Automated Targeted Screening of Leachables in Pharmaceutical QC Labs Using the Agilent LC/MSD XT and Agilent OpenLAB CDS

Application Note

Pharmaceuticals QA/QC

Authors

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Abstract

This Application Note describes a routine analysis workflow for pharmaceutical QA/QC labs. Organic nonvolatile leachables from ophthalmic drug products were identified and quantified using an Agilent Liquid Chromatograph/Mass Selective Detector XT (LC/MSD XT), based on single quadrupole technology, with the Agilent 1260 Infinity II LC system and Agilent OpenLAB CDS Software. The newly designed OpenLAB CDS has several advantages for targeted screening, such as reference spectral matching, quantification with quantifier and qualifier ions, and suspect identification using library searches. The software enables a complete 21 CFR Part 11 compliance solution that includes data security, integrity, and traceability.

Identification of organic nonvolatile leachables in ophthalmic drug products (ODP) using an accurate mass LC/Q-TOF instrument was described previously[1]. Selected compounds from that list were used to develop a targeted screening method for routine QA/QC analysis. The data analysis processing method was linked to the data acquisition method to give automated results. The identified compounds were quantified using a standard addition method, and qualifier ions were used to increase the level of confidence for identification. To help identify suspected leachables, the acquired spectra were matched against a custom NIST LC/MS library.

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Introduction

Extractables and leachables studies are typically performed using accurate mass, high-resolution mass spectrometers[1,2] to confidently identify compounds that are present in a wide range of concentrations. For routine QC analysis, the Agilent XT Liquid Chromatograph/Mass Selective Detector provides a robust, easy-to-use, and cost-effective analytical approach. The targeted method developed using an LC/UV/MSD system can perform not only targeted screening, but also suspect identification. Acquiring data in both SIM and SCAN modes, in positive and negative polarities allows multiple analytes to be identified in a single analytical run.

This Application Note demonstrates targeted screening of leachables to be easy and highly automated. Automated data processing with report generation helps to evaluate a large number of samples efficiently. The targeted screening results indicate leachable compounds that are present in samples and can be quantified. A standard addition approach was used to demonstrate quantification. Suspects include a wide range of compounds reported in the literature[3] which, unexpectedly, can be found in the sample. Suspected leachables populated in the in-house spectral library can be used to identify nontargeted peaks during routine testing.

In accordance with 21 CFR part 11 or EU Annex 11, QC laboratories operate under strict GMP guidelines where data security, integrity, and traceability are required. Data integrity is often the primary focus for inspection by regulatory agencies. It includes secure retention and retrieval of electronic records, controlled access, and electronic audit trails with date and time stamps. This work also demonstrates the compliance features of Agilent OpenLAB CDS Software. OpenLAB CDS Software offers a single software solution for liquid chromatography, gas chromatography, and mass spectrometry. It provides a new user interface with customizable and interactive reporting and drag-and-drop template creation. Key software features enable data security, integrity, traceability, and usage of electronic signatures per regulatory guidelines. A custom calculator feature within OpenLAB CDS can be used to assess compounds and determine if they meet threshold criteria for E&L studies, enabling calculations to be performed without exporting the data, thus maintaining data integrity.

Experimental

Reagents and standards

All reagents and solvents were LC/MS grade. Ammonium formate was from Agilent (p/n G1946-85021). Analytical standards and formic acid were purchased from Sigma-Aldrich (St. Louis, USA). Ultrapure water from a Milli-Q system (Millipore, USA) was used.

LC/MS System

LC separation was carried out using an Agilent 1260 Infinity II LC system, consisting of a binary pump (G7112B), a vial sampler (G7129A), and a diode array detector (DAD) (G7117C). The MS system used was the Agilent LC/MSD XT system (G6135CA) with an Agilent Jet Stream Source (G2735L). Agilent OpenLAB CDS (version 2.1) was used for data acquisition and analysis. The DAD was operated at multiple wavelengths. OpenLAB CDS was used to align UV and MS chromatograms. The UV signal at 280 nm of a blank sample was used to perform UV background subtraction. Table 1 shows the LC and MS experimental conditions.

Table 1. LC/MS experimental conditions.

Parameter	Value
Column	Agilent Poroshell 120 EC-C8, 3.0 × 150 mm, 2.7 µm (p/n 693975-306), operated at 45 °C
Needle wash	10 seconds (80 % methanol/20 % water)
Flow rate	0.6 mL/min
Injection volume	2 µL
Mobile phase	A) Water, 4.5 mM ammonium formate + 0.1 % formic acid (FA) B) 80 % MeOH + 20 % IPA (v/v), 4.5 mM ammonium formate + 0.1 % FA
DAD	214 ±4 nm, 230 ±4 nm, 254 ±4 nm, and 280 ±4 nm (reference 360 ±100 nm), at 10 Hz
Gradient	Time (min) %B 0 7 5 15 20 100 25 100
Stop time	25 minutes; post time: 5 minutes
MSD parameters	
Drying gas flow	10 L/min
Drying gas temperature	150 °C
Nebulizer pressure	40 psi
Capillary voltage	3,500 V (positive and negative modes)
Nozzle voltage	300 (positive and negative modes)
Fragmentor voltage	120 V (positive mode), 90 (negative mode)
Peak width	0.06 minutes
SIM/SCAN	SIM of expected standards and SCAN range 80–800 *m/z* in both positive and negative modes
Dwell time	200 ms

Sample preparation

Standard samples

Eight compounds (Table 2) were selected from a previous study on ophthalmic drug products[1]. These standards were dissolved in acetonitrile to give a stock concentration of 100 µg/mL. Further dilution was performed using a solution containing either 80 % mobile phase B and 20 % mobile phase A, or 50 % acetonitrile and 50 % water.

System suitability samples

Three compounds were selected from the 8-compound set, and used as standards to test for system suitability: phthalic anhydride, methyl 2-benzoyl benzoate, and 3,5-di-*tert* butyl-4-hydroxybenzyl alcohol. They were prepared at 0.5 µg/mL concentration. These were selected because of their varied LC elution times and polarities. Sample data were acquired using the targeted screening method using UV and SIM/SCAN modes.

Drug product preparation

An ophthalmic drug product (ODP) was obtained from local drug stores in India, and used for the leachables analysis. The ODP was centrifuged, and injected directly into the LC/MS system.

Standard addition method

The ODP was diluted with an equal amount of acetonitrile and centrifuged. Analytical standards to be quantified were dissolved in 100 % acetonitrile, and diluted to 0, 450, 750, 1,050, and 1,350 ng/mL using 50 % acetonitrile and 50 % water. The compound 2-ethylhexyl 4-(dimethyl-amino) benzoate was used as the internal standard (ISTD) and prepared at a concentration of 600 ng/mL. Five solutions, each containing the diluted ODP, the ISTD, and one of the five analytical standard concentrations, were prepared in separate vials and vortexed. These solutions were used to generate the standard addition calibration curve. The calibration curve was set as *linear*, and the *ignore origin* option, which bypasses the origin, was selected for generating the curve.

Table 2. List of standard compounds used in the experiment for targeted screening analysis. The UV (nm) and SIM (*m/z*) data show the experimental values used in the method.

Compound	Formula	Predominant charge	UV (nm)	m/z	SIM m/z	RT
Phthalic anhydride	$C_8H_4O_3$	$[M+H]^+$	280	149	149	9.73
Methyl-2-benzoyl benzoate	$C_{15}H_{12}O_3$	$[M+H]^+$	280	241.1	209.2	14.59
2,2-Dimethoxy-2-phenyl-acetophenone (Irgacure 561)	$C_{16}H_{16}O_3$	[M+H]+	280	257.1	225.3	16.29
3,5-Di-*tert* butyl-4-hydroxybenzyl alcohol	$C_{15}H_{24}O_2$	$[M-H]^-$ and $[M+H]^+$	280	235.1 (–)	219.0 (+) 235.1 (–)	17.03
Isopropyl-9H-thioxanthen-9-one, mixture of 2 and 4 isomers	$C_{16}H_{14}OS$	$[M+H]^+$	280	255.1	255.1	18.31
2-Ethylhexyl 4-(dimethyl-amino) benzoate (octyldimethyl PABA)	$C_{17}H_{27}NO_2$	$[M+H]^+$	280	278.2	278.2	19.64
1,3-Di-*tert* butyl benzene	$C_{14}H_{22}$	–	214	–	–	20.15
Bis (2-ethylhexyl) phthalate	$C_{24}H_{38}O_4$	$[M+H]^+$	280	391.3	391.3	21.13

Standard samples used for LC/MS library creation

Thirty five standard compounds commonly reported as extractables and leachables (suspects) were used to create an LC/MS spectral library containing information about molecular peaks, adducts, isotopes, and characteristic InSource Fragmentation products for fragile compounds. The library was built on NIST LC/MS software (NIST 14) using OpenLab CDS Software.

Analytical methodology

Figure 1 shows the workflow for the routine analysis of samples in a QC environment. To ensure that the LC/MS system meets instrument performance, a system suitability test mix was analyzed. Following the targeted screening analysis, appropriate target compounds were quantified, and suspect compounds identified.

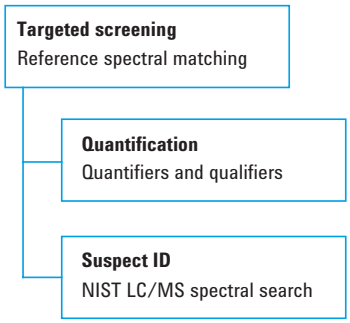


Figure 1. Targeted screening followed by quantification and suspect peak analysis.

Targeted screening

After system suitability runs, standards were selected from E&L studies performed on a Q-TOF system for routine analysis of DP[1]. The LC/MS separation method was developed using these standards in positive and negative SCAN modes. The mass spectra of the standards obtained from the SCAN data were added as reference spectra in the processing method. The predominant molecular ion was used as the SIM ion in the acquisition method, which was operated in SIM/SCAN mode with different polarities. The UV reference spectra of the standards were also stored in the processing method. To enable quick review of the results, the appropriate reports were added to the processing method. The processing method and customized reports were linked to the acquisition method during submission of the runs. Post acquisition, the data files were processed by the processing method, then reports were generated and automatically sent to the desired location.

Quantification

Based on the results of the targeted screening, a SIM-based quantification acquisition method was created. Representative qualifier and quantifier ions were selected for the compounds of interest. A standard addition method was used to quantify all observed leachables.

Suspect identification using library searching

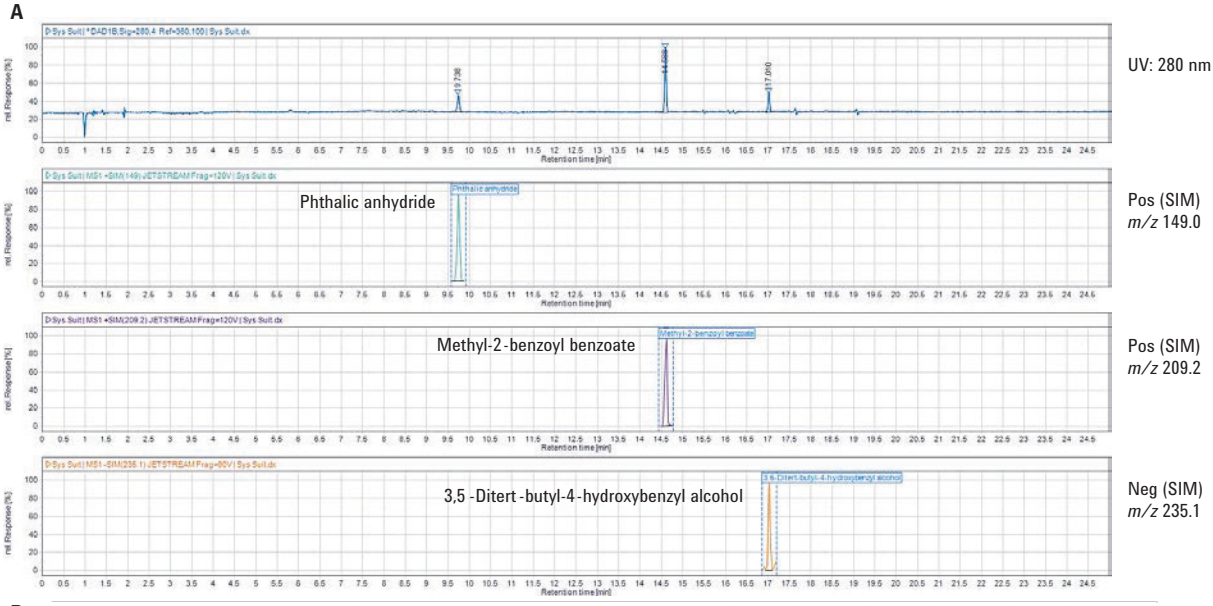
The targeted screening ODP samples were reprocessed with a custom calculator to identify MS and UV leachables that met a threshold based on an estimated analytical evaluation threshold (AET). Compounds meeting the AET were used to search the LC/MS library to confirm suspected leachables in the formulation.

Results and Discussion

System suitability test

The system suitability test mix was analyzed to determine the performance of the column and the instrument. The System Suitability functionality of the processing method was used, wherein the column details and signal-to-noise (S/N) criteria were entered. Figure 2A shows the chromatographic separation. Figure 2B shows the system suitability results, taken from the injection results summary page. The resolution of the compounds (calculated from the UV signals) and peak shape meet the in-house system suitability criteria, indicating that the system is suitable for further experiments. The processing method used in the system suitability samples can be re-used for future system suitability test samples.

A



B

Name	Signal description	RT (min)	Area %	Height %	Resol. USP	S/N	Symmetry	Tailing	Plates USP
Phthalic anhydride UV	DAD1B,Sig=280,4 Ref=360,100	9.738	22.181	16.91		78.54537	1.10045	0.88526	181,308.1393
Phthalic anhydride	MS1 +SIM(149) JETSTREAM Frag=120V	9.746	100	100			0.92425	1.02516	109,369.188
Methyl-2-benzoyl benzoate UV	DAD1B,Sig=280,4 Ref=360,100	14.588	59.466	64.37	62.87639	64.69265	1.02218	0.95606	865,456.5638
Methyl-2-benzoyl benzoate	MS1 +SIM(209.2) JETSTREAM Frag=120V	14.612	100	100			1.3698	0.87949	237,913.6697
3,5-Di-*tert*-butyl-4-hydroxybenzyl alcohol UV	DAD1B,Sig=280,4 Ref=360,100	17.01	18.353	18.72	38.76049	31.09572	0.97004	1.08979	1,192,262.442
3,5-Di-*tert*-butyl-4-hydroxybenzyl alcohol	MS1 -SIM(235.1) JETSTREAM Frag=90V	17.027	100	100			0.89068	1.28468	484,610.9334

Figure 2. UV (background subtracted) and MSD signals for the three compound suitability mix samples (A). The USP resolution, S/N, peak shape, and theoretical plates were determined by the system suitability functionality of the Agilent OpenLab CDS (B).

Automated targeted screening

A targeted screening method was developed for eight selected standards in SIM/SCAN positive and negative modes (Figure 3). The UV/MS reference spectra and retention times of these standards were stored in the master processing method. This was then updated, and linked to the acquisition method in the sequence table to enable automated processing of the data (Figure 3). ODP and blank samples were acquired using the targeted screening method, and processed with the linked processing method. The automated generation of Quant Reports indicates the number of targeted compounds identified. The results show that two compounds, methyl-2-benzoyl benzoate and Irgacure 561, were identified in the ODP by reference matching scores and LC retention times. Due to the complexity of the ODP sample and concentration of the compounds present, the reference spectra matching scores were low for Irgacure 561, while those for methyl-2-benzoyl benzoate were high. These target compounds were subsequently quantified in SIM mode using qualifier and quantifier ions.

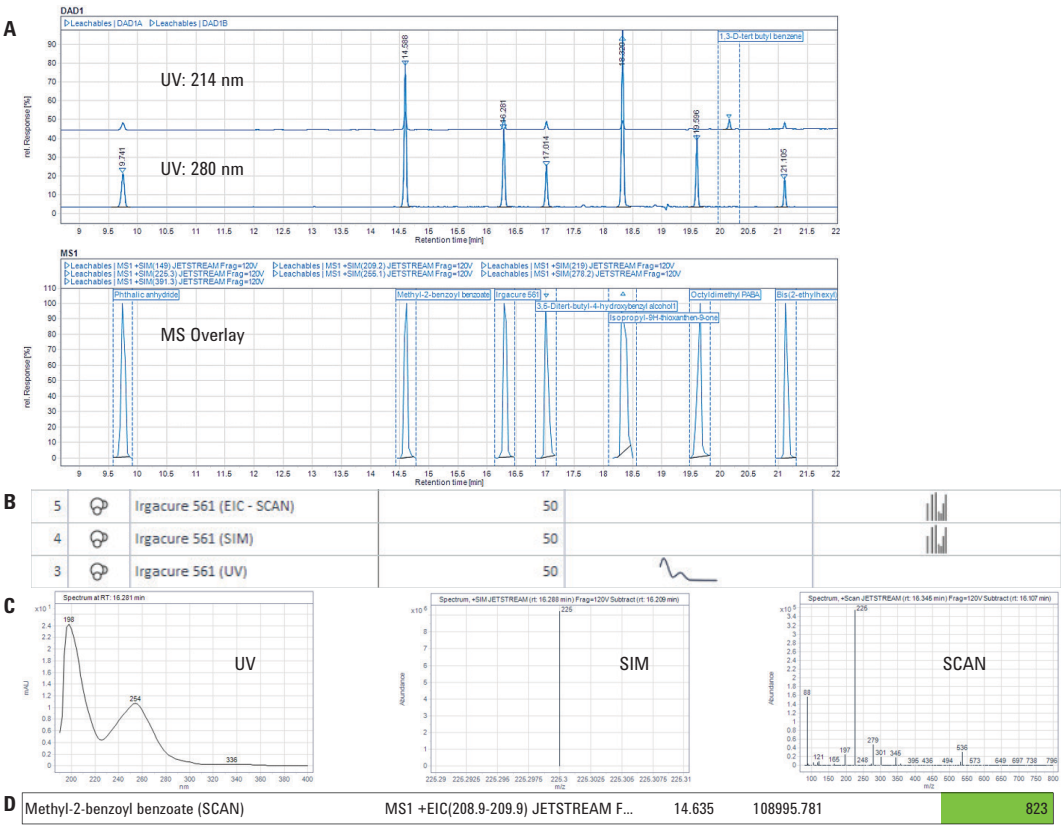


Figure 3. Separation of the standard mix of compounds using LC/UV/MS (A). The UV and MS (SCAN) and MS (SIM) reference spectra are stored in the processing method (B and C). The results of the targeted screening analysis are displayed with reference spectra matching scores (D).

Quantification of leachables

Confident identification of an impurity by the LC/MSD XT requires not only its molecular weight, but also additional unique characteristics of a compound such as retention time and qualifier ions. As per SANCO guidelines[4], for a unit mass resolution instrument, three or more diagnostic ions are required for the identification of pesticide residue in food and feed. Including the qualifier ion(s) adds additional confidence for compound confirmation during quantification. In addition to quantifiers, the isotope peak at 210 *m/z* and fragment ion 197 *m/z* were used as qualifier ions for methyl-2-benzoyl benzoate and Irgacure 561, respectively. A standard addition method was used to accurately obtain the quantification values (Figure 4). Quantification of ODP samples determined the concentration of methyl-2-benzoyl benzoate and Irgacure 561 to be 148.4 ng/mL and 149 ng/mL in the DP sample, respectively.

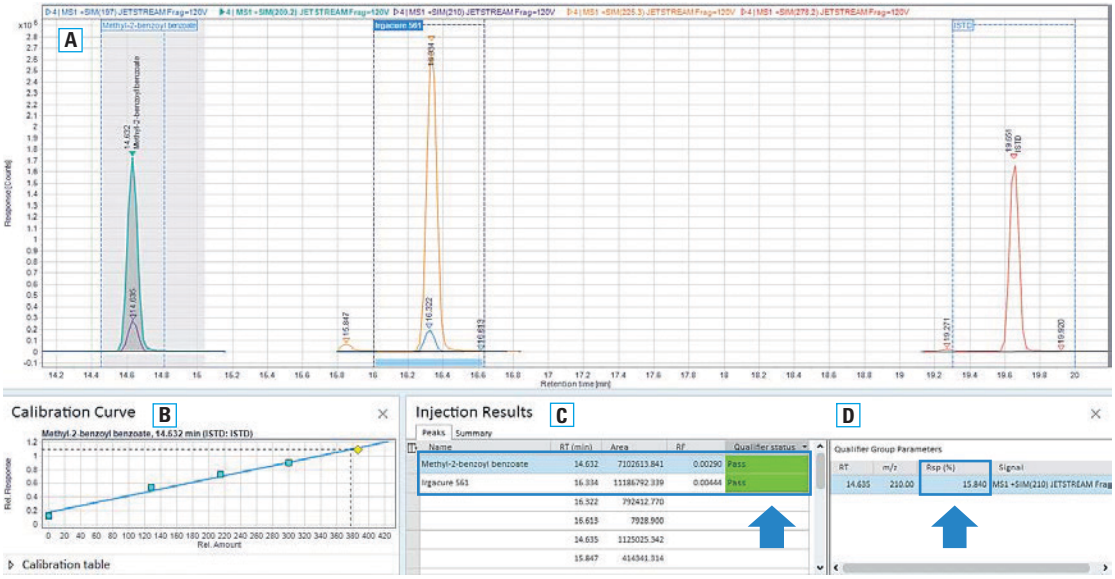


Figure 4. A) Drug formulation analysis showing SIM quantifiers of methyl-2-benzoyl benzoate. B) Calibration curve of the standard. C) Results of the analysis along with status of *Pass* for qualifier status. D) The qualifier response percentage achieved.

Confident suspect identification experiments

OpenLAB CDS Software effectively matches MS spectra with LC/MS spectral libraries. A suspect peak at 22.7 minutes was detected in the chromatogram of ODP. The spectra were extracted and matched to a customized LC/MS spectral library. The results identified erucamide with high library matching scores (Figure 5).

OpenLAB CDS compliance

OpenLAB CDS provides features for labs to achieve compliance for laboratory data acquisition and processing. Data security, integrity, traceability, and e-signature features of OpenLAB CDS are in accordance with the guidelines stated in 21 CFR part 11[5].

Data security

To ensure that access to information is protected, OpenLAB data – including methods and results – are stored in secured project folders. Only users with specific rights have access, thus providing security from data tampering.

Figure 5. A suspect peak from the DP sample detected (A) and its mass spectra extracted (B) and library matched to erucamide (C and D).

Data integrity

When data are exported out of a CDS to be analyzed by different software, the possibility exists that data accuracy could be compromised. OpenLAB CDS includes a customizable calculator that allows users to carry out data analysis such as application-specific formulae without the help of external software (Figure 6A). In E&L studies, compounds above AET thresholds are reported to regulatory authorities. In ODP, the AET values are based on concentration (µg/mL) as described[1]. In this example (Figure 6), an IF command was used to display MS compounds crossing a specific threshold area value of 100,000 (Figure 6A) and labeled as *MS Leachable*. Processes such as these, built within OpenLAB CDS, help to determine specific leachables without the need to export data to other software platforms.

Data traceability

Important components of data traceability include audit trails, activity logs, and method versioning. Audit trail reviews also play an important role. OpenLAB CDS can include confirmation and documentation of audit trail reviews as part of electronic records. Figure 7 shows a snapshot of the audit trail, indicating audit trails and their reviews in parallel.

e-Signatures

Electronic signatures are a legally binding equivalent of individual's handwritten signature (11.3(b)(7)). OpenLAB CDS links electronic signatures to records, embeds results, and ensures that they are present when records are displayed or printed (Figure 9). This speeds up data review and approval.

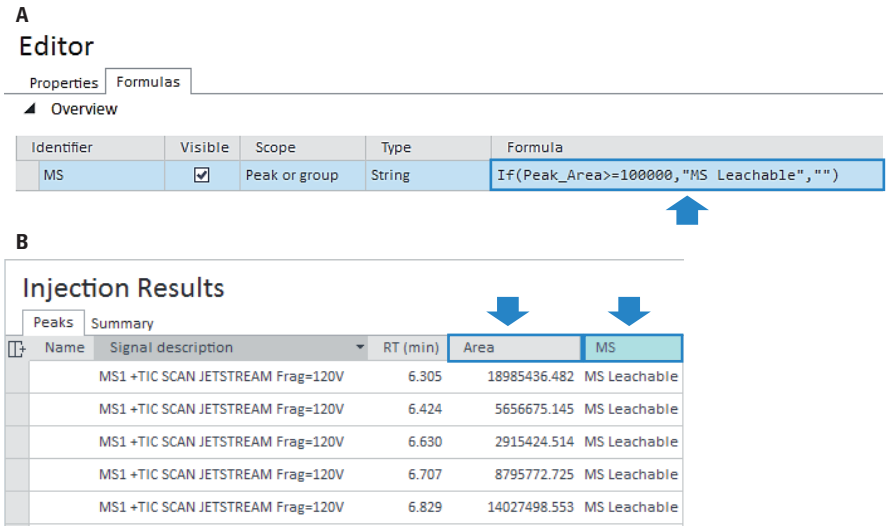


Figure 6. The custom calculator editor (A) used to define threshold criteria and label compounds as *MS Leachable* (B).

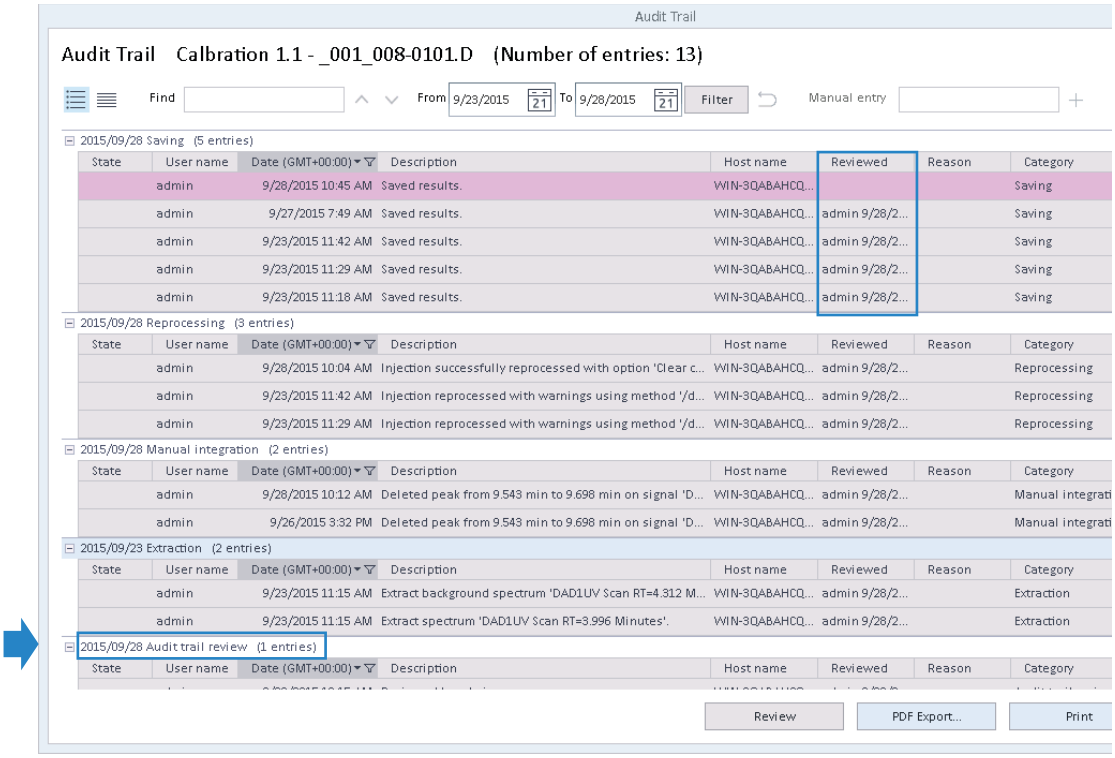


Figure 7. Audit trail and its review for data traceability.

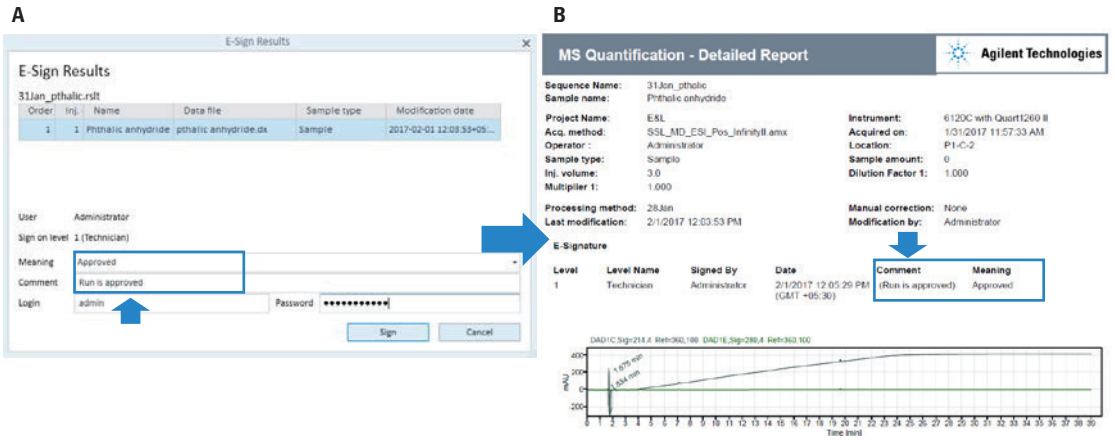


Figure 8. e-Signature entries (A) are displayed in the reports (B), enabling speedy data approval.

Conclusions

This Application Note describes a quick and efficient targeted screening of leachables from drug formulations in pharmaceutical QC labs using the Agilent LC/MSD XT controlled by Agilent OpenLAB CDS. The OpenLAB CDS predefined data processing methods, including UV and MS reference spectra, were used to set up the targeted analysis of leachable impurities. Quantifier ions, together with qualifier ions, were used to quantify analytes with added confidence. Suspect compounds were detected using the platform's capability to search unit mass LC/MS spectral libraries. Features such as the custom calculator within OpenLAB CDS were used to perform application-specific calculations. OpenLAB CDS provided the data security, integrity, and traceability features necessary for maintaining compliance in QC labs.

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