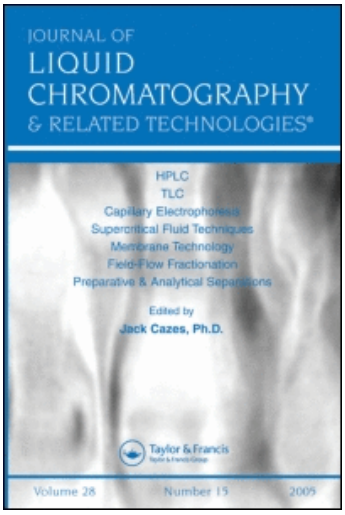

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A PHASE APPROPRIATE APPROACH TO RP-HPLC METHOD DEVELOPMENT FOR IMPURITIES ANALYSIS IN ACTIVE PHARMACEUTICAL INGREDIENTS VIA CONTINUOUS MANUFACTURING PROCESS UNDERSTANDING

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A PHASE APPROPRIATE APPROACH TO RP-HPLC METHOD DEVELOPMENT FOR IMPURITIES ANALYSIS IN ACTIVE PHARMACEUTICAL INGREDIENTS VIA CONTINUOUS MANUFACTURING PROCESS UNDERSTANDING

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□ *A concept of a systematic approach to the development of phase appropriate RP-HPLC methods for quantifying organic impurities in Active Pharmaceutical Ingredients (APIs) is presented. This dynamic and practical approach emphasizes the utilization of comprehensive chromatographic knowledge gained throughout the lifecycle of drug development based on continuous understanding of the API manufacturing process. At the beginning of a project, a chromatographic database can be built by scouting the relevant impurities and the API on carefully selected RP-HPLC columns, different organic modifiers and different pHs with only two gradient runs for each combination of RP-HPLC column, organic modifier, and pH. This comprehensive chromatographic database contains the experimental data and the predicted separations by chromatographic simulation software (e.g., DryLab®). Once this chromatographic database is established, an appropriate RP-HPLC method can be selected from the database based on pre-defined chromatographic method performance criteria (method attributes). More importantly, the database can be updated for any new impurities or obsolete impurities that are no longer relevant and can be taken out of the database throughout the lifecycle of the project based on continuous manufacturing process understanding, which facilitates the selection and development of appropriate RP-HPLC methods to achieve pre-defined method goals at different phases of drug development. A group of impurities identified in the manufacturing of pazopanib hydrochloride (API of Votrient, a recently approved anti-cancer medicine by the U.S. FDA) were used as an example to simulate the method development process and demonstrate the validity of this approach. Phase appropriate key chromatographic method attributes are proposed for each of the defined phases of drug development.*

Keywords chromatographic database, DryLab®, manufacturing process understanding, pazopanib hydrochloride (GW786034B, API of Votrient), phase appropriate, RP-HPLC method development

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INTRODUCTION

Organic impurities in an Active Pharmaceutical Ingredient (API)[1] are process or degradation-related unwanted organic compounds that can arise during the manufacturing process and/or the storage of the API. These unwanted organic impurities usually offer no benefit, but potentially pose risk to patient safety or drug efficacy.[2–6] The requirement on controlling organic impurities in an API for a new drug application is clearly defined in the ICH guideline.[1] Consequently, the detection, identification, quantification, and control of these impurities have become an important part of drug development in order to ensure product quality and ultimately patient safety.[6–10] Indeed, a drug-related impurities test is a key element of any API specifications starting at First Time in Humans (FTIH) clinical studies.[4]

Usually, drug development can be classified into three phases: early phase (e.g., FTIH), late phase (e.g., pivotal clinical studies), and new drug application/commercial manufacturing phase. The organic impurities that need to be controlled at each of the drug development phases usually vary because different impurities may be observed and overall knowledge of impurities grows with maturing manufacturing processes (e.g., changes made to the synthesis, formulation, or production procedure during drug development).[3] For instance, at the early phase of drug development, potential organic impurities usually are known starting materials, synthetic intermediates, reaction by-products, and degradation products. For the analysis of the API in early phase development, it is desirable that the analytical method should separate the API, the actual impurities in the API, and the intermediates and/or starting materials two stages back from the API forming stage (see example in Results and Discussion section). However, this should be evaluated case-by-case based on chemistry knowledge and process understanding.

As the project progresses to late phase development, usually more organic impurities will be identified and controlled in the API due to better process understanding. At this stage, some impurities that were controlled in the early phase of drug development may no longer be relevant; for example, an impurity controlled in the early phase may actually be transformed to a different impurity in the API. Once the manufacturing process is finalized and an active search for all possible impurities via impurity fate mapping[11] is completed, intrinsic knowledge of the impurity formation pathway and the process capability to purge these impurities are obtained. As a result, a comprehensive and scientifically-justified impurity control strategy is derived for the manufacturing process to progress to new drug application/commercial manufacturing phase.[11] At this stage, Quality by Design (QbD)[12–14] principles can be applied to impurities control

based on the comprehensive process understanding via impurity fate mapping. Only Critical Quality Attributes (CQAs)[13] need to be controlled in the API with simpler and more robust methods, while other relevant organic impurities to the overall API process can be controlled in the earlier synthetic steps (e.g., starting materials or intermediates) whenever possible.[11] Therefore, analytical methods are evolved along with the progress of manufacturing process understanding to meet different challenges and requirements at different phases of the drug development lifecycle, and knowledge of impurities accumulated during method development at earlier phases may benefit the development of analytical methods at later phases.

Reversed phase high-performance liquid chromatography (RP-HPLC) is by far the most widely used analytical technique for quantitation of organic impurities in APIs.[2,15] There have been many articles discussing RP-HPLC method development approaches for different purposes.[16–28] Before the QbD concept was widely applied, however, only a couple of books and a few recent review articles touched on the phase appropriate HPLC method development with the discussions of orthogonal HPLC method development.[9,10,29–32] Rasmussen et al. stated that HPLC method developed for early phase API is not expected to maintain applicability during late phases of development due to changes in synthetic routes and, as a result, the set of analytes that the method is required to separate may change.[29] Bynum described various roles of HPLC played in different phases of drug development and how HPLC methods evolved during the drug development process.[30] Rasmussen et al. suggested that the fundamental knowledge of the chemistry of drug substance gained during early development should translate into designing control methods for commercial supplies.[31] The importance of working closely during method transfer between the development lab and the receiving lab was also discussed.[32] However, the linkage between the HPLC method developed for each development phase and the improved manufacturing process understanding throughout the development lifecycle was rarely discussed, and there has been very limited practical guidance on how to utilize the chromatographic knowledge gained during the lifecycle of a drug candidate to develop the phase appropriate HPLC methods. Since the QbD concept was introduced, a greater emphasis was placed on the understanding of the origin and fate of organic impurities in API for manufacturing process development, and eventually devising a risk based strategy to control those impurities during the API commercial manufacturing.[12–14] To our knowledge, literature on systematic approach to phase appropriate RP-HPLC method development in QbD environment is limited. The interactive relationship between continuous manufacturing process understanding and the corresponding phase appropriate HPLC methods deserves more discussions.

In this paper, a concept of a systematic approach to the phase-appropriate RP-HPLC method development will be presented. Practical guidance on how to develop phase-appropriate RP-HPLC methods aided by building a chromatographic database throughout the lifecycle of drug development is provided. A case study on method development for an API of a recently approved anti-cancer medicine is followed to elucidate the interactive relationship between RP-HPLC methods and continuous manufacturing process understanding.

EXPERIMENTAL

Materials

Pazopanib hydrochloride (**API**), intermediates (**IM1 and IM2**), forced degradation product (**Deg1**) and other impurities (**Imp6** to **Imp15**) were synthesized in house by GlaxoSmithKline. The stage 3 starting material (**SM3**) was made by a contract manufacturer. HPLC grade Trifluoroacetic acid (TFA), acetonitrile and methanol were obtained from Sigma-Aldrich (St. Louis, MO, USA). HPLC grade acetic acid and ammonium acetate were from J.T. Baker (Phillipsburg, NJ, USA). De-ionized water was obtained from a Milli-Q system (Millipore, Bedford, MA, USA).

As shown in Table 1, the HPLC columns used in this study include Waters (Milford, MA, USA) Xterra MS C18 column (150 × 4.6 mm, 3.5 μm), Xbridge C18 column (150 × 4.6 mm, 3.5 μm), YMC-Pack Pro C18 (150 × 4.6 mm, 3 μm); Phenomenex (Torrance, CA, USA) Luna C18 (2) column (150 × 4.6 mm, 3 μm); Agilent (Wilmington, DE, USA) Zorbax SB C8 column (150 × 4.6 mm, 3.5 μm), and Zorbax Bonus-RP column (150 × 4.6 mm, 3.5 μm). Two additional C18 columns (i.e., Xterra MS C18 and Luna C18

TABLE 1 Method Components

Column	Agilent Zorbax Bonus-RP (polar embedded)	
	Waters Xbridge C18 (hybrid BEH C18)	
	Phenomenex Luna C18 (2) (conventional C18)	
	Agilent Zorbax SB C8 (conventional C8)	
	YMC YMC-Pack Pro C18 (conventional C18)	
	Waters Xterra MS C18 (hybrid HPT C18)	
pH (buffer)	2	0.1% (v/v) TFA
	3.2	0.1% (v/v) acetic acid
	4.8	10 mM ammonium acetate, pH adjusted with acetic acid
	5.4	10 mM ammonium acetate, pH adjusted with acetic acid
	6.8	10 mM ammonium acetate
	7.9	10 mM ammonium acetate, pH adjusted with ammonium hydroxide
Organic Modifier	Acetonitrile	
	Methanol	
	Acetonitrile/Methanol (1:1 v/v)	

(2)) were selected here based on the chemical properties and structures of the compounds and our previous experience.[28] Relatively large numbers of RP-HPLC columns and pHs were scouted in this experiment so that a comprehensive chromatographic behavior of each compound can be achieved in a wide range of each chromatographic component. This can provide an extensive chromatographic knowledge for developing phase appropriate RP-HPLC method development approach and also making a good recommendation for minimum combinations that one can start with.

All impurities were spiked into the **API** at certain concentrations. The mixtures were then used for scouting under the chromatographic conditions described in the following sections. Alternatively, a batch or several batches of the **API** that contain all impurities could be used, which would be a usual case for early phase method development when authentic reference materials of impurities might not be available.

High Performance Liquid Chromatography (HPLC)

Agilent 1100 HPLC systems (Palo Alto, CA, USA) equipped with diode array detectors were used for this work. Waters Empower software (Milford, MA, USA) was used to acquire, store, and process the chromatographic data.

Chromatographic Simulation Software

DryLab® 2000 plus from Rheodyne LLC (Rohnert Park, CA, USA) was used for chromatographic method simulation and optimization.

Method Scouting Procedure

The chromatographic conditions include the combinations of six HPLC columns, six pHs and three organic modifiers as listed in Table 1 with two gradients (5% v/v organic modifier to 95% v/v organic modifier in 20 min (short gradient) and 45 min (long gradient), respectively).[28] A column temperature of 40°C was used for all chromatographic separations. The flow rate was fixed at 1 mL/min. The injection volume of all chromatographic separations was 5 μL. Diode array spectra of all compounds were generated from 200 nm to 400 nm.

RESULTS AND DISCUSSION

Concept of Phase Appropriate RP-HPLC Method Development

In principle, a systematic approach to RP-HPLC method development with the emphasis of chromatographic knowledge gained by building a

chromatographic database[28] can be utilized to develop the phase-appropriate RP-HPLC methods based on continuous manufacturing process understanding.

At the beginning of a project, a chromatographic database (e.g., retention, tailing, resolution of the analytes) can be built by scouting (or called "screening," alternatively) key RP-HPLC components (e.g., column, organic modifier and pH as discussed later). Considering the fast turn-around time and the limited resource at early phase, a step-wise approach may be applied at the beginning of a project. However, initial method scouting should start with at least two columns (e.g., polar embedded and C18) based on the characteristics of analytes, two organic modifiers (e.g., acetonitrile and methanol), two pHs covering a reasonable range (e.g., pH 2 and pH 7) with two gradient runs. Biswas et al. showed the success for 40 pharmaceutical compounds and demonstrated method orthogonality by using similar and simple systematic screening procedures.[24] One should perform a risk-to-benefit evaluation based on the complexity of the required separation to examine if the minimum method scouting combinations could be selected. Though it may be labor intensive, the more the combinations are scouted, the better chance a desired separation is achieved. Additional scouting with more combinations of column/organic modifier/pH can always be performed later on if a desired separation cannot be achieved. In addition, commercially available chromatographic simulation software can be used to extend the experimental data by simulating separations beyond the scouting conditions to create a chromatographic database.

The purpose for building a chromatographic database starting at early phase development is to provide a foundation for method development from the beginning so that any chromatographic information can be obtained directly from the database to assist method development at later stages. Along with continuous manufacturing process understanding throughout the lifecycle of product development, any new impurities can always be added later into the database by additional scouting, or obsolete impurities that are no longer relevant can be removed from the database. In general, additional scouting for the additional impurities should be performed at the initial combinations of column/organic modifier/pH to complete the database and increase the understanding of the chromatographic behavior of all targeted compounds. This is very important for methods that are aimed for new drug application/commercial manufacturing. However, one may not need to scout the conditions for new impurities that are proven to be inadequate for the existing impurities to improve the efficiency of method development. This chromatographic database, with all previous knowledge about the analytes, would benefit method/knowledge transfer from analyst-to-analyst and from lab-to-lab.

Once the chromatographic database is established, the pre-defined method performance criteria, i.e., method attributes, can be developed to aid the selection of appropriate RP-HPLC method conditions at different development phases. In general, method attributes become more and more stringent as the project progresses from early phase to late phase and eventually to new drug application/commercial manufacturing phase due to different business and regulatory requirements.[29–30]

Rational for the Selection of Chromatographic Components for Scouting

RP-HPLC column, organic modifier, mobile phase pH, and temperature are widely used RP-HPLC components of method development and optimization.[15] Considering the combining effect and interaction of these components and, in order to improve the efficiency by minimizing the lab work and afterwards; the data analysis, a two-tier approach, is recommended for the selection of chromatographic components for scouting. It is noteworthy that chemical properties and structures of the targeted compounds should be thoroughly investigated and used as the basis for the selection of the initial scouting conditions.[24,28–30]

For the first tier, the most widely used bonded phase silica columns such as C18, polar embedded, and C8 columns,[15,33,34] frequently used organic modifiers such as acetonitrile and methanol, as well as moderate pHs, can be scouted as the initial input to the database. Column stability has to be taken into consideration for the selection of column and pH range during method scouting. It is known that traditional bonded phase silica columns are typically stable under moderate temperature (normally $\leq$60°C) and in pH between 2.5 to 7.5, but not at high pHs (e.g., 9–12). Therefore, moderate pH range 2–7 can be scouted under constant temperature (e.g., 40°C).

For the second tier, more column types (e.g., Phenyl, HILIC, Phenyl Hexyl or C30), higher pH (e.g., pH 10), broader temperature range, and some organic additives such as triethylamine (TEA) or tetrahydrofuran (THF) can be explored if desired method attributes could not be achieved at the first tier. Notably, Phenyl columns have a potential stability issue at pH $>$ 7; therefore, it is included at the second tier for scouting. At pH 10, bonded phase silica columns with acceptable stability are very limited. However hybrid columns are generally more stable especially under extreme pH conditions, thus are the columns of choice for the use up to pH 12. In addition, HPLC instrument performance may be negatively impacted at high pH, which may result in method reproducibility issue in drug production environment. Therefore, scouting at high pH (e.g., pH

10) is recommended when necessary and only with specially designed hybrid columns.

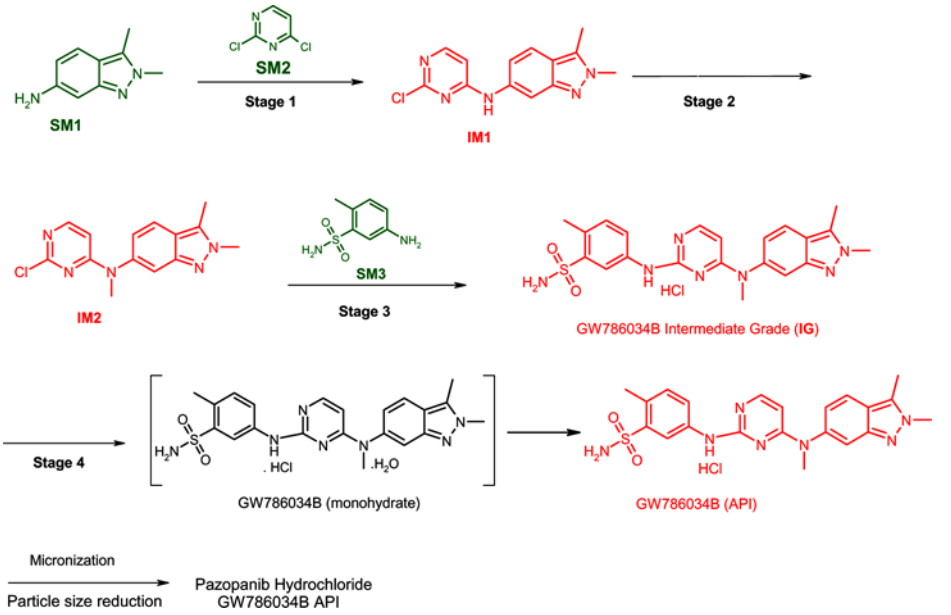
Case Study

In this paper, a group of impurities identified in the manufacturing of pazopanib hydrochloride are used as a case study to simulate the method development process at different phases of drug development and demonstrate the validity of this approach. The known impurities will be grouped into three phases of drug development based on process progression and understanding. The separation of the impurities in each group will be thoroughly discussed. Phase-appropriate key chromatographic method attributes will be proposed for each phase of the drug development defined in this paper.

Although the focus of this paper is a phase-appropriate approach to RP-HPLC method development for impurities analysis in API, it suggests that this phase appropriate and systematic approach with the emphasis of continuous chromatographic knowledge and process understanding can also be applied to the RP-HPLC method development for synthetic intermediates and starting materials with smaller numbers of method scouting combinations to begin with and less stringent method performance criteria as well as for drug product. Ideally, one should aim to develop a harmonized RP-HPLC method for both API and drug product if possible. The systematic approach discussed in this paper should be well suited for this task.

Selection of Impurities

Pazopanib hydrochloride (**GW786034B** in Scheme 1), a VEGFR tyrosine kinase inhibitor, is the active pharmaceutical ingredient of Votrient that was approved recently by the U.S. FDA for the treatment of advanced renal cell carcinoma.[35] The reason that pazopanib hydrochloride and its related impurities were chosen for this work is because thorough impurity fate mapping had been generated for the impurities in pazopanib hydrochloride[11] and authentic reference standards were made for its method validation. The structures of **API**, **IM1**, **IM2**, and **SM3** are shown in Scheme 1, but the structures of the other compounds (i.e., **Deg1** and **Imp6** to **Imp15)** are not shown in this paper because they are not critical to the discussion and have no impact on the conclusions made. As shown in Figure 1, these impurities may be divided into three groups based on the phases of drug development, and process progression and understanding, which will be discussed in detail in the following sections. Note that the



SCHEME 1 Manufacturing process of pazopanib hydrochloride (**GW786034B**, API) *Note*: Stage 1 refers to the coupling of **SM1** and **SM2** to obtain **IM1**. Stage 2 refers to the methylation of **IM1** to obtain **SM2**. Stage 3 refers to the coupling of **IM2** and **SM3** to obtain **IG**. Stage 4 refers to the final purification and form control step in which **IG** is recrystallized to obtain the monohydrate form, which is then converted to the desired Form of API. (Scheme available in color online.)

purpose of this work is not to re-develop RP-HPLC methods for pazopanib hydrochloride. Instead, the work presented here is to illustrate the concept and benefit of a systematic approach to RP-HPLC method development in order to arrive at phase-appropriate methods for quantifying organic impurities in any APIs.

It is very noticeable in Figure 1 that the CQA impurities have been actually observed throughout the drug development lifecycle of pazopanib hydrochloride, which means that efforts put in for the early phase development would significantly benefit the method development for the late phase and the new drug application/commercial manufacturing phase. Therefore, less resource may be needed for the project throughout the entire development lifecycle.

Method Scouting Summary

Once the scouting runs were completed, chromatographic experimental data (e.g., retention time, peak area, and peak tailing) were uploaded into DryLab® for simulation, prediction, and optimization to

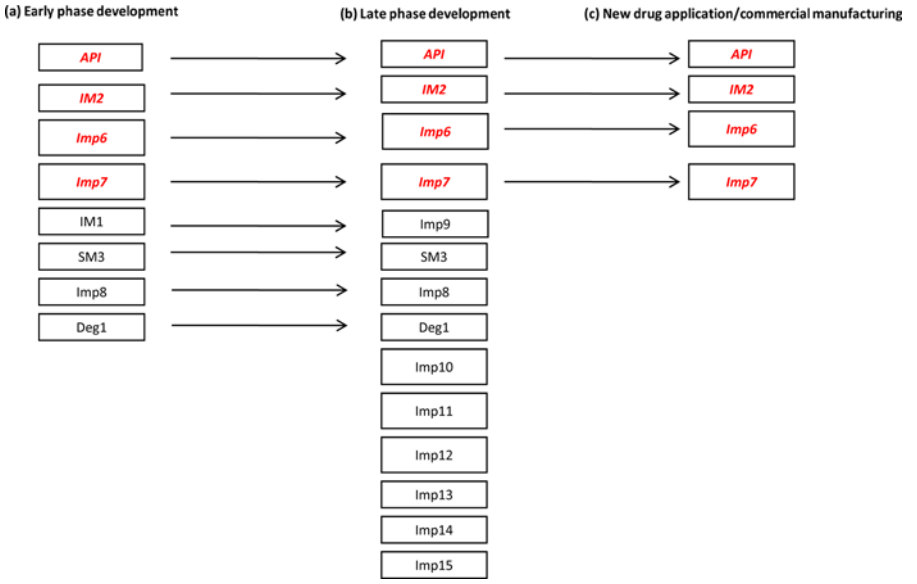


FIGURE 1 Compounds selection: Pazopanib hydrochloride related compounds (a) early phase development (based on batch data, there are eight compounds); (b) late phase development (based on impurity fate mapping, there are fourteen compounds); and (c) new drug application/commercial manufacturing phase development (based on the CQAs identified, there are four compounds). **IM2** in group (c) is for **IG** only. See details in the Selection of Impurities section. Note that the first four compounds of each group are **API** or CQAs with bold and italics fonts. (Figure available in color online.)

generate potential chromatographic conditions. The experimental data and predicted separations provided a comprehensive data set. It formed a chromatographic database that would assist with method understanding, optimization and selection.[28] Actual laboratory separations were carried out to confirm the promising conditions predicted by DryLab®. It is important to note that HPLC instruments with the same dwell volume and plumbing should be used throughout the scouting process because dwell volume and plumbing have significant effect on gradient elution,[15] which applies to the initial scouting and subsequent scouting with new impurities. In order to add the chromatographic data of any new impurities to an existing chromatographic data set, one should always include the API with the new impurities during the scouting of these impurities. Thus, the relative retention time of a new impurity to the API can be used to correct any slight shift of its retention time before adding it into the existing chromatographic database. The slight retention time shift observed in RP-HPLC at different times is due to minor variability of the chromatographic system (e.g., temperature, pH, age of column, flow rate, and other instrumental parameters) at the times of the scouting runs. Alternatively,

existing impurities in the database may be scouted with new impurities to update the existing chromatographic database in some situations (e.g., the separation of the mixture becomes very complex, dwell volume, and/or plumbing of the HPLC system is changed from the original setting, authentic reference material becomes available for previously unidentified peaks in the scouting performed before). Once the chromatographic database is built, it is very simple to remove any obsolete impurities that no longer need to be controlled in a method by unselecting them in DryLab®.

Early Phase Development

In the early phase of drug development (e.g., at FTIH), normally a less efficient synthetic process (e.g., adopted straight from drug discovery with or without modifications) is used to prepare API for clinical trials with little or no process understanding. Batch analysis data and knowledge on safety coverage of impurities are usually very limited. Potential organic impurities that need to be controlled in API usually are known starting materials, synthetic intermediates, reaction by-products and degradation products at the time. For the example of pazopanib hydrochloride, the seven potential impurities were selected based on the available batch data and the outcome of its stress degradation studies. **SM3** is the starting material for the API forming stage, while **IM2** is the stage 2 intermediate and also the other starting material for the API forming stage. **IM1** is the stage 1 intermediate. **Deg1** was the only major degradation product of the API under stress degradation conditions. **Imp6** was identified as a dimeric impurity of the API. **Imp7** and **Imp8** were unknown impurities at very low levels ($\leq$0.1% area) in the batches analyzed at the time.

SM1 and **SM2** were not included in this group of compounds because they were more than two stages back of the API forming stage and they were not observed in actual API batches at the levels that can be quantified by a RP-HPLC method with UV detection. In fact, **SM1** is a potential genotoxin and is always less than 1.7 ppm w/w in all of the API batches,[36] while, theoretically **SM2** does not participate in the remaining reactions.

At the early phase of drug development, the key chromatographic method attributes are usually focused on the resolution of the critical pair in the separation and the sensitivity of the **API**. For the separation of the eight compounds in pazopanib hydrochloride at early development phase, it is very desirable to have the resolution between the critical pair (i.e., **IM2** and **API** as shown in Figure 2) as not less than 1.5 and the signal to noise

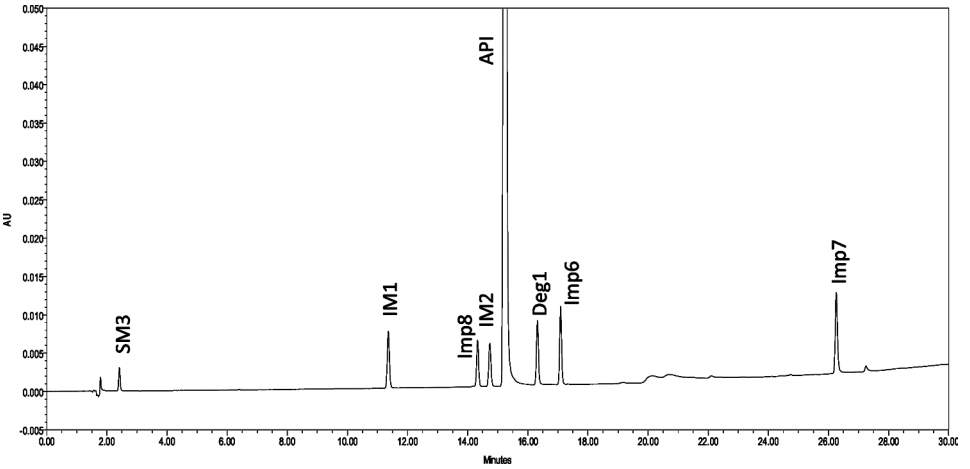


FIGURE 2 Separation of early development phase compounds. HPLC conditions: Column: Xbridge C18, 150 × 4.6 mm, 3.5 µm); mobile phase A: 10 mM ammonium acetate (NH_4OAc) in water; mobile phase B: methanol; gradient: 28%B to 100%B in 30 minutes; flow rate: 1 mL/min; UV detection: 268 nm; column temperature: 40°C; sample concentration: 0.2 mg/mL pazopanib hydrochloride spiked with the impurities in 50:50 acetonitrile and water; injection volume: 5 µL. Note that the resolution between **IM2** and **API** is 3.8.

ratio of at least 10 for a 0.05% w/w of **API** solution as the key chromatographic method attributes based on our experience. The API is used as a surrogate at the early phase development for sensitivity check of the method because well characterized reference materials for true impurities are generally unavailable at this stage of drug development. Usually method robustness, method ruggedness, and secure column supply are less critical at this phase of drug development. One can perform "paper evaluation" on method robustness for gradient rate, initial percentage of organic modifier, and flow rate using the chromatographic simulation software especially if a critical separation is observed.

Several promising chromatographic conditions were identified through DryLab® simulation and actual laboratory analyses were performed to confirm the predicted separations. A lead method with a single gradient at pH 6.8 (10 mM NH_4OAc in water) that employs Xbridge C18 column and methanol was selected as shown in Figure 2.

One could argue why a systematic approach to the RP-HPLC method development is needed for early phase method development as most of the early phase compounds would be terminated in later phases of drug development due to safety and/or efficacy reasons. Some would even prefer to just develop a "quick and dirty" method that usually uses low pH mobile phases (e.g., TFA) because of the convenience, TFA's compatibility with MS detection, and good stability of most of the

RP-HPLC columns at low pHs. In addition, non-systematic (e.g., "quick and dirty") approaches normally start with a single linear gradient for certain period of time (e.g., 5 to 95% organic modifier for 30 min), then manually "optimize" the method, which could be very labor intensive and time consuming.

The systematic approach to RP-HPLC method development discussed in this paper with the combination of carefully selected HPLC columns, different organic modifiers and pHs, and two gradient runs for each combination of HPLC column, organic modifier, and pH should significantly increase the chance of developing an acceptable method at the first try. Note that the method scouting runs described in this systematic approach can be automated using most of the modern HPLC systems. Although, non-systematic (e.g., "quick and dirty") approaches may seem to save some time (a large portion is instrument time) on paper and initially, significantly more resource may have to be spent later time due to lack of understanding of the chromatographic behavior of the compounds studied. Figure 3 shows one of the promising conditions for the separation of the eight compounds with a single gradient at pH 2 that employs YMC-Pack Pro C18 column and methanol, while the peak area and peak height of **SM3** (5-amino-2-methyl-benzenesulfonamide) in the initial scouting runs were much lower than those obtained at higher pHs, which will be

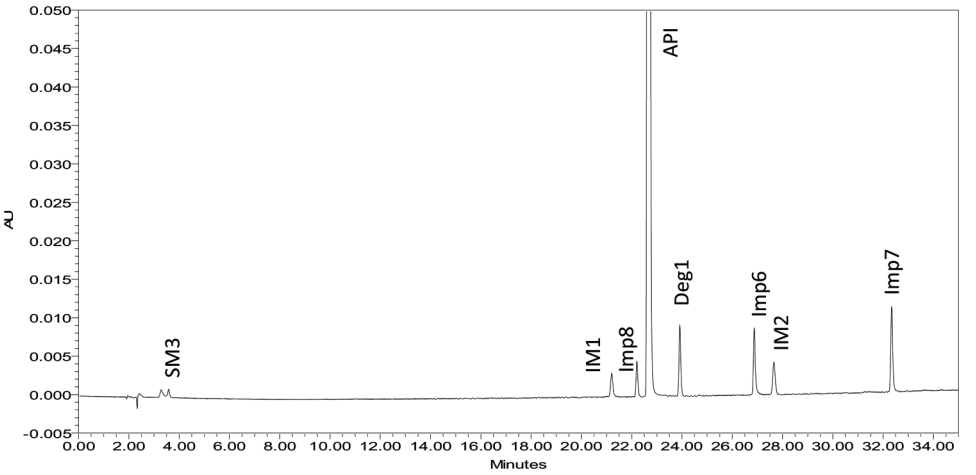


FIGURE 3 Separation of early development phase compounds at pH 2. HPLC conditions: Column: YMC-Pack Pro C18, 150 × 4.6 mm, 3 µm); mobile phase A: 0.1% v/v TFA in water; mobile phase B: methanol; gradient: 6%B to 70%B in 35 minutes; flow rate: 1 mL/min; UV detection: 268 nm; column temperature: 40°C; sample concentration: 0.2 mg/mL pazopanib hydrochloride spiked with the impurities in 50:50 acetonitrile and water; injection volume: 5 µL. Note that the resolution between **Imp8** and **API** is 3.5.

discussed later. This confirmed our previous experience on the poor sensitivity of **SM3** at pH 2 during the development of pazopanib hydrochloride. An initial look at the separation in Figure 3 showed that a comparable separation of the eight compounds was achieved as compared to that in Figure 2 except **IM2** peak eluted much later and the peak of **SM3** was split or distorted. It was found that a much weaker mobile phase was used in Figure 3 (6% methanol) as compared to that in Figure 2 (28% methanol) at the start of the gradient because **SM3** was much less retained at pH 2 since it is a very polar compound and potentially partially charged at pH 2. Therefore, if started with "quick and dirty" approach at low pH (i.e., TFA) only, additional resource would be needed to modify the method conditions in Figure 3 at pH 2 or start from scratch to redevelop a satisfactory method.

Figure 4 plots the peak area of **SM3** against pH and shows that **SM3** peak area at pH 2 is significantly less (at least 3.4 fold) than those at other pHs, which has negative impact on its sensitivity. The plot of **SM3** peak height against pH gives almost identical shape as that of **SM3** peak area against pH, thus it is not shown here. Diode array spectra of **SM3**, which used the same brand HPLC column, organic modifier, and gradient, but different pH (pH 2 vs. pH 6.8) as shown in Figure 5, confirmed the finding. Note that the scale of Y-axis in Figure 5b is 10 times more than that in Figure 5a. This highlights the importance of response factors which should be a consideration no matter what method development approach is used.

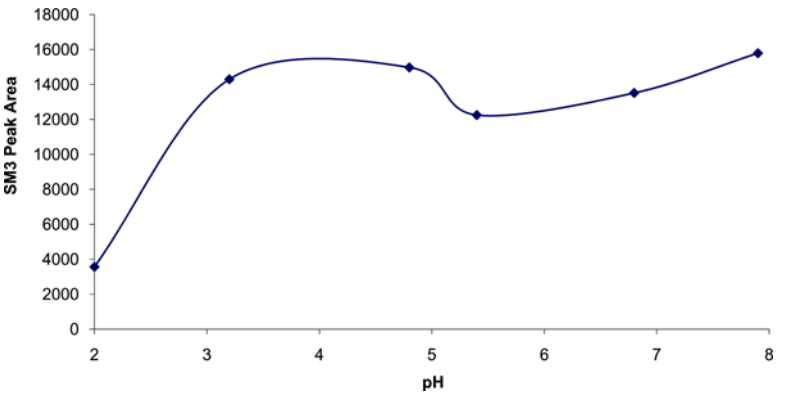


FIGURE 4 Effect of pH on **SM3** peak area. HPLC conditions: Column: YMC-Pack Pro C18, 150 × 4.6 mm, 3 µm); mobile phase A: buffer in water with pH of 2, 3.2, 4.8, 5.4, 6.8, and 7.9; mobile phase B: acetonitrile; gradient: 5%B to 95%B in 45 minutes; flow rate: 1 mL/min; UV detection: 268 nm; column temperature: 40°C; sample concentration: 0.2 mg/mL pazopanib hydrochloride spiked with the impurities in 50:50 acetonitrile and water; injection volume: 5 µL. (Figure available in color online.)

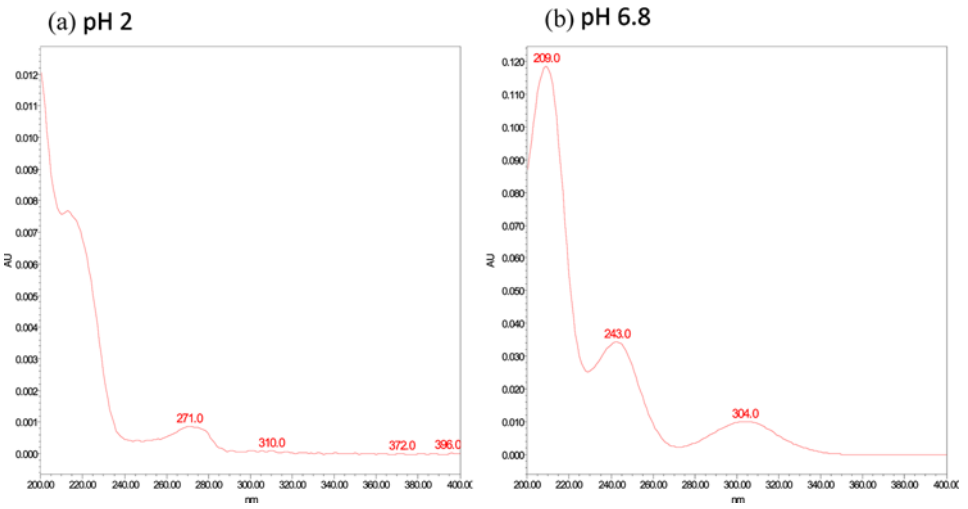


FIGURE 5 Effect of pH on diode array spectra of **SM3**. Column: YMC-Pack Pro C18, 150 × 4.6 mm, 3 µm); mobile phase B: acetonitrile; gradient: 5%B to 95%B in 45 minutes; flow rate: 1 mL/min; UV detection: 268 nm; column temperature: 40°C; sample concentration: 0.2 mg/mL pazopanib hydrochloride spiked with the impurities in 50:50 acetonitrile and water; injection volume: 5 µL. (a) pH 2 (mobile phase A: 0.1% v/v TFA in water); and (b) pH 6.8 (mobile phase A: 10 mM NH_4OAc in water). (Figure available in color online.)

Late Phase Development

As project progresses, better manufacturing process understanding may have been achieved in order to develop a more efficient and environmental friendly synthetic process with the aim of commercial manufacturing. As a result, more organic impurities than those in the early phase development for an API process will likely be discovered and identified even though the actual impurities observed in API batches may become less or their levels may be reduced. Once the manufacturing process is finalized, significant numbers of impurities are normally identified through active search for any possible impurities in an API process.[11] This means that analytical methods used for impurity testing at the early phase development may not be suitable for late phase development (e.g., pivotal clinical trials).[11]

At the late phase development, preferably one should aim to develop a single RP-HPLC method (instead of multiple RP-HPLC methods) for routine use that can separate all impurities (actual and potential) to support the impurity fate mapping activities. Many references stressed the importance of orthogonal methods.[24,29–32] One of the advantages of the chromatographic database described in this paper is that one can easily evaluate separations at different combinations of RPLC

columns, organic modifiers, and pHs; and select an orthogonal method to supplement the lead method if needed. The characteristic of the chromatographic database provides a build-in quality of the methods developed. The RP-HPLC method at this stage may be complex because the number of impurities involved is usually quite large. For the example of pazopanib hydrochloride, many more potential impurities were identified (**Imp9** to **Imp 15**). Interestingly, **Imp9** is the by-product derived from **IM1** in pazopanib hydrochloride. Thus, **Imp9** instead of **IM1** should be monitored at this phase of development, while **IM1** is no longer relevant.

The key chromatographic method attributes at this phase are usually focused on the resolution of the critical pair in the separation, good peak shapes for the API and the impurities, and sensitivities of the API and the impurities. For the separation of the fourteen compounds in the late phase group of impurities in pazopanib hydrochloride, it is very desirable to have the resolution between the critical pair as not less than 1.5, the tailing factor of the **API** of 0.8 to 1.5 and the signal to noise ratio of at least 10 for a 0.05% w/w of **API** and ideally for each impurity. For the methods selected, method robustness, method ruggedness, and secure column supply should be considered even though they are not critical. One can perform "paper evaluation" on method robustness for gradient rate, initial percentage of organic modifier, and flow rate using the chromatographic simulation software if a critical separation is observed. It is advisable that an appropriate reference standard for the observed impurities should ideally be prepared for the method scouting work at this phase of drug development. If one method could not meet the requirement, then additional methods may be developed and used. For example, a different method other than the main method routinely used may be needed for the better separation of a pair of closely eluting compounds.[29,31] The chromatographic database described in this paper is ideally suited for this purpose.

For the separation of pazopanib hydrochloride related late phase impurities, a few promising chromatographic conditions were identified through DryLab® simulation and actual analyses in the laboratory were performed to confirm the predicted separations. A lead method with a two-step gradient at pH 6.8 that employs Xbridge C18 column and methanol was predicted by DryLab®, however a resolution of 1.1 between **Imp13** and **Imp6** in the real laboratory run was observed as shown in Figure 6, while the predicted resolution was 1.8 between both compounds. Two reasons may explain why the real laboratory run could not achieve the predicted resolution by DryLab®. In theory, retention (log k') is generally non-linear over the volume fraction of the organic modifier (Φ) especially for poorly retained ($k' < 1$) and strongly retained compounds

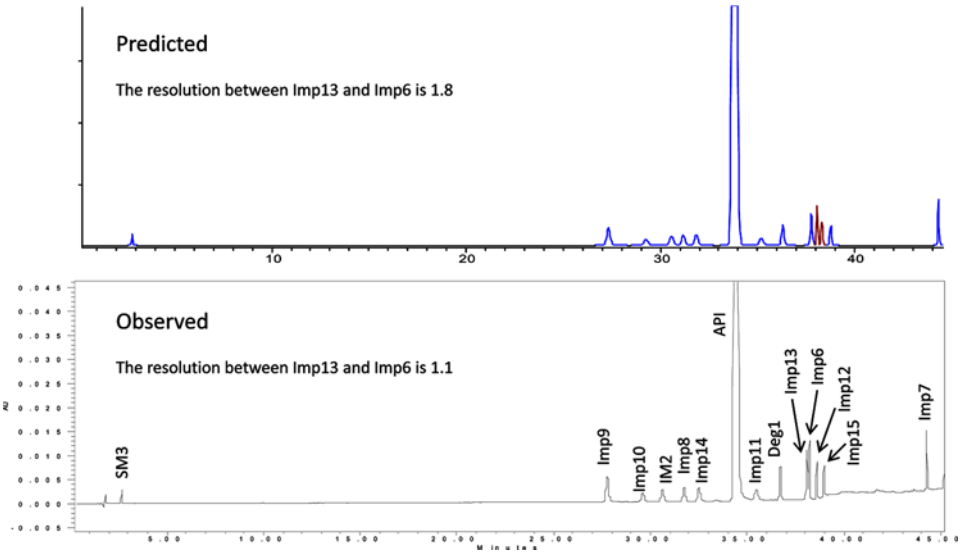


FIGURE 6 Separation of late phase development compounds. HPLC conditions: Column: Xbridge C18, 150 × 4.6 mm, 3.5 µm); mobile phase A: 10 mM ammonium acetate (NH_4OAc) in water; mobile phase B: methanol; flow rate: 1 mL/min; gradient: 25%B to 52%B in 32.5 minutes, then 52%B to 100%B in 12.5 minutes; flow rate: 1 mL/min; UV detection: 268 nm; column temperature: 40°C; sample concentration: 0.2 mg/mL pazopanib hydrochloride spiked with the impurities in 50:50 acetonitrile and water; injection volume: 5 µL. Note that the resolution between **Imp13** and **Imp6** is 1.1 in the observed separation. (Figure available in color online.)

($k' > 10$).[37–38] However, DryLab® assumes a linear relationship of log k' over Φ. Specifically for this separation, co-elution of compounds resulted in inaccuracy of their retention times generated.

An improved separation for the late phase development compounds was achieved by purposely increasing the predicted resolution between **Imp13** and **Imp6** while sacrificing the predicted resolutions between **IM2** and **Imp8** as well as **Imp6** and **Imp12** as shown in Figure 7. A two-step gradient was needed for the separation of the late phase development compounds. This reflects the complex nature of this group of compounds especially on the structural similarity and size of the molecules. As **Imp7** elutes very close to the end of the gradient in Figure 7, it is recommended to add a few minutes of holding time at 100% mobile phase B (methanol) to ensure all compounds would elute out during the real sample analysis.

It is noteworthy that the optimized chromatographic conditions as shown in Figure 7 for the late phase development compounds employ the same column, organic modifier, and pH, but have a different gradient compared to that of the early phase development compounds as shown in Figure 2. The knowledge and experience gained for the early phase development compounds should provide significant insight and help on

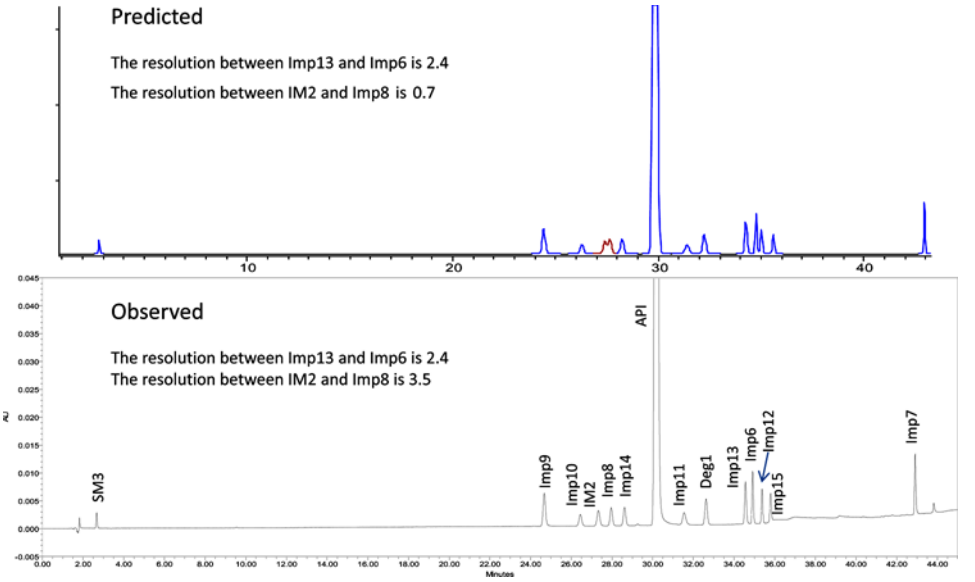


FIGURE 7 Improved separation of the late phase development compounds. Chromatographic conditions are the same as those in Figure 6 except that the gradient is 25%B to 55%B in 30 minutes, then 55%B to 100%B in 15 minutes. Note that the resolution between **Imp13** and **Imp6** is 2.4 in the observed separation. (Figure available in color online.)

arriving at optimized separation conditions for the late phase development compounds.

New Drug Application/Commercial Manufacturing Phase

At the new drug application/commercial manufacturing phase, full understanding should have been achieved on the origin, formation pathway, fate, and process purgeability of all of the impurities identified at the late phase development. Risk mitigation steps to reduce those impurities for the commercial API manufacturing process should have been developed and; finally, a comprehensive and scientifically-justified control strategy for the overall commercial manufacturing process must be in place.[11] Therefore, typical API batches manufactured via the commercial process usually contain less actual impurities (i.e., CQAs) at lower levels as compared to those manufactured via earlier processes. Consequently, the RP-HPLC method at this phase could be significantly simplified to control the CQAs only.

Our experience suggests that at this phase of drug development, the key chromatographic method attributes should focus on the resolution of the critical pair in the separation, good peak shapes for the API and

the CQAs, and sensitivities of the API and the CQA impurities. For the separation of the four compounds in the new drug application/commercial manufacturing phase group of impurities in pazopanib hydrochloride, one should achieve the resolution between any pairs of compounds as not less than 1.5 (ideally >1.8), the tailing factor of all compounds of 0.8 to 1.5 (ideally 0.9 to 1.5) and the signal to noise ratio of at least 10 for a 0.05% w/w of every compound. Method robustness, method ruggedness and secure column supply are critical to the method at this phase of drug development and must be considered when selecting methods from the database.[28] For the example of pazopanib hydrochloride, **Imp6** and **Imp7** are the only CQAs identified for the final API commercial manufacturing process. The development of a suitable RP-HPLC method for the **API** and both impurities has been thoroughly discussed.[28]

Note that **IM2** is a non-DNA reactive genotoxin. Based on thorough understanding of its process purgeability, **IM2** is controlled in stage 3 intermediate (**GW786034B intermediate grade**, or **IG**) at not greater than 0.6% w/w with the idea of "test early and analyze high" following QbD principles.[36] As a result, **IM2** was also included in the final RP-HPLC method development for the **IG** as shown in Figure 8.

The three methods developed for pazopanib hydrochloride in the previous sections used Xbridge C18 and Zorbax Bonus-RP, methanol and acetonitrile, and pH 7 and 2, respectively. Although only one example is shown

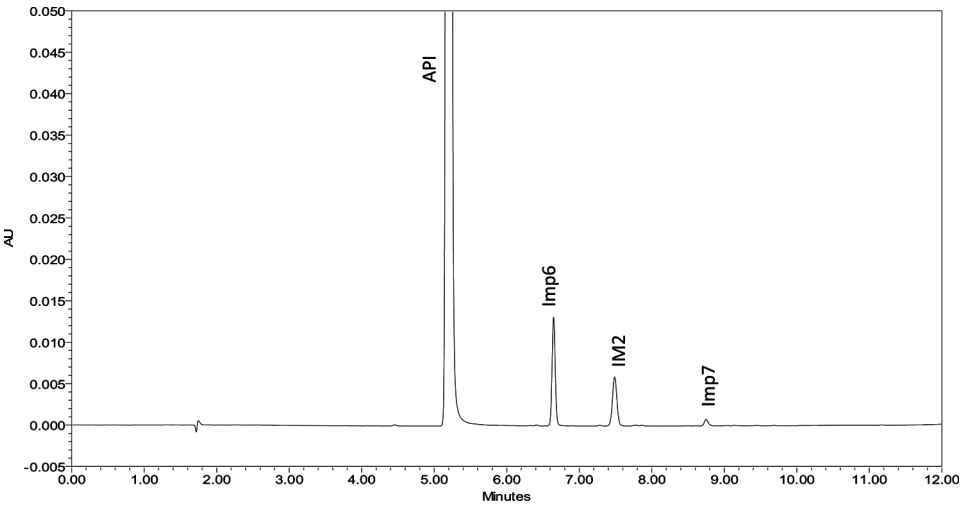


FIGURE 8 Separation of CQA impurities in **GW786034B IG**. HPLC conditions: Column: Zorbax Bonus-RP (150 × 4.6 mm, 3.5 µm); mobile phase A: 0.1% v/v TFA in water; mobile phase B: acetonitrile; gradient: 20%B to 56%B in 12 minutes; flow rate: 1 mL/min; UV detection: 268 nm; column temperature: 40°C; sample concentration: 0.2 mg/mL; injection volume: 5 µL.

in this paper, it clearly demonstrates the importance of starting method scouting with different combinations of RP-HPLC columns, organic modifiers, and pHs.

As discussed previously, different RP-HPLC methods would likely be developed for different phases of drug development. Correlation of the analysis data generated by these methods can be easily achieved by using the relative response factor of each impurity to the API during the lifecycle of the project.

CONCLUSIONS

A concept of a phase-appropriate approach to RP-HPLC method development for organic impurities analysis in API has been presented. Using pazopanib hydrochloride related impurities encountered during early, late, and new drug application/commercial manufacturing phases as a practical example, it has clearly shown that the advantage of this approach over a "quick and dirty" approach (i.e., only use mobile phases at pH 2 in method scouting), even at the early phase of drug development. For example, impurity **SM3** has poor sensitivity and elutes at very low concentrations of organic modifier that may cause peak splitting or distortion under pH 2 conditions. As a systematic approach, it is very important to utilize the comprehensive chromatographic knowledge gained throughout the lifecycle of the development of a drug candidate based on continuous understanding of the API manufacturing process. Once a chromatographic database was built at the beginning of a project with limited impurities, new impurities could be added to or obsolete impurities could be removed from the database throughout the project lifecycle.

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