

Tracking Fate and Purge of Impurities and Calculating Carryover

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Introduction

The purpose of process development in pharma is to select and optimize a synthetic route to produce the active pharmaceutical ingredient (API) by the safest, cheapest, fastest, and cleanest (by green chemistry where possible) route, following both Good Laboratory Practice (GLP) and Quality by Design (QbD) principles. As with any synthetic process impurities are generated, and for API development it is mandated by regulatory authorities (e.g., Federal Drug Agency—FDA, European Medicines Agency—EMA, etc.) that impurities are tracked and identified above a certain threshold, while genotoxic and mutagenic impurities must be reported at any level (as stated in the ICH Q7 guideline¹).

Route scouting data, in process development, is typically stored in electronic notebooks. Associated analytical information may be accessible as PDF images stored within an experiment record. Unfortunately, however, from the perspective of entity characterization and review, the analytical data is not dynamically linked with the individual stage(s) of the process route and is unsearchable and inaccessible.

Effective management of API development data, and particularly impurity tracking, is necessary for the development of an optimal impurity control strategy. In order to successfully track the fate and purge of impurities, many scientists currently gather LC/MS and LC area percent values for impurity entities in MS Excel® spreadsheets, in lieu of chemically intelligent software to manage this data. While spreadsheets are effective for handling and managing numerical data—helping formulate and understand relationships through sorting and plotting—they are a weak tool for relating chemical structures with the analytical spectra and chromatograms used to identify and characterize them. For example, Excel is unable to generate an impurity map from the synthetic route or calculate purge factor from the available analytical data.

Here, we discuss Luminata™—software designed to help project teams assemble synthetic routes, associated impurities, and all relevant analytical data for process development in a systematized and searchable manner. Luminata enables effective inter- and intra-departmental collaboration and automatically calculates purge factors directly from LC/MS and LC data. In this document, we focus on two manual and often tedious workflows: process optimization and fate & purge calculations.

Convenient Management of Process Route Information

Luminata² facilitates the import of the whole process route associated with a given dataset, including each synthetic stage. The resulting ‘process map’ enables clear visualization of the impurities at each route phase, and straightforward comparison of molecular composition across reaction steps.

Beyond incorporating good manufacturing practice (GMP) into drug substance production, Luminata also supports evaluation of in-process samples, filtrates, or any other entities to assist with synthesis

optimization. Figure 1 illustrates an example route optimization of sulfabenzamide, where in-process samples from the reaction, filtrate liquors from product isolation, and the final isolated product are documented.

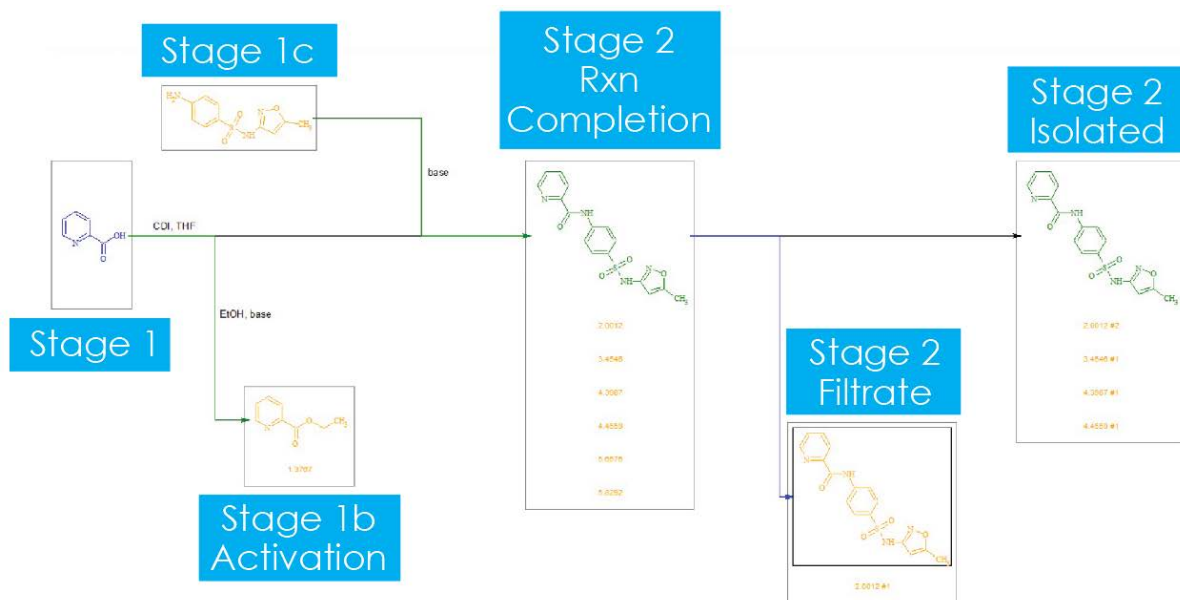


Figure 1 Optimized synthesis of Sulfabenzamide (green, Stage 2) mapped in Luminata. All steps in the reaction are tracked with starting materials (blue), intermediates/products (green), and stage-specific impurities indicated (orange).

Sulfabenzamide is an antibacterial substance that is synthesized through a two-step reaction. Within Luminata, this two-step reaction can be completely documented with all the stages involved. In Stage 1, the process chemist activates the carboxylic acid with Carbonyldiimidazole (CDI) to form the imidazolidine.³ Subsequently, the chemist checks how far the activation has progressed toward completion from Stage 1 via a quench conversion to the methyl ester (Figure 1, Stage 1b—Activation). The next substrate is then added (Figure 1, Stage 1c) and reaction completion tested (Figure 1, Stage 2—Rxn Completion). At this point all known or unknown impurities within the reaction can be sifted. Finally, the analyst proceeds through process work-up (Figure 1, Stage 2—Filtrate) and then purification of the compound (Figure 1, Stage 2—Isolated).

For all these individual stages, corresponding HPLC data can be associated with each step. Thus, the process map represents a powerful tool for the comparison of stages, specifically denoting the proportion of each impurity that is rejected into the respective filtrate, isolated, or reaction completion steps. The software helps to conveniently record and share information about the removal and carry-over of impurities throughout the process route.

Each set of reactions also forms an interactive record. Within a record, analysts can examine the impacts of different conditions (such as temperature or solvents) on process optimization. The analyst can, for example, assess whether altering a given condition will generate more impurities at any/each stage. Most process chemists currently use an electronic notebook (ELN) to store this chemical and analytical information where a vast amount of important data is abstracted into largely unsearchable, PDF documents.

Calculation of Fate & Purge

With all the process route information documented in the software we are now able to focus on Luminata's capability to link chemical information about impurity fate with all the relevant analytical data in turn, enabling dynamic calculation of carryover.

Once the connection of impurities between each stage has been defined by the user (by creating arrows to indicate a conversion or carryover), the corresponding purge factor is automatically calculated from the associated LC/MS data as illustrated in Figure 2.

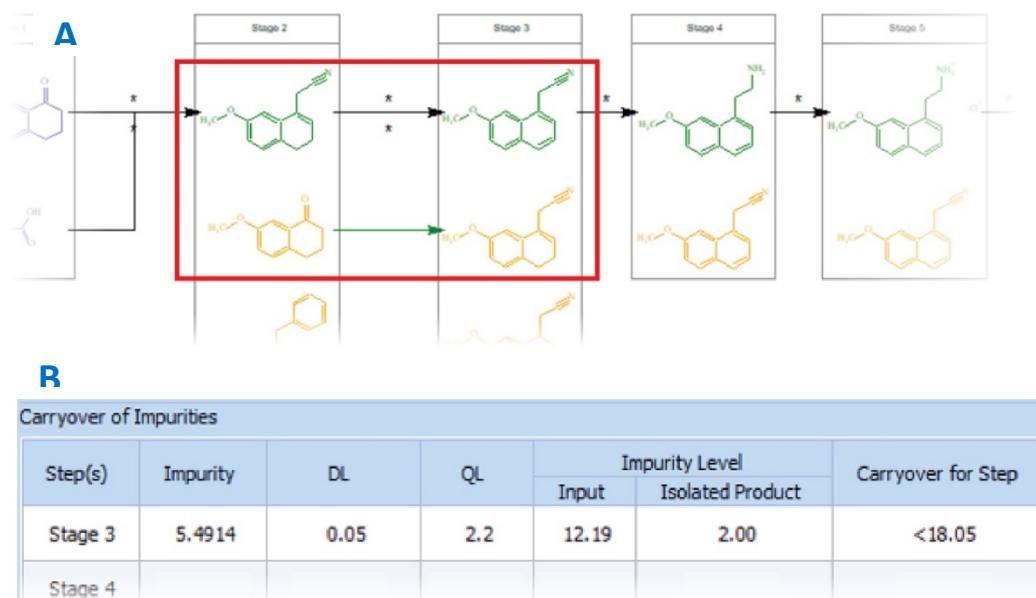


Figure 2 Creation of an arrow indicating conversion of an impurity from stage 2 to stage 3 in the Luminata process map (A) leads to automatic population of the corresponding detection limit (DL) and quantitation limit (QL) in the impurity carryover table (B).

Furthermore, beyond populating the purge factor at each stage, Luminata also automatically calculates a cumulative purge factor value for the entire reaction (Figure 3).

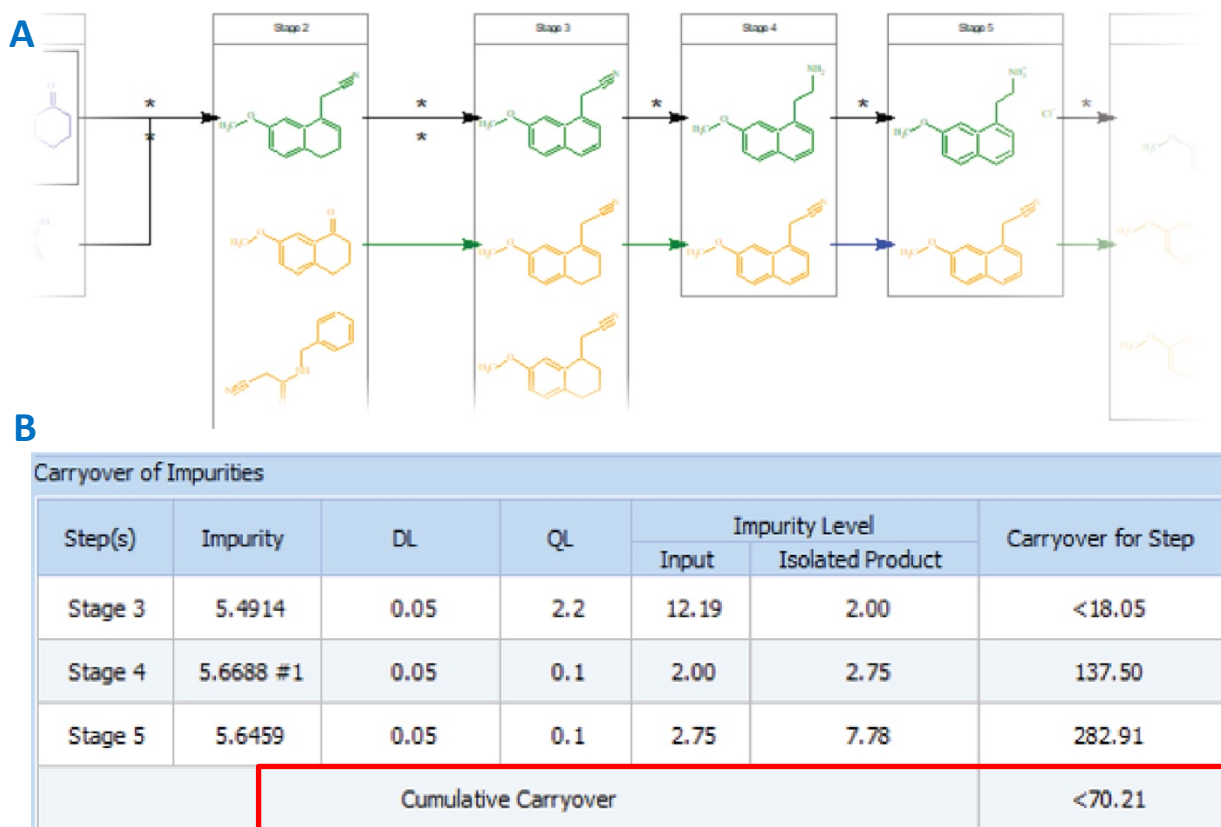


Figure 3 As the reaction pathway for Sulphabenzamide is defined in the Process Map in Luminata (A), the Carryover of Impurities Table (B) populates dynamically where 'DL' and 'QL' represent detection limit and quantitation limit respectively.

Calculating Carryover

Carryover is calculated using 'Area %' values for two consecutive stages:

$$\text{Carryover} = \left(\frac{\text{Area}\%_{\text{stage}(x)}}{\text{Area}\%_{\text{stage}(x-1)}} \right) \times 100$$

Cumulative carryover is calculated using the carryover calculated for each individual step in the route, for example:

$$\text{Cumulative Carryover} = \left(\frac{\text{Carryover}_{\text{stage1} \rightarrow \text{stage2}}}{100} \right) \left(\frac{\text{Carryover}_{\text{stage2} \rightarrow \text{stage3}}}{100} \right) \left(\frac{\text{Carryover}_{\text{stage3} \rightarrow \text{stage4}}}{100} \right) \times 100$$

Detection and quantitation limits (DL and QL, respectively) can be edited at each stage. The detection limit is defined as the lowest concentration of a substance measurable by the analytical instrument.

Quantitation limit represents the lowest detectable concentration of a substance that also meets certain statistical criteria defined by the investigator. For a given experiment these values may be equal, or the QL may be higher than the DL. The software relies on user-defined DL and QL values to calculate carryover. Values falling below these limits are denoted with a '<' to denote the imprecise nature of the calculated result—a practice widely used in the Pharma/Biotech industry.⁴

In addition to calculating cumulative carryover amounts for the fates of particular impurities, the software enables comparison of different batches within a total record set. For example, if an impurity has been spiked into several processes in varying amounts (i.e., 1%, 3%, and 5%) it may not be purged at the same final stage. Luminata allows the user to easily compare all these different spiked records, and create one cumulative carryover table (Figure 4).

Carryover of Impurities in Project "Agomelatine" for Processes "spiked 1%, spiked 2%"									
	Process	Record Identifier	Step(s)	Impurity	DL	QL	Impurity Level		Carryover for Step
							Input	Isolated Product	
<input checked="" type="checkbox"/>	spiked 1%	8019	Stage 3	5.4914	0.09	2.1	12.19	2.00	<17.23
<input checked="" type="checkbox"/>	spiked 1%	8019	Stage 4	5.6688 #1	0.05	0.1	2.00	2.75	137.50
<input checked="" type="checkbox"/>	spiked 2%	8020	Stage 5	5.6459	0.05	0.1	2.75	8.00	290.91
<input type="checkbox"/>	spiked 2%	8020	Stage 3	5.4914	0.05	2.2	12.19	2.00	<18.05
<input type="checkbox"/>	spiked 2%	8020	Stage 4	5.6688 #1	0.05	0.1	2.00	2.75	137.50
<input type="checkbox"/>	spiked 1%	8019	Stage 5	5.6459	0.05	0.1	2.75	7.78	282.91
Cumulative Carryover									68.91

Figure 4. Cumulative carryover table of two records with differing spiked impurity amounts in Luminata.

Purge factors for other impurities within the same record may also be calculated by selecting the impurity of interest (Figure 5).

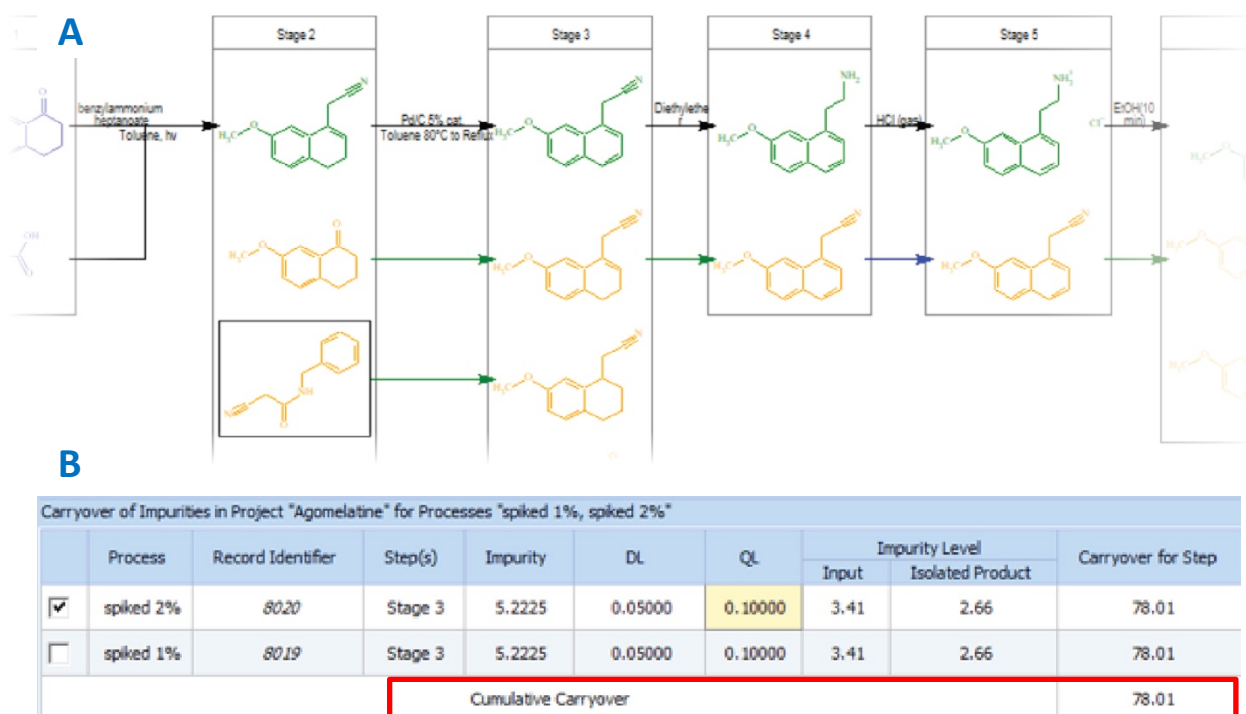


Figure 5 Selection of an impurity entity in Luminata (A) allows the purge factor to be calculated automatically from the associated LC/MS data in the Carryover of Impurities table (B).

Conclusion

Luminata supports effective workflow optimization for process chemists, and enables informed decision-making by automatically calculating quantitative purge factors for process-related impurities using associated analytical data.

References

1. ICH, Q7 Good Manufacturing Practice Guide for Active Pharmaceutical Ingredients (November 2000).
2. Luminata, version 2018.1, Advanced Chemistry Development, Inc., Toronto, ON, Canada, www.acdlabs.com/luminata, 2018.
3. Montalbetti, C.A.G.N.; Falque, V. (2005). Amide bond formation and peptide coupling. *Tetrahedron*, 61: 10827- 10852.
4. Armbruster, D.A.; Pry, T. (2008). Limit of Blank, Limit of Detection and Limit of Quantitation. *Clin. Biochem. Rev.*, 29(Suppl 1): S49–S52.