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Application Note 179

LC/MS CHROMASOLV® Solvents

In traditional liquid chromatography using ultraviolet (LC/UV) detection, certain criteria are placed on the mobile phase solvents and modifiers. For example the U.S. Pharmacopoeia (USP) defines "HPLC-Grade" water by setting maximum absorbance criteria at various wavelengths as well as limiting residue on evaporation.

In LC/MS the criteria for selection of solvents and modifier reagents are significantly different than in LC/UV. Phosphate buffers that are commonplace in LC/UV exhibit poor volatility and suppress ionization in LC/MS and thus are avoided. In much the same way, metal content of solvents, which do not significantly affect most LC/UV analyses, must be examined closely for use in LC/MS.

This report discusses the positive effect of controlling sodium and potassium metals in LC/MS solvents.

Investigation of Cluster Formation in Presence of Alkali Metal Ions

In LC/MS the formation of pseudomolecular ions $[M+H]^+$ is highly desired for ease in structural interpretation of the data. When significant concentrations of sodium and potassium ions are present in the mobile phase solvents, adducts are often formed. The formation of adducts can complicate data interpretation and adversely affect sensitivity. Human gastrin (MW=2097) is used as a model peptide for this series of LC/MS studies. The normal ionization product using electrospray ionization (ESI) is the protonated, doubly-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), few metal ion clusters appear, and the

Table 1. Specifications for LC/MS CHROMASOLV Solvents

LC/MS Solvent:	Water	Acetonitrile	Methanol	2-Propanol	Ethyl Acetate
Specifications					
Assay (GC) (min)	-	99.9%	99.9%	99.9%	99.7%
Fluorescence at 254nm (max)	1 ppb	0.5 ppb	1 ppb	1 ppb	-
Fluorescence at 365nm (max)	1 ppb	0.5 ppb	1 ppb	1 ppb	-
Chloride (Cl) (max)	0.000001%	-	-	-	-
Fluoride (F) (max)	0.000001%	-	-	-	-
Nitrate (NO_3) (max)	0.00001%	-	-	-	-
Sulfate (SO_4) (max)	0.00001%	-	-	-	-
Free acid (max)	-	0.001%	0.001%	0.001%	-
Free alkali (as NH_3) (max)	-	0.0002%	0.0005%	0.0005%	0.0005%
Non-volatile matter (max)	0.001 %	0.0002%	0.0005%	0.0005%	0.0005%
Water (Karl Fischer) (max)	-	0.01%	0.02%	0.05%	0.03%
Transmittance at 200nm (min)	95%	95%	-	-	-
Transmittance at 230nm (min)	99%	99%	75%	75%	-
Transmittance at 260nm (min)	-	-	98%	98%	50%
HPLC gradient (254nm) (max)	1 mAU	0.2mAU	2 mAU	2 mAU	-
Silver (Ag) (max)	0.1 ppm	0.1 ppm	0.1 ppm	0.1 ppm	-
Aluminum (Al) (max)	0.5 ppm	0.5 ppm	0.5 ppm	0.5 ppm	-
Barium (Ba) (max)	0.1 ppm	0.1 ppm	0.1 ppm	0.1 ppm	-
Calcium (Ca) (max)	0.1 ppm	0.1 ppm	0.1 ppm	0.1 ppm	0.1 ppm
Cadmium (Cd) (max)	0.05 ppm	0.05 ppm	0.05 ppm	0.05 ppm	-
Cobalt (Co) (max)	0.02 ppm	0.02 ppm	0.02 ppm	0.02 ppm	-
Chromium (Cr) (max)	0.02 ppm	0.02 ppm	0.02 ppm	0.02 ppm	-
Copper (Cu) (max)	0.02 ppm	0.02 ppm	0.01 ppm	0.02 ppm	-
Iron (Fe) (max)	0.1 ppm	0.1 ppm	0.1 ppm	0.1 ppm	-
Potassium (K) (max)	0.1 ppm	0.1 ppm	0.1 ppm	0.1 ppm	0.1 ppm
Magnesium (Mg) (max)	0.1 ppm	0.1 ppm	0.1 ppm	0.1 ppm	0.1 ppm
Manganese (Mn) (max)	0.02 ppm	0.02 ppm	0.01 ppm	0.02 ppm	-
Sodium (Na) (max)	0.1 ppm	0.1 ppm	0.1 ppm	0.1 ppm	0.1 ppm
Nickel (Ni) (max)	0.02 ppm	0.02 ppm	0.02 ppm	0.02 ppm	-
Lead (Pb) (max)	0.1 ppm	0.1 ppm	0.02 ppm	0.1 ppm	-
Tin (Sn) (max)	0.1 ppm	0.1 ppm	0.1 ppm	0.1 ppm	-
Zinc (Zn) (max)	0.1 ppm	0.1 ppm	0.1 ppm	0.1 ppm	-
Particle test	+	+	+	-	-
LC/MS suitability test	+	+	+	+	+

pseudomolecular ion mass response can easily be determined (Figure A). The observed clusters $[M+H+Na]^{2+}$ and $[M+H+K]^{2+}$ are in relatively low abundance.

When the content of sodium and potassium is higher (10ppm), the adducts formed with these metal ions become dominant (Figure B). The result is a complex set of responses due to the adduct formations possible. In this case responses at m/z 1049.9, 1060.9, 1068.9 (not labeled), 1072.1 and 1080.1 correspond to $[M+2H]^{2+}$, $[M+H+Na]^{2+}$, $[M+H+K]^{2+}$, $[M+2Na]^{2+}$ and $[M+Na+K]^{2+}$ adducts, respectively.

Conclusions

In this study we have shown that the alkali metal content of solvents used in LC/MS has a significant impact on the results. High levels of sodium and potassium, commonly found in traditional LC/UV solvents can both complicate mass spectral interpretation and decrease sensitivity. Such artifacts make spectral interpretation significantly more challenging. In addition, the relative response of the pseudomolecular ion is decreased 10-fold when the alkali metal concentrations are high.

Figure A. Mass Spectrum of Human Gastrin at Low ppm Alkali Metal Concentrations

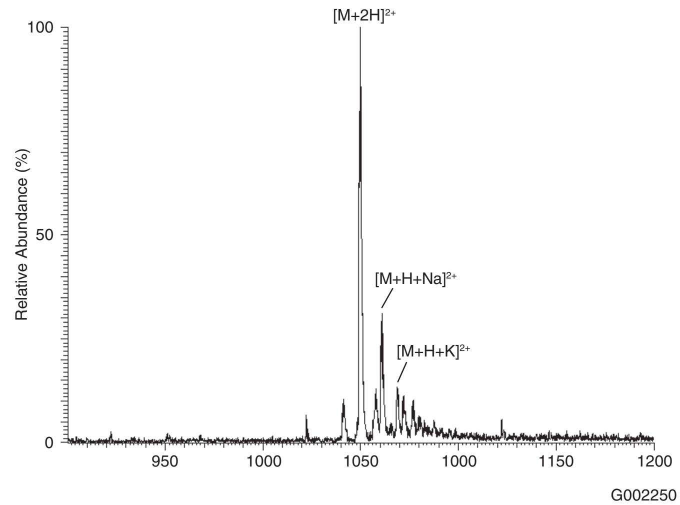
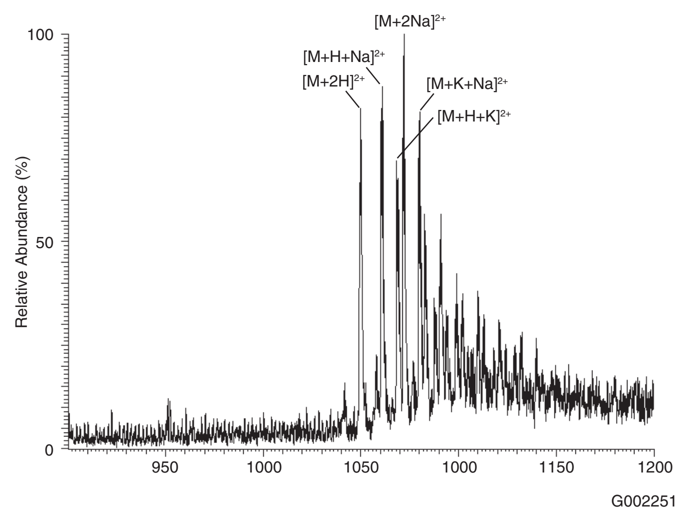


Figure B. Mass Spectrum of Human Gastrin at High ppm Alkali Metal Concentrations



Ordering Information

Other solvents and sizes are available. Please call or visit our website.

Description	Size	Cat.No.
Water, LC/MS	1L	**39253**
Acetonitrile, LC/MS	1L and 2.5L	**34967**
Methanol, LC/MS	1L and 2.5L	**34966**
2-Propanol, LC/MS	1L and 2.5L	**34965**
Ethyl Acetate, LC/MS	1L and 2.5L	**34972**
Gastrin 1 Human	250µg, 0.5mg, and 1mg	**G 9020**

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