Amino acids are vital to humans since they are involved in many important metabolic processes. In the middle of the last century, experts came up with the logical idea of diagnosing metabolic disorders by determining amino acid concentration levels. The ideal tool for the determination of amino acids in biological matrices is gas chromatography combined with mass spectrometry (GC/MS), but in order to meet the efficiency requirements of modern diagnostic laboratories, the analytical procedure needs to be highly automated.

Amino acids are equally important to human beings and animals, but they can only be produced by the organism to a limited extent. We humans have to obtain several amino acids with our food. Amino acids are building blocks for proteins and they perform a large number of other functions in our organism. The amino acid tyrosine, for example, is converted into catecholamine, i.e. into a hormone, that has a stimulating and stabilizing effect on the cardiovascular system; glutamate, another key player, is a neurotransmitter which handles vital communication in our body.

Due to their involvement in many different metabolic processes, amino acids are generally useful as markers for metabolic disorders, including, for example, congenital metabolic disorders, among which Phenylketonuria (PKU) is one of the most well-known. A person suffering from PKU is unable to metabolize phenylalanine; this means that the amino acid is accumulated in the body, adversely effecting mental development. A PKU test can be performed by simply determining whether the patient has a significantly elevated phenylalanine concentration level. Amino acid concentrations are determined in body fluids such as blood, plasma or urine.

Routine monitoring of amino acid concentrations in biological samples is frequently performed using dedicated amino acid analyzers; the analyzers are laboratory instruments that are based on cation exchange chromatography combined with post-column derivatization and UV detection. The main disadvantage of this type of analyzer is that the overall analysis is quite time-consuming. Gas chromatography with mass-selective detection (GC/MS) represents an attractive and efficient alternative; Amino acids are derivatized using propyl-chlorofor-mate; the resulting compounds are volatile and can be determined by GC/MS.

“We have completely automated the sample preparation, the method is suitable for high sample throughput”, says Katja Dettmer, Ph.D., Metabolomics Project Manager at Professor Peter Öfner’s Institute of Functional Genomics at the University of Regensburg, Germany. All sample preparation steps from addition of the internal standard (IS) to derivatization and finally introduction of the prepared sample into the GC/MS system, are performed automatically using the dual-rail PrepStation version of the GERSTEL MultiPurpose Sampler (MPS PrepStation). Dr. Dettmer: “Automation enabled us to minimize the amount of manual work, while at the same time improving reproducibility.”

A more detailed explanation: the MPS PrepStation has two robotic arms that operate independently of each other. One of these is, in this case, fitted with a 1 mL liquid syringe used to add derivatization reagent. The other robotic arm holds a 10 µL liquid syringe, used for adding internal standard (IS) and introducing the prepared sample to the GC/MS analysis system. Apart from these hardware details the key component of the system is the GERSTEL MAESTRO software. In the MAESTRO PrepBuilder, the user simply selects sample preparation steps by mouse-click from a drop down menu and adds them to the sample preparation method. Sample preparation and GC/MS analysis is then synchronized in a combined sequence table and performed in parallel for optimum productivity thanks to the PrepAhead function; and finally the Scheduler overview enables the user to check the progress of the analysis at any time. Clear information is provided at a glance on how much time
is required to finish the batch in order to help the laboratory workflow and facilitate planning.

**Sample preparation and GC/MS analysis**

This is the procedure Dr. Dettmer and her colleagues adopted in their amino acid determination project: blood or urine samples were placed in individual autosampler vials, which were sealed with crimp caps and placed in the cooled sample tray of the MPS PrepStation. All further steps were performed automatically, including transport of the vials to and from the vial agitator, which can stir, shake, heat or cool samples as per individual method requirements. Depending on the sample matrix, a sample volume of between 20 and 50 µL was used to perform the analysis. The following steps are performed automatically by the MPS PrepStation: 1. Add two characteristic mass fragments being recorded for each amino acid.

**Method description and validation**

Dettmer and her colleagues determined concentration levels of 33 amino acids and dipeptides using the MPS-CIS GC/MS method: Alanine, sarcosine, glycine, α-aminobutyric acid, valine, β-aminoisobutyric acid, leucine, alloisoleucine, isoleucine, threonine, serine, proline, asparagine, thiaprolin, aspartate, methionine, hippuric acid, hydroxyproline, glutamate, phenylalanine, α-aminoacidipinic acid, α-aminomelaminic acid, glutamine, ornithine, glycyl-proline, lysine, histidine, hydroxylysine, tyrosine, proline-hydroxyproline, tryptophane, cystathionine and cysteine.

Quantification was performed from calibration curves using a series of 13C and 15N labelled internal standards: Alanine, glycine, valine, leucine, isoleucine, threonine, serine, proline, asparagine, aspartate, methionine, glutamate, phenylalanine, glutamine, lysine, histidine, tyrosine, tryptophane and cysteine. In addition, [2H5] hippuric acid and [2,5,5-2H3] α-aminoacidipinic acid were used. Concentrations of amino acids for which isotopically labelled internal standards were not directly available were calculated based on the response from the isotopically labelled amino acid eluting closest to them”, explains Dr. Dettmer. The use of stable, isotopically labelled amino acids as internal standards resulted in a considerable improvement in both reproducibility and correlation coefficient of the calibrations. “The GC/MS analysis run for the 33 amino acids lasted less than ten minutes, a significant improvement over the conventional method”, the scientist is pleased to report.

Calibration curves for most of the amino acids were generated over the range from 0.3–2 µM based on 50 µL samples of biological fluid. The limit of detection (LoD) ranged from 0.03 µM for the amino acids alanine, glycine and tryptophane to 12 µM for glutamine and proline-hydroxyproline; the limit of quantification (LoQ) was in the range from 0.3 to 30 µM. Dr. Dettmer used the method successfully to analyze various body fluids, while also determining reproducibility. Human and mouse urine and human plasma were investigated, performing determinations in decaplicate. Relative standard deviation (RSD) ranged from 2.0 to 8.8% for human urine; from 0.9 to 8.3% for human plasma; and from 1.3 to 9.1% for mouse urine.

**Practical Applications**

Dr. Dettmer concludes: “Biological samples like urine, cell cultures, cell extracts, and plasma can be analyzed easily and reliably with our method”. The determination of amino acid concentrations in body fluids in order to diagnose congenital metabolic disorders, such as the ones listed earlier, is just one of the possible applications. The determination of amino acids plays a particularly important role not only in clinical diagnostics but also in food analysis. It is a well-established fact that the organism has to obtain essential amino acids through food under normal circumstances, these cannot be synthesized by the body. Dr. Dettmer notes that her MPS-CIS-GC/MS method could also be used to determine the concentration levels of amino acids in milk, beer and fruit juice. To demonstrate the applicability of their method to food analysis, Dettmer and her colleagues analyzed apple juice, beer and soy sauce. Incidentally: soy sauce is an Asian condiment consisting of water, soybeans, cereal and salt. The analysis revealed, that soy sauce contains numerous amino acids – in very large amounts in some cases – with glutamate dominating at 48 mM. The main amino acids in apple juice are alanine, proline, asparagine, aspartate and glutamate, with asparagine standing out at a concentration of 3.16 µM. The amino acids alanine (1.13 mM) and proline (3.56 mM) are the main components in beer. The analysis of a sample of whole milk. Amino acids for which an isotope labeled standard was available are marked red in the illustration.