WATERS LCMS CERTIFIED SAMPLE VIALS
We characterized the chemicals used in manufacturing by reversed-phase HPLC with 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 pp level.

Waters LCMS Certified Vials

In 2006, Waters® LCMS Certified Vials join our product line. To produce the best product for LC/MS applications, we first surveyed vials from around the world, including our current LC and GC certified vials. We then tested all products, including components not currently in use, by the same testing protocol.

Why Certified Vials?

- Vials are manufactured by glass artisans and engineers who don’t understand the requirements of auto samplers
- We understand the requirements for HPLC, specifically with respect to auto samplers and detectors. As a leading producer of mass spectrometers, we also understand the demanding requirements of MS.

You need to produce dependable results. Your time is too valuable to be chasing sources of contamination or ion suppression. For these reasons, we have introduced two 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 to ensure there were no residues or contaminants to interfere with your assays. The chemicals used in manufacturing or packaging are:

- Surfactants used to prevent glass scratches and breakage
- Lubricants used on machinery
- Antistatic, antioxidants or mold release agents that may be present in plastic wraps or packaging
- Low MW silicone polymers from septa improperly cured

At Waters we take all of these factors into consideration in the design, manufacture and delivery of our vials and accessories.

Figure 1: Flow path of a typical sample through an entire analysis scheme

Background

The figure above shows a simple analysis scheme, beginning with some form of sample preparation of the dissolved sample. The prepared sample is then put into a vial and placed in the LC auto sampler. In HPLC the sample is separated on the column and passes through an LC detector for analysis. In LC/MS, after separation on column, detection and further analysis are accomplished in the mass spectrometer. MS instruments commonly used with LC are equipped with an atmospheric pressure ionization interface [API]. Among various API interfaces currently available:, electrospray ionization [ESI] is the most commonly used technique.
As shown in Figure 2, the main function of the ESI source is to convert the liquid effluent from the LC into a stream of charged droplets. Upon heating and evaporation, the size of the droplet is reduced, and, consequently, the density of charges at the droplet surface increases. The repulsion forces between the charges increase until there is an explosion of the droplet. This process repeats until analyte ions are freed from the droplet into the gas phase. The ESI source is prone to phenomena called ‘ion suppression’ or ‘ion enhancement’ when the analytes of interest elute in the presence of certain mobile phase additives or residual matrix components. Impurities from any source may interfere with the ionization process. Impurities that can be charged in MS affect the limit of quantitation [LOQ] or contribute to a high relative standard deviation [RSD]. For more discussion on the topic, please review cited papers. Vials are overlooked as a potential source of problems. Yet they are an important link between the prepared sample and the final analysis. If the vial is produced under poor quality control, contamination from process [surfactants, lubricants] or human handling [lotions, cigarette smoke] sources is possible.

### Waters LC/MS Certification Process

We began by purchasing vials, caps and septa combinations from around the globe and submitted all vials thru a screening protocol which included:

- Soaking vials for 4 hours in different organic/water mobile phases
- Adding acid and base to some of the solvent combinations
- Analyzing the contaminants in these different solvents with ESI-MS and recording the results. One hundred scans were combined for each reference and sample.

The details of the experiment are included in a poster that can be obtained by contacting Waters.

### Results

In the following figures, we compare reference MS scans from the blank wash solvents to those obtained from the same solvents soaking in competitor’s standard vials and Waters LCMS Certified Vials. MS scans from 250 to 1000 m/z for a competitor’s standard vial [top] and the corresponding blank [bottom]. Note that the sensitivity of both scans is identical. Clearly, this vial/cap/septum combination exhibits significant, potentially interfering low mass contamination as well as clusters in the high mass range. This is evidence of a range of various contaminants such as oils, surfactants, and agents that bleed from the packaging material. Such a vial is not suitable for LC/MS analysis. This result is typical of the vials we purchased from sources around the world. Though levels varied widely, all the standard tested vials showed a significant contribution to the MS background.

![Figure 2. Electrospray Ionization Process](image)

![Figure 3: Determination of the MS background generated from a competitor’s vial](image)

In Figure 4, a similar experiment is run using a Waters Certified LCMS vial. Please note that the scales in Figure 3 and Figure 4 are identical. If a vial is 'clean', i.e., not leaching any contaminants, its scan should be similar to the reference. Clearly the vial analyzed in Figure 4 would be considered clean and acceptable for use in LC/MS.
This comparison shows that there are significant differences between the cleanliness of vials from different vendors. Waters is working with its suppliers to provide high-quality vials and septa with minimum levels of contamination. These efforts are based on a research program that identified the problem sources. This in turn helps our suppliers to eliminate potential for contamination in their manufacturing processes.

Conclusion

In our survey of competitive products, we saw that the quality of vials, when used for LC/MS applications ranged, from marginal to very poor quality. Many of these vials are not suitable for LC/MS applications. At Waters, our research into vials manufacturing and process controls completed during the certification project for LC Certified Vials was complimented by knowledge gained from our ongoing efforts in the area of MS. We have investigated signal suppression/enhancement with various mobile phase modifiers and researched the best materials of construction for sample prep devices with an eye toward extractables and their impact on MS analysis. Knowledge from the combined experience in research, manufacturing and applications work has been applied to the LCMS Certified Vials project. We have taken steps to ensure proper selection of materials for LCMS Certified Vials and the need to tightly controlled manufacturing processes and handling procedures so that we can deliver a consistently clean vial for LC/MS applications. We will continue to provide the best products for your demanding applications.

— Brian P. Murphy, Claude R. Mallet, Uwe D. Neue, Patrick D. McDonald

“All Waters LCMS Certified Vials are tested on a Waters® ZQ® Mass Spectrometer using MassLynx® control. The vials come packaged with a Certificate of Analysis showing the reference and vial scan for the manufacturing lot.

Reference


Figure 4: Experimental results typical of Waters LCMS Certified Vials