

“Adoption of this technology as the ‘first-line’ approach for *in vitro* metabolite profiling has resulted in the analysis of over 2800 samples operating only three days each week over a seven-month period; **as many as 21 new chemical entities in one day** from diverse structural classes and therapeutic programs have been analyzed in this fashion.”

TILLER PR ET AL. *RAPID COMMUN. MASS SPECTROM.* 2008 APR; 22 (7): 1053-61.†

## METABOLIC PROFILING SOLUTIONS

**GOOD METABOLIC PROFILING INFORMATION USED TO BE JUST ABOUT BETTER DATA.**

**NOW IT'S ABOUT GATHERING THE RIGHT DATA, AT THE RIGHT TIME, TO MAKE BETTER DECISIONS.**

Accurately detecting and identifying both candidate pharmaceuticals and their metabolites in biological fluids is essential to drug discovery and lead optimization efforts.

Waters UltraPerformance LC® (UPLC®) and SYNAPT™ Mass Spectrometry technologies are setting new standards in information content for metabolic identification and metabolomics – delivering superior chromatographic separations, increased sensitivity, higher throughput, and readily accessible answers.

But Waters' portfolio of laboratory solutions enables you to do much more than simply acquire and process data faster. We compile comprehensive information across an analytical workflow that helps you capture the best from our technologies in meaningful steps. Your results are harnessed by software platforms that help you rapidly turn data into knowledge that is critical to the characterization of drug metabolites and potential biomarkers. You can be more confident as your pharmaceutical discovery steps closer towards becoming a life-saving therapeutic drug.

### Waters system solutions for metabolic profiling

- Innovative UPLC chromatographic technology
- Exact mass with TOF-MS, and four dimensions of information with HDMS™
- Specialized, powerful software data mining and interpretation tools
- Algorithms for accurate and simple compound identification

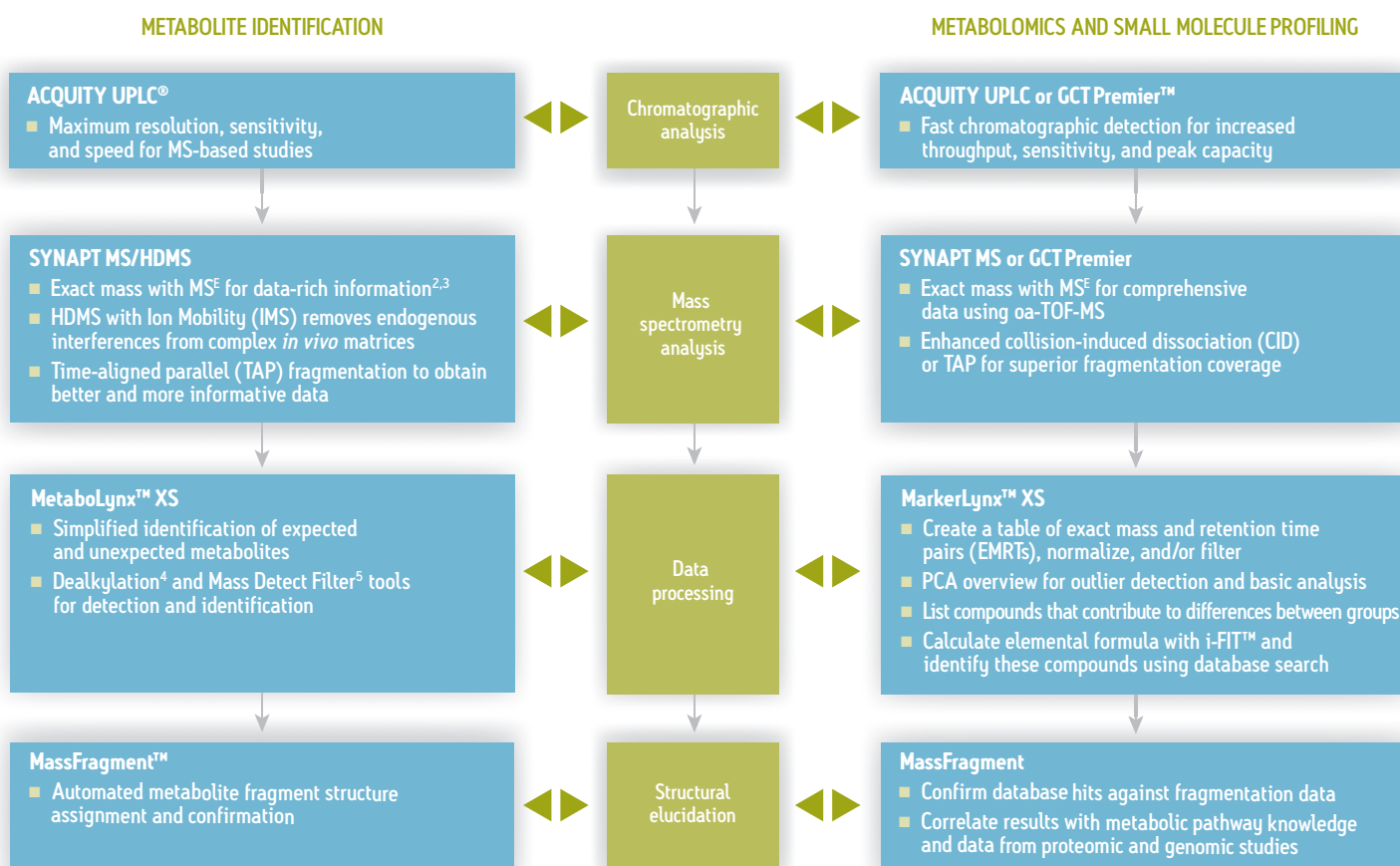


## ANSWER THE RIGHT QUESTIONS

Address these challenges early and accurately, and fewer drug candidates will fail late in development due to toxicity or poor pharmacokinetics:

- Where is a drug metabolized?
- What levels of metabolites exist?
- What is the exact structure of the metabolites?
- How quickly are the metabolites excreted?
- Where in the body are the metabolites formed?
- Are the metabolites toxic?

The complex nature of samples and the low levels of metabolites can make any of these activities difficult and time-consuming tasks. The Waters metabolite profiling workflow will improve the productivity and efficiency of your studies, while helping reduce the cost and risk involved in the drug discovery/development process.



“...the approach described here usually requires, in total, as little as one hour of acquisition time... and about one hour of data interpretation time by the project scientist. As a result, the **capacity for conducting preliminary metabolite identification** experiments **has increased by almost an order of magnitude**, and the turnaround time for metabolism data has been shortened to the point where the **information has a much greater impact on decision-making...**”



## UPLC: GROUNDBREAKING CHROMATOGRAPHIC PERFORMANCE FOR COMPLEX SAMPLES

Waters' proven UPLC technology is a key part of our pioneering solutions for metabolite profiling, delivering high-throughput separations with the sensitivity and resolution needed for robust MS/MS analysis.

Central to the Waters ACQUITY UPLC System is sub-2  $\mu\text{m}$  column particle technology, which operates at high linear flow rates to produce chromatograms with peak widths of less than two seconds at the base. The ACQUITY UPLC System maximizes separation performance with optimal throughput, minimal carryover, ultra-high resolution, and the robustness and reproducibility you can rely on.

Together, these attributes make the ACQUITY UPLC System ideal for analyzing complex biological mixtures. Chromatographic run times of just five minutes contain as much information as was previously produced in a 30-minute HPLC run. Data-rich results come with the sensitivity and resolution that ensure top-quality subsequent MS/MS analysis (Figure 1).

More peaks in less time, and dramatically greater resolution and sensitivity – the ACQUITY UPLC System optimizes laboratory analyses, helping you advance your research with greater confidence and productivity.

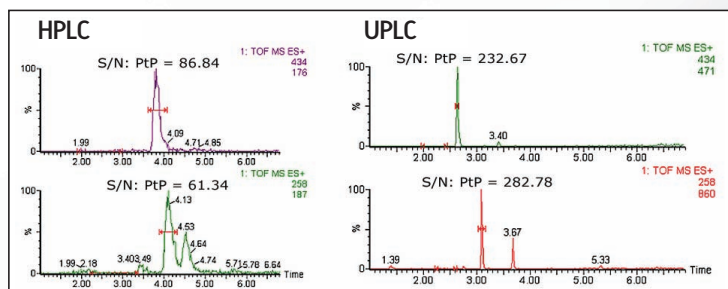


Figure 1. Comparing the total ion chromatogram from HPLC (left) and UPLC (right) separations of the metabolites of dextromethorphan. The high resolution of the ACQUITY UPLC System produces very narrow peaks, increasing assay sensitivity and reducing the possibility of co-elution of analytes, making the system an ideal inlet to MS.



“The real benefit for UPLC for metabolic profiling is the fact that older HPLC methods are easily transferred and the benefits are very clear; you only need to look at the chromatography and the quality of the data generated in a much shorter time frame. ... it is very important to detect metabolites that were not possible to see as in the case of HPLC due to co-elution.

“This solution has provided savings for the pharmaceutical company both in terms of time and resources invested in selecting successful candidate compounds.”

LAB MANAGER, DMPK LABORATORY FOR GLOBAL PHARMACEUTICAL COMPANY<sup>6</sup>



## SYNAPT MS AND GCT PREMIER: STREAMLINE HIGH-THROUGHPUT SCREENING WITH OA-TOF MS

SYNAPT Mass Spectrometry Systems complement UPLC separation technology with both API/MALDI ionization modes and exact mass MS with MS<sup>E</sup>. Combining this advanced hardware with chemically-intelligent informatics yields a structure-driven process rather than a mass-driven process that is uniquely configured to probe molecular structures, dramatically impacting the process of metabolic profiling.

Mass spectrometry is ideally suited to the search for metabolites or biotransformations of a parent compound in biological fluids, and has for years been the principal analytical tool in drug metabolism studies. Finding the exact structures of metabolites formed and what biotransformations occurred to the drug in the presence of the hundreds or thousands of compounds in urine or plasma samples is quite a challenge. The SYNAPT MS System, with its ability to accurately measure mass, greatly simplifies this search by allowing the user to efficiently extract information from the complex biofluid.

SYNAPT MS provides high sensitivity, full-scan exact mass measurement. The system allows researchers to not only find the metabolites, but to also probe the structure of the metabolites using MS<sup>E</sup> for enhanced fragmentation: all critical information about a drug metabolite can be obtained all from a single experiment (Figure 2).

### A complete report on the drug candidate's metabolism

MS<sup>E</sup> acquires molecular data – fragment ion, precursor ion, and neutral losses – in a single injection using parallel high and low collision energy MS acquisition, overcoming the limitations of conventional data-dependant approaches (Figure 3). UPLC/ MS<sup>E</sup> datasets are a comprehensive quantitative digital record of your sample that enables you to re-interrogate data instead of re-running a sample. Structural elucidation can be further streamlined using MassFragment and i-FIT isotope filtering software tools.

Using the SYNAPT MS for a UPLC/MS<sup>E</sup> approach to metabolism studies, or the GCT Premier for exact mass with GC for volatile and less polar samples, can literally shrink the elapsed time required to do these studies from weeks to, in some cases, just hours.

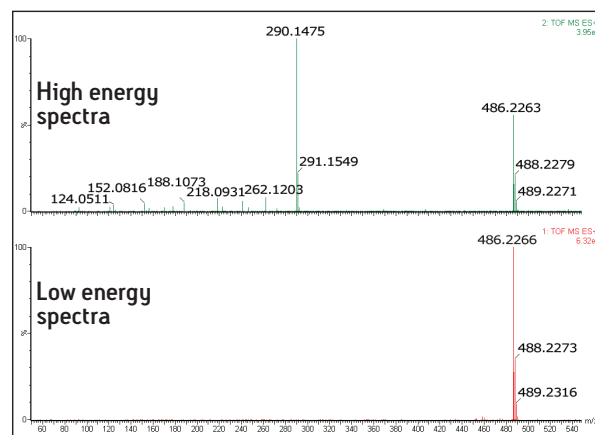
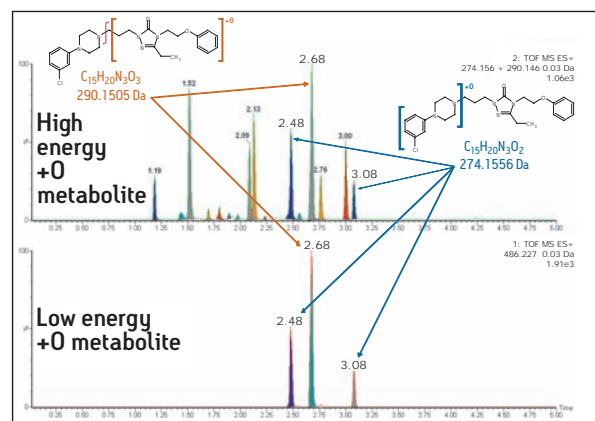


Figure 3. Observe the level of information that can be obtained by MS<sup>E</sup> in high-energy acquisition mode. Here, the key diagnostic fragment ions at m/z 274 and m/z 290 were used to confirm the metabolites in the low-energy acquisition mode, and to localize the most likely position of the biotransformation – all from a single injection.

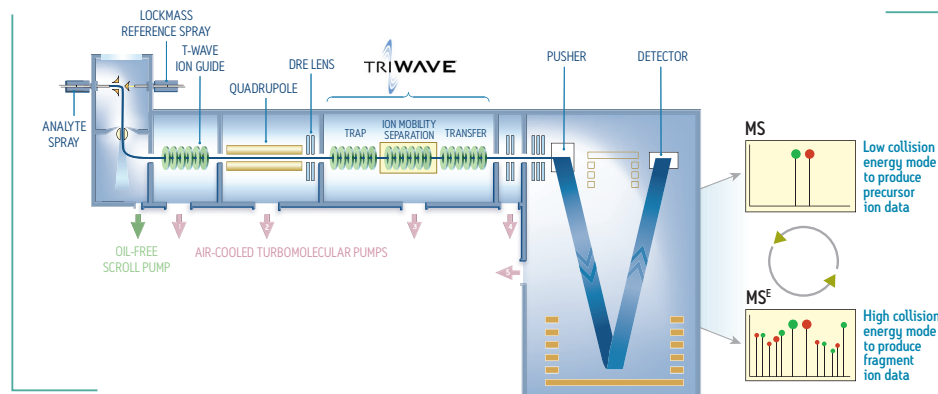


Figure 2. MS<sup>E</sup> acquires full scan data, collecting vital information for metabolic profiling assays in one analysis.



## SYNAPT HDMS: AN ADDITIONAL DIMENSION OF DATA

For researchers working at the boundaries of conventional mass spectrometry – who need to further define and characterize their samples – the SYNAPT High Definition MS™ (HDMS) System uniquely upgrades the functionality of the SYNAPT MS.

A typical challenge when running *in vivo* samples is that without using radiolabeled compounds, there are no reference points to use to look for xenobiotics and detect and identify low-level metabolites from a complex endogenous background.

### Redefining the boundaries of MS

Using Waters' patented Triwave™ technology, the SYNAPT HDMS System combines high-efficiency ion mobility measurements and separations with high-performance quadrupole, time-of-flight MS. This unique combination makes it possible for researchers to analyze samples that are differentiated by size, shape, and charge – as well as mass.

The versatile SYNAPT HDMS System can be operated in two modes:

- TOF mode includes simultaneous high and low energy collision MS<sup>E</sup> data acquisition for exact mass without any prior knowledge of the metabolites of interest
- IMS mode provides increased specificity and sample definition beyond that achievable by conventional mass spectrometers

Information generated by the SYNAPT HDMS System can be easily visualized and manipulated using DriftScope™ Mobility Environment Software (Figure 4), which separates background ions from real drug-related metabolites – allowing you to extract only the relevant drug-related information you need.

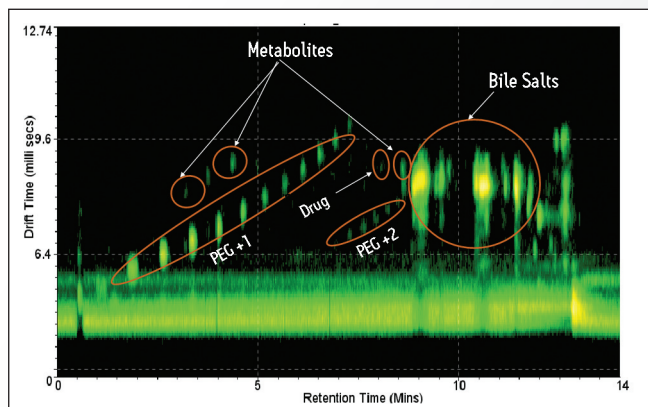


Figure 4. IMS separation of drug and metabolites from endogenous compounds (bile, 30 min to 1 h), displayed with DriftScope Software.

## ADDITIONAL METABOLIC PROFILING APPLICATIONS

- Potential biomarker screening
- Identification of markers of efficacy
- Toxicity screening
- Impurity profiling
- Academic medicinal chemistry research
- Industrial process characterization and production evaluation
- Agricultural process analysis
- Natural products analysis
- Profiling and monitoring quality of foods and beverages

In IMS mode, the SYNAPT HDMS System facilitates the discovery of putative metabolites, dissecting data with great specificity and using drift time to remove redundant peaks (Figure 5).

When used in conjunction with UPLC separations, the SYNAPT HDMS System provides an information-rich approach for metabolite detection and identification.

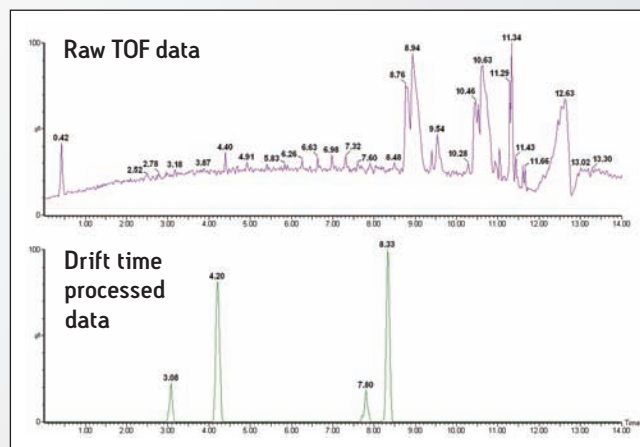


Figure 5. IMS-TOF excised data: a much clearer picture.



## DO MORE THAN PROCESS DATA. DEVELOP CRITICAL CHEMICAL INTELLIGENCE.

Waters has developed specialized data handling and interpretation tools for MassLynx™ Software that help you reach a more dynamic understanding of the information you have captured. Its MetaboLynx XS and MarkerLynx XS Application Managers are the ultimate tools for data analysis, mining, and interpretation for every metabolic profiling study.



## METABOLYNX XS FOR METABOLITE IDENTIFICATION

The MetaboLynx XS Application Manager facilitates data analysis in the detection and identification of expected and unexpected metabolites. The software detects putative biotransformations without requiring operator-supplied predictions of metabolic fate.

### MetaboLynx XS Application Manager features:

- Reports results via a data browser (Figure 6) that enables analysts to quickly review the evidence that support each automated metabolic assignment, either locally or remotely via a secure corporate network.
- Compares and contrasts each metabolized sample with a control sample.
- Samples from *in vitro* incubations or *in vivo* dosing experiments can be quickly analyzed by UPLC/MS, followed by a multi-dimensional data search that correlates retention time, m/z value, intensity, and components from alternative detection technologies (e.g. photodiode array UV or radiochemical monitoring).
- Exact mass data filtering capabilities with MSE allow you to set a low threshold and post-filter the data, easily removing false positives to ensure that important information is never missed.
- Mass Defect Filter (Figure 7), a post-acquisition data filtering technique used with a dealkylation algorithm tool, is based on the mass defect of the parent drug and its metabolites. It allows researchers to rationalize expected and unexpected metabolites more efficiently and minimize the number of false positives, and from this initial step, generate an extensive expected metabolite list.
- MassFragment, a chemically-intelligent structural elucidation tool, assigns structures by taking fragment ion spectra of the drug and/or metabolite, using it to automatically calculate fragments based on a series of novel chemically intelligent algorithms.

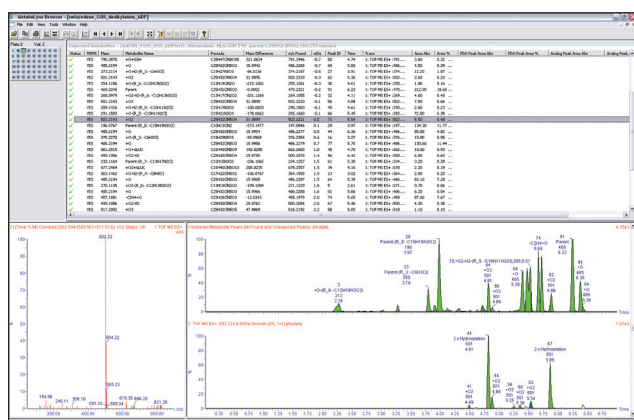


Figure 6. The MetaboLynx XS Application Manager browser.

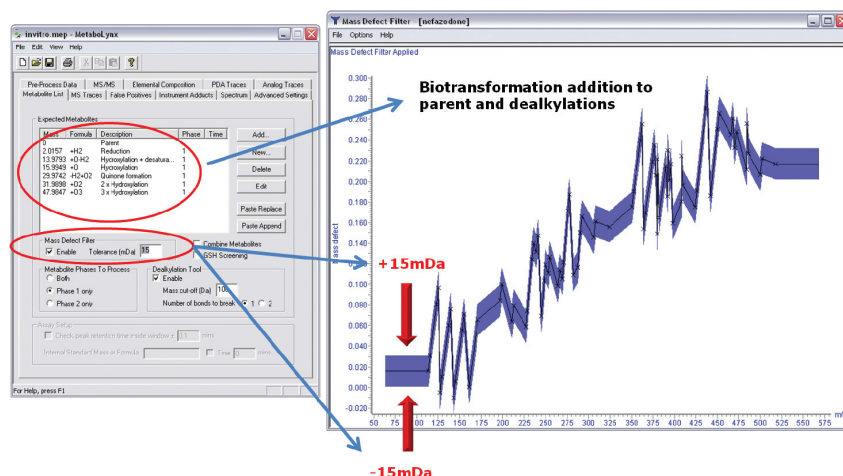


Figure 7. Mass Defect Filter provides automatic generation of metabolic cleavages.



## MARKERLYNX XS FOR METABOLOMICS AND SMALL MOLECULE PROFILING

Profiling complex mixtures of small molecules using MS is an imposing challenge due to the information density and complexity of the data. UPLC/MS or GC/MS metabolomics studies include six critical steps: peak detection, peak integration, peak alignment, statistical analysis, database searching, and confirmation by MS/MS. MarkerLynx XS Application Manager handles these steps by comparing and discriminating data sets using multivariate statistical analysis to see patterns and extract differences quickly and easily.

### MarkerLynx XS Application Manager features:

- Improved peak-picking and alignment routines, exact mass data filters, and metabolite and adduct search tools.
- The power of multivariate analysis with the simplicity of desktop software. A fully integrated multivariate statistical module featuring novel interactive reports and plots (Figure 8) allows the user to quickly mine complex data. Patterns, trends, correlations, and variability are displayed in easily-interpreted 2D and 3D plots.
- Provides sophisticated and comprehensive database tools via integration with the Database Manager (Figure 9), enabling the user to search local and online databases as well as ACD/Labs ChemFolder databases. Custom in-house databases can also be created using structure editors or directly from SDF files.



Figure 8. The MarkerLynx XS Application Manager browser includes interactive plotting options.

The screenshot displays the MarkerLynx XS Database Manager software interface. The top menu bar includes 'Database', 'Edit', 'Tab', and 'Help'. The main window is titled 'Compound List' and contains a table with the following columns: Name, Mass, Formula, Molecule, and Pathway. The table lists various compounds with their corresponding chemical structures and pathways.

| Name              | Mass     | Formula  | Molecule                           | Pathway   |
|-------------------|----------|----------|------------------------------------|---|
| L-proline         | 115.0... | C5H9...  | <chem>C1CCNC1</chem>               | Urea cycle and metabolism of amino groups; Arginine and proline metabolism; Nov...    |
| Maleate           | 116.011  | C4H4O4   | <chem>C1=CC=CC=C1</chem>           | Butanoate metabolism; Nicotinate and nicotinamide metabolism                          |
| 2-Methylaceto...  | 116.0... | C5H8O3   | <chem>CC(C)C(=O)O</chem>           | Valine, leucine and isoleucine degradation; Valine, leucine and isoleucine biosynt... |
| 2-Oxoisovaler...  | 116.0... | C5H8O3   | <chem>CC(C)C(=O)O</chem>           | Valine, leucine and isoleucine degradation; Valine, leucine and isoleucine biosynt... |
| 3-Oxopentano...   | 116.0... | C5H8O3   | <chem>CC(C)C(=O)O</chem>           | Valine, leucine and isoleucine degradation; Valine, leucine and isoleucine biosynt... |
| Hexanoic acid     | 116.0... | C6H12... | <chem>CCCCCCCC(=O)O</chem>         | Carboxylic acid derived from Hexane   |
| Aspartyl semis... | 117.0... | C4H7...  | <chem>CC(C)C(=O)O</chem>           | Lysine, isoleucine, Methionine, threonine synthesis; Aspartic acid pathway            |
| L-2-aminoacet...  | 117.0... | C4H7...  | <chem>CC(C)C(=O)O</chem>           | Glycine, serine and threonine metabolism  |
| L-aspartate-4...  | 117.0... | C4H7...  | <chem>CC(C)C(=O)O</chem>           | Glycine, serine and threonine metabolism; Lysine biosynthesis                         |
| N-Acetyl-L-gly... | 117.0... | C4H7...  | <chem>CC(C)C(=O)O</chem>           | HNCO peptide linkage  |
| Glycocyamine...   | 117.0... | C3H7...  | <chem>CC(C)C(=O)O</chem>           | Urea cycle and metabolism of amino groups; Glycine, serine and threonine metabo...    |
| Indole            | 117.0... | C8H7N    | <chem>C1=CC=C2C(=C1)C=CC=C2</chem> | Tryptophan metabolism; Phenylalanine, tyrosine and tryptophan biosynthesis            |

Figure 9. The MarkerLynx XS Database Manager, showing a local database.

“Researchers used UPLC-based metabolomics to streamline a toxicology study. With HPLC, processing time took seven days and required as many as 40 animals and up to 35 g of compound. The lab significantly reduced necessary resources with the ACQUITY UPLC System – a total of just eight animals and 500 mg of the drug candidate was needed for the UPLC toxicology study.”

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