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The WCAS Analytical Digest

WEST COAST ANALYTICAL SERVICE, INC.

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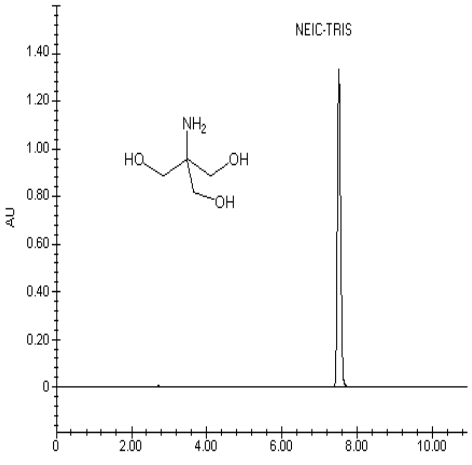
TRIS by HPLC

TRIS, also known as tromethamine, is a commonly used buffer and component in pharmaceutical formulations. This compound does not have a good UV-Vis chromophore for HPLC, and therefore it must be derivatized for quantification by UV absorbance. We have adapted a method from Weng & Ghodbane (J. Liq. Chrom. & Rel. Technol. 22(3):477-484 (1999)) for derivatization of TRIS with 1-(1-naphthyl)-ethyl isocyanate (NEIC). Samples are either dissolved in methanol, or for water containing samples, dried with nitrogen after addition of methanol, and reconstituted in methanol for direct derivatization with this reagent.

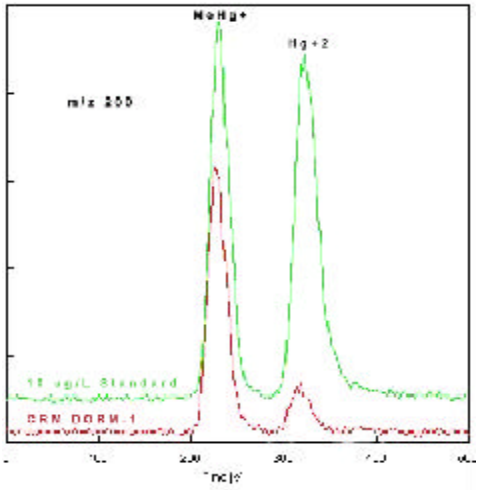
We have also used benzoyl chloride under the same conditions to derivatize TRIS. The TRIS derivatives are separated from other reaction products in an acetonitrile:water gradient on a C18 column. NEIC derivatives are measured at 280 nm (see web site for chromatogram) and benzoyl derivatives at 254 nm.

We were able to generate a linear standard curve for TRIS between 20 and 200 ppm in water. The estimated detection limit of TRIS in water is 1 ppm. We have also derivatized ethanolamine in methanol with NEIC, and many other amines with poor chromophores may work well with this technique. Volatile primary amines in methanol or nonvolatile primary amines in water or methanol should work in this assay. Call Jack Northington with questions.



Methylmercury

We've finally found a methylmercury method that we like. It is based on a method by Chiou, et al[1]. Biological tissue samples are extracted into a mixture of L-cysteine and 2-mercaptoethanol by microwave digestion. Mercury compounds are separated on a C8-column using the same mixture of L-cysteine and 2-mercaptoethanol. An ICPMS system with conventional pneumatic nebulization is used as the HPLC detector.



This method has been applied to the National Research Council of Canada Reference Material DORM-1 Dogfish Muscle. Concentrations of 0.70±0.03 µg/g methylmercury and 0.12±0.03 µg/g inorganic mercury have been found in very good agreement with the certified value for total mercury of 0.800±0.074 µg/g and a value of 0.721±0.033 µg/g for methylmercury determined by Beauchemin, et al[2].

Using this method a detection limit of about 0.3 µg/L for standard solutions is achievable. Detection limits for tissue samples are about 30 µg/kg. The option of vapor generation might give better detection limits by a factor of 10.

The chromatograms above show a 10 µg/L standard and an extract of DORM-1.

[1,2] Please visit our web site for references and more information by searching for methylmercury.

Quick Quotes

What a father says to his children is not heard by the world, but it will be heard by posterity.
Jean Paul Richter

The time to repair the roof is when the sun is shining.
John F. Kennedy

WCAS • 9240 Santa Fe Springs Road • Santa Fe Springs, CA 90670 • 562.948.2225 • fax 562.948.5850

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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.

We will be exhibiting at the AAPS Annual Meeting and Exposition from November 11th to the 13th at the Metro Toronto Convention Centre in Toronto, Canada. Our booth number will be 364 and we will have another nifty giveaway!! We will be available for technical, pricing, or any other questions that you may have. Hope to see you there!

The SupplySide West International Trade Show and Conference will be in Las Vegas on December 5th and 6th. We will be in booth 1273 and look forward to meeting a lot of you.

It was a pleasure meeting some of you at the Biotechnology Conference in San Diego this past June. Our booth gift was an Olympus Digital Camera and the winner was Dr. Jayanthi Rajagopalan from Akzo Nobel in Research Triangle Park. We hope he enjoys his prize!

Please remember to visit our web site at www.wcas.com as we add technical articles frequently. You can also request quotes and to be added to our mailing list from the web site.

The Association of California Water Agencies is holding its Fall Conference November 19th to the 22nd. We will be exhibiting from November 20th to the 21st at the Disneyland Hotel. Our arsenic speciation by IC-ICPMS as well as methylmercury will be of interest to many of you. Also, testing of water treatment chemicals is a specialty of ours. Much of the testing is wet chemistry just as for many of our pharmaceutical tests that we perform. Fax your list of chemicals and specifications over and we can put a quote together for you. We hope to see you there!

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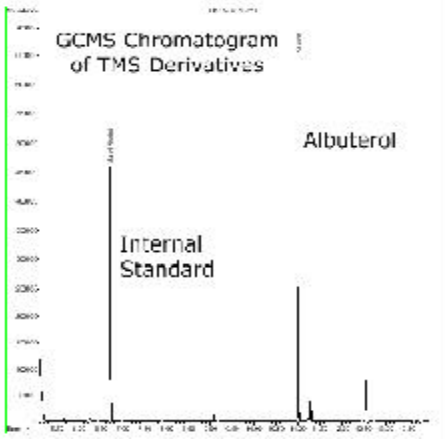
Albuterol by GCMS

Albuterol (or albuterol sulfate) is used for the treatment of asthma. It's generally taken as either tablets or from an inhaler. WCAS provides testing for albuterol according to USP monographs as well as in inhalers.

There are at least two methods for determining the dose of Albuterol from an inhaler. USP sets standards for albuterol tablets but not inhalers. The method for assaying tablets is based on reverse phase HPLC with an ion pair reagent with detection using UV absorbance at 276 nm. For legal cases, we prefer the following method using GCMS because it provides definitive identification of the albuterol active ingredient as well as the dose assay.

GCMS Method

First, the samples are prepared by removing the canisters from the mouthpiece and dispensing five actuations from each sample into water containing salicyl alcohol as an internal standard. An aliquot of the water is then evaporated to dryness and the TMS derivatizing agent is added to the residue. The solutions are then analyzed by GCMS. Quantitation is performed using the internal standard method against a five-point initial calibration. Additionally, one sample is prepared in duplicate. Results are given as free albuterol and albuterol sulfate per actuation from the valve.



Example Report:

Sample	Micrograms/Actuation as Albuterol	as Albuterol Sulfate
Conrol	100	120
Sample	115	138
Sample Duplicate	103	124
Detection Limit	4	5

Sample	Actuations taken	Weight before (g)	Weight after (g)	grams lost per Actuation
Control	5	28.2366	27.8609	0.0751
Sample	10	28.4295	27.5794	0.0850