

Agilent OpenLab CDS for Automated High Throughput Purity Assessment Using Mass Spectrometry

Part I: Sample Purity Determination using LC/MS

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Abstract

This application note presents the use of Agilent OpenLab CDS software to simplify and automate compound confirmation and sample purity assessment workflows. Data used in this application note was generated on a high-throughput Agilent InfinityLab Liquid Chromatography/Mass Selective Detector XT (LC/MSD XT) with Agilent OpenLab CDS software. In this simulated sample-purity experiment, a variety of pharmaceutical compounds were stored under refrigeration for four months to facilitate degradation and subsequently analyzed by LC/MS. To determine the sample purity of the degraded samples, the data obtained were processed, reviewed, and reported in a fully automated workflow. Color-coded batch summary views and reporting allow visualization of the purity assessment result and can quickly reveal the degraded samples in a batch.

Introduction

Purity assessment is an essential task in many laboratories. Organic chemists use purity analysis to confirm compound identity and to estimate yield for a synthesis process; medicinal chemists usually assess the purity of the synthesized pharmaceutical compounds before proceeding to biological studies; formulation scientists incorporate purity assessment in formulation optimization, as well as to specify storage recommendations; and pharmaceutical QA/QC labs routinely perform purity assessment to confirm that drug intermediates or final products are within the quality control limits.

LC/MS is used for purity assessment due to its sensitivity, response to wide ranges of compounds, and most importantly, selectivity to differentiate compounds based on the compounds' unique physical chemical property—mass to charge (m/z) ratio. Hence, purity assessment using LC/MS can significantly improve the analytical confidence of the measurements.

To speed decision-making, chemists would prefer to perform purity testing themselves, rather than rely on the supportive analytical services provided by central or contract labs. However, three major challenges have prevented chemists from running their own samples: 1) open access to LC/MS instrumentation, 2) the effort needed to learn how to operate an LC/MS system and interpret MS data, and 3) the laboratory bench space needed to accommodate a mass spectrometer. The solution presented here solves these challenges with an automated workflow built into OpenLab CDS that enables the chemist to quickly obtain purity results without need to interpret MS data. Due to its drastically reduced footprint, the mass selective detector can be stacked with LC modules to fit within a small lab bench space.

This Application Note describes how Agilent OpenLab CDS software can be used with an Agilent InfinityLab Liquid Chromatography/Mass Selective Detector XT (LC/MSD XT) to automate the compound confirmation and sample purity assessment workflow through a simulated storage degradation experiment for several pharmaceutical compounds. With this setup, the chemist can quickly determine the purity of samples.

Experimental

Standards and chemicals

Standards of Buspirone hydrochloride, Amitriptyline hydrochloride, Nefazodone hydrochloride, Clopidogrel hydrogensulfate, Paclitaxel, and Fosinopril sodium were purchased from Sigma-Aldrich (St. Louis, MO). The molecular weights of these compounds ranged from 277 to 853, demonstrating the broad applicability of LC/MS with regard to the size of the compounds.

Individual stock solutions of all standards at 1000 $\mu\text{g/mL}$ concentration were prepared in acetonitrile (ACN). The stock solutions were stored under refrigeration for four months before they were used to prepare working solutions at 20 ng/uL in 20% ACN in water. These working solutions were then analyzed to determine the sample purity after four months of storage.

LC/MS instrumentation and analysis

An Agilent single quadrupole InfinityLab LC/MSD XT system was used for the LC/MS analysis. This system includes an Agilent single quadrupole mass spectrometer equipped with an Electrospray (ESI) source with Agilent Jet Stream technology (AJS), an Agilent 1260 Infinity II Binary Pump, Sampler, and a Diode Array Detector.

A 4-minute high-throughput generic LC separation was performed on a 2.1 x 50 mm Poroshell 120 EC-C18 column, using water and ACN with 0.1% formic acid as the mobile phase. The LC/MSD system was operated in positive ESI mode with full-scan acquisition from 100-900 m/z at a rate of 2 scans per second.

Software

Agilent OpenLab CDS version 2.2 was used for data acquisition, processing, and reporting.

Results and Discussion

Compound confirmation

LC/MS is ideally suited for compound confirmation because, under normal conditions, it produces ions indicative of molecular weight with few fragment ions. When LC/MS analysis is performed in the positive ion mode, a compound of molecular weight M generally produces an ion formed by the addition of a proton $[M+H]^+$. For example, Paclitaxel, a compound with a molecular weight of 853.9, generally produces an ion at 854.3 m/z (protonated version of its most abundant monoisotopic species). Depending on the mobile phase used for LC/MS analysis, $[M+NH_4]^+$ or $[M+Na]^+$ may also be present, producing ions at 871.3 or 876.3 m/z , respectively. In negative ion LC/MS, hydride abstraction produces $[M-H]^-$ ions, with possible Cl^- or acetate adducts.

If the molecular weight is known, extracted ion chromatograms (EICs) can be used to determine whether the expected compound is present. If a single major peak in the EICs overlaps in time with the major peak in the Total Ion Chromatogram (TIC), then the expected compound can be confidently assumed to be present.

Sample purity estimation

Sample purity can be estimated by comparing the area sum of the peak(s) in the EIC(s) or the single peak area in TIC to the area sum of all the other peaks in the TIC. For example, if the EIC peak area is 950,000 counts and the area of all the other peaks in the TIC is 50,000 counts, the sample can be estimated to be 95% pure.

Because ionization efficiency (and thus MS response factor) can vary greatly from compound to compound, many chemists prefer to use a more “universal response” detector—for example a spectroscopic detector such as a UV or DAD—to estimate sample purity. Though UVDs are commonly used, Evaporative

Light Scattering Detectors (ELSDs) may also be used. When configured in series, the same compound eluting from a column will exhibit different retention times in the UVD, ELSD, and MSD chromatograms. Therefore, a signal delay needs to be applied between the MSD and the additional detector being used to calculate sample purity.

A compound's retention time in the MSD EIC is used along with the signal delay to find the compound's peak in the other detector. The area percent of that peak is the measure of sample purity. Figure 1 shows how the purity is calculated for a sample of Paclitaxel that has undergone degradation during storage. Based on the response in the UV signal, the Paclitaxel is still 97% pure.

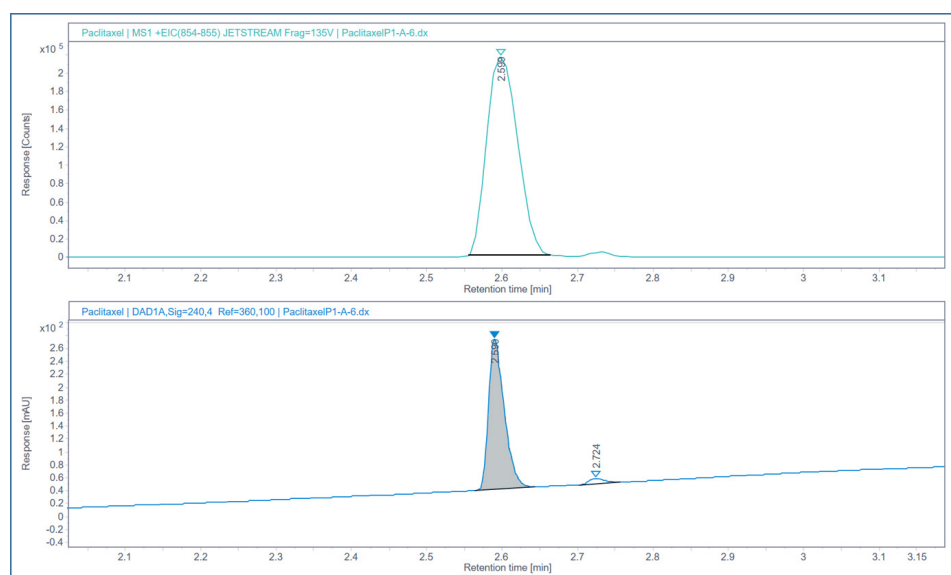


Figure 1. EIC for $[M+H]^+$ and UV chromatogram of Paclitaxel with retention times adjusted for the slight delay in MSD analysis.

Automated data processing and reporting

OpenLab CDS enables chemists with little data system training or experience in MS data interpretation to quickly obtain a report that confirms a compound's identity with an estimate of its purity. When submitting a sample or set of samples, the chemist specifies a molecular weight (most abundant monoisotopic species) or molecular formula for the target compound that the software will use to automatically process the data and generate the report (Figure 2).

The Processing Method window is used to specify the parameters for OpenLab CDS to compute sample purity, including the signal delay, integration parameters, potential adducts, purity cutoff, and the choice of which signal to use for the purity calculation (Figure 3). Though one generic processing method can be used for most sample purity assessments, the chemist has the option to define a different method to carry out their sample purity assessment when using OpenLab CDS. Chemists can also reprocess their data later by entering an updated molecular weight or chemical formula. This is very useful if a mistake was made during the initial data entry.

	State	Vial	Acq. method	Proc. method	Sample name	Target1	Volume
1	<input checked="" type="checkbox"/> Completed	PI-A7	Sample Purity.amx	Sample Purity.pmx	Solvent		3
2	<input checked="" type="checkbox"/> Completed	PI-A1	Sample Purity.amx	Sample Purity.pmx	Buspirone	385.2	2
3	<input checked="" type="checkbox"/> Acquiring	PI-A-2	Sample Purity.amx	Sample Purity.pmx	Amityryptylene	277.2	2
4	<input checked="" type="checkbox"/> Pending	PI-A-3	Sample Purity.amx	Sample Purity.pmx	Clopidogrel	C16H16ClN02S	2
5	<input checked="" type="checkbox"/> Pending	PI-A-4	Sample Purity.amx	Sample Purity.pmx	Nefazodone	C25H32ClN5O2	2
6	<input checked="" type="checkbox"/> Pending	PI-A-5	Sample Purity.amx	Sample Purity.pmx	Fosinopril	563.3	2
7	<input checked="" type="checkbox"/> Pending	PI-A-6	Sample Purity.amx	Sample Purity.pmx	Paclitaxel	853.3	2

Figure 2. Example of the sequence table. The column 'Target1' is where the chemist places either the chemical formula or the molecular weight of the expected compound.

The screenshot shows the 'Processing Method' window with the following settings:

- General:**
 - Calculate sample purity
 - Minimum purity limit: 80.00%
 - Base signal: MS
 - Calculation to use: TIC % (for MS base signal only) EIC/TIC %
- Positive Ions:**
 - Charge carriers:** -electron, +H, +Na, +K, +NH4
- Negative Ions:**
 - Neutral losses:** H2O
- Charge states, if not known:** Min: 1, Max: 1
- Aggregates:** Dimers, Trimers

Figure 3. Processing Method setup for determining sample purity. Here, sample purity will be a TIC-based calculation with an 80% cutoff that considers both the protonated and sodiated adducts.

After sample analysis and data processing, the results for a batch of samples can be reviewed in a summary table (Figure 4), or sample-by-sample to examine the individual EICs, TICs, and UV signals (Figure 5.) Color-coding quickly points to problematic samples. As shown in Figure 4, Buspirone, Amitriptyline, Nefazodone, and Clopidogrel, met the 80% purity cutoff, while Paclitaxel and Fosinopril did not. As shown in Figure 5, reviewing a particular sample provides the exact calculated purity, as well as the mass spectra of all the peaks integrated in the TIC. The mass spectra can provide useful information about the nature of any impurities present.

Sample Purity Results

Order	Sample name	Data file	Overall targets found	Overall purity
1	Solvent	SolventP1-A7.dx	N.A.	N.A.
2	Buspirone	BuspironeP1-A1.dx	Yes	Pure
3	Amitriptyline	AmitriptylineP1-A-2.dx	Yes	Pure
4	Clopidogrel	ClopidogrelP1-A-3.dx	Yes	Pure
5	Nefazodone	NefazodoneP1-A-4.dx	Yes	Pure
6	Fosinopril	FosinoprilP1-A-5.dx	Yes	Impure
7	Paclitaxel	PaclitaxelP1-A-6.dx	Yes	Impure

Figure 4. Sample purity results summary table. Color-coding shows that Paclitaxel and Fosinopril sodium did not meet the specified 80% purity threshold.

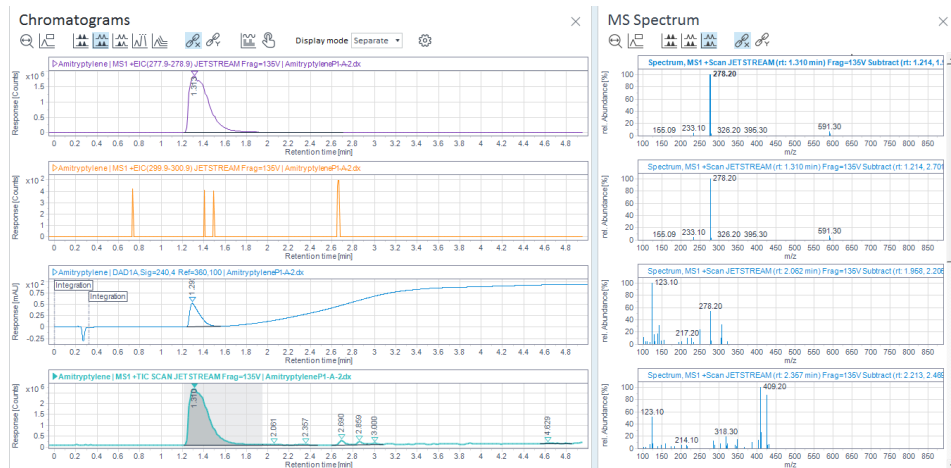


Figure 5. Sample specific data review of Amitriptyline shows the EIC, TIC, UV signal and mass spectra of the peaks integrated in the TIC.

A sample purity report indicating whether the compound was found, along with the sample purity estimate, can be generated using a predefined report template, automatically generated after sample analysis (Figure 6). The use of color in the report makes it easy to determine if the compound was found and whether it met required purity levels.

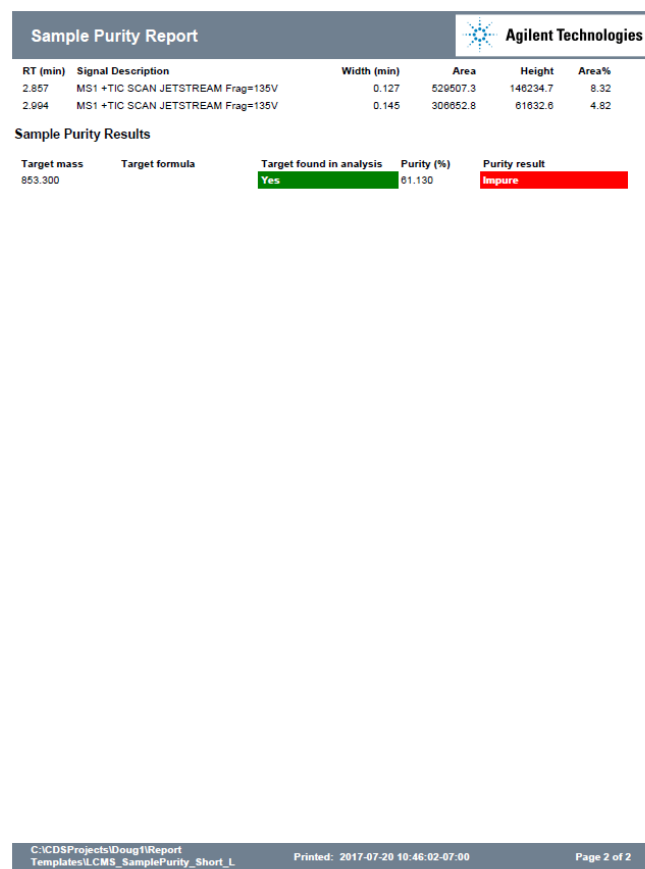
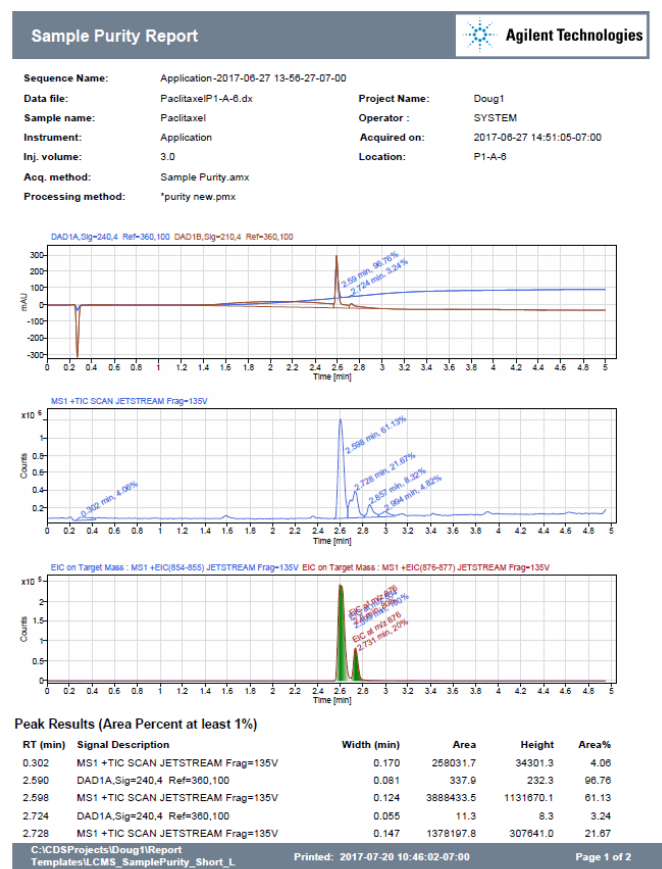


Figure 6. Typical sample purity report. In this case, an 80% purity cutoff based on the TIC signal was specified. Paclitaxel was found (shown in green), but the purity of Paclitaxel in the sample was 61%, and thus below the cutoff (shown in red).

Workflow automation

OpenLab CDS can be used to automate sample purity workflows. As shown in Figure 2, sample information, target masses, and processing method can be easily managed with the sequence table. Report definition is highly customizable to suit different purposes using the built in OpenLab CDS reporting module. The reporting template(s) of choice can be integrated in the processing method for automated report generation and printing, as shown in Figure 7.

Conclusion

OpenLab CDS provides a simple, automated solution for sample purity assessment. Chemists with little training or experience in MS data interpretation can quickly obtain sample purity results without relying on external analytical services, which improves laboratory throughput.

For large sample batches, the sample purity results summary table with color-coding makes it easy to spot impure samples. Sample-by-sample review allows the chemist to inspect individual EICs, TICs, and UV signals, the exact calculated purity, and the mass spectra of all the peaks integrated in the TIC to learn more about any impurities

present. Powerful, flexible OpenLab CDS reporting templates with built-in calculations and color-coded results-flagging summarize the sample purity results (whether the compound was found and estimated purity) in an easy-to-view format. The sample purity report can be automatically generated after sample analysis.

The chemist can easily change the information entered for a sample, for example if they entered the wrong formula. The sample can then be quickly reprocessed without the need to re-inject it. The entire sample purity workflow can be automated within OpenLab CDS, significantly improving the productivity of high-throughput laboratories.

Processing Method

LCMS Sample Purity

- General
- Properties
- Signals
- Extraction
 - Chromatogram
 - Spectrum
- Integration Events ChemStation
 - Standard
 - Advanced
 - Manual Integration
- Compounds
 - Identification
 - Calibration
 - Spectra
- System Suitability
 - Properties
 - Column
- Tools
 - Custom Calculation
- Reports
 - Injection Report

General | Scaling

First report

Report template: LCMS_SamplePurity_Short.rdl [Browse...]

Report destination: None Printer File

File format: PDF (*.pdf) Excel workbook (*.xlsx)
 Word document (*.docx) Plain text (*.txt)
 CSV (Comma delimited) (*.csv)

Copy report to folder: None Storage Windows file system

Destination folder: [] [Browse...]

Second report

Report template: SequenceSummary_Short.rdl [Browse...]

Report destination: None Printer File

File format: PDF (*.pdf) Excel workbook (*.xlsx)
 Word document (*.docx) Plain text (*.txt)
 CSV (Comma delimited) (*.csv)

Copy report to folder: None Storage Windows file system

Destination folder: [] [Browse...]

Figure 7. Report definition for workflow automation.

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