

EVALUATION OF AN INTEGRATED LC-MS QUANTITATION WORKFLOW FOR FORENSIC TOXICOLOGY APPLICATIONS

Marijn Van Hulle¹, Victoria Starkie², Rachel Lieberman³
¹Waters Corporation, Antwerp, Belgium. ²Waters Corporation, Wilmslow, United Kingdom. ³Waters Corporation, Milford, MA, USA

Introduction

Forensic toxicology laboratories require quantitative LC–MS workflows that are reproducible, efficient, and defensible under accreditation and legal scrutiny. Challenges commonly include manual data handling between acquisition and quantitation platforms, inconsistent method setup, and the significant analyst time required for chromatographic review of large multi-analyte datasets. Integrated software environments that consolidate acquisition, optimization, data processing, and review may address these limitations while supporting data integrity requirements.

Objectives

The objective of this work was to evaluate the applicability of the waters_connect™ Software Platform with MS Quan application for routine screening and quantitative LC–MS workflows typical of forensic toxicology laboratories.

Methods

Representative qualitative and quantitative multi-analyte assays were acquired and processed using waters_connect Software with MS Quan application. Method development, including automated retention time updating for 100's of analytes, MRM optimization via infusion, automated source optimization, data acquisition, data processing, and review were conducted within the same software environment. Data were assessed using a combination of full manual review and configurable **exception-focused review** (XFR) rules reflecting commonly used forensic acceptance criteria (e.g., ion ratios, retention time tolerance, signal-to-noise thresholds, blanks, internal standards, and Calibration/QC performance).

Results

- 98 analytes, each with 2–3 MRM transitions, plus internal standards (1–2 transitions each)
- 55-sample sequence (~25,000 chromatograms) processed in under 60 seconds
- Structured, workflow-based review across the key MS Quan pages

Cals & QCs page

- 588 calibration and 392 QC points assessed for linearity and deviation in minutes
- Manual interactions reduced by more than 90% using XFR
- Only 3 exceptions across all 98 analytes: one R² exception and two QC deviations (Figure 1)

Review calibration curves faster and focus attention where it matters. Remove an individual calibration point directly from the curve, then open the detailed view (Figure 2) to review or re-integrate associated peaks. XFR filters reduce routine checking while full-curve visibility remains available. A combined calibrator and QC chromatogram view makes comparisons and exception investigation easier.

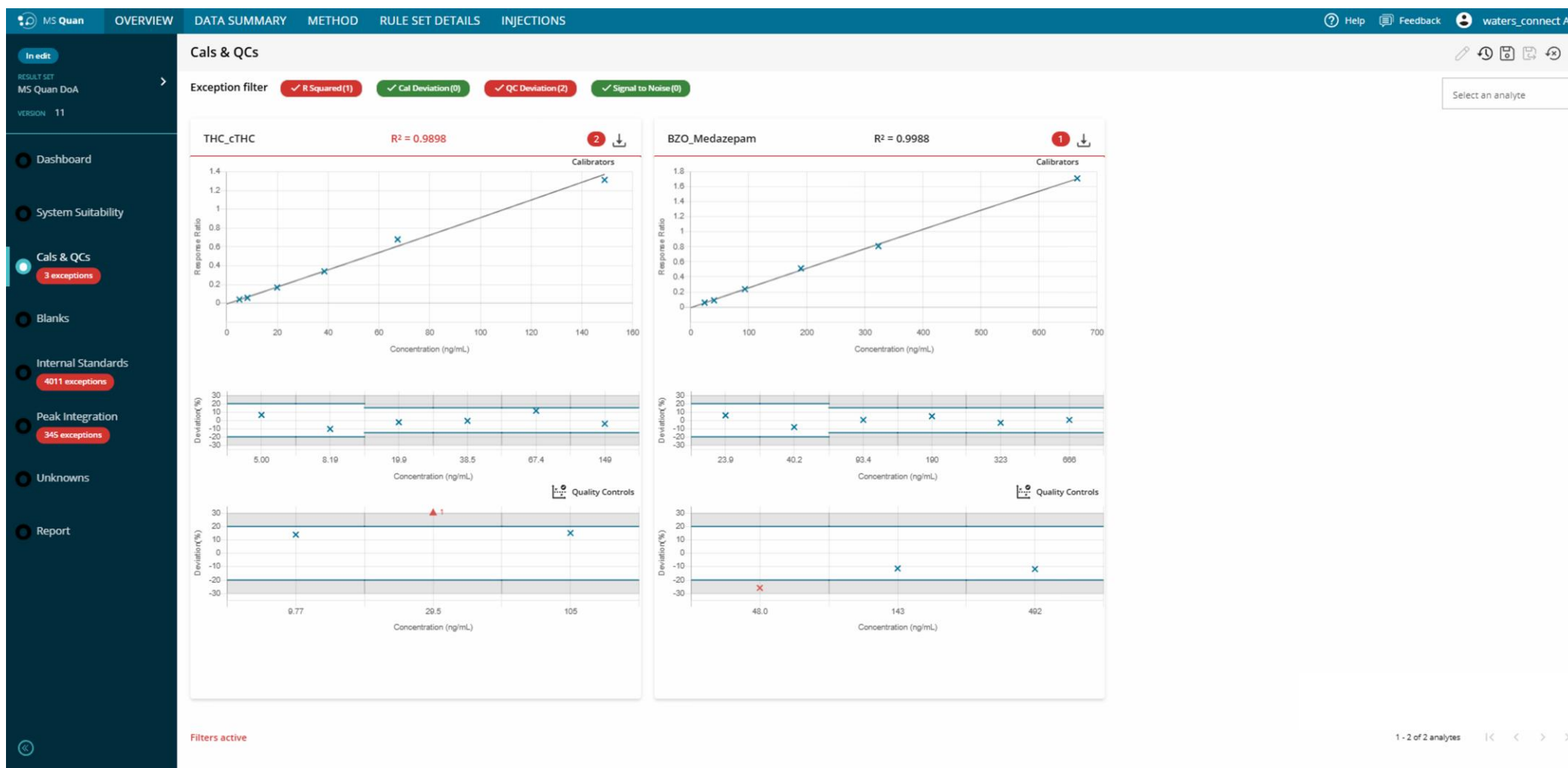


Figure 1: Cals & QCs page with XFR enabled, resulting into a significant reduction of time needed to evaluate all calibration curves.

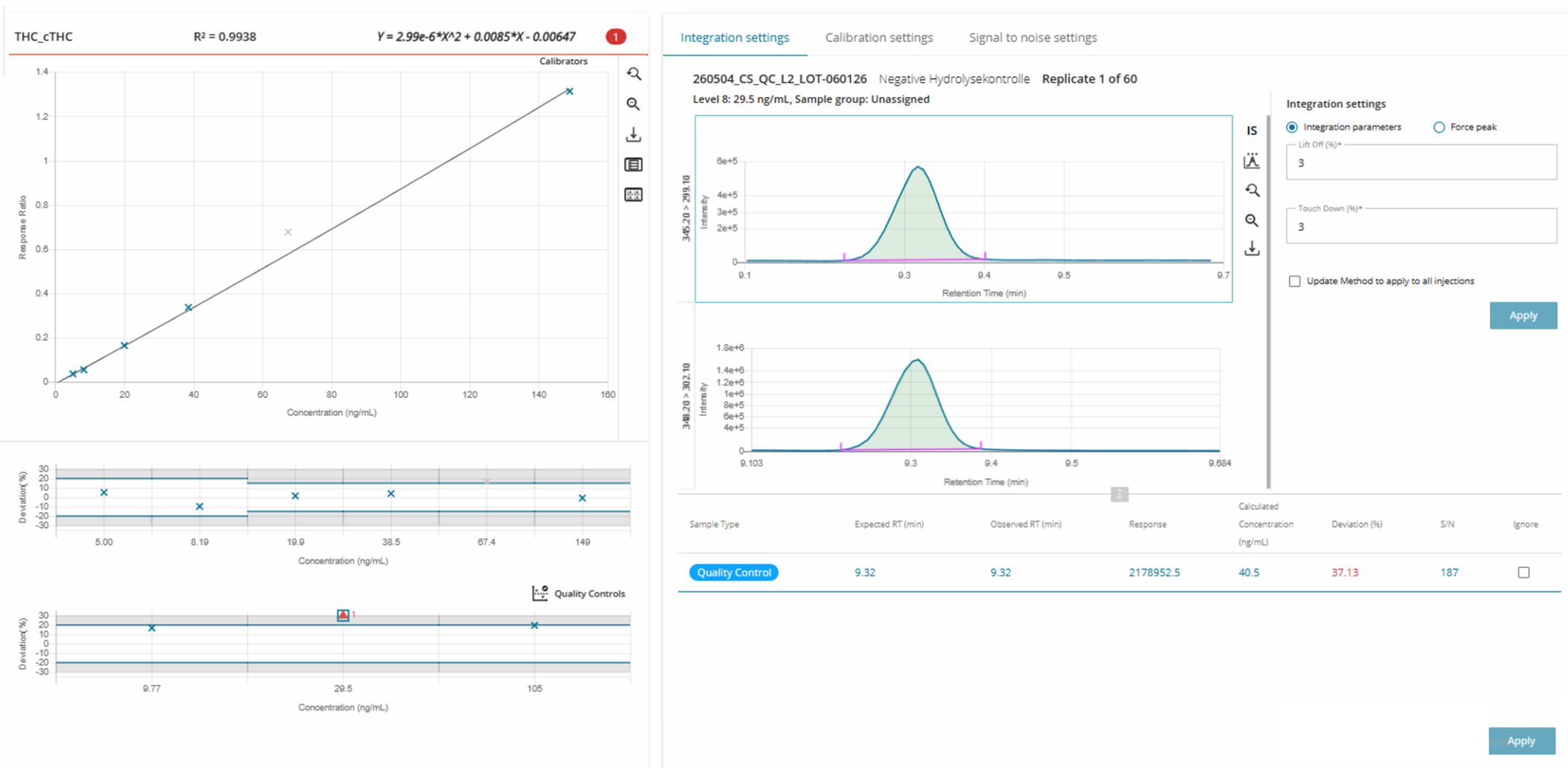


Figure 2: Detailed view of the Cals & QCs details for a specific analyte, allowing editing of the method settings and modifying the integration.

Internal Standards page

Monitor internal standard performance across every injection at a glance (Fig. 3). Trend plots flag retention time or response results outside rule-based acceptance criteria, helping users identify issues such as ion suppression early and focus only on affected injections. Double-clicking a data point opens the detailed peak view for rapid integration review and correction, reducing manual checks, speeding troubleshooting, and increasing confidence in results.

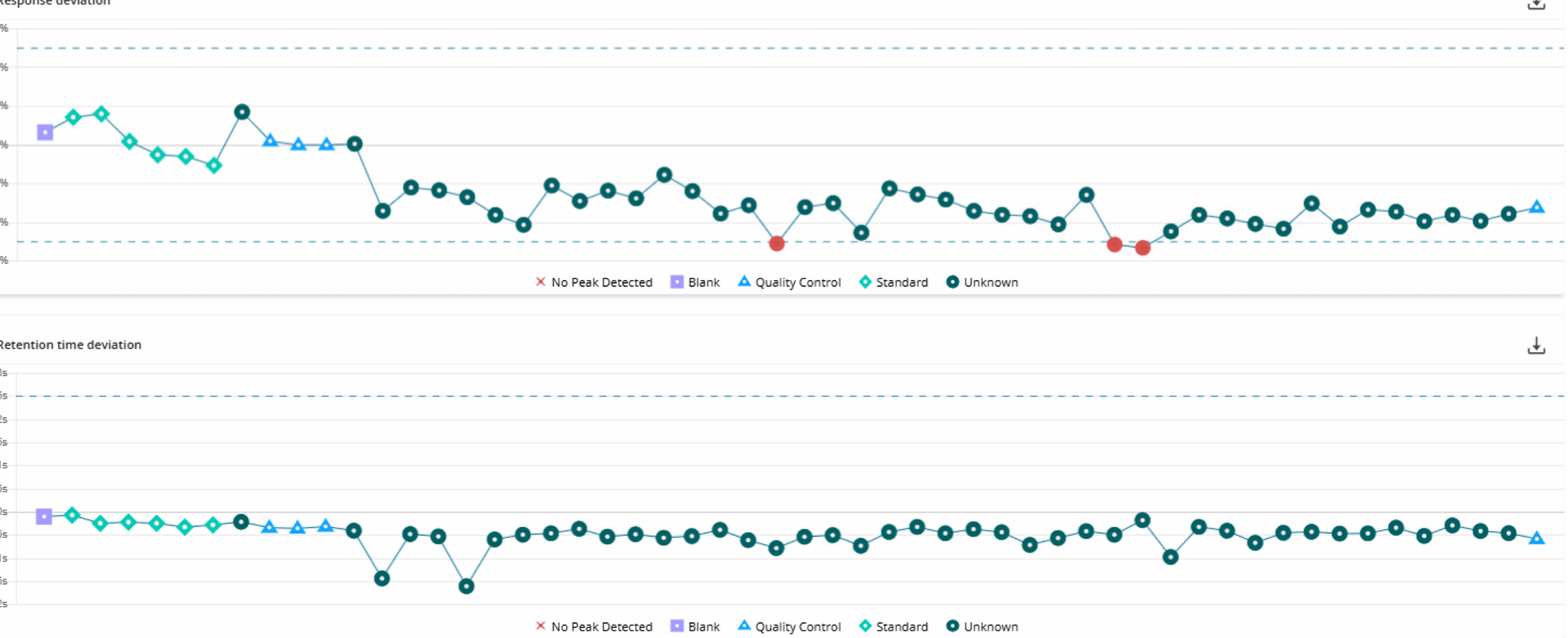


Figure 3: Trend plot of Response deviation (top) and Retention Time deviation (bottom) for the internal standard of Cocaine.

Peak Integration page

The Peak Integration page plots for up to 100 chromatograms on a single screen. To streamline data evaluation, users may restrict the display to results containing exceptions and apply additional filters based on sample type.



Chromatograms can be organized by analyte or by injection.

Analyte-centric	Injection-centric
Few analytes	Large panels
Link between Drugs & metabolites	

See figure 4 for an injection-centric chromatogram view. Chromatograms with associated exceptions are marked with red circle. Restricting data review to flagged peaks decreases total processing time by approximately 50%. Double-clicking on a chromatogram opens the detailed view for further inspection.

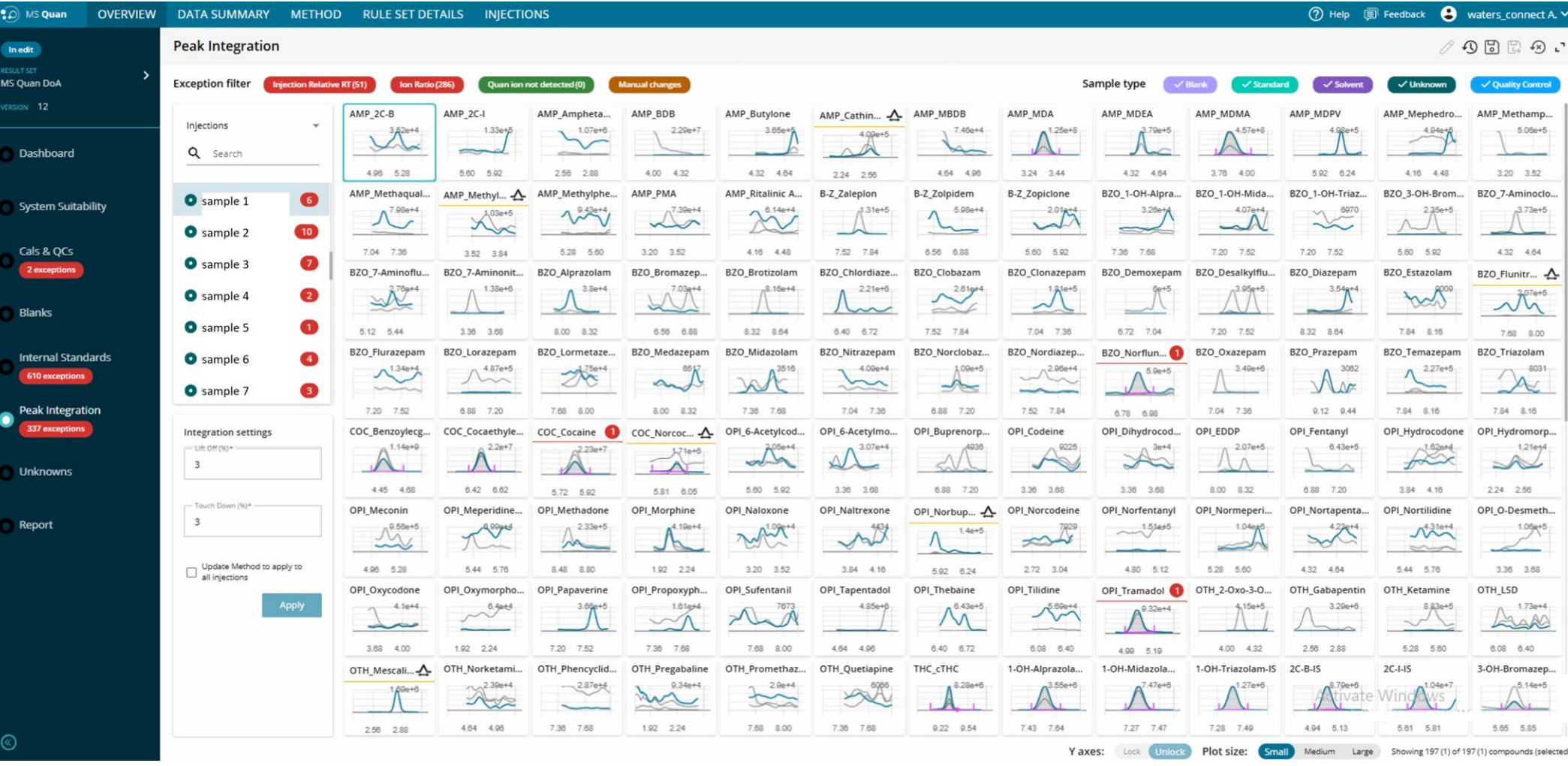


Figure 4: Per injection view of the Peak Integration page.

Figure 5 shows how XFR assists in evaluating false positive results. It shows a detailed view of Mescaline, which was highlighted as a RT and ion ratio exception.

RT exception generated as a result of wrongly assigned peak
Ion ratio exception reported because qualifier is not found
Deleting incorrectly integrated quantifier peak automatically resolves both exceptions.

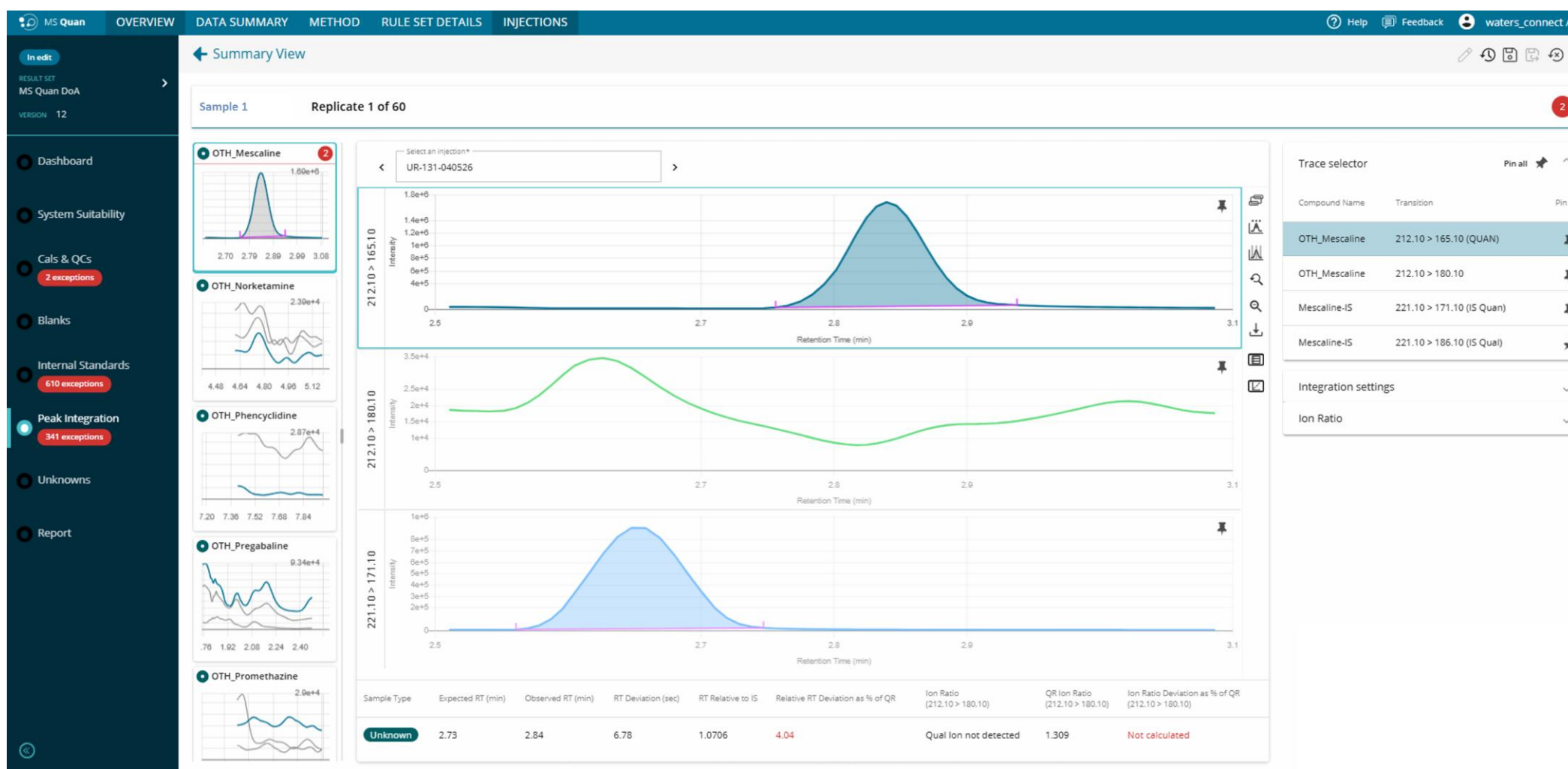


Figure 5: Detail of exception for Mescaline in the Peak Integration page.



Waters™

Discussion

- Workflows:** waters_connect Software provides an end-to-end integration of system control, acquisition, quantitation, and reporting for both method development (Fig. 6) and routine analysis (Fig. 7) workflows. The arrows indicate integrated workflows between software tools.
- Method Development:** Centralized access to instrument tuning and MRM optimization data facilitates consistent method development and transfer, with retention times and dwell times automatically incorporated into acquisition methods.

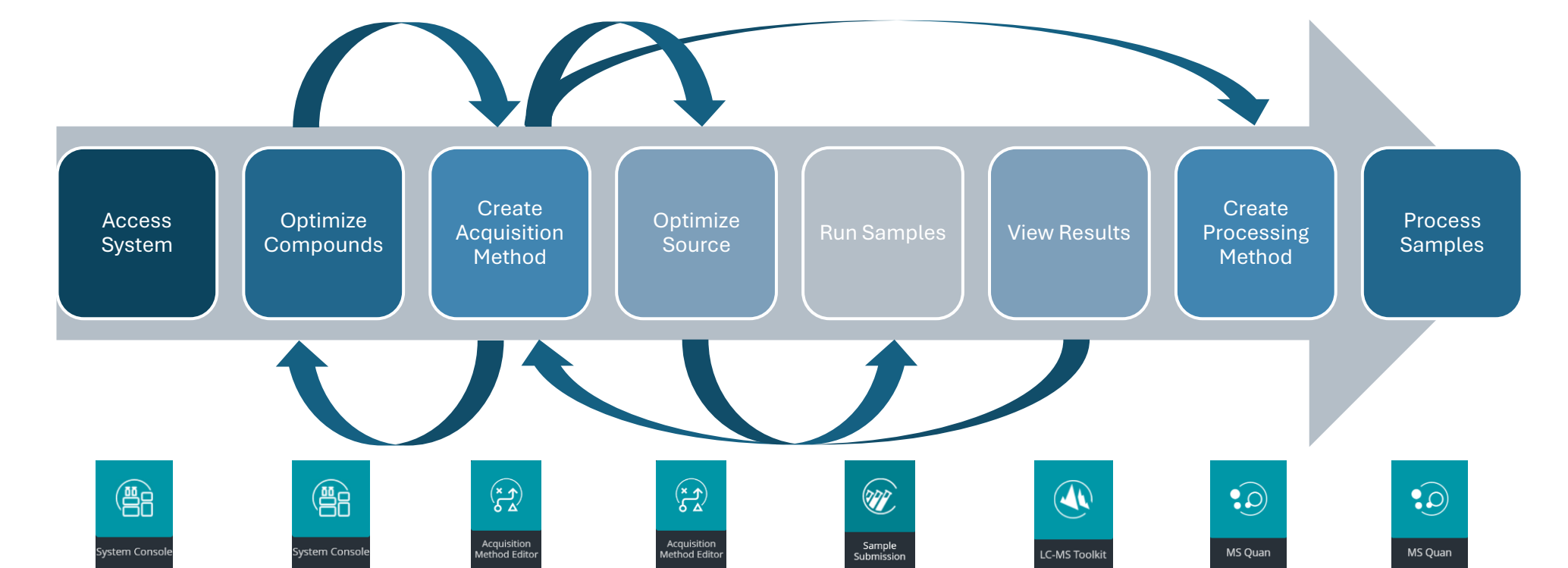


Figure 6: Method development workflow in waters_connect Software.



Figure 7: Routine analysis workflow in waters_connect Software.

- Faster, focused review:** MS Quan application flags XFR exceptions and brings multi-trace evidence together, helping analysts identify carryover, integration, and matrix effects while retaining full chromatogram access.
- Greater confidence in casework:** Automated system suitability checks document performance before samples are analyzed, protecting result quality and reducing avoidable rework.
- Controlled, transparent workflows:** Version control, audit trails, user access management, and change traceability strengthen oversight and defensibility.
- Less manual handling:** waters_connect software and MS Quan application unite acquisition, optimization, processing, and review to improve efficiency and support defensible quantitative LC–MS results.