Reversed-phase point-of-use purifier for the removal of trace organics in high purity water

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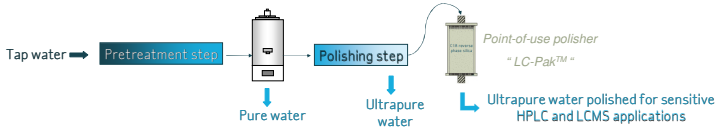
Introduction

Recent developments of Liquid Chromatography instrumentation, such as UHPLC coupled with mass spectrometry, have increased the separation efficiency and sensitivity. Unfortunately, there are some unwelcome consequences. One of these is the detection of trace contamination in the solvents used to prepare mobile phases. Our aim was to design a cartridge which produces high water quality for very sensitive LC-MS applications. A new point-of-use purifier based on reversed-phase chemistry was developed with the purpose of removing traces of organic contamination just before water is dispensed.



Scheme 1: Water purification chain

In this poster are shown:
- **Results of tests performed during the polisher's validation**
- **LC-MS baselines of ultrapure water with or without the polisher**
- **Comparisons of water filtered with the specific polisher and LC-MS Grade bottled water**

Validation of the polisher

Reserpine test

Reserpine is an alkaloid often used as a standard for LC-MS's suitability tests.

▪Experimental method:

Ultrapure water produced by a water purification system and the polishing cartridge was mixed with methanol HPLC grade (50/50). The reserpine solution (10 µg/L) was then infused in the MS detector for 3 min at 10 µL/min. The detector scans masses between *m/z* 100 and 1000 amu in electrospray positive mode. MS spectra are acquired and combined during 3min on Waters® ZQ2000™.

▪Results:

To pass the test no signal should be greater than 10ppb $[M+H]^+$ 609$^+$ (ESI^+) on the spectrum. Figure 1 shows that the water tested passed the test.

	LC-Pak 1	
	254nm	365nm
200L	< 10ppb	< 10ppb
500L	< 10ppb	< 10ppb

Table 1

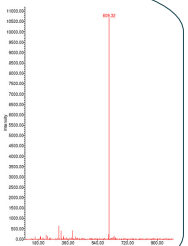


Figure 1

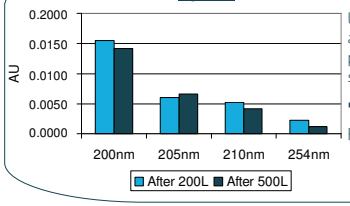
The LC–Pak™ cartridge was extensively validated to ensure that the water quality at the outlet matches the demanding requirements. Our criteria to validate this new polishing cartridge are listed in figure 2.

Parameter	Specification of LC-Pak ultrapure water	Comments
HPLC gradient test Absorbance of highest eluted peak	At 210nm < 0,006AU At 254nm < 0,002AU	Concentration of 60mL water at 1mL/min prior to elution
	At 210nm < 0,003AU At 254nm < 0,001AU	No water preconcentration
Optical properties Absorbance in UV range	UV 200nm < 0,05AU UV 205nm < 0,01AU UV 210nm < 0,01AU UV 254nm < 0,005AU	According to ISO 3696 procedure
Fluorescence as Quinine	At 254nm < 1ppb At 365nm < 1ppb	
Compliance with suitability for LC/MS Reserpine test	No peak higher than 10ppb of reserpine at *m/z*609 in ESI+	
Residue after evaporation	< 0,0001% w/w	Test performed as specified in ISO 3696 procedure

Figure 2

UV absorbance

Figure 3



▪ Experimental Method:

UV absorbance of water produced by the water purification system and the polisher was measured at 4 wavelengths according to the procedure described in ISO 3696 using a Shimadzu® UV spectrometer UV1700 Pharma Spec.

▪ Results:

Results on figure 3 show that:
- at 200 nm abs. ‹ 0.05 Abs. Unit
- at 205 nm abs. ‹ 0.01 AU
- at 210 nm ‹ 0.01 AU
- at 254 nm ‹ 0.005 AU

LC-MS baselines during the polisher's lifetime

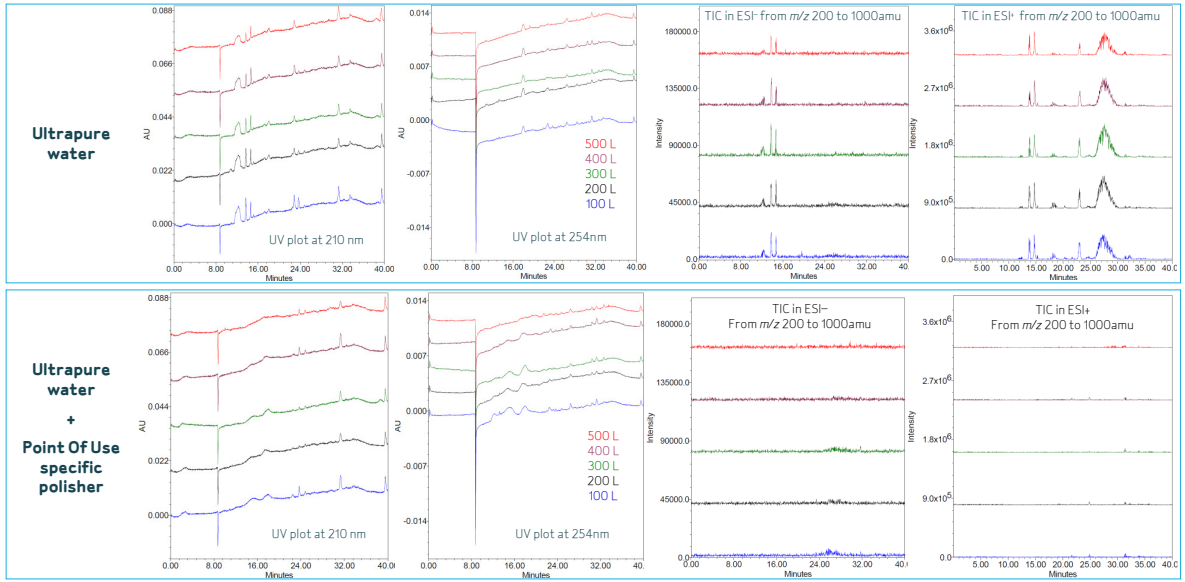


Figure 4: Overlay of chromatograms at 210nm and 254nm, and overlay of total ionic current plot in ESI^+ and ESI^-

Experimental Method:

The polishing cartridge was adapted to the outlet of an ultrapure water purification chain. A water sample was collected before and after the polisher, after every 100L of water dispensed, up to 500L. HPLC and MS chromatograms of water samples collected at inlet and outlet of the LC-Pak™ are compared on figure 4.

▪Instrumentation :

HPLC system : Waters Alliance® 2695
Pre-column Column : C18 XTerra®; 3µm, 4.6x30mm
Column : C18 Atlantis®; 3 µm, 2.1x150mm
Detectors : PDA Model 2996 and Waters ZQ2000™

▪ Mobile phase:

HPLC Grade acetonitrile and water to be tested

▪ Procedure:

Sample preparation : 60mL of water sample were pre-concentrated on a X-Terra® MS column (C18, 4.6x30 mmx3.5 µm) before being analyzed by HPLC. The following gradient profile (water : acetonitrile) was used :
- 0% to 100% ACN in 30 min
- hold at 100% ACN for 10 min

Results:

✓ There was no peak higher than 0.006 AU at 210 nm and no peak higher than 0.002 AU at 254 nm.

✓ The polishing cartridge removes most of the organic contamination in UV plots as well as in the total ionic plots in Mass Spectrometry.

LC–Pak versus LC-MS bottled water

In order to verify that fresh ultrapure water produced by the polishing cartridge matched or exceeded the quality of commercially available bottled water for LC-MS applications, 60 mL of different water samples were pre-concentrated on a C18 reversed phase chromatography column, then eluted and analyzed by LC-MS (PDA ,single quadrupole). Examples of the results obtained are shown below (figure 5).

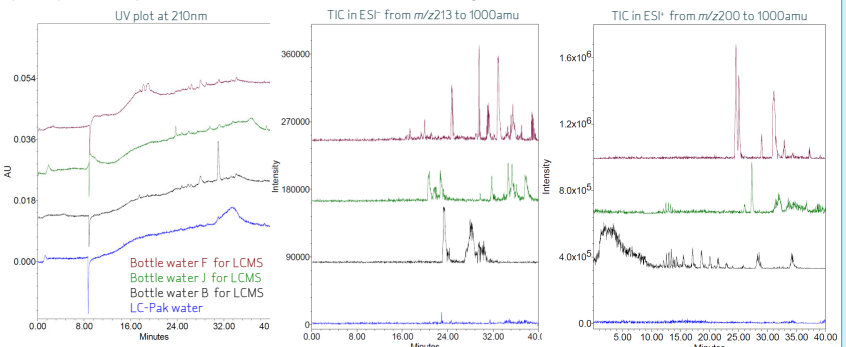


Figure 5: Overlay of chromatograms at 210nm, and overlay of total ionic current plot in ESI^+ and ESI^-

Conclusion

✓ **Freshly produced ultrapure water polished by this new cartridge (LC-Pak™) contains very low level of organic contaminants.**

✓ **A C18-based polishing cartridge is designed to answer the demands of UHPLC, LC-MS and LC–MS/MS analytical techniques used for organic traces and ultra-traces analysis.**

✓ **The water produced by a complete purification chain equipped with the C18 polisher is similar or superior in quality to commercially available bottled LC-MS water**

✓ **The purifier was qualified to deliver 500L of organics-free ultrapure water.**

✓ **A certificate of quality is delivered with the LC-Pak™**