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The WCAS Analytical Digest

WEST COAST ANALYTICAL SERVICE, INC.

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ICPMS, an alternative to Heavy Metals by USP <231>

Heavy metals by USP <231> generally requires large samples (2 g) for a 10 ppm detection limit, too much for some very expensive drugs produced in small quantities such as proteins and peptides. In addition, <231> is a limit test and as such is not quantitative or qualitative. While it detects elements with insoluble sulfides [lead (Pb), mercury (Hg), bismuth (Bi), arsenic (As), antimony (Sb), tin (Sn), cadmium (Cd), silver (Ag), copper (Cu), molybdenum (Mo)], it does not identify which element is present.

Alternatives include some of the spectroscopic techniques such as GFAA, ICP-OES, and ICPMS. Of these, ICPMS is the method of choice. ICPMS is

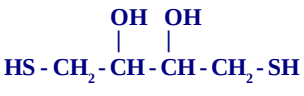
- the most sensitive technique, having the lowest detection limits (0.01-1 µg/L in solution),
- a fast, multi-element technique (up to 60 elements in a 2 minute scan)
- capable of definitive, multiple isotope identification (less prone to interferences)

Typical detection limits for ICPMS are 0.01-1 µg/L (ppb) in solution. Sample preparation usually consists of simply diluting the sample in 1% nitric acid although we offer a variety of digestion techniques. Since ICPMS is the most sensitive technique for trace elements in solution, a 10 mg sample will give detection limits for most elements in the range of 0.01-1 µg/g (ppm). Also, more than 60 elements can be determined in the same analysis which is a big advantage over GFAA where each element is done separately.

Some proteins can also bind metals and therefore the metal assay is important for QC. Here again, using only very small samples, ICPMS can accurately assay the metal content. We offer a variety of options including single element, multi-element (including just the <231> responding heavy metals), and complete (>60 element) metals screens.

Dithiothreitol

1,4-Dithiothreitol (DTT) is commonly used in biochemical research to protect sulfhydryl groups from oxidation or reduce disulfide linkages to free sulfhydryl groups in proteins and enzymes. DTT is also known as Cleland's reagent.



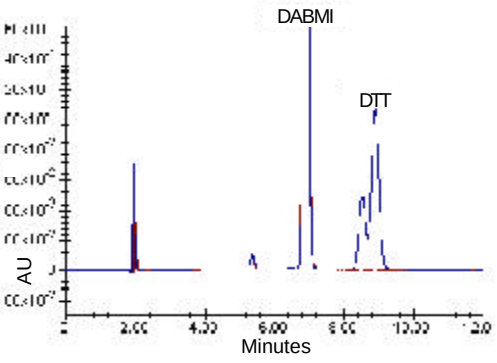
DTT

1,4-Dithiothreitol

(1,4-dimercapto-2,3-butanediol)

Dithiothreitol by HPLC

To determine DTT by HPLC, the DTT is first derivatized with a maleimide (DABMI, Molecular Probes). The reaction involves addition of the thiol across the maleimide double bond to form a thioether.[1] This forms a derivative with a strong



visible absorption at 419 nm. The reagent and the DTT derivative are separated on a C18 reverse phase column with an acetonitrile:water eluent (chromatogram above). Detection limits are approximately 0.1 µg/mL.

Two peaks are observed in the chromatogram probably due to the diastereomers produced from the racemic standard which has two optically active centers. This test should work equally well for other compounds with sulfhydryl groups.

[1]Nakashima K, Umekawa C, Yoshida H, Nakatsuji S, Akiyama S. J Chromatogr 414, 11-17 (1987) PN5869.

Quick Quotes

May you live all the days of your life.
Jonathan Swift

Change is one thing, progress is another. "Change" is scientific, "progress" is ethical; change is indubitable, whereas progress is a matter of controversy.
Bertrand Russell

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WCAS Laboratory Tidbits

For more information on any of the following call 562-948-2225 and ask for the person mentioned or Eric Lindsay.

Our web site, www.wcas.com, has a whole new look! You will also note that new technical articles have been added. Many of our SOPs, forms, and manuals are available in PDF format. If you need an Analytical Request Form an editable PDF version is available under *Forms*. Visit the site frequently as it is always changing and growing.

New art work in front of our building! Santa Fe Springs requires a percentage of the cost of buildout to be applied to art. We don't have the slightest idea what it is supposed to be.

The WCAS Analytical Digest can be emailed to you as a PDF attachment instead of being mailed through the post office. Just email your request to eric.lindsay@wcaslab.com and we will make the changes. This way you can get your delivery early, can save it on your hard drive for easy access, and we save costs!

The AAPS and SupplySide West shows went very well for us. It was a pleasure meeting some of you in Toronto and Las Vegas. As usual, we will keep you updated as to when we will be at future shows.

The City of Santa Fe Springs gave commendations to Samina Hussain, Charlie Jacks, and Mary Hammons for coming in over the Labor Day weekend to respond to a drinking water emergency. They worked most of the night and into the next day testing tap water to show that a contaminant had been eliminated and that citizens could again feel safe in drinking their water. We would also like to thank them again for helping out our local community. Each of them received a plaque suitable for framing. As a matter of fact, they are now framed and on their office walls!

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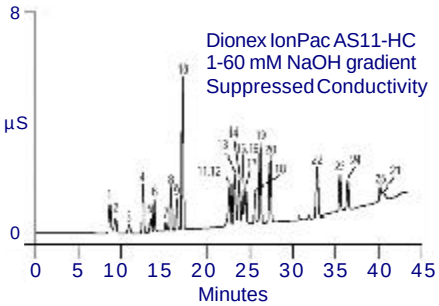
Organic Acids

Organic acids can be analyzed by gas chromatography (GC), liquid chromatography (HPLC), or ion chromatography (IC). The separation of free carboxylic acids by GC works for the smaller, monocarboxylic acids, although the polarity of the molecule makes the peak tail on nonpolar columns. For the best chromatography, organic acids are generally derivatized to an ester. HPLC works better on larger acids, but the specificity may decrease with the size of the alky group and many acids behave better as ester derivatives. Ion chromatography can measure a wide variety of organic anions in aqueous solution without derivatization.

Organic Acids Screen

The chromatogram below shows the separation of 25 organic and inorganic anions by ion chromatography. Each anion is present at 10 mg/L (ppm). This chromatogram was taken from the Dionex Application Note 123. The application involved the analysis of fermentation broths, a very complex mixture of sugars, alcohols, glycols, organic acids, nutrients, cells, and cell debris.



1. Lactate
2. Acetate
3. Propionate
4. Formate
5. 2-keto-D-gluconate
6. Pyruvate
7. Valerate
8. Chloroacetate
9. Bromate
10. Chloride
11. Phenylacetate
12. Bromide
13. Nitrate
14. Malate
15. Methylmalonate
16. Carbonate
17. Malonate
18. Maleate
19. Sulfate
20. Oxalate
21. Trichloroacetate
22. Phosphate
23. Citrate
24. Isocitrate
25. Pyrophosphate

This approach has some drawbacks. The gradient requires long run times, high concentrations of one or more anions can overload the column, some components elute very close to others, and the gradient produces a sloping baseline compromising some detection limits. In many cases, testing for specific organic acids may best be performed in an isocratic run on different columns. But overall this approach provides an excellent screen for a large number of organic acids that are difficult to measure any other way.

EPA 1664

Unless explicitly specified by a client we will be using EPA 1664 in place of EPA 413.1, 413.2, and 418.1 on all future samples received by WCAS. We will also be using EPA 1664-SGT when appropriate. EPA 1664 uses hexane instead of Freon so our charges will also be less due to the tremendous difference in costs.

Calcium Carbonate

Calcium carbonate is used in a large variety of products: antacids, adhesives, cement, paint, plastics, paper, pharmaceuticals, mineral supplements, and much more.

Calcium carbonate is one of the materials we test most frequently. Originally we started when CA Prop 65 limited the lead (Pb) in calcium supplements. Now we perform all kinds of tests for calcium carbonate, from assay to impurities, for producers and users. The methods we use are summarized below:

Tests per USP, EP, FCC, ACS, and others

Identification: wet chem. for Ca &carbonate
Assay by EDTA titration
Fluoride by ion selective electrode
Heavy Metals by sulfide precipitation
As, Ba, Fe, and Pb by various wet methods
Organic Volatile Impurities by headspace
Chlorides and Sulfates by wet methods
Magnesium & Alkali Salts by wet methods
Acid Insoluble Substances by wet methods

Tests per WCAS Methods

Lead (Pb) for CA Prop 65 by ID-ICPMS
>60 Element Screen by ICPMS
As, Hg, Cd, etc. by ICPMS