Journal of Pharmaceutical and Biomedical Analysis 50 (2009) 144–150

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

journal homepage: www.elsevier.com/locate/jpba

Analytical control of genotoxic impurities in the pazopanib hydrochloride manufacturing process

David Q. Liu, Ted K. Chen, Michael A. McGuire, Alireza S. Kord*

Chemical Development, GlaxoSmithKline Pharmaceutical Research and Development, 709 Swedeland Road, King of Prussia, PA 19406, USA

ARTICLE INFO

Article history:
Received 13 February 2009
Received in revised form 30 March 2009
Accepted 1 April 2009
Available online 10 April 2009

Keywords:
Genotoxic impurity
Pazopanib hydrochloride
Analytical control strategy
Spiking/purging
Trace analysis

ABSTRACT

Pharmaceutical regulatory agencies are increasingly concerned with trace-level genotoxic impurities in drug substances, requiring manufacturers to deliver innovative approaches for their analysis and control. The need to control most genotoxic impurities in the low ppm level relative to the active pharmaceutical ingredient (API), combined with the often reactive and labile nature of genotoxic impurities, poses significant analytical challenges. Therefore, sophisticated analytical methodologies are often developed to test and control genotoxic impurities in drug substances. From a quality-by-design perspective, product quality (genotoxic impurity levels in this case) should be built into the manufacturing process. This necessitates a practical analysis and control strategy derived on the premise of in-depth process understanding. General guidance on how to develop strategies for the analysis and control of genotoxic impurities is currently lacking in the pharmaceutical industry. In this work, we demonstrate practical examples for the analytical control of five genotoxic impurities in the manufacturing process of pazopanib hydrochloride, an anticancer drug currently in Phase III clinical development, which may serve as a model for the other products in development. Through detailed process understanding, we implemented an analysis and control strategy that enables the control of the five genotoxic impurities upstream in the manufacturing process at the starting materials or intermediates rather than at the final API. This allows the control limits to be set at percent levels rather than ppm levels, thereby simplifying the analytical testing and the analytical toolkits to be used in quality control laboratories.

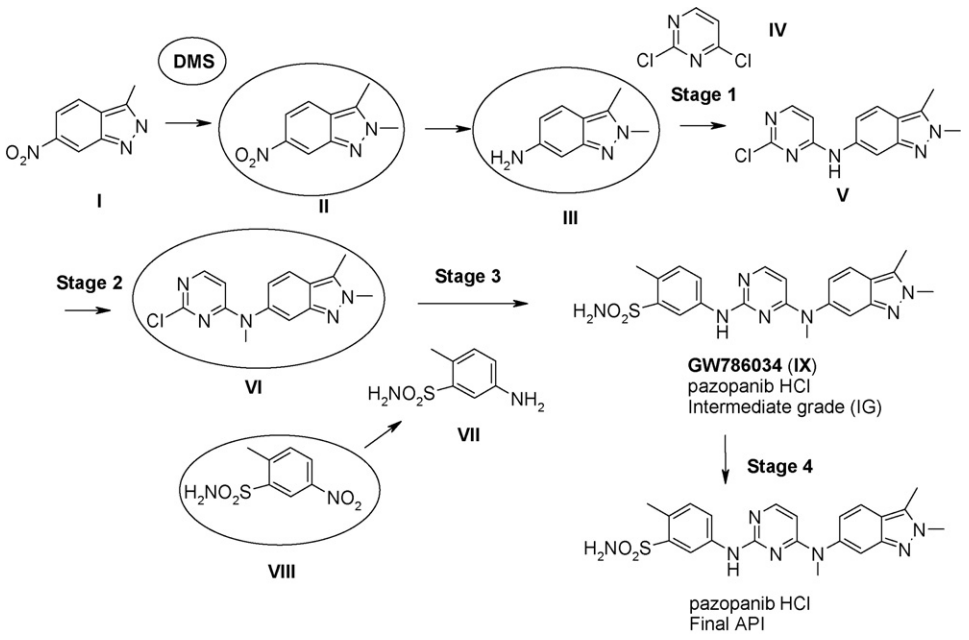
1. Introduction

Genotoxic impurities in chemically synthesized drug substances may arise from the use of reagents and starting materials, from process intermediates that are carried over to the final active pharmaceutical ingredient (API), and from the formation of reaction by-products. Analysis and control of genotoxic impurities is not explicitly covered in the International Conference on Harmonization (ICH) guidelines Q3A(R2), Q3B(R2), or Q3C(R3). Thus, a 'threshold of toxicological concern' (TTC) approach has been proposed by the European Medicines Agency (EMEA) to limit the daily patient exposure to no more than (NMT) 1.5 μg/day for supporting a market application [1]. Recently, a staged TTC has been proposed by the PhRMA (Pharmaceutical Research and Manufacturers of America) White Paper [2], which has become the basis for the recently published draft US FDA guidance for industry [3]. Based on these proposed TTC guidelines, genotoxic impurities in drug substances need to be controlled at low ppm level (weight/weight, this is used throughout the manuscript unless noted) to ensure patient safety at typical doses. Testing and control of genotoxic impurities at trace levels presents challenges to the pharmaceutical industry with regard to both analytical and process controls [4,5]. Furthermore, the US FDA has challenged the pharmaceutical industry to develop products using advanced technologies that will result in products incorporating quality-by-design (QbD) rather than quality-by-testing (QbT) [6]. The aim of this initiative is to have the process understanding and control needed so that the product quality is built into the manufacturing process. Since such a process can produce consistent quality product over the time and the control of the product quality does not solely rely on analytical testing of final API, the release testing becomes just one aspect of a multi-faceted approach to controlling the product quality.

Since the levels of genotoxic impurities are often critical quality attributes (CQAs) of drug substance, they must also be considered as part of a complete QbD approach. Practical examples of genotoxic impurity control strategies are lacking, leaving little guidance for the pharmaceutical industry. Recent publications by Dobo et al. [7], Argentine et al. [8], and Pierson et al. [9] attempt to address the issue by discussing hypothetical case studies. The purpose of the current paper is to communicate a practical case study describing the strategies for the analysis and control of five genotoxic impu-

* Corresponding author at: GlaxoSmithKline, UW2960, P.O. Box 1539, 709 Swedeland Road, King of Prussia, PA 19406, USA. Tel.: +1 610 270 5064.
E-mail address: alireza.s.kord@gsk.com (A.S. Kord).

0731-7085/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.jpba.2009.04.002



Scheme 1. Manufacturing process of pazopanib HCl (**GW786034**) showing the five genotoxic impurities (circled).

rities in the manufacturing process of pazopanib hydrochloride, **GW786034 (IX)** (Scheme 1), a VEGFR tyrosine kinase inhibitor currently in Phase III clinical development for the treatment of renal cell carcinoma [10]. The analytical control strategy presented herein was derived from a QbD view of process understanding, using spiking and purging of various genotoxic impurities into relevant reaction stages. To successfully support this, sensitive analytical methods for various genotoxic impurities at trace levels must first be developed and validated to test the drug substance intended for clinical studies, and to ensure product quality and patient safety. These trace analysis methods are typically based on liquid chromatography–mass spectrometry (LC/MS) instrumentation, which is also the technique of choice for guiding the spiking/purging studies. However, these hyphenated MS-based trace analysis methods are non-routine, costly, and difficult to implement in quality control laboratories in a manufacturing environment; a strategy to simplify the analytical testing is therefore imperative. Furthermore, controlling these impurities upstream in the synthetic process, either at the starting materials (SM) or the relevant intermediate stages, is favorable. In cases where batches fail genotoxic impurity specifications, re-work can be carried out on a SM or an intermediate rather than on the final API, providing for a more cost effective solution while also offering greater process flexibility.

This paper details the analytical testing and control strategies for five genotoxic impurities encountered in the pazopanib HCl manufacturing process. The control strategy was derived from a combination of process spiking/purging data and batch history data generated using trace analysis methods, with the goal of moving to less sensitive but more routine HPLC/UV methods wherever possible by developing a deep understanding of the fate of the genotoxic impurities. The data generated in this study were essential for finalizing the analytical methods and overall analytical control plan, as well as for setting specification limits for the registered starting materials (RSM), isolated intermediates, and the final API.

2. Experimental

2.1. Trace analysis LC/MS methods

An Agilent 1100 HPLC/MSD system (Wilmington, DE, USA) with electrospray ionization (ESI) operated in positive ion mode was used for all LC/MS analyses, except in analyses of **VIII**, where negative ion atmospheric pressure chemical ionization (APCI) was used. Detection was performed using single ion monitoring (SIM) at *m*/*z* 288 for **VI**, *m*/*z* 162 for **III**, *m*/*z* 192 for **II**, *m*/*z* 215 for **VIII**, and *m*/*z* 116 for dimethyl sulfate (**DMS**), respectively. The ion monitored for the detection of **DMS** was that of the derivatization product, triethylmethyl ammonium ion, as a result of triethylamine derivatization. Because of analyte reactivity of **DMS**, direct analysis by gas chromatography–mass spectrometry (GC/MS) was impractical. Therefore, a derivatization LC/MS method was developed where **DMS** was derivatized by triethylamine to form triethylmethyl ammonium for enhanced detection [11]. For the analysis of **II**, **III**, and **VI**, chromatographic and mass spectrometric conditions are similar, all using a Phenomenex Luna C18(2) column (50 mm × 2.0 mm, 3.5 μm) (Torrance, CA, USA) with ESI positive ion detection. However, due to the high requirements of method specificity and sensitivity at the trace levels, chromatographic and mass spectrometric parameters were optimized individually with subtle differences. The chromatographic conditions were for **II**, isocratic elution using 73% of A (0.1% TFA in water) and 27% B (acetonitrile); for **III**, isocratic elution using 88% of A (0.1% heptafluorobutyric acid in water) and 12% B (acetonitrile); and for **VI**, isocratic elution using 74% of A (0.1% formic acid in water) and 26% B (acetonitrile), respectively. Compound **VIII** is an arylsulfonamine lacking an easily protonated functional moiety in the structure. Therefore, APCI negative ion LC/MS was employed to take advantage of its deprotonation potential. Isocratic elution using 83% of A (0.1% formic acid in water) and 17% of B (methanol) was employed for chromatography. The complete experimental details and conditions are omitted here since they are not the main focus of the current discussion. All the LC/MS methods were validated; the results are summarized in Table 1.

2.2. HPLC/UV methods

DMS was derivatized by an aqueous solution containing 5 mg/mL of 2-mercaptopyridine, and the derivatization product was detected by UV at a wavelength of 315 nm. A SunFire C18 column (50 mm × 2.1 mm, 3.5 μm) (Waters, Milford, MA, USA) was used for separation. The mobile phases were 0.1% heptafluorobutyric acid in water (A) and ACN (B). The impurity profile method

Table 1
Validation results of the trace LC/MS methods for analyzing five genotoxic impurities in pazopanib HCl API.

Parameters[a]	DMS	VIII	II	III	VI
Sensitivity (LOQ in ppm)	0.5	0.2	0.6	0.6	0.2
Accuracy (% recovery)[b]	85	98	91	88	96
Injection precision[b] (% RSD, $n = 6$)	1.9	2.1	5.8	2.6	0.3
Linearity and range[c] (ng/mL)	1–100	3–100	2.4–60	3–100	2–1000

[a] The method specificity for all methods was demonstrated by no more than 10% interference in the blank.
[b] At the target concentration levels of 1.7 ppm for **DMS**, **VIII**, **II**, **III** and 115 ppm for **VI**.
[c] $R^2 > 0.9995$ for all methods.

of **III** was used for control of **II** where a Zorbax SB-C8 column (150 mm × 4.6 mm, 3.5 μm) (Agilent, Wilmington, DE, USA) was employed. A gradient of 10 mM NH_4OAc in water (A) and ACN (B) was used for elution, and the UV detection wavelength was set at 297 nm. The impurity profile method of **V** was used as a control of **III**. The method employs a Waters XBridge C18 column (150 mm × 4.6 mm, 3.5 μm) for separation, a gradient of 10 mM NH_4OAc in water (A) and ACN (B) for elution and UV at 242 nm for detection. The impurity profile method of **VII** was used to control **VIII**. A Zorbax Bonus RP column (150 mm × 4.6 mm, 3.5 μm) with a mobile phase of 0.1% $HClO_4$ in water (A) and methanol (B) was used for separation. The detection wavelength was set at 220 nm. The impurity profile method for intermediate grade (IG) **GW786034 (IX)**, Stage 3 isolate, for control of **VI** also uses the Zorbax Bonus RP column. The elution mobile phases are 0.1%TFA in water (A) and acetonitrile (B), respectively with a detection wavelength set to 268 nm. The complete experimental details and conditions are omitted here since they are not the main focus of the current discussion. A brief summary of the validation results of all HPLC/UV methods is presented in Table 2.

2.3. Sample preparations

2.3.1. Sample preparations for the LC/MS methods

All samples of API or intermediates were prepared by dissolving 4–5 mg of solid materials in 1 mL of diluent solvents which are typically mixtures of water and acetonitrile. The spike recovery samples were prepared in the same fashion except they were spiked with aliquot of analyte standard solutions at the target concentrations. The above prepared solutions were subjected to LC/MS analysis directly.

2.3.2. Sample preparations for the HPLC/UV methods

For the analysis of **II**, **III**, **VIII** and **VI** in the starting materials or intermediates **III**, **V**, **VII**, and IG pazopanib **IX**, respectively, the samples were prepared at concentrations ranging from 0.2 to 0.6 mg/mL for direct injection onto the HPLC/UV systems. For the analysis of **DMS** in **III**, the samples were prepared at 2.5 mg/mL in acetonitrile (12–14 mg in 5 mL of acetonitrile in a 9-mL vial), to which 1 mL of derivatization reagent (5 mg/mL of 2-mercaptopyridine aqueous solution) was added. After mixing, 1 mL of this solution was transferred into a 2-mL HPLC vial which was heated at 60 °C for 1 h before injecting onto HPLC.

3. Results and discussion

3.1. Genotoxic impurities in the pazopanib HCl manufacturing process

The manufacturing process for pazopanib HCl API is shown in Scheme 1 [10]. As a result of a synthetic route risk assessment for structural motifs known to be genotoxic [12,13], five genotoxic or potential genotoxic impurities were identified, including dimethyl sulfate (**DMS**), **II**, **III**, **VI**, and **VIII**, and they were all designated as drug substance CQAs (Fig. 1). **DMS** is used in the synthesis of **II**, while **II** is the precursor of **III**. Compound **III** is the Stage 1 RSM. Compound **VIII** is used in the synthesis of **VII** which is one of the RSM for Stage 3 reaction. **VI**, on the other hand, is the Stage 2 intermediate which is the precursor for Stage 3 process. **DMS** is a known genotoxin. Compound **II** is only a 'structure alert' as a result of DEREK screen; thus the latter is a potential genotoxin. Compounds **III** and **VIII** were positive in Ames tests; therefore, they are genotoxic. The fifth genotoxic impurity, **VI**, has been shown to be non-DNA reactive; therefore, a higher TTC limit was justified [14]. Trace analysis LC/MS methods were developed, validated, and used to ensure that four of the five genotoxic impurities, namely **DMS**, **II**, **III**, and **VIII**, did not exceed the 1.5 μg/day TTC limit in drug substance, which corresponds to a limit of NMT 1.7 ppm each for a dose up to 800 mg/day. The fifth genotoxic impurity, **VI**, has a limit of NMT 115 ppm in the drug substance because of its non-DNA reactive nature. Prior to the implementation of the control strategy, final APIs are tested and released for clinical use according to the proposed TTC limits (Table 3).

3.2. The fate of genotoxic impurities in the pazopanib HCl manufacturing process

The fate of each genotoxin during the manufacturing process has been determined (Fig. 1). Each genotoxin is either a reagent or an intermediate in the synthesis. It is chemically highly improbable that any of the genotoxins can form during the manufacturing process or by degradation under stability testing conditions.

DMS is mainly eliminated in the mother liquors and washes in Stage 1. Trace-level conversion to diethyl sulfate and methyl ethyl sulfate is possible but these products are eliminated in the same manner. Compound **II** is not reactive in Stage 1 or 2 and is eliminated in the mother liquors and washes. Compound **III** is a starting material and principally reacts to yield **V**. Excess is eliminated in

Table 2
Validation results of the HPLC/UV methods for controlling the five genotoxic impurities in the pazopanib HCl manufacturing process.

Parameters	DMS	VIII	II	III	VI
Sensitivity (LOQ)	0.022%	0.03%	0.05%	0.03%	0.05%
Accuracy[a] (% recovery)	67.5	95.5	105.3	113.4	99.3
Repeatability[b] (% RSD)	1.7	1.5	4.0	0.2	5.4
Linearity and range[c]	0.05–0.5%	0.05% to 640% of spec limit	0.05% to 370% of spec limit	0.03% to 150% of spec limit	0.05% to 250% of spec limit

[a] Demonstrated at the specification limits, see Table 3.
[b] Demonstrated at 80–120% specification limits, $n = 6$ or more.
[c] $R^2 > 0.9995$ for all five methods.

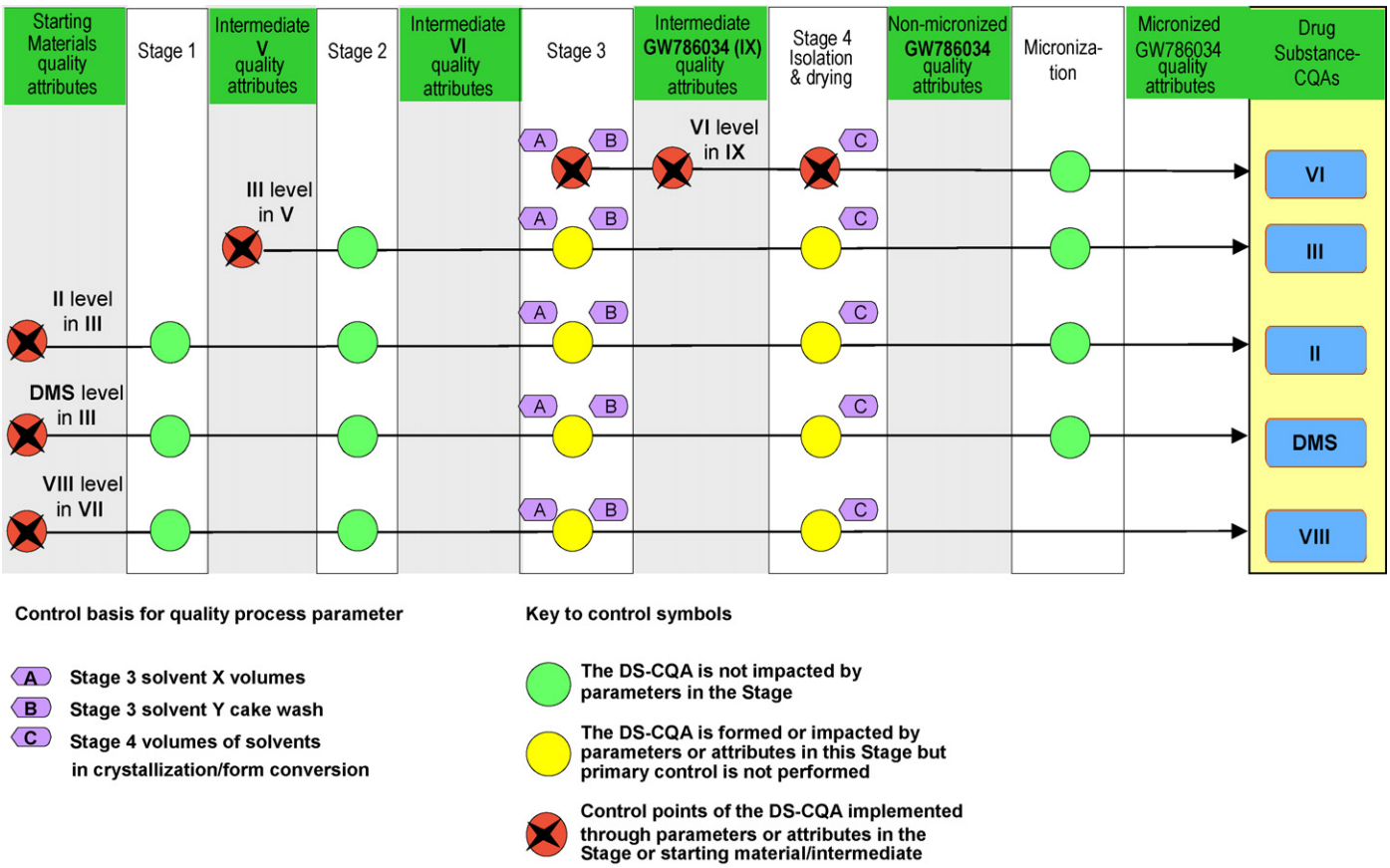


Fig. 1. Genotoxin control strategy for pazopanib hydrochloride.

the mother liquors of Stage 1, and typically less than 0.2% of **III** is found in isolated **V**. This small amount of **III** reacts in Stage 2 slowly to yield mono and dimethylated products which are completely eliminated in the mother liquors of Stage 2. Compound **VI** is the Stage 2 intermediate and is mainly consumed by reaction to yield intermediate grade **IX** in Stage 3. However a typical amount of NGT 0.6% **VI** is trapped in the crystals of intermediate grade **IX**. This is purged in the mother liquors and washes of Stage 4. Process parameters of Stages 3 and 4 are important for purging **VI**, and because of this they have been designated quality process parameters (Fig. 1). Compound **VIII** does not react in Stage 3 or 4 and is eliminated in the mother liquors and washes of Stages 3 and 4.

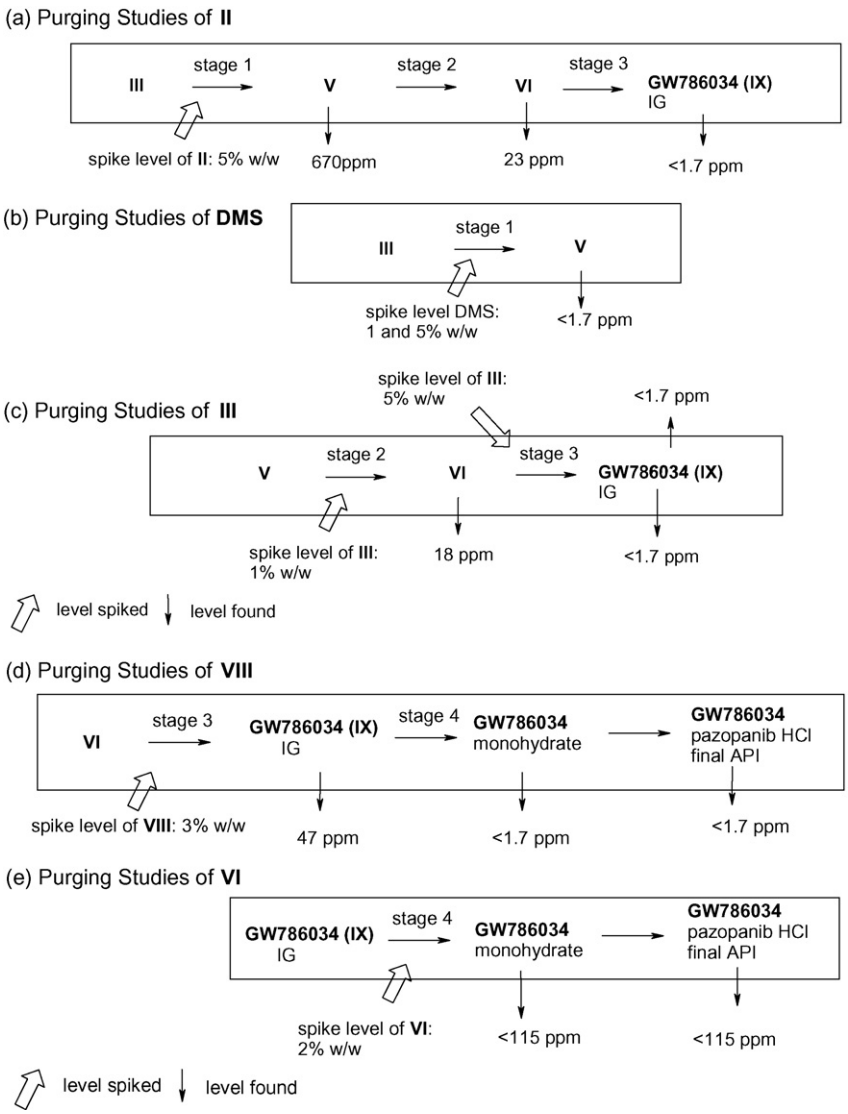
It has been demonstrated that the quality process parameters of Stages 3 and 4 which affect purging, when run at the lower end of the parameter's purging ability (lower end of the proven acceptable range), are sufficient to purge all genotoxins to less than the TTC when they are added to the Stage 3 reaction in an amount which assumes no purging in Stages 1 and 2.

3.3. Analytical control of genotoxic impurities in pazopanib HCl

Pharmaceutical impurities are conventionally tested in samples of final API. From the QbD perspective, however, the product quality (genotoxic impurity levels in this case) should be built into the manufacturing process. We present herein the process understanding and control of the genotoxic impurities in the pazopanib HCl process. As a result, the control points were defined upstream in the manufacturing process at the RSMs or intermediates instead of at the final API. This control strategy offers multiple benefits. First, it allows for higher testing and specification limits for all genotoxic impurities, because it generally allows for control at easily achieved percentage levels (e.g. 0.1%, w/w or peak area) rather than more challenging ppm levels. With higher specification limits at earlier stages, conventional HPLC/UV impurity methods rather than trace analysis LC/MS methods can be implemented, simplifying the analytical methods to be used in manufacturing facilities. Trace analysis LC/MS methods are generally undesired methodology in the manufacturing environment because of their complexity, cost, and potential lack of robustness. Second, this control strategy provides greater process flexibility; for example, in the case of a batch failure caused by a genotoxic impurity level not meeting a specification, re-work options can be applied at the RSM or intermediate stages rather than to the costly final API. In order to deliver this strategy to ensure the ultimate control of all five genotoxic impurities, comprehensive spiking/purging experiments were conducted for the

Table 3
Proposed genotoxin TTC limits in drug substances and the specification limits in the starting materials and intermediates (the control points).

Genotoxic impurities	TTC Limits in API (ppm)	Control points	Specification limits at the control points (% w/w)
DMS	1.7	Stage 1 SM, **III**	0.1%
II	1.7	Stage 1 SM, **III**	0.1%
III	1.7	Intermediate, **V**	0.6%
VIII	1.7	Stage 3 SM, **VII**	0.1%
VI	115	Intermediate, IG pazopanib, **IX**	0.6%



Scheme 2. Spiking/purging studies of the five genotoxic impurities.

pazopanib HCl process to map the fate of genotoxic impurities. In addition, actual batch data were also collected. After comprehensive review of the purgeability data in combination with the actual batch data, control points of all genotoxic impurities were established for the pazopanib HCl process as discussed in detail below.

Compound **II** is the synthetic precursor of **III** (Scheme 1) and is a potential genotoxic impurity. To ensure that the pazopanib HCl clinical supplies contained NMT 1.7 ppm of **II**, about 79 batches of drug substance were tested by the trace analysis LC/MS method (see Table 1 for the validation results), and the level of **II** was found to be below this limit. This suggests that the manufacturing process can purge the impurity effectively. In order to gain an understanding of the process tolerability of this impurity, spiking/purging studies were conducted. As illustrated in Scheme 2a, when levels as high as 5% (w/w) of **II** were spiked into **III**, the level of **II** was reduced to 670 ppm in Stage 1 product, 23 ppm in Stage 2 product, and less than 1.7 ppm in Stage 3 product, intermediate grade (IG) pazopanib HCl (**IX**). Furthermore, 16 batches of **III** were analyzed. The levels of **II** in **III** were found to be less than 1.7 ppm in 11 batches and less than 24 ppm in 5 others. Based on these data, a strategy to control **II** in **III** at a limit of NMT 0.1% (w/w) was proposed. This eliminates the need for testing for this impurity in the final drug substance. Consequently, a simple HPLC method with UV detection with an LOQ of 0.05% (w/w) **II** in **III** was developed as the analytical control method. The validation results of the method are summarized in Table 2.

DMS is used in the synthesis of **II**, a precursor of **III** (Scheme 1), which is of known genotoxic concern [15]. To ensure the control of **DMS** in the drug substance to NMT 1.7 ppm, **DMS** was tested in about 79 batches of pazopanib clinical supplies using the trace analysis LC/MS method [11], and they were found to contain NMT 1.7 ppm in all batches tested. The data suggest that **DMS** can be purged effectively by the manufacturing process. To understand the process tolerability, **DMS** was spiked into Stage 1 at 1% and 5% (w/w) levels respectively and tested in the corresponding Stage 1 product **V** (Scheme 2b). The results for both spiking levels showed that the **DMS** concentration in **V** was less than 1.7 ppm following typical isolation processes. This indicates that the process can tolerate up to 5% (w/w) of **DMS** in **III**. To examine the actual levels of **DMS** in typical batches of **III**, 20 batches from the specified supplier were analyzed by the trace analysis LC/MS method (see Table 1 for the validation results). In all cases the actual level of **DMS** in **III** was NMT 1.7 ppm. Based on this knowledge and process understanding, a strategy to test and control **DMS** in RSM **III** at a limit of NMT 0.1% (w/w) and not to test

the final API was proposed. Consequently, an HPLC method using UV detection following the derivatization with 2-mercaptopyridine was developed for analyzing **DMS** in **III** as a means of analytical control. The validation results of the method are described in Table 2.

Compound **III** is a proposed RSM used in Stage 1 for manufacturing **V** (Scheme 1) and is also a genotoxic impurity. To ensure pazopanib HCl clinical trial supplies contained NMT 1.7 ppm of **III**, 79 batches of drug substances were tested using the trace analysis LC/MS method (see Table 1 for the validation results). All batches passed the limit test of 1.7 ppm. In order to understand the process purgeability, **III** was spiked into Stages 2 and 3, respectively, followed by testing the intermediates isolated using typical processes. The results are summarized in Scheme 2c. After spiking 1% (w/w) of **III** into Stage 2, 18 ppm and less than 1.7 ppm were found in Stage 2 isolates **VI** and Stage 3 isolates **IX** (IG pazopanib HCl), respectively; when spiking 5% **III** into the Stage 3 reaction, the level of **III** in the Stage 3 isolate **IX** was NMT 1.7 ppm. Therefore, a strategy to control **III** to a limit of NMT 0.6% (w/w) in Stage 1 product **V** was proposed. This eliminates the needs to test **III** in the final API using the trace analysis LC/MS method; instead, a HPLC/UV impurity method was developed for testing and controlling **III** in **V**, the Stage 1 product. The validation results of the method are listed in Table 2.

Compound **VIII** is the nitro precursor of **VII**, the proposed Stage 3 RSM (Scheme 1). To understand the process tolerability of this impurity in Stages 3 and 4, up to 3% (w/w) (equals to 5% peak area) of **VIII** was spiked into the Stage 3 reaction (Scheme 2d). Using the trace LC/MS method (see Table 1 for the validation results), the levels of **VIII** were determined in the Stage 3 product **IX**, the Stage 4 product (monohydrate), as well as the final pazopanib HCl API. The levels of **VIII** were found to be 47 ppm in the Stage 3 isolate **IX**, and NMT 1.7 ppm in both the monohydrate and the final API, respectively. These data demonstrate that up to 3% (w/w) of **VIII** in **VII** RSM can be purged effectively by the process. To assess the typical levels of **VIII** in **VII** RSM from the existing suppliers, 20 representative batches were tested using a validated HPLC/UV method. Two of the 20 batches were found to contain approximately 0.05% (peak area) of **VIII**, and the remaining 18 contained less than 0.05%, well within the process tolerability. Due to relatively late recognition of this compound as a genotoxic impurity, the levels of **VIII** in actual final API were tested only in limited batches and they were all found to be NMT 1.7 ppm. Together with the purging data, it is reasonable to conclude that **VIII** will not exceed 1.7 ppm in any final API batches. In short, a control strategy to limit **VIII** in **VII** (the proposed Stage 3 RSM) to NMT 0.1% (w/w) by an HPLC/UV impurity method can be implemented. The validation results of the impurity method are described in Table 2.

Compound **VI** is the Stage 2 product that feeds into Stage 3 for manufacturing IG **IX** (Scheme 1). The TTC limit for **VI** in drug substance was set to NMT 115 ppm based on the genotoxin risk assessment. All drug substances were tested by the LC/MS limit test method, and all released clinical batches met the TTC limit. It has been demonstrated that **VI** can be purged effectively at Stage 4. Approximately 70 batches of IG **IX** containing **VI** ranging from 0.1% to 0.6% (w/w) have gone through the Stage 4 recrystallization/purification commercial processes; the levels of **VI** were reduced to NMT 115 ppm in all batches of final API. In order to examine the tolerability of **VI** in IG **IX** (Stage 4 purgeability), an experiment was performed where 2% (w/w) **VI** was spiked into IG **IX** and subsequently processed through the Stage 4 workup. It was established that the concentrations of **VI** in **IX** monohydrate and the final API were well below 115 ppm (Scheme 2e). Therefore, a strategy to control **VI** at a limit of NMT 0.6% (w/w) in IG **IX** was proposed. This eliminates testing of **VI** in the final API which requires the trace LC/MS method. Instead, a simple HPLC/UV impurity method was developed to test and control **VI** in IG **IX**. The validation results of the HPLC/UV impurity method are listed in Table 2.

The overall pazopanib HCl genotoxic impurity analytical control points and the control limits are summarized in Table 3 and Fig. 1. Both **DMS** and **II** are controlled in Stage 1 RSM **III** at NGT 0.1% (w/w). Compound **III** is controlled to NMT 0.6% (w/w) in the Stage 1 product **V**. **VIII**, on the other hand, is controlled in the Stage 3 RSM **VII** at NMT 0.1% (w/w). Lastly, compound **VI** is controlled at IG **IX** at NMT 0.6% (w/w). In summary, the implemented analytical strategy involves testing and controlling all five genotoxic impurities in SMs or intermediates rather than in API. As a consequence, five sophisticated LC/MS methods for testing these impurities at low ppm level in final API were eliminated. Since the analytical limit is at % level, simplified HPLC/UV methods are instead applicable, providing for a more robust and cost effective analytical approach for transfer to manufacturing facilities. From the QbD perspective, the pazopanib HCl genotoxic impurity control strategy has been built into the manufacturing process, and consistent product quality in terms of genotoxin levels is ensured.

4. Conclusions

Analysis and control of trace-level genotoxic impurities formed during drug development and manufacturing has presented a challenge to the pharmaceutical industry in recent years. However, specific guidance is currently lacking with respect to how to cope with this challenge; if it is not tackled strategically, it may ultimately impede productivity and inflate costs by drawing extensive analytical resources into drug development and manufacturing. In an effort to gain control over the genotoxic impurities encountered in the manufacture of pazopanib hydrochloride, we have developed a successful analytical control approach based upon the process understanding data as well as actual batch data. Five trace analysis LC/MS methods were developed and validated for testing all the genotoxic impurities in the pazopanib drug substances intended for clinical use, to ensure patient safety. These methods provided batch data that were critical elements of the specification setting and provided a basis for the design of the control strategy.

To map the fate of the various genotoxic impurities in the pazopanib HCl manufacturing process, comprehensive spiking/purging studies were conducted. The process tolerability data obtained from these studies provided the premise for formulating the analytical control strategies of the five genotoxic impurities. By setting the control points early in the chemical manufacturing process, these genotoxic impurities can be monitored at percent level rather than ppm level. Consequently, simplified HPLC/UV impurity methods become amenable for testing and control of various genotoxic impurities in SMs or intermediates, respectively. It is worth noting that in the event of a change in the SM supplier or synthetic route, representative batches would have to be tested thoroughly to ensure continued conformance to genotoxic impurity specifications. Similarly, if the commercial manufacturing process is modified, the validity of the spiking/purging studies would have to be validated by additional experiments.

Much of the genotoxic impurity discussions in recent literature focus on analytical methodologies of trace analysis. While developing trace method without a doubt poses a real challenge to the analytical community, controlling genotoxic impurities in drug substance relying on analytical testing using sophisticated analytical tools such as hyphenated mass spectrometry technologies may not be the ultimate goal. The case study presented in this report shows a practical implementation of a strategy for controlling five genotoxic impurities in the commercial process developed for a new chemical

entity. It is the authors' hope that the present study will serve as a model for similar issues encountered during process development. This control strategy has been discussed with the US FDA during a face-to-face meeting and is an important part of the recent NDA filing.

Acknowledgements

The authors would like to thank Drs. Yan Li, Shawn Yang, Mingjiang Sun, Jianguo An, Ravinder Sudini, Dharmesh Bhanushali, Mr. Lin Bai and Sheng Tang of Chemical Development, GlaxoSmithKline, for their contributions to the analytical data generation and spiking/purging experiments. We also thank Drs. Gerald Terfloth, Fred Vogt, and Vance Novack of Chemical Development, GlaxoSmithKline, for valuable discussions during the preparation of this manuscript.

References

[1] Committee for medicinal products for human use (CHMP), European Medicines Agency (EMEA), London, 28 June 2006 (CPMP/SWP/5199/02, EMEA/CHMP/QWP/251344/2006).
[2] L. Müller, R.J. Mauthe, C.M. Riley, M.M. Andino, D. De Antonis, C. Beels, J. Degeorge, J.A.G.M. De Knaep, D. Ellison, J.A. Fagerland, R. Frank, B. Fritschel, S. Galloway, E. Harpur, C.D.N. Humfrey, A.S. Jacks, N. Jagota, J. Mackinnon, G. Mohan, D.K. Ness, M.R. O'Donovan, M.D. Smith, G. Vudathala, L. Yotti, Regul. Toxicol. Pharmacol. 44 (2006) 198–211.
[3] US Food and Drug Administration CDER Guidance for industry, http://www.fda.gov/cder/guidance/7834dft.pdf (accessed 14 January 2009).
[4] E.J. Delaney, Regul. Toxicol. Pharmacol. 49 (2007) 107–124.
[5] T. McGovern, D. Jacobson-Kram, Trends Anal. Chem. 25 (2006) 790–795.
[6] L.X. Xu, Pharm. Res. 25 (2008) 781–791.
[7] K.L. Dobo, N. Greene, M.O. Cyr, S. Caron, W.W. Ku, Regul. Toxicol. Pharmacol. 44 (2006) 282–293.
[8] M.D. Argentine, P.K. Owens, B.A. Olsen, Adv. Drug Deliv. Rev. 59 (2007) 12–28.
[9] D.A. Pierson, B.A. Olsen, D.K. Robbins, K.M. DeVries, D.L. Varie, Org. Process Res. Dev. 13 (2009) 285–291.
[10] L.A. Sorbera, J. Bolós, N. Serradell, Drugs Future 31 (2006) 585–589.
[11] J. An, M.J. Sun, L. Bai, T. Chen, D.Q. Liu, A. Kord, J. Pharm. Biomed. Anal. 48 (2008) 1006–1010.
[12] C.D.N. Humfrey, Toxicol. Sci. 100 (2007) 24–28.
[13] C.G. Mohan, T. Gandhi, D. Garg, R. Shinde, Mini Rev. Med. Chem. 7 (2007) 499–507.
[14] H.M. Bolt, H. Foth, J.G. Hengstler, G.H. Degen, Toxicol. Lett. 151 (2004) 29–41.
[15] IARC Monographs on the Evaluation of Carcinogenic Risks to Humans: Dimethyl Sulfate, vol. 71, 1999, pp. 575–588.