

# Broader Coverage and Automatic Mass Calibration Using the TripleTOF™ 5600 System with DuoSpray™ Ion Source

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## The DuoSpray™ Ion Source

Traditionally, a limiting step in LC/MS analysis has been that only one ionization technique can be used at a time. Switching ionization modes often means physically changing the source. The AB SCIEX DuoSpray™ Ion Source combines the two most common ionization techniques – an Electrospray Ionization (ESI) probe and an APCI (Atmospheric Pressure Chemical Ionization) probe integrated in a single source housing with Turbo V™ Source functionality. This allows flexibility in method development and sample analysis for a wider range of analytes. On the TripleTOF™ 5600 system, this also enables online automatic mass calibration using the optional Calibrant Delivery System (CDS).

## The TripleTOF™ 5600 System

The AB SCIEX TripleTOF 5600 System is the first accurate-mass, high-resolution system to deliver rapid and sensitive qualitative and quantitative analysis in a single platform. It provides high sensitivity for low level quantitation with a dynamic range comparable to a high performance triple quadrupole mass spectrometer. Using the highly innovative EasyMass™ calibration system, mass accuracy of ~2 ppm RMS can be routinely achieved with a simple user friendly external calibration.



**Figure 1. The DuoSpray™ Source.** The DuoSpray Source combines ESI and APCI probes in a single Turbo V™ Source housing for highest functionality.



The TripleTOF 5600 System enables multiple advanced workflows such as fast acquisition of high resolution product ion spectra on many precursors in the same scan cycle. Information dependent acquisition (IDA) can be performed for simultaneous quantitation and qualitative identification. Selectivity in highly complex samples can be achieved with advanced techniques such as real time multiple mass defect filtering and high resolution MRM (MRM<sup>HR</sup>).

The TripleTOF 5600 system achieves high speed, resolution, sensitivity, mass accuracy, and a wide linear dynamic range all at the same time.

## Key Benefits of the DuoSpray™ Source with the TripleTOF 5600 System

- Perform ionization in ESI or APCI without switching sources.
- When combined with CDS, enables fully automatic and unattended EasyMass™ external mass calibration.
- No interference from calibrant ions during data acquisition.
- Flexible configuration enables more efficient method development and sample analysis, with broader compound coverage.

## Flexible Configuration Options Using the DuoSpray™ Ion Source with the TripleTOF™ 5600 System

The DuoSpray™ Source extends the flexibility for different workflows and front-end configurations. Sample analysis can be performed in ESI while the APCI probe is used for calibration, or vice versa. In the first configuration, the column is connected to the ESI probe inlet while the APCI probe inlet is connected to the Calibrant Delivery System (CDS, Figure 2) for introducing the calibration solution. This provides ultimate flexibility for assay conditions, and eliminates the need for valve switching to introduce the calibrant into the sample stream

The CDS pump is controlled directly from Analyst® Software and supports up to 4 different calibration solutions which can be automatically selected from the software.

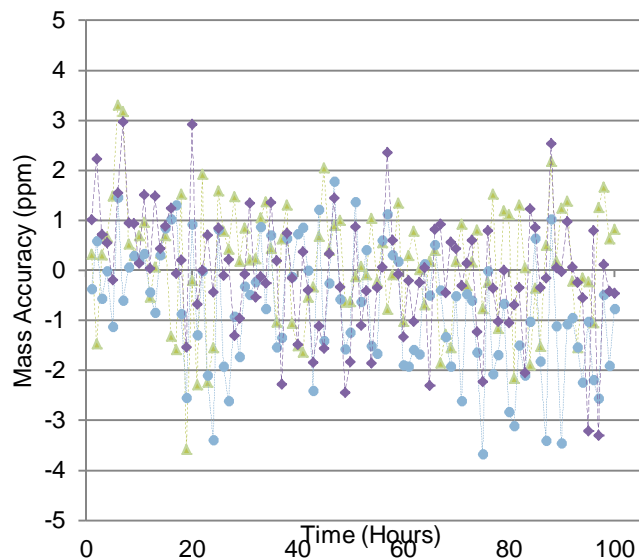
### EasyMass™ Automatic External Calibration

External calibration is automatically scheduled and run during sample batches using Analyst® Software. The entire calibration process is completed in 2 minutes. The software automatically activates the CDS which introduces the calibration solution through one of the probes on the DuoSpray™ Source. Data is automatically acquired, mass calibration is performed, and the system calibration automatically updated. The software then shuts off the CDS and proceeds with the next sample injection.

With this approach, the mobile phase flow is never interrupted. Furthermore, the calibration solution is never introduced into the system during sample data acquisition. This insures the calibration solution components will not interfere with full scan



**Figure 2. The Calibrant Delivery System (CDS).** The CDS can accommodate up to four different solutions for calibration and tuning. Automatic external mass calibration is performed without operator intervention during sample acquisition.



**Figure 3. Stability of Mass Accuracy for Long Term Operation.** 100 hours of acquisition with the TripleTOF™ 5600 System utilizing the Calibrant Delivery System (CDS). Mass Accuracy RMS = 1.69

data acquisition. The operator only needs to specify the frequency of calibration (e.g. every 20 sample injections).

This method has the added advantage of allowing the system to calibrate TOF MS and TOF MS/MS modes separately. The software automatically analyzes the data acquisition method to determine if MS, MS/MS or both are being used. The correct scan modes are calibrated, without altering ion source conditions or interrupting mobile phase flow. The result is a user friendly automated mass calibration with no mechanical valve switching required and no carryover or interference from the calibrant ions.

### DuoSpray™ Source Configuration

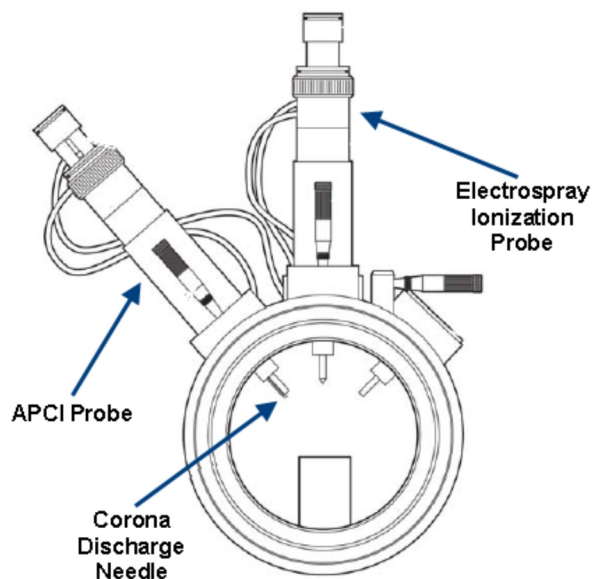
The ESI and APCI probes enable ionization across a wide range of molecular weights and polarity. The combination of both ionization probes in one source housing allows greater flexibility in method development, sample analysis, and in the case of the TripleTOF™ 5600 system, flexibility in instrument experimental design.

In Electrospray ionization, the ionized liquid sample is sprayed into fine droplets. Through the process of desolvation, the liquid from the solvent droplets evaporates as the charged ions in the gas phase are pulled into the mass spectrometer. In Atmospheric Pressure Chemical Ionization (APCI), the sample is introduced and vaporized using a combination of gas and heat, then ionized using a corona discharge. ESI is applicable to a wide range of polar compounds including peptides and heat labile small molecules, while APCI can be used for less polar thermally stable analytes.

The DuoSpray™ Source consists of an ESI probe in the center based on Turbo V™ Source geometry (Figure 4). The APCI probe is mounted to the side and also functions as one of the heaters for ESI. The spray voltage (for ESI) and the corona needle current (for APCI) are always powered. The ionization mode is simply determined by which probe is used for sample introduction, resulting in total flexibility based on assay requirements.

## Using CDS in an Acquisition Batch

The Calibrant Delivery System, in conjunction with Analyst® Software has the ability to insert calibration runs automatically into an acquisition batch. This reduces or eliminates the need to perform additional calibration runs manually as the Auto-Cal process periodically inserts calibration runs and executes them unattended. Auto-Cal uses pre-defined reference tables to calibrate both MS and MS/MS scan modes in between sample analysis runs.



**Figure 4. DuoSpray™ Source.** The DuoSpray Source is designed with the ESI probe in the centre and the APCI probe on the left side. On the right side is a heater for rapid desolvation even at higher flow rates, the APCI probe also functions as the second heater.

## Alternative Calibrant Delivery by ESI

In the default CDS configuration, the ESI probe is used for sample analysis and the APCI probe is used to deliver the calibrant solution. When APCI is required for sample analysis, the ESI probe can alternatively be used for calibrant introduction.

## Conclusions

- Combining the DuoSpray™ Source with the TripleTOF™ 5600 System enables flexible system configuration and EasyMass™ calibration.
- Using the Calibrant Delivery System (CDS), the calibration solution can be delivered automatically through either ESI or APCI.
- External mass calibration is performed in two minutes or less between sample injections in a completely automated and unattended manner.
- By using the DuoSpray™ Source, mobile phase flow is never interrupted during mass calibration; and the calibrant is never introduced during data acquisition. This eliminates any potential interference from the calibrant ions.

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Publication number: 3470211-01



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