

# Waters®Alliance® HT LC/MS System

## Inlet Considerations for Flow Injection Analysis:

### Increased Sample Throughput and High Quality Results In MS Applications:

Analytical laboratories are frequently asked to screen a large number of samples for one or more compounds in as short a time as possible. A simple determination of the molecular weight of the analytes contained in the sample is often sufficient. Depending on the complexity of the sample, the need to perform a chromatographic separation may not be necessary. Flow injection analysis (FIA) has gained increased popularity as a sample introduction technique for mass spectrometry (MS). The Waters Alliance HT System coupled to a Waters ZSpray™ Mass Detector (ZMD) is ideally suited for medium to high throughput, fast flow injection mass spectrometric analysis. This Performance PerSPECTive discusses why the Alliance HT System is an ideal inlet for high throughput MS applications by addressing capabilities including: sample handling and climate control, low sample carryover between injections, fast cycle times and configuration flexibility.

### Flexible Sample Handling Options

The XYZ-style sample manager in the Alliance HT System provides expanded sample capacity for processing up to 1,536 samples in a single programmed operation. Samples can be submitted for analysis in standard 96-position shallow or deep-well as well as from high-density, 384-position microtiter plates. Custom plate configurations can be accommodated and microcentrifuge tubes or 2.0 mL sample vials can also be used. Maximum sample handling flexibility is possible since different sample container formats are easily combined within the sample manager.

### Optimal Sample Environment Control

The sample manager component of the Waters Alliance HT System provides precise control of the environmental conditions where the samples reside prior to analysis. Sample temperature is controlled by a built-in Peltier heater / chiller. Light-sensitive samples are protected from light while contained in the sample manager compartment. In combination, these features minimize evaporation, and reduce temperature or light-induced sample degradation ensuring the quality and confidence in the collected results.

### Low Sample Carryover Between Injections

Sample carryover is always a concern when performing high sensitivity, mass spectrometric analyses. A previous Performance PerSPECTive (WPP300) detailed the low sample carryover performance characteristics of the Alliance HT System when used for HPLC or LC/MS applications. Results from this study (see LC/MS data on page 2) also support the exceptionally low carryover characteristics of the Alliance HT System when used as an inlet to a mass detector. In the series of 96 repetitive injections of the 50 ng of trimipramine and 50 ng of lidocaine sample, every 4th well and injection (24 in total) contained a blank solution of 50% water/ 50% methanol. Neither trimipramine nor lidocaine were detected in any of the "blank injections" using single ion recording (SIR) MS monitoring.

### Parallel Processing Translates Into Increased Sample Throughput

The data contained in this study involved the MS analysis of 96 samples contained in a standard 96-well microtiter plate. Analyses were performed using the Alliance HT System's parallel operation mode. Using parallel mode, the total cycle time which included sample introduction, SIR analysis, sample manager cleaning, and sample pick-up for next injection was approximately 1.5 minutes. This translates to more analysis per day since only 2 hours and 23 minutes is required to complete the flow inject MS analysis of all 96 samples.

## Easy Configuration for MS Applications

The Alliance HT System can be configured with an optional, factory installed 3-port column selection valve designed for FIA MS applications. The valve can be configured so that the Alliance HT System can be used for traditional HPLC applications or for FIA by the use of a restrictor bypass loop. Valve position selection is controlled through embedded software on the Alliance HT System or through MassLynx™ software. This makes switching from HPLC to FIA applications quick and easy. In the example shown below, the restrictor bypass loop from the Alliance HT System was connected directly to the Atmospheric Pressure Chemical Ionization (APCI) probe of the ZMD using 48 inches of 0.009 in. diam. PEEK tubing.

## Matched Mass Detector to High Throughput Inlet

The Waters ZMD Detector is a fully featured single quadrupole benchtop mass spectrometer which offers many advanced features. Its rugged ZSpray source is well suited for high-throughput FIA analysis. The ZSpray source design permits prolonged LC/MS analysis of a wide range of samples including complex biological or environmental matrices. The ZSpray can even tolerate nonvolatile HPLC buffers to allow for many hours of uninterrupted analysis. The entire system is under the control of MassLynx software.

## Flow Injection MS Analysis of Trimipramine and Lidocaine

### Alliance HT System Conditions:

Plate: 96-well, shallow microtiter plate (300 µL volume per well)  
Sample Temp.: 5 °C in dark environment  
Inj. Vol.: 5 µL (full loop, 4X overfill) of Trimipramine (10 ng/µL) and Lidocaine (10 ng/µL)  
Inj. Mode: Parallel  
Mobile Phase: 50% Water, 50% Methanol @ 1.0 mL/minute  
Wash Solvent: Methanol  
Wash Protocol: Needle interior for 3 sec., Needle exterior and Injector port for 7 sec.  
Purge Solvent: 50% Water, 50% Methanol (300 µL needle wash replacement volume)

### ZMD MS Conditions:

Analysis Mode: +APCI  
Source Temp: 130 °C  
Probe Temp.: 500 °C  
Capillary Voltage: 3.39 volts  
Cone Voltage: 35 volts  
Gas Flow: 600 L/hr

Single Ion Recording (SIR) at m/z 235.1 (Lidocaine)  
Single Ion Recording (SIR) at m/z 295.1 (Trimipramine)

## Comparison of Samples with Water / Methanol Blanks Using FIA

Figure 1: Typical SIR FIA Chromatogram (+APCI, m/z = 235.1) from a 5 µL injection of Lidocaine and a methanol/water blank.

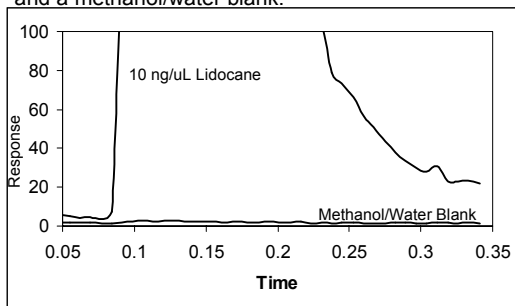
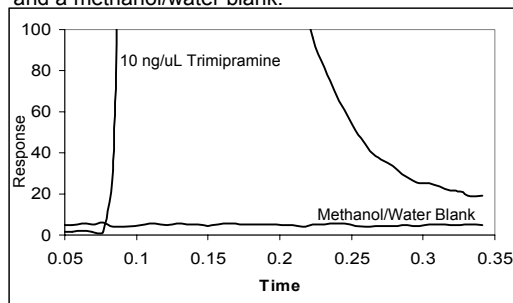


Figure 2: Typical SIR FIA Chromatogram (+APCI, m/z = 295.1) from a 5 µL injection of Trimipramine and a methanol/water blank.



### Summary:

- The ability to obtain high quality mass spectrometric results on a large number of samples in the shortest amount of time has placed new demands on the inlet of a LC/MS system.
- The Alliance HT System was specifically designed to exceed performance expectations in LC/MS applications where sample handling, climate control, low sample carryover between injections, fast cycle times, and configuration flexibility are important.
- Compared to traditional LC/MS systems, the Waters Alliance HT System with the ZMD helps reduce the cost of analyses by increasing sample throughput while maintaining high quality MS results.