Hidden Motif Scientific

Innovative Non-Invasive Technologies for Time-Resolved Human Metabolism Profiling

Proprietary innovations drive the development of cost-effective diagnostic devices for personalized medicine applications.

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Summary

Our proprietary technology overcomes key technical and methodological challenges in current metabolomics-based diagnostics, offering cost-effective, eco-friendly, and user-friendly health monitoring solutions, democratizing access to high-precision metabolic data. By recording robust, time-resolved metabolic profiles, it aligns with physiological and psychological changes, adapting to each patient's unique metabotype while remaining unaffected by lifestyle or nutrition.

Initially validated for identifying the body's metabolic response in neural shock caused by metabolic imbalance (e.g., withdrawal syndrome), its nature as a continuously evolving platform signifies immense potential for future development, offering diverse healthcare insights currently beyond the reach of other methodologies.

The platform shows capacity to detect subtle yet important physiological changes. It can find broad application in such areas like the nervous system, metabolic disorders (e.g., diabetes), immunity (e.g., immune-based cancer treatment), and inflammatory processes.

To uncover patterns in large patient groups, extensive use of AI-based approaches is intended.

Keywords

- Non-invasive disease diagnostics
- Time-resolved metabolic profiling
- Artificial intelligence in personalized medicine

Introduction

Metabolomics is essential for linking genotype and phenotype, and it is increasingly used to identify and validate targets for personalized health improvement.

However, the conventional approach of studying biomarker patterns at a single time point often leads to limited practical outcomes and insights, as it fails to capture the full dynamic picture of metabolism.

It is crucial to recognize that metabolism is a dynamic process regulated by kinetic mechanisms. Therefore, to fully unlock the potential of metabolic-based health diagnostics, time-resolved metabolomics – where metabolites are observed over time – is essential.

Time-resolved metabolomics relies on the concept of metabolic homeostasis, referred to as 'metabostasis,' which represents the baseline state that living systems strive to maintain. The metabostasis pattern is not uniform across individuals and is, to some extent, personalized. It is also widely believed, although not definitively proven, that there are several major human metabolic subtypes known as metabotypes.

Working Hypothesis Statement

Instead of seeking general metabolic patterns specifically linked to diseases, which may vary among individuals and can be challenging to extract directly from complex metabolic data, we adopt a more reliable and straightforward approach. This involves focusing on the specific 'ground state' metabostasis pattern inherent to each individual or, presumably, to specific groups within a metabotype.

Changes in the metabostatic pattern – whether disruptions or restorations – can be recorded over time for specific physiological or psychological changes. This approach allows for the identification of individual-specific (or metabotype-specific) reactions of human metabolism, referred to as metabolic signatures. In this context, a biomarker is not merely a single metabolite but a comprehensive time-resolved metabolic signature that reflects deviations from the baseline metabostatic state. The specific values of the parameters describing these metabolic patterns are inherently individual from the outset.

The working hypothesis of time-resolved human metabolomics is that by understanding the full range of specific metabolic signatures, we can eventually identify broader patterns linked to certain diseases or disorders. For several classes of disorders, the likelihood of this approach yielding accurate insights is considered to be very high.

Specific metabolic signatures are significantly altered in individuals with various metabolic disorders. Furthermore, brain function is critically dependent on regulated energy metabolism, making metabolic disturbances particularly relevant within the central nervous system due to the brain's high energy demands. Many metabolites also play essential roles in regulating central nervous system functioning and immune responses.

It is widely accepted that metabolism responds acutely to various conditions, including intoxication, inflammation, hormonal fluctuations, withdrawal syndrome, and both short-term and long-term stress. Substantial evidence indicates that metabostasis is disrupted in cases of cardiovascular diseases, while metabolic reprogramming leads to significant alterations in metabolic processes in cancers, even at early stages.

To make significant strides in this field, it is essential to obtain informative and comprehensive time-resolved metabolic signatures that reflect various physiological and psychological changes, captured with high accuracy – namely, extremely robust data. Gathering such data from extensive patient cohorts allows for effective processing by advanced AI algorithms, which are now highly developed and supported by accessible AI services.

But AI can perform effectively only when it is combined with both high-quality and high-quantity data. Success also hinges on the availability of high-quality data that is informative and accurately captured, enabling AI to draw meaningful conclusions and identify key patterns. So, equally important is the ability to gather large volumes of this data, as it is the only way to ensure the robustness of the insights generated.

That is why the scalability of the technology of time-resolved metabolic profiling relies critically on making it affordable, eco-friendly, and user-friendly. We prioritize these aspects in our approach because they are fundamental to achieving our goals.

Overall Strategy

There are several options for selecting bodily fluids to monitor metabolism, including blood, urine, saliva, and sweat. However, the latter two contain too few metabolites, so metabolism is generally studied through blood or urine analysis. Thus, the real choice is always between these two.

For our purposes, in developing a metabolic analyzer for widespread use, blood is not an ideal option, while urine proves to be nearly perfect. Four key considerations led to this conclusion.

1. The analysis should be non-invasive, meaning the bodily fluid must be easy to collect.
2. Sample preparation should not be required.

These two essential requirements clearly favor urine as the preferred sample, ruling out blood. Additionally, there are two broader factors that further support urine-based diagnostics.

3. Urine generally contains more metabolites than blood plasma. Most metabolites found in blood are also present in urine, though in different concentrations. Additionally, urine contains a vast number of unique metabolites not found in blood. While many of these are poorly studied and their roles are not well understood, this is advantageous for us, as it increases the likelihood of discovering new, previously undescribed metabolic patterns.
4. Metabolic homeostasis is regulated more tightly in blood than in urine, which is not ideal for our purposes. This makes it harder and less likely to detect meaningful metabolic deviations related to the physiological or psychological changes being studied. Simply put, for time-resolved metabolomics, blood is less informative than urine.

Technological Innovation

Currently, metabolomics-based diagnostics face significant challenges due to the lack of appropriate technical solutions for time-resolved metabolomic research.

Chromatographic methods coupled with mass spectrometry, such as HPLC/MS and GC/MS, present several drawbacks:

- They require extensive sample preparation.
- They demand highly skilled professional chemists for operation.
- Their processes are not environmentally friendly.
- They are expensive, making them less accessible.

High-end spectral methods like NMR and HRMS have their own limitations:

- They do not detect signals from individual substances, meaning they cannot measure individual metabolites.
- They also require highly skilled professional chemists for operation.
- Their high cost further restricts their use.

As a result, while some metabolomic research can be conducted in specialized centers by highly skilled chemists using these advanced tools, the scale required for time-resolved metabolome analysis remains out of reach. The current data generated by these methods is small, disorganized, and varies in accuracy, making it insufficient for deriving reliable conclusions. Additionally, this limited and consistent data cannot be effectively utilized by AI for further analysis and development.

Our HPLC-based technology leverages a unique combination of conventional (non-MS) HPLC detectors with proprietary separation techniques. This approach overcomes the previously mentioned technical challenges while maintaining affordability, with significant potential for cost reduction in large-scale production.

Methodological Advances

Based on a review of literature on metabolomic analysis, we identify at least four significant methodological issues researchers encounter:

1. Studies do not account for the multiple metabolic subtypes in humans. Reliable methods to identify these subtypes or adjust measurements accordingly are lacking, overlooking this crucial factor.
2. Measurements are not time-resolved; patients are only tested once, with no follow-up assessments.
3. Fragmented, inconsistent urine samples are used, compiled in an unreliable manner, without averaging, etc.

4. Patients are grouped together without considering disease stage, progression over time, or the treatments they receive.

Our technology eliminates the drawbacks listed in points 1-3. In future experiments and clinical trials, we will also address the issue noted in point 4.

Experiment Overview and Discussion

To explore the connection between metabolism and both the physiological and psychological aspects of well-being, we chose the highly relevant healthcare topic of acute antidepressant withdrawal syndrome. Our goal was to observe and document the anticipated development of a significant metabolic imbalance within three days, followed by a relatively rapid restoration of metabostasis upon resuming the medication.

The experiment was conducted over ten days. The SSRI antidepressant, taken daily at 10 a.m., was discontinued on the third day and resumed at noon on the fifth day, when all typical physiological and psychological effects of antidepressant withdrawal syndrome were clearly pronounced.

Each day, one composite urine sample was analyzed, consisting of three samples: two collected at different times during the day and one from the previous evening. The metabolic composition of the urine was analyzed using a beta version of an analyzer based on innovative proprietary technology developed in-house.

A total of 36 distinct signals from major urine metabolites were identified, with high signal specificity (see Fig. 1) ensuring maximum accuracy in metabolic analysis.

For the paired relationships of the obtained signals, dependencies over time were established. Based on the nature of their changes over time, the metabolic relationships were categorized into three types (see Fig. 2):

A. stable but unresponsive to the studied process,

B. stable and responsive to the process,

C. unstable.

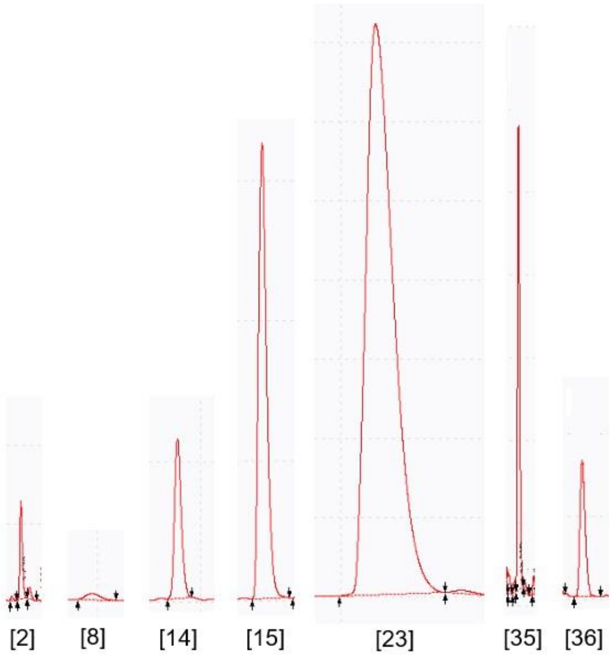

Figure 1. *Signal profiles used to identify individual metabolites (2, 8, 14, 15, 23, 35, and 36) demonstrate exceptional specificity, ensuring highly accurate measurements.*

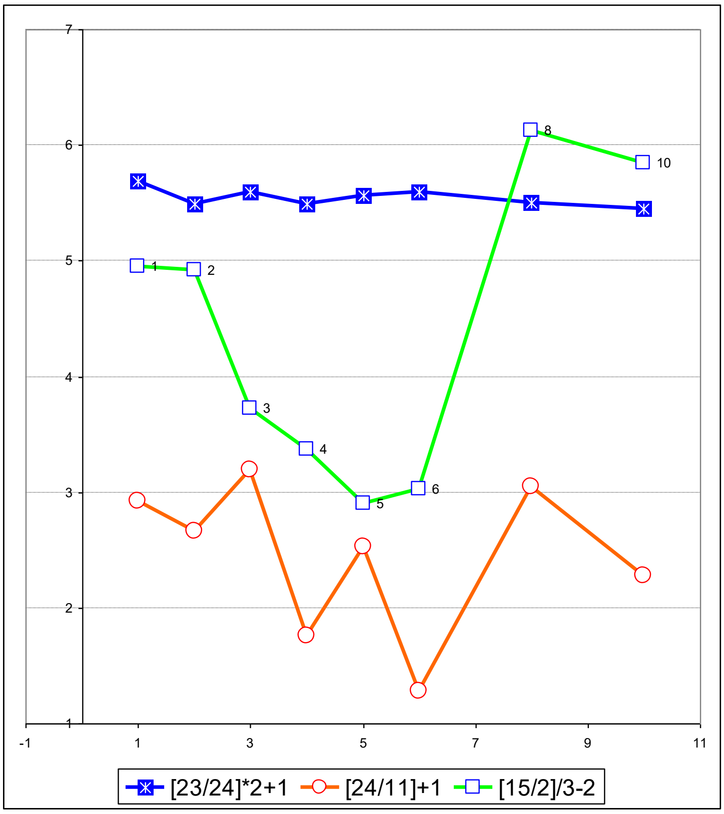

Figure 2. *Three categories of metabolic relationships: stable but unresponsive to the studied process [23/24], stable and responsive to the process [15/2], and unstable [24/11]. The presence of stable metabolic relationships over time (i.e., parameters of types A and B) serves as a cornerstone validating the effectiveness of our measurement methodology.*

This confirms its capability to detect metabostasis in the body through the analysis of selected samples using our chosen approach.

Additionally, the presence of type B parameters that respond to the process of antidepressant withdrawal syndrome demonstrates the method's suitability for tracking metabolic imbalance by observing both the disruption and restoration of metabostasis. This suggests that urine-based diagnosis using time-resolved urine metabolomics, through our proprietary innovative approach, is a viable and effective technology.

The varying nature of the parameters that describe the process is illustrated in Figure 3. Some parameters react sharply, demonstrating a very close correlation with fluctuations in well-being, including both deterioration and improvement ([35/36]). Others are more balanced, exhibiting slight inertia ([15/8]). Some respond with significant inertia, showing delays of one to two days or more, which allows us to observe the impact on the body through metabolism even after well-being has already returned ([15/2], [15/14]). Thus, the delay in the parameter's response to the stimulus increases in the order of [35/36] > [15/8] > [15/2] > [15/14], ranging from the minimal possible delay (from a few hours to a day) to several days or more.

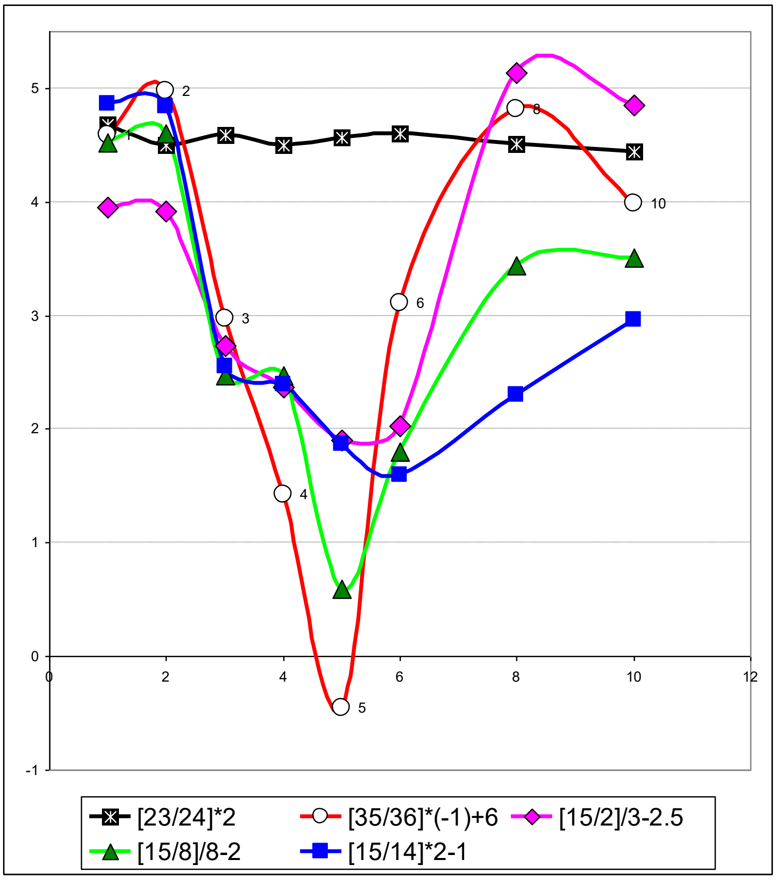

Figure 3. *Various parameter types categorized by their response levels: responsive [35/36], responsive with slight inertia [15/8], responsive with high inertia [15/2], [15/14], and non-responsive [23/24]. In practice, all three types of parameters prove to be useful. Parameters with the least inertia are well-suited for assessing the current psycho-emotional state, while those with moderate delays are effective for evaluating the overall physiological condition of the body. Meanwhile, parameters with significant inertia are valuable for testing for intoxication and other extreme impacts on the body, which cannot be concealed even over the course of a week.*

By thoughtfully combining metabolic relationships with known metabolic pathways, we can generate additional metabolic parameters with valuable properties. For instance, combining parameters [15/2] and [15/14] into a derived parameter [15/2]*[15/14] has proven effective. This combination yields a smoother time-resolved profile (see Fig. 4), allowing us to pinpoint the moment of extreme impact on the body with high precision, even up to a week after the event.

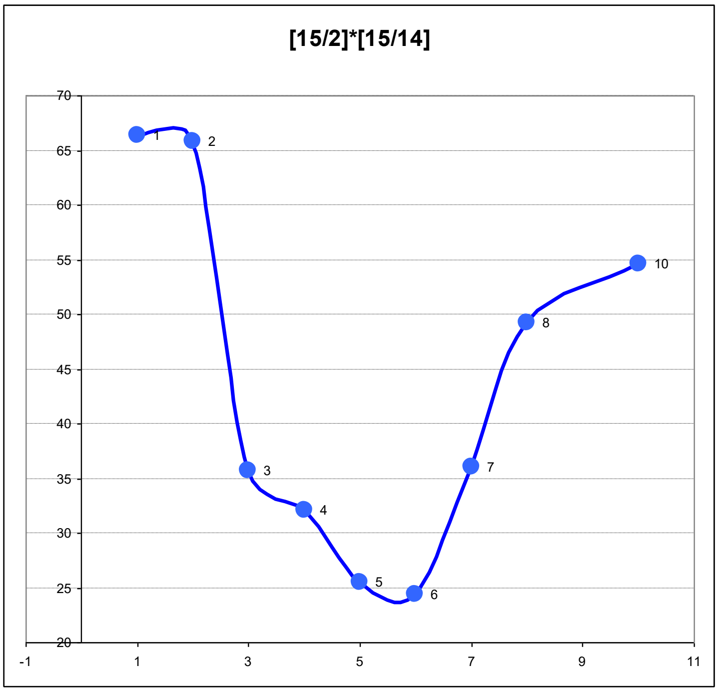

Figure 4. *The derived parameter [15/2]*[15/14] has proven effective, producing a smoother time-resolved profile. This enables precise identification of the moment of peak impact on the body, even up to a week after the event.*

The full set of stable metabolic ratios in a "neutral" undisrupted metabolic state, which can be measured using the analyzer in its current configuration, technically encompasses the observed metabolic pattern. The time-resolved metabolic signature of the process can then be expressed as a matrix, where the rows represent the metabolic pattern parameters that respond to the process, and the columns correspond to the time intervals of observation (e.g., days).

We will visualize the time-resolved metabolic signature for this case using the exact values of parameters [35/36], [15/8], [15/2], [15/14], and [15/2]*[15/14] (see Fig. 4-5).

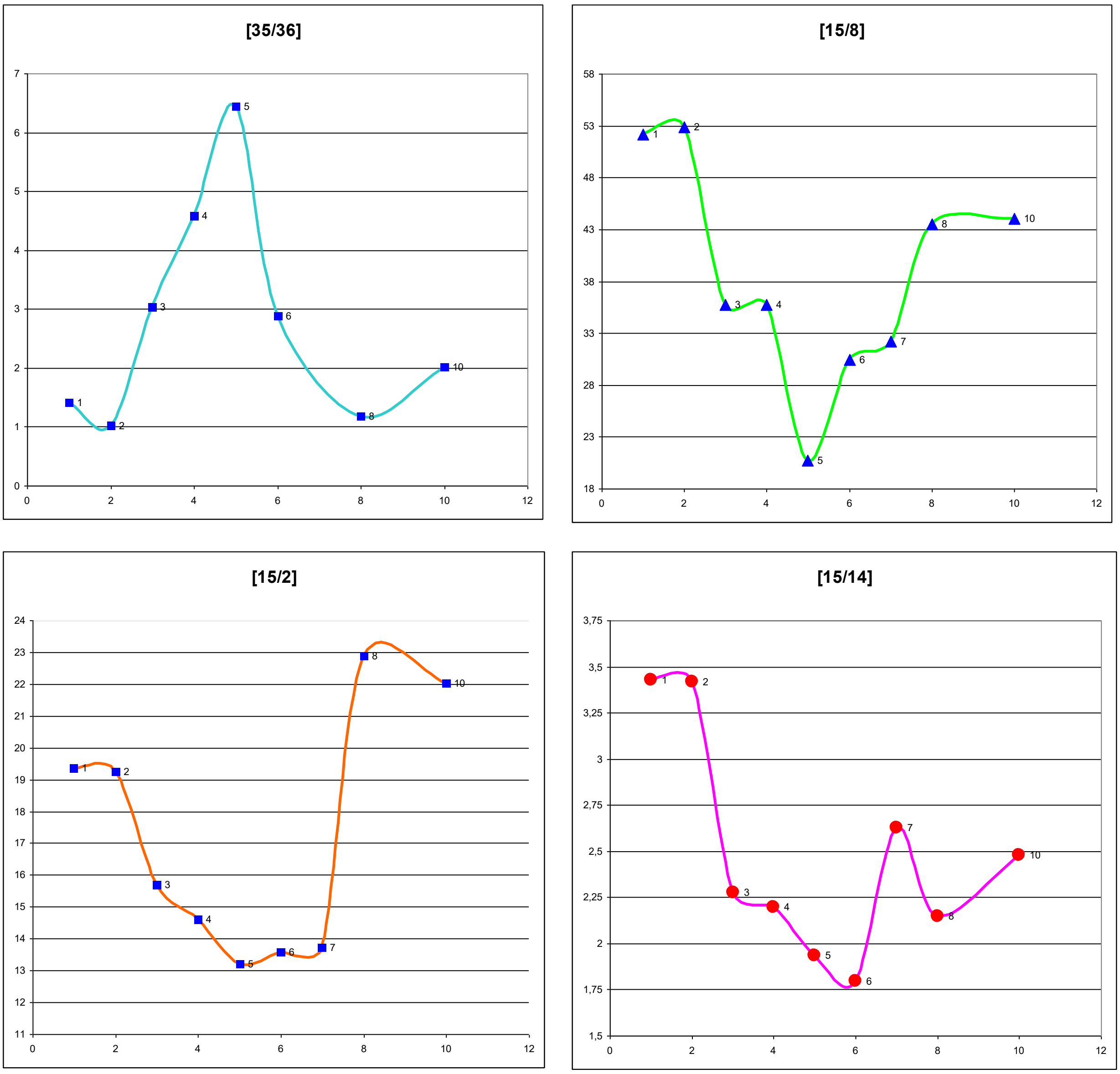

Figure 5. *Time-resolved profiles of metabolic relationships: [35/36], [15/8], [15/2], and [15/14].*

To do this, we first categorize them into four classes based on the degree of metabolic imbalance – ranging from green (no imbalance) to red (maximum imbalance). The matrix, composed of numerical categorization criteria (see Tab. 1), essentially reflects the individual's metabotype. We will refer to this as the "metabotype scores matrix."

Table 1. Metabotype score matrix for withdrawal syndrome

[15/8]	>45	38-45	28-38	<28
[15/2]	>17	14-17	13.5-14	<13.5
[15/14]	>2.75	2.4-2.75	2.0-2.4	<2.0
[15/2]*[15/14]	>60	45-60	30-45	<30
[35/36]	≤2	2-4	4-6	>6

Next, we will present the time-resolved metabolic signature matrix in a more intuitive format, replacing numerical values with the four levels of metabolic imbalance (Tab. 2).

Table 2. Time-resolved metabolic signature matrix for withdrawal syndrome

metabolic pattern	**DAYS**								
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**10**
[15/8]									
[15/2]									
[15/14]									
[15/2]*[15/14]									
[35/36]									
pill: yes/not in time/no									
how one feels									

Potential Applications and Future Prospects of the Technology

Leveraging time-resolved metabolic signatures and metabotype scores matrices unlocks exciting possibilities for medicine and pharmacology.

Metabotype scores matrices provide detailed insights into personalized metabolic patterns, offering a transformative approach to healthcare. With the potential for AI to swiftly analyze these scores across thousands of patients, we will gain profound insights into human metabolic patterns, paving the way for advancements in metabotype diagnostics and, consequently, personalized disease treatment.

In turn, time-resolved metabolic signatures help visualize key psychological and physiological changes, while also serving as a real-time indicator of overall well-being. They may also respond to factors such as infections, inflammation, allergic reactions, and various immune disorders, warranting further investigation.

This technology is expected to be vital for assessing drug toxicity, tracking patient status during treatment, and monitoring remission or relapse in clinical settings. Its initial implementation could focus on drug development through organ-on-a-chip technologies, where regulatory barriers for rapid adoption are minimal.

Additionally, it holds potential for monitoring psychological states and cognitive performance in high-risk professions, such as civil and military aviation pilots, astronauts, nuclear station operators, and similar fields.

In this application of the technology, the time-resolved metabolic signature matrix should be analyzed before the occurrence of these adverse events, which is feasible in a clinical setting.

AI is expected to play a significant role in the development of this technology, focusing not only on the categorization of metabotypes but also on the classification of metabolic signatures. Additionally, AI will facilitate the rapid adaptation of the technology to new practical applications and emerging challenges.

In the medium term, we envision a pivotal step toward the seamless integration of our technology into smart toilets, further enhancing routine health monitoring.

Looking to the long term, we believe that these advanced tools for continuous health monitoring, used in both clinical and outpatient settings, will provide enough data to identify universal metabolic signatures associated with various acute age-related diseases, including neurodegenerative conditions and cancers.

About Authors

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- 30+ years in developing commercial analytical chemistry solutions.
- Over 17 years of entrepreneurial leadership.
- Served corporate clients including Sanofi, Novartis, Danone, Dohler, Cargill, and Merck.
- Author of three books on HPLC technology, available at www.hplc.today, with approximately 10,000 copies sold, supported by Shimadzu and Thermo Scientific.
- Developed a series of professional courses on HPLC technology and method development, attended by 500+ R&D professionals from 70+ companies.
- Authored 30+ scientific articles and possesses extensive expertise in statistical and numerical methods in chemistry; the Ph.D. thesis advisor was Nobel Prize nominee Prof. V. Davankov.

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- Built 12 years of experience in the Israeli pharma and startup ecosystems, transitioning from analytical chemist to HPLC method developer and lab manager.
