Waters TruView LCMS Certified Sample Vials

Waters Corporation, Milford, MA, USA



WHY CERTIFIED VIALS?

As a leading producer of analytical instruments and chemical consumables, Waters understands the dimensional tolerances of autosamplers and the demanding chemical requirements of all consumables due to more sensitive detection limits of the instruments. We understand you are required to produce dependable results. Your time is too valuable to be chasing sources of contamination, ion suppression, or analyte loss. For these reasons, Waters has introduced three lines of vials certified for their intended use.

LC/GC CERTIFIED VIALS



Waters introduced LC/GC Certified Vials in 2004. In this certification program, we covered dimensional specifications and chemical cleanliness. We reviewed chemicals used in all phases of manufacturing and packaging to ensure there were no residues or contaminants to interfere with your assays.

Waters characterized the chemicals used in manufacturing by reversed-phase HPLC with ultra-violet (UV) detection, with the intention of developing a test to detect residues or contaminants in the final product. The final test is run using product packaged for a minimum of four days. The test detects residues from manufacturing or contaminants released from the plastic wrap or packaging. An active glass surface will pick up contamination from the packaging very quickly. The test detects known processing residues at the parts per million (ppm) or µg/mL level.

LCMS CERTIFIED VIALS



In 2006, Waters introduced LCMS Certified Vials. For this product line, the investigation went beyond known contaminants and looked for mass spectrometry interferences regardless of the origin. In comparing vial products sourced around the world, we saw a wide range of results. The MS scans showed evidence of contaminants such as oils, surfactants, and agents that bleed from packaging1. Many of the vials were not suitable for LC-MS analysis.

Our research in vials manufacturing and process controls completed during the certification project for LC/GC Certified Vials was complemented by knowledge gained from our ongoing efforts in the area of MS2. The combined experience in research, manufacturing, and applications work was applied to the LCMS Certified Vials project.

Waters selected the best materials for LCMS Certified Vials and worked to tightly control manufacturing processes and handling procedures to deliver a consistently clean vial for LC-MS applications. In 2006, the most widely used mass spectrometer in LCMS was a single quadrupole MS with most scientists working in the 10's to 100's ng/mL analyte concentration. These vials are perfectly suited for these concentrations.

TRUVIEW LCMS CERTIFIED VIALS



The newest addition to the vials product line, TruView™ LCMS Certified Vials, include the stringent dimensional tolerances, UV and MS cleanliness tests required of the LC/GC and LCMS Certified Vials lines. The additional product attribute of TruView vials is the glass surface exhibits low polar analyte adsorption. The vials are manufactured under tightly controlled process conditions (patent pending) that limit the concentration of free ions on the surface of glass. Low levels of free ions on the surface of glass results in low analyte adsorption.

Applications involving sensitive MS/MS spectrometers operate at analyte concentrations of ng/mL to pg/mL levels. At these concentrations, analyte adsorption will compromise analytical results. Waters TruView LCMS Certified Vials are tested for high recovery of analyte at 1 ng/mL concentration using UPLC™-MS/MS (MRM) and yield little adsorption. These vials exhibit the lowest adsorption of autosampler vials in the market.

BACKGROUND

To show the influence that the glass surface can have on samples at low concentrations, we used the analyte nortriptyline at 1 ng/mL concentration (1 ppb). We added the sample to 2 mL autosampler vials purchased around the world3, including vials from other leading manufacturers. The test instrument consisted of a Waters ACQUITY™ UPLC with a tandem-quadrupole mass spectrometer run in ESI+ mode. The column used was an ACQUITY UPLC BEH C_{18}, 1.7 µm with a 10 mM ammonium acetate pH 5 with 5% acetonitrile for mobile phase A, and 95% acetonitrile for mobile phase B. The system was run from 5-95% mobile phase B in a gradient. The sample was injected immediately after adding

to the vial (time initial) from a stock solution, and re-injected repeatedly, recording the lapse times the sample was in contact with the glass. System suitability standards were run to ensure the test system was operating properly.

RESULTS

To test for the rate of adsorption, the sample was injected repeatedly from the same competitor's vial. The MS peak area diminished rapidly as analyte adsorbed on the vial surface (Figure 1). This same analyte adsorption was observed across most of the vials tested by this method. After a period of time, three hours or more, the rate of adsorption ceased as the analyte had saturated the free ion surface and no additional analyte was adsorbed.

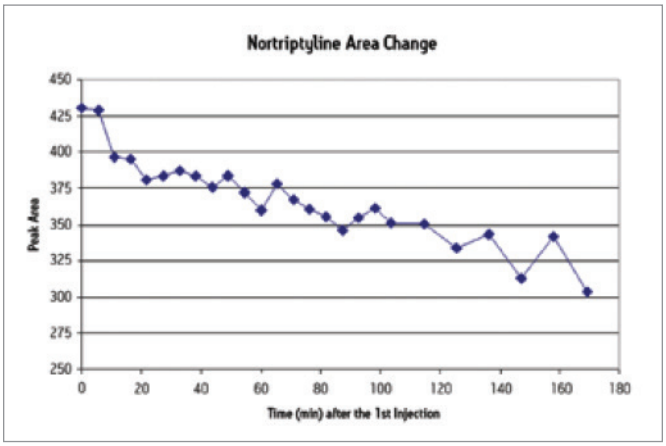


Figure 1. Nortriptyline at 1 ng/mL concentration injected repeatedly over a three hour time period from the same vial.

A similar test was run with fewer injections over a longer time span; initial, 4, 8, and 24 hours. The results showed little to no adsorption beyond the four hour period. From this analysis, a protocol was established for a larger sampling study. The test times were initial (controlled within minutes of adding sample to the vial) and repeated at four hours.

For the larger test study, 10 vials from each manufacturer's package were tested. In some cases, more than one lot of vials was tested when we had multiple manufacturers' lots. The peak area of the initial injection (time initial) was compared to the peak area from the four hour injection. The results reported were the average for the lot with a sample size of 10 (Table 1).

Manufacturer	Clear Glass Average Analyte Loss	Amber Glass Average Analyte Loss
Vendor A	55%	69%
Vendor B	25%	25%
Vendor C	54%	74%
Vendor D	18%	59%
Vendor E	75%	Not tested*
Waters TruView LCMS Certified Vial	1%	6%

Table 1. The average analyte loss shown in this table is the calculated loss from the initial and the four hour injections. The sample size is 10 vials per manufacturer's lot.

**Amber glass vials were not available from Vendor E at the time of testing.*

For a visual image of adsorption, Figure 2 shows overlaid UPLC-MS/MS chromatograms; time initial and time 4 hours from the same vial. The tall peak is the time initial peak, injected within a minute of adding the sample to the vial. The four hour peak is overlaid with the initial peak. In most cases, the four hour peak is considerably smaller due to adsorption of the analyte on the glass surface.

The first two chromatograms in Figure 2 are representative of results we saw with the vials we tested from other manufacturers. The last chromatogram is an overlay of peaks for the WatersTr uView LCMS Certified vial. For this lot of TruView vials, there was a 1% sample loss.

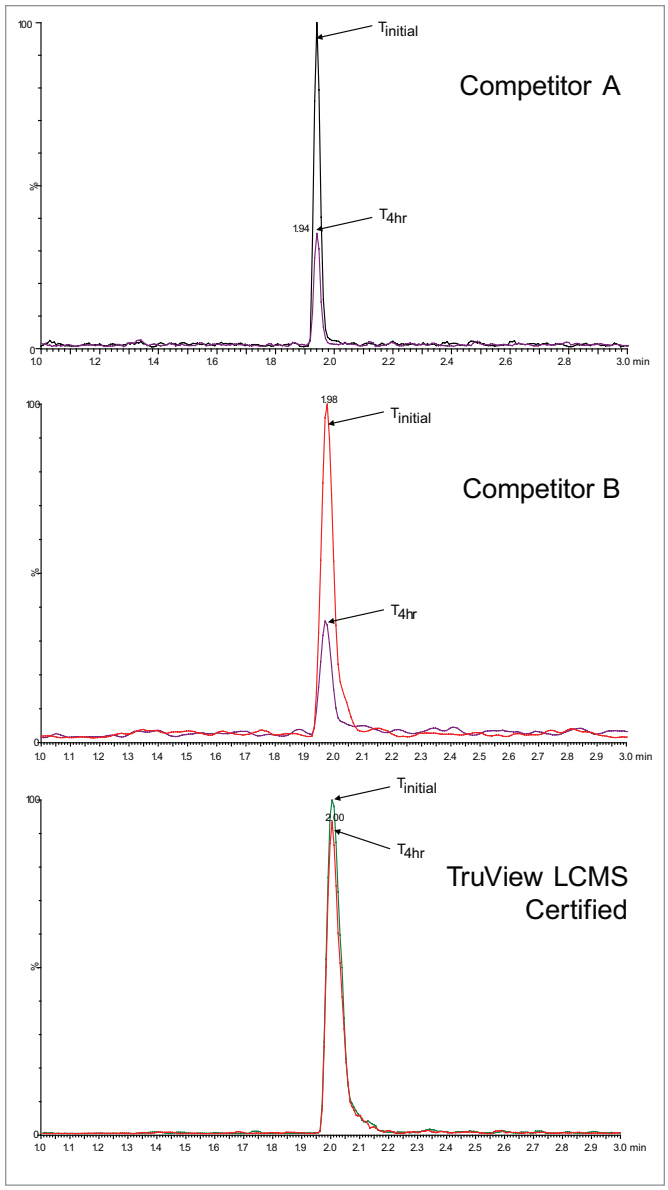


Figure 2. Nortriptyline at 1 ppb concentration in several vials. The tall peak is the initial injection; the smaller peak (overlaid) is the four hour injection. The first two chromatograms are competitor's vials. The third chromatogram is for the Waters TruView LCMS Certified vial.

DISCUSSION

Glass is inorganic with a certain level of free ions4. In the manufacturing process of preparing vials, the ions can bloom and be in higher concentration on the surface. There are several ways the higher concentration can affect analytical results. The ions can solvate and go into solution in an aqueous sample. Solvating the ions can raise the pH of the sample and exposes ion exchange sites on the glass. If the analytes in the sample have polar sites, there can be adsorption to the glass surface. Adsorption will have a greater effect on accuracy of results when analyte concentrations are lower; pg/mL. At high concentrations, adsorption will have less influence on analytical results as there is a limited area

of glass surface. Once the sites on the surface are saturated or coated with analyte, no more analyte can adsorb.

If you are running samples at low concentration today, you might not have noticed adsorption in your results. This could mean the analyte is not interacting with and/or being adsorbed on the surface. Alternatively, you may be testing after the adsorption has occurred. Hours often pass between the time samples are prepared/added to the vials and the time samples are actuallyi njected and analyzed on the instrument.

Instrument development is yielding more sensitive mass spectrometers. With more sensitive instruments, analytes can be detected at pg/mL levels. At these concentrations, adsorption issues need to be understood (and avoided) for good quantitative results.

RECOMMENDATION

Analyte Concentration	Detection Source	Recommended Product
µg/mL	UV, RI (non-ms)	LC/GC Certified Vials
100's ng/mL	Single quadrupole and older MS/MS	LCMS Certified Vials
1 ng/mL and lower	MS/MS	TruView LCMS Certified Vials

- For non-MS applications and sample concentration ≥ µg/mL, Waters LC/GC Certified Vials are a good choice. Adsorption would have little effect on reported results due to high analyte concentration and low glass surface area.
- For LC-MS applications, with sample concentrations in the 10-100's ng/mL, Waters LCMS Certified Vials are a good choice. The LCMS Certified Vials have fewer masses seen by MS and high analyte concentration would have less effect on results.
- For MS applications with sample concentration in the ng/mL and lower range, Waters TruView LCMS Certified Vials are the best selection. This vial combines low adsorption and few masses seen by MS.

** Note: For any compound that is prone to adsorption, test to determine if a vial change is necessary.*

WATERS TRUVIEW LCMS CERTIFIED VIALS

The glass vials used in the TruView LCMS Certified product line are manufactured using tightly controlled process limits. The process yields a glass surface that has a lower concentration of free ions on the surface. The packaged product comes delivered with a certificate of analysis showing the overlay chromatograms of the control and vial from the lot. Vials are also tested for cleanliness by the UV test and an MS scan.

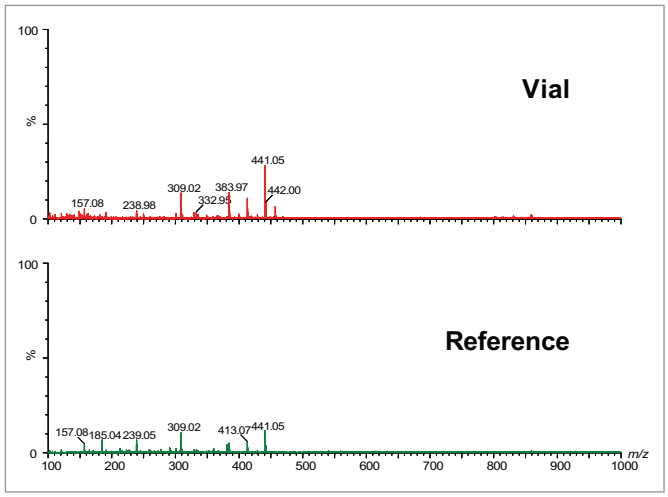


Figure 3. MS scan for cleanliness.

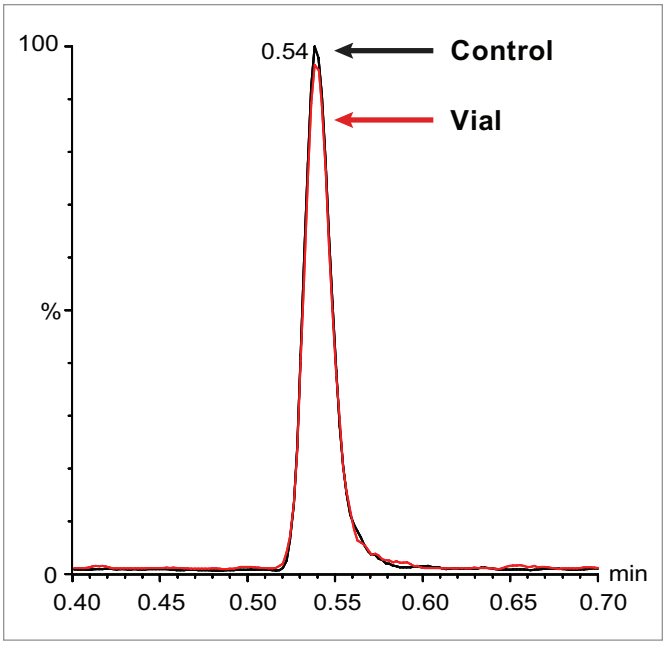


Figure 4. UPLC-MS/MS MRM for adsorption test.

References

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