APPLICATION NOTE

ACD/LABS [ADVANCED CHEMISTRY DEVELOPMENT, INC.]

Tracking Fate and Purge of Impurities and Calculating Carryover

Joe DiMartino
Jesse Harris
Sanjivanjit K. Bahl

Introduction to Fate and Purge and Carryover

The purpose of process development in pharmaceutical research is to select and optimize a synthetic route to produce the active pharmaceutical ingredient (API) by the safest, cheapest, fastest, and cleanest pathway. This method should also follow both Good Laboratory Practice (GLP) and Quality by Design (QbD) principles. As with any synthetic process, impurities are generated. Regulatory authorities such as the Food and Drug Administration (FDA) and the European Medicines Agency (EMA) require that impurities are tracked and identified above a certain threshold. Genotoxic and mutagenic impurities must be reported at any level (as stated in the ICH Q7 guideline[1]).

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, analytical data is not dynamically linked with the process route's individual stage(s). It is unsearchable and inaccessible.

Effective API development data management and impurity tracking are necessary to develop an optimal control strategy. To successfully track the fate and purge of impurities, many scientists gather LC/MS and LC area percent values for impurity entities using Excel® spreadsheets. While spreadsheets are adequate for handling

and managing numerical data, they are a weak tool for relating chemical structures with the analytical spectra and chromatograms used to identify and characterize them. For example, Excel cannot map chemical routes, search for compounds based on molecular structure, or process analytical data.[2]

Here, we discuss Luminata®—software designed to help project teams map synthetic routes, track impurities, and access analytical data for process development in a systematized and searchable manner. Luminata enables effective inter- and intra-departmental collaboration and automatically calculates carryover values directly from LC/MS and LC data. In this document, we describe two workflows that are often tedious and time-consuming without Luminata—process optimization and carryover calculations.

Convenient Management of Process Routes

Luminata 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 a straightforward comparison of molecular composition across reaction steps.

Beyond incorporating good manufacturing practice (GMP) into drug substance production, Luminata also allows users to evaluate in-process samples, filtrates, or 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.

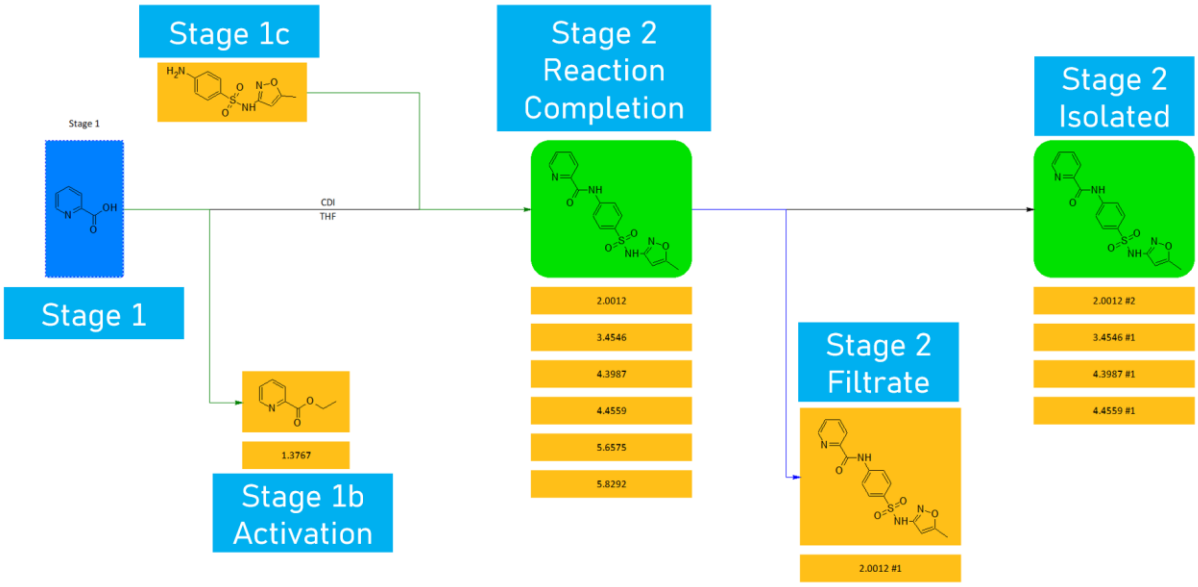


Figure 1. *Optimized synthesis of Sulfabenzamide (green, Stage 2) mapped in Luminata. All steps in the reaction are tracked with starting materials (blue), intermediates and products (green), and stage-specific impurities indicated (orange).*

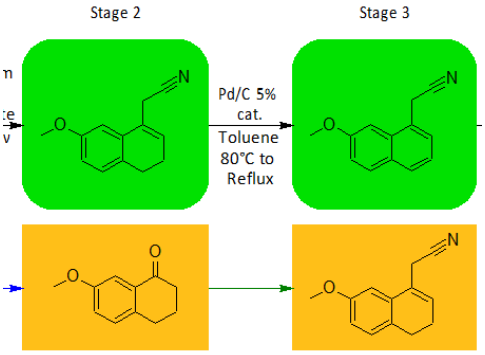
Sulfabenzamide is an antibacterial substance that is synthesized through a two-step reaction. Within Luminata, this two-step reaction can be documented with all the stages involved. In Stage 1, the process chemist activates the carboxylic acid with carbonyldiimidazole (CDI) to form the imidazolide.[3] The chemist then 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 added (Figure 1, Stage 1c), and reaction completion is tested (Figure 1, Stage Reaction Complete). At this point, all known or unknown impurities within the reaction can be separated. 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 is a powerful tool for comparing stages, denoting the proportion of each impurity rejected at each stage. The software helps conveniently record and share information about the removal and carryover of impurities throughout the process.

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. For example, the analyst can assess whether altering a given reaction 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 massive amount of valuable data is hidden in largely unsearchable PDF documents.

Calculating Carryover

In addition to storing development information in one place, Luminata can link chemical information about impurity fate with all the relevant analytical data. This enables 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 carryover value is automatically calculated from the associated LC/MS data, as illustrated in Figure 2.

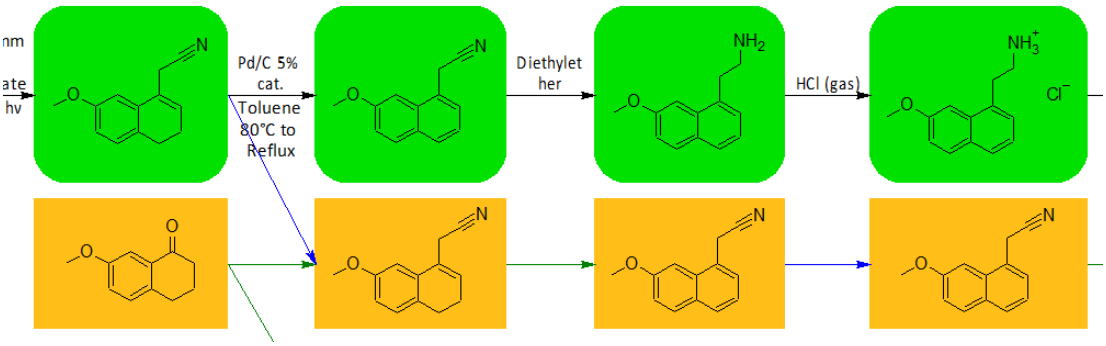


Carryover of Impurities

Step(s)	Impurity	DL	QL	Left		Right		Carryover (%)
				Measured Input Amount	Input Stage Sample ID	Measured Output Amount	Output Stage Sample ID	
Stage 3	Entity 4	0.05	0.1	77.22	Sample 1	85.06	Sample 2	110.15
Stage 2	Entity 3	0.05	0.1	ND	-	77.22	Sample 1	ND
Cumulative Carryover								110.15

Figure 2. *Creating an arrow indicating conversion of an impurity in the Luminata process map leads to automatic population of the corresponding detection limit (DL) and quantitation limit (QL) in the impurity carryover table.*

In addition to calculating the carryover at each stage, Luminata also automatically calculates the cumulative carryover value for the entire reaction (Figure 3).



Carryover of Impurities

Step(s)	Impurity	DL	QL	Left		Right		Carryover (%)
				Measured Input Amount	Input Stage Sample ID	Measured Output Amount	Output Stage Sample ID	
Stage 3	5.4914	0.05	0.1	77.22	Sample 1	2.00	Sample 2	2.59
Stage 4	5.6459	0.05	0.1	2.00	Sample 2	7.78	Sample 3	389.00
Stage 5	5.6459 #1	0.05	0.1	7.78	Sample 3	11.91	Sample 4	153.08
Stage 6	5.8443	0.05	0.1	11.91	Sample 4	ND	-	<0.42
Stage 2	Entity 3	0.05	0.1	ND	-	77.22	Sample 1	ND
Cumulative Carryover								<0.06

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

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

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

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

$$Cumulative\ Carryover = \left(\frac{Carryover_{stage1\rightarrow2}}{100}\right)\left(\frac{Carryover_{stage2\rightarrow3}}{100}\right)\left(\frac{Carryover_{stage3\rightarrow4}}{100}\right) x\ 100$$

Detection and quantitation limits (DL and QL, respectively) can be edited at each stage. The software relies on user-defined DL and QL values to calculate carryover. Values falling below these limits are denoted with a '<' to indicate the imprecise nature of the calculated result—a practice widely used in industry.[4]

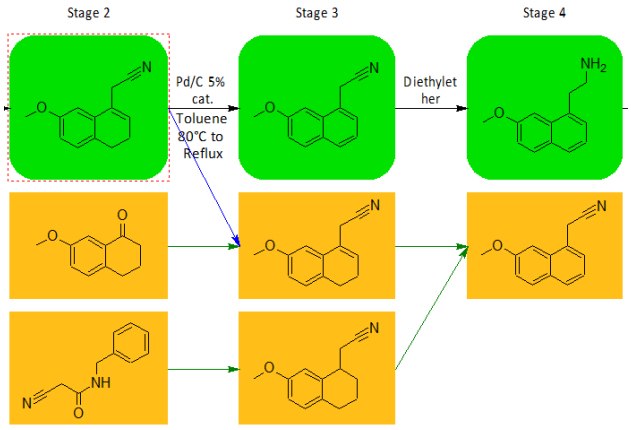
In addition to calculating cumulative carryover amounts for the fates of each impurity, the software enables the comparison of different batches within a complete record set. One use for this functionality is "spike and purge" experiments, where an impurity is spiked into test batches in varying amounts (i.e., 1%, 2%, 3%, 4%, or 5%) to determine if it is purged at the same final stage. Luminata allows users to compare all these different spiked records and create one cumulative carryover table (Figure 4).

Carryover of Impurities in Project "Agomelatine" for Processes "spiked 2%, spiked 3%"

	Process	Record Identifier	Step(s)	Impurity	DL	QL	Left		Right		Carryover (%)
							Measured Input Amount	Input Stage Sample ID	Measured Output Amount	Output Stage Sample ID	
☑	spiked 2%	*9507*	Stage 2	Entity 3	0.05	0.1	ND	-	77.22	Sample 1	ND
☑	spiked 3%	*8363*	Stage 3	5.4914	0.05	0.1	77.22	Sample 1	2.00	Sample 2	2.59
☑	spiked 2%	*9507*	Stage 4	5.6459	0.05	0.1	2.00	Sample 2	7.78	Sample 3	389.00
☑	spiked 3%	*8363*	Stage 5	5.6459 #1	0.05	0.1	7.78	Sample 3	11.91	Sample 4	153.08
☑	spiked 2%	*9507*	Stage 6	5.8443	0.05	0.1	11.91	Sample 4	ND	-	<0.42
☑	spiked 2%	*9507*	Stage 2	Entity 3	0.05	0.1	ND	-	77.22	Sample 1	ND
Cumulative Carryover											<0.06

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

Carryover calculations for other impurities within the same record can also be determined by selecting the impurity of interest (Figure 5).



Carryover of Impurities

Step(s)	Impurity	DL	QL	Left		Right		Carryover (%)
				Measured Input Amount	Input Stage Sample ID	Measured Output Amount	Output Stage Sample ID	
Stage 3	5.2225	0.05	0.1	3.41	Sample 1	2.66	Sample 2	78.01
Stage 4	5.6459	0.05	0.1	2.66	Sample 2	7.78	Sample 3	292.48
Stage 5	5.6459 #1	0.05	0.1	7.78	Sample 3	11.91	Sample 4	153.08
Cumulative Carryover								349.27

Figure 5. *Selection of an impurity in Luminata allows the carryover value to be calculated automatically from the associated LC/MS data in the Carryover of Impurities table.*

Conclusion

Luminata supports effective workflow optimization for process chemists. This enables informed decision-making by automatically calculating quantitative carryover values for process-related impurities using associated analytical data.

References

1. ICH, Q7 Good Manufacturing Practice Guide for Active Pharmaceutical Ingredients (2016). Link

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