room temperature in the inverted position.

Figure 2 shows the LC/MS analysis of a control, a competitor vial + closure and an MS Certified vial+closure. The LC/MS chromatograms for the control and the MS Certified are very similar, suggesting that there is minimal interference from the vial. However, the Total Ion Chromatogram (TIC) obtained for the competitor vial + closure shows a substantial amount of contamination. Combining the MS spectra of the latter part of the chromatogram reveals that the majority of the peaks in the positive ESI spectra are 74 units apart, suggesting the presence of polydimethylsiloxane (C₂H₆OSi)_n.

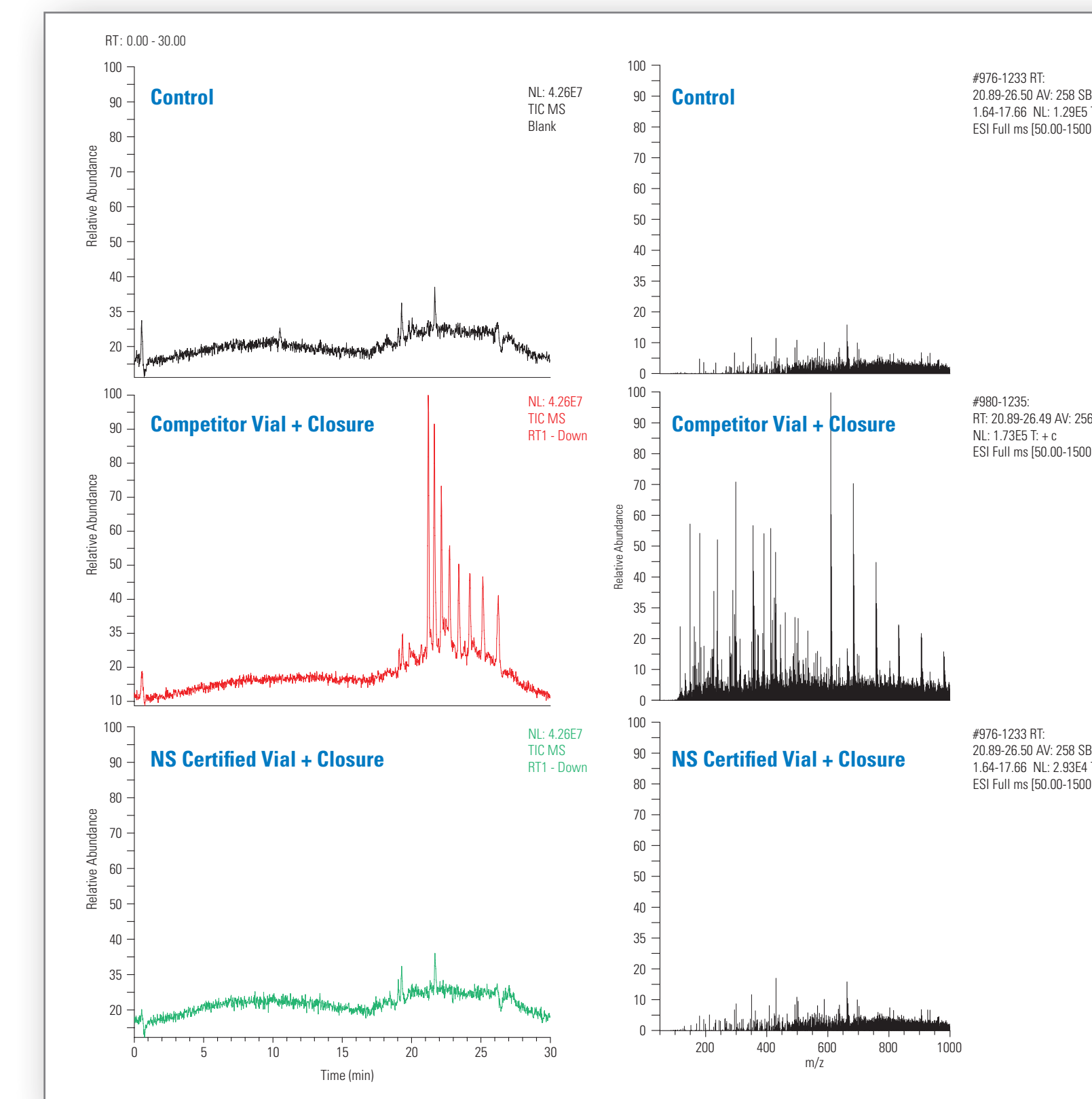


FIGURE 2. LC/MS +ve ESI chromatograms obtained for different sample types. The MS spectra are obtained by summing the spectra over the last portion of the chromatogram.



Figure 3 shows the results of the GC/MS analysis of the MS certified vials compared to the control. Both sets of chromatograms and spectra clearly demonstrate that there is minimal difference between the MS certified vials and the control.

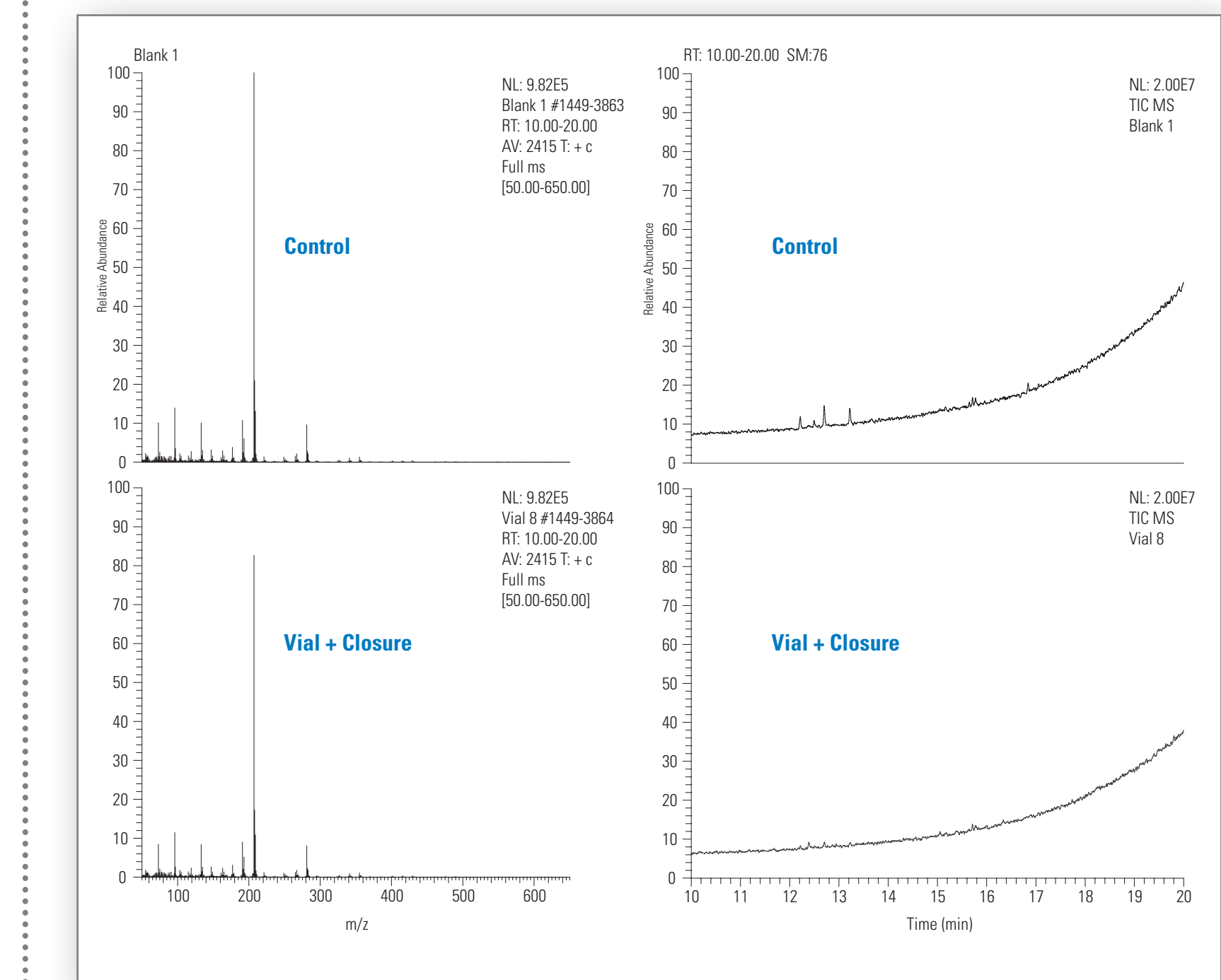


FIGURE 3. GC/MS data for the MS certified vials. Comparison to control TIC and spectrum across the full time span, demonstrate that there is minimal difference between the two.

Conclusions

- UV detection is generally not sensitive enough to detect trace amounts of non-volatile organic contaminants present in autosampler vials.
- Some commercially available autosampler vials can leach organic compounds into the sample, particularly if the sample solvent comes into contact with the closure.
- The National Scientific Mass Spec Certified vials showed no evidence of sample contamination in LC/MS with ESI or GC/MS with EI ionisation, even when the sample solvent came into contact with the closure.

For more information or to request your FREE sample pack, visit www.nationalscientific.com/cleanvials

¹Thermo Fisher Scientific, Runcorn, UK, ²Thermo Fisher Scientific, Rockwood, USA

©2010 Thermo Fisher Scientific Inc. All rights reserved. All trademarks are the property of Thermo Fisher Scientific Inc. and its subsidiaries