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Updating the RADIAN™ ASAP™ Seized Drug Reference Library

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For forensic use only.

INTRODUCTION

Forensic drug chemistry laboratories are constantly challenged by the large number of confiscated samples requiring analysis, and the need for fast turnaround times. Industry guidelines stipulate that two independent analytical techniques are required to analyse a sample, therefore typical workflows comprise a presumptive screening method, followed by a more selective method.[1] RADIAN ASAP combines the simplicity of the Atmospheric Solids Analysis Probe (ASAP) with the specificity of Mass Spectrometry (MS), and has been demonstrated to be effective for rapid, accurate triage of samples received by forensic drug chemistry laboratories.[2] Another significant challenge for drug chemistry laboratories is the pressure to respond appropriately to the ever-changing trends within the illicit drug market and to ensure that their analytical methods and reference libraries remain up-to-date. Therefore, the ability for laboratories to quickly update and expand their analytical methods and reference libraries, with new and emerging analytes, would be of benefit to drug chemistry laboratories and drug enforcement agencies. Here we demonstrate the process of updating the RADIAN ASAP seized drug reference library with a series of benzimidazoles.

PROCEDURE FOR UPDATING THE RADIAN ASAP SEIZED DRUGS LIBRARY

An overview of the procedure used for the addition of a new compound to the RADIAN ASAP library is displayed in Figure 1. To illustrate the steps involved in the process, we have used the addition of isotonitazene, a benzimidazole.

Certified reference material (CRM) for a series of benzimidazoles, including isotonitazene, were obtained as 1 mg solid material from Cayman Chemical (Michigan, USA). Each CRM was dissolved in 1 mL of methanol to obtain individual stock solutions at 1 mg/mL. Prior to RADIAN ASAP analysis, the CRM stock solutions were further diluted with methanol to a concentration of 50 µg/mL.

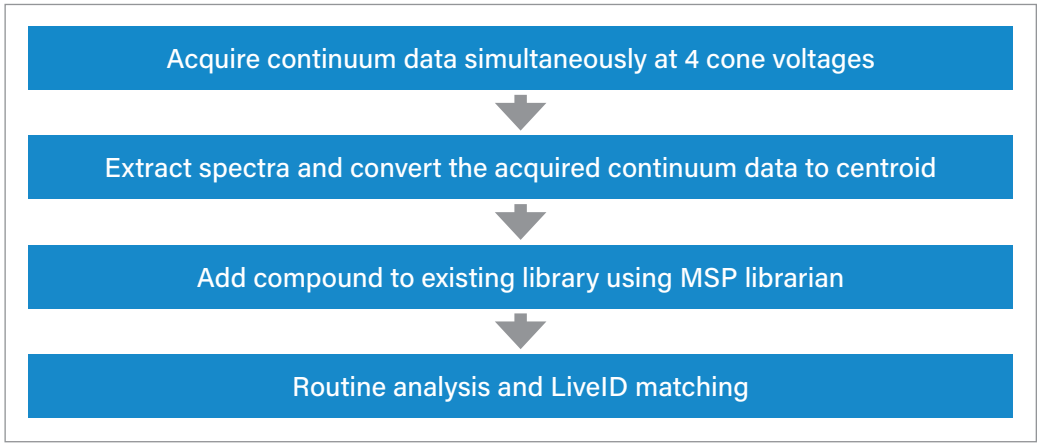

Figure 1. Overview of the steps required for updating the RADIAN ASAP library with new compounds.

STEP 1: ACQUIRE CONTINUUM DATA AT MULTIPLE VOLTAGES

Isotonitazene was analysed using RADIAN ASAP; a 'dipping' method was used for the sampling procedure.[2] For each sample, a new glass capillary was initially cleaned using the supplied automated bakeout procedure. After cooling, the capillary was dipped into the isotonitazene solution (50 µg/mL) for 5 seconds before inserting into the RADIAN ASAP source which automatically triggers data acquisition. Data was acquired using the RADIAN ASAP in positive ionization mode, with mass detection performed using full scan MS over the range *m/z* 50-600 in continuum mode (Figure 2). ASAP ionization is similar to the Atmospheric Pressure Chemical Ionization (APCI) process and typically results in $[M+H]^+$ ions for most polar drugs. The data was acquired simultaneously at four differing cone voltages (15, 25, 35, 50V) to generate a spectral fingerprint, which includes the precursor and product ions.

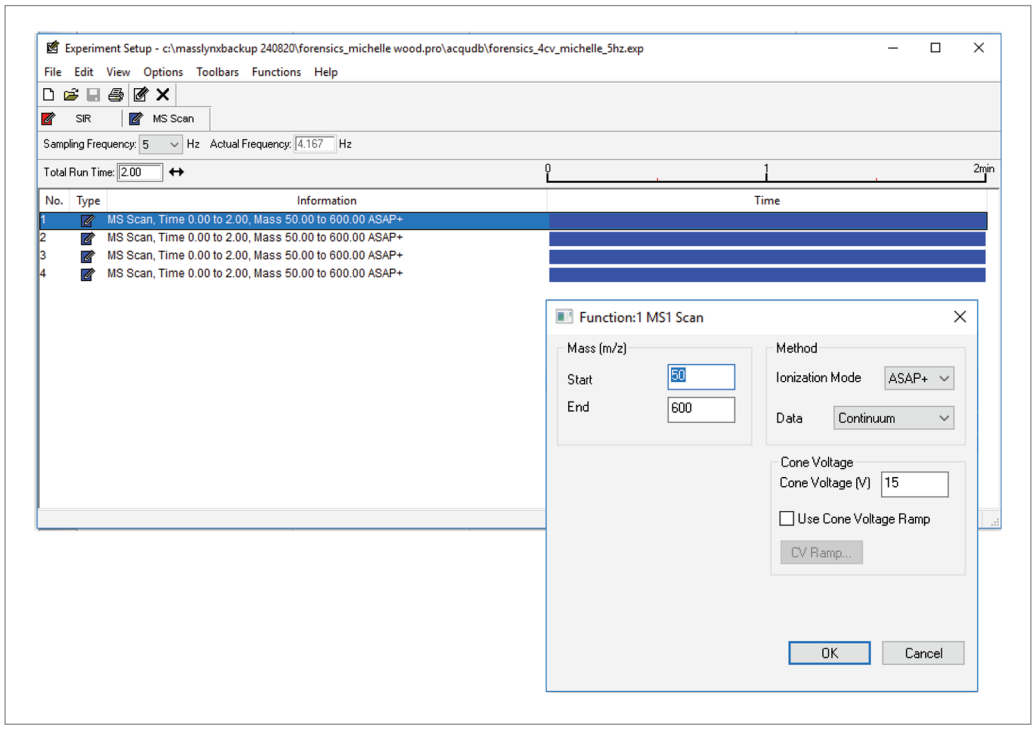

Figure 2. MS method parameters used to acquire spectral data at four increasing cone voltages. Further details of the method are shown for the first function i.e., Function 1 which collects the data at 15V.

Figure 2 shows the acquisition method used and Figure 3 details the resultant data for isotonitazene. Panel 3A displays the four individual total ion chromatograms (TIC) that are generated for each analysis and the corresponding response obtained for each voltage.

STEP 2: EXTRACT AND PROCESS SPECTRA

The software that is used for routine processing of seized drug data is LiveID™ and provides automated comparison of the spectral data, for the material, with a reference library. The LiveID software and the updated seized drug reference library can be downloaded from Waters Marketplace, in the LiveID resources section (https://marketplace.waters.com/apps/170156/liveid#!resources).

The matching algorithm used to compare spectral data uses centroid data, therefore each continuum spectrum must be converted to centroid before use. The continuum spectra were converted by firstly combining the spectra across the peak for each cone voltage (Figure 3B) and then applying the mass measure parameters which are shown in Figure 3C. Thus, the final output was a centroid spectrum for each cone voltage, which was ready to use to create a library entry (Figure 3D).

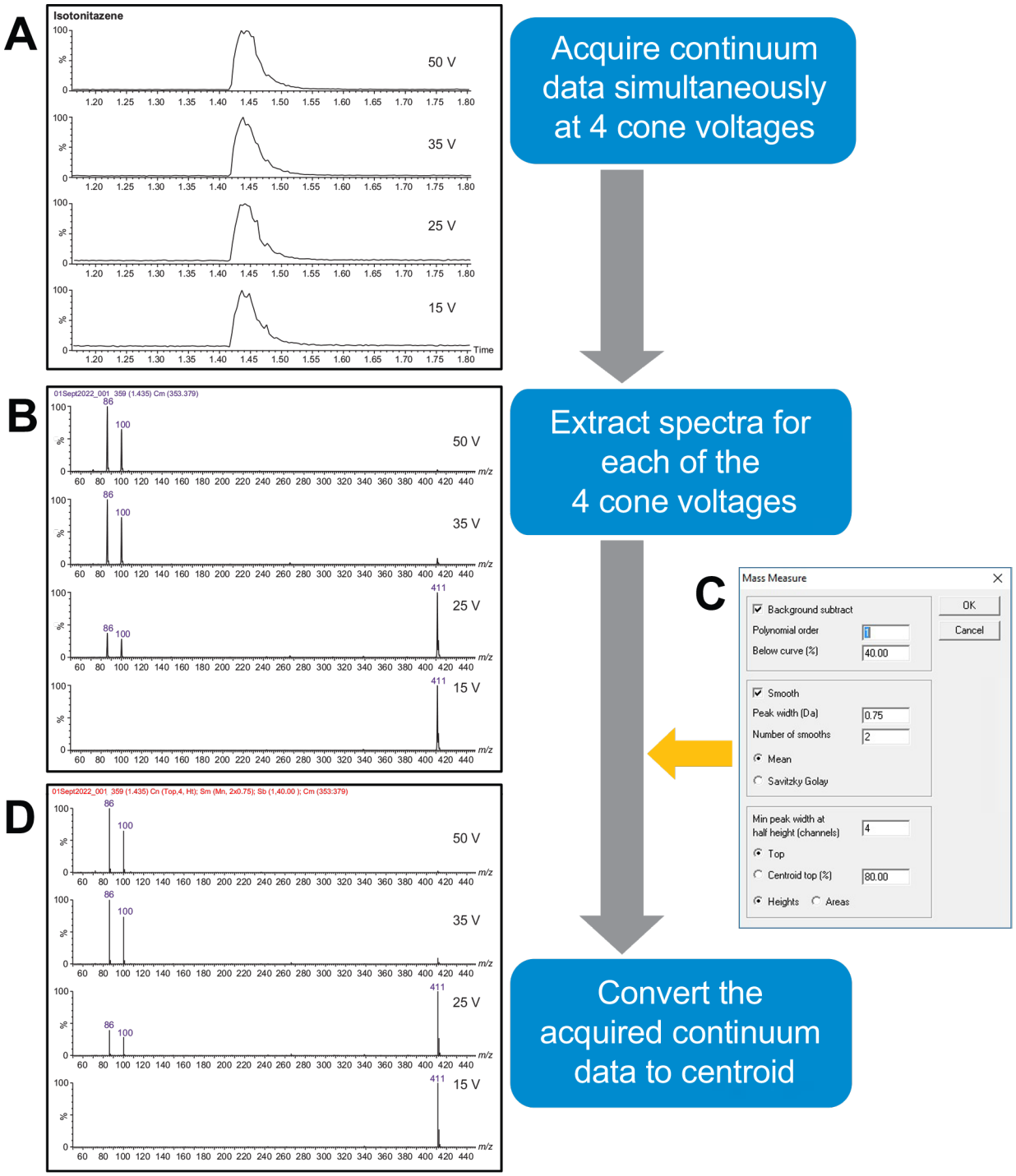

*Figure 3. Panel **A** shows the TIC for isotonitazene for the four differing cone voltages (15, 25, 35, 50V). Panel **B** shows the continuum data (display must be set to integer mass) for four differing cone voltages (15, 25, 35, 50V). The precursor ion (m/z 411) can be seen in the lower cone voltages, with smaller fragments being generated as the cone voltage increases. Panel **C** shows the mass measure parameters which were applied to convert to centroid data. Panel **D** shows the final output ready for use for the library entry addition.*

STEP 3: ADD CENTROID DATA TO THE LIBRARY

MSP Librarian is an open-source software which enables users to create, edit and update existing libraries for use with LiveID software. It can be downloaded from https://sourceforge.net/projects/msp-librarian/. Ensure the LiveID plugins are also downloaded and activated using the Plugins function – this is a one-time operation. Further information can be found from https://sourceforge.net/p/msp-librarian/wiki/Plugins/.

The most efficient way to create a new library entry is to first duplicate an existing library entry and then to edit this by providing the new information. Therefore, for this study, the current library was opened in MSP librarian and a compound entry selected and duplicated (Figures 4A and 4B). The original compound information was then amended to reflect the new compound using the edit feature (Figure 4C). The information included name i.e., isotonitazene, and chemical formula i.e., $C_{23}H_{30}N_4O_3$. Note the addition of the latter, automatically populates the mass and the exact mass fields. The cone voltage information, which is in the comments field, was also amended to reflect the spectra (Figure 4D). The centroid spectrum prepared earlier in MassLynx (Step 2, Figure 3D) was copied using the 'Copy Spectrum List' function, found in the 'Edit' menu and then added into the MSP Librarian library entry using the 'Paste' function (Figure 4E). This can result in a very large peak list. To adjust the number of ions to be used in the entry (typically to a maximum of 15 ions), the percentage intensity cut off was increased, (Figure 4F). This procedure was then repeated for each of the four cone voltages and once completed, the library was saved.

The above process was repeated for the remaining eleven benzimidazoles to update the RADIAN ASAP seized drug library. In summary, for each analyte there is a separate library entry for each of the four cone voltages, which contains the analyte name, molecular formula, and mass spectra in positive ionization mode.

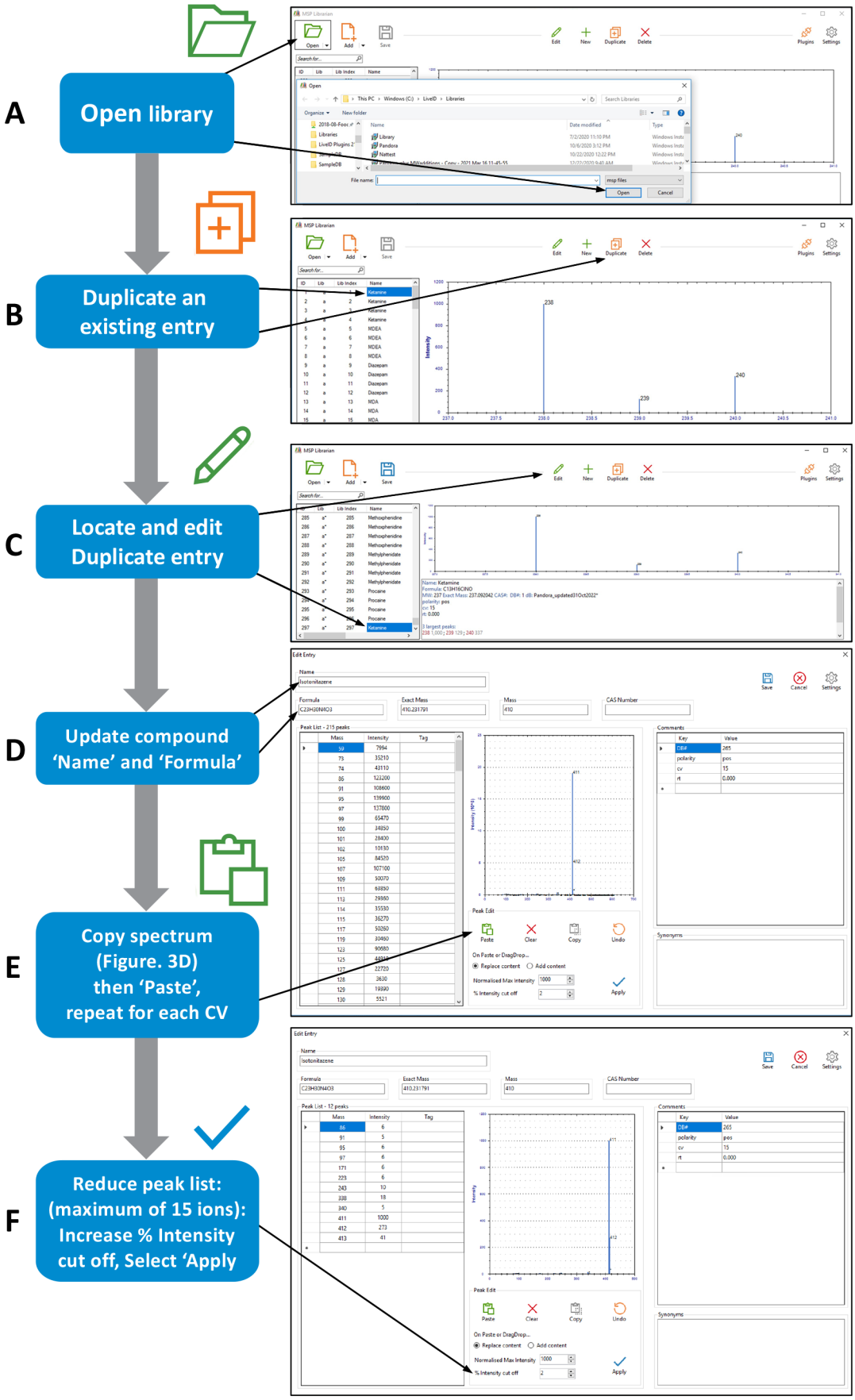

*Figure 4. Panel **A** displays opening the library to be updated in MSP Librarian. Panel **B** shows the duplicate function and panel **C** shows the edit function. Panel **D** displays the edit screen in MSP Librarian where the compound details can be updated. Panel **E** displays the spectrum and function to be used to copy from MassLynx to MSP Librarian, these must be displayed as integer masses. Panel **F** demonstrates how to reduce the peak list once the spectrum has been copied.*

STEP 4. ROUTINE ANALYSIS AND LIVEID MATCHING

In LiveID, the reference library was updated to the most recent version by selecting “Load new library”, then selecting the updated library. Once loaded, samples can be matched against the new, updated library, including previously acquired samples.

Following the update of the library, to evaluate the new entries, solutions of the twelve benzmidazoles were reanalysed using the standard analytical procedure. The dipping method of sampling was again used as detailed in the experimental section, and the data was automatically processed using LiveID 2.0 library matching software, in conjunction with the updated library as detailed in this white paper. A minimum match factor of 850 was used as the reporting cut off for a sample to be deemed positive. When reanalysed, all 12 compounds gave match scores >900 (range 924 to 999). The reanalysed LiveID library search results achieved for etonitrazene are shown in Figure 5.

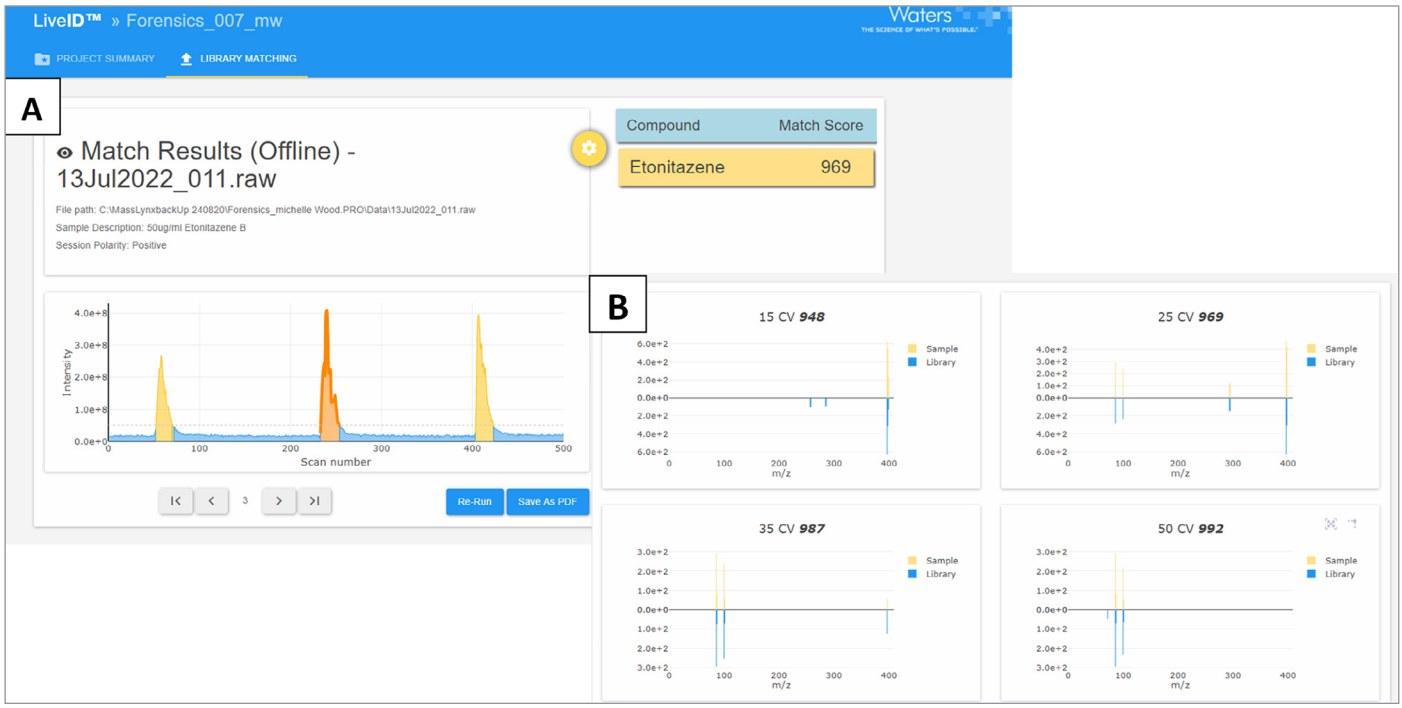

*Figure 5. LiveID analysis of etonitazene CRM. Panel **A** shows replicate analysis for etonitazene reference material with a match factor (969) for the second triplicate. Panel **B** displays the spectral match (reverse-fit model) with the analysed sample against the new library entry.*

CONCLUSIONS

MSP Librarian, an opensource software allows customers to edit existing libraries or to create their own reference library. The software uses the centroid data generated in MassLynx software, to create a separate library entry for each of the four cone voltages for each analyte, which can be added to the reference library. LiveID software can then be used with the updated library to compare both newly and previously acquired data against, generating a match score using a reverse-fit model. Allowing customers to keep their screening methods up to date with any trending or emerging analytes in the illicit drug market.

This white paper demonstrates the ease with which new emerging drug analytes, can be added to the RADIAN ASAP drug screening method and the accompanying reference library. Due to the serious health risks these analytes pose, a rapid screening of seized drugs can enable drug chemistry laboratories to enable drug chemistry laboratories to keep their screening methods up to date and relevant in a constantly evolving seized drugs landscape.

REFERENCES

1. Scientific Working Group for the Analysis of Seized Drugs (Recommendations) -Edition 8, 13 June 2019. https://www.swgdrug.org/approved.htm (accessed 24 Aug 2022)
2. Wood M. Radian ASAP with LiveID – Fast, Specific, and Easy Drug Screening. Waters Application Note. 720007125. 2021

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