Targeted Exposomics: Profiling Urinary Organic Acids

Application Note

Clinical Chemistry, Emerging Omics Technologies

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Abstract

Measuring the exposome or totality exposures over individuals' lifetime, offers new insight into exposure-response relationships (biological epidemiology) by determining and differentiating the causal pathways (exposure biology) and reactive pathways (systems biology) in chronic human disease. Through the application of discovery-based omics and targeted methodologies to measure the exposome, exposomics will identify new biomarkers of disease, guide methods to mitigate exposures, and ultimately lead to more personalized medical interventions. Volatile organic solvent exposure, such as benzene or toluene, is an example of an exposure biomarker (the causal disease pathway) that leads to primary and secondary disease traits as evidenced by perturbations in the urinary organic acid profile (the reactive disease pathway). This application note presents a top-down, targeted approach of measuring the urinary organic acid exposome using GC/MS and post-acquisition mass spectral deconvolution with targeting library searching.

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Introduction

The exposome

An exposome is a quantity that measures individuals' lifetime bioaccumulation of exposure (including the prenatal term) and encompasses not only exogenous exposures (food, air, water, ionizing radiation, and lifestyle choices) but also endogenous exposures (metabolic processes, xenobiotics, and the activity of gut microbiota). With respect to endogenous biochemistry, it should be noted that even naturally occurring chemicals can become toxic if homeostasis is not maintained. An excellent example of this is given by Maple Syrup Urine Disease (MSUD), wherein three amino acids critical for protein synthesis are not properly metabolized and, therefore, buildup to toxic levels in the blood stream. Although MSUD is rare in the general US neonate population (1 in 180,000 births), it is greater than 1,000-fold more prevalent in the Amish and Mennonite population and many states require clinical screening of organic acids (biomarkers for MSUD and other diseases) within 24 hours of birth [1].

Exposomics

Exposomics is the application of omics tools (chromatography, spectrometry, spectroscopy, bioinformatics, and so forth) to measure the exposome and correlate the data to the genome, the environment, and the onset of disease. To this end, Stephen M. Rappaport [2] describes a top-down approach of biomonitoring tissue, blood, urine, and other bodily fluids and tissues to measure the exposome rather than a bottom-up approach of monitoring the environment (air, water, and so forth) for exposure risk potential.

One example of targeted top-down exposomics is biomonitoring of urinary organic acids using single quadrupole mass spectrometry and post data acquisition tools. These tools include: mass spectral deconvolution, identification using retention time locked mass spectral libraries and statistical analysis.

Organic aciduria

Organic acidurias are typically autosomal recessive disorders wherein one copy of the defective gene is inherited from each parent. There is a 25% chance that their offspring will possess two copies of the defective gene and manifest the disease. More than 60 metabolic perturbations are known to have a distinctive organic aciduria profile. In the case of MSUD, acidic urinary biomarkers include: 2-oxoisocaproic acid, 2-hydroxyisocaproic acid, 2-hydroxyisovaleric acid, 2-oxoisovaleric acid, 2-hydroxy-3-methylvaleric acid, and 2-oxo-3-methylvaleric acid.

Although most organic acidurias are directly correlated to genetic anomalies, many are also precipitated by exogenous and iatrogenic sources and gastrointestinal dysbiosis. For example, exposure to xylene isomers (commonly found in paint solvents) results in increased levels of methyl benzoate and methylhippurate [3]. Administration of valproic acid, a common anticonvulsant, can result in iatrogenic increases in 3-hydroxyisovalerate, 5-hydroxyhexanoate, 7-hydroxyoctanoate, *p*-hydroxyphenylpyruvate, and dicarboxylic acid levels in urine [4]. Gastrointestinal dysbiosis is evidenced by increased urinary levels of citramalic acid, 5-hydroxy-methylfuroic acid and tartaric acid arising respectfully from *Saccharomyces*, *Aspergillus,* and *Candida* species overgrowth in the bowel. With respect to tartaric aciduria from yeast overgrowth in the bowel, there has been at least one report of the manifestation of Autism-like features in a study of twin brothers [5]. As evidenced in these examples and those above, organic acidurias arise from both endogenous and exogenous sources. As sources and levels of exposure change over time, composite exposomes can be constructed by analyzing specimens obtained during various life stages [2]. In this sense, baseline urinary organic acid levels collected at birth represent one chemotype in the exposome that can be can be monitored over the course of a lifetime for comparative analysis and disease correlation.

Measuring the urinary organic acid exposome

Chen, *et al.* described a single quadrupole GC/MS method for analysis of urinary organic acids (UOA) that implements Retention Time Locking (RTL), Agilent Deconvolution Reporting Software (DRS) and backflush Capillary Flow Technology (CFT) [6]. With the 2013 introduction of the Agilent 7890B GC with an Agilent 5977A Series GC/MSD that operates on the Agilent MassHunter Software platform, this same method can be implemented and improved upon by the added sensitivity and features inherent in the new system and software platform. Chen's method used a 30 m × 0.25 mm, 0.25 µm Agilent HP-5MS column and the Agilent QuickSwap CFT device to allow for post analysis backflush and no-vent column exchange and maintenance.

Agilent now offers Ultra-Inert (UI) consumables such as syringes, septa, liners, and columns to greatly reduce the potential of flow path activity and improve chromatographic performance. Ultra Inert consumables can be used not only on the inert flow-path Agilent 7890B GC, but also on earlier generations of the n890 GC series and, in this example, the HP-5MS column can be replaced with the HP-5MSUI column. Familiar tools such as automated RTL are readily applied and the 7890B is designed to integrate CFT into analytical methods with advanced electronic pressure control, software wizards that quickly add backflush to any properly configured

method, and pneumatic calculators that aid in method development and translation.

Agilent has phased-out the QuickSwap device and now offers the more flexible Purged Ultimate Union (sometimes referred to as the pressure controlled tee (PCT)). With the PCT, the analyst is not locked into post-column backflush configurations that only allow post-run backflush as defined earlier using the QuickSwap device. The PCT can be placed in pre-analytical column, mid-column, and post-column configurations. Concurrent backflush conditions (concomitant backflush and data collection) can be implemented for the former two configurations to reduce cycle time and increase productivity even more than standard post-run backflush. The 5977A Series GC/MSD with a complete inert pathway, extraction ion source, high-performance turbomolecular pump, and triple axis detector is the most sensitive and robust mass selective detector to date with improved signal-to-noise specifications, thus lowering detection limits. Running on the MassHunter Software platform, the acquisition software has a similar look and feel of MSD Productivity ChemStation acquisition for efficient creation, update and use of GC/MS methods, and analysts have the option to use MassHunter Data Analysis or classic MSD ChemStation Data Analysis. With MassHunter, organic aciduria data analysis can be managed in either the MassHunter Quantitative or Qualitative packages with built-in deconvolution algorithms to search a user created, custom RTL spectral library. Using classic MSD ChemStation Data Analysis, the analyst can implement DRS using the same RTL spectral library. In either case, UOA's are accurately and robustly identified, quantified, and reported. The data can be exported to Agilent Mass Profiler or Mass Profiler Professional (MPP) for normalization, alignment, and statistical and pathway analysis. MPP facilitates identification of important differences in the type and relative amount and profile of UOA's present in different samples using statistical techniques such as analysis of variance (ANOVA), principal component analysis (PCA), clustering, volcano plots, hierarchical clustering, enrichment, and class prediction. Figure 1 illustrates a PCA analysis of several human samples and the clustering of chemotypes in the defined chemical space.

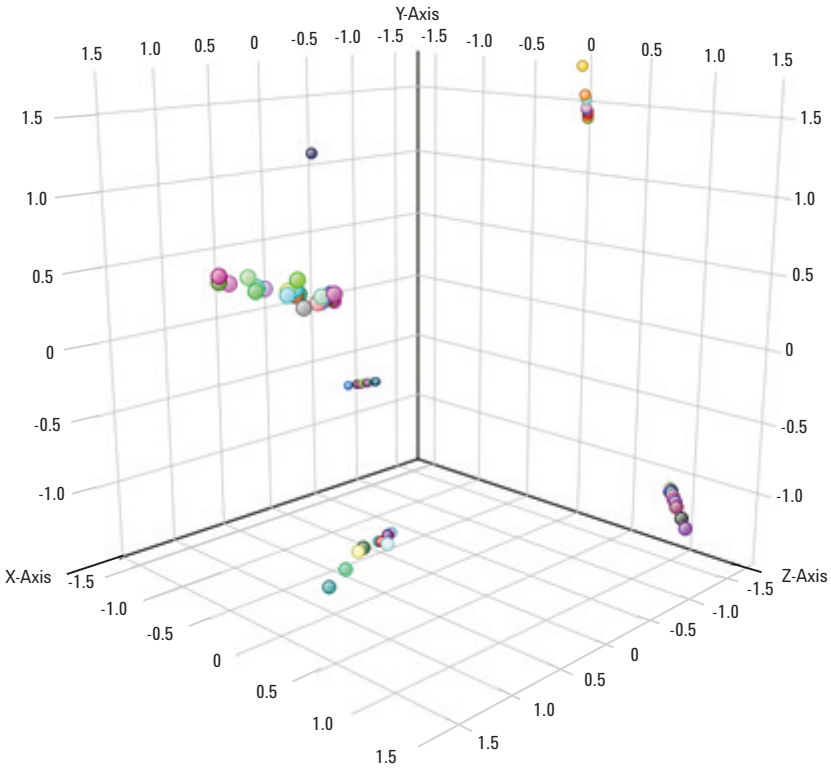

Figure 1. PCA analysis using MPP.

Conclusion

It is estimated that 70% to 90% of chronic human diseases are caused by environmental factors and are not attributable to inherited genetic variability [7,8]. Profiling urinary organic acids using single quadrupole GC/MS offers the ability to identify and quantify organic acidurias and establish a profile of the UOA exposome at any life stage. Baseline data can be collected at birth and compared to later data to monitor disease onset and progression. Large cohort studies of individual exposomes can be evaluated and compared to determine new UOA biomarkers using statistical analysis. Monitoring the exposome for chemicals such as UOA can facilitate the discovery of risk factors and identify new biomarkers for most of the diseases responsible for human morbidity and mortality. This model can further aid in the understanding of the genome × environment relationship in disease.

References

1. Learn. Genetics. The University of Utah, Retrieved March 12, 2013 from, http://learn.genetics.utah.edu/content/disorders/whataregd/msud/
2. S.M. Rappaport, *J. Expo. Sci. Environ. Epidemiol*. (2011) **21**, 5–9
3. R.S. Lord and J. Alexander Bralley, *Alt. Med. Rev*. (2008) **13**, 205-215
4. A. Kumps, P. Duez, and Y. Mardens, *Clin. Chem*. (2002) **48**, 708–717
5. W. Shaw, E. Kassen and E. Chaves, *Clin. Chem.* (1995) **41**, 1094-1104
6. J. Chen, C-K. Meng, S.B. Narayan, *et al., Clin. Chim. Acta*, (2009) **405**, 53-59
7. P. Lichtenstein, N.V. Holm, P.K. Verkasalo, *et al*., *N. Engl. J. Med*. (2000) **343**, 78–85.
8. T.A. Manolio, F.S. Collins, N.J. Cox, *et al., Nature* (2009) **461**, 747–753.

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Printed in the USA
November 22, 2013
5991-3541EN

Agilent Technologies