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Analytical control of process impurities in Pazopanib hydrochloride by impurity fate mapping

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ABSTRACT

Understanding the origin and fate of organic impurities within the manufacturing process along with a good control strategy is an integral part of the quality control of drug substance. Following the underlying principles of quality by design (QbD), a systematic approach to analytical control of process impurities by impurity fate mapping (IFM) has been developed and applied to the investigation and control of impurities in the manufacturing process of Pazopanib hydrochloride, an anticancer drug approved recently by the U.S. FDA. This approach requires an aggressive chemical and analytical search for potential impurities in the starting materials, intermediates and drug substance, and experimental studies to track their fate through the manufacturing process in order to understand the process capability for rejecting such impurities. Comprehensive IFM can provide elements of control strategies for impurities. This paper highlights the critical roles that analytical sciences play in the IFM process and impurity control. The application of various analytical techniques (HPLC, LC–MS, NMR, etc.) and development of sensitive and selective methods for impurity detection, identification, separation and quantification are highlighted with illustrative examples. As an essential part of the entire control strategy for Pazopanib hydrochloride, analytical control of impurities with 'meaningful' specifications and the 'right' analytical methods is addressed. In particular, IFM provides scientific justification that can allow for control of process impurities up-stream at the starting materials or intermediates whenever possible.

1. Introduction

Active pharmaceutical ingredients (APIs) are manufactured by scale-up of synthetic chemical processes. Development of synthetic chemical process can lead to the generation of unwanted organic impurities. These usually include the un-reacted starting materials (SMs), impurities originating from the SMs, un-reacted intermediates (IMs), reaction by-products, and degradation products [1–3]. These undesired impurities usually provide no benefit to patients and can pose risks to patient safety or drug efficacy [4,5]. Thus, the detection, identification, quantification, and control of such impurities originating in the manufacturing process have become an important element of drug development in order to ensure product quality and ultimately patient safety [6–8]. Regulatory agencies also explicitly regulate the control criteria for these drug related substances in APIs and subsequent drug products by providing guidance for the pharmaceutical industry [1,9].

In the traditional framework, product quality is ensured predominantly by restricting flexibility in the manufacturing process and end product testing (so called quality-by-testing, QbT) [10]. Under QbT, process impurities are usually tested against specifications set using the observed data from a number of batches. However, this places little or no emphasis on the process understanding and the design of an effective and efficient impurity control strategy. In light of the recent quality by design (QbD) initiative by the U.S. Food and Drug Administration (FDA) [11], increasing attention has been drawn to the application of the QbD principles [12,13] to impurity investigation and control, emphasizing process understanding based on sound science and risk management [10,14,15]. Under the new QbD paradigm, impurities should not only be tested in the final API, but rather be proactively controlled in the manufacturing process. By developing a product with this goal in mind, end product testing would be solely used for the confirmation of product quality since the process understanding and/or process control provides sufficient evidence that batches will meet the specification if tested [10]. However, only a handful of discussions with regard to a systematic approach to the investigation and control of process impurities have been made available over the past few years [7,8,16–18]. Particularly, the development of a comprehensive QbD approach to impurity investigation in order to actively seek out drug quality is not widely discussed in the literature.

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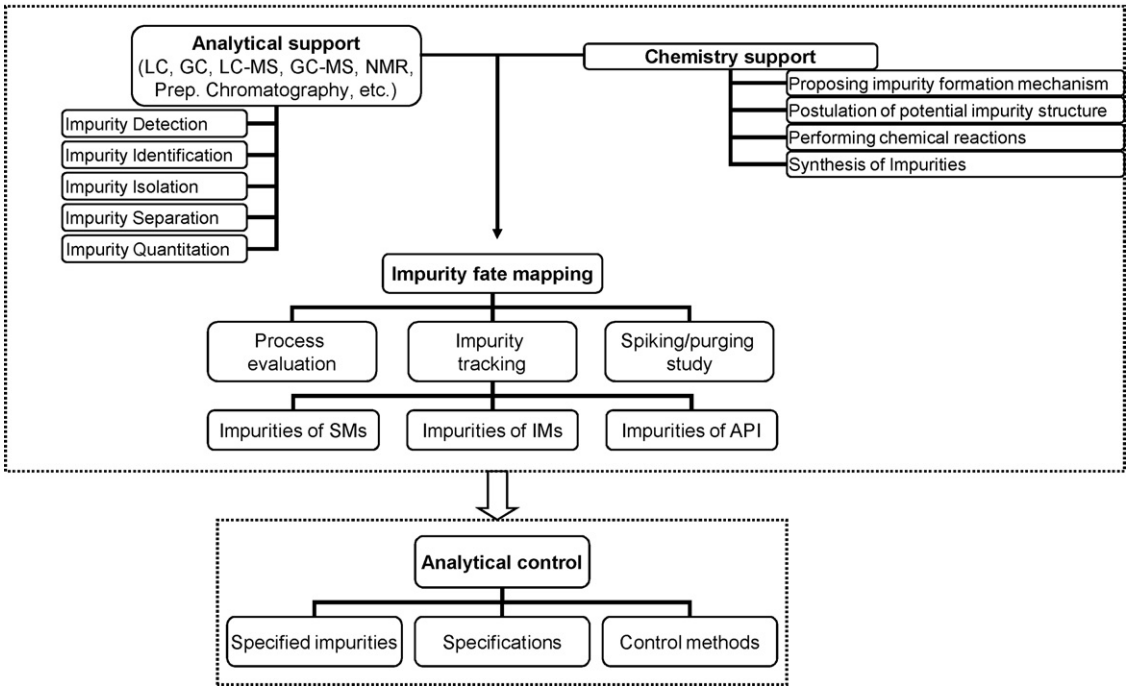


Fig. 1. General outline of the impurity fate mapping framework.

A comprehensive and systematic approach to analytical control of process impurities by *impurity fate mapping* (IFM) is reported in this paper. This approach is taken to actively search for possible impurities in an API process, obtain intrinsic knowledge of the origin, formation pathway, fate, and process purgeability of impurities, design risk mitigation steps to reduce those impurities during reactions, and finally derive a comprehensive and scientifically justified control strategy for the overall process [19,20]. A general outline of such an IFM framework is provided in Fig. 1, which is a joint effort from a cross-functional team involving analytical scientists, synthetic chemists, and process engineers. This report highlights the analytical perspective of the IFM process.

To successfully support IFM activities, the application of various complementary analytical techniques for impurity detection, structural identification, separation/isolation, and quantification are essential [21]. For instance, liquid chromatography (LC) with UV detection is an essential tool for separation and determination of impurities in drugs [2,22] which is backed up with gas chromatography (GC) when impurities are not amenable to LC, whereas the orthogonal techniques, LC–mass spectrometry (LC–MS) and GC–MS are universal tools for impurity tracking and identification [23–25]. When definitive structures are required, isolation by preparative chromatography followed by nuclear magnetic resonance spectroscopy (NMR) analyses is a powerful technique for characterization of impurity structures [6,21]. The use of these orthogonal techniques as appropriate helps to maximize the impurities that can be detected and the structures that can be confirmed. Typically IFM commences at the late development stage when the final route of synthesis and an optimal process are finalized where a comprehensive impurity control strategy is needed. This means that analytical methods used for impurity testing at the early development stage may not be suitable for IFM, because of changes in impurity profiles caused by changes in the synthetic route from early to late development stages as well as the greater number of impurities identified through IFM itself. Therefore, evolution of analytical methods becomes a key aspect of IFM, which can pose significant challenges to the analytical method development. Furthermore, analytical control strategy of process impurities can be derived based on the knowledge gained throughout the IFM. As part of the overall control strategy (including the effective and efficient control of quality process parameters and analytical control of impurities), the analytical control is usually comprised of three key aspects as shown in Fig. 1: (1) designation of specified impurities, (2) setting of scientifically justified specifications, where IFM can make significant contributions, and (3) development of simple, robust and rugged analytical control methods for use over the product lifecycle.

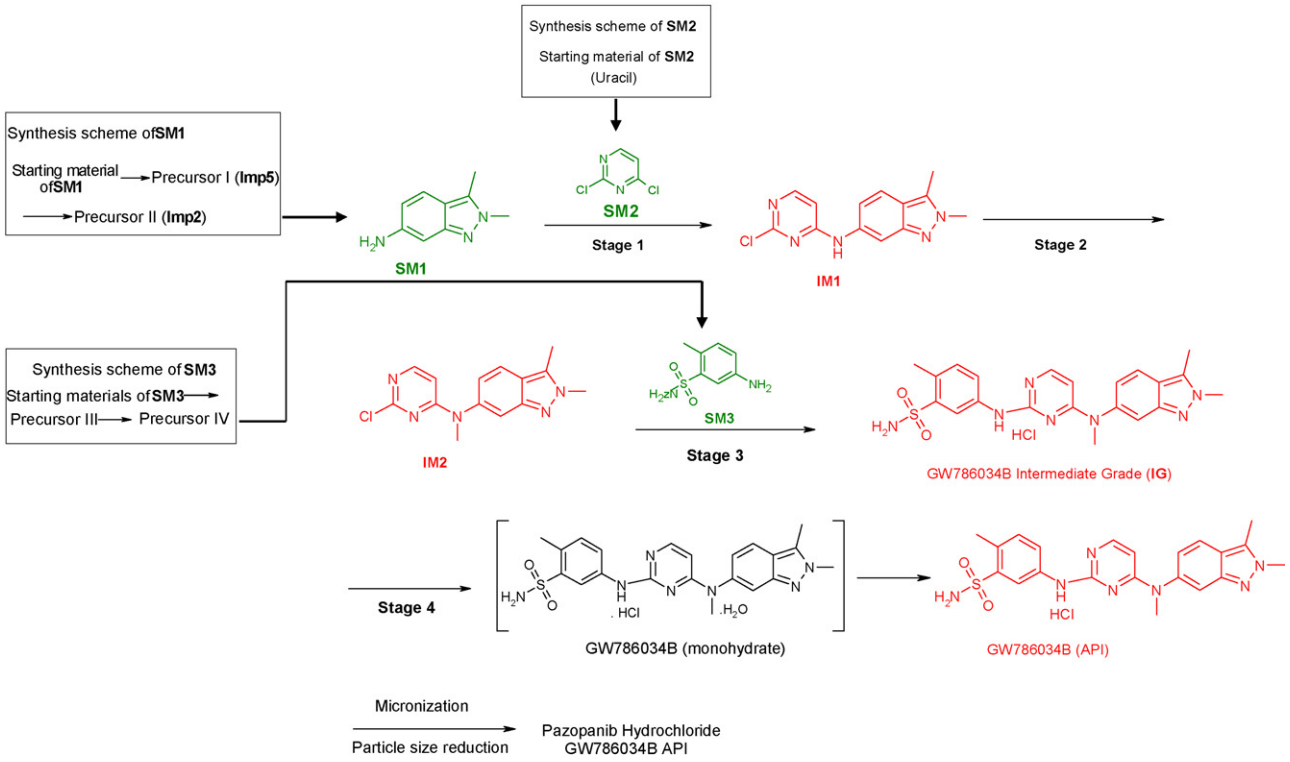
By emphasizing process understanding and risk management, the IFM approach outlined in Fig. 1 has been successfully applied to the understanding and control of process impurities in Pazopanib hydrochloride (**GW786034B** in Scheme 1), a VEGFR tyrosine kinase inhibitor approved recently by the U.S. FDA for treatment of renal cell carcinoma [26]. The work presented in this paper illustrates how QbD concepts are applied to the analytical control of process impurities in API. The analytical support to the IFM of Pazopanib hydrochloride and analytical challenges encountered during this process are addressed in this article.

2. Experimental

2.1. Materials

Drug substances, intermediates, and impurities were synthesized or isolated by preparative chromatography in house at GlaxoSmithKline. The starting materials (SMs) were obtained from various vendors: three vendors V1a, V1b and V1c for **SM1**; two vendors V2a and V2b for **SM2**; and three vendors V3a, V3b and V3c for **SM3**. Trifluoroacetic acid (TFA), formic acid, ammonium acetate, and HPLC grade acetonitrile were all from J.T. Baker (Phillipsburg, NJ, USA). De-ionized water was obtained from a Milli-Q system (Millipore, Bedford, MA, USA).

The HPLC columns used in this study include Waters (Milford, MA, USA) Xterra MS C18 column (150 mm × 4.6 mm, 3.5 μm), Xbridge C18 column (150 mm × 4.6 mm, 3.5 μm), Xbridge phenyl column (150 mm × 4.6 mm, 3.5 μm), Sunfire C18 column (150 mm × 4.6 mm, 3.5 μm), and SymmetryShield RP18



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 **IM2**. 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.

(150 mm × 4.6 mm, 5 μm) column; Phenomenex (Torrance, CA, USA) Luna C18 (2) column (150 mm × 4.6 mm, 3 μm) and Luna C18 (2) column (50 mm × 2.0 mm, 3 μm); Agilent (Wilmington, DE, USA) Zorbax SB C8 column (150 mm × 4.6 mm, 3.5 μm), and Zorbax Bonus-RP column (150 mm × 4.6 mm, 3.5 μm). An Akzo Nobel/Kromasil (Brewster, NY, USA) Kromasil C18 column (250 mm × 10 mm, 10 μm) was used for preparative chromatography.

In general, LC–UV conditions were purposely selected to be compatible with MS for LC–MS investigation. However, for certain LC–UV method, which may need to use non-MS friendly conditions (e.g. perchloric acid in method F). In such a case, a secondary MS compatible method was developed for LC–MS investigation.

2.2. High performance liquid chromatography (HPLC)

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

2.3. Mass spectrometry

For chemical reaction monitoring, an Agilent (Palo Alto, CA, USA) ion trap mass spectrometer equipped with an electrospray ion source was used, which was operated in the positive ion mode. Chromatographic separations were achieved by using an Agilent binary HP1100 system with a Phenomenex Luna C18 (2) column (50 mm × 2 mm, 3 μm). The exact mass LC–MS experiments were performed on a Q-TOF Premier mass spectrometer (Waters, Manchester, UK) coupled to an Agilent 1100 HPLC system controlled by MassLynx 4.1 software. The electrospray ionization source was operated in the positive ion mode with a spray voltage of 3.5 kV. The source and desolvation gas temperatures were set to 120 and 300 °C, respectively. The desolvation gas flow rate was 600 L/h, and the sample cone voltage was set to 30 V. Argon was used as the collision gas at 0.45 mL/min with a collision energy of 10 eV for MS and 30 eV for MS/MS experiments. A solution of leucine-enkephalin at *m/z* 556.2771 was used as the lock mass, which was introduced via a Lockspray™ device.

2.4. Nuclear magnetic resonance spectroscopy

NMR experiments on the preparative isolates and synthesized materials were conducted on a Bruker DRX700 NMR spectrometer (Bruker Instrument, Billerica, MA, USA) using a 5 mm Bruker broadband inverse (BBI) probe in DMSO-d_6 at temperatures of 60 °C. NMR structure elucidation was performed using standard 1D (^{1}H and ^{13}C) and 2D NMR experiments including short and long-range heteronuclear correlation with the heteronuclear single-quantum coherence (HSQC) and heteronuclear multiple-bond coherence (HMBC) experiments, respectively. ^{1}H and ^{13}C NMR spectra were referenced to the solvent resonances.

3. Results and discussion

3.1. Process evaluation

A thorough process evaluation was first conducted at the beginning of the IFM of Pazopanib hydrochloride. This assessment was used to provide initial input of known and potential impurities that may be generated in the process, and help the planning of further experiments. This was mainly a paper exercise including an analytical evaluation and a chemistry assessment. Close collaboration between the two disciplines is essential to the initiation of the IFM.

The purpose of the analytical evaluation was to collect any available analytical information of known impurities identified prior

to IFM. All the analytical information with respect to the process impurities in Pazopanib hydrochloride, such as batch analysis history acquired at early development stages, impurities in SMs identified by chemists while working with the vendor's synthetic routes, and degradants that were discovered during storage or previous stability studies, were collected and evaluated.

In parallel, the chemistry assessment was conducted based upon the chemistry of the synthetic route so that a list of potential impurities can be postulated for further investigation. A single route of synthesis, which uses well-established chemical transformations and has been used throughout the development of Pazopanib hydrochloride, was selected and refined to produce the final commercial manufacturing process. A schematic representation of the synthetic route is given in Scheme 1. It is a four-stage synthesis involving three SMs (**SM1**, **SM2**, and **SM3**), three isolated solid IMs (**IM1**, **IM2**, and **IG**), and a separate particle forming and size reduction step to produce Pazopanib hydrochloride drug substance (API). Since the last micronization step only affects physical properties and not the impurity content, the IFM was focused on stages 1–4.

As many impurities could arise from the purchased materials such as SMs, good understanding of multiple sources of SMs along with the vendor's synthetic routes and previous knowledge on impurities in the samples from different SM suppliers are of particular importance to the success of IFM. For example, materials provided by different vendors may have various impurity profiles. So if impurities that arise from a certain SM were not included in IFM due to the uncertainties of SM sources and their synthetic routes, a targeted analytical method cannot be obtained, thus placing the analytical method and specification at risk at late development stage. To minimize the risk of uncertainties associated with SMs for late phase assets, it is imperative for the project team to identify multiple SM sources as soon as the route of synthesis is fixed and evaluate all the synthetic schemes. One of the challenges faced by IFM is that many suppliers are reluctant to share their knowledge with regard to the pertinent route for the synthesis of SMs or have limited understanding of the potential impurities. In fact, the results of IFM for SMs can help vendors to control impurities in their processes, which is a true benefit that may not be recognized by many vendors, and may need to be communicated to enhance vendor's understanding of IFM. Through the assessment of the synthetic route of registered SMs, it was found that the suppliers for each SM used a similar synthetic scheme as shown in Scheme 1, which greatly facilitated the IFM of Pazopanib hydrochloride. Besides the synthetic route, any impurity information based on vendor and our knowledge were also collected if available.

3.2. Impurity tracking

Based on the initial input on potential impurities by the process evaluation described in Section 3.1, impurity tracking from SMs, through IMs and finally to API of Pazopanib hydrochloride manufacturing process was carried out. The applications of appropriate analytical techniques such as LC–UV, LC–MS, and NMR for impurity detection, identification, isolation and quantification during this process, along with the evolution of analytical methods are discussed in the following sections.

3.2.1. Evolution of analytical methods

It is likely that the fit for purpose analytical methods at the early development stages may not be suitable for the tracking of all potential impurities identified in IFM due to the limited knowledge of impurities prior to IFM. As IFM progressed for Pazopanib hydrochloride and more impurities were identified, these analytical methods had to be modified or changed entirely in order to accommodate the analysis of various impurities discovered. As such, the development of appropriate analytical methods is critical. It is highly desirable that impurity standards/markers are made available, either isolated by preparative chromatography or synthesized chemically, to assist HPLC method development and validation as well as further impurity spiking/purging studies (Section 3.3). However, for cases where impurity standards are difficult to obtain, alternative approach can be pursued (see Section 3.3). To be mentioned is that once IFM is completed and overall control strategy is defined, more straightforward, robust and rugged analytical control methods targeting on specified impurities can then be developed to be suitable for the use in manufacturing environment. The evolution of analytical methods for SMs, IMs, and API is summarized in Table 1. The detail examples are given in Sections 3.2.2–3.2.4 for analytical methods supporting IFM and Section 3.4.3 for analytical control methods that will be used over the product lifecycle.

3.2.2. Impurities of the SMs

Impurities originating in SMs can be carried to the next stage, and can also be transformed by the process into new impurities in the IMs and/or eventually in the API. Impurity tracking was therefore conducted starting from the three registered SMs (**SM1**, **SM2**, and **SM3**) of Pazopanib hydrochloride.

Batches of SMs manufactured by different vendors were obtained and analyzed by the LC–UV methods that were initially used to test the impurities known at the early development stages. With the assistance of LC–MS tool, impurities present in the batches of SMs were identified. Special attention was given to the predicted potential impurities which were proposed at the time of process evaluation. In addition, stability or forced degradation studies were also conducted for the SMs, as the analysis of the degraded samples was helpful to reveal new degradation impurities that may not have been detected or predicted [27]. For instance, such an exercise for the three Pazopanib hydrochloride SMs led to the discovery of degradation impurities including uracil and **Imp9** for **SM2** (structure not disclosed).

As a result of collaborative exercise between chemistry and analytical, nine potential impurities which are believed to present a risk to the purity of **SM1** were proposed (structures not shown, one of which is a genotoxic impurity dimethyl sulfate). Similarly, three and seven potential impurities that could arise from **SM2** and **SM3** were proposed, respectively. Such impurities were then included in the scope of the analytical method development for SMs. This ensures the right analytical method was used for impurity tracking. In order for analysts to check if the methods developed at the early development stages were adequate for all impurities proposed by IFM and the need to develop a new method, authentic impurity markers were obtained, either by chemical synthesis or isolation by preparative chromatography. Taking **SM1** as an example, method A (Fig. 2a) had been developed and initially used based on the limited knowledge of impurities available at early development stages. After the thorough IFM assessment for **SM1**, nine potential impurities were proposed and obtained to evaluate this method. It was found that the two potential impurities (two **SM1** precursors **Imp2** and **Imp5**) were co-eluting under such method conditions (Fig. 2a). This was initially not recognized due to two reasons: (a) lack of comprehensive knowledge of **SM1** synthetic process and its impurities at the early development stages; and (b) all **SM1** batches tested were so clean (total impurities $\leq$0.1%) that none of them contained these two precursors at the measurable level (<0.05%). Because of the inadequate separation between these two impurities, a new HPLC method (method B) that could separate all impurities of concern was developed for **SM1** (Fig. 2b). Among the nine potential impurities in **SM1**, dimethyl sulfate (a genotoxic impurity in **SM1**) was analyzed using a separate method [28], and therefore not discussed here.

Table 1
Evolution of analytical methods.

Process material	Initial method	Method for IFM	Control method
SM1	**Method A** Phenomenex Luna C18(2) column (50 mm × 2 mm, 3 μm); wavelength 220 nm; flow rate 1 mL/min; temperature 40 °C; mobile phase: A = 0.05% (v/v) TFA in water, B = 0.05% (v/v) TFA in acetonitrile; gradient: 0–95% B in 8 min	**Method B** Zorbax SB-C8 column (150 mm × 4.6 mm, 3.5 μm); wavelength 297 nm; flow rate 1 mL/min; temperature 40 °C; mobile phase: A = 10 mM ammonium acetate in water, B = acetonitrile; gradient: 16–40% B in 25 min	**Method B**
SM2	**Method C** Zorbax SB phenyl column (150 mm × 4.6 mm, 3.5 μm); wavelength 259 nm; flow rate 1 mL/min; temperature 40 °C; mobile phase: A = 0.05% (v/v) TFA in water, B = 0.05% (v/v) TFA in acetonitrile; gradient: 0–95% B in 30 min	**Method D** Zorbax SB phenyl column (150 mm × 4.6 mm, 3.5 μm); wavelength 259 nm; flow rate 1 mL/min; temperature 30 °C; mobile phase: A = 0.05% (v/v) TFA in water, B = 0.05% (v/v) TFA in acetonitrile; gradient: 0–86% B in 18 min	**Method D**
SM3	**Method E** Symmetry Shield RP18 column (150 mm × 4.6 mm, 5 (m); wavelength 242 nm; flow rate 1 mL/min; temperature 30 °C; mobile phase: A = 20 mM ammonium acetate in water, B = 20 mM ammonium acetate in water/acetonitrile (20/80, v/v); gradient: holding 10% B for 10 min (0–10 min), then ramp up to 100% B in 20 min (10–30 min)	**Method F** Zorbax Bonus-RP column (150 mm × 4.6 mm, 3.5 μm); wavelength 220 nm; flow rate 1 mL/min; temperature 40 °C; mobile phase: A = 0.1% (v/v) perchloric acid in water, B = acetonitrile; gradient: 2–45% B in 24 min	**Method F**
IM1	**Method A**	**Method G** Zorbax SB phenyl column (150 mm × 4.6 mm, 3.5 μm); wavelength 220 nm; flow rate 1 mL/min; temperature 40 °C; mobile phase: A = 0.05% (v/v) TFA in water, B = 0.05% (v/v) TFA in methanol; gradient: 30–95% B in 30 min	**Method H** Waters Xbridge C18 column (150 mm × 4.6 mm, 3.5 μm); wavelength 242 nm; flow rate 1 mL/min; temperature 40 °C; mobile phase: A = 10 mM ammonium acetate in water, B = acetonitrile; gradient: 16–47% B in 20 min
IM2	**Method A**	**Method I** Phenomenex Luna C18 (2) column (150 mm × 4.6 mm, 3 μm); wavelength 220 nm; flow rate 1 mL/min; temperature 40 °C; mobile phase: A = 0.05% (v/v) TFA in water, B = 0.05% (v/v) TFA in acetonitrile; gradient: from 15% to 50% B in 20 min, then from 50% to 95% B in 5 min	**Method J** Agilent Zorbax Bonus-RP column (150 mm × 4.6 mm, 3.5 μm); wavelength 290 nm; flow rate 1 mL/min; temperature 40 °C; mobile phase: A = 0.1% (v/v) TFA in water, B = acetonitrile; gradient: 18–68% B in 17 min
IG	**Method K** Phenomenex Develosil RP–Aqueous –3 column (150 mm × 4.6 mm, 3.5 μm); wavelength 214 nm and 268 nm; flow rate 1 mL/min; temperature 35 °C; mobile phase: A = 0.1% (v/v) TFA in water/acetonitrile (90.5/9.5), B = 0.1% (v/v) water/acetonitrile (40.5/59.5); gradient: holding 0% B for 1 min (0–1 min), then ramp up to 90% B in 30 min (1–31 min)	**Method K**	**Method L** Agilent Zorbax Bonus-RP column (150 mm × 4.6 mm, 3.5 μm); wavelength 268 nm; flow rate 1 mL/min; temperature 40 °C; mobile phase: A = 0.1% (v/v) TFA in water, B = acetonitrile; gradient: 20–56% B in 12 min
API	**Method K**	**Method K**	**Method L**

Second example on SM impurity tracking leading to method evolution can be taken from **SM3**. The HPLC method (method E) as shown in Fig. 3a was demonstrated appropriate for testing **SM3** impurities identified prior to IFM and was used initially. With the progress of the IFM, more impurities were identified for **SM3**. A potential impurity (**Imp14**, structure not shown), an isomer of **SM3**, was proposed and synthesized for evaluating this method. Since it was a structural isomer of **SM3**, this adds new challenges to the analytical method. Indeed, it was found that the separation between **Imp14** and **SM3** was poor with a resolution less than 1.5 by using the initial method E (Fig. 3a). Therefore, a new HPLC method (method F) was developed to separate all potential **SM3** impurities identified via IFM (Fig. 3b).

The investigation of impurities in **SM2** was done in the similar manner as for **SM1** and **SM3** thus not discussed in detail here. The above case studies of SMs highlighted the importance of selective analytical methods in the process understanding and control of impurities. Without the right method to measure the impurities, potential impurities could be inevitably missed. This may lead to incomplete IFM and ultimately impact API quality. It is worth mentioning that all analytical methods used to support impurity tracking of SMs had been developed using a previously reported science-based and risk-based HPLC method development approach [29] and fully validated. The details of method validation are out of the scope of this paper.

3.2.3. *Impurities of the IMs*

Following the impurity mapping of the SMs, impurity tracking continued to stage 1 through stage 4. The potential impurities of each stage postulated during process evaluation (Section 3.1) were used as the basis for this exercise. When performing impurity tracking in IMs, special attention was given to the search for these potential impurities either by LC–MS via the expected molecular ions or by LC–UV using impurity markers.

Firstly, the isolated product of each stage was analyzed thoroughly. Any impurities detected by LC–UV (method A) at levels

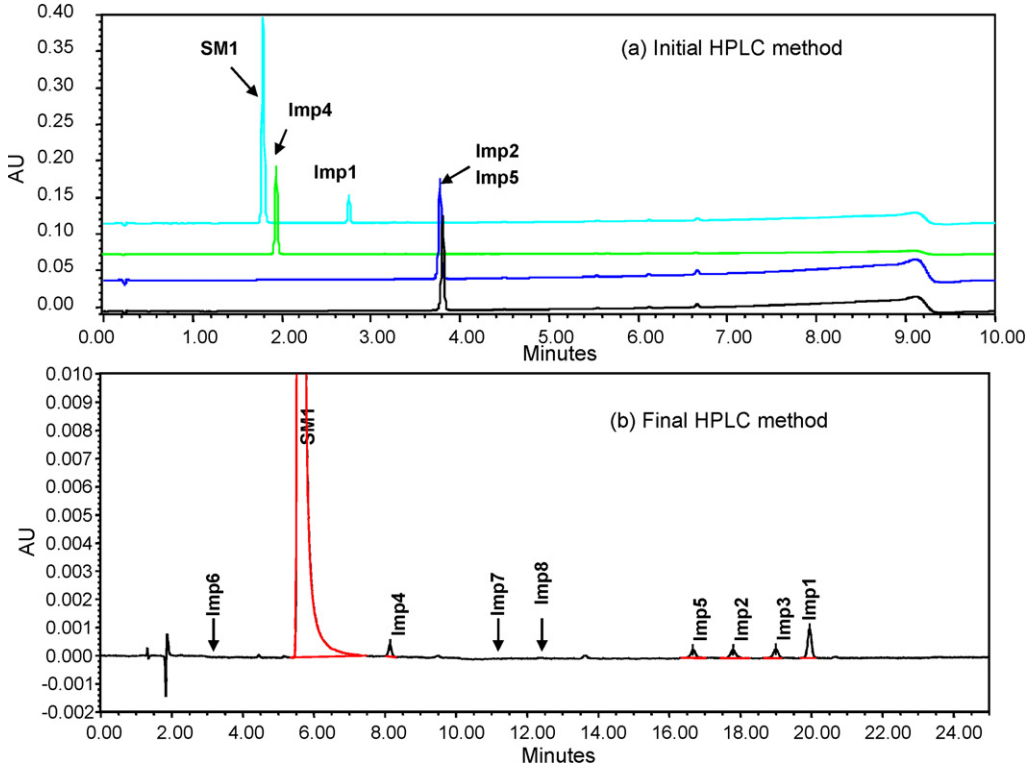


Fig. 2. Chromatograms of (a) the initial HPLC method for **SM1** (see method A in Table 1 for HPLC conditions) and (b) the final HPLC method for **SM1** (see method B in Table 1 for HPLC conditions).

higher than 0.05% area under typical manufacturing conditions were subjected to structural identification by LC–MS, and tentative structures were proposed. Secondly, the reaction process of each bond forming stage was stressed by running for extended times to generate the potential impurities. The reaction mixtures at various time points were sampled and analyzed by LC–MS (with UV detection in tandem) to monitor the reaction progress and impurity formation. An example of the chemical reaction monitoring is given

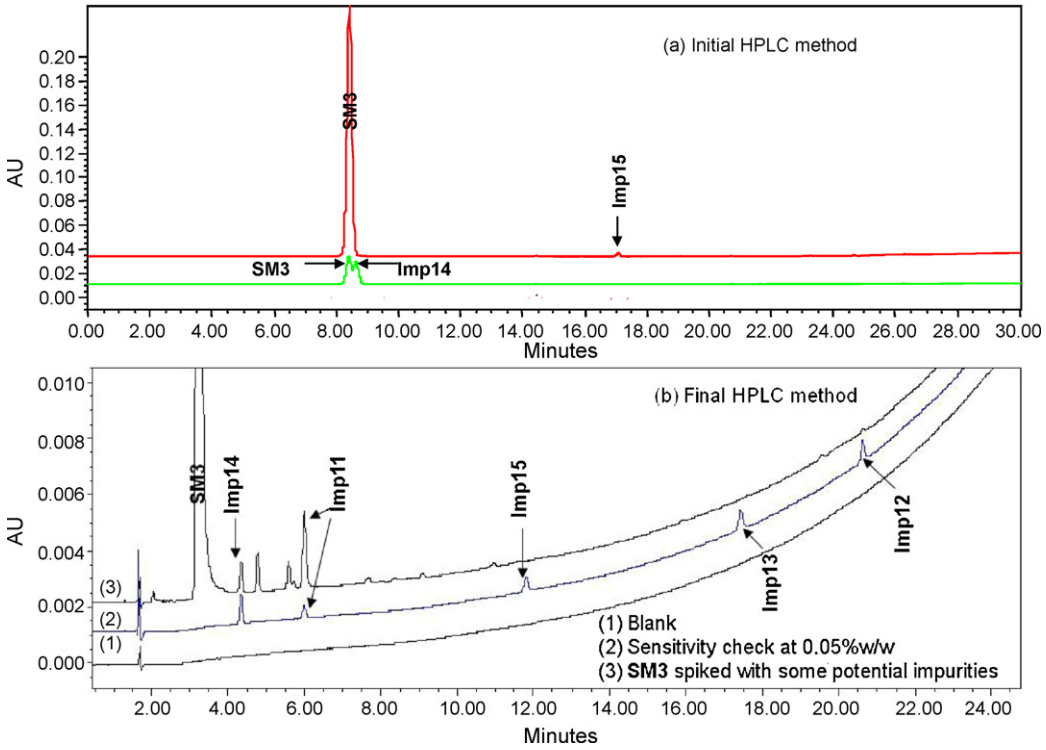


Fig. 3. Chromatograms of (a) the initial HPLC method for **SM3** (see method E in Table 1 for HPLC conditions) and (b) the final HPLC method for **SM3** (see method F in Table 1 for HPLC conditions).

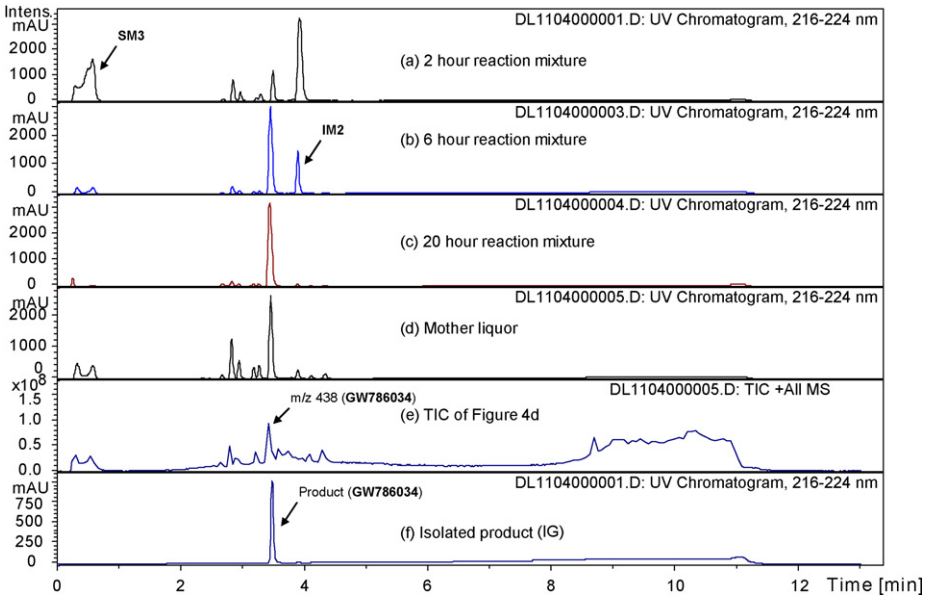
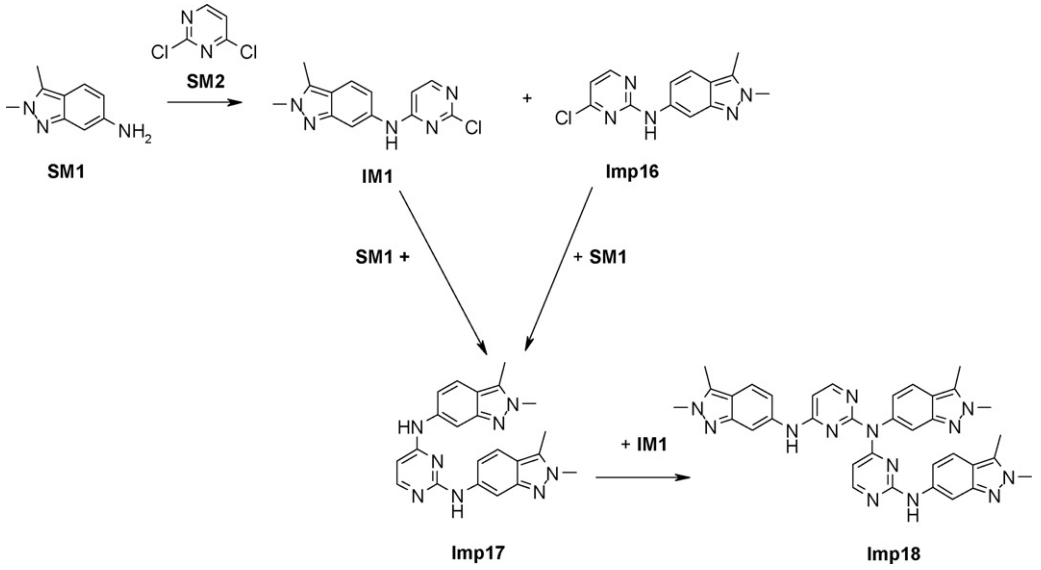


Fig. 4. Chromatograms of LC–MS for stage 3 chemical reaction monitoring at various time intervals. LC conditions: see method A in Table 1, extend gradient by holding at 95% B for 30–60 min.

in Fig. 4 where stage 3 reactions were performed. Fig. 4a–c shows the UV trace of the LC–MS analyses of the stage 3 reaction mixture at selected time points 2, 6 and 20 h. The UV chromatograms of the mother liquor and the isolated product are shown in Fig. 4d and f, respectively. As an example, Fig. 4e shows a total ion chromatogram of the LC–MS analysis of the mother liquor indicating the complex impurity profiles. Normally, one would think it was unnecessary to study the impurity peaks in the mother liquor wash. However, in the context of IFM, there is benefit from understanding the fate of the impurities in the mother liquors as well. Understanding the impurity profile of the wash helped confirm the amount of impurities that could be removed from the wet cake. Furthermore, many impurities were enriched in the mother liquor, which facilitated structural identification and resulted in increased understanding of their removal from the desired product. Since the MS data were collected on an ion trap mass spectrometer in the data-dependent mode, MS^n data (multistage MS/MS) were acquired automatically [30]. Thus, after a single injection, tentative structures of most of these impurities can be established rather quickly. The short 8 min gradient LC–MS chromatographic conditions may not guarantee the complete elution of highly lipophilic impurities, if existed. Thus, confirming the absence of such late eluting peaks was imperative and was achieved by a 30–60 min column wash at high organic content (usually 95% acetonitrile). In some instances when LC–MS alone was not sufficient for positive identification of impurity structures, additional on-line analytical techniques such as LC–NMR were needed for further structural confirmation. Should the impurity levels be too low for LC-NMR structure elucidation, the impurity was isolated from the mother liquors by preparative LC for tube NMR studies. This example is not detailed here as similar examples will be provided in Section 3.2.4.

The investigation into the impurities of IMs could help reveal potential impurities that might be overlooked or hidden under other peaks by the analytical methods used at the early devel-



Scheme 2. **Imp17** was proposed as the precursor of **Imp18** in stage 1.

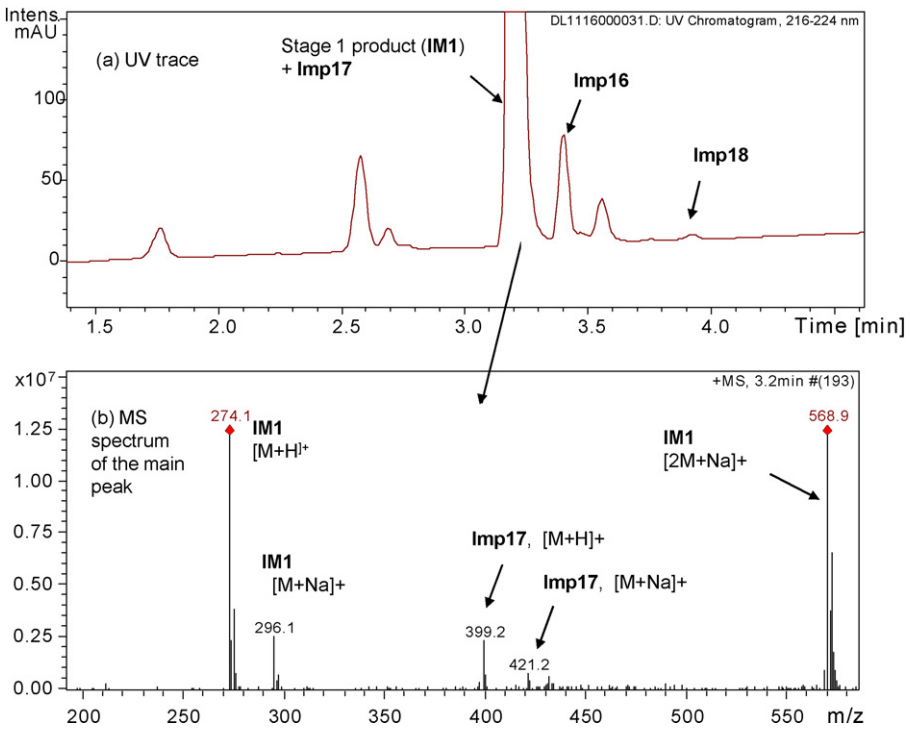


Fig. 5. Discovery of **Imp17** hidden under the main peak **IM1** by LC–MS investigation. LC conditions: see method A in Table 1, extend gradient by holding at 95% B for 30–60 min.

opment stage. Taking the chemical reaction monitoring of stage 1 reaction as an example (Scheme 2), **Imp 17** was initially undetected due to co-elution with the main peak **IM1**. The most abundant impurity in **IM1** (Fig. 5) was **Imp16**, which was identified as a regioisomer of **IM1**. It gave an $[M+H]^+$ ion at m/z 274 indicating a molecular weight of 273 Da. **Imp18** (the pseudo trimer of **IM1**) was detected at very low level in a crude reaction mixture, which gave a protonated molecule $[M+H]^+$ at m/z 636. Its structure was proposed as shown in Scheme 2. The detection of this impurity, however, led to the identification of **Imp17**. The hypothesis was that intermediate **Imp17** (the pseudo dimer of **IM1**) must exist and it must have served as a precursor to **Imp18** as illustrated in Scheme 2. **Imp17** can be generated via addition of a second molecule of **SM1** onto **IM1** or **Imp16**. Indeed, careful examination of the LC–MS data led to the discovery of **Imp17** hidden under the main peak **IM1** when using the initial LC conditions for reaction monitoring (Fig. 5a). The MS spectrum of the main peak at 3.2 min is shown in Fig. 5b. Besides the three ions at m/z 274 $[M+H]^+$, 296 $[M+Na]^+$, 569 $[2M+Na]^+$ belonging to **IM1**, two additional ions at m/z 399 $[M+H]^+$ and 421 $[M+Na]^+$ were observed which belong to **Imp17**. Furthermore, it was found that several additional potential impurities proposed by chemical reaction monitoring cannot be sufficiently separated by the initial LC method (method A). Therefore, an improved method (method G) was developed and used for impurity tracking of **IM1**. This method as shown in Fig. 6 could adequately separate all impurities of concern in the stage 1 reaction and was compatible to MS instrumentation. To ensure accurate data generated for IFM, the method was appropriately validated and the detail validation requirements will be discussed in Section 3.3. This analytical approach was applied to the chemical reaction monitoring of all bond forming stages of the Pazopanib hydrochloride process.

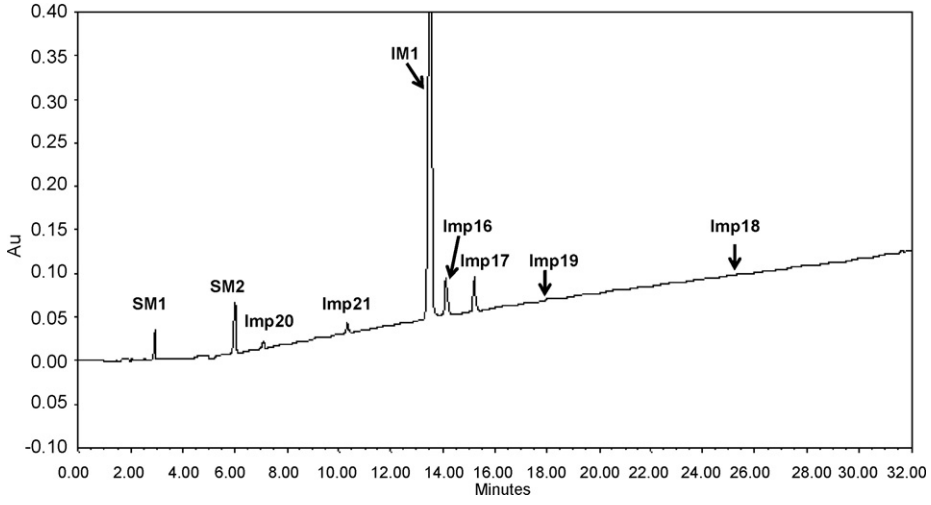


Fig. 6. Chromatogram of the improved stage 1 impurity profile method. HPLC conditions: see method G in Table 1.

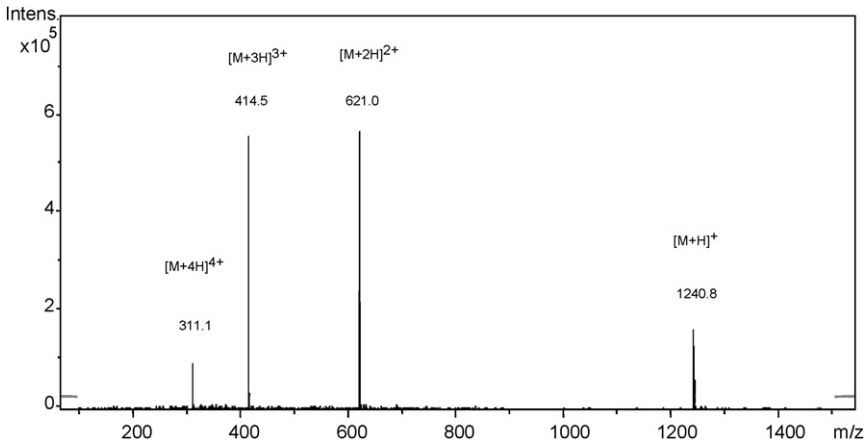


Fig. 7. MS spectrum of **Imp35**.

3.2.4. *Impurities of API*

Tracking the impurities from the API to the SMs was conducted in parallel to the tracking of impurities in SMs and IMs (Sections 3.2.2 and 3.2.3). In current Pazopanib hydrochloride batches manufactured so far, only two impurities (**Imp34** and **Imp35**) were detected by LC–UV at low levels. In such a case, the scope of tracking impurities of API was somewhat limited. However, it was a critical element of the IFM because any impurity found in API would typically be defined as a critical quality attribute (CQA). The identification of **Imp35** in a specific batch of API is a good example for this approach.

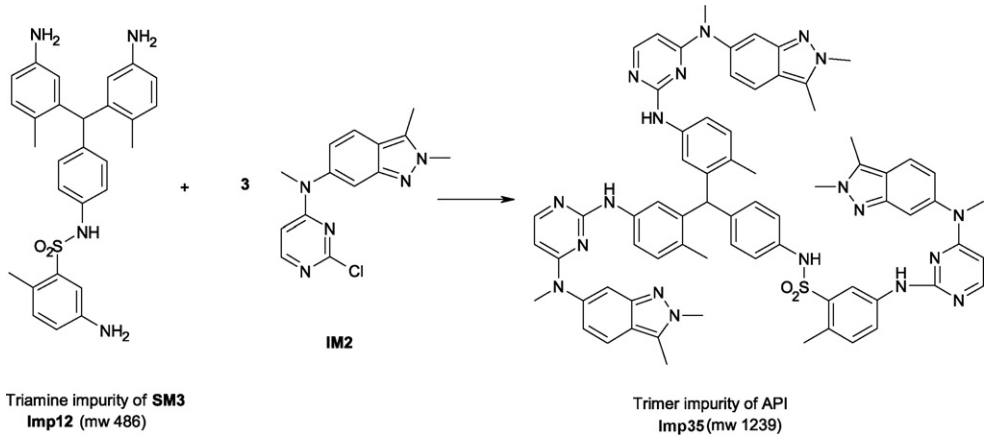
Imp35, referred to as the trimer of API, was detected at 0.06% area level in one of API batches. The trimer impurity had a relative retention time to API of 1.6 (by method K), indicating that the compound was either large or very hydrophobic. By LC–MS analysis, the protonated molecule of **Imp35** was measured at *m/z* 1240.8. This impurity also gave intense doubly- and triply-charged ions at *m/z* 621.0 and 414.5 respectively, as illustrated in Fig. 7. An in-depth NMR analysis had to be undertaken to determine the molecular structure of **Imp35**. As the relatively low level of this impurity presents a sensitivity problem for LC-NMR analysis, preparative chromatography was initiated to purify a small quantity for the NMR analysis. The purified compound was studied by ^{1}H NMR, ^{13}C NMR, and 2D NMR experiments, as well as high resolution mass spectral analysis (spectra not shown). The results suggested that the structure of **Imp35** is composed of three **IM2** units and a residual core portion of 486 Da as indicated by the MS fragmentation data.

In order to solve the missing piece of the puzzle (486 Da), targeted LC–MS analysis was performed on the specific batch of **SM3** purchased from V3-a, which was used for manufacturing of this particular batch of API. As a result, a precursor **Imp12** (triamine of **SM3**) having an *m/z* of 487 $[M+H]^+$ was identified in the batch of **SM3**. A plausible formation mechanism of one molecule of **Imp12**, which served as a core building block for **Imp35**, reacting with three molecules of IM2 at the stage 3 was postulated (Scheme 3). **Imp35** was not observed in API batches synthesized using the corresponding **SM3** batches that did not contain **Imp12**, which clearly suggested that **Imp12** was a crucial building block for the formation of **Imp35**. Finally, an authentic compound of **Imp35** was synthesized and subjected to MS and NMR spectral analysis as the structural confirmation (Figs. 7 and 8).

Tracking the impurities in the API enabled the discovery of the **Imp35** which was identified as a CQA of the drug substance and can be traced back to Imp12 in **SM3**. Knowing the structure and the origin of **Imp35** can help set an appropriate control strategy in an effort to minimize the presence of **Imp35** in future production batches. The same approach was used to investigate **Imp34**, which can be traced back to **Imp11** in **SM3** and was identified as another CQA of the drug substance.

3.3. *Impurity spiking/purging study*

Various impurities were spiked into the appropriate stages of the Pazopanib hydrochloride manufacturing process at much higher levels than typically observed to determine their fate



Scheme 3. Formation mechanism of **Imp35**.

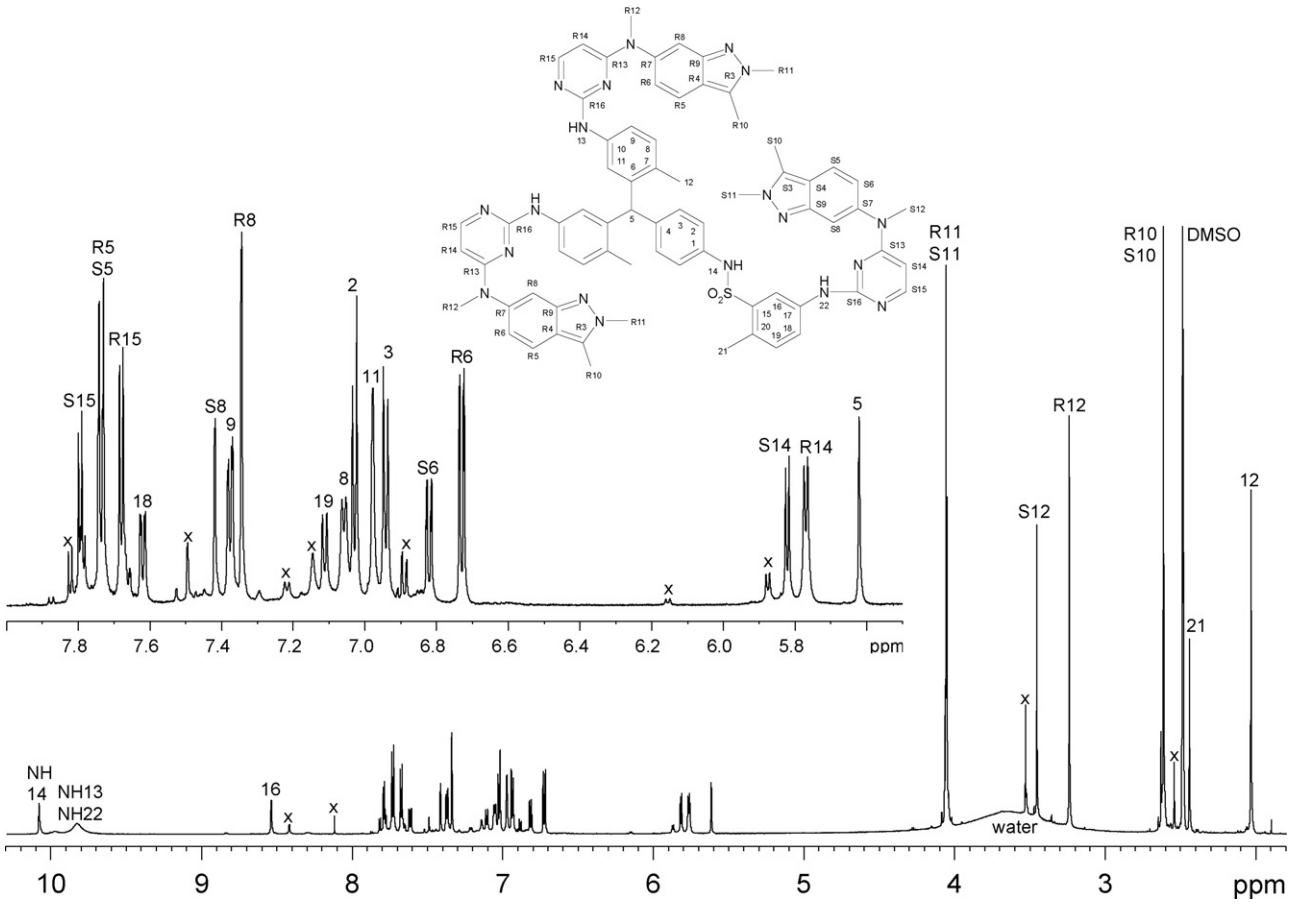


Fig. 8. ^{1}H NMR spectrum of **Imp35** in DMSO-d_6 at 60 °C.

through the process. This was referred to as a spiking/purging study. This study was aimed to: (1) check if the impurity can be completely purged through the process, otherwise it should be controlled as the specified impurity in the isolated product of that stage (see Section 3.4.1); (2) determine the level to which the specified impurity was purged by the subsequent reaction steps, and this provided sound scientific justification for development of suitable specifications for the analytical control of specified impurities at appropriate points (see Section 3.4.2). With the assistance of selective and sensitive analytical methods, greater insights into the ability of the manufacturing process for impurity removal were achieved.

Based on a risk assessment (detailed in Section 3.4.1), selected impurities were subjected to the spiking/purging exercise. Impurities were considered purged when the level of the impurity in isolated product was below 0.05% area by HPLC analysis. If the impurities are of genotoxic potential, they were considered purged when the levels became below the TTC (threshold of toxicological concern). For Pazopanib hydrochloride process impurities, generally good purgeability was observed except for **Imp34** and **Imp35** which displayed limited purgeability. The output of spiking/purging experiments for some of the specified impurities in **SM3**, **IM1** and **IG** are exemplified in Table 2. Examples for the spiking/purging studies of the two **IM1** impurities (**Imp16** and **Imp17**) are detailed below.

As mentioned, **Imp16** was the isomer of **IM1** formed during stage 1 reaction (see structure in Scheme 2). In the isolated **IM1**, it was typically detected at no more than 2.0% area. Spiking/purging experiments were performed at 1.5%, 3%, and 5% (w/w) (equivalent to 2, 4 and 6% area) of **Imp16**. The results showed that **Imp16** and its methylated stage 2 product (**Imp22**) were purged completely in the stage 3 output. **Imp17** (see structure in Scheme 2) is a pseudo dimer of **IM1** produced under the stage 1 reaction conditions from the reaction of 2 equivalents of **SM1** with **SM2**. Spiking/purging experiments of **Imp17** were conducted at the 2 and 5% (w/w) levels (equivalent to 3 and 8% area). It was found that **Imp17** and its by-products were eliminated in stage 3 outputs at a level as high as 5% (w/w).

Since the analytical methods were of critical importance to the accuracy of the spiking/purging data, which were ultimately used in the development of the overall control strategy, all methods supporting IFM were appropriately validated to demonstrate the required specificity, linearity, sensitivity, etc. It should be emphasized that the limit of quantitation (LOQ), typically at 0.05% level, and the relative response factors (RRFs) of impurities were determined to evaluate the actual amount of impurities (%, w/w) that can be purged through the process (usually by LC–UV, % area). The values of RRF and LOQ were usually determined when appropriate amount of authentic impurity standards became available. When the amount of authentic impurity standards were insufficient for multiple weight/point validation as required for formal validation [31], the single weight/point validation was acceptable at this stage. In other instances, although mixtures with enriched impurities were obtained and used to check method specificity, it was quite difficult to get authentic impurity standards even for single weight/point validation of RRF and LOQ. Under this circumstance, analysts can alternatively estimate LOQ and RRF by comparing the structures and UV spectra of the testing impurities to other known impurities having similar structures and UV spectra. For example, the HPLC method (method G) as demonstrated in Fig. 6 was used as an in-process monitoring method to follow the stage 1 reaction. To ensure the method was able to detect the purging of various impurities in **IM1**, rather than simply providing false negatives

Table 2
Outputs of the spiking/purging study and batch data for exemplified impurities.

Process material	Impurity	Spiking/purging data[a] (% area, unless specified)	Proposed specification limit (% area, unless specified)	Typical ranges in batches (% area, unless specified)
SM3	**Imp11** (diamine)	Stage 3 derivative **Imp34** has limited purgeability in stage 3/4	NGT 0.14	<0.05–0.11
	Imp12 (triamine)	Stage 3 derivative **Imp35** has limited purgeability in stage 3/4	NGT 0.14	<0.05–0.18[b]
IM1	**Imp16** (isomer)	6% in stage 2 is purged by stage 3 output	NGT 3.0	0.39–2.0
	Imp17 (pseudo dimer)	8% in stage 2 is purged by stage 3 output	NGT 4.0	0.08–2.1
IG	**Imp34** (dimer)	Limited purgeability in stage 4	NGT 0.15% (w/w)	<0.05–0.13% (w/w)
	Imp35 (trimer)	Limited purgeability in stage 4	NGT 0.15% (w/w)	<0.05–0.07% (w/w)

[a] Impurities were considered purged when the level of the impurity was <0.05% area by HPLC. A genotoxin impurity was considered purged when the level was below the TTC (threshold of toxicological concern). The unit (%, w/w) was converted to % area using RRFs of impurities by the final control methods.
[b] A single batch at 0.18% area was received early in development and appropriate controls have been put in place to assure NGT 0.14% area.

due to the inability of the method to detect the impurities, the RRFs and LOQs of the potential impurities were either validated or estimated. Because of limited **Imp20** and **Imp21** markers available, the RRFs and LOQs of these two impurities were obtained based on single weight/point validation. In the case of **Imp18** and **Imp19**, where pure compounds were unavailable, their RRFs were estimated by comparing the structures and UV spectra of **Imp18** (pseudo trimer of **IM1**) and **Imp19** (dimer of **IM1**) to the known impurity **Imp17**. As shown in Fig. 9, the UV spectra of **Imp18** and **Imp19** obtained from the reaction mixtures were similar to that of **Imp17**, and these two impurities have more conjugated aromatic rings than **Imp17** (pseudo dimer of **IM1**). Therefore, they should have stronger UV absorbance (more sensitive) than **Imp17**. It was reasonable to estimate that impurity **Imp18** and **Imp19** can easily achieve the same RRF and LOQ of 0.05% (w/w), which had been validated for **Imp17**. By taking the worst case scenario, it was safe to use the RRF of **Imp17** to calculate the actual amount of these impurities that can be purged. For the remaining impurities shown in Fig. 6, linearity, RRFs and LOQs were all validated following regulatory requirement [31]. It should be mentioned that the single weight/point validation and the estimation approach should only be applied at this stage of IFM where all potential and hypothetical impurities were included in the exercises. When the analytical control strategy was developed to target on the specified impurities (see Section 3.4.1), it was highly desirable to make the authentic standards of the specified impurities available for formal validation.

In addition, specific analytical methods for the quantification of trace level genotoxic impurities were also developed and validated to support the spiking/purging studies [28].

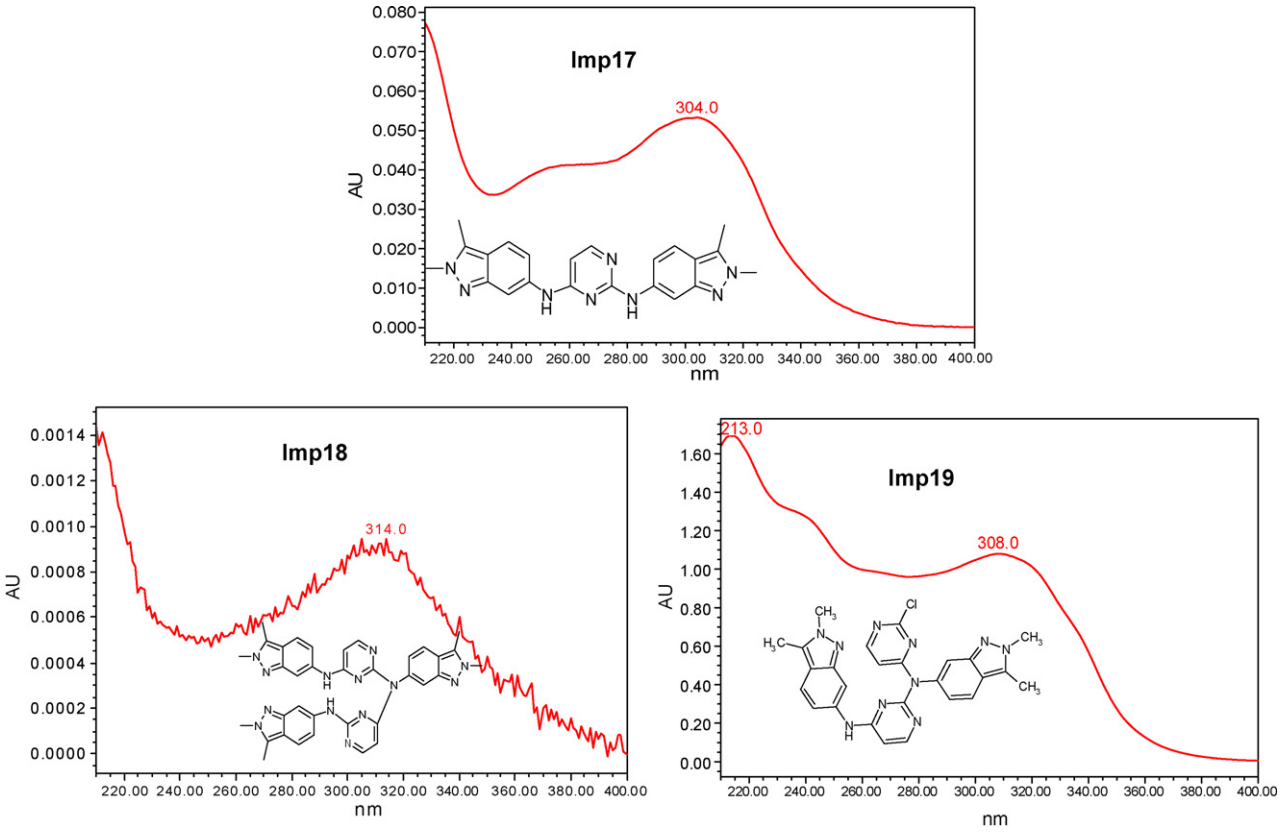


Fig. 9. Structures and UV spectra for estimation of RRFs and LOQs of **Imp18** and **Imp19**.

3.4. Analytical control of Pazopanib hydrochloride process impurities

The analytical control of process impurities is comprised of three essential parts: definition of specified impurities, specifications, and analytical control methods (Fig. 1) as discussed in the below sections. The comprehensive IFM of the Pazopanib hydrochloride manufacturing process conducted as described above adds to the overall control of the API quality by providing a scientific rationale for specification setting.

3.4.1. Definition of specified impurities

As the results of IFM, potential impurities were identified. However, some of them may just be hypothetical (difficult to form), or too reactive to survive, or purged completely in the isolated materials. Therefore, these potential impurities were further evaluated through a risk assessment [19,20] in order to determine the impurities that will be included as part of the specifications of Pazopanib hydrochloride, isolated IMs, or SMs (so called 'specified' impurities here). When an impurity was detected at >0.05% area in a SM, IM or API prepared under the routine or stretched manufacturing process conditions, or was a suspected genotoxin regardless of whether or not it was detected in development batches, it was defined as a specified impurity. However, if it was always converted to a downstream impurity which was controlled in the subsequent stages and/or purged completely and was not present at >0.05% area in isolated IM, it would not pose a risk to the product quality and therefore was not defined as a specified impurity. Among the specified impurities, the impurities found in API and known/potential genotoxic impurities are defined as CQA impurities that could impact on patient safety. The impurities found in SMs and IMs are not CQAs because they were evaluated by risk assessment and were found to be easy to control.

The result of the risk assessment helped narrow down the list of specified impurities to ~20. For example, among the eight potential impurities proposed for the stage 1 isolated material **IM1** (Fig. 6), five impurities (**SM2**, **Imp18**, **Imp19**, **Imp20** and **Imp21**) were never detected in the isolated **IM1** and/or do not participate in the next stage reaction thus can be purged completely downstream. Thus, they were eliminated from the impurity list for **IM1**, and only the three impurities (**Imp16**, **Imp17** and **SM1**) were determined to be specified impurities in IM1.

As a result, a 'risk-assessed' impurity fate map for Pazopanib hydrochloride synthetic stages 1 through 4 was then drafted (Fig. 10). This risk-assessed impurity fate map includes specified impurities from registered SMs, IMs and API. As shown in Fig. 10, the lines connecting the impurities show the origin and fate of each impurity as it proceeds through the process. All materials on the map were synthesized or isolated from mother liquors and their structures were characterized and confirmed by appropriate analytical methods as demonstrated in the previous sections. The specified impurities will be the targets of specifications and analytical control methods for each isolated process material. This risk-assessed impurity fate map provides valuable information about process understanding of the Pazopanib hydrochloride impurities with regard to their formation and fate and is essential to the development of the effective control strategy. In addition, this comprehensive knowledge of the process impurities had been shared with the product manufacture sites which should help process troubleshooting during the product lifecycle management.

3.4.2. Specification setting

Establishing appropriate impurity specifications for registered SMs, isolated IMs, and API is an important component of the commercialization and registration of drug substances. Knowledge gained through IFM enabled us to set meaningful specifications

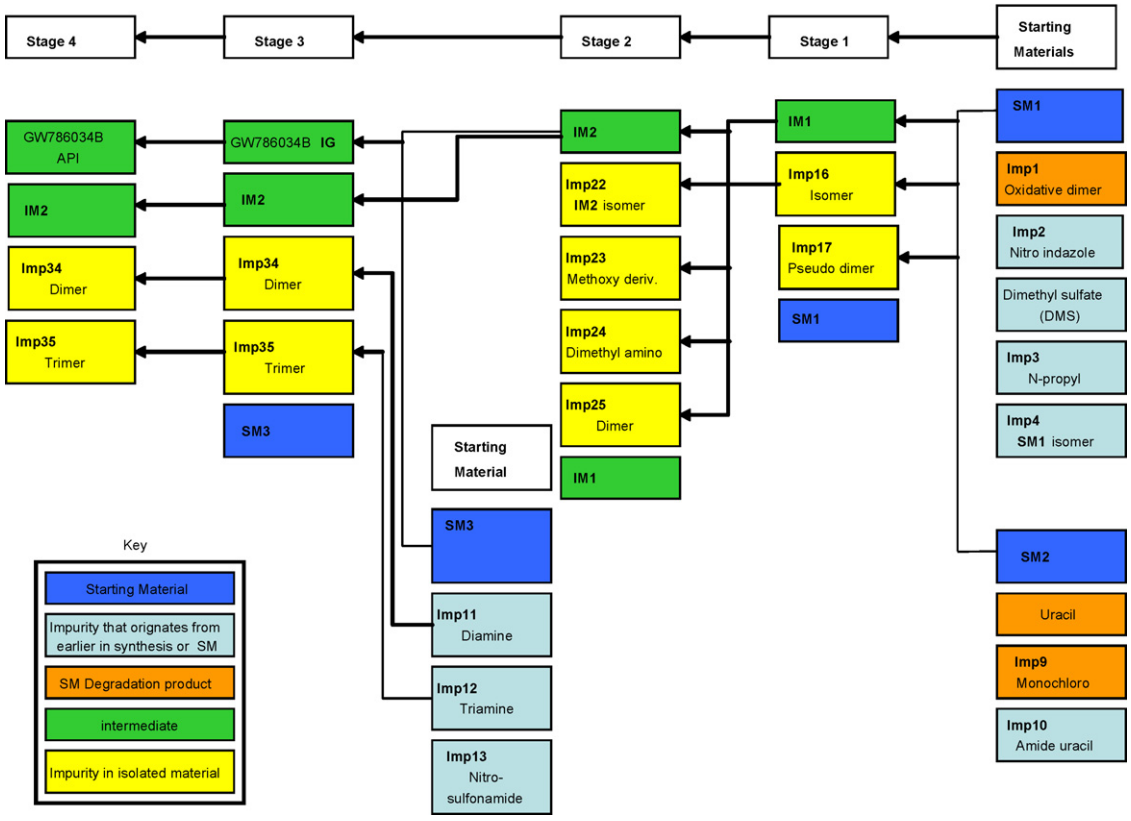


Fig. 10. Simplified risk-assessed impurity fate map.

for such materials of Pazopanib hydrochloride. The results of the spiking/purging study and historical batch data as exemplified in Table 2 were employed to define the control limits of the specified impurities. A very conservative approach was used, which allowed only a fraction of the process maximum tolerated level for each impurity. Following the examples of the spiking/purging experiments conducted for **Imp16** and **Imp17** in Section 3.3, the specifications for **Imp16** and **Imp17** in **IM1** were set at no greater than (NGT) 3.0% area and NGT 4.0% area, respectively, which are at a level of 1/2 the highest purged amount. It should be noted that the specification limits of impurities in SMs and IMs were set using % area for ease of operation. These values were correlated to the purging results (%, w/w) based on the RRFs of impurities by final control methods.

Whenever possible, the levels of impurities originating from SMs or IMs should be controlled up-stream by the specifications of the impurities or precursor impurities at the previous stages through appropriate in-process control, rather than by testing in the final materials. This is a QbD approach, which does not necessarily mean less analytical testing, but is about the right analysis at the right time based on a high degree of process understanding via IFM. This approach is preferred, because (1) expensive rework of API or even earlier IMs can be avoided based on the knowledge and understanding of impurities via IFM, (2) the limits for these impurities in the isolated intermediates potentially can be higher, thus allowing for less challenging analytical methods using routine analytical instrumentation, and (3) it offers opportunity for greater process flexibility and sourcing flexibility with cost saving.

To provide an example of this approach, the CQA impurities in API were tightly controlled up-stream by the specifications of their precursor impurities in starting material. As the result of the spiking/purging study in Section 3.3, it was known that two API CQA impurities (**Imp34** and **Imp35**), which were generated in stage 3 from two **SM3** impurities (**Imp11** and **Imp12**), could not be completely purged. Therefore, the precursor **Imp11** and **Imp12** were controlled to NGT 0.14% area in the **SM3** buy-in specification (Table 2), which will in turn control the impurity **Imp34** and **Imp35** carried into the next step (**IG**) and further to the API. The control of impurities up-stream is also exemplified by the genotoxin control strategy in the API [28]. Developing extremely sensitive and robust analytical methodologies that can adequately monitor genotoxic impurities at low ppm levels poses significant challenges on the pharmaceutical industries. Furthermore, transferring such highly technical methodologies to manufacturing site QC laboratories may not be practical since the modern analytical instrumentation and scientific expertise are not readily accessible. The results of spiking/purging studies of the IFM work enabled the development of the control strategies for the genotoxic impurities in Pazopanib hydrochloride API. Total six known/suspected genotoxic impurities were controlled in either the SMs or isolated IMs at higher limits, thus favoring the analytical method development and the use of simpler analytical instrumentation. These impurities include dimethyl sulfate, **Imp2**, **SM1**, **Imp13**, and **IM2**, which had been reported separately [28], and **Imp4** (the isomer of **SM1**). In summary, dimethyl sulfate, **Imp2**, and **Imp4** are controlled in stage 1 **SM1** at NGT 0.1% (w/w). Genotoxin **SM1** is controlled to NGT 0.6% (w/w) in stage 1 product **IM1**. Genotoxin **Imp13**, on the other hand, is controlled in **SM3** at NGT 0.1% (w/w). Lastly, the genotoxin **IM2** is controlled in **IG** at NGT 0.6% (w/w) [28].

3.4.3. Analytical control methods

As an element of the overall analytical control strategy, analytical methods are of critical importance to the testing of the process materials to meet the specifications. This section details the development of final analytical control methods for Pazopanib hydrochloride SMs, IMs, and API, which will be used in the manufacturing environment during the product lifecycle.

Although several analytical methods have been developed (Table 1) for better impurity tracking during IFM, those methods may not be ideal as the final control methods in the manufacturing environment. One of the key aspects of the analytical QbD approach is to implement the right method at the right time, based on science and risk assessment. According to the above IFM and process understanding, only the specified impurities defined in Section 3.4.1 are of importance to the Pazopanib hydrochloride manufac-

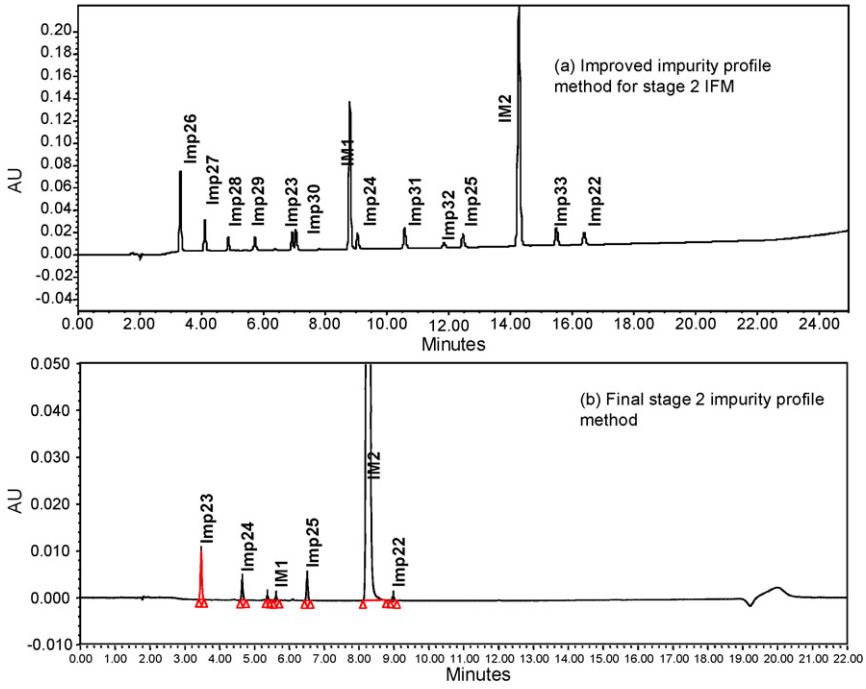


Fig. 11. Chromatograms of (a) the improved impurity profile method for stage 2 IFM (see method I in Table 1 for HPLC conditions) and (b) the final stage 2 impurity profile method for IM2 (see method J in Table 1 for HPLC conditions).

turing process. Therefore, the final control methods should only target on these impurities and such simplified analytical methods facilitate the transfer to the manufacture QC laboratories. Moreover, the methods have to be robust and rugged for the use over the product lifecycle.

Following this rationale, a systematic HPLC method development approach emphasizing predefined objectives, proven development tools, scientific understanding, method control, and risk management was developed [29] and applied to the development of the impurity methods for Pazopanib hydrochloride SMs, IMs and API. During this process, the overall control strategy defined based on the spiking/purging study, and the knowledge gained during IFM such as the definition of specified impurities, authentic standards/markers, and impurity physicochemical characteristics (e.g. p*K*a, UV chromophore, solubility, and stability), were extremely valuable for the method development and validation. Since most of the impurities are controlled up-stream in the process as part of the overall analytical controls, the final control method for API only needs to test for two CQA impurities (**Imp34** and **Imp35**) in the final API, a very simple, robust, and rugged reversed-phase HPLC method (method L) was developed using the systematic method development approach [29]. The development of the stage 2 impurity method can also be taken as an example to demonstrate the right method at the right time. As the initial LC method (method A) was insufficient for the stage 2 IFM, an improved HPLC method (method I) was developed to separate all potential impurities in stage 2 (Fig. 11a). Following the definition of specified impurities based on IFM, it was found that only five impurities needed to be specified in stage 2 isolated material **IM2**, though none of them would be carried to the final API. Using the systematic method development approach, the final control method for **IM2** (method J) was developed focusing on the separation of the five specified impurities and **IM2**. A typical chromatogram generated by this method is shown in Fig. 11b.

In summary, all final control methods for the SMs, IMs, and API as listed in Table 1 were developed using the systematic method development approach [29], and were proven to be robust and reproducible to meet the needs of the manufacturing environment.

4. Conclusion

A comprehensive approach to analytical control of process impurities by IFM has been established and successfully applied to the understanding and control of impurities in the manufacturing process of Pazopanib hydrochloride. The ultimate goal of IFM is to design a comprehensive impurity control strategy to ensure that product quality (impurity levels in this case) is built into the manufacturing process. This approach aligns well with the FDA's QbD initiative by emphasizing process understanding and risk management. The analytical control strategy developed for Pazopanib hydrochloride process impurities consisted of three key aspects: (1) defining specified impurities that are of critical importance to the process, (2) establishing meaningful specifications for SMs, IMs and API, and (3) developing and validating targeted analytical methods. Alignment with QbD is achieved by controlling impurities up-stream in the process whenever possible, via the specifications of the SMs or IMs, and using simpler analytical methods than those that might be needed to perform testing in the final API. A similar principle was demonstrated in the case of several genotoxic impurities, where more sophisticated analytical methods employing LC–MS to test the final API were eliminated based on process understanding, while simpler LC–UV methods were implemented as the control methods [28]. In the present case, the IFM approach of clarifying the method goal and simplifying the analytical methods allows for final control methods that are more easily implemented in the manufacturing quality control environment.

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