

1 Introduction

The study presented here details the use of UHPLC-TOF mass spectrometry to detect and quantify a class of compounds known as benzodiazepams.

Screening of these compounds is done using immunoassays, which suffer from non-specificity and poor sensitivity requiring, additional confirmation with GC-MS assays. The GC-MS assays require derivatization of analytes which can be time consuming. However, LC/MS based techniques do not require derivatization. Among the LC techniques, LC/MS/MS is often used to quantitate benzodiazepams in biological fluids however, these assays are only suitable for targeted analysis.

2 Experimental conditions

A PerkinElmer Flexar™ FX-15 LC pump with a PerkinElmer AxION® 2 TOF was used for UHPLC separation and detection of the compounds. The separation was achieved on a PerkinElmer Brownlee SPP C-18, 2X50 mm, 2.7 µm column using a mobile phase gradient of water and acetonitrile containing 0.1% formic acid. The TOF was operated in positive mode.

Benzodiazepam standards were purchased from Cerilliant (Round Rock, TX, US). Calibration curves were set up for urine by spiking urine with varying concentrations of analyte compounds and diluted with methanol (1:1) and injected on column (5 µL). For serum samples, after spiking with varying concentrations of compound mixture, protein was precipitated with acetonitrile containing 1% acetic acid, centrifuged, supernatant diluted 1 to 2 with water and 10 µL injected on column.

3 Results

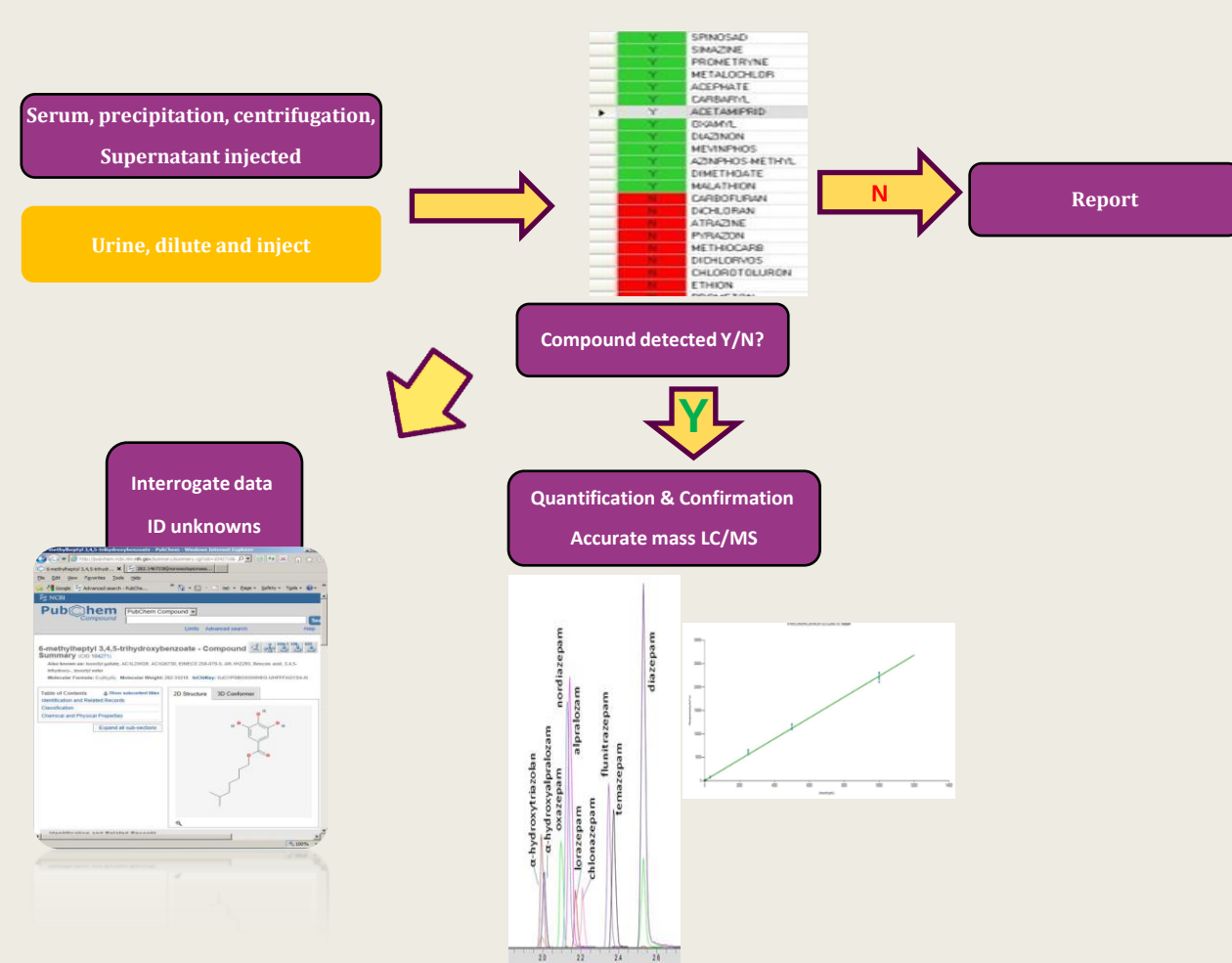


Fig. 1. Workflow for testing, identification and quantification of benzodiazepines in urine and serum

Testing

To rapidly identify the presence or absence of compounds in large batches of samples, AxION Solo™ software was used. AxION Solo provides quick visualization of the presence or absence of analytes in the samples (Figure 2). The presence of individual analytes can be coded with a specific color for ease of identification. The software identifies the presence of a compound based on accurate mass and isotope profile ratio as shown in Figure 3. In addition to searching against spectral information, the software can also search for target analytes based on user defined retention time windows which further improves the specificity of detection. The list of target analytes can be quickly and easily added to as previously unknown analytes are detected in samples. The analysis of benzodiazepines was completed in < 3 min (Figure 4) with all peaks eluting before 2.7 minutes.

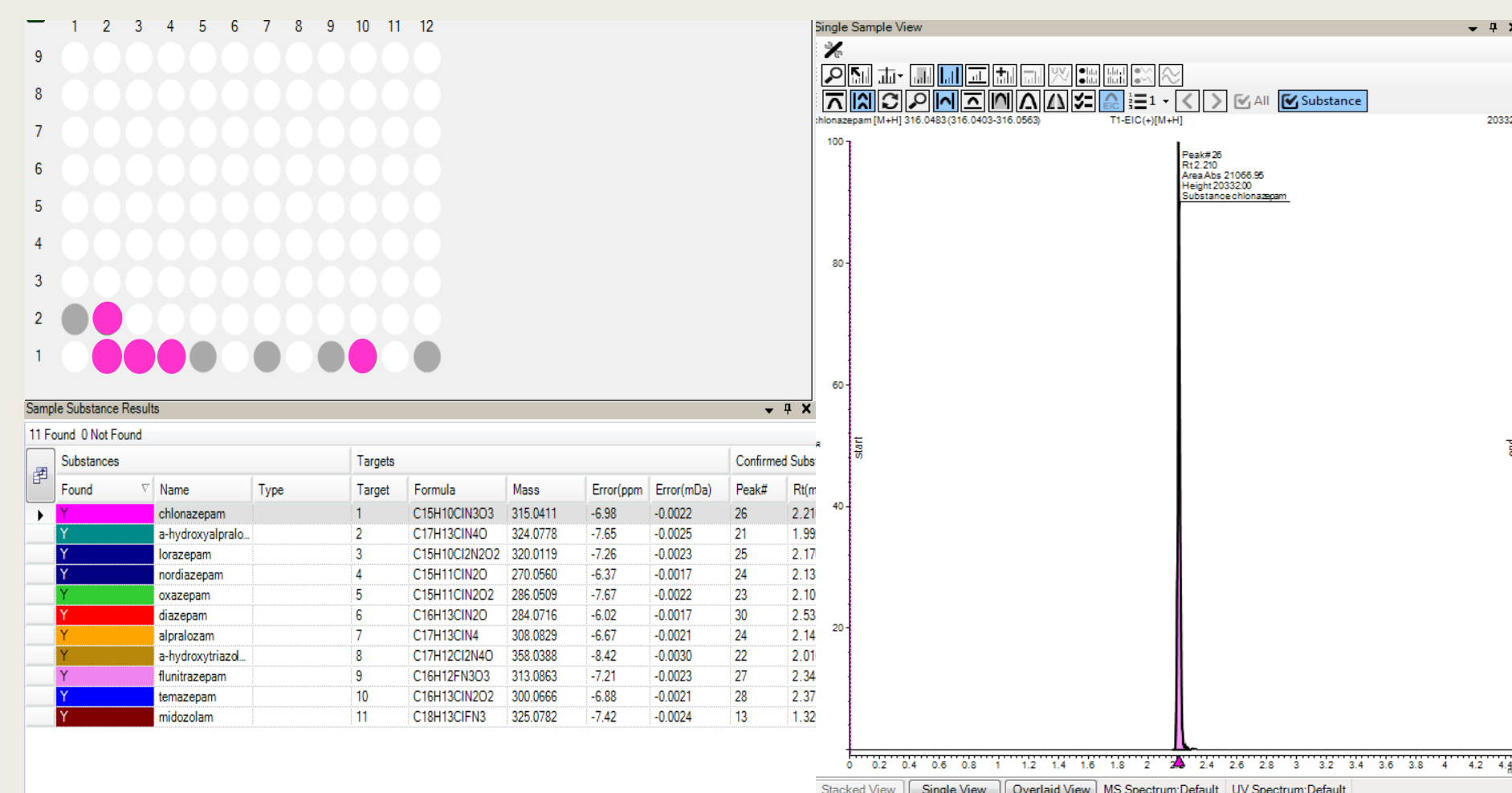


Fig. 2. Axion Solo Software: The top left hand corner shows the presence (pink) and the absence (grey) of chlonazepam in different samples (vials). The remaining benzodiazepines detected in the selected vial are displayed in the table (bottom left).

