Finding the correct dosage of medication by breath analysis

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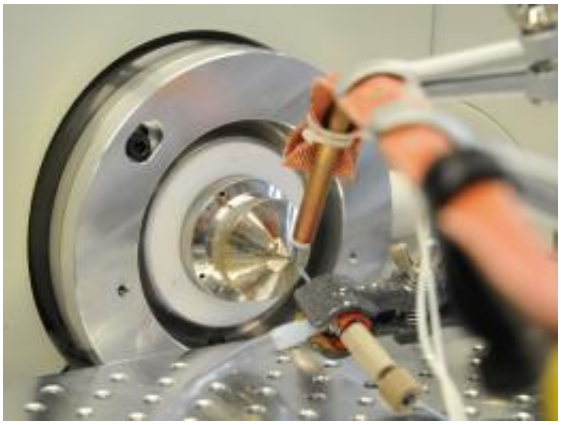

The cone (center of image) aspirates the ionised breath and feeds it into the mass spectrometer. Credit: Peter Rüegg/ETH Zurich

Using a mass spectrometric method, ETH Zurich researchers are able to measure metabolites of a common epilepsy medication directly in exhaled breath. This simplifies testing of patients and represents a step towards personalised medicine.

Spoiled meat, pesticides on vegetables or fruit, melamine in milk: there are very few fields of chemical analysis which ETH Zurich Professor Renato Zenobi has not already investigated using mass spectroscopic methods. He has now added another analysis method, which is based on mass spectrometry, to this series: breath analysis to track down metabolites of a widely used drug for treating epilepsy.

“Breath contains hundreds of chemical substances”, says Zenobi, and adds that breath analysis has great potential for medical diagnostics. However, the Professor of Analytical Chemistry at ETH stumbled upon the topic of epilepsy by chance. He says that one of his former coworkers was dependent on the anti-epileptic valproic acid (VPA). This active ingredient suppresses epileptic seizures. However, to clarify the correct dose of the medicine for a patient, the latter must have blood samples taken every few weeks to measure the VPA concentration. In the future, this tedious procedure could be replaced with non-invasive breath analysis, because Zenobi has shown that VPA metabolites are detectable in patients’ breath. Medicines and their metabolites can leave the body through the kidneys or, to a certain extent, via the lungs.

Obtaining test results directly and easily

The test the method, developed by Zenobi’s former postdoctoral associate Gerardo Gamez and in collaboration with researchers from China and Bremen and the Swiss Epilepsy Center, is similar to previous analytical methods originating from this group at ETH Zurich. All that is needed to test a person’s breath is for them to blow into a small tube. The breath passes through a heated Teflon tube to an area where the exhaled air is ionised by protonation. The chemical compounds, now carrying a charge, are aspirated through a small orifice into the mass spectrometer, where they are then separated and measured according to their mass. There is no need to pre-treat or store the samples.

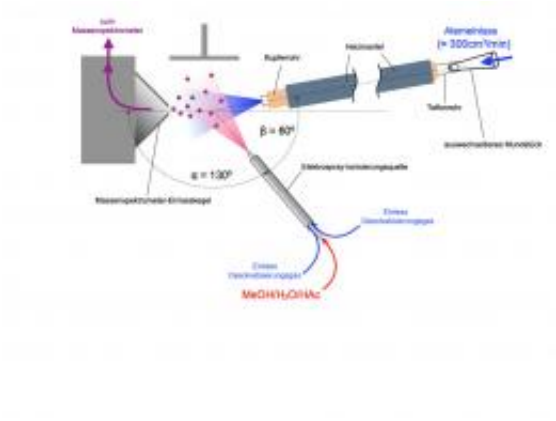

Diagram of direct breath analysis by mass spectrometry. Credit: provided by R. Zenobi / ETH Zurich

Currently, however, the analysis instrument is still unwieldy and nowhere near as practical as an alcohol testing device used by the police. This is because a mass spectrometer also contains pumps, which generate the vacuum needed inside the instrument. “Nevertheless, this method is direct and simple, non-invasive, painless and represents an important step towards personalised medication”, says the ETH Zurich professor.

First test scores a direct hit

First of all, the researchers tested Zenobi’s coworker who suffers from epilepsy, as well as healthy persons as controls – and scored a bull’s-eye. The spectra produced by the mass spectrometer for the breath of epilepsy patients showed two distinct signals not found in the breath of healthy persons. However, if a dose of VPA was administered to healthy volunteers, the same signals were found in their breath as in the case of persons being treated with VPA.

Further detailed investigations showed the researchers that the peak caused by the heavy molecule was attributable to a VPA metabolite with

an ammonium group bound to it. The second peak corresponded to exactly the same compound after ammonia had been split off. The VPA metabolite was previously unknown to the research world. Thus the ETH Zurich chemists have, in one stroke, discovered both a new metabolite and a new biomarker for VPA in breath.

Non-invasive diagnosis is increasingly important

Furthermore, the researchers compared the VPA values from blood tests with the breath analyses. This revealed that patients treated with VPA always exhale the metabolite, and that its concentration in exhaled breath correlates with that of free VPA in blood: the higher the blood value, the stronger the signal in the mass spectrometer. Finally, the MS analysis also yielded information on how quickly the body degrades VPA. According to Zenobi, the VPA level decreases exponentially with time.

The importance of non-invasive diagnosis in modern medicine is growing strongly. It is often easier to apply and is less stressful for patients than invasive methods such as blood sampling or biopsies. Renato Zenobi is also convinced that his new diagnostic method has a future for patients treated with VPA.

“However, the method is so widely and universally applicable that it can also deal with many other substances in breath”, he says. The ETH Zurich professor can therefore well imagine that someday every medical practice will have a mass spectrometer that will enable a wide variety of diagnoses.

Epilepsy

There are around 70,000 epileptics in Switzerland, 15,000 of whom are children. Five percent of the whole population suffer an epileptic seizure

at least once in their lifetime, and one percent become ill with epilepsy. There is not just one form of epilepsy; doctors distinguish up to 30 different types. Up to 70 percent of all types of epilepsy can be treated well with medication. Valproic acid is an anti-epileptic which was developed around 1970. Together with carbamazepine, it is the medicine most commonly used to treat epilepsy: more than half of all the epileptics in Europe are still treated with these two drugs. However, many new anti-epileptics have been developed and introduced in the last 10 years.

More information: Gamez G, Zhu L, Disko A, Chen H, Azov V, Chingin K, Krämer G & Zenobi R. Real-time, in vivo monitoring and pharmacokinetics of valproic acid via a novel biomarker in exhaled breath. *ChemComm*., 2011. Doi:10.1039/c1cc10343a

Provided by ETH Zurich