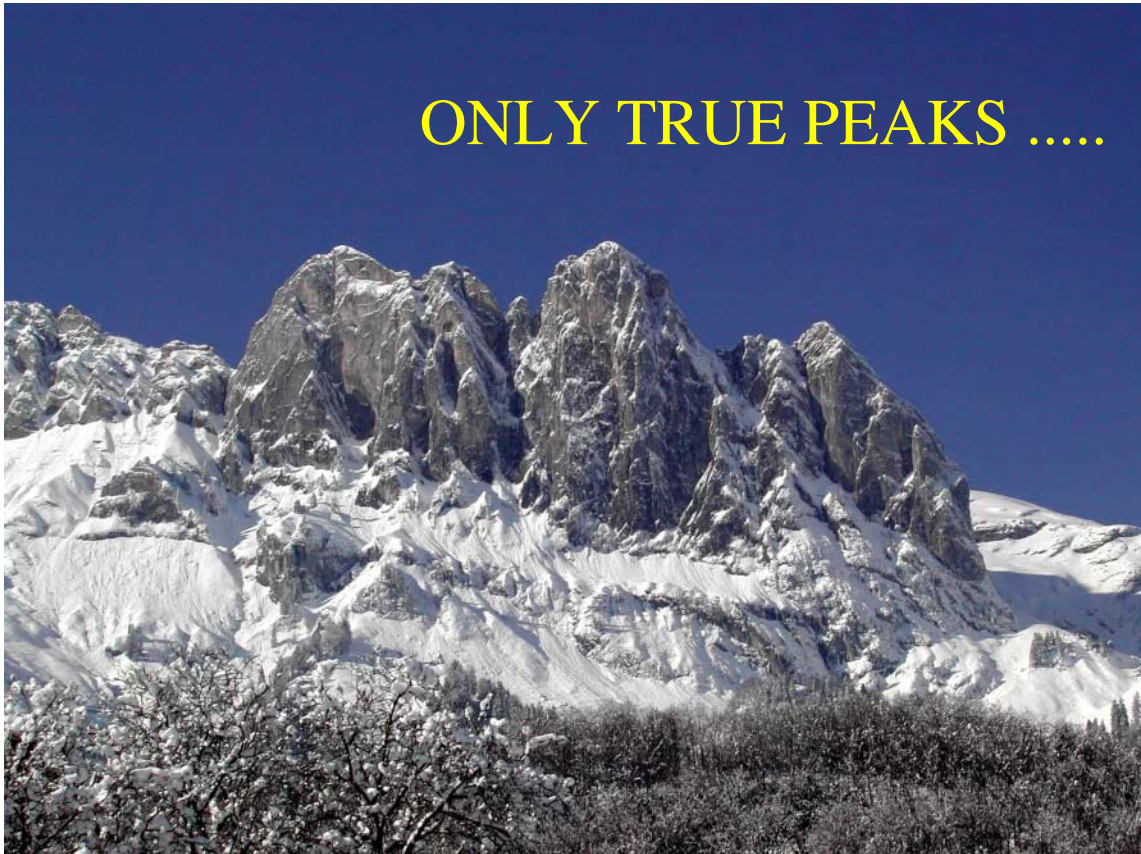


ONLY TRUE PEAKS



LC-MS CHROMASOLV® Solvents

Riedel-deHaën®

Over the past decade improvements of LC-MS systems have made great progress. The combination of classic Liquid Chromatography (LC) with Mass Spectrometry (MS) has lead to a remarkable increase of analytical applications.

Today LC-MS is an important technique for identification and quantification of metabolites in pharmaceutical laboratories, as well as for pharmaco kinetics and protein, peptide and oligonucleotide analysis. However, modern LC-MS ionization methods such as Electrospray (ESI) and Atmospheric Pressure Chemical Ionization (APCI) require a new generation of solvents, fulfilling the special demands of atmospheric pressure interfaces (API).

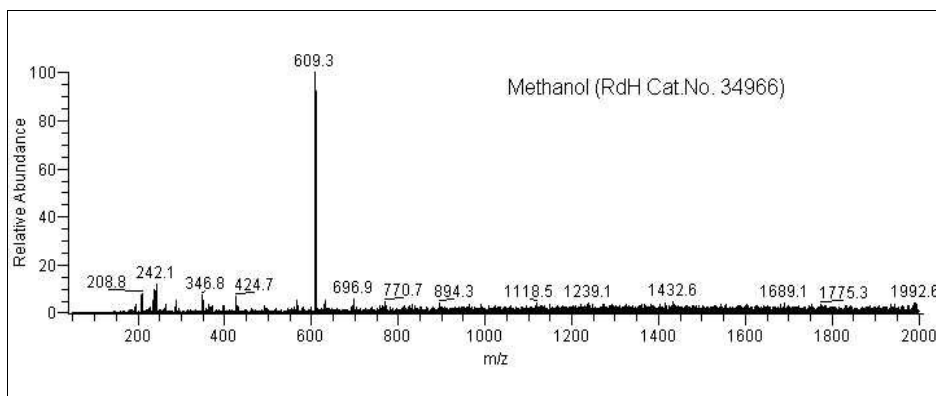


Figure 1: Reserpine Test: reserpine spectrum measured in Methanol (Cat.No.34966); no signals should be greater than $[M+H]^+ = 609$ (100 ppb reserpine; ESI, positive mode).

The most important specification parameter is a very low amount of metal ions, especially sodium and potassium. When operating ESI in positive ion mode the preferred ionization reaction is the formation of $[M+H]^+$ -ions, for peptides the double charged $[M+2H]^{2+}$ -ion. In the presence of sodium and potassium these metal ions form dominating clusters with the peptide, which causes difficulties in the correct determination of the molecular ion for further MS-steps.

Analyzing peptides with Riedel-de Haen LC-MS CHROMASOLV® Solvents will give you only the “true peaks” (see below).

Other important parameters for LC-MS Solvents include particles, which could clog up the inlet filter of a Nano-LC instrument, and a suitability test for LC-MS, based on the reserpine specification test of many instrument suppliers (see Figure 1).

The new Riedel-de Haen LC-MS CHROMASOLV® Solvents guarantee all these parameters in addition with a high UV-transmittance and an excellent gradient baseline for combination with UV or Diode Array Detection in line.

Completing the Riedel-de Haen LC-MS CHROMASOLV® Solvents, we have developed a water quality (Cat. No.39253), which is suitable for both gradient HPLC and MS applications as well.

The LC-MS CHROMASOLV Water offers a tremendous advantage over other quality grades. It can be used in both detection methods UV and MS, without any compromise. A comparison of LC-MS Water (Cat.No. 39253) and a non-gradient grade was made (Figure 2) and the differences are evident.

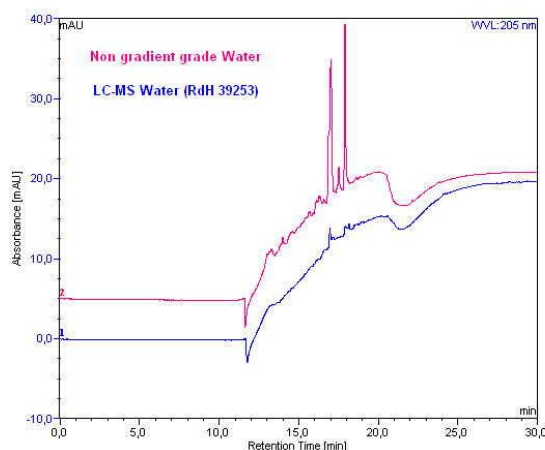


Figure 2: UV gradient at 205 nm, LC-MS Water (Cat. No. 39253) and non gradient grade water.



Investigation of Cluster Formation in Presence of Alkali Ions

In our R+D laboratories we have investigated the influence of metal ions on the quality of LC-MS spectra. The results lead us to develop a specification of the LC-MS CHROMASOLV® Water with a very low content of the alkali elements sodium and potassium. Human gastrin ($M=2097$) was used as a model peptide for LC-MS. The normal ionization product with electro spray is the protonated, double charged molecular ion with $m/z = 1049.8$ $[M+2H]^{2+}$. When human gastrin is dissolved in water (0.2% formic acid) with a very low amount of sodium and

potassium ions (< 0.1ppm), only few metal ion clusters appear, and the molecule mass peak can be determined, isolated and further fragmented to yield the amino acid (AA) sequence. The observed clusters are $[M+H+Na]^{2+}$ and $[M+H+K]^{2+}$ in a relatively low abundance. The sample was infused with a micro syringe thus modeling the conditions of a capillary nano spray.

When the content of sodium and potassium is higher (10ppm), the clusters with these metal ions become dominating. The result is a “fence” of double and multicharged ions. Such an artefact makes it difficult to determine the “true” peak, the protonated double charged molecular ion for further investigation.

Besides that, the relative sensitivity is decreased 10-fold. The major problems with that occur in automatic MS/MS mode, where the most abundant peaks are selected for further collisions to give the AA sequence. Figure 3 shows the unspecific mass spectrum obtained when using water with a 10 ppm content of Na/K. A clear determination of gastrine $[M+2H]^{2+}$ is not possible.

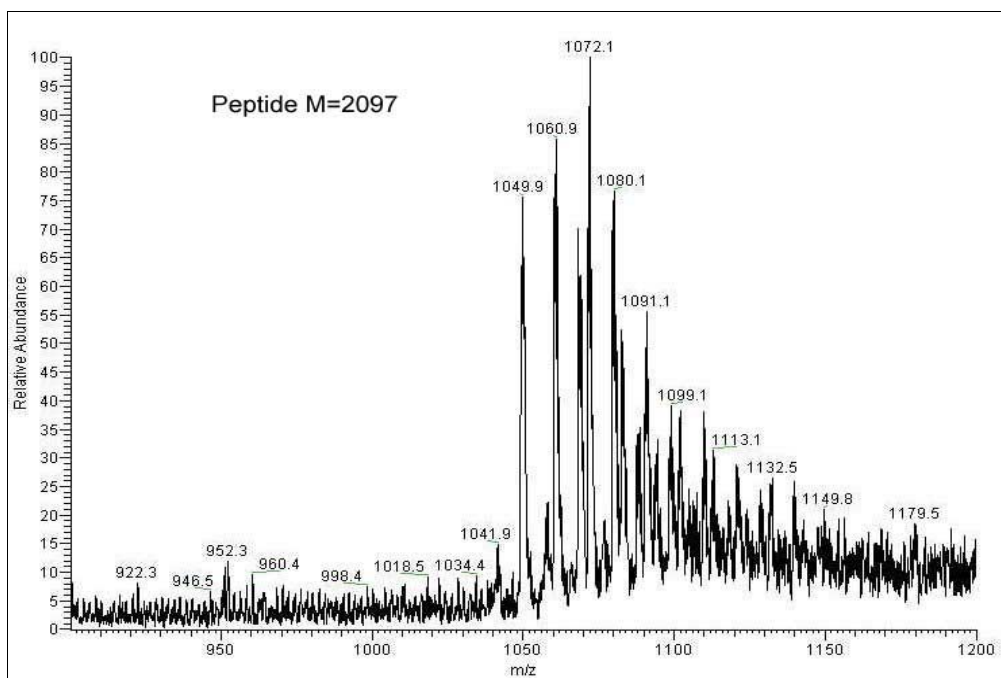


Figure 3: ESI mass spectrum of a peptide (gastrine) in water with Na / K = 10 ppm. Many clusters, 10 fold decreased sensitivity.

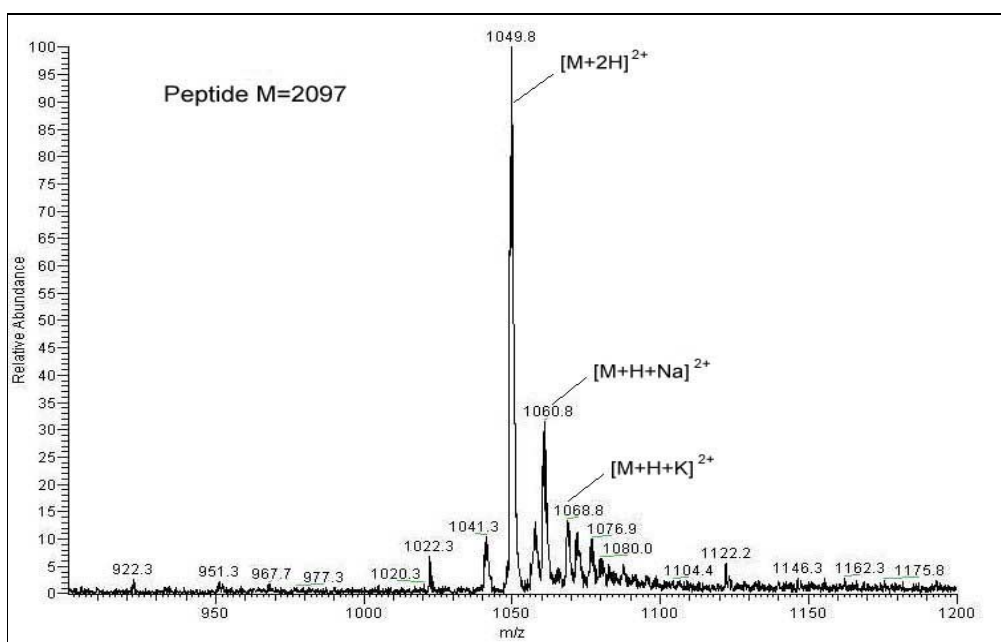


Figure 4: ESI mass spectrum of a peptide (gastrine) in water with Na / K < 0.1 ppm (only few clusters).

In high contrast to the above is the gastrine mass spectrum shown in Figure 4, obtained using LC-MS CHROMASOLV[®] Water 39253 containing sodium and potassium not higher than 0.1 ppm each. Analyzing peptides with Riedel-de Haen LC-MS CHROMASOLV[®] Solvents, which are thoroughly analyzed for metal ions, will give you only the **TRUE PEAKS**.

If you want to be sure in your protein research to avoid obstacles like this, you must test our LC-MS CHROMASOLV® Water (Cat.No.39253).

In accordance to these results we decided to upgrade our already existing LC-MS Solvents to the same high specifications, except Ethyl acetate, which is used only for sample preparation (see Table 1).

SPECIFICATIONS / PRODUCT RANGE

LC-MS Solvent	Water	Acetonitrile	Methanol	2-Propanol	Ethylacetate
productnumber	39253	34967	34966	34965	34972
packsizes	1 L	1 L / 2.5 L	1 L / 2.5 L	1 L / 2.5 L	1 L / 2.5 L
assay (GC)	-	min. 99.9 %	min. 99.9 %	min. 99.9 %	min. 99.7 %
fluorescence at 254nm	max. 1 ppb	max. 0.5 ppb	max. 1 ppb	max. 1 ppb	-
fluorescence at 365nm	max. 1 ppb	max. 0.5 ppb	max. 1 ppb	max. 1 ppb	-
Chlorid (Cl)	max. 0.000001%	-	-	-	-
Fluorid (F)	max. 0.000001%	-	-	-	-
Nitrate (NO ₃)	max. 0.00001%	-	-	-	-
Sulfate (SO ₄)	max. 0.00001%	-	-	-	-
free acid	-	max. 0.001 %	max. 0.001 %	max. 0.001 %	-
free alkali (as NH ₃)	-	max. 0.0002 %	max. 0.0005 %	max. 0.0005 %	max. 0.0005 %
non-volatile matter	max. 0.001 %	max. 0.0002 %	max. 0.0005 %	max. 0.0005 %	max. 0.0005 %
water (Karl Fischer)	-	max. 0.01 %	max. 0.02 %	max. 0.05 %	max. 0.03 %
transmittance at 200nm	min. 95%	min. 95%	-	-	-
transmittance at 230nm	min. 99%	min. 99%	min. 75%	min. 75%	-
transmittance at 260nm	-	-	min. 98%	min. 98%	min. 50%
HPLC gradient (254nm)	max. 1 mAU	max. 0.2 mAU	max. 2 mAU	max. 2 mAU	-
Silver (Ag)	max. 0.1 ppm	max. 0.1 ppm	max. 0.1 ppm	max. 0.1 ppm	-
Aluminum (Al)	max. 0.5 ppm	max. 0.5 ppm	max. 0.5 ppm	max. 0.5 ppm	-
Barium (Ba)	max. 0.1 ppm	max. 0.1 ppm	max. 0.1 ppm	max. 0.1 ppm	-
Calcium (Ca)	max. 0.1 ppm	max. 0.1 ppm	max. 0.1 ppm	max. 0.1 ppm	max. 0.1 ppm
Cadmium (Cd)	max. 0.05 ppm	max. 0.05 ppm	max. 0.05 ppm	max. 0.05 ppm	-
Cobalt (Co)	max. 0.02 ppm	max. 0.02 ppm	max. 0.02 ppm	max. 0.02 ppm	-
Chromium (Cr)	max. 0.02 ppm	max. 0.02 ppm	max. 0.02 ppm	max. 0.02 ppm	-
Copper (Cu)	max. 0.02 ppm	max. 0.02 ppm	max. 0.01 ppm	max. 0.02 ppm	-
Iron (Fe)	max. 0.1 ppm	max. 0.1 ppm	max. 0.1 ppm	max. 0.1 ppm	-
Potassium (K)	max. 0.1 ppm	max. 0.1 ppm	max. 0.1 ppm	max. 0.1 ppm	max. 0.1 ppm
Magnesium (Mg)	max. 0.1 ppm	max. 0.1 ppm	max. 0.1 ppm	max. 0.1 ppm	max. 0.1 ppm
Manganese (Mn)	max. 0.02 ppm	max. 0.02 ppm	max. 0.01 ppm	max. 0.02 ppm	-
Sodium (Na)	max. 0.1 ppm	max. 0.1 ppm	max. 0.1 ppm	max. 0.1 ppm	max. 0.1 ppm
Nickel (Ni)	max. 0.02 ppm	max. 0.02 ppm	max. 0.02 ppm	max. 0.02 ppm	-
Lead (Pb)	max. 0.1 ppm	max. 0.1 ppm	max. 0.02 ppm	max. 0.1 ppm	-
Tin (Sn)	max. 0.1 ppm	max. 0.1 ppm	max. 0.1 ppm	max. 0.1 ppm	-
Zinc (Zn)	max. 0.1 ppm	max. 0.1 ppm	max. 0.1 ppm	max. 0.1 ppm	-
particle test	+	+	+	-	-
LC-MS suitability test	+	+	+	+	+

Table 1: LC-MS Solvents and specifications

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