KATZ ANALYTICAL SERVICES

Providing analytical solutions for complex problems

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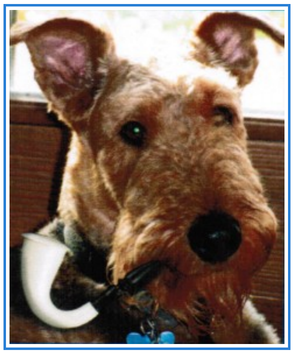
cGMP *Bioanalytical GMP* *GLP*

Sherlock Speaks…



Hi All and GREETINGS!

I hope that everyone had a great summer and are looking forward to the Fall as much as me and my buddies. Great weather, changing colors, and most important:

ARE YOU READY FOR SOME FOOTBALL!!

Vikings have a new coach, new QB, and my season tickets just arrived – nothing better than a stadium brat with a cold one.

Which reminds me of one of my favorite riddles – you know how much I love puzzles:

If you have a barrel of beer (yum) and you need to measure out one gallon, how do you do it if you only have a three gallon container and a five gallon container?

E-mail me your answer:

Sherlock@katzanalytical.com

The first winning answer wins my stupid brother who just tracked mud all over the house– just kidding – the winner gets,

LUNCH WITH ME!

In This Issue:

Upcoming Events:

October 19 - KAS Hosts the Fall **M**innesota **C**hromatography **F**orum Meeting

KAS Labs, 2499 Rice St. Suite 90 Roseville, MN

6:30 pm- Hors d'oeuvres (Mancini Catering)

7:00 pm- Joan Stevens from Agilent Technologies will speak on QuEChERS Method

Hope to see you there!!!

October 24-26 - BioInterface Conference 2011 Hotel Sofitel in Bloomington, MN

Come visit us at our Booth!!!!

October 26 - Bill Katz to Speak at BioInterface Conference- ***Session 4: Vascular Stent and Related Metallic Implants***

ANALYSIS OF METALS IN BIOLOGICAL MATRICES USING INDUCTIVELY COUPLED PLASMA/MASS SPECTROMETRY (ICP/MS)

Wendy Fleming and Bill Katz

The use of implanted materials can be seen throughout the history of man with early discoveries of bone embedded implants linked to the Mayan civilization over 1,350 years ago. Excavations of these Mayan burial sites in Honduras unearthed fragments of tooth shaped shell pieces placed into the mandible dating from about 600 AD.

More recently, beginning in the 1930's, we start seeing more sophisticated implants as artificial hips become more common with the use of steel and chrome joints.

Today, metallic implants are used in many clinical applications with examples including:

1. Bone and Joint Replacement – about one million patients worldwide are treated annually for replacement hip and knee joints.
2. Dental Implants- a titanium “root” is introduced into the jaw bone where the superstructure of the tooth is then built.
3. Maxillo and Cranial/Facial Treatment- artificial parts may be required to restore the ability to speak or eat, as well as, for cosmetic appearance.
4. Cardiovascular Devices- includes devices such as pacemakers and defibrillators, replacement heart valves, and intra-vascular stents.

These implants are constructed from various metals each having unique physical properties for specific applications. Common implant materials include stainless steel, cobalt-chromium alloys, titanium and titanium alloys, gold, and tantalum.

In spite of the wide application of metal implants, concerns regarding corrosion and wear resulting in the production of metal ions and debris is a growing issue. While the specific effect of these metallic contaminants is a topic of current study, researches have suggested that these species may enter the cell, disrupting inter-cellular processes causing alteration of the immune system and hypersensitivity. Others have raised concerns about chromosomal damage, chronic inflammation, necrosis of bone marrow, and carcinogenic effects.

Clearly, release and accumulation of metal debris/ions is a potential problem requiring further study. But, how does one quantitatively analyze such species at low levels in tissue, blood, and urine?

Inductively coupled plasma-mass spectrometry (ICP-MS) is an extremely sensitive method of determining metals. With proper sample preparation and low blank dissolution techniques, ICP-MS can provide analyses of solutions from parts per billion (ppb or ng/mL) to parts per trillion (ppt or pg/mL) levels in a variety of matrices including blood, tissue, urine, and many other biological matrices.

What sets this technique apart from other elemental analyses including atomic absorption and optical emission techniques is speed, sensitivity, precision, a wide dynamic range, ability to perform simultaneous multi-elemental analysis, and the capability to do isotopic comparison. The highly sensitive detection capability is essential when determining trace level metals from implants in biological fluids and tissue making this technique favored above all others for this application.

Figure 1 shows a schematic representation of an ICP-MS.

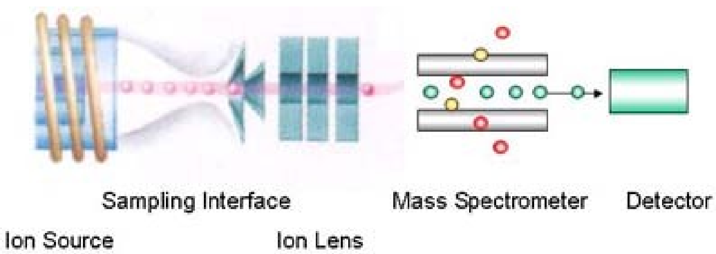


Figure 1

With ICP-MS, as illustrated in Figure 1, the sample is introduced into the argon plasma as an aerosol from the nebulizer. Once the aerosol is introduced, it is completely desolvated and the elements in the aerosol are converted into gaseous atoms and then positively ionized. These ions are then focused by a series of lenses and separated with a quadrupole mass spectrometer with respect to their mass to charge ratio with subsequent detection by an electron multiplier.

Data is only as good as the sample; therefore, selecting the correct sample preparation technique is essential to obtaining quality data. There are a variety of sample preparation techniques specific to biological matrices to choose from that will dissolve the elements of interest into solution: closed vessel microwave digestion, acid digestion, sample ashing, and chemical chelation. Having a good understanding of the chemistry of the sample matrix and the element(s) of interest is the best approach to selecting the sample preparation technique.

The study of the potential effects of metals accumulation is currently a topic of investigation in many laboratories. While much of the work to date is considered preliminary in scope, various research groups have published results in different types of animal models.

One such study is from J.L. Woodman at Rush-Presbyterian -St Luke's Medical Center (Journal of Orthopaedic Research 1:421-430, Raven Press, New York, 1984) where the effects of titanium, aluminum, and vanadium implants were studied in baboons. In a study of seven living baboons, they reported higher titanium levels in urine from an implanted animal as compared with the control, 14 ng/ml of titanium versus 2 ng/ml in the control animal. No differences in either the aluminum or vanadium concentrations were noted. In the same study analyzing the serum, they find significantly higher aluminum in the implant animal as compared with the control, 472 ng/ml and 264 ng/ml, respectively and neither titanium nor vanadium serum levels increased.

While the topic of metals accumulation is currently receiving significant attention, clearly more work is required before the long-term clinical effects are fully understood. Because of the potential biological impact low metal levels can have, ICP-MS is certainly a technique that will provide the sensitivity and accuracy required for such investigations.

HPLC Method Development Tips and Tricks #1:

Neutral Compounds

Merlin Bicking, Ph.D. ACCTA, Inc.

The first of a four part series on method development covering neutrals, acids, bases, and zwitterion compounds.

Introduction

These days many laboratories are asking less experienced staff to perform HPLC method development, an activity that 20 years ago was only performed by experienced chromatographers with advanced degrees. While the instruments are much easier to use now, the scientific challenges are still just as complex.

So, while we can't replace an advanced degree or years of experience with one article, we can give you some advice to get you started. In this series we will briefly discuss how to get started, by discussing the challenges for several compound classes – neutrals, acids, bases, and zwitterions. Let's start with the neutrals.

About the Columns:

C18: this reversed phase type separates by carbon number using an aqueous/organic mobile phase.

PFP: this is a unique phase that shows both reversed phase effects along with retention of positively charged molecules, when used with a buffer and an aqueous/organic mobile phase.

Silica: this phase is often called "HILIC" or "aqueous normal phase" (ANP). Retention order is often the opposite of the reversed phase materials when used with a buffer and aqueous/organic mobile phase.

Other columns to consider if these don't work:

- Polar embedded phase
- Aqueous stable phase
- Biphenyl

HPLC Method Development for Neutral Compounds

These compounds have no significant acid or base properties, and no charge. They include the normal organics, like benzene derivatives (toluene, ethylbenzene, etc.), but also may include more polar groups such as aliphatic alcohols, carbonyls, esters, ethers, etc.

If your molecule has no or few polar groups, your job is usually pretty easy. Any reversed phase column (C18, C8, etc.) would be a good place to start. With an organic/water mobile phase, these columns retain molecules based on the number of carbons in the molecule – more carbons means more retention. You simply adjust the organic content to control retention – less organic means less retention. Adjustment of the pH is not necessary, unless it is needed for other components in the sample. Note: the silica (HILIC) phase is not a good choice, since neutral compounds show very little retention.

Getting Started

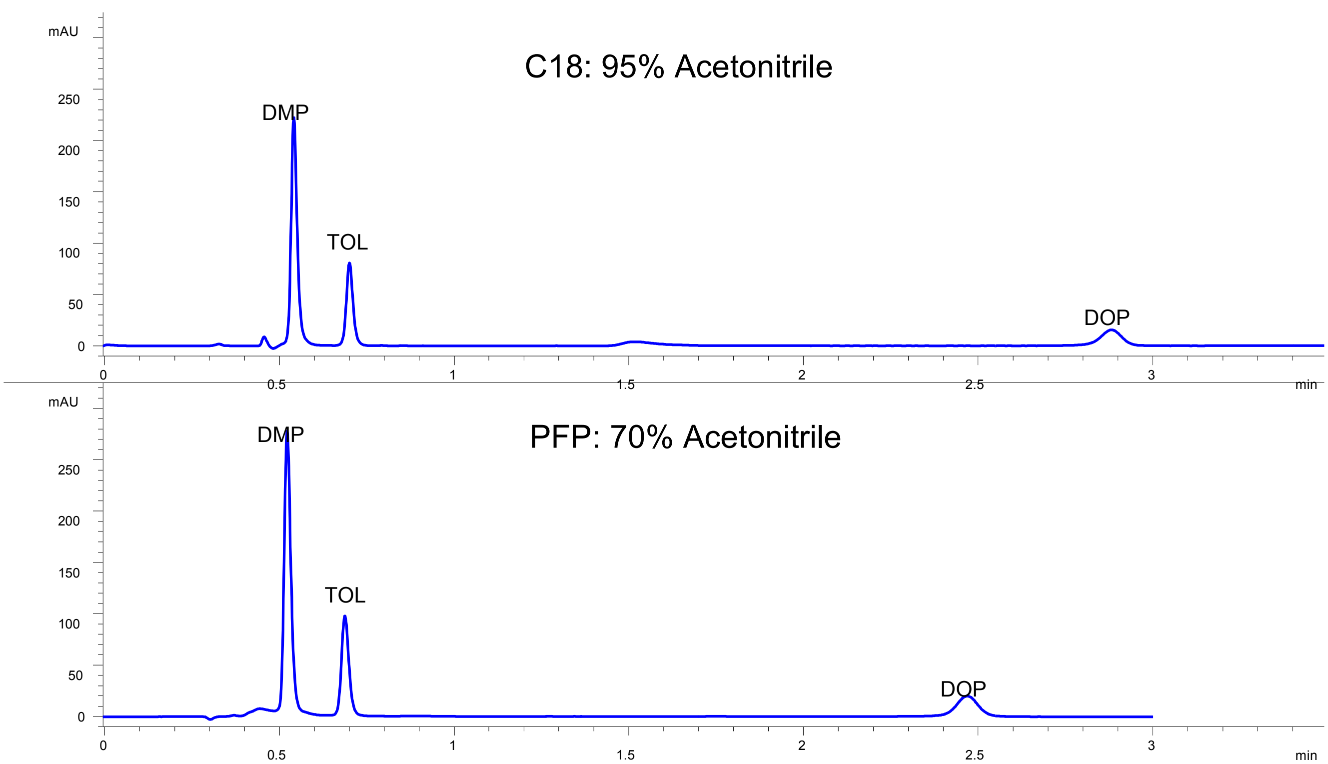
Column: choose either C18 or PFP .

Mobile phase: start with 90:10 organic:water, and adjust as needed to produce desired retention. Either acetonitrile or methanol may be used, but realize that there may be some selectivity differences between these two solvents, and less acetonitrile is needed to produce the same retention.

Sample solvent: dissolve in mobile phase when possible, or match as closely as possible. If your mobile phase has less than 50% organic, your sample solvent should contain about 50% organic or less. Avoid 100% organic when possible.

Example

In this example, we are separating dimethyl phthalate (DMP), toluene (TOL), and dioctylphthalate (DOP). The two columns produce the same retention order, but C18 is more retentive and requires more acetonitrile.



Merlin Bicking, Ph.D. Senior Analytical Scientist at ACCTA, Inc., is a guest contributor to the KAS Newsletter

For additional information on ACCTA, Inc. visit, www.accta.com

This information is a summary from a recent publication:

M. Bicking, R. Henry, "A Global Approach to HPLC Column Selection using RP and HILIC Modes: What To Try When C18 Doesn't Work," LCGC North America, Vol.28 (3), 234-244.

You can read a short summary or view the digital version .