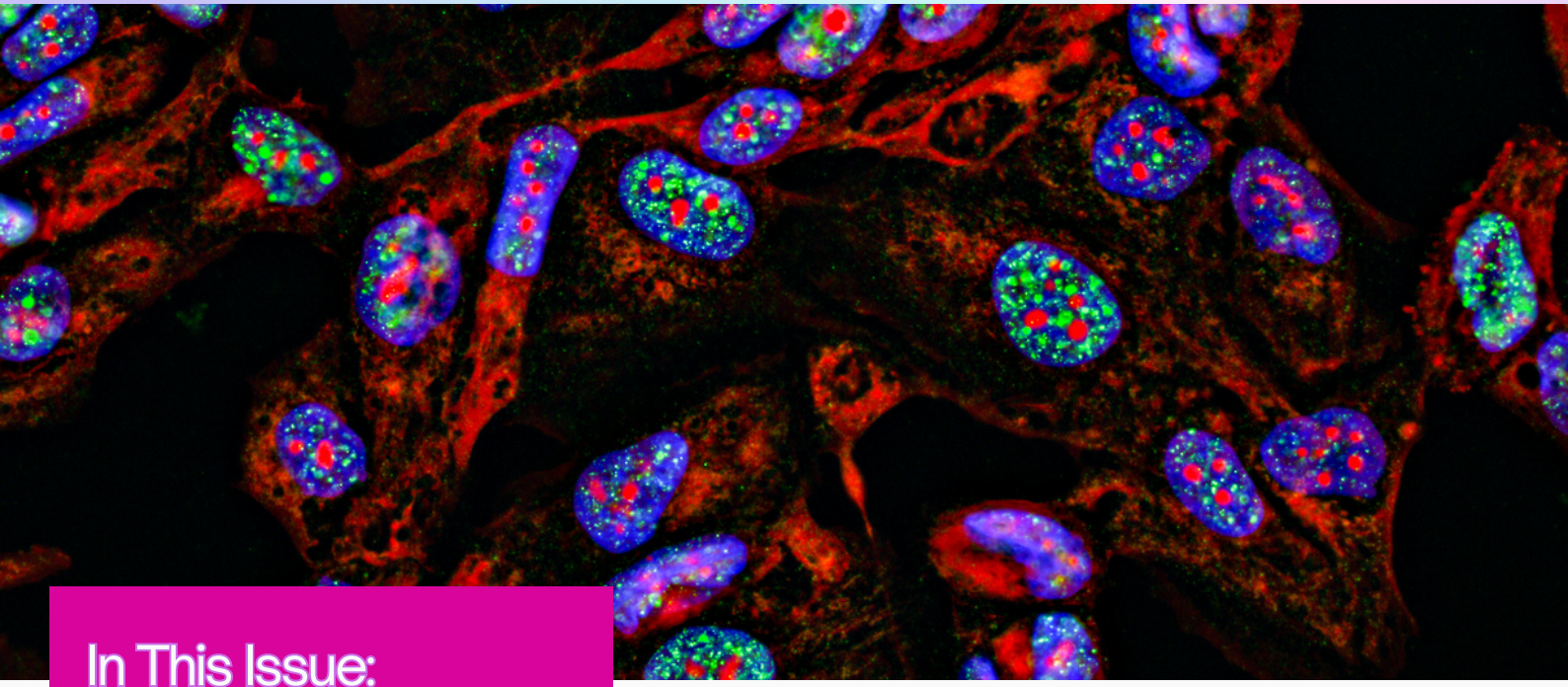
Issue 1/2024 January 2024

PRESCRIPTION

Latest news and updates from the Faculty of Pharmacy



In This Issue:

High-Throughput NMR-Based Metabolomics: A Robust Toolbox for Searching Human Disease Biomarkers

Metabolomics deals with the whole ensemble of metabolites (the metabolome). It is a holistic approach to studying systematic metabolic changes in biospecimens such as cells, tissue, organs, or organisms. It is a valuable tool that uniquely connects all "omics" techniques and best represents the phenotype. As one of the -omic sciences, it relates to biology, physiology, pathology and medicine, but metabolites are chemical entities, small organic molecules or inorganic ions. Therefore, their proper identification and quantitation in complex biological matrices require a solid chemical ground [1]. Metabolomics monitors the global outcome of all exogenous and endogenous factors without making assumptions about the effect of any single contribution to that outcome. It makes metabolomics a perfect technique for investigating and understanding the molecular mechanisms of human health and disease.

Metabolomics can contribute to precision medicine for the comprehension of individual susceptibility to drug administration, nutrition, and lifestyle interventions, the influence of environmental factors, as well as to the characterisation of the metabolic signature of diseases for diagnostic and prognostic purposes and for the understanding of the biochemical causes based on different pathophysiological conditions [2,3].

Thus, we need standard operating procedures, good chemical skills in sample preparation for storage and subsequent analysis, accurate analytical procedures, a broad knowledge of chemometrics and advanced statistical tools, and a good knowledge of at least one of the two metabolomic techniques, Mass Spectrometry (MS) or Nuclear Magnetic Resonance (NMR). Chemists traditionally cultivate all these skills. NMR and MS are the analytical techniques used to observe and quantify metabolites from biological samples, and each technique has advantages and disadvantages. NMR spectroscopy offers the unique potential to screen hundreds of metabolites holistically. It has already proven a powerful technique capable of providing a global picture of various metabolic processes underlying complex and multifactorial diseases, such as neurodegenerative and neurodevelopmental diseases [4] (Fig. 1).

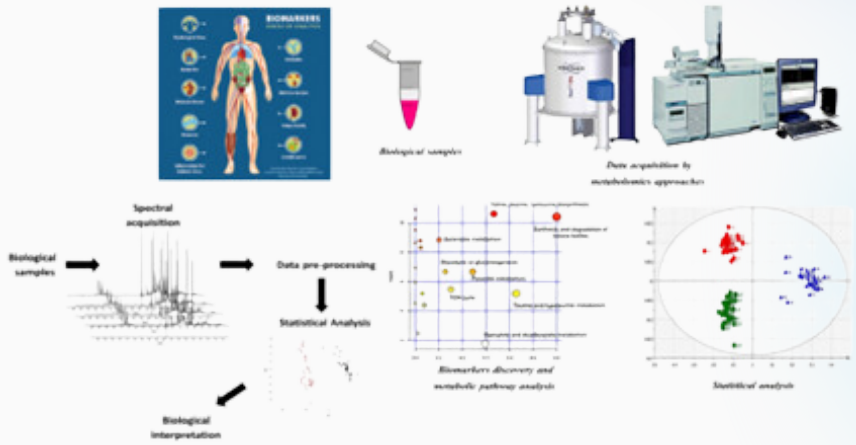


Figure 1

NMR-based metabolomics has emerged as a reliable high-throughput analytical technique that has been extensively applied to nearly every scientific field, including biomedicine, biomarker discovery and medical diagnosis, drug discovery and development, environmental science, agriculture, nutrition, food science, plant science, renewable energy, and systems biology. High-throughput NMR-based metabolomics techniques present several advantages to the clinician, including non-destructive, fast, and reproducible data acquisition, low cost per sample, and minimal sample preparation or intervention compared to MS. The most crucial feature of NMR-based metabolomics is its ability to provide highly accurate and reproducible quantification of metabolites from complex metabolite mixtures [6]. 1H NMR metabolomics provides a single snapshot of all the NMR-detectable compounds in a biospecimen. These approaches can identify the differences in low molecular weight molecules that exist in states of health or disease, which contributes to understanding cellular and physiological metabolism, helping to identify many unexpected biochemical causes for several important chronic and complex diseases, such as cancer, cardiovascular disease, diabetes, and obesity. One of the most striking aspects of metabolomics is the ability to detect alterations in the metabolome at the systemic level, which correlate with pathological states even for those diseases that are not immediately associated with metabolism. Such sensitivity, most probably involving immune mechanisms, is particularly promising to monitor the individual response to illness [7]. NMR-based metabolomics has the most ambitious objective of detecting early metabolic perturbations even before the manifestation of disease symptoms. The most common sample types in metabolomics studies include various biological samples such as urine, plasma, tissues, saliva, milk, seminal fluid, and sputum. Depending on the nature of the biospecimen (biological tissue, biofluid) or cell extract under study and the focus of the study (e.g., polar, nonpolar metabolites, and lipids), the resulting NMR spectrum corresponds to tens of thousands of molecules, mainly belonging to the class of amino acids, carbohydrates, alcohols, and organic acids [8].

Automated methods for the analysis of lipid and lipoprotein particles (LDL, HDL, VLDL, etc.) have been developed using NMR-based metabolomics. The breakdown of cholesterol into its component parts, called lipoprotein particles, involves the breakdown of proteins and lipids that include cholesterol. Therefore, lipid metabolism and metabolomics rely heavily on lipoprotein profiling [3].

Different metabolomes characterise different types of cells, but the measurable molecules are end-products or intermediates of the main metabolic pathways. Intensity variations in intracellular metabolites (endo-metabolome) induced by drug treatment, protein overexpression, genetic manipulation, etc., can directly relate to the up or downregulation of specific pathways. The NMR spectra of tissues reflect organ-specific biochemistry. However, tissue samples are extremely valuable as direct reporters of the diseased organ, where variations in the metabolome concerning a healthy state are expected to be most evident—the saliva metabolome changes in correlation with oral disorders and the presence of distant pathologies. A vital contribution to the metabolome of faecal extracts originates from metabolites resulting from the gut microbiota [7].

Coeliac disease (CD) is a multifactorial and complex disorder, including genetic and environmental factors that induce autoimmune response and nutrient malabsorption, which can have a significant potential impact on metabolism. The NMR-based metabolic signature of CD in serum and urine detected important alterations in the metabolic profiles of patients compared to healthy controls, mainly in ketone body metabolism and gut microbiota alterations [8].

NMR-based metabolomics of faecal material has been studied to monitor the metabolic effects of gut microbial ecology involved in dietary interventions and to investigate gut diseases such as inflammatory bowel disease, ulcerative colitis, and colorectal cancer. The metabolic activity in the colon may help identify new biomarkers related to the health or disease status and molecular regulation of the complex gut system. Over 60 metabolites have been identified from the obtained 1H NMR spectra. The 1H NMR spectra contained resonances of short-chain fatty acids (predominantly acetate, propionate, and butyrate), branched-chain fatty acids (isovalerate, isobutyrate), biogenic amines (trimethylamine and dimethylamine), organic acids (succinate, fumarate), carbohydrates (predominantly glucose), and amino acids (leucine, isoleucine, valine, tyrosine, phenylalanine, and others) [7].

Lung cancer is the most lethal among all types of cancer, and this has led to increased attention on its investigation. A recent study focused on discovering diagnostic biomarkers for lung cancer by examining tissue using ex vivo HRMAS NMR and in vitro NMR. NMR was used with targeted rapid-resolution liquid chromatography to obtain serum metabolite profiles for Stage I lung cancer patients (n=25) and matched healthy controls. Twenty-five metabolites were identified, which could distinguish the two groups with 100% sensitivity and specificity. This study highlighted the potential of NMR-based metabolomics for the early-stage detection of lung cancer. Additionally, another study detected 17 metabolites in serum associated with disease progression and 18 metabolites that differentiated between lung cancer patients and healthy controls [10,11].

Numerous NMR-based metabolomics investigations have focused on studying diabetes in human and animal models. In particular, it showed evidence for a significant correlation between several metabolites, including N-carbamoyl-β-alanine and glucose; N-carbamoyl- β -alanine was indicated to be a potential diabetes marker. In another study, plasma from a total of 71 patients with diabetes, coronary heart disease, diabetic coronary heart disease or healthy controls were investigated to assess the metabolic risk for diabetic coronary heart disease. The study found higher concentrations of five metabolites, including fatty acids, valine, isoleucine, phenylalanine, and lactate, in nondiabetic obese subjects compared to nondiabetics with low BMI. Further, 19 metabolites, including saturated fatty acids, several amino acids, lactic acid, 3-hydroxybutyric acid, choline, 3,7-dimethyluric acid, pantothenic acid, myoinositol, sorbitol, glycerol, and

glucose, were found to be higher in diabetic subjects compared to controls. Saliva metabolic profiling provides a non-invasive, beneficial method, such as in small children's investigations. By utilizing this approach, an analysis of type I diabetes was conducted in small children (<6 years of age) and found that many metabolites associated with glycolysis and the TCA cycle were altered between the diabetic and control groups [4,12].

Most viruses that cause illnesses tend to alter the metabolic profile of the target cells. This includes SARS-CoV, Zika, Dengue, Chikungunya, Influenza, and Hepatitis C. In patients infected with SARS-CoV, lipid metabolic changes and elevated levels of cysteine, alanine, aspartic acid, succinic acid, and lactate are observed persistently in their serum. Researchers have also found that microglia phospholipid and carboxylic acid levels are altered after Zika virus infection. West Nile virus infection increases sphingolipid and glycerophospholipid levels while decreasing cholesterol esters, whereas dengue virus infection increases fatty acids, acylcarnitines, glycerolipids, fructose, and hydroxyl ketones. Recently, a study used NMR-based metabolomics to deduce COVID-19 plasma and serum metabolomic profiles. The researchers compared healthy individuals' amino acid, carbohydrate, fatty acid, and glycerophospholipid profiles with patients diagnosed with varying degrees of COVID-19 severity. Additionally, another cross-sectional investigation found plasma profiles related to distinct COVID-19 outcomes, indicating that plasma changes occur at different phases of the disease [13].

NMR metabolomics identifies intermediate metabolites impacted by SARS-CoV-2. Over 200 biomolecules have been found in whole saliva, including metabolites, electrolytes, lipids, and proteins. These metabolites maintain the balance of oral tissues and reflect local and systemic conditions. Whole saliva also contains antiviral components, such as defensins, immunoglobulin A, gp340, lactoferrin, lysozyme, and cathelicidins [13].

NMR metabolomics is one of the most recent omics technologies that can describe the comprehensive measurement of the whole ensemble of metabolites present in a biological specimen, the metabolome. The metabolome can be considered the final product of the dynamic and evolving interaction of genetic expression, transcriptional changes, post-translational modifications of proteins, and various other external factors such as environment, diet, drug administration, diseases, lifestyle, and age [14]. NMR-based metabolomics is a well-established technique that has been applied to many areas of biomedical research to characterise the metabolomic profile/fingerprint of diseases for diagnostic and prognostic purposes and to unravel biomarkers/fingerprints that can predict individual responses to treatments (pharmacometabolomic).

ABOUT THE AUTHOR:

Assoc. Prof. Dr. Syed Adnan Ali Shah completed his Master of Science in Chemistry from the University of Karachi, Pakistan, in 2000. He obtained his PhD in Structural Organic Chemistry from the ICCBS, University of Karachi, Pakistan, in 2005. He then pursued a postdoctoral fellowship at the Institute of Analytical and Radiochemistry, University of Innsbruck, Austria. He became a Senior Lecturer in the Department of Pharmacology and Chemistry at the Faculty of Pharmacy, Universiti Teknologi MARA in 2007. He has published several articles in peer-reviewed journals and authored two books. He has ten US patent applications and four granted US patents in the field of natural product chemistry. He has also secured several research grants. His field of research included Natural product chemistry, NMR-based metabolomics and synthetic organic chemistry.

Questions
Let's dive deeper into the article and evaluate your comprehension. We have 6 questions for you here.

References

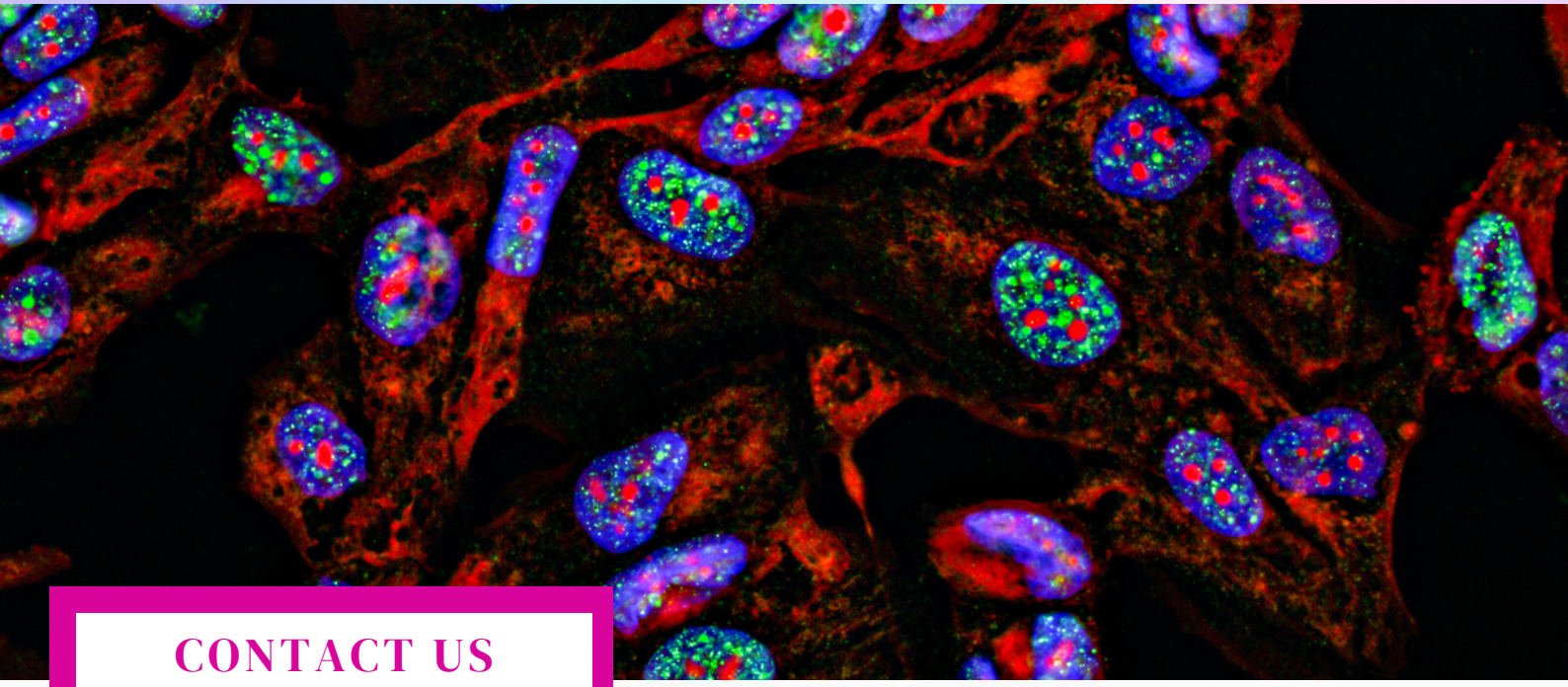
1. Vignoli, Al.; Ghini, V.; Meoni, Gaia.; Licari, C.; Takis, P.G.; Tenori, L.; Turano, P.; Luchinat, C. High-Throughput Metabolomics by 1D NMR. Angew. Chem. Int. Ed. 2019, 58, 968 – 994.
2. Chandra, K.; Al-Harthi, S.; Almulhim, F.; Emwas, A.; Jaremko, Ł.; Jaremko, M. The robust NMR toolbox for metabolomics. Mol. Omics, 2021, 17, 719–724.
3. Copié, V.; Edison, A.S.; Eghbalnia, H.R.; Hoch, J.C.; Gouveia, G.J.; Pathmasiri, W.; Powers, R.; Schock, T.B.; et al. NMR and Metabolomics—A Roadmap for the Future. Metabolites 2022, 12, 678. https://doi.org/10.3390/ metabo12080678.
4. Gowda N.G.A.; Raftery, D. Recent Advances in NMR-Based Metabolomics. Anal. Chem. 2017, 89, 490−510.
5. Bingol, K. Recent Advances in Targeted and Untargeted Metabolomics by NMR and MS/NMR Methods. High-Throughput 2018, 7, 9; doi:10.3390/ht7020009.
6. Edison, A. S.; Colonna, M.; Gouveia, G. J.; Holderman, N. R.; Judge, M. T.; Shen, X.; Zhang, S. NMR: Unique Strengths That Enhance Modern Metabolomics Research. Anal. Chem. 2021, 93 (1), 478−499.
7. Lamichhane, Santosh.; Yde, C.C.; Schmedes, M.S.; Jensen, H.M.; Meier, S.; Hanne Bertram, C. Strategy for Nuclear-Magnetic-Resonance-Based Metabolomics of Human Feces. Anal. Chem. 2015, 87, 5930−5937.
8. Medina, S.; Domınguez-Perles, R.; Gil, J. I.; Ferreres, F.; Gillzquierdo, A. Curr. Med. Chem. 2014, 21, 823−848.
9. McCartney, A.; Migliaccio, I.; Moretti, E.; Malorni, L.; Luchinat, C.; Biganzoli, L.; Tenori, L. Precision Oncology via NMR-Based Metabolomics: A Review on Breast Cancer. Int. J. Mol. Sci. 2021, 22, 4687.
10. Singh, A.; Prakash, Ved.; Gupta, Nikhil.; Kumar, A.; Kant, R.; Kumar, D. Serum Metabolic Disturbances in Lung Cancer Investigated through an Elaborative NMR-Based Serum Metabolomics Approach. ACS Omega 2022, 7, 5510−5520.
11. Vignoli, A.; Santini, G.; Tenori, L.; Macis, G.; Mores, N.; Macagno, Fr.; Pagano, F.; Higenbottam, T.; Luchinat, C.; Montuschi, P. NMR-Based Metabolomics for the Assessment of Inhaled Pharmacotherapy in Chronic Obstructive Pulmonary Disease Patients. J. Proteome Res. 2020, 19, 64−74.
12. Hasanpour, M.; Iranshahy, M.; Iranshahi, M. The application of metabolomics in investigating anti-diabetic activity of medicinal plants. Biomedicine & Pharmacotherapy. 2020, 128, 110263.
13. Junior G.C.S.; Pereira C.M.; Fidalgo T.K.S.; Valente A.P. Saliva NMR-Based Metabolomics in the War Against COVID-19. Anal. Chem. 2020, 92, 15688−15692.
14. Charris-Molina, A.; Burdisso, P.; Hoijemberg, P.A. Multiplet-Assisted Peak Alignment for 1H NMR-Based Metabolomics. J. Proteome Res. 2024, 23, 430−448.

Issue 1/2024

January 2024

PRESCRIPTION

Latest news and updates from the Faculty of Pharmacy



CONTACT US

@pharmacyuitm

@pharmacy_uitm

Faculty of Pharmacy UiTM

https://pharmacy.uitm.edu.my/

+603-3258 4645

korporatff@uitm.edu.my

NEWSLETTER EDITORIAL TEAM

PRESCRIPTION
Faculty of Pharmacy,
Universiti Teknologi MARA,
Kampus Puncak Alam,
42300 Bandar Puncak Alam, Selangor.