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Application potential for tandem mass spectrometry MS/MS in routine clinical diagnostics

The principles of mass spectrometry were published by Joseph J Thompson (1908 Nobel Prize) in the Philosophical Magazine as long ago as 1899. Application of this technique to clinical diagnostics was unimaginable at the time. Whilst the sensitivity of mass spectrometry was recognised, it was for many decades considered a purely research tool and not appropriate for routine use. The technique was, however, extensively automated in the 1990s, equipping it for the performance of high throughput screening. MS came increasingly to be used in the clinical chemistry arena, with neonatal screening and forensic applications being pioneering areas.

Mass spectrometry in diagnostics

Today, MS has proven its appropriateness for routine use and is ideal for high throughput applications in clinical diagnostics where the sensitivity, specificity and simplicity of the technique can be put to use.

Following on from the established areas of neonatal screening and forensics, MS is increasingly being used for high throughput assays of drug concentrations in human matrices, replacing conventional immunoassays which have proved unable to overcome the problems of cross-reactivity and the time scales associated with developing antibodies for new drugs.

Tandem mass spectrometry is an extremely important tool in neonatal diagnostics. A number of congenital, in some cases life-threatening metabolic disorders are diagnosed with this method within a very short time, ensuring that newborns can get the treatment they need right away. LC-MS/MS can detect around 40 different clinical disorders in neonates on the basis of a minimal amount of whole blood blotted on filter paper.

Routine diagnostics requirements

However, the adoption of MS for routine diagnostics revealed a drawback. Whilst commercial sample preparation methods and quality control material were available for other, conventional methods, none existed specifically for MS. Availability of these aids would not only address newcomers' needs but also provide significant support to established, professional MS laboratories following international standardisation.
MS has lost its aura of complexity and difficulty and is used in many laboratories as a welcome alternative for specific applications.

Chromsystems products for LC-MS/MS

The strengths of LC-MS/MS described above can only be realised however if the technique can be applied to relevant parameters and if the sample throughput justifies the investment required. Hence conventional high performance liquid chromatography (HPLC) will maintain its current role for a wide range of routine diagnostic applications. Although Chromsystems is extending its range of activities to include LC-MS/MS, this applies only to selected assays.
These new reagent kits and quality control material are the latest in a long line of high quality, user friendly Chromsystems products. First of these is a kit for the analysis of various immunosuppressant drugs, produced for, and in co-operation with, the Waters Corporation of Milford, MA. Chromsystems is presently developing, and will shortly be launching, its own reagent kits and quality control material for clinical diagnostic applications.

The Application of Mass Spectrometry in Clinical Analysis

Dr. Mike Morris, Business Manager Clinical Mass Spectrometry Group, Waters, UK

Technical Backgroud

Mass spectrometers operate by manipulating the ions formed from analyte molecules in electric and magnetic fields under a vacuum. By carefully controlling the way the fields are applied, it is possible to separate different species according to their mass-to-charge ratios, and thus it is possible to discover many characteristics of the molecules under investigation.

A mass spectrometer can be considered to comprise of four distinct units, those of:

a) Sample introduction: Inlet
b) Ion formation: Ion source
c) Separation of the ions according to their m/z values: Mass analyser
d) Detection of the ions: Detector

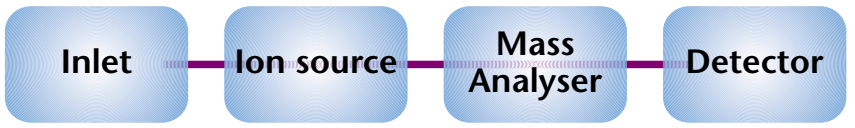

Figure 1: Schematic representation of a mass spectrometer.

Sample Introduction

For the purposes of this article, the samples under consideration will primarily be solution based, and the logistics of introducing the analytes into the mass spectrometer require the manipulation of liquids. In the simplest experiment, a sample might be introduced by a loop injection into a flowing mobile phase. In more complex experiments an HPLC column may be introduced. In both cases, though, the net result is that the sample passes with the mobile phase into the ionisation source of the mass spectrometer. The use of liquid samples introduces the possibility of further efficiencies by utilising the developments and throughput capabilities that have been introduced into modern HPLC instrumentation.

Ionisation

There are a number of different ways of forming ions in the gas phase, but those most suited to the ionisation of the (typically) thermally labile molecules encountered in biological systems are the so-called 'soft' ionisation techniques such as fast-atom bombardment (ref), or the atmospheric ionisation techniques (electrospray, atmospheric pressure chemical ionisation, atmospheric pressure photoionisation, etc). In these techniques, molecular-related ions are typically formed in solution by the addition (or removal) of small solution-based ionic species, such as the proton (H^+), the sodium ion (Na^+) and the ammonium ion (NH_4^+). Thus, if analysing a compound in solution, one might expect to observe a combination of these species, depending on the exact nature of the mobile phase in which the solution has been prepared, and the conditions within the ionisation source (e.g. temperature, gas flows, etc).

Mass Separation

There are a variety of different mass analysers in use that can be used to separate the charged particles by manipulation in electric and/or magnetic fields. For the purposes of this article, we will only consider quadrupole mass filters in which radio-frequency and direct current voltages are applied to a series of electrodes in order to allow the stable passage of ions of a certain mass-to-charge ratio. In this respect, the quadrupole is directly analogous to a light filter that only allows the passage of a narrow band of wavelengths.

Ion Detection

A number of different ion detection systems are in general use in mass spectrometry, all of which serve to convert the ions to a measurable electrical signal.
In an ideal situation, the signal from the detector will be proportional to the number of ions striking the detector, thus the measurement will be quantitative.
In quadrupole-based instruments, continuous dynode electron multipliers and photomultiplier detectors are typically used.

General

Once the ions are transferred into a tandem mass analyser, it leads to a number of interesting possibilities - firstly, it is possible to separate components according to their individual mass-to-charge ratios. This means that it is possible to introduce mass-selection into the compound detection process.

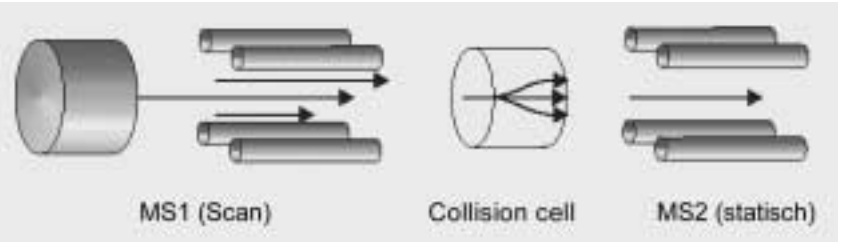

Figure 2: A schematic representation of the parent or precursor ion scan in which the first mass analyser (MS1) is scanned and the second mass analyser (MS2) is set to pass only one specific fragment (MS1 can alternatively be static and MS2 scanning).

In tandem quadrupole mass spectrometers, two mass analysers are connected in series, and are separated by a collision region in which ions selected by the first analyser may be fragmented by colliding them with a high pressure region of neutral gas molecules. This process leads to the formation of structurally significant ionic or neutral sub-units of the mass-selected molecule, and can be used to interrogate the molecular structure, or add an extra dimension of specificity to the analysis of specific compounds.

Applications

Mass spectrometry developed as an analytical method of choice in the petroleum industry, and has, for many years, been viewed with some scepticism in the Life Sciences community. Developments in soft ionisation techniques over the last decade have allowed for the application of mass spectrometry in the direct analysis of biological materials. In addition, developments in electronics and computer technology now make modern mass spectrometers considerably easier to use than in times past, and allow for the performance of complex analytical experiments without the requirement for years of operator training.

Research

Mass spectrometry is used extensively in the discovery and development of new drugs, as well as being used routinely during toxicological studies and clinical trials. It seems natural that mass spectrometry (LC-MS and LC-MS/MS) should evolve into a tool useful in the clinical analysis arena for the detection and quantification of physiological compounds, including administered drugs and their metabolites.

Diagnostics

In the last few years, mass spectrometry has been applied to a growing number of problems of clinical significance. Perhaps the most significant breakthrough has been in the application of mass spectrometry for high-throughput (in mass spectrometry terms) screening for endogenous metabolites from dried blood spots.
Methods have been developed for the rapid determination of amino acids [1], acylcarnitines [2,3] and bile acids [4] with minimal sample preparation. The common features of the molecular structure in related chemical species lead to predictable decomposition within the mass spectrometer that may be used to isolate and measure individual species from complex biological matrices with minimal sample clean-up. Neonatal screening for phenylketonuria, maple syrup urine disease and medium-chain acyl coenzyme A dehydrogenase deficiency (MCAD), to name but a few, is being performed on some million babies around the world annually, and is likely to increase.

TDM

However, the same instrument that is used for high-throughput screening of specific metabolites may be applied to a diverse range of applications, including the analysis of the immunosuppressive drugs - cyclosporine (Cyclosporin A) [5,6], tacrolimus (FK506) [7], and sirolimus (rapamycin) [8].

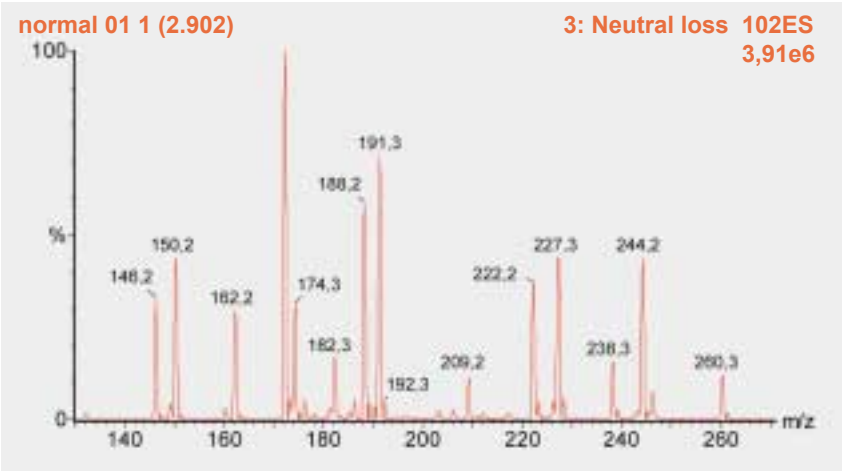

Figure 3: Neutral amino acid profile from a healthy patient

These compounds are administrated to transplant patients, post-transplant, for the lifetime of the patients. There is not a defined therapeutic concentration for these drugs, but individual concentration ranges are required for each patient and each transplant type.

Comparison

Two critical issues for the transplanted patient are organ failure owing to chronic rejection and toxicity as a result of chronic immunosuppression, and it is for these reasons the drugs must be carefully monitored. The current common assays to monitor these medications are immunoassays and HPLC. The current immunoassays are very expensive, and the linear analytical range does not cover the concentrations ranges required by new therapeutic guidelines, thus requiring a sample dilution which delays reporting, and can affect the accuracy of the result. The HPLC assays for immunosuppressives are tedious to perform and very aggressive on the HPLC columns. Improvements in the LC-MS/MS technology has allowed large transplant centres to assay large volumes of all three drugs in an economical manner.

We have, however, only scratched the surface with regard to the number of potential applications of mass spectrometry in clinical laboratories. It is clear that these techniques will play an increasingly important role in clinical investigations as the 21st century develops.

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A Study of Oxidative Stress

Gabriel Erlenfeld, MSc, Chromsystems

Molecular oxygen O_2 is essential for cellular respiration. It is reduced to water as a result of energy production in the mitochondria ($6O_2 + 24H^+ + 24e^- \rightarrow 12H_2O$). Three to 10% of the oxygen is not metabolized to water but converted to reactive oxygen species or ROS. These oxygen species (see Table 1) are in many cases free radicals, or molecules with unpaired electrons which are highly reactive and unstable as a result. Unlike the oxygen we breathe in, which is reduced in the organism in a controlled fashion and has a fixed place in the redox chain of the mitochondria, ROS are "loose cannon" in a reaction and may cause serious cell damage in human tissue through the oxidation of unsaturated fatty acids, proteins or DNA, possibly resulting in significant dysfunction of organs and organ systems.

Radical chain reaction, endogenous and exogenous factors

When free radicals react with other substances, free radicals may be produced which in turn are extremely reactive and attack other molecules. A radical chain reaction occurs. The production of free radicals is not limited to the mitochondrion but may be promoted by other endogenous factors. Granulocytes and macrophages produce ROS in a targeted fashion as part of their immune response. The ROS are released by cell lysis and combat infections caused by bacteria, protozoa, parasites etc.
Natural enzymatic reactions involving oxidases, peroxidases and oxidoreductases also give rise to ROS as by-products. In addition to these endogenous factors, a number of exogenous inductors have also been identified: ultraviolet radiation, X-rays, and exposure to ozone, toxic chemicals (environmental toxins), nicotine and alcohol may promote the production of ROS.

Physiological antioxidative resources

Endogenous production of free radicals is a normal physiological process. Accordingly, the body can tap into a repertoire of antioxidative resources. These may be classified in two groups (Table 2): firstly, antioxidants, which provide a "buffer" against the oxidative pressure of ROS on other molecules by their own slight amenability to oxidation, and secondly, other enzymes that catalyze the conversion of oxidants and reduce the presence of these free radicals. These resources enable a healthy organism to protect itself against free radicals and maintain a healthy metabolic state.

Oxidative stress

Free radicals get dangerous only if the balance between antioxidative and oxidative processes shifts to the detriment of the former. If that happens, antioxidative measures may no longer be able to cushion the oxidative pressure, resulting in progressive damage to fatty acids, proteins and DNA. This state is called oxidative stress. As time passes, an unfavourable interaction of oxidative stress with other factors causes this damage which manifests itself in tissue ageing and degenerative conditions such as cardiovascular disease, cataracts, Alzheimer's, arteriosclerosis, arthritis, chronic inflammatory disease, allergies and so on.

Measuring oxidative status

For preventive management of oxidative stress and its effects, it is necessary to determine the oxidative status of an organism and its tissues. This is important not only for athletes, whose highly elevated metabolism converts more oxygen and therefore produces more ROS, but for all those people whose diet (in particular) and lifestyle (in general) draw heavily on antioxidative resources and whose tissues are exposed to oxidative stress.

Chromsystems products

Chromsystems supplies a range of HPLC reagent kits for the analysis of various markers of oxidative stress. Specially developed, dedicated test kits are available for assaying malondialdehyde or vitamin E, vitamin C or beta-carotene, coenzyme Q10 or glutathione.
A matching HPLC column is available for each product. A selection of controls for quality assurance purposes completes the range.

OXIDANTS		EFFECTS
$^1O_2$	Singlet oxygen	Reactive oxygen species (ROS) which may form free radicals.
H_2O_2	Hydrogen peroxid	
$O_2^{-\cdot}$	Superoxide anion	Free radicals, possess one or several unpaired electrons, highly reactive, may in their turn generate free radicals by reacting with other molecules (radical chain reaction).
HO·	Hydroxyl radical	
ROO·	Peroxyl radical	
RO·	Alkoxyl radical	
NO·	Nitrous oxide	

Table 1: Examples of oxidants.

ENDOGENOUS ANTIOXIDATIVE REPERTOIRE	EFFECTS
ENZYMATIC	
Se-glutathione peroxidase (GSH-Px), a selenium-containing enzyme, catalyzes the oxidation of reduced glutathione (GSH) to oxidated glutathione (GSSG), using up H_2O_2 or organic peroxides in the process.	$ROOH + 2GSH \rightarrow GSSG + ROH + H_2O$
Catalase	$2H_2O_2 \rightarrow 2H_2O + O_2$
Superoxide dismutase (SOD)	$2O_2^{-\cdot} \rightarrow H_2O_2 + O_2$
NON-ENZYMATIC	
Glutathione	Glutathione peroxidase, antioxidant
Coenzyme Q_{10}	Radical scavenger, liopophilic
Liponic acid	Radical scavenger
Uric acid	Uric acid → allantoin + CO_2 (with involvement of singlet oxygen or superoxide)
EXOGENOUS ANTIOXIDATIVE REPERTOIRE	**EFFECTS**
Vitamin C	Hydrophilic radical scavenger, regenerates vitamin E
Vitamin E	Lipophilic radical scavenger
Phytochemicals (carotenoids, flavonoids, sulphides, phenolic acids, phytoestrogens)	Various
Various food additives	Various
β-Carotene	$^1O_2$ scavenger

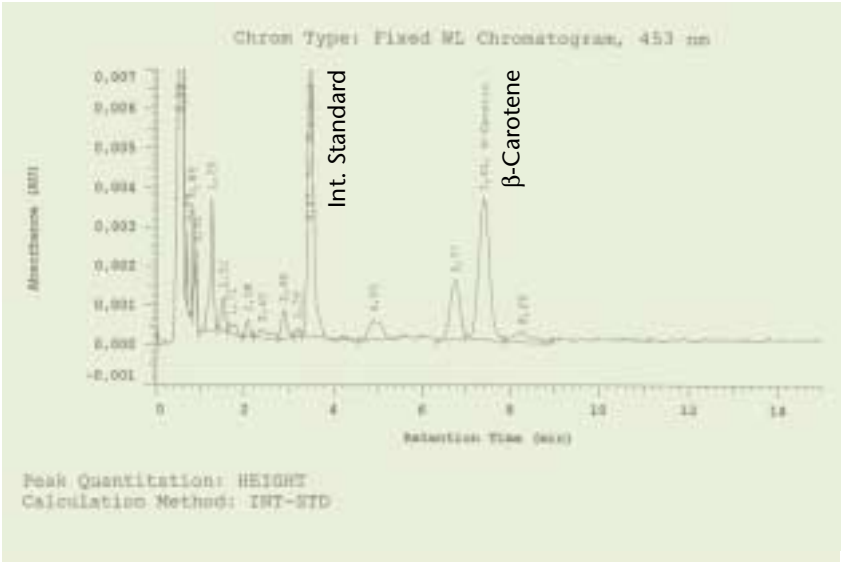
Table 2: Examples of the endogenous and exogenous (acquired from outside the body) antioxidative repertoire.

β-Carotene – more than an Antioxidant

Dr. Hans-Ulrich Koch, Institute for Laboratory Medicine, Berlin

β-carotene is one of 500 naturally occurring carotenoids. It is a plant pigment of a special kind in that it is a precursor of retinol or true vitamin A. β-carotene is a fat-soluble substance which is up to approximately 50% absorbed in the small intestine, depending on the fat content, and broken down in the intestinal mucosa into retinol with the aid of the enzyme β-carotene-15,15`-dioxygenase and the involvement of molecular oxygen. The remainder reaches the liver and fatty tissue where it is stored. β-carotene from these depots is activated as needed, thereby counteracting any excessive vitamin A intake.

β-carotene has other protective properties in addition to its provitamin A function. It increases the activity of the B and T lymphocytes responsible for cellular immune response. It is a potential radical scavenger with its site of action in lipid-containing cell membranes (in conjunction with coenzyme Q_{10} and vitamin E), where its main feat is to inactivate the singlet oxygen that is extremely destructive to the cells. Finally, it helps protect the organism from damage by ultraviolet light. The new recommended daily allowance for β-carotene is stated as being 2 to 4 mg. However, studies have long shown that extreme surplus intake of β-carotene increases the risk of cancer in smokers and reportedly stimulates intestinal polyp growth. This prompted the German medicines evaluation agency (BfArM) to add a warning to prescribing information and patient information leaflets pointing out the elevated risk of cancer in smokers. The final verdict on these risks remains to be issued, however.



The figure shows the chromatogram of a patient sample containing normal levels of β-carotene. The chromatogram was produced using a reagent kit supplied by Chromsystems, Munich. The sample was prepared by rapid combined precipitation and extraction. Subsequent chromatographic assay is done using an isocratic HPLC system with UV/VIS detection.

In view of the substance's broad spectrum of action, monitoring of β-carotene concentrations is recommended. Over the past number of years, β-carotene assay has been recommended as an oxidative status marker, but many experts propose using β-carotene as a marker for intestinal disorders, steatorrhoea in particular. This application is based on β-carotene's fat solubility. Lipid absorption disorders (coeliac disease, Whipple's disease, short bowel syndrome, lactose intolerance, etc.) result in lipid accumulation in the bowel and an associated elevated solubility of β-carotene and other fat-soluble vitamins. β-carotene levels start diminishing just one week after development of lipid absorption disorders and malabsorption (reduced secretion of digestive enzymes, for example in subjects with pancreatic exocrine dysfunction), and this is very easily detected by serum assay. There is a reciprocal correlation between the serum β-carotene concentration and the degree of lipid absorption dysfunction or malabsorption. In many cases, therefore, the unpleasant and methodologically challenging van de Kamer faecal lipid assay method involving three 24-h faeces collection sessions can be replaced by the more rapid and more reliable HPLC method.

Defining a reference range is not unproblematic, however. Whereas photometric assays measure not just β-carotene but various other carotenoids to a greater or lesser extent, HPLC has been shown to provide exact detection of β-carotene. Serum or plasma may be used.
The current reference range for this method is 150–1250 ng/ml. More readings should be taken however in order to verify the reference range.

Literature provided by the author upon request.

Applications for HPLC analysis

Vitamin B1 and B2 Assay in Beer

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Vitamins in the Brewing Process

Barley and malt are high in vitamins which are localized in living tissues of the germ and aleuronic layer and are involved as a prosthetic group in enzyme construction. Among B-complex vitamins, vitamin B_1 is present in barley at a volume of 1.2–7.4 mg/kg of barley dry substance. The vitamin B_2 concentration is raised during germination to 1.5 times the barley content. This corresponds to a concentration of 1–3.7 mg/kg of malt dry substance.

Vitamins are required in addition to numerous other organic substances for yeast proliferation and fermentation. The main vitamins involved are thiamine (vitamin B_1), riboflavin (vitamin B_2), niacin (Vitamin B_3), pantothenic acid (vitamin B_5), pyridoxine (vitamin B_6), biotin (vitamin H) and inositol. Organic growth substances act as functional metabolic elements of important cell enzymes. Niacin is contained in coenzyme I, which acts as a hydrogen carrier in the phosphoglycerine aldehyde dehydrogenase system in the yeast cell metabolism. Niacin is present in coenzyme I in the form of nicotinic acid amide. Pantothenic acid is an element of coenzyme A, which plays a key role in carbohydrate metabolism.

Pyridoxal-5´-phospate is the active coenzyme form of pyridoxine and of key importance in amino acid metabolism. In addition to biotin, which is important for yeast growth and serves as a coenzyme in all ATP-dependent carboxylation processes, thiamine and riboflavin also support yeast metabolism as growth substances. As a carboxylase coenzyme, thiamine plays a key role in carbohydrate metabolism. Riboflavin is involved in oxide reaction processes as a flavin mononucleotide in the prosthetic groups of dehydrogenases. Beer contains only small amounts of thiamine, but brewer's yeast is rich in thiamine as it absorbs this vitamin very rapidly from the wort. Beer however contains larger amounts of riboflavin because this enzyme (as already described) is affected by the malting process and mainly comes from the malt. The yeast absorbs little riboflavin from the wort during fermentation.

Riboflavin supports a number of processes that accelerate beer ageing, as photooxidation of riboflavin promotes Strecker degradation. This results in proliferation of volatile substances, including a fairly large number of longer-chain, in some cases unsaturated carbonyls which are believed to be the main cause of beer's ageing taste. Similarly to Strecker degradation, the oxidation of higher alcohols is accelerated during exposure to light in the presence of riboflavin. Oxidation of the side chains of isohumolones is likewise enhanced by light and the presence of riboflavin. Photoactivation of riboflavin hence results in the production of carbonyl compounds and mercaptans. 3-methyl-2-buten-1-thiol (light taste) also occurs during the formation of numerous mercaptans. However, riboflavin slows down the autooxidation of longer-chain fatty acids to shorter-chain aldehydes.

	Empfohlener Tagesbedarf RDA (recommended daily allowance)	Vorkommen in Pilsner Lagerbier	Bedarfsdeckung durch 1 Liter Pilsner Lagerbier in %
B_1 Thiamin	1,0–1,3 mg	0,029 mg/l	2,3
B_2 Riboflavin	1,2–1,5 mg	0,335 mg/l	20,9
B_3 Niacin	13–17 mg	7,733 mg/l	46,9
B_5 Pantothensäure	6 mg	1,490 mg/l	24,8
B_6 Pyridoxin	1,2–1,6 mg	0,619 mg/l	36,4
B_9 Folsäure	400 µg	86 µg/l	38,2
B_{12} Cobalamin	3–4 µg	0,82 µg/l	27,3

Figure 1: Recommended daily B-vitamin allowance for an average adult and B-vitamin levels in Pilsner beer.

Possible correlations between the vitamin B_1 and B_2 contents of wort and beer and the biochemical processes involved in beer production

A Chromsystems GmbH/Munich reagent kit for assaying vitamin B_1 and B_2 in whole blood was employed for analysis of vitamin B_1 and B_2. Chromsystems is specialized in producing reagent kits for clinical applications. The applications are easy to perform and undemanding in terms of equipment, a simple isocratic HPLC system being sufficient. The application was modified in cooperation with the company in response to the amended sample matrix (mash, wort and beer). Applications for transfer to other available products are currently being developed. This includes adaptation of HPLC analysis for assay of vitamin B_6.

The vitamin assay enables rapid and easy determination of vitamin B_1 and B_2 concentrations and might serve to allow the use of vitamins as analytical indicators in the beer production process.

Vitamin B_1

Thiamine is a colourless, bitter tasting, water-soluble and heat-sensitive vitamin. It consists of a pyrimidine ring bound to a thiazole ring via a methylene group. Thiamine is in the form of the thiamine pyrophosphate coenzyme of the pyruvate dehydrogenase complex, transketolase, phosphoketolase and 2-oxoglutaratdehydrogenase and transfers active aldehyde groups. The associated enzyme activities are reduced as a result of thiamine deficiency. In view of its heat sensitivity, thiamine also has potential as an analytical indicator for thermal effects during the beer production process. The thiamine content could be used in the mash and wort process steps to measure thermal exposure, or in the filling process to evaluate exposure to heat during short-term heating or pasteurization.

Vitamin B_2

Riboflavin is a yellow, fluorescent, slightly soluble in water, bitter-tasting, photosensitive but thermally stable substance. Like all B-vitamins, it contains a heterocyclic ring system, the isoalloxazine system. Riboflavin is rarely present in foods in a free state and is usually protein-bound as a flavin mononucleotide (riboflavin-5-phosphate/FMN) or flavin adenine dinucleotide (FAD), i.e. as a flavoprotein. FMN and FAD are also the derivatives that take effect in the organism as coenzymes of oxidases and dehydrogenases. Riboflavin's photosensitive properties make it potentially suitable for use as a marker for light exposure. Given that a high riboflavin content has a negative impact on the stability of the taste of beer, riboflavin might also be used as a marker to monitor storage conditions and to investigate the impact of the type of container (e.g. bottle colour, type of plastic).

Transfer of medical applications for use in determining B-vitamin levels in wort and beer

For effective use in a production process, an analytical method has to meet certain requirements. It needs to be rapid, easy, reproducible and highly accurate. Reliable analysis of vitamins is a major challenge. Conventional microbiological growth tests are still used today alongside modern chromatographic methods. Few labs have the capacity to perform accredited vitamin analysis. Vitamin analysis by HPLC in wort and beer is particularly difficult because of the very low concentrations involved.

HPLC is successfully used to analyze vitamin-containing alcohol-free beverages, but these methods cannot in most cases be transferred to the wort and beer matrix. Applications for very low concentration ranges are more likely to be found in the medical area where fast and accurate analysis is a must.
A 5-point calibration was performed in a Pilsner wort (12°% by wt) and beer for vitamin calibration purposes. Pure thiamine and riboflavin were supplied by Sigma-Aldrich Germany. The coefficient of variation for the analyses was 3.1% for vitamin B_1 and 1.8 % for vitamin B_2 (n=7).

Examples of vitamin analysis applications in the brewing room

According to Narziß, thiamine, along with niacin, is the most important vitamin for the fermentation process. Some tests have already been performed to investigate the changes in vitamin B_1 and B_2 concentrations during the boiling process, in order to describe the thermal impact of the

boiling process on vitamin B_1 and B_2 content. These studies confirmed that the vitamin B_1 concentration declines as a function of thermal exposure, in this instance through various boiling periods at 100°C. The extent to which tracing the vitamin B_1 concentration during boiling is a suitable marker for thermal exposure must be clarified in further tests. In contrast, levels of riboflavin, a fairly thermally stable vitamin, did not show any changes during the boiling process within the analytical method's range of precision. Figure 2 shows the relative change in vitamin B_1 and B_2 content during the boiling process.

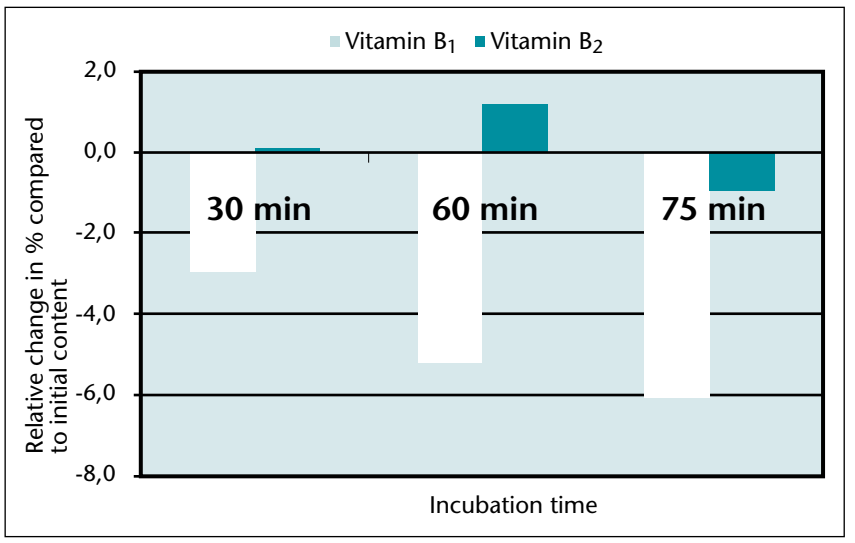

Figure 2: Change in vitamin B1 and B2 content during the boiling process due to thermal impact.

Summary

In cooperation with Chromsystems GmbH, a reagent kit to assay vitamin B_1 and B_2 in whole blood has been successfully transferred to the matrices of mash, wort and beer. The workability of this easy-to-use, precise analytical method enables uncomplicated HPLC assay to determine vitamin levels in the brewing process despite very low concentrations. The B-vitamins in the wort are of key importance firstly as yeast growth substances. Secondly, vitamins such as riboflavin may affect the stability of the beer taste.
In addition, the physicochemical properties of vitamins also make them potentially suitable as analytical markers in the beer brewing process, for example to evaluate thermal exposure. This opens up numerous interesting new areas of application for vitamin analysis in the brewing process.

We wish to thank Chromsystems Instruments & Chemicals GmbH, Munich for the support and cooperation provided.

Outlook

Further studies into vitamin analysis are to be conducted in a research project starting at the Chair of Brewing Technology I with the intention of gaining further insights into the fate of vitamins in the brewing process. An objective is to establish technological means of influencing the process and enhancement technologies that comply with the Bavarian Beer Purity Act.

This text has been abridged. The original article has been published in the journal "Der Weihenstephaner", issue 1/2005. References will be cited on request.

Chromsystems Upgrades

Urinary VMA, HVA, 5-HIAA (Order No. 1000/B)

New Mobile Phase Order No. 1011
Replaces Mobile Phase Order No. 1001

> ***Improved separation of VMA and internal standard, mobile phase recirculable as before***
> ***Minimum deactivation of ECD working electrode***
> ***Shorter chromatogram run times***

The sample preparation setup and HPLC separation column are unchanged.

Plasma homocysteine (Order No. 45000)

New Internal Standard
Order No. 45099
Replaces former Int. Standard Order No. 45005

> ***Optimum reproducibility***
> ***Reduced interferences in samples from dialysis patients***
> ***Long column residence times***

***It is extremely important** to use the new Internal Standard Order No. **45099** only with Mobile Phase Order No. **39001**. Order No. 39001 replaces the former Mobile Phase Order No. 45001. The HPLC column is unchanged.*

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Part 2: Customer Services Department

Dear Reader, our new series of articles called "Chromsystems in Closeup" tells you more about Chromsystems and some of the processes in our company.
Each issue of DIALOG takes a closer look at one of our departments: Customer Services, Dispatch/Warehouse, Research and Development, Production and Quality Management, Sales and Marketing. In short, we're inviting you to join us on a tour through our company.

So welcome to Chromsystems.

The Customer Services Department.

Customer Services is the central department whose responsibility is to accept orders coming in from various sources, make an electronic record, and initiate further processing of the order. In addition to the numerous different export regulations, our customer services experts must know about the products in detail. That's why all our customer services staff undergo compulsory training in processing consignments involving hazardous substances. Although the hazardous substance content is in most cases so low as to be almost non-existent, they still need to be declared according to proper procedure using numerous forms that must be completed in a professional manner. Finally, the Customer Service team also acts as a switchboard for incoming calls, answering many callers' queries every day on technical issues, delivery periods, availability, etc.

Margit Paschiller took over as head of the Customer Services department in mid-2003. An experienced industrial business expert, she gained most of her broad and diverse expertise in the processing of orders intended for export. Ms. Paschiller sees one of her challenges in keeping track of the very diverse special wishes that Chromsystems concedes to its customers.

Unusually for this line of business, Chromsystems routinely allows customers to order tailor-made special configurations and special volumes. The huge takeup on this option in dozens of different countries imposes a huge responsibility on each staff member. With regard to export processes in particular, close contact with Marketing and Sales colleagues is necessary as details are often arranged specifically for a particular order. Hazardous goods declarations are similarly challenging. Despite uniform regulatory provisions, the requirements are interpreted differently by different freight companies. The positioning of a label may sometimes decide whether a correctly declared package is delivered or not, which is critical aspect.

As the interface between the company and freight organizations, the Customer Services department is responsible for guaranteeing punctuality and top quality delivery. After product packaging in the warehouse, the Customer Services department issues the shipping papers and invoice. Collection of the shipment usually marks the end of the process.

Now you know who's on the other end the next time you dial the Chromsystems number + extension 0.

Events

Chromsystems is represented at the following national and international trade shows coming up in 2005:

> 26–29 June
HOMOCYSTEINE 2005, Milan, Italy

> 26–28 July
AACC Clinical Lab Expo, Orlando, USA

> 1–3 September
ADMA-Conference, Mersin, TR

> 6–8 September
HOSPIMEDICA, Bangkok, THA

> 14–15 September
Vitamins 2005, Pardubice, Czech Republic

> 14–16 September
Sports Medicine Conference, Hamburg, Germany

> 4–7 October
AACB Conference, Sydney, AUS

> 6–8 October
DGKL, Jena, Germany

> 11–14 October
MedLab 2005, Rom, Italy

> 16–19 November
Medica, Düsseldorf, Germany

> 24–25 November
Journé Internationales de Toxicologie Hospitalière, Liége, Belgium

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