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ANALYTICAL CHEMISTRY FOR API PROCESS ENGINEERING

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30.1 INTRODUCTION

The role of the analytical chemist in API process development is critically important in the pharmaceutical industry. The analysis and the analytical data they provide are the "eyes" on the process. Without accurate analytical results, the process would be running blind. Often the process engineer and chemist know what to expect. But without reliable analytical data, it is impossible to know if the processes have quantitatively met expectations.

The level of importance placed on the analytical data highlights how critical it is that the data be sound and truly representative of the process.

Occasionally the analytical results may be confounded with unquantified or unseparated components or simply may be nonrepresentative due to oversight on the part of the chemist, engineer, analyst, or a combination of the three. This breakdown in the quality of the analytical results is traced back to a breakdown in the communication between the parties involved. Information that one or all parties are unaware of can directly impact the quality of analytical results. The entire process team needs to be cognizant of information such as the stability of reaction components, composition of samples (in addition to starting materials, intermediates, and products), and what level of precision is required of the results.

This chapter will deal directly with what a process engineer should know about the analytical data. This includes information around what is required to insure that the data that are produced, be it by an analyst or engineer, is of the highest quality needed for a particular study. Details around what each analytical technique is tracking and what are its limitations, common mistakes that may confound analytical results, and coupling analytical methods to overcome these limitations will all be covered in this chapter. Finally, it will be shown through examples how this level of understanding of the analytical techniques can be leveraged by the engineer to solve the problems of mass balance and estimate kinetic parameters.

High-quality analytical data are paramount if one wishes to accurately know how a process is truly performing. In most cases, certain assumptions are made during the application of the analytical data and understanding the validity of the assumptions is important. Information in this chapter will help the engineer be aware of these typical assumptions and their applicability.

30.2 USE OF ANALYTICAL METHODS APPLIED TO ENGINEERING

Occasionally the analyst and the engineer can feel that the other is speaking different languages. For example, the terms potency and purity are commonly used and can be a source of confusion without clarification around what these numbers mean and how their values were arrived at. Both potency and purity refer to a measure of the active or desired ingredient relative to the sample. The details of how purity and potency are actually determined are important to understand and are the subject of the next section.

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30.2.1 Purity

A strict definition of percent purity would require qualifying what the purity basis is, that is, purity percent by weight or purity percent by HPLC (high pressure/performance liquid chromatography) area at 254 nm. Often in the pharmaceutical industry purity percent by HPLC area is shortened to just purity and when the more rigorous definition is applied, purity percent is stated as purity by wt%.

The term purity typically is based on area percent values alone.

$$\text{Purity} = \left[\frac{\text{Active}_{\text{area}}}{\text{Active}_{\text{area}} + \text{Other}_{\text{area}}}\right] \times 100\% \qquad (30.1)$$

where "$\text{Other}_{\text{area}}$" refers to the peak areas of all the other peaks in the chromatogram. Thus, any impurity is assumed to have the same response factor as that of the main component. The reason area percent purity is reported is one of timing. In early development of a new chemical entity, there are usually no standards. As area percent purity is something that can be reported from the first injection, a meaningful metric can be generated without a lot of work to develop standards. The area percent purity values can be used to compare the historical samples with each other to compare different chemical approaches to the project. Later on in the project when standards have been made and characterized, percent purity by mass values can also be reported. This percent purity by mass relative to the standard (also referred to as potency) taken with the percent purity by area value is a good indicator of how well the standards are characterized. For the remainder of this chapter, the percent purity by area will be referred to as just purity.

30.2.2 Potency

$$\text{Potency of A}^{\text{s}} = \left[\frac{\text{Mass}_{\text{A}}^{\text{S}}}{\text{Mass}_{\text{total}}^{\text{S}}}\right] \times 100\% \qquad (30.2)$$

where

$$\text{Mass}_{\text{A}}^{\text{S}} = \text{Area}_{\text{A}}^{\text{S}} R_{\text{f}_{\text{A}}} \qquad (30.3)$$

and

$$R_{\text{f}_{\text{A}}} = \frac{\text{Mass}_{\text{A}}^{\text{STD}}}{\text{Area}_{\text{A}}^{\text{STD}}} \qquad (30.4)$$

Here, S denotes sample, STD denotes standard, and A denotes material A.

The term potency in equation 30.2 is a bit deceiving at first glance as it appears that when samples are reported at a given percent potency, this is a percent by mass intrinsic to the test sample. This is not the case. Actually, this value is a percent active compared to an external standard as shown in equation 30.3 through the use of a proportionality constant called a response factor designated by R_{f}. This response factor is generated from a reference standard as shown in equation 30.4 by taking the ratio of the response, HPLC area in this case, to the mass of the sample. The reference standard is typically a well-characterized purified sample of the desired material used to calibrate HPLC peak area to mass of the sample. In most cases in the pharmaceutical industry, the reference standard is not commercially available and has to be purified through crystallization or preparative-scale chromatography.

Response factor can be simplified as the ratio of the output response to the input material and is used in most analytical techniques with linear responses such as mid-IR spectroscopy, mass spectroscopy, or in this case UV spectroscopy. After close inspection of equations , it becomes apparent that the accuracy of the potency value hinges on the quality of the standard used to generate the response factor. As all reported values are relative to the standard, it is possible to have a test result indicating a potency greater than 100%, showing the sample is more potent than the standard.

Early in development, this standard may be nothing more than the most pure obtained sample to date. The limited characterization of the standard consists of analysis for residual solvents including water and residue on ignition testing (ash). Anything that is not ash or solvent is then attributed to the material of interest. So to reiterate, potency refers to the % active component in a given sample relative to an external reference standard for that active component.

EXAMPLE 30.1

(A) An isolated sample is submitted for the typical purity and potency analysis. The results reported were the following:

Purity: 99.5%
Potency: 97.3%

What do these results tell us about the sample?

Solution

A typical mass balance for the reference standard is the following:

$$\text{Mass}_{\text{total}}^{\#} = \text{Mass}_{\text{A}}^{\#} + \text{Mass}_{\text{residual solvent}}^{\#} + \text{Mass}_{\text{ASH}}^{\#} + \text{Mass}_{\text{impurities}}^{\#} \qquad (30.5)$$

Where the superscript # can be either STD or S if the mass balance is for the standard or the sample, respectively. The term $\text{Mass}_{\text{A}}^{\#}$ refers to the mass of the desired compound of interest in the standard or the sample. Writing equation 30.5 in terms of the sample and solving for $\text{Mass}_{\text{A}}^{\#}$ results in equation 30.6.

$$\text{Mass}_{\text{A}}^{\text{S}} = \text{Mass}_{\text{total}}^{\text{S}} - \text{Mass}_{\text{residual solvent}}^{\text{S}} - \text{Mass}_{\text{ASH}}^{\text{S}} - \text{Mass}_{\text{impurities}}^{\text{S}} \quad (30.6)$$

Substituting equation 30.6 into equation 30.2 results in the following equation:

$$\text{Potency of A}^{\text{S}} = \left[\frac{\text{Mass}_{\text{total}}^{\text{S}} - \text{Mass}_{\text{residual solvent}}^{\text{S}} - \text{Mass}_{\text{ASH}}^{\text{S}} - \text{Mass}_{\text{impurities}}^{\text{S}}}{\text{Mass}_{\text{total}}^{\text{S}}}\right] \times 100\% = 97.3\% \quad (30.7)$$

From equation 30.7, it become apparent that the terms for residual solvent, ash, and impurities are why the potency is less than 100%. At first glance, it would be easy to assume that because the purity value is 99.5%, the $\text{Mass}_{\text{impurities}}^{\text{S}}$ term is low. Remember though that the percent purity is actually percent purity by area percent. If there are any impurities that have a drastically larger response factor than the desired material, then they will be underreported. At best, the purity value of 99.5% infers what are the dominate terms that are reducing the potency of the sample. Submitting the sample for further analysis for residual solvents and ash is the only way to identify if the missing 2.7% is due to residual solvents and ash or underreported impurities.

(B) Consider the example where the magnitudes of purity and potency are reversed; that is, the sample has a higher potency than purity:

Purity: 95.3%

Potency: 103.2%

What does this tell us about the product and, more importantly, about the standard?

Solution

The potency value of 103.2% means that the sample is more potent than the standard. Because potency is a relative activity of a sample compared to the standard, it is possible to have values greater than 100%. What this indicates is that the mass balance around the standard is not fully closed. From equation 30.5, the mass of the active material in the standard is determined by difference, so

$$\text{Mass}_{\text{A}}^{\text{STD}} = \text{Mass}_{\text{total}}^{\text{STD}} - \text{Mass}_{\text{residual solvent}}^{\text{STD}} - \text{Mass}_{\text{ASH}}^{\text{STD}} - \text{Mass}_{\text{impurities}}^{\text{STD}} \quad (30.8)$$

Substituting equations 30.3, 30.4 and 30.8 into equation 30.2 results in the following equation:

$$\text{Potency of A}^{\text{S}} = \left[\text{Area}_{\text{A}}^{\text{S}} \frac{\text{Mass}_{\text{total}}^{\text{STD}} - \text{Mass}_{\text{residual solvent}}^{\text{STD}} - \text{Mass}_{\text{ASH}}^{\text{STD}} - \text{Mass}_{\text{impurities}}^{\text{STD}}}{\text{Area}_{\text{A}}^{\text{STD}}} / \text{Mass}_{\text{total}}^{\text{S}}\right] = 103.2\% \quad (30.9)$$

If the areas and total mass of both the sample and the standard are accurate, then from equation 30.8 to have potency greater than 100% the only way is that the characterization of the reference standard around ash, solvent, or impurities is off. The source of the failure to close the mass balance of the standard is most likely due to the impurities not being fully characterized, as residual solvent and ash are standard analysis. If the purity of the standard (UV area percent) was near 100%, then there may be impurities that are not showing up at the wavelength that the detector is set at, or they may not be UV active. In such a case, further purification of the standard by chromatography or recrystallization is needed to better close the mass balance of the standard and gain an accurate R_{f}.

To further complicate the issue, a sample purity value of 95.3% indicates that the R_{f} is too high due to a poorly characterized standard, and the samples' total impurities of 4.7% indicate that some of these impurities have higher molar absorption coefficient relative to the desired and thus will appear to be present in higher concentration. The assumption with area percent values is that everything has the same R_{f} as the main peak. If any of the impurities have a lower R_{f} than the main peak, then the impurities will be overreported by the area percent value. The various scenarios discussed above have been summarized in Table 30.1:

For comparing processes with each other based solely on isolated yield and relative potency, a less than fully characterized standard still allows relative comparison; that is, 103% potent material is better than 95% potent material. For work that would require a more stringent mass balance, kinetics, or process understanding, the mass balance should be closed by utilizing a combination of complementary analytical techniques such as quantitative H^1 NMR and HPLC. Two areas where analytical data are most frequently needed by the API process development engineer are data to close the mass balance and data to develop kinetic models. These two utilizations of the data are not independent of each other, as it is necessary to have a reasonable mass balance before attempting to develop a kinetic model. As such, it is imperative to have analytical techniques available that can both "see" what needs to be tracked and give values of concentrations that are needed for both the mass balance and the kinetic model.

30.3 METHODS USED AND BACKGROUND

What follows is a brief overview of the most common analytical techniques used and some concepts that need to

TABLE 30.1 Possible Scenarios of Purity and Potency Values

	Purity: $\text{purity(area\%)} = \frac{(A_{\text{area}})}{(\sum \text{UVactive}_{\text{area}})} \times 100$	Potency: $\text{potency(wt\%)} = \frac{(A_{\text{area}})(R_{\text{fA}})}{(\text{Sample}_{\text{mass}})} \times 100$
Purity = potency	Can occur if the response factors of all the UV active components including impurities are very similar so that an area percent of A is equivalent to a wt%. It also requires that the reference standard for the potency determination be highly accurate	
Purity < potency	If we assume the reference standard is accurate, then this situation can arise if the impurity peaks have a higher extinction coefficient and higher absorbance than the desired component A. This translates to artificially high impurity count and lower purity by area percent	
Purity > potency	If we assume the reference standard is accurate, then purity will exceed potency when there are non-UV components present that are not being detected by HPLC. This will contribute to lower potency values and higher HPLC area percent—for example, if the sample has high salt content (ash). This will look pure by HPLC because the ash is not detected	
Potency > 100%	Reference standard likely not well characterized with respect to wt% ash, residual solvent, or impurities	

be kept in mind when attempting to analysis the data generated. A more through discussion about each technique can be found elsewhere [1].

All methods of column chromatography rely on the same basic principles. First, there is a sample that is made up of a mixture of components. This mixture is loaded onto a column that separates the individual components as they partition between two phases, the mobile and stationary phases. In liquid chromatography (LC), the partitioning is driven by the polarity of the components and the differing polarity of the mobile phase versus the stationary phase, absorbing and deabsorbing onto the stationary phase down the length of the column. In gas chromatography, the partitioning is driven by the relative volatility of the components as it alternates between the gas phase and dissolution into the stationary phase. The net effect of any chromatographic system is to separate the components of the sample mixture. It is the detector attached to the outlet of the chromatographic system that allows one to see the relative concentrations of each species in the sample. As such, the type of detector used will dictate what is "seen" by the analytical method. Table 30.2 lists the types of detectors available, what type of chromatographic system they are most often paired with, and what they are capable of detecting.

The underling similarity in all these methods of detection excluding FID is that the resulting signal is proportional to the concentration. The important thing to remember is that for every component of a sample that is being analyzed, there is a proportionality constant that is unique to that compound. So in the example of the UV detector, the most common detector for different LC methods, this proportionality constant is the molar absorptivity ε. The relationship of ε to concentration and absorption is described by Beer–Lambert law as shown in the following equation:

$$A = \varepsilon bc \tag{30.10}$$

where A is the absorption (dimensionless), ε is the molar absorptivity (L/(mol cm)), c is the concentration (mol/L), and b is the detector path length (cm).

In the case of mass spectrum detectors (MS), the proportionality constant is the ionization potential, and in electrochemical detection, it is the redox potential. Even in the case of nonchromatographic methods, the idea of proportionality constants should always be remembered. As an example, quantitative NMR has relaxation times that can be thought of as proportionality factors. So for every sample analyzed, be it with chromatography or not, the individual components will have a unique proportionality constant that may or may not be similar to other components in that sample. This is why most detectors are not universal detectors; all species that are chemically different will have different proportionality constants. In many instances, if the components are all structurally similar, then their proportionality constants may be very similar as well, but this is not always the case. This is why taking area percent values as direct replacements for concentration can lead to erroneous results. At best, these area percent values can be used to indicate relative abundances, but care around the possibility of different response factors must be taken if area percent values are used as replacements for concentration values for calculating mass balances or kinetic profiles. The following example illustrates this point.

EXAMPLE 30.2 RESPONSE FACTORS VERSUS AREA PERCENT

A high-temperature coupling reaction was evaluated with potassium hydroxide in a high boiling solvent. Initial reaction completion HPLC looked promising with apparent conversion of >80% although long reaction times were required (Figure 30.1). However, the isolated yields were

TABLE 30.2 Properties of Most Common Detector Types [2]

	Chromatography Method	Detection	Sensitivity	Notes
Ultraviolet (UV)	LC	Absorption of UV light by pi–pi bonds, that is, conjugation	Dependent on ε of the analyte	Variation of ε on the order of 100- to 1000-fold is possible
Mass spectrometer (MS)	LC/GC	Charged particles	μM concentrations	Does not detect mass but rather mass to charge (m/z) ratio
Flame ionization detector (FID)	GC	Ionized particles from combustion of organic compounds	ppm–ppb	Signal proportional to the number of carbon atoms, that is, signal proportional to mass not concentration
Conductivity	LC	Charged ions by measuring resistance in detection cell	5×10^{-9} g/mL	Most often used for ion exchange chromatography
Electrochemical	LC	Current generated by oxidation or reduction of sample	Order of magnitude more sensitive than UV	More selective and sensitive than UV, but detector not as rugged as UV
Refractive index (RI)	LC	Variations in refractive index	0.1×10^{-7} g/mL	Universal detector, poor detection limit, and sensitivity to external condition (temperature, dissolved gas, etc.) limit practical use
Evaporative light scattering detector (ELSD)	LC	Nonvolatile particles of analyte scattering light	0.1×10^{-7} g/mL	Analyte needs to be nonvolatile whereas mobile phase needs to be volatile
Florescence	LC		Low ng/mL range	Typically require derivatization with fluorophore reagents

low (<50%), but this was attributed to a laborious workup. The workup involved two extractions followed by distillation and then crystallization. An extensive amount of time was spent trying to optimize the reaction with an eye toward fixing the workup and increasing isolated yield after the reaction was optimized.

The "conversion" was calculated in the lab as:

$$\text{Conversion} = \frac{\text{Area\% product}}{(\text{area\% starting material} + \text{area\% product})} \tag{30.11}$$

There was some concern around this approach, but the argument against pulling samples for quantitative HPLC was that the reaction was very thick and heterogeneous, making it hard to sample representatively. Provide a solution to the approach.

Solution

To get accurate quantitative HPLC data and potency values, the sampling limitation was avoided by not sampling. A reaction was run and the quantitative HPLC sample made by using the entire reaction in a volumetric flask. By doing this, there would be no sampling error as the entire reaction would be used.

In this instance, the quantitative HPLC conversion was calculated as

$$\text{Conversion} = \frac{\text{moles product}}{(\text{moles starting material at } T_0)} \tag{30.12}$$

where moles of product are calculated as

$$\text{moles product} = \text{Area}_{\text{product}} \times R_{\text{f}}^{\text{mole}}{}_{\text{product}} \tag{30.13}$$

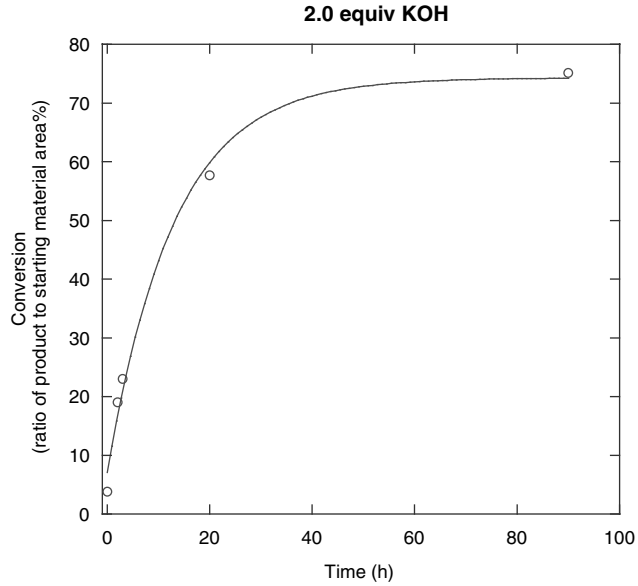


FIGURE 30.1 Conversion as calculated by the chemist in the lab by equation 30.11 only taking the ratio of starting material and product area percents into account.

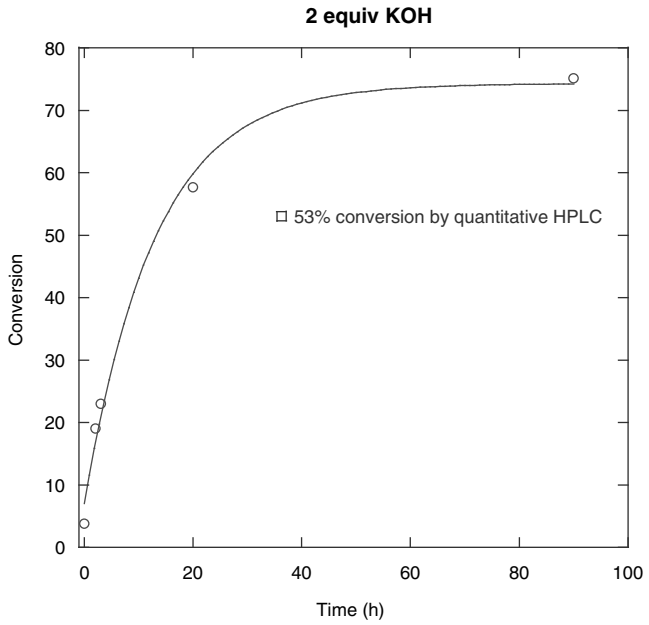


FIGURE 30.2 Conversion calculated by equation 30.11 (solid line) compared with conversion calculated by equation 30.12 (empty box). This discrepancy between the two values at the 48 h time point indicates that the forced mass balance of equation 30.11 elevated the product concentration by not taking into account a possible side reaction of the starting material that did not result in product.

R_f^{mole} is calculated from equation 30.4 on a mole basis.

Quantitative HPLC showed there to be much less product after 45 h than originally assumed (Figure 30.2). Low yield was not due to product loss in workup, but rather was never formed to begin with. The starting material was reacting/degrading to something other than product. Forcing the mass balance on area percent (between starting material and product), it appeared higher than it actually was. Quantization resulted in the decision to discontinue further development on these conditions.

30.3.1 Mechanics of HPLC and UPLC

HPLC and the more recent UPLC (ultrapressure/performance liquid chromatography) are considered the standard lab equipment when it comes to understanding what is going on in a synthesis or process. The difference between these two techniques lies in the size of the solid phase packing in the columns as well as the pressures that are employed, and hence the high/ultra descriptors. For HPLC, the solid-phase packing is between 5 and 3 μm and 200–400 bar pressure, whereas for UPLC, solid phase is below 3 μm and pressures above 1000 bar.

A quick aside about the equation that governs the efficacy of both techniques, as well as any other column chromatography, the van Deemter equation in its simplified version (equation 30.14) [3].

$$H = A + \frac{B}{u} + Cu \tag{30.14}$$

where H (sometimes shown as HETP) is the variance per unit length, also referred to as height equivalent to a theoretical plate; u is the volumetric flow rate; A is the term describing the multipaths in the packed bed; B is the term describing longitudinal diffusion; and C is the term describing resistance to mass transfer.

This hyperbolic function relates the variance per unit length to particle size, mass transfer between the stationary and mobile phases, and the linear velocity of the mobile phase. This relationship was the first result from applying rate theory to the chromatography process and was originally developed to describe gas chromatography. It has been extended to describe liquid chromatography as well with modifications to the lumped parameters terms A, B, and C in equation 30.14. A typical van Deemter plot is shown in Figure 30.3.

The van Deemter equation is useful in describing the theory and mechanism of the chromatography process, not only for the small analytical chromatography used in analysis, but also for large-scale separations done on large pilot plant and commercial scale. This equation explains why the problem of unresolved peaks cannot be solved by just going to a longer column at the same flow rate. The increased resolving power of more packing in a longer column is lost to the increase of the B term in equation 30.14 (increase of eddy and longitudinal diffusion) due to increased time spent on the column. Thus, the number of theoretical plates is less for the longer column (when held at the same flow rate) even though it is longer with more packing because the height of the plates, the H term in equation 30.14, is larger as well. This is where UPLC comes into its own. By decreasing the packing size and increasing the pressure, the linear velocity is kept high and the increased resolving power of more packing is

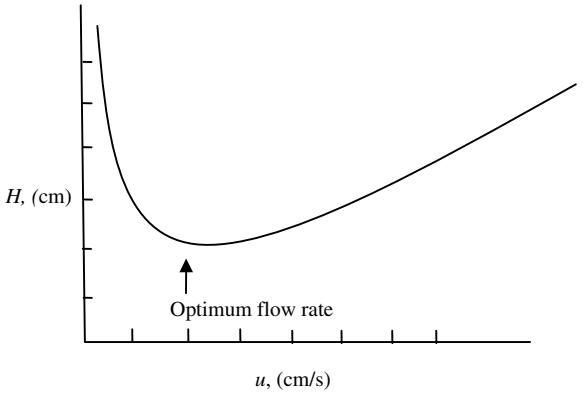


FIGURE 30.3 Characteristic van Deemter plot shape illustrating the presence of an optimum flow rate to maximize column efficiency.

not lost to increased diffusion, thus giving higher plate counts for a given time on the column. The net result is increased resolution and throughput for the analyses.

Both methods, HPLC and UPLC, are only a tool for separating individual components from a mixture and feeding them to a detector will give a response that is proportional to concentration. It is concentration data that are typically the most applicable to the engineer and their accuracy is of foremost importance.

30.4 THINGS TO WATCH OUT FOR IN LC AND GC

30.4.1 Injections have Everything in Them, Not Just the Desired Reactants

The first thing that must be communicated to the analyst, or kept in mind for those who are acquiring their own data, is to account for what is in the reaction mixture. The most common mistake in LC that everyone makes once and hopefully only once is the toluene mistake. This is what happens when people forget that toluene, unlike most organic solvents, has a chromophore and is retained on most LC columns. I cannot tell you how many bright analytical chemists have come running down in a panic telling everyone that there is this major new impurity only to find out that the project has switched to toluene in the process and had not notified the analyst. Worse yet are the chemists who report that they have excellent *in situ* yield only to be looking at a nonexistent reaction because they assumed that large peak that was not starting material was product when in reality it was the toluene peak. This can lead to wasted development time chasing a nonexistent reaction.

In GC, the major concern is nonvolatiles, that is, salts. If a lot of reaction mixtures are injected that contain a large percentage of salts, then the injector may become plugged, necessitating the cleaning of the injector before accurate analysis can resume. What is more complicated is when the product or reactants are salts, that is, charged species. These will not "fly" on the GC and will require some sort of quench to run on the GC. Most often this is a neutralization of the reaction mixture to quench the charge on the desired compounds so as to facilitate GC analysis.

30.4.2 Unplanned/Planned Modification of Stationary Phase

If running one's own analysis, one must be cognizant of possible changes to the HPLC column due to history. Depending on the nature of the mobile phase being used, "conditioning" of the column may take place such that the results may not be repeatable or representative of differing HPLC systems. This opens the possibility of analysis that cannot be duplicated, leading to confusion around what results are accurate. A prime example of conditioning of the column is ion-pairing mobile phase, such as sodium dodecyl sulfate, or a weak ion-paring agent such as perchloric acid. In the case of ion-pairing mobile phases, the stationary phase is modified or conditioned over time to be more retentive of polar species such as primary amines due to the stationary phase being modified by the mobile phase containing the ion-pairing agents. There is a memory effect now for this column that will still maintain the effect even if the mobile phase is switched to a more traditional acidic mobile phase. This has the greatest impact when someone develops a method with such a conditioned column as it will be impossible to replicate these results without this preconditioned column.

The other extreme is when the column is conditioned negatively or destroyed by running samples of reaction mixtures that destroy the resolving power of the stationary phase. This is most often seen with samples from reactions such as hydrogenations that contain metal species that bind to the stationary phase resulting in reduced resolving power. If a column is suspected of being conditioned either negatively or positively, then the only option available is to replace the column and see if the previous analysis is replicated. Thus, it is always good to periodically run a system suitability test to check analytics with a reference mixture to confirm retention times/peak shapes.

30.4.3 Product Stability/Compatibility with Analysis Method

Stability of the reaction mixture or products to the chromatographic conditions is another major concern that needs to be addressed before a strategy for analyses can be agreed upon. The majority of aqueous mobile phases utilized in UPLC and HPLC are acidic. This regularity of acid mobile phases is due to two major factors. The first factor is that until recently the silicon support for the column mobile phase was not stable to high pH values as silicon is soluble at pH levels above 11. The second factor is that if the mobile phase pH is near the pK_a of any of the sample components, then slight variations in the pH of the mobile phase can change the polarity of the components. This change in polarity will then change retention time and order of elution of the components.

Because of these two factors, nearly 80% of the mobile phases are acidic (pH < 1) to both maintain the stability of the mobile phase and prevent any change in the analysis due to pH variations. The idea is to protonate everything and prevent pH gradients from forming on the column that may cause chromatographic artifacts.

This proclivity of mobile phases to be acidic makes stability to acid aqueous conditions one of the main stability concerns. As the amount of sample that will be loaded on the column for each injection is so miniscule, on the order of microliters, the sample gets swamped by the mobile phase. If the sample is not stable to aqueous/acid conditions, then

degradation will be taking place as the sample travels and elutes from the column. The net effect is that the sample that was representative of the reaction at a given time or point in the process is scrambled by the analysis method, rendering the results no longer representative. This is unfortunate if one lab-scale reaction is lost because of this, devastating if three weeks of DOE experimentation is rendered useless because of this, and both have happened.

In GC, the major issue with stability of the reaction mixture is that of thermal stability. Remembering that the standard injector temperature for GC is 280°C, this is the temperature that the reaction samples have to "endure" just to get on the column.

If stability is a problem in LC or GC, then quenching the reaction samples (if this improves stability) or some sort of derivatization method may be required.

30.4.4 Derivatization

Derivatization is the process by which a reaction sample is further reacted to form a new compound as part of the sample preparation. This may be done for various reasons such as increasing reactant stability to the analysis method, modifying the components of a sample to make them detectable such as attaching a chromophore, or increasing volatility for GC analysis [4]. The major issue with derivatization is that this is a second reaction that is in series with the desired reaction. The net effect of this is that if the reaction of interest is to be accurately characterized, then the derivatization reaction needs to be quantitative in reaction completion and at a reaction rate that is orders of magnitude faster than the desired so as not to skew the analysis. For a system that a kinetic model is being developed and derivatization is required, then a quench of the reaction should be used before derivatization, or a derivatization that also quenches the reaction. This is to insure that the analysis is representative of the time the sample was taken, not the time the sample was analyzed.

30.5 USE OF MULTIPLE ANALYTICAL TECHNIQUES

Oftentimes when trying to understand a process by developing both a mass balance and a kinetic model, it is best to start at the beginning. In most cases, the beginning is a full characterization of the feedstocks going into the process. It will be very difficult to close the mass balance if one is not aware of what is going into the process. The use of two or more complementary analytical techniques can greatly aid in fully understanding the inputs for a process. The following Case Study 1 illustrates this point.

30.5.1 Case Study 1: Mass Balance Around Starting Materials to Develop a Kinetic Model

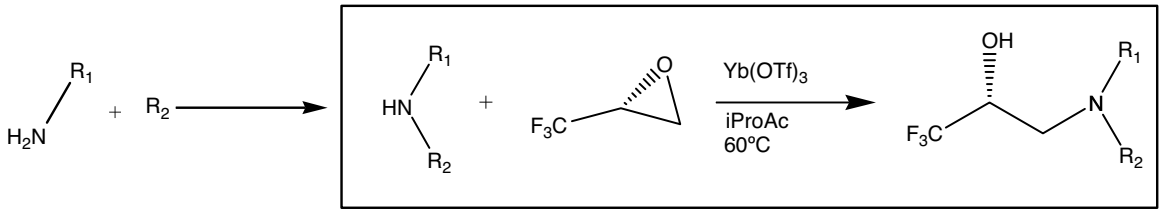
A process involves coupling secondary aniline with a volatile chiral epoxide utilizing an ytterbium catalyst in isopropyl acetate at 60°C. The secondary aniline was synthesized from the primary aniline as shown in Scheme 30.1:

The secondary aniline was telescoped into the reaction with the chiral epoxide with some residual primary aniline present. The observation was that when the process to coupling the secondary aniline and epoxide was first scaled up in a kilo-scale facility, a second charge of the epoxide reagent was required to drive the reaction to completion. The reaction had been run in a sealed reactor so as to limit losses of the epoxide with its very high vapor pressure. Owing to the sealed reactor configuration, the time for the reaction to complete was believed to be 16 h but had not been confirmed as reaction completion samples were not taken during this initial 16 h over fears of venting the epoxide during sampling. In addition, the incoming secondary aniline reagent was in a solution of isopropyl acetate that was known to have residual primary aniline from the first reaction. The question was what the impact of residual primary aniline was on the desired reaction of the secondary aniline with the epoxide.

It was decided to undertake a kinetic study of this reaction to identify the answers to the following questions:

1. How long does the reaction take?
2. Why did the first scale-up require the second charge of epoxide to drive the reaction to completion?
3. What is the impact of residual primary aniline?

The first step to developing a kinetic model was to fully characterize the two incoming reagent streams. The



Scheme 30.1 The desired reaction of the secondary aniline (the reaction in the box) that is synthesized from the primary aniline.

aniline reagent stream had an unknown potency as standards were not available for quantitative HPLC. The epoxide reagent had a certificate of analysis (COA) from the vendor, but its potency would be reevaluated to confirm these numbers.

To gain a handle on the composition of the aniline reagent stream, a H^1 NMR was taken that resolved the isopropyl acetate from the aniline compounds. Figure 30.4 shows the H^1 NMR with the peaks assigned to the structure. From this figure, it becomes apparent that there was not enough resolution between the primary and secondary aniline compounds with NMR to decouple their individual concentrations. Using the HPLC area percent, the concentration of the different anilines was calculated.

The assumption in this approach was that the only other components in the stream besides the secondary aniline were the isopropyl acetate and the primary aniline. The second assumption was that the NMR relaxation times for all the components were on the same timescale. The third assumption was that the response factors for the two anilines were similar enough to be able to use the area percent values directly. The calculation for the composition of the starting material is shown below:

From Figure 30.4, the ratio of isopropyl acetate to aniline compounds

isopropyl acetate $\frac{71.1}{3H} = 23.7$

where H is the proton

aniline compounds $\frac{28.9}{3H} = 9.63$

The values 23.7 and 9.63 represent the relative number of moles of IPAC and aniline compounds. So the mol% isopropyl acetate is easily calculated

$$\frac{23.7}{23.7 + 9.63} = 71.1\%$$

and the mol% aniline compounds is

$$\frac{9.63}{23.7 + 9.63} = 28.2\%$$

The step of taking the ratio of the area to the number of protons in this case is redundant as both peaks in the NMR being compared are for methyl groups (three protons), but this is an important step that can often be missed.

The area percent values from the HPLC in Figure 30.5 were used to decouple the concentration of aniline compounds.

Secondary aniline 84.6%

Primary aniline 15.4%

So on a mol% the composition is

$0.846 \times 0.282 = 0.239 \times 100 = 23.9\%$ secondary aniline

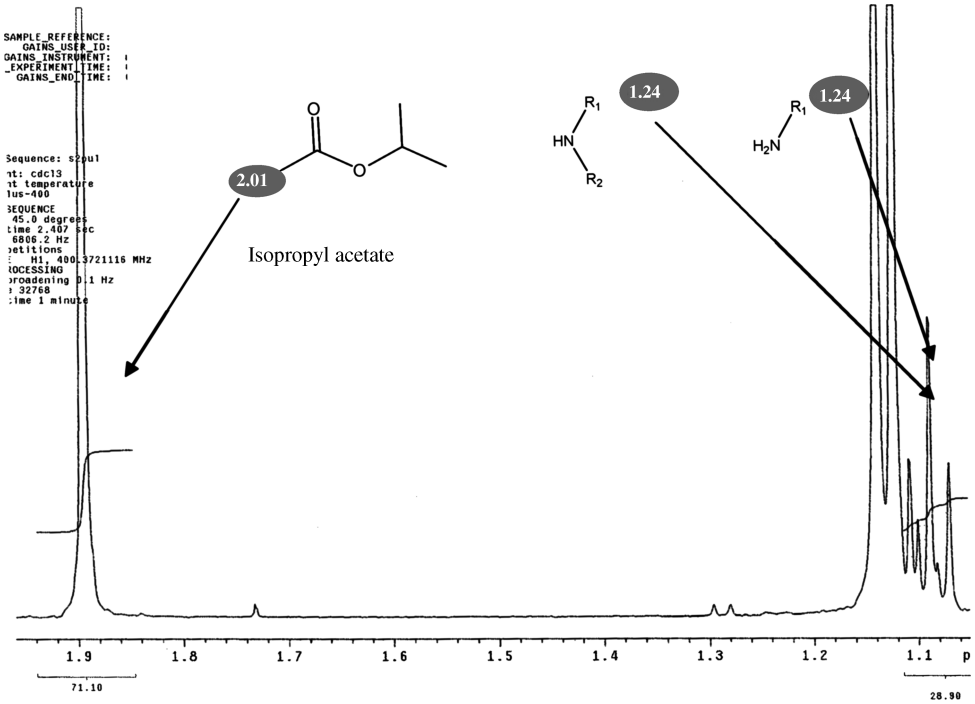


FIGURE 30.4 The NMR scan of the secondary aniline solution with the methyl group protons integrated for both the primary and secondary aniline compounds, as well as the methyl protons for the isopropyl acetate.

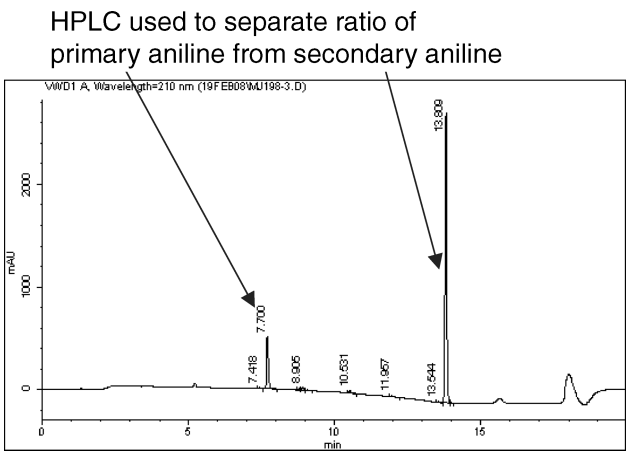


FIGURE 30.5 HLPC of the secondary aniline starting solution showing the primary aniline present at 15% by area.

$$0.154 \times 0.282 = 0.043 \times 100 = 4.3\% \text{ primary aniline}$$

Using molecular weights and assuming a basis of 1 mol

$$0.711 \text{ mol} \times 102.13 \text{ g/mol} = 72.61 \text{ g isopropyl acetate}$$

$$0.239 \text{ mol} \times 453.86 \text{ g/mol} = 108.47 \text{ g secondary aniline}$$

$$0.043 \text{ mol} \times 247.72 \text{ g/mol} = 10.65 \text{ g primary aniline}$$

$$72.61 \text{ g} + 108.47 \text{ g} + 10.65 \text{ g} = 191.73 \text{ g total}$$

Weight percent

$$\frac{72.61 \text{ g}}{191.73 \text{ g}} \times 100 = 37.87 \text{ wt\% isopropyl acetate}$$

$$\frac{108.47 \text{ g}}{191.73 \text{ g}} \times 100 = 56.57 \text{ wt\% secondary aniline}$$

$$\frac{10.65 \text{ g}}{191.73 \text{ g}} \times 100 = 5.55 \text{ wt\% primary aniline}$$

This characterizes the incoming aniline reagent stream, and now the same process is repeated with the epoxide reagent stream. By the certificate of analysis from the vender the epoxide, which is a liquid, it is known to have methyl *tert*-butyl ether present at 12% by weight. As this compound cannot be detected with HPLC with a UV detector, H^1 NMR was again used to characterize the material as shown in Figure 30.6. Taking the area of the MTBE compared to the area of the epoxide peaks, we are able to calculate the mol%

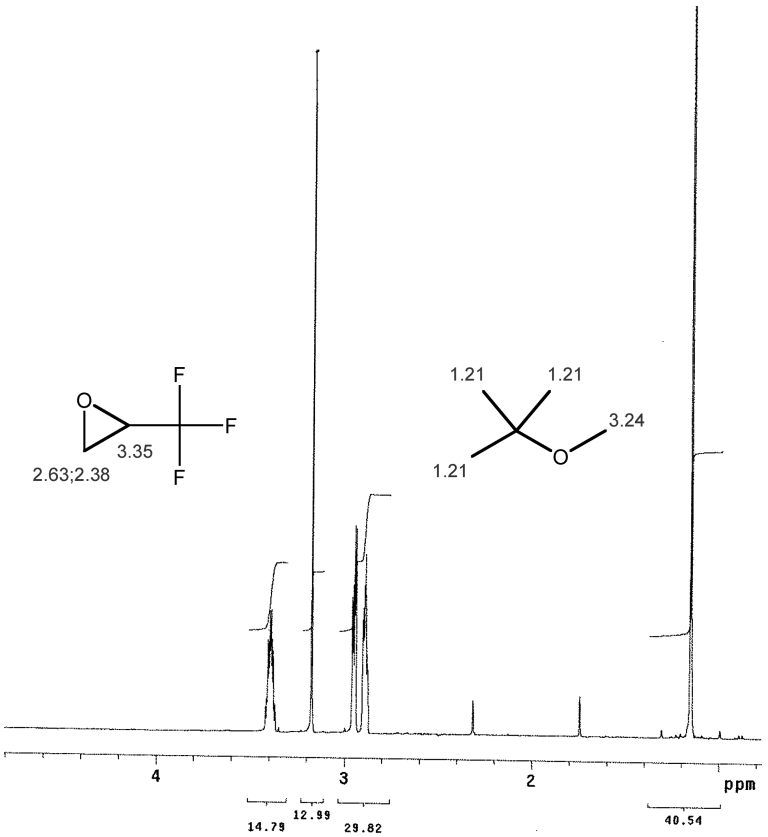


FIGURE 30.6 H^1 NMR of epoxide starting material with three separate peaks, each representing one proton, while the two singlets at 1.21 ppm (nine protons) and 3.24 ppm (three protons) indicate the presence of MTBE.

of each as the following:

$$\frac{12.99 + 40.54}{12\text{H}} = \frac{4.46\%}{\text{H}}$$

$$\frac{14.79 + 29.82}{3\text{H}} = \frac{14.87\%}{\text{H}}$$

$$\frac{14.87}{4.46 + 14.87} \times 100 = 76.93\% \text{ epoxide}$$

$$\frac{4.46}{4.46 + 14.87} \times 100 = 23.07\% \text{ MTBE}$$

Using molecular weights and assuming 1 mol total solution to convert to weight percent

$$0.7693 \text{ mol} \times 112.05 \text{ g/mol} = 86.20 \text{ g}$$

$$0.2307 \text{ mol} \times 88.15 \text{ g/mol} = 20.34 \text{ g}$$

$$20.34 \text{ g} + 86.20 \text{ g} = 106.54 \text{ g total}$$

Weight percent

$$\frac{86.20 \text{ g}}{106.54 \text{ g}} \times 100 = 80.91\% \text{ epoxide}$$

$$\frac{20.34 \text{ g}}{106.54 \text{ g}} \times 100 = 19.09\% \text{ MTBE}$$

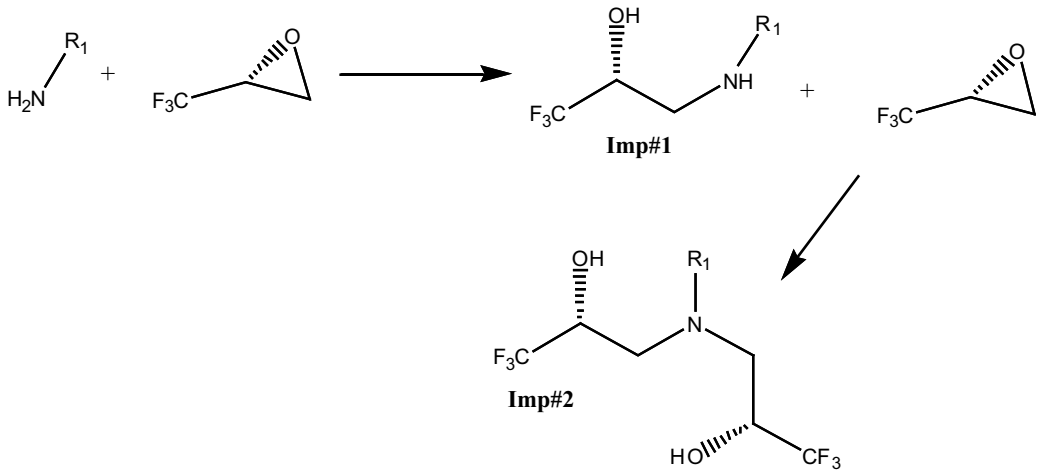
Remembering that from the COA at the time the epoxide was received, it was 12 wt% MTBE, but due to the volatile nature of the epoxide, every time the container was opened, it had been concentrating the MTBE to nearly 20% by evaporation of the epoxide. This is most likely why when it was first scaled up, an additional amount of epoxide had to be charged as the first charge was effectively an undercharge due to lower than expected potency of the epoxide.

With these characterized reagent streams, a kinetic model could now be developed for the system. Reactions were set up in small septum-capped vials at three temperatures and two catalyst loadings. The septum caps allowed sampling of the reaction mixtures without venting the epoxide. The HPLC of these IPC samples showed the fate of the aniline species when reacted with the chiral epoxide. Over time, the secondary aniline reacts with the epoxide as the desired reaction, but so does the primary aniline according to Scheme 30.2:.

The primary aniline reacts with the epoxide to form impurity 1 that then reacts with a second mole of the epoxide to form impurity 2. This reaction progression is shown in the HPLC traces in Figure 30.7. The shoulder peak on impurity 2 is in fact the diastereomer that is formed when the second chiral epoxide is added. Standard reverse phase HPLC column packing is not capable of separating enantiomers but can separate diastereomers.

To calculate the concentration over time, the area percent values from the HPLC were used. Each reaction system (Schemes 30.1 and 30.2) had the area percent values normalized only for that system. The normalized values were then used to calculate the concentration at that time point by multiplying by the initial starting concentrations. To illustrate this process, Table 30.3 lists the area and normalized values for each reaction system for the 60°C reaction using the standard catalyst loading.

The data from Table 30.3 were then transformed into concentration data by multiplying the normalized area values for each reaction system with the starting concentrations. The secondary aniline starting concentration of 0.797 M was used for the desired reaction system of Scheme 30.1 and the concentration of the primary aniline of 0.145 M for the



Scheme 30.2 Undesired reaction pathway for primary aniline with epoxide.

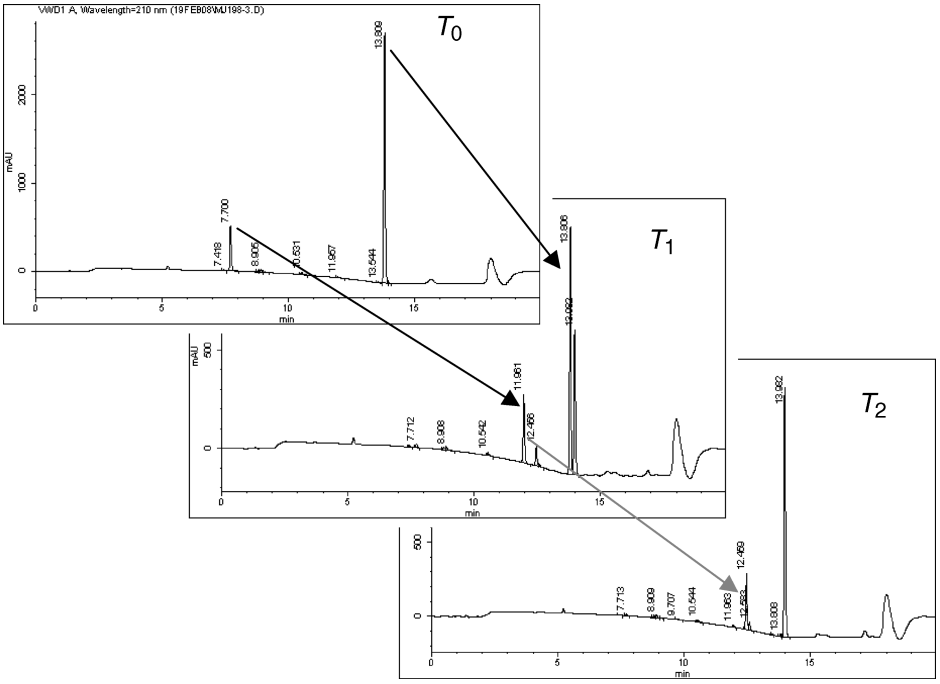


FIGURE 30.7 HPLCs over time showing both desired and undesired reactions. The desired reaction is secondary aniline at 13.8 min going to product at 13.9 min, while the undesired reaction is primary aniline at 7.7 min going to impurity 1 at 11.9 min, which further reacts to impurity 2 at 12.4 min.

TABLE 30.3 HPLC Area and Normalized to Each Reaction System

Time (s)	Secondary Aniline Area	Desired Product Area	Secondary Aniline Area	IM1 Area	IM2 Area	Normalized Area for System of Scheme 30.1		Normalized Area for System of Scheme 30.2		
0	11763.66	0.00	2138.86	0.00	0.00	1.00	0.00	1.00	0.00	0.00
720	7701.32	755.84	636.18	1281.50	40.90	0.91	0.09	0.32	0.65	0.02
4680	3884.43	4631.85	23.46	1202.17	864.00	0.46	0.54	0.01	0.58	0.41
9420	1771.80	6652.65	15.72	639.57	1432.61	0.21	0.79	0.01	0.31	0.69
16380	619.75	7895.18	25.21	289.47	1830.73	0.07	0.93	0.01	0.13	0.85
19380	292.27	6106.68	25.17	160.02	1451.73	0.05	0.95	0.02	0.10	0.89
78240	15.82	6365.85	0.00	0.00	1626.31	0.00	1.00	0.00	0.00	1.00

TABLE 30.4 Concentrations Versus Time for All Components in Schemes 30.1 and 30.2

Time (s)	Secondary Aniline (mol/L)	Desired Product (mol/L)	Secondary Aniline (mol/L)	Imp#1 (mol/L)	Imp#2 (mol/L)
0	7.97E − 01	0.00E + 00	1.45E−01	0.00E + 00	0.00E + 00
720	7.25E − 01	7.12E − 02	4.71E − 02	9.48E − 02	3.02E − 03
4680	3.63E − 01	4.33E − 01	1.63E − 03	8.33E − 02	5.99E − 02
9420	1.68E − 01	6.29E − 01	1.09E − 03	4.44E − 02	9.94E − 02
16,380	5.80E − 02	7.39E − 01	1.70E − 03	1.95E − 02	1.24E − 01
19,380	3.64E − 02	7.60E − 01	2.23E − 03	1.42E − 02	1.28E − 01
78,240	1.97E − 03	7.95E − 01	0.00E + 00	0.00E + 00	1.45E − 01

undesired reaction system of Scheme 30.2. This resulted in the concentration versus time data as shown in Table 30.4.

With this understanding of the reactions involved, the temperature-dependent kinetic model could be developed using DynoChem software as shown in equation 30.15.

$$\mathrm{Yb}(OTf)_3 + \text{secondary aniline} + \text{epoxide} \xrightarrow{k_2} \text{product} + \mathrm{Yb}(OTf)_3$$

$$\mathrm{Yb}(OTf)_3 + \text{primary aniline} + \text{epoxide} \xrightarrow{k_2} \text{imp 1} + \mathrm{Yb}(OTf)_3$$

$$\mathrm{Yb}(OTf)_3 + \text{imp 1} + \text{epoxide} \xrightarrow{k_3} \text{imp 2} + \mathrm{Yb}(OTf)_3 \tag{30.15}$$

where the temperature-dependant rate constants $k_{\#}$ are defined as follows:

$$k = k_{\mathrm{ref}} \times \exp^{-E_a R}(1/T - 1/T_{\mathrm{ref}}) \tag{30.16}$$

The fit in DynoChem resulted in the values given in Table 30.5.

TABLE 30.5 The Output Values for the Kinetic Model with the Confidence Interval (CI)

	Final Value	Units	CI (%)
$k_{1\mathrm{ref}}$	0.0031	$L^2/(mol^2\,s)$	11.157
$k_{2\mathrm{ref}}$	0.0173	$L^2/(mol^2\,s)$	7.745
$k_{1\mathrm{ref}}$	0.0029	$L^2/(mol^2\,s)$	9.346
E_{a1}	59.554	kJ/mol	10.953
E_{a2}	57.586	kJ/mol	7.521
E_{a3}	56.99	kJ/mol	9.932

Equation 30.15 with the values from Table 30.5 results in the predicted versus actual plots of reaction progression as shown in Figure 30.8. The first reaction of the primary aniline with the epoxide was found to have a rate constant ($k_{2\mathrm{ref}}$) that was an order of magnitude faster than the desired reaction rate constant ($k_{1\mathrm{ref}}$), further explaining why additional epoxide was needed to consume all the secondary aniline starting materials.

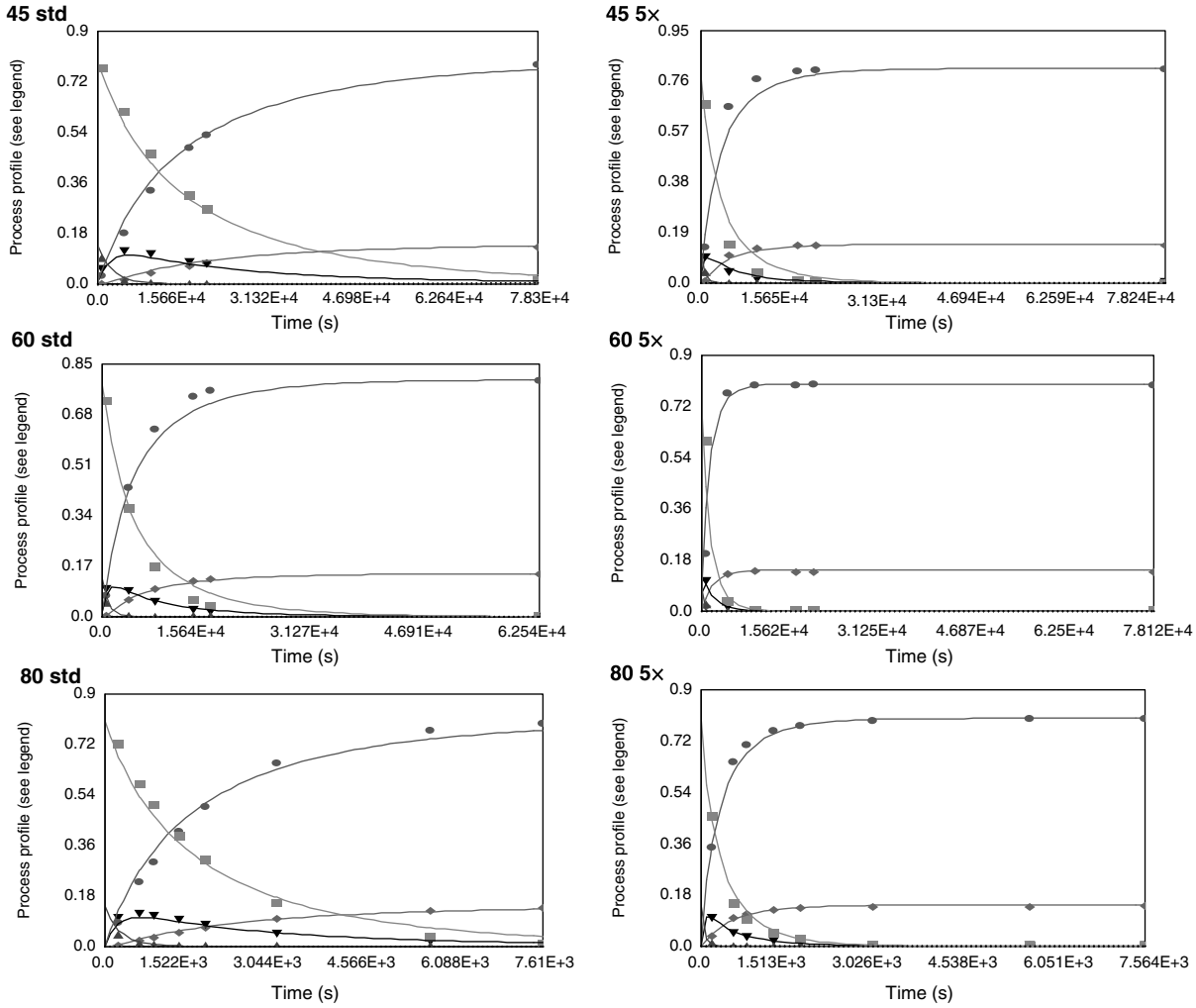


FIGURE 30.8 Predicted versus actual values for reaction progression from kinetic model.

From Case Study 1, we see that using the complementary analytical techniques of NMR and HPLC-UV, it was possible to fully characterize the starting materials. Once this was done, the area percent values were used to calculate the concentration over time that was then used to develop the kinetic model that gave the necessary process understanding. The assumption in this case was that all the species in a reacting system, that is, secondary aniline to the desired product for one system and primary aniline to impurity 1 onto impurity 2 for the other reactive system, had the same response factor and that area percent could be used without response factors. This is a reasonable assumption to make as the epoxide that was being added to the molecules did not have a chromophore and the electronics of the UV chromophore between starting materials and products were not changing much; that is, the responds of primary aniline differs slightly from that of impurity 1 and 2, as did the responds of secondary aniline to the desired product. But what if this assumption about starting materials and products having the same response cannot be made, what is the course of action? This problem is explored in Case Study 2.

30.5.2 Case Study 2: Process Understanding for Development of Continuous Process

Two reactions in series need to have CSTR reactors sized for a given annual throughput. To do this reactor sizing, absolute reaction rates as a function of temperature are needed. These reaction rates have to track the impurity levels throughout the process, not only the desired reaction, so as to arrive at an optimum reactor configuration. In the first reaction, referred to as reaction A, "feed" reacts with the "starting material" forming "product" and a series of impurities. This system is further complicated as the reagent "feed" can exist as two different tautomers, with only one of which is reactive. The six reactions in this system are shown in equation 30.17.

$$\begin{aligned}
&\text{"Feed"} + \text{TEA} = \text{"Feed"}^* + \text{TEA}\\
&(\text{Feed and Feed}^* \text{ are tautomers})\\
&\text{Starting material} + \text{"Feed"}^* \rightarrow \text{Product}\\
&\text{"Feed"}^* + \text{TEA} \rightarrow \text{decomp}\\
&\text{Product} + \text{"Feed"}^* \rightarrow \text{ImpA}\\
&\text{ImpA} \rightarrow \text{ImpB}\\
&\text{Product} + \text{H}_2\text{O} \rightarrow \text{Hydrolysis product}
\end{aligned} \tag{30.17}$$

In this case, HPLC data were available for comparison with external standard response factors to arrive at wt% of each species. In order to get these wt% data, all reaction samples were made up as quantitative samples, that is, mg/mg reaction in samples in volumetric flasks.

With these data it is now possible to close the mass balance around the incoming limiting reagent "starting material." This mass balance at time t was calculated by comparing to initial starting material (SM_0) with equation 30.18.

$$\frac{SM_t + prod_t + impA_t + impB_t + Hydrolysis_{prod_t}}{SM_0 * 100} = \%\text{measured\_mass\_balance} \tag{30.18}$$

Calculations using equation 30.18 were performed for every sample taken from the reaction over the course of the reaction to give the mass balance. Mass balance for step A around the starting material including the impurities that are being tracked is shown in Figure 30.9. This mass balance illustrates that for three reactions at three different temperatures and over the course of each reaction, the mass balance fluctuates near 100%. In this case, the mass balance is not being forced to 100% by taking ratios but is calculated from external standards. What we can conclude from this data is that there is not an unaccounted for reaction as this would cause a systematic drain on the system as a function of temperature or over the course of the reaction. The variability of the mass balance around the 100% point is most likely due to variability in sampling.

The data can be smoothed by reprocessing with relative molar response factors, resulting in smoothed data that has had the sampling error removed. This is done by setting one of the compounds, usually the starting material or the product, as the reference and having a relative response factor of one. The other compounds then have their response factors calculated as a fraction of this reference response factor by equation 30.19.

$$R_{\text{f comp}}/R_{\text{f product}} = R_{\text{f relative}} \tag{30.19}$$

The relative response factors can now be used to adjust the area values of individual components. These area values are then summed and used to calculate new response corrected

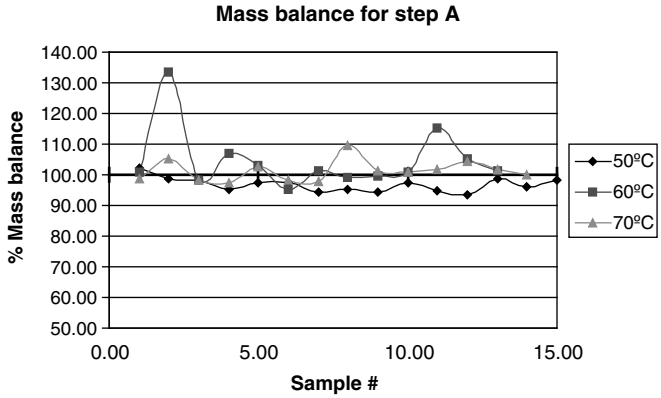


FIGURE 30.9 Mass balance from quantitative HPLC over the course of the reaction at three temperatures.

TABLE 30.6 Relative Response Factors Used to Smooth the Data by Converting Area into Fraction T_0 Concentration

Component	A	B	C	D	E
Relative response factor	1	0.4415	0.2628	0.4173	1.9011
Area	980,582.93	138,507.25	118,161.23	1125.98	2110.25
RRF corrected area	980,582.93	313,730.71	449,665.07	2698.10	1110.04
Fraction of $[T_0]$	0.755	0.242	0.314	0.002	0.001

area percent values. These area percent values are then used in conjunction with the starting material concentration at time zero to calculate individual component concentrations.

An example of these calculations for one time point are shown below for the reaction where B and C are reacted together, with C in excess to give product A and impurities D and E shown in Table 30.6.

The fraction of T_0 concentration is calculated by taking the relative response factor corrected areas summed together resulting in 174,7786.85 total area counts. The subtle point now is to make sure that the reagent in excess is not double counted. So product and impurities fraction of T_0 concentration values are calculated as shown in equation 30.20:

$$\frac{\text{Area}_{\text{A,B,D,E}}}{(\text{Area}_{\text{total}} - \text{Area}_{\text{C}})} = \text{Fraction\_}T_0 \qquad (30.20)$$

where the reagent in excess, reagent C, is calculated by equation 30.21:

$$\frac{\text{Area}_{\text{C}}}{(\text{Area}_{\text{total}} - \text{Area}_{\text{B}})} = \text{Fraction\_}T_0 \qquad (30.21)$$

These fractions of T_0 values can now be multiplied by the T_0 concentrations (limiting reagent concentration for all but the reagent in excess, which is multiplied by its T_0 concentration). The results of these calculations are shown in Table 30.7.

The profiles of the starting material and the product calculated with external standards versus calculated with relative response factors are shown in Figure 30.10. These smoothed data were then used to develop the kinetic model with DynoChem software package. The model versus predicted data from this model is shown in Figure 30.11.

TABLE 30.7 Final Conversion of T_0 Concentration to Concentration at this Time Point

Component	A	B	C	D	E
$[T_0]$	0	0.512	0.603	0	0
Fraction of $[T_0]$	0.755	0.242	0.314	0.002	0.001
Concentration at this time point	3.869E − 01	1.238E − 01	1.890E − 01	1.065E − 03	4.380E − 04

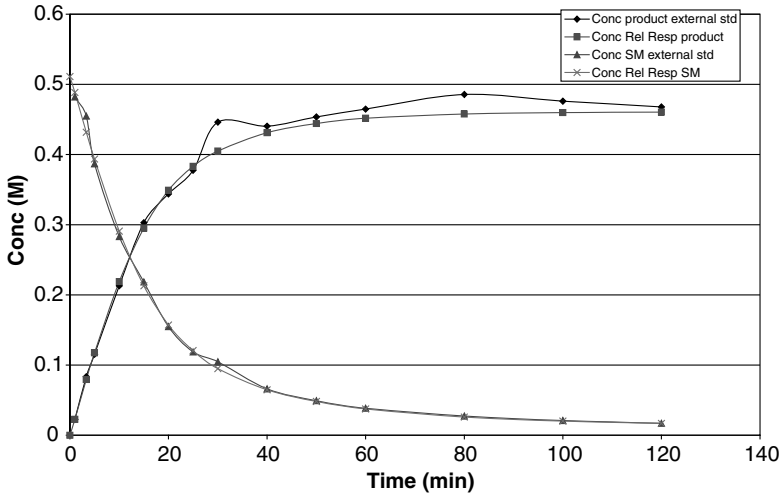


FIGURE 30.10 Starting material and product profiles comparing external standards and relative responds factors. The effect of smoothing the data and indicating where possible errors in sampling may have occurred is relatively straightforward once displayed graphically.

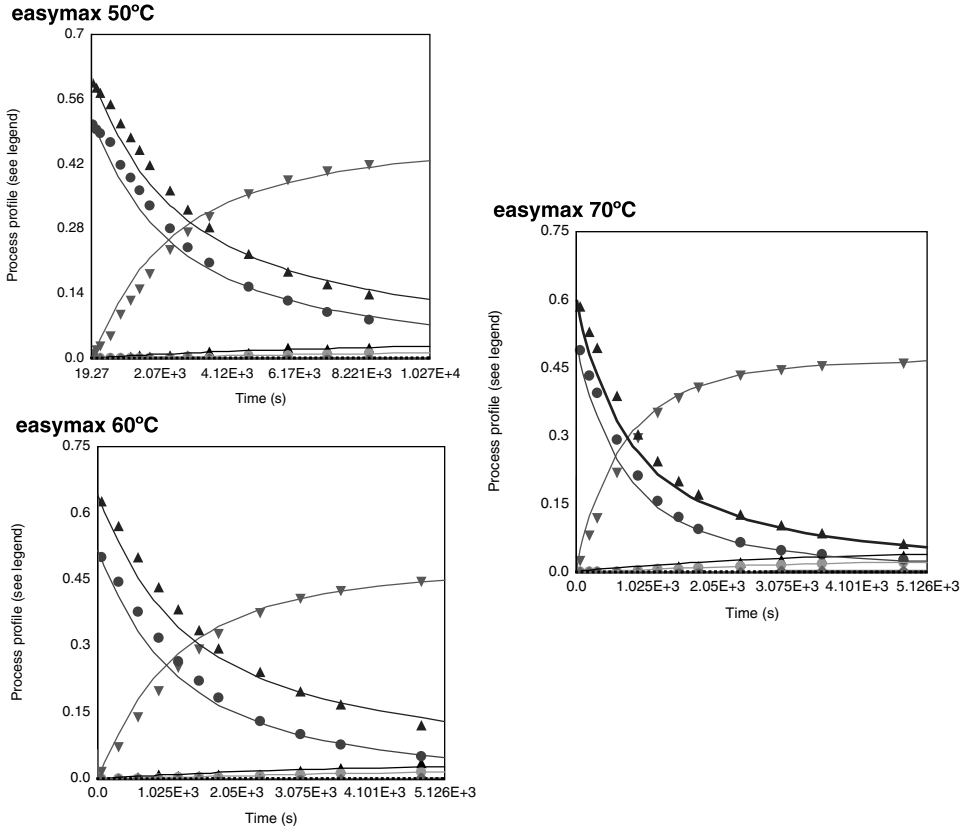


FIGURE 30.11 Kinetic model predicted versus actual concentrations at three different temperatures.

The model for step A was then validated by comparing the model to a semi-batch reaction done in a Mettler 0.5L RC-1 reactor in which the data were not calculated with relative response factors. These data are shown in Figure 30.12.

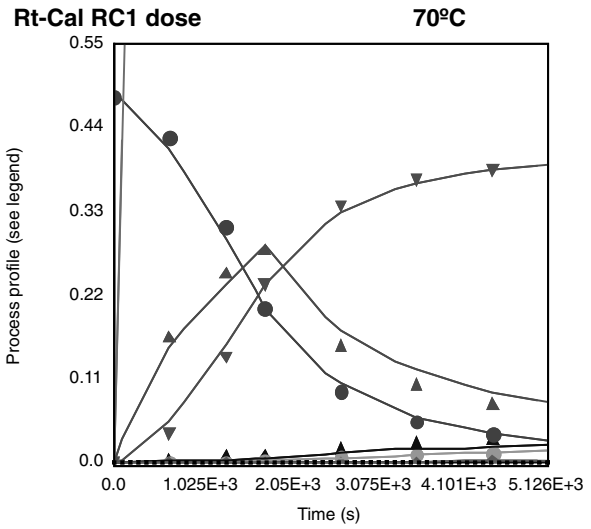


FIGURE 30.12 Validation semibatch reaction with 30 additions done in Mettler RC-1 reactor.

This process was repeated with the second reaction, reaction B, to develop a kinetic model. These models for both reactions A and B were then used to optimize a design for a series of CSTR reactors that would allow for the appropriate annual production.

It quickly become apparent that the data from the relative response factors are much smoother and better suited for fitting kinetic parameters. One could be tempted to utilize this approach from the beginning of the analysis. The importance of first verifying that the mass balance is closed before using relative response factors must be understood, as the use of relative response factors is a normalization of the data that forces the closure of the mass balance. If there had been a secondary reaction pathway that was not accounted for, then the kinetic model would have not represented the process and any reactor configuration that was designed would not have preformed as expected.

30.6 CONCLUSION

The use of analytical methods to elucidate process parameters, be it mass balance or ultimately kinetic information,

can be full of assumptions. Oftentimes, in the pharmaceutical industry tight timelines prevent the investigation into every assumption. Being aware of the assumptions is the only way that one is ever going to be able to test the ones that will have the biggest impact on the data. The key to understanding what assumptions are being made is being aware of what each analytical method is looking at, what it is proportional to, and understanding the complementary test methods that can give a clearer picture of the problem.

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