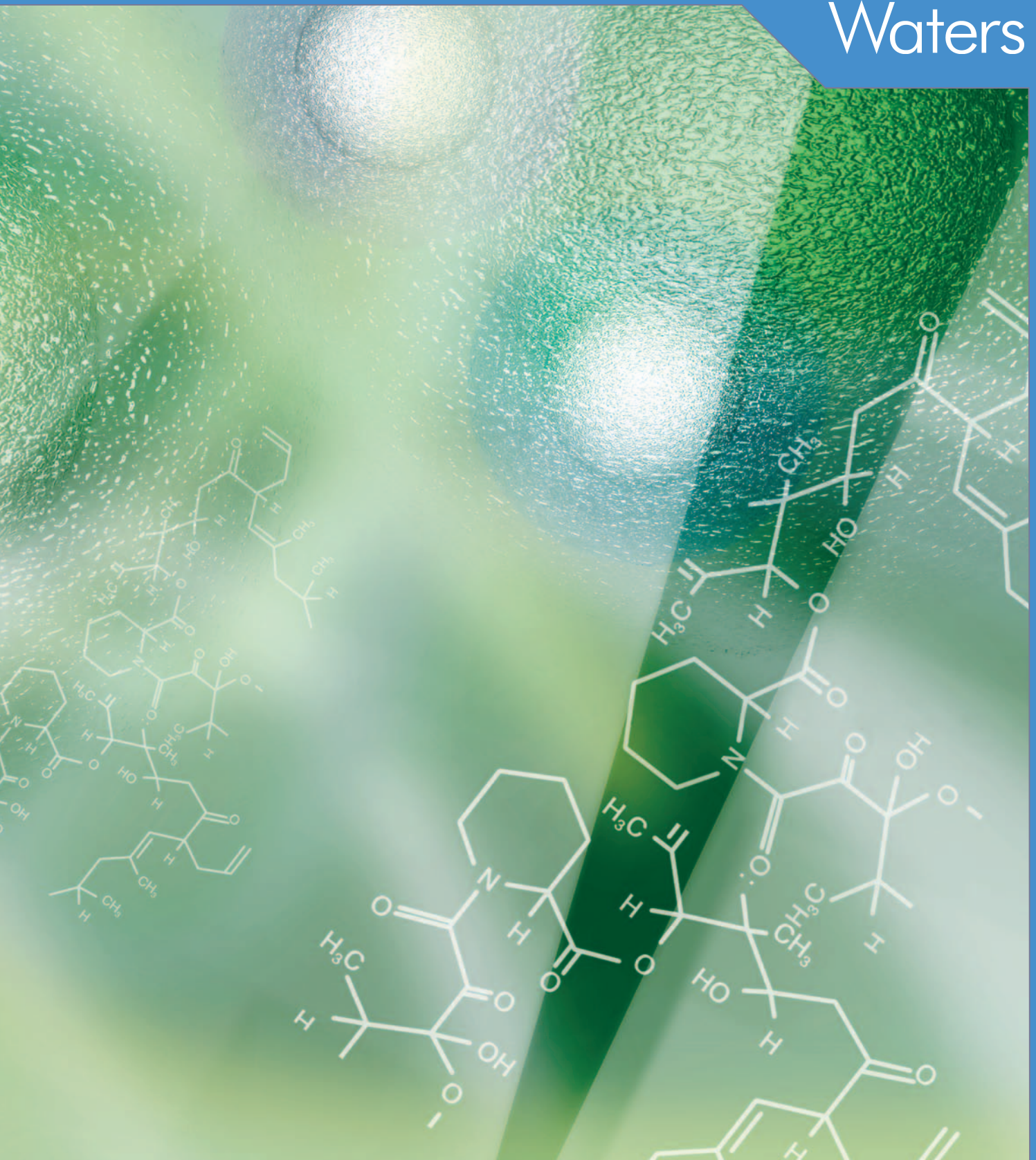


Waters



► **THE KEYS TO SUCCESS IN METABONOMICS: RAPID ANALYTE DETECTION. HIGH SENSITIVITY AND RESOLUTION. ROBUST DATA PROCESSING. GET ALL THE ANSWERS YOU NEED WITH WATERS SYSTEM SOLUTIONS.**

▶ BIOMARKERS AND METABONOMICS



METABONOMICS AS TOOL FOR BIOMARKER DISCOVERY

For pharmaceutical companies in drug discovery, the keys to unlocking new drug therapies are found in biomarkers. With the identification of a biomarker, researchers can dramatically improve their understanding of toxicity, monitor efficacy, and dramatically accelerate the development of new medicines.

Biomarker identification is an integral part of the field of metabolomics, which is now an essential component of the drug discovery process with the potential to significantly impact new molecular entity (NME) characterization. The correct implementation of metabolomic information will help pharmaceutical, biotechnology, and industrial companies streamline their discovery, development, and manufacturing processes by enabling scientists to visualize and identify fundamental differences in sample sets.

The successful deployment of metabolomics requires the development of reliable and efficient assays. This allows for the rapid detection of as many analytes as possible with a high degree of sensitivity and resolution in complex matrices. Equally important is the need for researchers to be able to easily process and interpret this large amount of data to convert it into meaningful information.

Waters addresses these challenges with an unrivalled suite of system solutions for metabolomics analysis. From our innovative Ultra Performance LC™ (UPLC™) system technology and our high-resolution exact mass Premier family of mass spectrometers, to our specialized Masslynx™ Software Application Manager for multivariate analysis and database searches, Waters offers the integrated tools that will give you complete confidence in your biomarker discovery and identification applications.

Groundbreaking chromatographic performance for complex samples

Introduced in 2003, Waters led the way in developing an HPLC/MS-Time-of-flight (ToF) platform specifically configured for metabolomics studies. We continue to provide pioneering solutions for metabolomics biomarker identification by incorporating our innovative UPLC technology in our suite of Metabolomics Systems.

Central to the Waters® ACQUITY UPLC™ System is a 1.7 μm bridged ethane hybrid particle. These new low-particle-diameter stationary phases operate at high linear flow rates to produce chromatograms with peak widths of less than one second at the base.

The ACQUITY UPLC System has been holistically designed to take full advantage of these sub-2 μm innovations, providing maximum chromatographic performance for optimized throughput, carryover, resolution, and robustness.

Together, these attributes make the ACQUITY UPLC System ideal for the analysis of the complex mixtures typical for metabolomics. Chromatographic run times of just one minute (Figure 1) contain as much information as was previously produced in a 10-minute HPLC run. With longer UPLC analysis times, information-rich LC/MS chromatograms are realized (Figure 2). These chromatograms typically have a peak capacity greater than 250 and result in a more than four-fold increase in sensitivity over traditional HPLC.

More peaks in less time, and greater sensitivity—the ACQUITY UPLC System is the optimal instrument solution for metabolomics biomarker discovery.

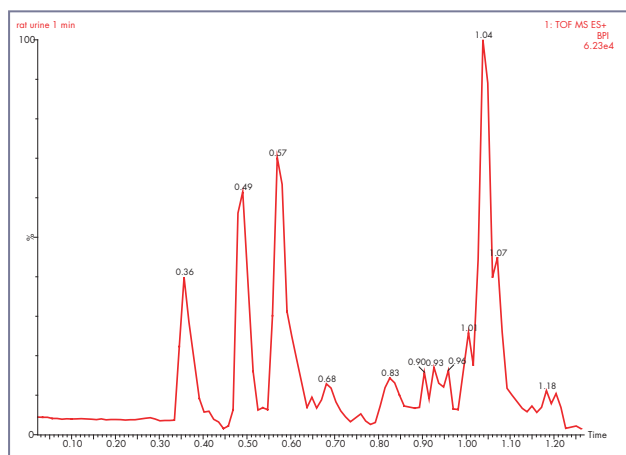


Figure 1. Rat urine analyzed on an ACQUITY UPLC BEH 2.1 x 50 mm, 1.7 μm column, eluted with a 0–95% aqueous:acetonitrile gradient over one minute at 1 mL/min, positive ion ESI ToF-MS.

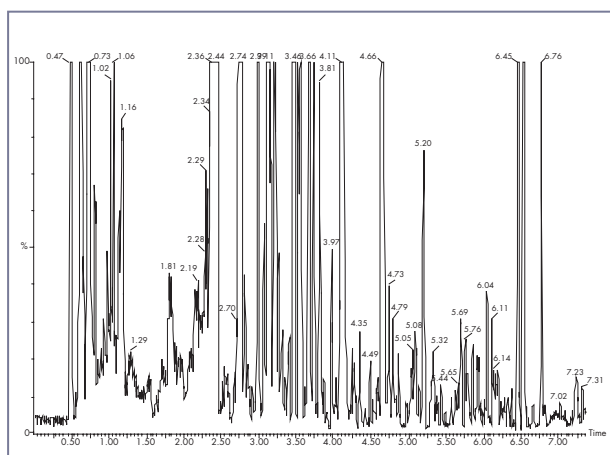
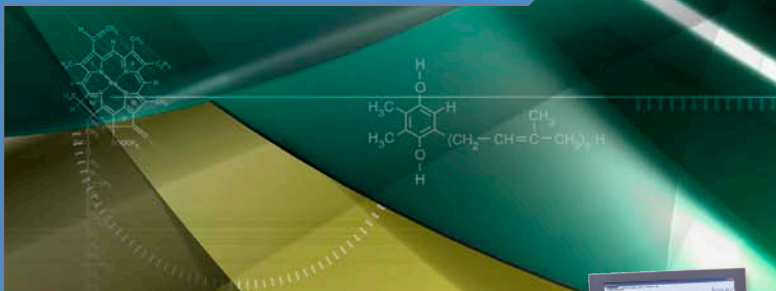


Figure 2. UPLC/MS separation of rat urine, using a linear gradient of 0–95% acetonitrile vs. 0.1% aqueous formic acid at 500 $\mu\text{L}/\text{min}$. Detection by positive ion ESI ToF-MS.

▶ BIOMARKER SCREENING



High-throughput biomarker screening with UPLC/MS

Metabonomics samples contain a chemically diverse range of compounds with a wide concentration range—all of which need to be measured accurately.

To complement the narrow peaks generated by the ACQUITY UPLC System, the Waters Micromass[®] LCT Premier[™] has been specifically designed for rapid detection and identification of components in complex mixtures.

The LCT Premier's dual-stage transfer optics, combined with its W-Optics[™] and the ZSpray[™] source, gives this mass spectrometer unrivalled sensitivity, mass accuracy, and long term reliability. The LCT Premier's mass accuracy is assured by automated exact mass measurement that uses a continuously infused internal lockmass delivered by LockSpray[™] source technology.

A unique feature of this mass spectrometer is its ESCi[®] and positive ion/negative ion mode switching, allowing electrospray/APCI high-resolution exact mass data to be acquired in both polarities, maximizing the amount of information you can produce from a single run (Figure 3).

To facilitate the multivariate statistical approach that is essential to metabonomics analysis, a linear response from the detector is required to ensure high quality results. The LCT Premier delivers up to four orders-of-magnitude exact mass data, for reliable quantitative information.

Altogether, the features that the LCT Premier Mass Spectrometer brings to exact mass UPLC/MS runs make it ideal for biomarker screening.



Waters ACQUITY UPLC System with the Waters Micromass LCT Premier Mass Spectrometer.

UPLC/MS METABONOMICS APPLICATIONS

- Toxicity screening
- Academic medicinal chemistry research
- Agricultural process analysis
- Industrial production evaluation
- Monitoring food quality

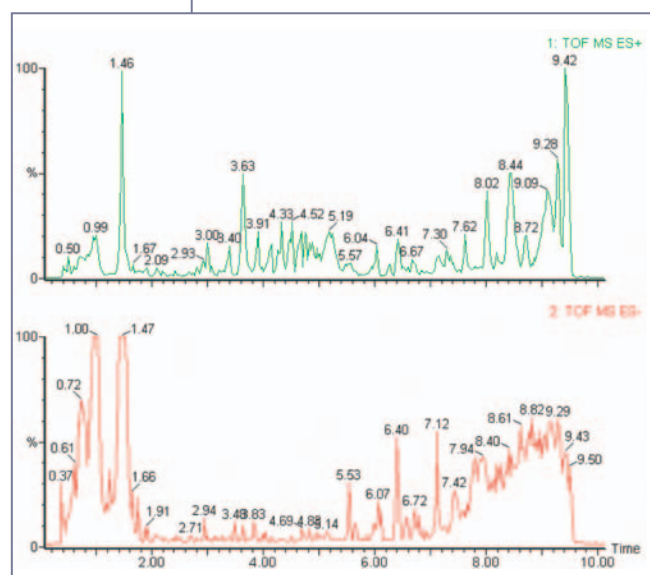


Figure 3. Positive ion/negative ion switching capabilities of the LCT Premier.



High-sensitivity biomarker identification with UPLC/MS/MS

The ability to characterize and identify biomarkers is critical to understanding the reasons for disease progression and drug toxicity. The identification of unknowns requires high-sensitivity detection, elemental composition, and fragmentation information; the Waters Micromass Q-ToF Premier™ Mass Spectrometer has been specifically designed for these tasks.

The Q-ToF Premier offers unsurpassed mass accuracy over a wide dynamic range and the flexibility of multi-mode ionization, generating mass resolution greater than 17,500 FWHM and a mass accuracy of 3 ppm.

The detection of biomarkers requires maximized sensitivity to generate the high-quality data required for structural elucidation using MS and MS/MS fragmentation information. The Q-ToF Premier includes our i-FIT isotope pattern algorithm for simplified elemental composition calculations, and our new Enhanced Duty Cycle (EDC) technology. During EDC operation, the mass acquisition range of the instrument is focused on a narrow m/z region that increases the MS or MS/MS sampling rate and produces up to a 10-fold improvement in sensitivity (Figure 4).

The mass accuracy of the Q-ToF Premier combines with unrivalled sensitivity in its MS or MS/MS mode and a linear response of over four orders of magnitude to enable the rapid and easy identification of biomarkers (Figure 5).

Waters ACQUITY UPLC System with the Waters Micromass Q-ToF Premier Mass Spectrometer.

UPLC/MS/MS METABONOMICS APPLICATIONS

- Probing mechanisms of toxicity
- Identification of markers of efficacy
- Characterization of industrial processes
- Profiling foods and beverages

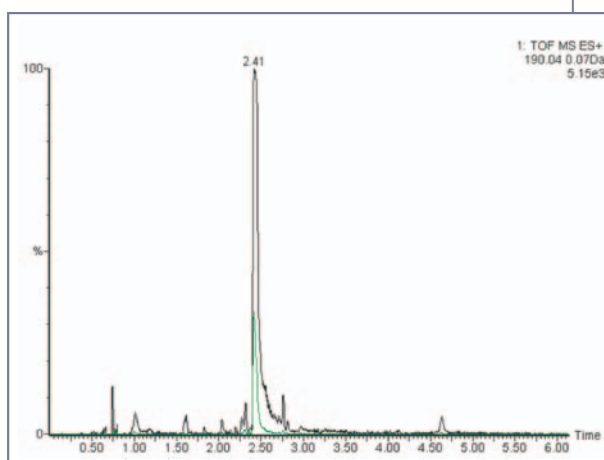


Figure 4. Comparison of Q-ToF Premier sensitivity with EDC mode off (green trace) and on (black trace) for the analysis of xanthurenate in rat urine.

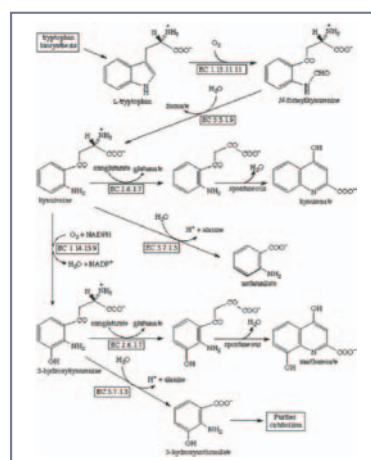


Figure 5. MS/MS and exact mass analysis of 4,8-dihydroxyquinoline-2-carboxylic acid in rat urine.

▶ METABOLOMICS

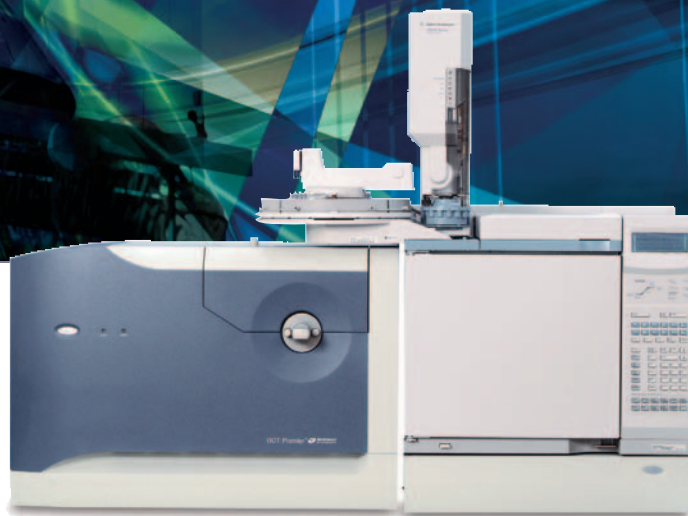


High-quality metabolomic screening with GC/MS

For metabolomics applications, capillary GC coupled to time-of-flight mass spectrometry is simple to use and provides highly sensitive analyses. This technique is ideally suited for the volatile, highly polar compounds often encountered in complex agricultural and industrial samples.

The Waters Micromass GCT Premier™ is a powerful orthogonal acceleration time-of-flight (oa-TOF) GC/MS system offering fast acquisition of full spectrum data, with high sensitivity and extended dynamic range. Exact mass capability facilitates elemental composition determination, offering a significant advantage over traditional GC/MS. The high full-scan sensitivity further extends its capability to profiling complex chromatograms.

The elemental composition information produced by the GCT Premier Mass Spectrometer, when combined with the EI spectrum it generates, allows easy identification of unknowns via NIST library searching (Figure 6). This, together with the instrument's sensitivity, scan speed, linearity, and exact mass capability, makes the GCT Premier an ideal GC/MS solution for metabolomics.



Waters Micromass GCT Premier Mass Spectrometer.

GC/MS METABOLOMIC APPLICATIONS

- Analysis of genetic strains in agriculture
- Fingerprinting of cosmetics
- Identification of biomarkers of disease

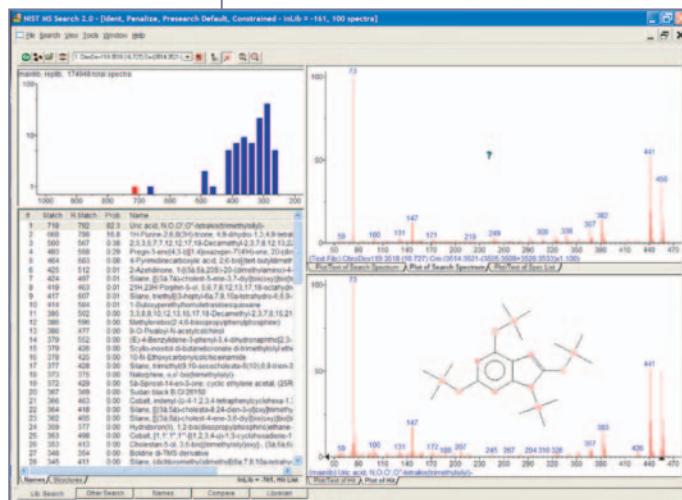


Figure 6. NIST Library search generated from the GCT exact mass MS analysis of a uric acid derivative from rat urine.

Meeting the specialized data processing needs of metabonomics

The utilization of LC/MS and GC/MS data for metabonomics analysis includes five critical steps: peak detection, peak integration, peak alignment, statistical analysis, and database searching.

These steps are particularly time-consuming and prone to errors when performed manually, especially with the large data sets generated. MarkerLynx™ db Application Manager for MassLynx Software has been specifically designed to address the task of GC/MS and UPLC/MS data processing for metabonomics.

MarkerLynx db employs state-of-the-art data integration algorithms, including ApexTrack™ and automated, spectral analysis tools for reliable peak detection. Prior to statistical analysis the data is automatically aligned, normalized, and drug metabolites or known impurities are excluded from the data set.

The resulting data is then be subjected to multivariate statistical Principal Components Analysis (PCA) using common tools such as mean centering and data scaling. The results are displayed using an intuitive browser (Figure 7), which allows easy data review and interpretation, with a dynamic link back to the raw data. Results can also be easily exported to other statistical packages for further investigation.

Finally, the marker ions of interest can be searched against an integrated, Oracle database using highly reliable exact mass information to view the structures of the potential biomarkers (Figure 8). Once identified, the biomarker results are automatically returned to the browser view, allowing consolidated, comprehensive review and reporting. All these features combine to make MarkerLynx db the ultimate tool for data analysis, interpretation, searching, and archiving for every metabonomics study.



Figure 7. The MarkerLynx db Application Manager browser.



Figure 8. Identification of a potential biomarker using MarkerLynx db's dynamic database searching.

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