Products

♦ Bioanalytical Systems, Inc. has just introduced a unique system, CHADS for Vials™, for labeling lab samples legibly, quickly and easily.

Chads are thin, flexible, pre-numbered tags, printed on waterproof and solvent-resistant material. They use no adhesive and are available in a variety of colors.

Chads adhere to the vials using friction. The hole in the Chad is slightly smaller than the diameter of the vial, so when the vial is inserted into the hole, the Chad is stretched tightly around the vial. When the vial is lifted, the Chad clings to its neck, whether the vial is capped, stopped, open or closed. The Chads come in sheets, so you can label one vial at a time, or label an entire rack of 192 vials (enough for two 96-well plates) in one motion. Load them into a fraction collector, a centrifuge, autosampler or freezer; when the vials are thawed, the Chads will be just as legible as the day they were applied. You will have no more tedious hand labeling or smudged, illegible labels with the Chads system.

For more information or to request a free sample and a copy of the company's 3-minute demonstration CD, go to ***www.culex.net***.

♦ Automated Serial Blood Sampler for Research Animals

Bioanalytical Systems, Inc. has introduced the Culex®Automated Blood Sampler (ABS). The system provides for robotic sampling of blood (10-250 µL per sample) from cannulated rats for research in pharmacokinetics, drug metabolism and drug safety assessment.

Four freely-moving animals are housed on a wheeled cart with refrigerated microfraction collectors, as well as collectors for urine and feces. Sterilized tubing sets maintain aseptic transfer of blood and replacement saline. A notebook computer enables the user to program independent collection protocols for each animal. Time and volume of blood draws are designated, along with the option to dilute the sample with specified volumes of heparinized saline. Food and water are provided *ad lib* and simultaneous drug infusions, microdialysis or implanted biosensors are feasible. Samples are collected in vials compatible with 96-well plates for centrifugation and sample preparation. Both serum and plasma can be managed. The blood sampling process does not stress the animals. For example, sleep is not disturbed. Animals can be maintained on the system for five days or more with excellent catheter patency. ***(www.culex.net)***

♦ Brain Microdialysis Probes for Transgenic Mice

New brain microdialysis probes with intracerebral cannulae have been developed by Bioanalytical Systems, Inc. (BAS) for use in transgenic mice, rats and other rodents. The MBR line of probes expands the offerings of microdialysis probes available from BAS for studies in brain, dermis, bile, blood vessels and other tissues.

These lightweight probes are secured in the guide by a low-insertion force elastomeric fit which accommodates the fragile skull of a mouse. Their small size makes them ideal for implanting multiple probes in a single animal. Guide cannulae can be implanted as close as 3.2 mm on center. Probes implanted without a guide can be placed within 2.4 mm of one another. Multiple MBR probes can be implanted in a rat, and depending upon the targets, two MBR probes may even be implanted in a single mouse.

MBR probes are available with 1 or 2 mm membrane lengths. The membrane offers a 38,000 MWCO (molecular weight cutoff) and is suitable for a broad range of neurotransmitters and drugs. A 24-karat gold coating on both the probe and guides provides an inert surface compatible with tissues and biological fluids.

In the LC Literature

Compiled by:
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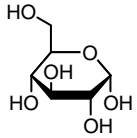
♦ Carbohydrates in Honey

Honey Carbohydrate Analysis by HPLC, with Electrochemical Detection, using a Ni-Cr Alloy Electrode

M.I. Mora and J.M. Marioli, J. Liq. Chrom. & Rel. Technol. 24 (2001) 711-720.



α-D-Glucose

Analysis of carbohydrates in honey, particularly those present as minor components, may be useful for the determination of the floral origin of the honey, and thus is of great value for investigating adulteration and fraud. The standard LCEC detection scheme for carbohydrates is separation on an anion-exchange column at high pH, followed by pulsed amperometric detection (PAD) at a gold or platinum electrode. Pulsed detection (alternating cleaning potentials with detecting potentials) is needed to prevent reduced response due to electrode fouling, but has the disadvantages of reduced sensitivity and the need for specialized equipment.

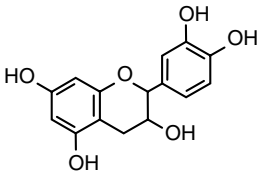
This paper investigates the use of a Ni-Cr (80:20) alloy electrode held at a constant potential (+500 mV vs. saturated calomel, equivalent to +550 mV vs. Ag/AgCl) to detect carbohydrates. Honey samples were diluted in water and filtered, then separated on a Hamilton RCX-10 column with a 0.1 N NaOH mobile phase. The authors speculate that detection is based on the cata-

lytic oxidation of sugars at an oxide-hydroxide layer on the surface of the electrode. Unlike gold and platinum electrodes, and similar to a Cu electrode, the Ni-Cr electrode showed no reduction in response for at least 21 days. Response was linear between 1 mM and 1 µM concentrations.

♦ Antioxidants in Tea

Identification and Determination of Polyphenols in Tea by Liquid Chromatography with Multi-Channel Electrochemical Detection

H. Long, Y. Zhu, T. Huang, L.A. Coury, and P.T. Kissinger, J. Liq. Chrom. & Rel. Technol. 24 (2001) 1105-1114.



(+) - Catechin (C)

Tea varieties contain a spectrum of antioxidants, many of which display anti-cancer properties in the laboratory. LCEC can be used to characterize these compounds and how they vary with tea variety, growth conditions, and processing. The following catechins were determined from hot-water extracts of tea: catechin, epigallocatechin, epicatechin, epigallocatechin gallate, and catechin gallate. Separation was on a 150 x 2 mm C_8 column, with an isocratic mobile phase composed of 20 mM monochloroacetic acid (pH 2.8) and 11% acetonitrile. Detection was by a BAS epsilon detector equipped with a radial-flow, 4-glassy-carbon array working electrode.

The detection limit for all analytes was about 1 ng/mL at a potential of +900 mV (vs. Ag/AgCl). The results were linear between 5-1000 ng/mL. Multi-channel detection proved useful for identifying the particular compounds: using the peak areas obtained at two different applied potentials, a unique ratio was obtained for each compound.

Technical Note

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♦ Determination of Histamine in Rat Microdialysates with Automated Precolumn Derivatization

Histamine (***F1***) is a heterocyclic primary amine known for its mediation of allergic response (1). Stored in mast cells and peripheral blood basophils, its release plays a role in allergy, inflammation, gastric acid secretion, microcirculation and neurotransmission (2). Its determination in experimental animals is of interest to pharmaceutical development in all of these areas.

Histamine has no significant chromophore or electrophore, making detection a challenge. Methods exist using radioenzymatic assay, bioassay, radioimmunoassay, gas chromatography, fluorometry, and LCMS (1-3) but these suffer from either low sensitivity/specificity, or require highly skilled operators. From a practical standpoint, the most successful assays rely on precolumn derivatization followed by liquid chromatography and either fluorescence or electrochemical detection.

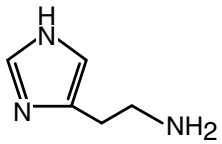
T1

Derivatization and injection protocol for BAS Sample Sentinel autosampler.

Derivatization		
Template		1
Description		Dilution
# Dilution Cycles		1
Load	100 µL	Solvent S1
Pickup	6 µL	Reagent A
Add	4 µL	Sample
Mix For	1.0	Minute
Overlap Enable		No
Injection		
Injection Volume		10 µL
Cycle Time		8.9 Minutes
Injection Type		Pull

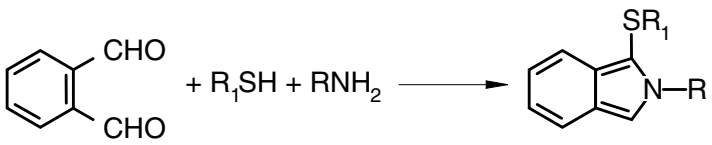
F1

Structure of histamine.



F2

OPA derivatization of a primary amine.



Method

The method used here, modified from (3), relies on derivatization with *o*-phthalaldehyde (OPA). In the presence of a thiol, OPA rapidly reacts with primary amines to form alkylthiolisoindoles (***F2***), which are electrochemically active, fluorescent, and UV-absorbing. This is the basis of the BAS Amino Acid Kit (MF-8905), which provides reagents and protocols for derivatizing and separating the neurotransmitter amino acids. I used only Reagent A from this kit, which combines OPA, an appropriate thiol, and pH control, for rapid derivatization and the formation of a stable product.

The derivatization steps outlined in ***T1*** took slightly more than five minutes to perform on the BAS Sample Sentinel autosampler. Since the chromatographic run lasted 15 minutes, a cycle time of 8.9 minutes on the autosampler allowed for subsequent samples to begin derivatizing, and be ready to inject about 30 seconds before they were required by the chromatograph.

Derivatization took place in 300 µL glass autosamper vials (MF-5270) with teflon-lined crimp caps (MF-5272). These vials may be used in BAS fraction collectors, providing a convenient way to collect and process samples with minimal handling. Resusable adapters (SS-0025) are required to use these vials in the autosampler. It is important that all samples and standards have

the same volume, as differing volumes would cause dilution errors when Reagent A is added. I used a 20 µL sample volume, with a 4 µL addition of Reagent A.

Results

OPA will derivatize all primary amines in the sample, so it's important that the chromatographic separation resolve histamine from any endogenous amines and amino acids. Good resolution was achieved, both with standards (***F3***) and with a microdialysate sample from rat anterior hypothalamus (***F4***). The determination of histamine by precolumn derivatization with OPA appears linear: a calibration curve for histamine standards is shown in ***F5***.

The limit of detection for histamine, based on a signal-to-noise ratio of 3, and the standards separation of ***F3***, was about 1 nM (0.01 pmoles injected). For real-world samples, where baselines have slopes and analyte peaks are crowded by nearby peaks, the limit of quantitation was about 15 nM (0.15 pmoles injected).

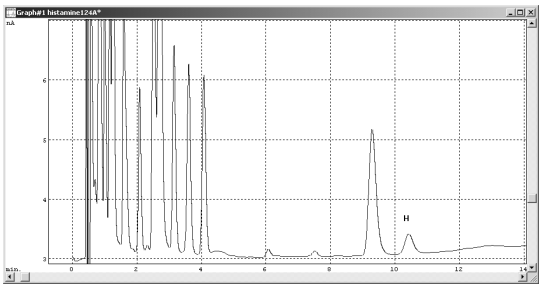
The automated determination of histamine in microdialysis samples presented here has several advantages. By using the same vials, you can easily collect and process samples while minimizing the extra steps (and possibilities for errors) involved in manual fluid transfers and manual derivatization. Keeping the odiferous (thiol) reagent in sealed vials dramatically reduces the stench. (No one came into my lab looking for gas leaks, as has happened in the past when I performed manual derivatizations.) And the autosampler performs each derivatization just before injection, allowing the queued samples to remain in their natural state as long as possible.

T2

Chromatographic Conditions.

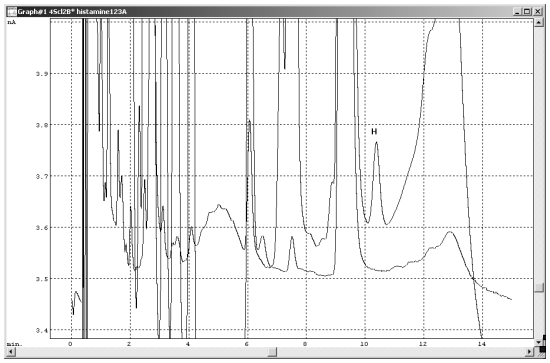
System: BAS 592e liquid chromatograph
Electrode: Radial-flow 3 mm glassy carbon
Potential: +700 mV vs. Ag/AgCl
Column: 3 x 100 mm, 3 µm C_{18} (MF-8954)
Column Temperature: 35 °C
Mobile Phase: 5.9 g $NaH_2PO_4 \cdot H_20$, 1.1 g Na_2HPO_4, 186 mg EDTA brought to 500 mL H_20, adjusted to pH 6.4. Combine 480 mL with 240 mL acetonitrile and 280 mL methanol.
Flow Rate: 1 mL/min
Loop Size: 20 µL
Injection Volume: 10 µL

F3



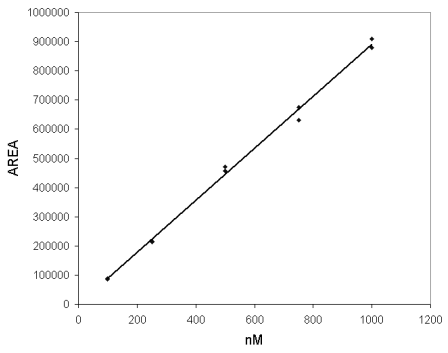
Separation of 100 nM each histamine (H) and 20 common amino acid standards (Ala, GABA, Arg, Asp, Asn, Gln, Glu, Gly, His, Ile, Leu, Lys, Met, Phe, Ser, Tau, Thr, Trp, Tyr, Val).

F4



Separation of histamine (H) from rat anterior hypothalamus microdialysate. Upper trace is dialysate, lower trace is perfusion fluid (Ringer's solution) alone. Peak corresponds to 50 nM histamine.

F5



Linearity of histamine standards. $R^2 = 0.997$

References

1. *D. Egger, G. Reisbach, L. Hultner, Simultaneous determination of histamine and serotonin in mast cells by high-performance liquid chromatography. J. Chromatogr. B 662 (1994) 103-107.*
2. *C.M.C.J. van Haaster, W. Engels, P.J.M.R. Lemmens, G. Hornstra, G.J. van der Vusse, Rapid and highly sensitive high-performance liquid chromatographic method for the determination of histamine and 3-methylhistamine in biological samples. J. Chromatogr. 617 (1993) 233-240.*
3. *D.R. Lowe, C. March, J.E. James, H.T. Karnes, A high performance liquid chromatographic method for histamine in plasma using solid phase extraction and fluorescamine derivatization. J. Liq. Chromatogr. 17 (1994) 3563-3570.*
4. *T.B. Jensen, P.D. Marley, Development of an assay for histamine using automated high-performance liquid chromatography with electrochemical detection. J. Chromatogr. B 670 (1995) 199-207.*

Miscellany

♦ Errata

Listed below are corrections to Current Separations Vol. 19, No. 2 (Dec. 2000). The article in its corrected form is found at *www.currentseparations.com/issues/19-2/19-2b.pdf*.

Page 41, second paragraph should read: Since...pre-IMER (ChO/peroxidase and ChO/catalase, respectively) set prior... The H_2O_2 is then converted by peroxidase/catalase to H_2O... The extent of decomposition of H_2O_2 in the pre-IMER may vary based on the activity of the catalase... ...interference has been shown to be minimal as long as the catalase remains active (***F3***).

Page 42, top of page: ...BAS consists of ChO/catalase...

♦ Fifth Workshop on Biosensors and Biological Techniques in Environmental Analysis

May 31-June 4, 2002
Cornell University
Ithaca, New York USA

This will be the Fifth Workshop on Biosensors and Biological Techniques in Environmental Analysis, organized by the International Association of Environmental Analytical Chemistry (IAEAC) and Cornell University. Previous Biosensor Workshops were held in Paris, France in 1994, in Lund, Sweden in 1996, in Las Vegas, Nevada (USA) in 1998, and in Mao, Menorca (Spain) in 1999. The meeting will be an opportunity for scientists in all areas of environmental analytical chemistry to discuss special analytical techniques of common interest.

♦ Third International Symposium on Microdialysis in Drug Research and Development

June 19-22, 2002
Minneapolis, Minnesota, USA
Preceded by a course on Basic and Advanced Aspects of *In Vivo* Microdialysis. June 18-19, 2002

The application of microdialysis is rapidly expanding in the field of pharmacokinetic and drug disposition studies. Both the First and Second International Symposia on Microdialysis in Drug Research and Development (1998, Netherlands and 2000, Sweden) proved to be valuable to attendees, allowing opportunities for in-depth discussions of the microdialysis technique and providing an overview of recent advances involving microdialysis.

The Third International Symposium will be held in Minneapolis, Minnesota, USA. The general topics to be addressed are listed below. Poster and podium sessions will be held in which participants may present their research. The symposium will be preceded by a Microdialysis course planned for June 19, 2002.

Proposed Sessions

Analytical and methodological aspects of microdialysis • Clinical microdialysis • Microdialysis in the skin and subcutis • Microdialysis in ADME research • Applications in preclinical drug development

Further Information

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www.pharmacy.umn.edu/resgrad/pceutics/thirdintsymp/index.html

♦ Second North American Bioanalytical Forum: NABF 2002

September 29-October 2, 2002
Lawrence, KS/Kansas City, MO
(Site to be announced.)

The objective is to bring together bioanalytical chemists from the pharmaceutical and biotechnology industries, contract research organizations, government laboratories and academic scientists in an atmosphere that encourages the exchange of information and informal discussion on the latest scientific advances in drug bioanalysis and related areas.

To foster and maximize scientific interchange while maintaining the informal nature of the meeting, registration will be limited to 130 individuals. The meeting will feature single-stream presentations, poster presentations, discussion sessions and several evening social events. Short courses will precede or possibly follow the scientific sessions, and a tabletop exhibition will provide information on recent developments in analytical instrumentation, products and services.

Members of the Scientific Organizing Committee for the forum are being named at this time. For information about the preliminary program, registration and travel arrangements, visit *www.ku.edu/~pbasymp* or contact Forum Chairman, John F. Stobaugh, Ph.D., 2095 Constant Avenue, Lawrence KS, 66047 USA, *stobaugh@ku.edu*.

♦ New Conference! INTERACT 2002

The Analytical Chemistry Division of the Royal Australian Chemical Institute (equivalent to the American Chemical Society in US) holds its conference once every two years. The next conference (16th Analytical Chemistry Conference) will be conducted jointly with the Environment Division, the Pharmaceutical Science Group of New South Wales (in Australia) of RACI, as well as the Australasian Ecotoxicology Society,and the International Chemometric Society. We have decided to call this conference "INTERACT 2002". We estimate 500-600 local and international delegates will attend the conference.

For more information, visit: *www.pco.com.au/interact2002*.

A trade exposition will be part of the conference. You can get more information about this, from the website above.

♦ BAS participates in the Purdue Botanicals Center for Age-Related Diseases

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Many people have become interested in the health benefits of botanical products. These products are advertised widely in the media. Some of them may have very powerful drug-like effects, but because they are natural products they are not regulated by the Food and Drug Administration (FDA) in the same way drugs are regulated. In many cases there have been no scientific studies either to substantiate or to refute the popular claims made for these products.

The National Institutes of Health (NIH) has established five centers throughout the United States to study botanicals and determine by scientifically valid methods which botanicals are beneficial and in what doses. They are located at the University of Illinois at Chicago, University of Arizona, Purdue University, UCLA, and the University of Missouri at Columbia. These centers will develop analytical methods to validate the identity of the original plant material, and to quantitate the active components in products. Both animal and human clinical testing will be done to determine the physiological and pharmacological effects of botanicals and their component chemicals.

Bioanalytical Systems, Inc. (BAS) is a participant in the Purdue Botanical Center for Age-Related Diseases, which is headed by Dr. Connie Weaver, a renowned researcher in the area of calcium metabolism and bone health. This center is the collaborative effort of a number of investigators and several different institutions including: the University of Alabama at Birmingham, University of Illinois, Rutgers University and Indiana University School of Medicine. There are a number of research areas related to botanicals and age-related diseases. Dr. Weaver is investigating the potential use of isoflavones for the prevention of osteoporosis at Purdue University. Drs. James and Dorothy Morré are studying the use of polyphenols in the prevention and cure of cancer at Purdue University. Dr. Helen Kim is studying the potential of polyphenols for prevention of the neurodegenerative diseases of aging at the University of Alabama. Dr. Stephen Barnes is investigating the polyphenols and inflammatory diseases at the University of Alabama, and Dr. Elsa Janle is studying the effect of green tea catechins on obesity and type 2 diabetes at BAS.

In addition to the research projects, the center has several supporting cores to provide services to the center. These core services include administration, botanicals, statistics, education, analytical services, and *in vivo* testing services. Bioanalytical Systems is contributing heavily to three of these core services. BAS is providing analytical support, *in vivo* testing support and educational support. BAS has developed many methods for the analysis of botanical components. Our epsilon instrumentation is uniquely suited to the analysis of the complex mixtures of compounds found in botanicals, particularly antioxidants. Our *in vivo* testing facilities allow for simultaneous sampling of blood and interstitial fluid, metabolic studies and activity monitoring. For these studies we employ our Culex® automated blood sampling system as well as our microdialysis and ultrafiltration probes, which can sample the interstitial fluid of a variety of tissues. This allows us to do pharmacokinetic studies, distribution studies, physiological and metabolic monitoring. We also have the capability to analyze botanical products for dissolution and stability. In addition, BAS conducts a training course to teach other members of the botanical research community the analytical and *in vivo* sampling techniques. These services are available not only to the Purdue Center for Age-Related Diseases but to all researchers studying botanicals.

More information about the Purdue Botanical Center for Age-Related Diseases can be obtained at the center's web site at *http://fsp.cfs.purdue.edu/bot*.

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♦ BAS Profile

With this issue, we are reviving the BAS Profile *column in Current Separations. Each time, we will feature a staff member (or perhaps more) who has made a significant contribution to science and to the work done at BAS. It is appropriate that our first Profile of this new series is of Dr. Adrian Bott, who has written countless articles, Capsules and newsletters to educate BAS customers, staff and the scientific community.*

Adrian grew up in Brighton, England and matriculated at Cambridge University where he received a Ph.D. in inorganic chemistry. His ambition to study in the U.S. was realized when he had an opportunity to do post doctoral research at Purdue University in West Lafayette, Indiana, working in the Chemistry

Department on a collaborative project with the School of Pharmacy. This proved to be a serendipitous association.

Adrian's research used electrochemistry to look at and measure redox potentials of copper complexes to determine whether there is a correlation between those properties and physiological properties. To facilitate that research, the project participants purchased a BAS 100-A system. Along the way, Adrian became an expert at using the equipment and worked with some BAS people, so when his post-doc studies ended in 1991 BAS seized the opportunity to add him to the staff. Adrian not only found BAS but also met his wife through his research, as she was working in the Purdue School of Pharmacy.

Much of Adrian's career at BAS has been devoted to helping customers understand how to interpret EC data, explaining how that data is best used, and learning and teaching other applications for the EC instruments. He has written countless Capsules, articles and newsletters and has reached thousands of scientists around the world. Every 6 to 8 weeks, Adrian publishes a new EC e-newsletter that is sent to over 2,000 recipients. He also coordinates the LC and *In Vivo* and Analytics newsletters and in all, reaches over 5,000 people on a regular basis.

"The most stimulating aspect of my career at BAS," says Adrian, "has been the constant change and opportunity for new knowledge on an ongoing basis. My work now has a much greater emphasis on pharmaceutical applications, while maintaining support for our epsilon and other EC products."

Adrian and his wife Amy live near West Lafayette in the small community of Fowler, where Amy is active on the Park Board and in other city beautification projects. Adrian has taken the troubleshooting skills he developed at BAS and applies them to home and auto repair projects, always finding great satisfaction in developing new knowledge and skills. He and Amy enjoy taking their two daughters, Katherine and Maggie, ages 6 and 10, for weekends in Indianapolis, where they explore the Children's Museum and the Zoo.