MASS SPECTROMETRIC ANALYSIS OF EXHALED BREATH FOR NON-INVASIVE DIAGNOSIS

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Abstract

This article examines the physicochemical principles of mass spectrometric analysis of exhaled breath and its potential applications in biomedical diagnostics. Particular attention is given to the properties of volatile organic compounds (VOCs), including molecular mass, ionization energy, proton affinity, polarizability, and chemical reactivity, which influence their detection and analytical performance. [4] The fundamental stages of mass spectrometric analysis, including sample introduction, ionization, mass-to-charge ratio separation, and ion detection, are discussed in relation to the physical principles governing ionization efficiency and ion motion in electric and magnetic fields. The diagnostic significance of selected breath biomarkers, such as acetone, isoprene, ethane, nitric oxide, and formaldehyde, is also considered, highlighting their potential associations with metabolic processes, oxidative stress, and airway inflammation. Furthermore, a comparative analysis of gas chromatography–mass spectrometry (GC-MS), proton-transfer-reaction mass spectrometry (PTR-MS), and selected-ion flow-tube mass spectrometry (SIFT-MS) is presented, focusing on their operating principles, sample preparation requirements, measurement speed, quantitative capabilities, and analytical limitations. The findings discussed indicate that exhaled breath analysis represents a promising non-invasive approach for investigating physiological and pathological processes. However, the reliable clinical application of breath biomarkers requires standardized analytical procedures, appropriate calibration, consideration of environmental and metabolic confounding factors, and further clinical validation. The integration of advanced mass spectrometric techniques with statistical analysis and machine-learning methods may improve biomarker identification and support the development of sensitive, rapid, and non-invasive diagnostic approaches.

Keywords: Exhaled breath analysis, mass spectrometry, volatile organic compounds (VOCs), breath biomarkers, GC-MS, PTR-MS, SIFT-MS, ionization mechanisms, mass-to-charge ratio, non-invasive diagnostics, oxidative stress, metabolic processes, machine learning. The following is an English version of the additional scientific material, written in an academic style suitable for a research paper, coursework, or a graduation thesis.

Physicochemical Principles of Mass Spectrometric Analysis of Exhaled Breath

The physicochemical properties of volatile organic compounds (VOCs) play a crucial role in the analysis of exhaled breath. Their molecular mass, ionization energy, proton affinity, polarizability, and chemical reactivity determine the efficiency of their detection. Some breath biomarkers originate from metabolic processes in the lungs, bloodstream, and body tissues. These compounds can enter the exhaled air through gas exchange between the blood and the alveoli. Mass spectrometric analysis generally consists of four main stages: sample introduction, ionization, separation of ions according to their mass-to-charge ratio, and detection. The mass-to-charge ratio is expressed as: m/z where m represents the ion mass and z represents its charge in elementary charge units. [1] The mass analyzer separates ions according to this ratio, producing a mass spectrum that shows ion signal intensity as a function of mass-to-charge ratio. The efficiency of ionization depends on the ionization cross-section, the relative velocity of interacting particles, gas pressure, and the electric field within the ion source. Under simplified conditions, the probability of an ionization event can be expressed as:

$$P=1-\exp(-n\sigma l)$$

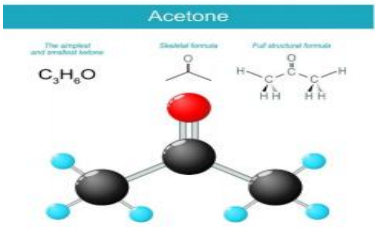
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where nn is the number density of the relevant collision partners, σσ is the effective ionization cross-section, and ll is the interaction path length. This equation represents a simplified model; actual ionization processes may also depend on particle energy, gas composition, and competing reactions.

The motion of ions in electric and magnetic fields is described by the Lorentz force:

$F=q(E+v\times B)$

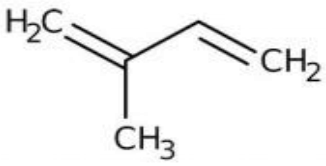
where qq is the ion charge, $\vec{E}$ E is the electric field, $\vec{v}$ v is the ion velocity, and $\vec{B}$ B is the magnetic flux density. This relationship forms part of the physical foundation of ion manipulation and separation in mass spectrometers. Major Biomarkers in Exhaled Breath and Their Diagnostic SignificanceVOCs in exhaled breath can reflect changes in biochemical processes occurring within the human body. Some compounds are produced during oxidative stress, lipid peroxidation, energy metabolism, and microbial activity. Consequently, breath analysis has emerged as a promising approach for the early investigation of various pathological conditions. [2]



Acetone (C_3H_6O)

Acetone is associated with fatty acid breakdown and ketone-body metabolism. Its concentration in exhaled breath may change in diabetes, fasting, and ketosis. However, dietary intake, physical activity, and other metabolic factors can also influence its concentration.

Isoprene (C_5H_8)

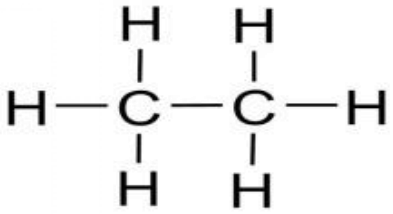


Isoprene is associated with cholesterol biosynthesis and lipid metabolism. Its breath may vary with physical activity, metabolic conditions.

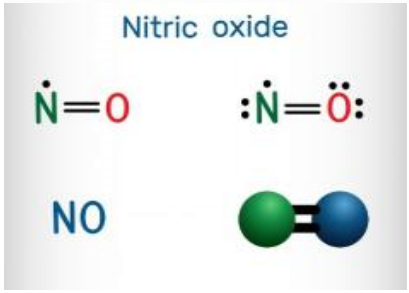
concentration in exhaled circulatory changes, and

Ethane (C_2H_6)

Ethane can be during lipid peroxidation therefore investigated as a indicator of oxidative stress.



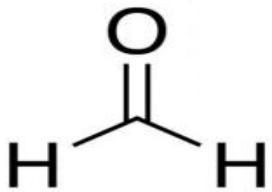
produced and is potential



Nitric oxide (NO)

Exhaled nitric oxide is an important biomarker for assessing airway inflammation, particularly in certain cases of asthma. Unlike VOCs, nitric oxide is an inorganic gaseous compound and is usually measured using specialized gas analyzers.

Formaldehyde (CH_2O)



Formaldehyde can be produced metabolic processes. However, its breath can also be affected by smoke, and other external sources. as a disease biomarker requires caution. value of breath biomarkers can often effectively by analyzing several rather than relying on a single substance. Statistical models and machine-learning algorithms can be used to identify patterns in complex VOC profiles. Nevertheless, most of these compounds are not independently sufficient for diagnosis and require further clinical validation. [3]

through endogenous concentration in exhaled environmental pollution, Therefore, its interpretation The diagnostic be evaluated more compounds simultaneously

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Comparative Analysis of GC-MS, PTR-MS, and SIFT-MS

The analytical performance of these techniques depends on their operating principles, ionization mechanisms, and measurement speed.

Characteristic	GC-MS	PTR-MS	SIFT-MS
Fundamental principle	Chromatographic separation followed by mass spectrometry	Proton-transfer reactions	Chemical reactions with selected reagent ions
Sample preparation	Often required	Usually minimal	Usually minimal
Measurement speed	Depends on chromatographic separation time	Near-real-time	Near-real-time
Quantitative analysis	Calibration and internal standards	Reaction kinetics and calibration	Reaction-rate constants and calibration
Compound separation	High when chromatographic separation is effective	Limited for some isomers and isobaric compounds	Depends on reagent-ion and reaction selectivity
Main limitation	Analysis time and sample preparation	Difficulty distinguishing some isomers	Reaction selectivity, spectral overlap, and instrument complexity

Gas Chromatography–Mass Spectrometry (GC-MS)

GC-MS combines gas chromatographic separation with mass spectrometric identification. The gas chromatograph separates the components of a complex mixture according to their interactions with the stationary phase and their volatility. The separated compounds then enter the mass spectrometer, where they are ionized and detected. [5]

Electron ionization (EI) at 70 eV is widely used because it produces reproducible fragmentation patterns for many compounds. These patterns can be compared with spectral libraries to support compound identification.

The main advantages of GC-MS are its high analytical specificity, established identification libraries, and ability to distinguish many isomers through chromatographic separation. [18] Its limitations include relatively long analysis times, possible analyte losses during sample preparation, and the need for suitable sampling and preconcentration procedures when VOC concentrations are very low.

Proton-Transfer-Reaction Mass Spectrometry (PTR-MS)

PTR-MS is based on chemical ionization, commonly using hydronium ions as reagent ions. When the proton affinity of an analyte is sufficiently high, a proton-transfer reaction can occur:

$$H_3O^+ + voc \rightarrow VOCH^+ + H_2OH_3O^+ + VOC \rightarrow VOCH^+ + H_2 \quad O$$

Here, VOCH+VOCH+ represents the protonated analyte.

Because this process often produces relatively limited fragmentation, PTR-MS can detect intact protonated molecules. [17] Its major advantages include rapid measurements, minimal sample preparation, and the ability to monitor concentration changes during successive breathing cycles. Depending on the instrument configuration and analyte, PTR-MS can achieve very low detection limits, sometimes within the parts-per-trillion-by-volume (pptv) range or lower. However, its performance is

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compound-dependent, and isomers or other compounds producing ions at the same nominal mass may be difficult to distinguish without additional separation or chemical information.

Selected Ion Flow Tube Mass Spectrometry (SIFT-MS)

SIFT-MS uses selected reagent ions, typically H_3O^+, NO^+, and O_2^+, to react with target compounds under controlled conditions. The resulting product ions are measured, and their formation rates can be used to calculate analyte concentrations.

If the relevant reaction-rate constants, reagent-ion concentrations, and product-ion signals are known, quantitative analysis can be performed using a kinetic model.

The principal advantages of SIFT-MS include rapid analysis, limited sample preparation, and the possibility of direct quantitative measurements. Using different reagent ions can improve selectivity for certain compounds. However, its analytical performance depends on reaction pathways, ion detection capabilities, and the extent of spectral interference. [6]

PTR-TOF-MS combines proton-transfer-reaction ionization with a time-of-flight mass analyzer. The mass-to-charge ratio of an ion is determined from its flight time through the analyzer. This technique offers rapid acquisition, high mass resolution, and the ability to monitor a broad range of compounds. High mass resolution can help distinguish ions with the same nominal mass but different exact masses. Nevertheless, it cannot reliably distinguish all structural isomers, especially those with identical elemental compositions and exact masses.

Sensitivity, Detection Limits, and Mass Resolution

The analytical performance of mass spectrometers is evaluated using several parameters: [16]

- Sensitivity: The change in measured signal in response to a change in analyte concentration.
- Limit of detection (LOD): The lowest concentration that can be reliably distinguished from background noise under specified conditions.
- Limit of quantification (LOQ): The lowest concentration that can be measured with acceptable accuracy and precision.
- Mass resolution: The ability to distinguish ions with similar mass-to-charge ratios.
- Mass accuracy: The closeness of the measured mass to its theoretical or reference value.
- Repeatability: The agreement between measurements performed under the same conditions.

Mass resolution is commonly expressed as:

$R=m/\Delta m$

where mm is the mass-to-charge ratio of the ion and m/Δm is the separation between two nearby peaks according to a specified reolution criterion. [7] For example, high-resolution PTR-TOF-MS can distinguish certain ions with the same nominal mass but different exact masses. However, high resolution alone cannot distinguish isomers that have identical elemental compositions and exact masses. [14] Additional chromatographic separation, chemical ionization strategies, or other analytical techniques may be necessary.

Because VOC concentrations in exhaled breath can be extremely low, background signals, detector noise, and analyte losses during sampling must be carefully controlled. Sensitivity should therefore be assessed not only using manufacturer specifications but also under realistic analytical conditions. [8] Breath Sampling and Sample Preparation Techniques. Sample collection is a critical step in breath analysis. Even highly sensitive instruments may produce unreliable results if samples are contaminated, improperly stored, or collected inconsistently.

Breath sampling is an essential stage in mass spectrometric analysis because sample quality directly affects the accuracy and reliability of analytical results. Several sampling techniques are used to collect exhaled breath, including direct online sampling, collection in inert sampling bags, and the use of sorbent tubes. The choice of method depends on the analytical technique, the chemical properties of the target compounds, and the required detection limits.

Direct online sampling allows exhaled breath to be introduced into the analytical instrument in real time. This approach minimizes sample storage effects and enables the monitoring of changes in

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VOC concentrations during breathing. In contrast, sampling bags provide a convenient method for collecting breath for subsequent laboratory analysis. [13] However, adsorption onto container surfaces, leakage, and chemical reactions during storage may alter the concentrations of certain compounds.

Sorbent tubes are commonly used when volatile compounds must be concentrated before analysis. In this approach, VOCs are retained on a suitable sorbent material and subsequently released by thermal desorption before entering the analytical system. [12] This procedure can improve detection sensitivity for compounds present at low concentrations, although recovery efficiency depends on the sorbent material and the chemical properties of the analytes. To minimize contamination, sampling systems should be appropriately cleaned, and background air should be evaluated when necessary. Environmental VOCs, humidity, temperature, breathing patterns, and the time of sample collection may influence the measured concentrations. Standardized sampling protocols are therefore important for obtaining reproducible results and comparing data between studies.

Future Perspectives

Recent developments in mass spectrometry have increased interest in the use of breath analysis for medical diagnostics and personalized healthcare. The integration of high-resolution mass spectrometry with advanced data-processing methods can facilitate the detection of complex VOC patterns that may be associated with specific physiological or pathological conditions. Machine-learning approaches may further support biomarker selection and the classification of breath profiles. [9]

Nevertheless, several challenges remain, including the lack of universally standardized sampling procedures, differences between analytical platforms, and the influence of individual variability on biomarker concentrations. Future studies should focus on improving analytical reproducibility, establishing reference ranges, and validating candidate biomarkers in large and diverse clinical populations. Combining breath analysis with other diagnostic methods may also improve the reliability of clinical interpretation.

Conclusion

Mass spectrometric analysis of exhaled breath represents a promising, non-invasive approach for investigating human metabolism and identifying potential biomarkers of various pathological conditions. The physicochemical properties of volatile organic compounds (VOCs), including molecular mass, proton affinity, ionization energy, and chemical reactivity, play a fundamental role in determining their detection and quantification. Understanding the principles of ionization, ion separation, and detection is therefore essential for the reliable interpretation of breath analysis results. Among the major analytical techniques, gas chromatography–mass spectrometry (GC-MS), proton-transfer-reaction mass spectrometry (PTR-MS), and selected ion flow tube mass spectrometry (SIFT-MS) offer complementary advantages. GC-MS provides effective chromatographic separation and reliable compound identification, whereas PTR-MS and SIFT-MS enable rapid measurements with minimal sample preparation. Furthermore, PTR-TOF-MS combines rapid analysis with high mass resolution, facilitating the differentiation of certain compounds with similar nominal masses. [10] Breath biomarkers such as acetone, isoprene, ethane, nitric oxide, and formaldehyde may provide valuable information about metabolic activity, oxidative stress, and airway inflammation. However, their concentrations can be influenced by physiological conditions, environmental exposure, diet, and physical activity. Consequently, the detection of a single compound is generally insufficient to establish a definitive diagnosis. The simultaneous analysis of multiple biomarkers, supported by statistical methods and machine-learning algorithms, may improve diagnostic performance and enable the identification of more reliable biomarker patterns.

Finally, the accuracy and clinical applicability of breath analysis depend not only on the analytical capabilities of mass spectrometers but also on appropriate sampling procedures, contamination control, calibration, and validation under realistic conditions. Further research and large-scale clinical studies are required to establish standardized protocols and confirm the diagnostic value of specific VOC profiles. [11] Overall, mass spectrometry-based breath analysis has considerable

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potential to contribute to early disease detection, non-invasive monitoring, and the development of personalized diagnostic strategies.

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