Drilling Deep **into Earth's History**

Life on Earth stagnated for billions of years in the stage of primitive single-celled organisms. Only when cells acquired a nucleus did things really take off, leading to diversification and the dazzling variety of life forms we see today. **Christian Hallmann** and his team at the **Max Planck Institute for Biogeochemistry** in Jena are investigating how, when and where that happened.

TEXT **DIRK LIESEMER**

Christian Hallmann, a geologist from Bremen, had to travel thousands of miles in a cross-country vehicle before finally reaching the Pilbara Craton, a particularly ancient piece of Earth's crust in the northwest of Australia. Only a few shrubs are able to survive in this parched area. On the rust-red plain, he and a small international team are drilling hundreds of meters into the 2.7-billion-year-old rock – delving down into a unique archive of Earth's history to obtain information in the form of rock samples. The scientists then examine the samples in search of minute traces of early life forms.

For months, Christian Hallmann and his colleagues from the US and Australia prepared the drilling that would be performed with machines as tall as houses. They are the cleanest drill holes of their kind ever sunk. Meticulous precautions were taken to recover the rock samples. The aim was to settle a controversy in historical geology. Among other things, the researchers wanted to clarify when eukaryotes arose in the oceans. Eukaryotes are life forms that pack their genetic material in a cell nucleus. Originally consisting only of single cells, over the course of Earth's history the eukaryotes gave rise to all complex multicellular organisms, including plants and animals. The first appearance of nucleated cells therefore marks a key point in evolutionary history.

LIPIDS AS MOLECULAR FINGERPRINTS

Until now, there have been conflicting theories in this regard. The first known microfossils of eukaryotes are approximately 1.5 billion years old. Yet in 1999, several researchers reported discovering 2.7-billion-year-old traces of eukaryotes in rock samples from the Pilbara Craton. They believed that they had found steroid hydrocarbons, or more specifically the remnants of eukaryotic lipids, which probably served in early eukaryotes to stabilize cell membranes and create separate compartments such as the cell nucleus. Thanks to this compartmentalization of cells, biochemical processes could run more efficiently, especially in large cells, which was a prerequisite for the development of more complex life forms. Today, these particular lipids serve as molecular fingerprints of eukaryotes. Critics, however, soon warned that the detected hydrocarbon abundances were much too low to provide reliable results. In addition, they claimed, over the course of Earth's history, the rocks experienced exceedingly high temperatures, which would have destroyed these telltale molecules.

Were the putative traces of eukaryotes in fact impurities? Christian Hallmann set out to answer that question. "We had to work as cleanly as possible in Australia," says the geologist. As early as the planning stage, he and his colleagues considered how to protect the rock samples from contamination, especially with hydrocarbons from the lubricants used in the drilling equipment. Lubricants are used to prevent damage to the machines, speed up the drilling process and reduce noise, but their petroleum-derived residues could contaminate samples and easily be confused with original eukaryote traces. Roger Buick from the University of

Photo: MPI for Biogeochemistry

Meters and meters of Earth's history: Christian Hallmann and his colleagues store the bulk of core samples from the Pilbara Craton in aluminum crates.

» How did the Earth develop so that complex life forms were able to spread at the end of the Precambrian?

Washington in Seattle, who organized the drilling for the study, therefore spent weeks looking for a company that would drill without such lubricants. That's what research problems can boil down to – especially when dealing with such intangible matters as early life forms.

That's precisely what Christian Hallmann deals with. At MARUM, the Center for Marine Environmental Sciences at the University of Bremen, he leads the Organic Paleobiogeochemistry Research Group, which is affiliated primarily with the Max Planck Institute for Biogeochemistry in Jena. Hallmann focuses on the Precambrian, the eon that spans more than 85 percent of Earth's earliest history. It began four and a half billion years ago, while the Earth was forming, and ended 541 million years ago, immediately before the explosive diversification of complex multicellular life forms.

The four billion years of the Precambrian were characterized by enormous changes. Nutrient cycles developed, while the chemical makeup of the oceans and atmosphere fluctuated wildly and the climate repeatedly swung from one extreme to another. The planet was probably covered in ice several times, making it seem like a giant snowball.

The factors that led to extreme glaciation are one of the questions that occupy Christian Hallmann and his American and Australian colleagues. They also want to know how the Earth

To drill cleanly into the Earth's crust, the researchers used water to which they had added fluorescent microparticles and isotopically labeled hydrocarbons. In this way, they were able to tell how deeply contaminants penetrated into the rock.

Photo: MPI for Biogeochemistry

Office in the outback: Christian Hallmann (left) and his cooperation partners spent nearly one and a half months in the Pilbara region. The researchers washed the core samples obtained from the ground with organically clean water (right). They looked for 2.7-billion-year-old biomarkers of eukaryotes in only a small proportion of the samples, which were immediately sealed. The majority, as seen here, were sent to colleagues around the world for various analyses.

developed during the Precambrian, leading to the burgeoning of complex life forms at the end of the eon. How did the Earth develop from an inhospitable environment with no oxygen in the atmosphere and vast quantities of iron in the oceans to the planet it is today? "I want to know, for example, when, why and under what environmental conditions the first eukaryotes arose," says Hallmann. "And how these life forms ultimately brought about today's Earth system – complete with oxygenated air and modern nutrient cycles."

For a long time during the Precambrian, only simple single-celled organisms lived, such as bacteria and, later, eukaryotes. The Precambrian is therefore still regarded as a time of evolutionary stagnation. Only during the final era of the Precambrian, known as the Neoproterozoic, did more complex multicellular organisms, plants and the metazoa (from the Greek for multicellular animals) evolve. The latter include all the animals we know today, and they all originated from the first simple forms. At the beginning of the Cambrian, 541 million years ago, all the precursors of today's animal phyla developed almost simultaneously within an astonishingly brief geological period of a few million years.

Christian Hallmann is particularly interested in the ecological conditions that facilitated the Cambrian species explosion. After all, eukaryotes, including plants and metazoa, require oxygen to produce energy. For a long time, only the precursors of cyanobacteria in the oceans produced this elixir of higher life. "From an environmental perspective, eukaryotes could, theoretically, have emerged 2.45 billion years ago, because sediments deposited at that time show evidence of the first oxygen in the atmosphere," says Hallmann.

BIOMARKERS SURVIVE FOR BILLIONS OF YEARS

To gain new insights into the evolution of eukaryotes, metazoa and their environments, Christian Hallmann is collecting rock samples from various sites formed during different epochs of Earth's history. His Australian samples come from the very early phase of our planet; some samples from Brazil and Siberia come from the time before the Cambrian species explosion. Analyzing rocks from various regions around the world is important for developing a sense of whether environmental changes were global or regional.

In the samples from the Pilbara Craton in Australia, Christian Hallmann and his colleagues are searching for the hydrocarbon remnants of biological lipids – especially steroids. Unlike other biological molecules that can also be very typical for specific life forms, these hydrocarbon biomarkers can theoretically survive in sediments for billions of years. In contrast, the genome, probably the most reliable molecular fingerprint of organisms, degrades rapidly and vanishes without a trace. "It's only in science fiction films that researchers find DNA originating from the primordial Earth," says Hallmann.

But even steroids and other lipid remnants can survive the passing eons only under ideal conditions. Above all, as little oxygen as possible should be present in the water during deposition of the sediments – like a park pond that has lost its oxygen because of too much duck feed. Clay minerals and some limestones can preserve the structure of the molecules, even as the molecules themselves are structurally converted within those minerals. Porous sandstone, by contrast, is completely unsuitable for the preservation process. Arne Leider, a colleague of Christian Hallmann, is investigating how the original molecules are transformed within various rock types. Only if these underlying processes are properly understood will researchers be able to interpret the molecular traces in their samples correctly – that is, if they find any.

For the search to be successful, it is not enough for the biomarkers to be enclosed in suitable rock. There are very few places on our planet that have re-

Photos: MPI for Biogeochemistry (2)

Searching for traces in the laboratory. Arne Leider (left) and Christian Hallmann interpret chromatographic analyses in order to identify biomarkers of eukaryotes and their breakdown products.

mained unchanged since the Precambrian. No oceanic sedimentary rocks exist at all from the first billion years of Earth's history. They are weathered, or the organic material they once contained has been converted into graphite that no longer contains any biological information. Or the rock has been recycled back into the interior of the Earth by tectonic activity and has melted. Even rocks from later epochs have frequently been overprinted by elevated temperatures as a consequence of tectonic movements, to the extent that any organic molecules they may have contained have been cracked or destroyed. To determine whether information on the original substance can nevertheless be gleaned for altered molecules and their fragments, Arne Leider is also investigating what happens to relevant biomarkers under the effects of heat.

The oldest known traces of petrified life occur in 3.5-billion-year-old rocks from Australia. These onion-like structures, known as stromatolites, were formed by bacteria that grew layer upon layer. Today, actively growing stromatolites can be found in only a few places that offer the right conditions, such as in a bay in Western Australia where larger grazing animals are absent due to elevated salinity.

CORE SAMPLES WERE OFTEN LEFT EXPOSED FOR YEARS

Geologists know of only two regions containing two- to three-billion-year-old rocks that might still harbor molecular traces of primordial eukaryotes: the Pilbara Craton in Australia and the Kaapvaal Craton in South Africa. Only at these two sites have the rocks not been excessively heated over the course of time. If eukaryotes already existed 2.7 billion years ago, their traces should be found here, the scientists reasoned.

In the summer of 2012, Hallmann and his colleagues prepared the boreholes in Australia. The work was largely funded by the Agouron Institute in Pasadena, in the US. His coworkers were from MIT, the University of California, Riverside, Macquarie University in Sydney, and the Australian National University in Canberra.

Geochemists still find it extremely difficult to obtain rock samples from the Precambrian that are free of impurities and to correctly analyze them. Accordingly, few groups have succeeded in doing so. In the past, many researchers have used core samples extracted from the ground by mining and petroleum prospecting companies. Not only do these companies typically use huge quantities of synthetic lubricants, but some core samples had been stored openly exposed to the air for years, allowing dust or diesel fumes to settle on them. Only then were they collected by or passed on to scientists.

Even at the Pilbara Craton site, it was uncertain whether the drilling would be successful. No one knew, for example, whether the freezers would maintain a constant temperature of minus 20 degrees in the heat of the outback in order to prevent volatile substances from escaping from the samples. And, of course, the purity of

Photo: Tom Pingel

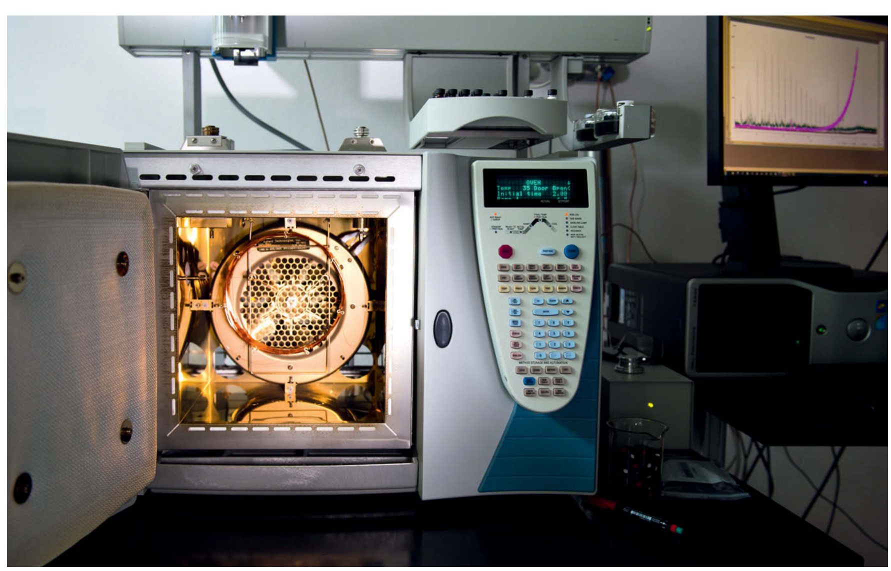
the samples was crucial. The researchers first cleaned the core barrels with synthetic detergents. They then drilled several hundred meters down into volcanic rock that contained no organic matter. In the process, any residual impurities on the drill rod were rubbed off by abrasion. The only lubricant allowed was groundwater.

Originally the researchers wanted to bring enough organically clean water for the drilling operation with them from their base in Perth. But that proved impossible: the distance was simply too great. Instead, the team searched on site for days for an underground source of water. They then sank a well and pumped groundwater above ground, where they stored it in a tank to allow any particles to settle to the bottom. Organically clean water was brought to the field site only in sufficient quantities to wash the samples.

They then added a fluorescent substance to the water, to dye it a luminescent green, as well as synthetically labeled hydrocarbons. These would later tell them how deeply water – and therefore potential impurities – had penetrated into the rock samples. Similar fluorescent beads had previously only been used in studies of the present-day 'deep biosphere' to determine how deeply bacteria of the same size as the beads can penetrate into core samples. The search for early eukaryotes in the outback marks the first time that organic geochemistry researchers have exercised such meticulous care in obtaining core samples.

They soon realized just how difficult, loud and slow drilling without lubricant can be – particularly since the drilling rods had to penetrate extremely hard rock strata. Only after 100 meters was the rock devoid of signs of oxidation and weathering, which affects minerals and destroys organic material. Below that level, the rock has essentially remained unchanged for billions of years.

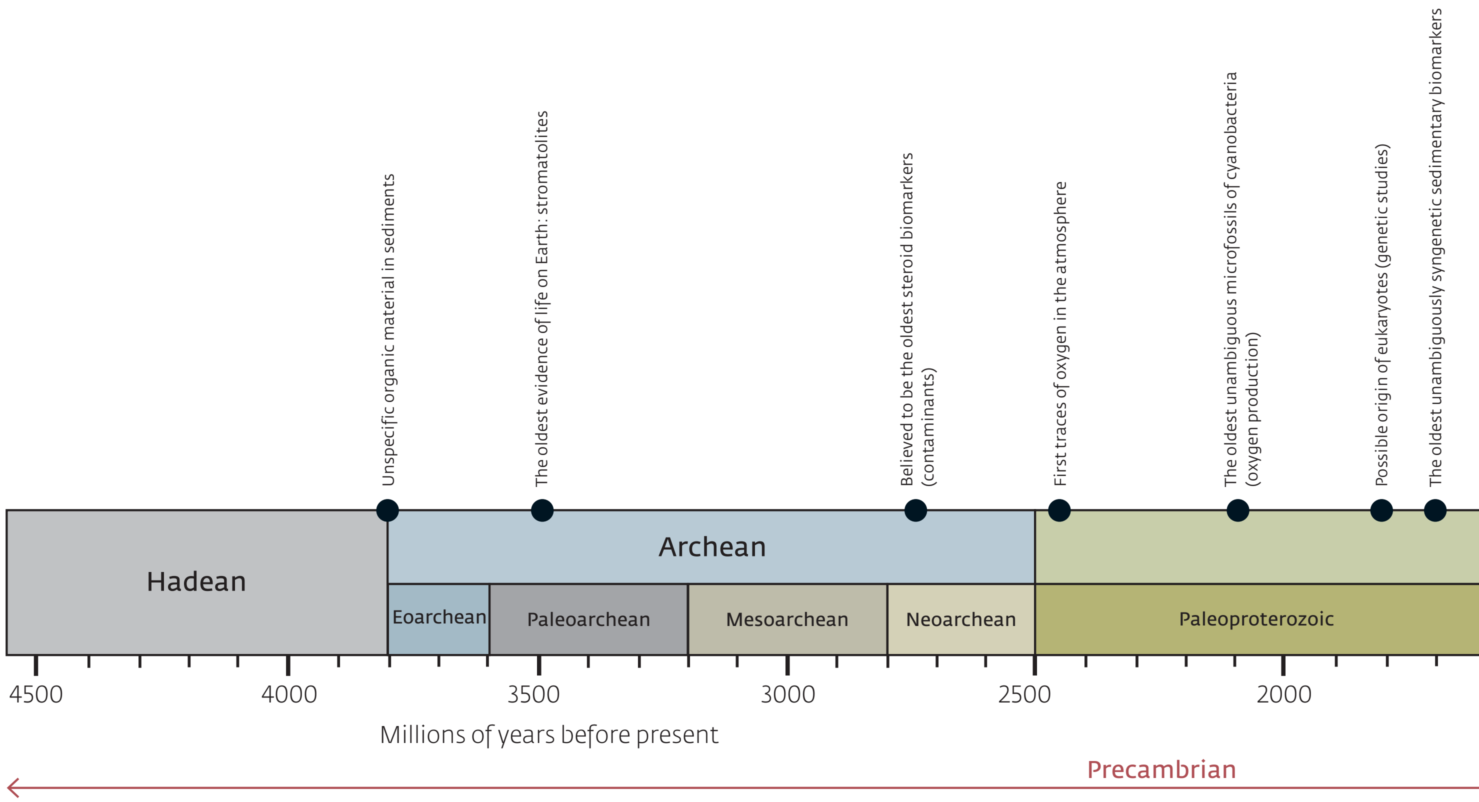
Gradually, the samples were brought up to the surface. The researchers opened up the five-meter-long core barrels and carefully transferred their treasure to aluminum boxes. They then rinsed the core samples with organically clean water and carefully broke it up into sections with a hammer. Then they used clean aluminum foil to lift out the pieces that appeared interesting for their



Above: A rock core of black shale is sawn into portions to allow the researchers to determine whether impurities have penetrated beyond the outer layers.

Below: A view into the open oven of a gas chromatograph shows the rolled-up capillary tube, in which a mixture of substances, such as lipids, is separated.

Photos: Tom Pingel (2)



study and placed them in Teflon bags that had previously been boiled in acid to remove any organic contaminants. After that, they filled the bags with the inert gas argon so that the samples would not be exposed to the oxidizing effects of the air. Quickly sealed, the bags were placed in a freezer, where they remained at minus 20 degrees.

The scientists had originally reckoned that the expedition should take no longer than three weeks to complete. In the end, they had to spend nearly one and a half months in the desert. Hallmann sees the fact that the drilling was a success as a milestone. The samples were safely transported to Perth, although the drive with the heavy freezer on the bed of a pickup truck proved pretty bumpy. During the three-day trip, they had to stop every few hours to cool down the freezer with their own generator, and then from the power supply at camping sites.

The samples were later transported on dry ice from Perth to Canberra by a courier. In Jochen Brocks' laboratory at the Australian National University, the researchers split up their treasure with a clean saw and conducted the first analyses. Katherine French took some of the samples to MIT, while Christian Hallmann took others to Bremen. Hallmann's coworkers used a gas chromatograph and a tandem mass spectrometer to analyze the samples. The former separated out the thousands of organic compounds, while the latter structurally identified the molecules that emerged from the gas chromatograph at various times.

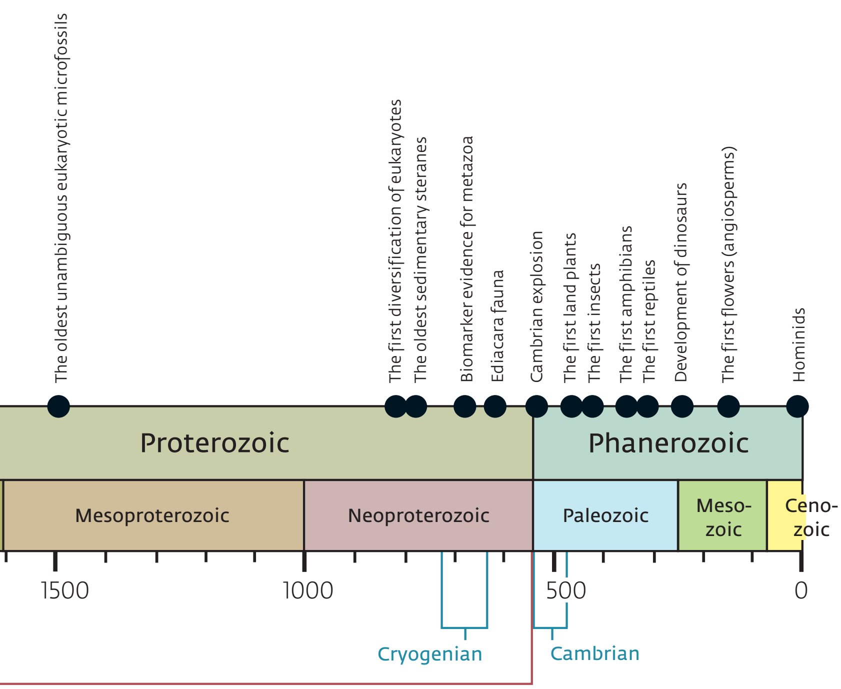
NO TRACES OF EUKARYOTES IN THE PILBARA CRATON

The research teams from the US and Germany analyzed the samples independently of each other, but the results were the same: the ancient rock from the Pilbara Craton contained no biomarkers for eukaryotes 2.7 billion years ago. This was concluded in the summer of 2015. "We now know that the entire region was so hot at least once in the course of its history that we can no longer detect any steroids, even if they were once present," says Christian Hallmann. However, that is unlikely. "We know with relative certainty that eukaryotes have existed for the past 1.5 billion years," Christian Hallmann says. This finding is based on microfossils and is consistent with genetic "molecular clock" analyses. The first single-celled eukaryotes probably arose in coastal water into which rivers carried nutrients.

Moreover, eukaryotes probably played an ecologically relevant role for the first time only around 750 million years ago. At that point, eukaryotic algae experienced a strong diversification and spread across the planet. Today, many eukaryotic algae produce certain volatile chemicals that can attract water droplets around them when they enter the atmosphere. The abundance of such cloud condensation nuclei, as they are called, could have drastically increased at this point in time. More clouds formed, allowing less sunlight to reach the cooling Earth. When the supercontinent Rodinia broke apart, vast quantities of freshly generated rock underwent rapid weathering and drew so much carbon dioxide from the atmosphere that the Earth cooled dramatically and disappeared under a mantle of ice and snow. This period is appropriately known as the Cryogenian.

"For a while I've had this idea that eukaryotes might have contributed to the Earth becoming a snowball," says Hallmann. He recently presented such a scenario together with Georg Feulner and Hendrik Kienert of the Potsdam Institute for Climate Impact Research.

Graphic: Christian Hallmann/MPI for Biogeochemistry



Evolutionary puzzle: Paleontologists and paleobiogeochemists are working to piece together when various life forms arose. They divide the various eons of Earth's history into periods, such as the Cryogenian and the Cambrian. Christian Hallmann is particularly interested in the conditions under which eukaryotes arose, and the explosion of species in the Cambrian.

The two scientists simulated the role of eukaryotes in the Earth's early climate. They found that the spread of eukaryotic life forms could indeed have contributed to a cooling of the climate and ushered in the subsequent ice age.

These findings are underscored by the fact that the supposed traces of 2.7-billion-year-old eukaryotes turned out to be contaminants. The lipids found in 1999 were so complex that they looked like the signature of a modern algal community. This would have suggested that eukaryotes had already differentiated and disseminated widely by 2.7 billion years ago. Cumulative findings of sedimentary steroids and especially microfossils, however, are starting to indicate that this didn't happen until 800 to 750 million years ago. "That's highly consistent with the extreme cooling that occurred around 700 million years ago," says Hallmann.

Thus, with the help of paleobiogeochemistry, the researcher has discovered something about the change in the primeval conditions for life. He continues to delve deep into Earth's history to uncover further information about this tumultuous phase of life that stands at the root of our very own existence.

TO THE POINT

- **The appearance of eukaryotes paved the way for more complex multicellular life forms to evolve – including, eventually, ourselves. When, where and under what conditions this occurred is still not entirely clear.**
- **An international team headed by researchers from the Max Planck Institute for Biogeochemistry has developed an extremely clean method for collecting and correctly analyzing rock samples that are billions of years old, in order to shed light on the origin of eukaryotes and the change in environmental conditions on Earth.**
- **According to the preliminary results, eukaryotes didn't originate 2.7 billion years ago, as has long been suggested, but probably only around 1.5 billion years ago.**
- **The rapid diversification and spread of eukaryotic algae may have contributed to the occurrence of at least one extreme ice age on Earth around 700 million years ago.**

GLOSSARY

Eukaryotes: Organisms whose cell contains a nucleus, and today, also organelles. The organization of cells into subunits allowed cellular processes to run more efficiently. As a result, single-celled organisms could evolve into more complex multicellular life forms.

Gas chromatography: In this chemical separation method, mixtures of gaseous substances are separated as they move through a long, thin capillary tube. The inside of the capillary tube is coated with a material to which the various substances have different affinities. Different substances therefore flow through the capillary tube at different rates, depending on their polarity and volatility, and separate into a number of fractions.

Craton: Ancient continental rock that has mostly been altered by pressure and heat in the course of Earth's history.

Metazoa: This group comprises all complex and multicellular animals.

Tandem mass spectrometry: An analytical method that links two mass spectrometers (MS) into one. In the first MS, substances are ionized with low energies and then selectively separated according to their mass-to-charge ratios. They are subsequently fragmented, and individual fragments are further separated in the second MS. The fragments can then be used to identify the original substances present with high sensitivity and selectivity.