Organic Process Research & Development **2010**, *14*, 977–985

A Systematic Method Development Strategy for Determination of Pharmaceutical Genotoxic Impurities

Mingjiang Sun, David Q. Liu, and Alireza S. Kord*

Analytical Sciences, GlaxoSmithKline Pharmaceutical R&D, 709 Swedeland Road, King of Prussia, Pennsylvania 19406, U.S.A.

Abstract:

Trace level genotoxic impurities (GTIs) in pharmaceutical products require precise, accurate, and robust analytical methodologies for their analysis and control. The need to control most genotoxic impurities to the low ppm level in combination with the very often reactive and labile nature of genotoxic impurities presents significant analytical challenges. This article reports a systematic GTI method development strategy (MDS) based on our successful experiences in GTI analysis in recent years and quality by design (QbD) principles emphasizing the expected method performance being built into the method. It starts with a predefined method goal, followed by method understanding and a risk control strategy. Due to the nature of the GTI analysis, sophisticated analytical methodologies such as chemical derivatization and mass spectrometry detection are often developed, especially in the research and development (R&D) phase of drug development. Such methods usually consist of more variables than conventional methods in pharmaceutical analysis. Therefore, sound scientific understanding and risk control strategies are of great importance to ensure the method performance for trace GTI analysis. For methods to be implemented in manufacturing quality control laboratories that are lacking sophisticated instrumentation and skilled analysts, method simplicity, robustness, and ruggedness become more prominent in addition to method accuracy. This article describes QbD approaches for developing such methods using real world case studies including dimethyl sulfate analysis of a recently approved drug, pazopanib HCl (Votrient).

Introduction

Pharmaceutical genotoxic impurities (GTIs), which may potentially increase cancer risks in patients, have recently received considerable attention from regulatory bodies and pharmaceutical manufacturers.[1] The European Medicines Agency (EMEA) has issued initial guidelines regarding the control of potential genotoxic impurities in drug substances and drug products,[2] and the U.S. Food and Drug Administration (FDA) also published a draft guidance document online recently.[3] As such, pharmaceutical manufacturers are actively seeking strategies to monitor and control the levels of GTIs in drug substances and drug products. Potential genotoxic impurities could be process impurities and/or degradants at trace levels that are generated during manufacturing processes and storage. Unless the toxicological thresholds are unequivocally established, potential genotoxic impurities are controlled at a generic 'threshold of toxicological concern' (TTC) of 1.5 μg per day in marketed products according to the guidance documents. For clinical investigations, however, a staged TTC applies where greater daily intake can be allowed for shorter dosing durations (Table 1). For marketed drugs with a daily dose of 1 g, the generic allowable level of 1.5 μg per day would require a control level of 1.5 ppm (ppm, μg GTI per g API), which is several hundred-fold lower than the classic impurity level of 0.05% encountered in pharmaceutical analysis. Considering such a low concentration, reliable analytical methods must be a critical element of the control strategies of genotoxic impurities.

Despite great advances in developing sensitive methods for individual or classes of genotoxic impurities in recent years,[4−6] a systematic strategy for developing robust and rugged methods is still lacking. Furthermore, the current regulatory focus on quality by design (QbD) of pharmaceutical products mandates a closer look at the analytical method development processes in genotoxic impurity analysis.[7−10] Successful applications of QbD principles and strategies to the method development of

* Author to whom correspondence may be sent. Fax: 610-270-6727. E-mail: Alireza.S.Kord@gsk.com.

(1) Friscia, O.; Pulci, R.; Fassio, F.; Comelli, R. *J. Environ. Pathol.* **1994**, *13*, 89.

(2) *Guideline on the Limits of Genotoxic Impurities*, CPMP/SWP/5199/02, EMEA/CHMP/QWP/251344/2006; Committee for Medicinal Products (CHMP), European Medicines Agency (EMEA): London, 28 June 2006.

(3) Genotoxic and Carcinogenic Impurities in Drug Substances and Products: Recommended Approaches; U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER): Silver Spring, MD, U.S.A., December 2008; http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm079235.pdf.

(4) David, F.; Jacq, K.; Vanhoenacker, G.; Sandra, P.; Baker, A. *LCGC Eur.* **2009**, *22*, 552.

(5) Liu, D. Q.; Sun, M.; Kord, A. S. *J. Pharm. Biomed. Anal.* **2010**, *51*, 999.

(6) Yuabova, Z. Y.; Holschlag, D. R.; Rodriguez, S. A.; Qin, C.; Papov, V. V.; Qiu, F.; McCaffrey, J. F.; Norwood, D. L. *J. Liq. Chromatogr. Relat. Technol.* **2008**, *31*, 2318.

(7) *International Conference on Harmonisation (ICH) Guidance for Industry: Pharmaceutical Development Q8*, (R2); U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER): Rockville, MD, Aug, 2009, http://www.fda.gov/RegulatoryInformation/Guidances/ucm128028.htm.

(8) International Conference on Harmonisation (ICH) Guidelines, Q9: Quality Risk Management, Nov. International Conference on Harmonisation (ICH) Guidance for Industry: Quality Risk Management Q9; U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER): Rockville, MD, Nov, 2005, http://www.fda.gov/RegulatoryInformation/Guidances/ucm128050.htm.

(9) Borman, P.; Chatfield, M.; Nethercote, P.; Thompson, D.; Truman, K. *Pharm. Technol.* **2007**, *31*, 142.

10.1021/op100089p
Published on Web 05/03/2010

Table 1. **U.S. FDA and EMEA recommended acceptable qualification thresholds for genotoxic impurities in pharmaceuticals in clinical studies**

	TTC limits corresponding to the duration of dosing					
control threshold (μg/day)	120	60	20	10	5	1.5
duration US FDA	<14 days	14 days to 1 month	1−3 months	3−6 months	6−12 months	>12 months
duration EMEA	1 day	≤1 mon	≤3 months	≤6 months	≤12 months	>12 months

HPLC and Karl Fisher titration have been presented in recent publications from our laboratory.[11,12] The aim of the current article is to discuss a systematic method development strategy (MDS) for developing robust and rugged methods for genotoxic impurities in drug substances or products. The GTI MDS presented herein follows the same scientific and risk-based approach developed in the previous publications as exemplified in the flowchart in Figure 1. Real-world drug development examples are provided.

Method Goal

Before commencement of the method development for genotoxic impurity analysis, the first step is to clarify the purpose of testing, which determines where the method will be implemented and how the results will be used. The purpose of testing defines requirements on method sensitivity and accuracy. During method goal setting, several factors must be considered including (1) whether the method is for batch release testing or for experimental investigation only (e.g., spiking/purging studies, stability monitoring, etc.); (2) the requirement for method sensitivity, accuracy, and precision (whether a limit or quantitative test is needed); and (3) end user and method complexity considerations (whether the method is being developed for research and development (R&D) or manufacturing analytical laboratories).

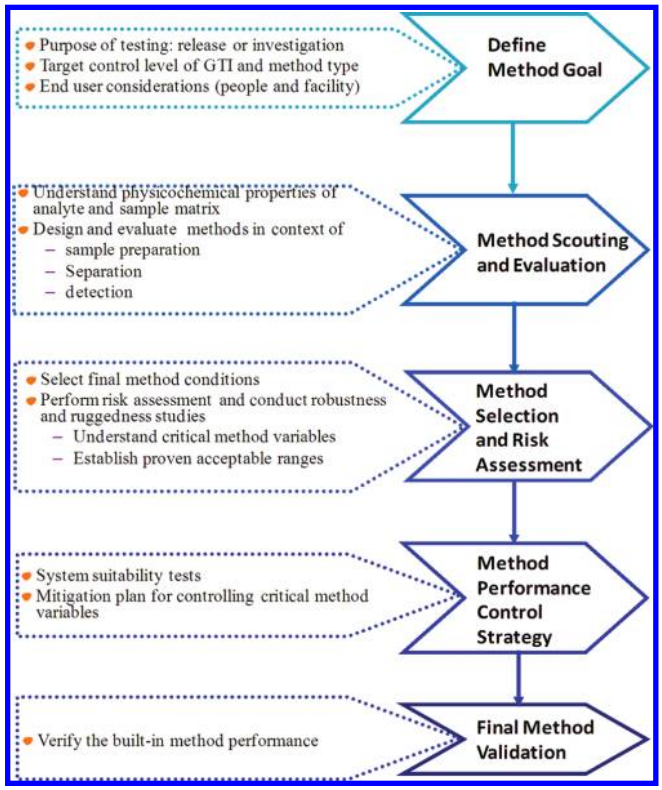

Figure 1. **Systematic approach to GTI analysis method development in a pharmaceutical QbD environment.**

For release testing of final drug substances or drug products, per regulatory requirements, the requisite method sensitivity is very often in the ppm range. And if necessary, a method for testing commercial products may need to be implemented in manufacturing quality control (QC) facilities. This would have implications for the method complexity with regard to instrument availability as well as analysts' expertise.[13] For instance, instruments such as mass spectrometers may not be readily available in typical QC laboratories, and the analysts may not be trained in operating such instruments or complex sample preparation procedures. Therefore, simple and conventional methods are preferred in manufacturing QC laboratories. Complex and nonconventional methods, e.g. LC/MS, may require additional investment, which needs to be discussed among stake holders as early as possible.

QbD pharmaceutical manufacturing advocates the quality being built into the process, i.e., genotoxic impurity levels can be controlled in-process on the basis of process understanding rather than solely relying on testing of the final products. This requires extensive investigation into the manufacturing processes from which an opportunity to control genotoxic impurities upstream can be identified, e.g. controlling the genotoxic impurities in starting materials or synthetic reaction intermediates.[14] To support the process understanding, significant spiking/purging experiments need to be conducted. For this type of analysis, the GTI levels in intermediates are normally much higher than that required for active pharmaceutical ingredient (API). Therefore, less sensitive and less robust methods might be sufficient. Because such spiking/purging experiments are usually conducted in lab scale in R&D, method complexity in terms of instruments as well as analyst expertise is generally not a concern.

Both limit tests and quantitative tests are currently used in GTI testing and have different requirements for method sensitivity and accuracy. A limit test in essence is a comparison of the concentration of an analyte in a sample to that of a known standard, and results are reported as not greater or greater than (pass or fail) the concentration of the standard. This is different from the quantitative analysis where the level or concentration of analyte is numerically reported. Limit test methods are not validated as vigorously as quantitative methods.[15] For APIs committed to commercial production, the actual level of a certain GTI in API should have been well characterized. If the

(10) Yu, L. X. *Pharm. Res.* **2008**, *25*, 781.
(11) Li, Y.; Terfloth, G. J.; Kord, A. S. *Am. Pharm. Rev.* **2009**, *12*, 87.
(12) Zhou, L.; Socha, J. M.; Vogt, F. G.; Chen, S.; Kord, A. S. *Am. Pharm. Rev.* **2010**, *13*, 74.
(13) Borman, P. J.; Chatfield, M. J.; Crowley, E. L.; Eckers, C.; Elder, D. P.; Francey, S. W.; Laures, A. M. F.; Wolff, J. C. *J. Pharm. Biomed. Anal.* **2008**, *48*, 1082.
(14) Liu, D. Q.; Chen, T. K.; McGuire, M. A.; Kord, A. S. *J. Pharm. Biomed. Anal.* **2009**, *50*, 144.
(15) International Conference on Harmonisation (ICH) Guidelines, Q2(R1): Validation of Analytical Procedures: Text and methodology, Jun. 1995.

actual level of GTI is far below the specification, limit tests should be sufficient for final product-release analysis. Quantitative analysis is generally preferred for samples of which genotoxic impurity level is approaching the specification or the genotoxic impurity is a degradant which may elevate in the course of manufacturing or storage. Furthermore, quantitative methods might be required for monitoring actual levels of GTIs in the intermediate steps during process understanding; this is especially true for late-stage development compounds.

In summary, a method goal should include but is not limited to purpose of testing, level of testing, method type, accuracy requirement, end user, and instrument capability and constraints of the receiving laboratory. The following examples describe how a typical method goal is developed.

Case Study 1. *Method Goals of Analysis of Dimethyl Sulfate (DMS) in API of Pazopanib.* Genotoxic dimethyl sulfate (DMS) is a reagent used in the synthesis of a starting material for manufacturing pazopanib.[14] During clinical development of pazopanib, a sensitive method was required to demonstrate the absence of DMS as an impurity in the final drug substance. In addition, in order to support the development of a process control strategy of DMS via spiking/purging studies, a sensitive and selective quantitative method to assay the levels of DMS at 1.7 ppm (w/w) in API is required.[16] Since this method is to be used in the R&D and is for investigational purposes, method and instrument complexity is not a major concern, whereas method sensitivity and selectivity are the key factors in method development. Therefore, the method is not constrained in terms of sample preparation procedures, separation technique, and detection strategy. A method goal can be set to develop a quantitative method to assay DMS in API with a limit of quantitation (LOQ) of 1.7 ppm or better.

Case Study 2. *Method Goals of Analysis of Dimethyl Sulfate (DMS) in a Starting Material of Pazopanib.* On the basis of process knowledge from purging/spiking studies, up to 5% (w/w) DMS in the starting materials can be purged effectively in the final manufacturing process.[14] As such, a specification of 0.1% (1/50 of the highest tested level) is set as the control limit of DMS in the starting material. In this case, the analytical control of DMS was shifted upstream to the starting material, where a second method able to analyze 0.1% DMS in the starting material was required.[14] As such, the method goal changes completely in several aspects compared to the previous case. First, the target control level becomes the percentage level instead of ppm level. Second, the method must be transferred to manufacturing QC laboratories, and thus, instrument availability and method simplicity as well as method robustness and ruggedness are of critical importance. Third, since the typical DMS level is well below 0.1% in the starting material and adherence to method simplicity desirable, a limit method will suffice the project needs. Therefore, a method goal can be set for this method to develop a limit test method that can detect DMS at 0.1% level, which should be readily implemented in the designated manufacturing QC laboratories.

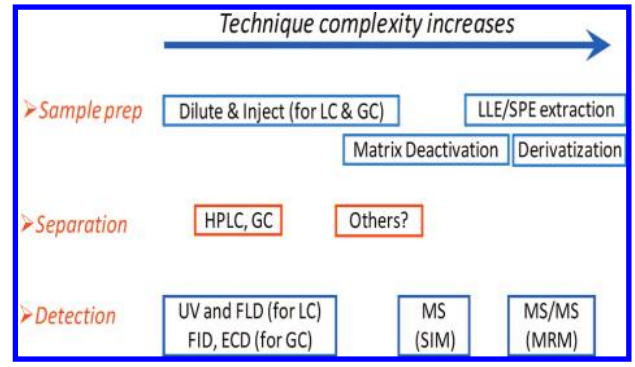

Figure 2. **Ranking of techniques according to their complexity.**

Method Scouting and Evaluation

Genotoxic impurities consist of a broad range of chemical structures. Varying physicochemical properties require a variety of analytical approaches in order to achieve the desired method sensitivity. For instance, some GTIs are small volatile molecules such as alkyl halides, while others are nonvolatile synthetic intermediates; some are chemically reactive, while others are relatively stable. In addition, the sample matrix (including API, minor impurities, excipients, and solvents) may have great influence on analytical method selection. Understanding analyte properties, a function of molecular structure, is the key to the successful design of a fit-for-purpose method. The properties of analyte and sample matrix dictate what kind of sample preparation, separation, and detection techniques to be used. Therefore, the method scouting and evaluation process herein is the combination of on-paper design and laboratory verification. The paper exercise is an economical and efficient way to start. However, the approach will inevitably rely on the analysts' knowledge and experience with the analyte and sample matrix. The output of the design process is a list of potential approaches which are ranked from the most to least promising on the basis of their sensitivity, complexity, accuracy, and the constraints of the end user. From an analytical procedure perspective, a trace analysis method can be dissected into three technical unit operations including sample preparation, separation, and detection, although they are not completely independent, e.g. certain sample preparation procedures are designed for a particular detection method. Each unit operation may have multiple options that require thorough evaluation individually. The relative degree of complexity of each unit operation is described in Figure 2.

Sample Preparation. Sample preparation is important for GTI analysis, because matrix effects in trace analysis are magnified, causing loss of sensitivity, abnormal recovery, and analyte instability.[17] The simplest sample preparation is 'dissolve and inject' (or 'dilute and inject' for liquid samples) in both liquid chromatography (LC) and gas chromatography (GC). For GC methods, headspace injection is a preferred introduction technique to avoid injection of a high concentration of API. These techniques are routinely accessible in most laboratories. Matrix deactivation, on the other hand, is a special sample treatment technique developed in our laboratory for stabilizing

(16) An, J.; Sun, M.; Bai, L.; Chen, T.; Liu, D. Q.; Kord, A. *J. Pharm. Biomed. Anal.* **2008**, *48*, 1006.

(17) Sun, M.; Bai, L.; Terfloth, G. J.; Liu, D. Q.; Kord, A. S. *J. Pharm. Biomed. Anal.* **2010**, *52*, 30.

certain reactive and unstable analytes.[17] Matrix deactivation makes it possible for such analytes to survive GC or LC chromatographic conditions. A good understanding of the degradation mechanism of the analyte or, at a minimum, an informed guess based on chemical structure is the prerequisite. Matrix deactivation assumes that the degradation of a certain analyte is caused or catalyzed by certain chemicals in the sample matrix, and removal or reduction of the reactivity of the interfering chemicals will improve the stability of analyte. The approach simply adds a selected reagent to sample diluents and thus does not increase complexity for routine operation of the analytical method. Sample extraction techniques including liquid−liquid extraction (LLE) and solid-phase extraction (SPE) are useful tools for enrichment of analyte or removal of interfering sample matrix, widely used in bioanalysis. These sample extraction techniques, however, add labor-intensive steps into the analysis, and the resulting method requires extra validation. As such, sample extraction is used only as a last resort.[18] Nonetheless, it may be potentially beneficial in analysis of drug products when dealing with complex excipients. Lastly, chemical derivatization of analytes is a versatile technique that can be used to modify physicochemical properties of analytes. By chemical derivatization, labile analytes can be stabilized, or a unique structural motif can be incorporated into poorly detectable analytes to improve detection and/or separation.[19,20] Selection of derivatization reagents is largely determined by functional groups within the analyte and the intended separation and/or detection techniques.

Choosing a Separation Technique. The most common and versatile separation techniques in pharmaceutical analysis are HPLC and GC, whereas others such as ion chromatography (IC), capillary electrophoresis (CE), and supercritical fluid chromatography (SFC) have not demonstrated usefulness in GTI analysis in the literature. GTIs can be divided into two groups based on their volatility, and HPLC and GC are chosen, depending on analytes' volatility. For nonvolatile analytes, reversed phase (RP)-HPLC is the most popular chromatography mode. Many types of RP-HPLC column stationary phases are well established for separation of various pharmaceutical molecules. Hydrophilic interaction liquid chromatography (HILIC) is complementary to RP-HPLC and is useful for separation of very polar analytes.[16]

Volatile compounds, on the other hand, can be separated by GC. In GC analysis, headspace injection adds another dimension of separation alongside chromatographic separation, where nonvolatile API is not introduced into the GC system. Therefore, headspace is the preferred mode of sample introduction for thermally stable volatile compounds. Direct injection, on the other hand, inevitably introduces a large amount of API into the GC system and thus may contaminate the injection port, GC column, or detector. More frequent instrument cleaning might be required. Recently, two-dimensional (2-D) GC techniques such as back flush and heart cut analysis have been applied to GTI analysis, which only introduce a fraction of the volume containing the analyte of interest into the MS detector or second column, and thus minimize instrument contamination.[21]

Choosing a Detection Method. In pharmaceutical analysis, UV is by far the most commonly used detector for HPLC, whereas flame ionization detector (FID) is the standard detector for GC. From the instrument simplicity, stability, and availability points of view, these standard instruments should be considered as the first-intent method whenever the method is intended to be used in manufacturing QC laboratories. However, often they may not offer sufficient sensitivity for certain analytes in trace GTI analysis. Electron capture detection (ECD) can be a good substitute for FID in GC when GTIs consist of halogens, which provides an additional level of selectivity in detection. It is evident from the recent literature that sensitive and specific mass spectrometry detection plays a dominant role in GTI analysis. Quadrupole mass analyzers are standard for quantitation, and selected ion monitoring (SIM) or multiple reaction monitoring (MRM) are the most commonly used detection modes. SIM is typically achieved on single quadrupole MS instruments, while MRM requires more sophisticated triple quadrupole MS instruments. In terms of method transfer into manufacturing QC laboratories, the disadvantages of using mass spectrometric detection are obvious, where higher capital investment is anticipated and specially trained operators are a prerequisite. Thus, in general, mass spectrometry detection for GTI analysis in manufacturing laboratories should be a last resort, and MRM methods on high-end triple quadrupole should be avoided if possible, even though it may add method robustness in terms of sample variation. Transferring mass spectrometry based methods into QC laboratories is possible but not desired;[13] alternative methods should be thoroughly explored when the drug development program approaches the late stage. During clinical development stages, however, mass spectrometry has been playing increasing roles in pharmaceutical analysis since instrument availability (especially single quadrupole MS) is not an issue. Taking advantage of high selectivity and sensitivity, MS detection facilitates GTI method development. Use of other detectors such as the evaporative light-scattering detector (ELSD) and the charged aerosol detector (CAD) for HPLC have been attempted in recent publications, but the true benefit is yet to be realized.[6] The application of fluorescence detector (FLD), while it appears promising, has not been reported.

Method Design and Evaluation. Method design and evaluation are achieved by considering the following factors holistically: the defined method goal, the properties of the analyte and sample matrix, and the capability of analytical techniques in each unit operation. In general, the simplest technique should be chosen for each unit operation. For example, conventional analytical instrumentation in pharmaceutical analysis such as HPLC with UV detection (for typical nonvolatile analytes) or GC with FID detection (for volatile small molecules) should be employed as the standard first attempt for GTI analysis. However, the resulting method may not necessarily offer the desired sensitivity defined in the method

(18) Zheng, J.; Pritts, W. A.; Zhang, S.; Wittenberger, S. *J. Pharm. Biomed. Anal.* **2009**, *50*, 1054.
(19) Bai, L.; Sun, M.; An, J.; Liu, D. Q.; Chen, T. K.; Kord, A. S. *J. Chromatogr., A* **2010**, *1217*, 302.
(20) Vanhoenacker, G.; Dumont, E.; David, F.; Baker, A.; Sandra, P. *J. Chromatogr., A* **2009**, *1216*, 3563.
(21) David, F.; Jacq, K.; Sandra, P.; Baker, A.; Klee, M. S. *Anal. Bioanal. Chem.* **2010**, *396*, 1291.

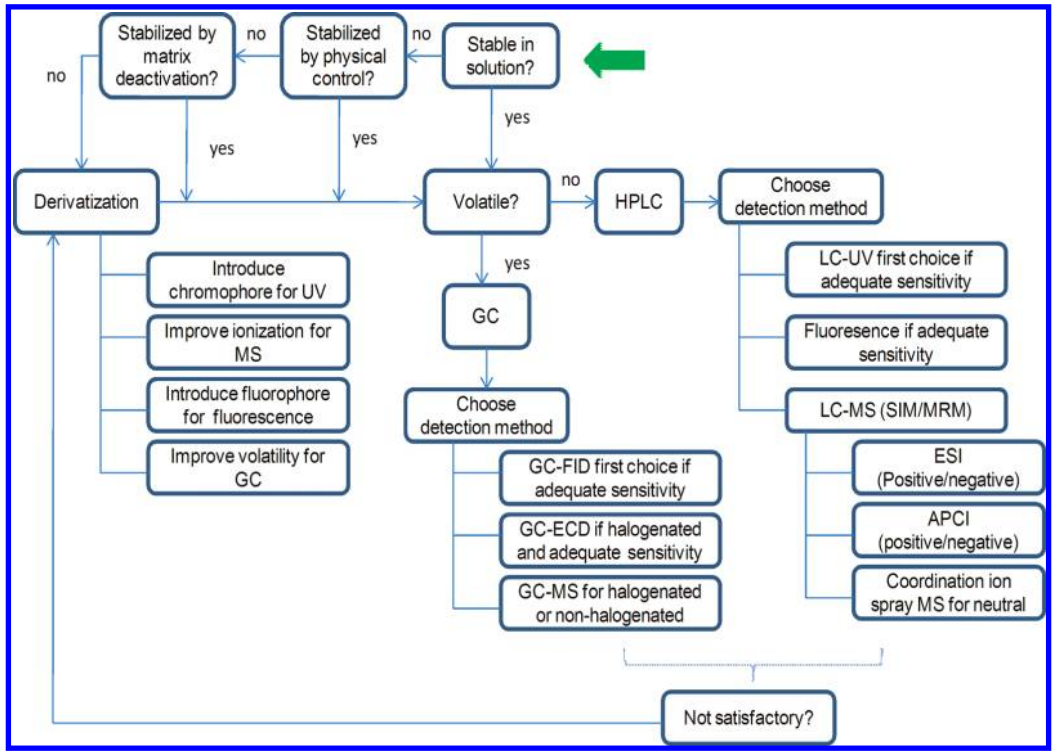

Figure 3. **Decision tree for designing a GTI analysis method.**

goal. Often, multiple approaches can be proposed on the basis of the paper exercise and analysts' knowledge, as well as literature reports. The potential methods should be ranked according to their sensitivity and complexity. We have developed a systematic decision tree as shown in Figure 3 to assist the method design and evaluation process. The decision tree starts by considering the solution stability of the analyte in the presence or absence of API. The stability of the analyte in solution determines whether alternative sample preparation procedures are required instead of 'dissolve and inject'. In the case where the analyte stability is poor, the stability can often be improved by physical measures such as storing sample at low temperatures or protection from light. More effective chemical measures such as matrix deactivation or chemical derivatization approaches may be used depending on the property of the analyte and sample matrix.[17,19,22] The next step is to examine the volatility of the analyte (or its derivatization product), which determines if it is more amenable to GC or LC. By the same token, proper detection techniques can be selected based upon molecule properties. Should none of the detection options meet the requirement of the method goal (sensitivity in particular), different chemical derivatization approaches would need to be considered. Various types of derivatives can be prepared in order to make the analyte amenable to the desired separation and detection techniques.[19] Sample enrichment techniques, such as LLE and SPE, are not included in the decision tree because they are not preferred due to the complexity of the procedures. However, this does not preclude their use in special cases when necessary.[18] It is worth mentioning that the three unit operations are not completely independent and should always be evaluated holistically.

Following the method ranking, the most promising method should be experimentally evaluated. For instance, if derivatization is involved, the derivatization reaction should always be tested for proof of concept. The derivatization product can be evaluated quickly by a generic GC-MS or LC-MS method, so that the characteristics of the derivatization can be fully understood. Fast and selective reactions with high yield of the desired product and minimal by-products are desired. In addition, the separation of the derivative from the API peak or other interferences has to be assessed. Ideally, the resolution should be better than 4 to prevent any adverse effect of the large amount of API, especially when the analyte elutes after the API. The resolution with other interfering peaks should be better than 2 if a nonspecific detection method is used. Furthermore, the sample preparation should be evaluated to examine whether the analyte has sufficient stability during analysis. If not, the matrix deactivation approach should be explored to stabilize the analyte. In our experience, this is critically important as a means to ensure the desired sensitivity, linearity, and accuracy of the method. Eventually, method sensitivity, linearity, accuracy and precision should be evaluated. Issues arising during method evaluation such as unexpected solution instability of the analyte, should be addressed accordingly, and the resulting modified method should be re-evaluated in the same manner.

Case Study 3. *Design and Evaluation of Dimethyl Sulfate (DMS) Methods for Pazopanib API and Starting Materials According to Method Goals.* Taking the above DMS analysis as an example, since the analyte itself is very unstable, derivatization is a preferred approach based on literature knowledge.[5,23,24] Although 'dilute and inject' sample introduc-

(22) Sun, M.; Bai, L.; Liu, D. Q. *J. Pharm. Biomed. Anal.* **2009**, *49*, 529.

(23) Elder, D. P.; Teasdale, A.; Lipczynski, A. M. *J. Pharm. Biomed. Anal.* **2008**, *46*, 1.

tion approach has been explored, it has certain limitations. The choice of derivatization reagents must be considered in the context of separation and detection techniques. For instance, Jacq et al. used pentafluorothiophenol as the derivatization reagent to convert DMS into volatile methyl pentafluorothiophenol, which can be analyzed using headspace GC/MS,[24] and the authors recommended using an internal standard (IS) to ensure method accuracy. An and colleagues used triethylamine to convert DMS into quaternary ammonium to be analyzed by HILIC/MS analysis.[16] Liu and colleagues developed a 2-mercaptopyridine derivatization approach for analysis of DMS at the 0.1% level which can be readily implemented on conventional HPLC/UV instruments.[14] The first two methods deliver low ppm level sensitivity and superior selectivity because of the use of MS-based detection techniques. The MS-based methods can be easily implemented in R&D settings, but they are not preferred in manufacturing QC laboratories. The third derivatization LC/UV method, on the other hand, affords 0.1% level sensitivity but uses simple separation and detection techniques. The above MS-based or UV-based methods need to be evaluated in the context of method goals. As a result, the MS-based method was selected for API analysis to establish process understanding during process development, while the UV-based method was used for testing and control of DMS in starting materials in the QC laboratories at manufacturing sites in keeping up with the method goals described in case studies 1 and 2, respectively.[14]

Method Selection and Risk Assessment

Following the above method evaluation, which includes the paper exercise supported by preliminary proof of concept testing, a method can now be selected for further investigation. This is essentially a method optimization step, wherein various method parameters are to be optimized on the basis of the method goal. Factors that may affect method performance should be proposed and grouped and subjected to risk assessment and robustness and ruggedness studies.[11] Risk assessment followed by robustness and ruggedness studies allows for effective investigation of method variables and understanding of their relative significance to method performance. This would lead to the identification of critical method parameters that should be closely monitored and tightly controlled throughout the method life cycle. It is worth mentioning that risk assessment and robustness and ruggedness studies are meant for methods to be used in a manufacturing facility throughout the life cycle of the product rather than for methods used for investigational purpose. However, this does not preclude the use of risk assessment tools in method optimization for R&D use.

Case Study 4. *Robustness and Ruggedness Studies for the DMS HPLC/UV Limit Method.* On the basis of process understanding, i.e., the purgeability of DMS in the manufacturing process of pazopanib, it was established that DMS can be controlled upstream in the starting material at the 0.1% level.[14] Therefore, the derivatization LC/UV method designed in case study 3 was selected to be used in manufacturing facilities. The derivatization involves the use of 2-mercaptopyridine reacting with DMS to produce 2-methylthiopyridine, which can be readily monitored by a HPLC/UV method (see Table 2 for method details).

Table 2. **Conditions of the HPLC/UV method for determination of DMS in starting material of pazopanib**

column details (column type, particle size and column dimensions)		Waters SunFire, C18, 50 mm × 2.1 mm ID, 3.5 μm	
column temperature		40 °C	
mobile phase A		0.1% HFBA (v/v) in Water	
mobile phase B		100% MeCN	
flow rate		0.4 mL/min.	
gradient profile	time (min)	%B	flow rate (mL/min)
	0	6.0	0.4
	5.0	6.0	0.4
	5.1	6.0	1.0
	6.9	60.0	1.0
	7.0	6.0	1.0
	10.0	6.0	1.0
	10.1	6.0	0.4
	11.0	6.0	0.4
detector wavelength	315 nm		
injection volume	2.0 μL		
sample concentration	2.5 mg/mL		

In order to identify the method variables, a risk assessment using a fishbone diagram was conducted (Figure 4). The variables were grouped into six categories. Each of the three unit operations is one category, and people, material, and facility and equipment are the others. Each variable was assigned as controlled (C), experimental (X), or noise (N). The controlled variables will be controlled through laboratory practices. The critical experimental variables will be fed into robustness studies, performed using design of experiment (DOE)[11] or single-variable experiment protocols.[13] The noise variables will be evaluated during ruggedness studies using measurement system analysis (MSA).[11] For the DMS derivatization LC/UV method, three high-risk experimental variables were identified according to their scores assigned by four analysts during the fishbone diagram exercise. They are heptafluorobutyric acid (HFBA) concentration in the mobile phase, derivatization reaction temperature, and column stability. These variables were fed into robustness studies, where the variables were evaluated within a defined range against method attributes such as retention time, sensitivity, recovery, peak resolution, and peak tailing factor. As a result, the proven acceptable ranges of the three variables were established. The acceptable derivatization reaction temperature range was determined to be 55−65 °C; the percentage of HFBA (±20%) had no effect on the results; the column lasted at least for 200 injections. The noise variables were evaluated by the ruggedness tests using five samples, two analysts, two columns, and two instruments on two different days. The ruggedness results are summarized in Table 3 and 4. The results indicated the noise variances have no significant impact on the result of DMS in the samples. Thus, it can be concluded that a robust and rugged method was achieved.

(24) Jacq, K.; Delaney, E.; Teasdale, A.; Eyley, S.; Taylor-Worth, K.; Lipczynski, A.; Reif, V. D.; Elder, D. P.; Facchine, K. L.; Golec, S.; Oestrich, R. S.; Sandra, P.; David, F. *J. Pharm. Biomed. Anal.* **2008**, *48*, 1339.

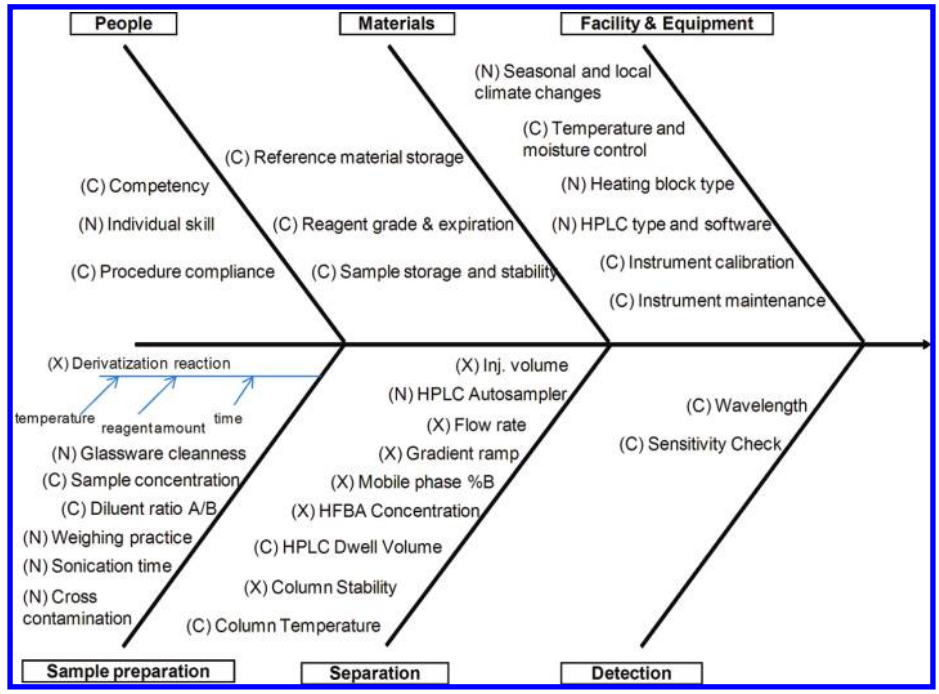

Figure 4. **Fishbone diagram of the HPLC/UV method for determination of DMS in starting material of pazopanib.**

Table 3. **Demonstration of the intermediate precision of the method for DMS: five replicate preparations of a starting material of pazopanib spiked with 0.1% (w/w) of DMS**

sample no.	analyst_1% recovery	analyst_2% recovery
1	65.3	79.2
2	65.2	75.3
3	69.1	73.1
4	67.4	75.6
5	70.4	76.0
Mean	67.5	75.8
%RSD	3.4	2.9

Table 4. **Demonstration of the intermediate precision of the method for DMS: six injections for one of the preparations of a starting material of pazopanib spiked with 0.1% (w/w) of DMS**

parameters (n = 6)	analyst_1	analyst_2
injection repeatability (%RSD)	1.7	1.4
RT (min)	2.7	3.2
% RSD	0.7	0.4
T_f value	1.1	0.9
% RSD	1.4	2.2
S/N (0.1%)	51	60
% RSD	19.1	1.0

Method Performance Control Strategy

As a result of robustness and ruggedness studies, the overall understanding of method performance under various conditions can be improved. Consequently, an analytical method performance control strategy along with appropriate system suitability criteria can then be defined to manage the method risks and to ensure that the method delivers the required performance. Typical system suitability criteria may include tests listed in Table 5. The variables that are not directly related to the method are also important. Since GTI analysis methods deal with trace analytes, good laboratory practices by analysts are of critical importance during method execution. In particular, a systematic control strategy to prevent sample contamination that could risk the analytical results should be in place. In our experience, it is imperative to establish a controlled sample segregation system and a spill containment/prevention procedure. Also, glassware cleanliness, proper pipet operation, and status of personal protection equipment such gloves and cleanliness, can never be overly emphasized. For volatile GTI analytes, evaporation of analytes may cause sample cross contamination. In such cases, precautions to separate the preparation of standard and samples in time (sample first, standard second) and/or space (separate chemical hoods) are highly recommended.

Additional Considerations for MS-Based Methods. It is known that adding an internal standard (IS) to samples can compensate for variations in sample preparation, separation, ionization, and instrument performance for MS-based methods.[25] The approach has also been used to solve analyte stability issues to some extent.[24] Both stable isotopically labeled and structurally related analogues could serve the purpose. MS-based methods with isotopically labeled IS offer the most reliable results for trace analysis when the level of the IS is within the range of the analyte by a factor of 10,[26] and accurate results can be achieved even with single-point calibration.[27] Thus, using IS in a GTI analysis method may build additional data accuracy and method robustness into the methods. IS is recommended for MS-based methods, especially when the level of GTI is close to the testing limit and the method may potentially be used in manufacturing QC laboratories. In this case, single-point calibration may serve the purpose for routine analysis for either quantitative or limit lest methods. In cases where IS is unavailable, additional risk assessment needs to be conducted to identify the potential risks, and a method performance control plan should be developed to mitigate the risks.

(25) Boyd, R. K.; Basic, C.; Bethem, R. A. *Trace Quantitative Analysis by Mass Spectrometry*; Wiley: Chippenham, 2008.
(26) Baldwin, R.; Bethem, R. A.; Boyd, R. K.; Budde, W. L.; Cairns, T.; Gibbons, R. D.; Henion, J. D.; Kaiser, M. A.; Lewis, D. L.; Matusik, J. E.; Sphon, J. A.; Stephany, R. W.; Trubey, R. K. *J. Am. Soc. Mass Spectrom.* **1997**, *8*, 1180.
(27) Zhurkovich, I. K.; Mil'man, B. L. *J. Anal. Chem.* **2009**, *64*, 986.

Table 5. **Example system suitability tests**

system suitability tests	parameter addressed	criteria
retention time	separation	within a range
resolution with interfering peaks	separation	better than 2
sensitivity	sensitivity of system	*S*/*N* at testing level not less than 10
standard deviation of response factors of replicate injections of standard solution at testing level	precision of system	less than 20%
MS detector calibrated	detector	pass tune check
recovery	sample preparation	better than predefined value

MS-based methods, owing to the high specificity and sensitivity of mass spectrometry, generally provide additional robustness and ruggedness compared to nonspecific detection techniques (e.g., UV) in terms of tolerance of sample variations. However, mass spectrometry detection involves multiple steps, including sample introduction, ionization, ion transmission, ion separation, and detection. Each stage of an instrument from different vendors is uniquely designed, and thus transferring a method between instruments from different vendors may require optimization of multiple instrumental parameters. The instrument in the receiving laboratory, even if it is the same model, may not perform exactly the same as the one used for method development because of the inherited differences in instrument performance as well as the instrument history. Therefore, the two instruments in the method development laboratory and the receiving laboratory should be standardized whenever possible. If not, early communication between the two laboratories and involvement of the receiving laboratory early during method evaluation and conducting a good risk assessment are essential. In short, for MS-based methods, instrument variables should be at the top of the list during risk assessment.

Case Study 5. *Transfer Method between Mass Spectrometers from Different Vendors.* During transfer of a limit test LC/MS method to a contract manufacture QC lab, challenges were encountered due to instrument differences between the two laboratories. The original method uses coordination ion-spray ionization to improve the ionization efficiency of a neutral analyte.[17] The analyte was separated on a C18 RP HPLC column, eluting with a mixture of aqueous potassium acetate (0.1 mM) and acetonitrile, and was monitored by SIM at *m*/*z* 260 $[M + K]^+$ on an Agilent MSD single quadrupole instrument. The method demonstrated excellent performance in the original lab with an *S*/*N* of 23 at the target testing concentration of 0.4 ppm. However, the analyte at this concentration could not be detected in the QC lab using a Waters ZQ single quadrupole instrument. Due presumably to the differences in instrument design, the Waters ZQ instrument did not handle the trace amount of potassium salt in the same way; thus, the method was overhauled. On the basis of the knowledge obtained during method evaluation, ammonium was selected as the alternative coordination ion. Both acetonitrile and methanol were screened as the organic mobile phase, and the concentration of ammonium buffer was optimized. Ultimately, a method that can perform similarly in both laboratories was developed. The final mobile phase was chosen to be 4 mM aqueous ammonium acetate and methanol. The method was subsequently validated in the contract lab and used to support batch releases and NDA filing.

During implementation of MS-based methods, day to day instrument stability should be carefully monitored and controlled via system suitability tests. Ionization is a critical step in MS analysis and can be interfered with by components in samples and mobile phases.[28] Ion suppression (or enhancement) has to be investigated thoroughly prior to method validation.[29,30] Testing multiple batches of samples should help to take into account the potential for ion suppression from various samples. Ion suppression can also be caused by instrument contamination, which can be minimized by diverting the unwanted interfering peaks (e.g., API) into the waste stream rather than into the mass spectrometer. This requires adequate resolution between the analyte and interfering peaks so that the small fraction consisting of only the analyte peak enters the MS. As such consistent retention time is an important attribute of the method, to ensure that the peak of interest elutes within the detection window, the method should be sufficiently robust and able to tolerate a small change in HPLC conditions such as the initial %B. Because of the QbD process used to develop the method, and the resulting understanding of the relationships between method variables and method performance, small modifications of parameters should not require revalidation. The concept has already been adopted by a technique of a GC/MS instrument, retention time locking,[31] by which instrument parameters are changed to keep the analyte's retention time constant using a locking compound. The maximum number of batches that can be analyzed continuously without deteriorating the instrument performance should also be assessed; i.e. the length of the analytical sequence should be controlled in routine analysis to ensure method stability and accuracy, especially when direct liquid injection GC/MS is used and a large amount of sample is injected.[17]

Case Study 6. *Instrument Response Instability Caused by Instrument Contamination within a Sequence.* The genotoxic impurity is a degradation product of an API, for which a MS-based analytical method was developed to determine its levels in the drug product (tablet formulation). After continuous injection of nine samples, serious ion suppression occurred, and the peak area lost a third of its intensity compared to the initial value within the same sequence. After cleaning of the ionization source the MS signal was restored. It was believed that the large amount of API and excipients injected onto the mass spectrometer caused deterioration of the MS signals. Therefore, the signal loss problem was ultimately solved by simply diverting the unwanted LC effluent to waste and narrowing the data collection window.

(28) Jessome, L. L.; Volmer, D. A. *LCGC North Am.* **2006**, *24*, 498.
(29) US FDA, Guidance for Industry, bioanalytical Method Validation, May 2001.
(30) Van Eeckhaut, A.; Lanckmans, K.; Sarre, S.; Smolders, I.; Michotte, Y. *J. Chromatogr., B* **2009**, *877*, 2198.
(31) Etxebarria, N.; Zuloaga, O.; Olivares, M.; Bartolome, L. J.; Navarro, P. *J. Chromatogr., A* **2009**, *1216*, 1624.

Final Method Validation

At this point, relationships between method parameters and method attributes are established. Key method risks have been identified and understood during method design, evaluation, and risk assessment, and have been thoroughly investigated during robustness and ruggedness studies. A method performance control strategy of the method has been developed. The final method validation is to be conducted to further verify the method performance that is built into the method and to gain further insight into the method. It is foreseeable that the final validation of the method at this stage will be successful.

The extent of validation should depend on the purpose of the method. For example, full validation should be performed for methods intended to be transferred to manufacturing sites, while limited validation is needed for investigational methods. Full validation may include specificity, sensitivity, linearity, precision (standard, sample, intermediate precision), accuracy (spiking recovery), and stability. The validation will also satisfy current requirements from regulatory bodies and should follow ICH guidelines. Different degrees of validation are required for limit test and quantitative methods; e.g., the ICH guidelines require only specificity and detection limit for limit test methods, whereas full validation is recommended for quantitative methods.[15]

Conclusions

A method development strategy for analysis of pharmaceutical GTIs at trace levels has been developed. The systematic approach begins with predefined method goals and emphasizes method understanding by identifying key method variables through risk assessment followed by robustness and ruggedness studies. This in turn allows for the proven acceptable ranges of the critical method parameters and method performance control strategy to be established. Consequently, method performance is built into the method based on sound scientific understanding and risk mitigation approaches.

The proposed generic method evaluation and selection flowchart can be used as a reference for most classes of GTIs. The flowchart was developed primarily on the basis of real-world experiences in our laboratories and built upon the premise of physicochemical properties of commonly encountered GTI analytes. It starts with good understanding of analyte stability and volatility, a key point for any method development. This leads to the evaluation of the three unit operations consisting of *sample preparation*, *separation*, and *detection* individually and then holistically in the context of predefined method goals. By following the proposed processes, success in developing sensitive and selective GTI methods is greatly enhanced. Since GTI analysis methods are often complex, greater understanding of the method variability is of critical importance. In short, by following QbD principles, a science- and risk-based control strategy can be developed to ensure more robust GTI methods.

Nonetheless, GTI methods should be put into the context of drug development phases. For early-phase projects, "fit for purpose" methods with reduced validation should be considered as long as good method understanding is established for even relatively complex methods (e.g., derivatization or MS-based methods). For late-phase projects, on the other hand, designing a QbD impurity control process strategy should precede the development of a QbD analytical method since in the QbD paradigm, product quality control should be shifted upstream whenever possible to reduce or eliminate end-product testing.

Acknowledgment

We thank Lin Bai, Shen Tang, Robert Reid, Josephine Vega, Jianguo An, Ted Chen, Gerald Terfloth, Yan Li, and Sarah Chen for their contribution to the method development of the examples. We also thank Fred Vogt for his critical review of the manuscript.

Supporting Information Available

Derivatization reaction procedure of dimethyl sulfate using 2-mercaptopyridine. This material is available free of charge via the Internet at http://pubs.acs.org.

Received for review March 30, 2010.

OP100089P