Pharmacokinetics of Eucalyptol Oil in Exhaled Breath Monitored by the Vocus CI-TOF

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The analysis of exhaled breath is a promising approach for pharmacokinetic studies aiming to understand the transformations, distribution, and fate of exogenous compounds in the human body. In comparison to conventional blood or urine sampling, which involves long processing times, measurement of volatile and semi-volatile organic compounds in breath enables non-invasive and real-time monitoring of metabolic processes. Law enforcement has already discovered the benefits of real-time breath analysis, with the widespread use of “breathalyzer” for ethanol detection. Medical research can similarly benefit from a far more chemically detailed analysis. The Vocus Chemical Ionization Time-of-Flight Mass Spectrometer (CI-TOF) can simultaneously and quantitatively monitor hundreds of compounds in human breath present at ultra-low concentrations (ppt) providing a promising online tool for detection of rapid changes in the human metabolome.

Experimental Design

In a proof of concept measurement, the breath of three healthy adult volunteers was monitored after the ingestion of capsule containing 300 mg of eucalyptol oil (Gelomyrtol, Alpinamed). Eucalyptol oil is a common active pharmaceutical ingredient that helps to clear the airways during an inflammation. After ingestion, the capsule is dissolved in the gastrointestinal system and eucalyptol is transferred and distributed via the blood stream. In the lungs it passes the blood-air barrier and is exhaled via breathing. Volatile compounds resulting from eucalyptol metabolism can also pass the blood-air barrier and be exhaled. Therefore, the online monitoring of breath concentration of volatiles allows assessing blood concentration changes of those compounds.

The Vocus CI-TOF was deployed to monitor the breath profile of each of the three participants 15 min before and every 15-30 minutes after the capsule ingestion. Human breath was directly sampled into the Vocus via a specialized heated breath inlet. The instrument was switched between traditional PTR (H_3O^+) mode and NH_4^+ reagent ions for optimized detection of oxygenated molecules. The data were acquired with 2Hz time resolution.

Results

Figure 1 shows the concentration of acetone and eucalyptol in thirteen exhaled breaths. Acetone, a product of lipolysis, is the most abundant ketone universally present in human breath. Eucalyptol (1,8-cineole) is the main compound found in eucalyptol oil. The concentrations were determined from direct calibration of 1,8-cineole solution via liquid calibration system.

Mass spectra recorded in PTR and NH_4^+ mode before and after the capsule ingestion are shown in **Figure 2**. The most abundant VOCs in breath before capsule ingestion are acetone and isoprene. Five hours after ingestion, intense peaks at the mass-to-charge ratio (m/Q) 172.17, 154.16 137.13, in NH_4^+ mode are observed. These correspond to molecules of

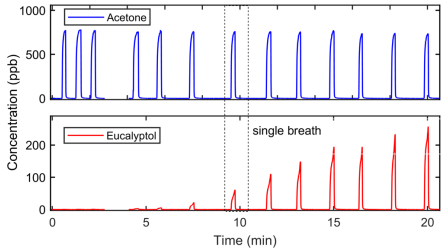

Figure 1 *Example of a measurement set of exhalations sampled via breath inlet coupled to VOCUS CI-TOF. Concentrations (ppb) of acetone and eucalyptol are shown. The dashed area region highlights one single breath exhalation. Time zero corresponds to ~3 hours after the capsule ingestion.*

eucalyptol ($C_{10}H_{18}O$) and monoterpenes ($C_{10}H_{16}$) such as α-pinene, β-pinene, Camphene or Limonene which are to lesser extent present in eucalyptus oil or formed via the metabolism of eucalyptol molecule. The analysis of pure eucalyptol standard in NH_4^+ mode shows less than 2% of $C_{10}H_{16}NH_4^+$ ion detected at m/Q 154, therefore contribution of eucalyptol fragmentation to monoterpene peak can be neglected. The opposite is true for PTR mode where the most significant peak is observed at m/Q 137 ($C_{10}H_{17}^+$) with small abundance of parent ion signal for eucalyptol at m/Q 155 ($C_{10}H_{19}O^+$). The use of NH_4^+ mode therefore provides significant advantage in comparison to PTR due to largely suppressed fragmentation and so easier identification of eucalyptol and its metabolites.

Figure 3 depicts the measured quantity of eucalyptol in exhaled breath of one subject

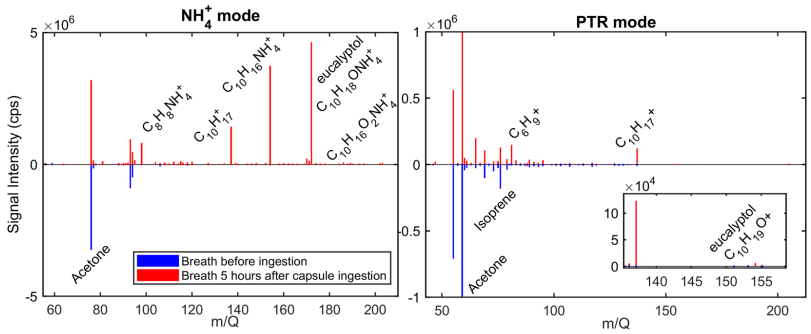

Figure 2 Mass spectra recorded in NH_4^+ and PTR mode for one individual before and after eucalyptol oil ingestion.

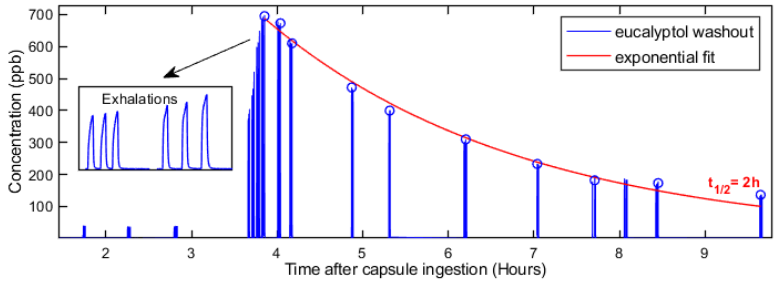

Figure 3 Washout of eucalyptol from one of the subjects over 10 h after an ingestion of a eucalyptol oil containing capsule. Three exhalations were measured each 15-30 minutes for better statistics.

over a 10-hour period. The maximum value reached was ~700 ppb with elimination rate constant of 0.33 h^{-1} using a simple exponential fit. The same subject was measured on the following day via the same measurement protocol: the maximum value reached ~650 ppb with elimination rate constant of 0.25 h^{-1}. Taking the average value, the half life of eucalyptol in breath for this individual would be ~2.3 hours. The breath concentration reflects the venous blood levels and after 4 - 5 half-lives the drug level should be ~3% of its maximum value and its effect is expected to be negligible. In our case the concentration after 8 half-lives is still ~ 45 ppb (~7% of maximum value). This suggests that a two-phase model with fast and slower exponential decay would describe the eucalyptol removal from the body more accurately. In **Figure 4** simple and double exponential fits are shown for two individuals. In both cases the elimination of eucalyptol is slower than the single-exponential distribution. It would take up to approximately 2 days until eucalyptol returns to its initial concentration.

In **Figure 5** the temporal

profiles of depicted compounds in breath of 3 subjects are shown. The T_{max} for eucalyptol in the breath varies between 2 – 4 hours, depending on the test subject. The main metabolites of eucalyptol are dehydrocineol ($C_{10}H_{16}O$), oxocineol $C_{10}H_{16}O_2$ and hydroxycineole ($C_{10}H_{18}O_2$) (*1-3*). Indeed, a delay in the kinetics was observed for these molecules in the exhaled breath showing the potential of Vocus CI-TOF application in pharmacokinetics studies.

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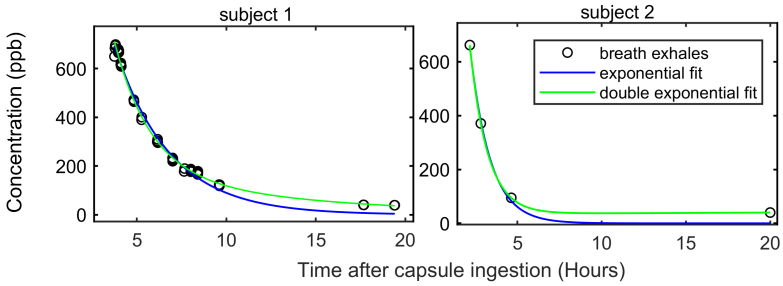

Figure 4 Washout kinetics for eucalyptol from two individuals after an ingestion of a eucalyptol oil containing capsule.

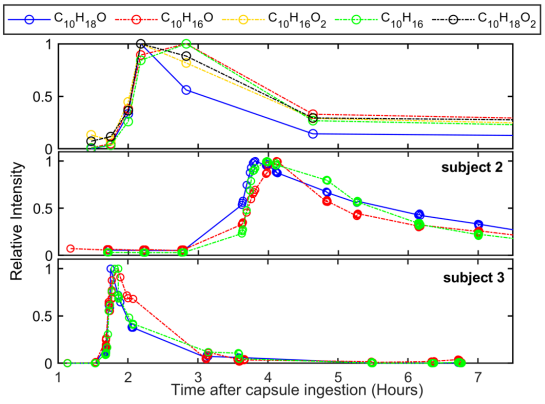

Figure 5 *Temporal profiles of signal intensities normalized to its maxima for eucalyptol (C10H18O), monoterpene (C10H16) and in breath elevated oxygenated species (C10H16-18O1-2). Solid lines represent eucalyptol present in the original capsule whereas dashed lines represent possible metabolites.*

References

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