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Evaluation and Control of Mutagenic Impurities in a Development Compound: Purge Factor Estimates vs Measured Amounts

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ABSTRACT: Using the Teasdale method, purge factor estimates for six impurities identified as mutagenic alerts in the synthesis of MK-8876 are compared to actual measured amounts of these impurities determined via appropriate analytical methods. The results from this comparison illustrate the conservative nature of purge factor estimates, meaning that overprediction of mutagenic impurity purging is unlikely when using this method. Industry and regulatory acceptance of the purge factor estimation method may help minimize analytical burden in pharmaceutical development projects.

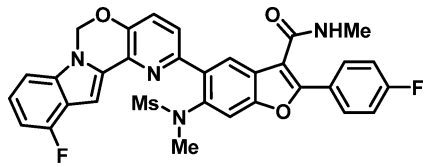
INTRODUCTION

The understanding, detection, and control of mutagenic impurities (MIs) in active pharmaceutical ingredients (APIs) have received increasing industry and regulatory attention over the past decade.[1] Since the early guidance on MI control issued by the EU in 2004,[2] there have been several updates and refinements, culminating in the recent International Conference on Harmonization M7 document released in 2014.[3] Across this time frame, the pharmaceutical industry has responded mainly by generation of extensive analytical data to support the absence of MIs from APIs manufactured for use in human clinical trials.

Recognizing the growing resource burden created by this situation, an alternative approach was initially outlined in a paper by Teasdale at AstraZeneca in 2010[4] and then further developed in a subsequent, more extensive paper by the same author in 2013.[5] In these papers, Teasdale proposed a purge calculation tool for the semiquantitative evaluation of MIs in process intermediates and APIs based on physicochemical properties and processing conditions. Using a series of case studies, it was shown that predicted values for residual MIs obtained using the purge calculation were generally in good agreement with actual analytical data. Importantly, it was established that the purge calculation tends to afford a more conservative estimate of MI purging than what is actually observed via analytical measurement; this is by intention and serves to minimize any risk to API quality when applying the calculation. Furthermore, it was suggested for other researchers to test the purge factor calculation in other "real-world" case studies to help establish validity of the approach. The current Letter is intended to contribute in this area by describing a case study of a development compound at Merck where several potential MIs were identified in the synthetic route and both purge calculations and experimental data are compared.

DISCUSSION

MK-8876 (Figure 1) was a compound in development for the treatment of hepatitis C virus. The expected dose and duration



MK-8876

Figure 1. Development compound MK-8876.

of treatment was 300 mg per day and 1−12 months, respectively, leading to a threshold of toxicological concern (TTC) of 67 ppm of MI acceptable in the API (M7 guidance). To support initial toxicology and clinical studies, the Process Chemistry department was engaged to deliver several kilograms of API under cGMP conditions. The final stages of the synthesis used for this initial API delivery are shown in below (Scheme 1).

Evaluation of these synthetic steps using the industry/agency accepted in silico analysis software (DEREK, MCASE)[6] revealed multiple intermediates and reagents alerted as potential MIs. From a reagent standpoint, 1-ethyl-3-(3-(dimethylamino)propyl)carbodiimide (EDC), iodomethane, chloroiodomethane, bis-boronic acid (BBA), and carbazole were identified as potential concerns. In terms of synthetic intermediates, the arylboronic acid **6** was noted as a structural alert. Following the format established by Teasdale, Table 1 provides a summary of the predicted and measured purge factors for each of these MIs across the relevant synthetic steps. The discussion section provides a brief description of the appropriate process details and rationale for purge estimation for each individual MI in question.

EDC. EDC (1.5 equiv) was used as the coupling agent in the formation of the *N*-methylamide **2**, and there are four isolations after the use of this reagent. For this MI, analytical data was obtained for arylboronic acid **6** (after only two of these

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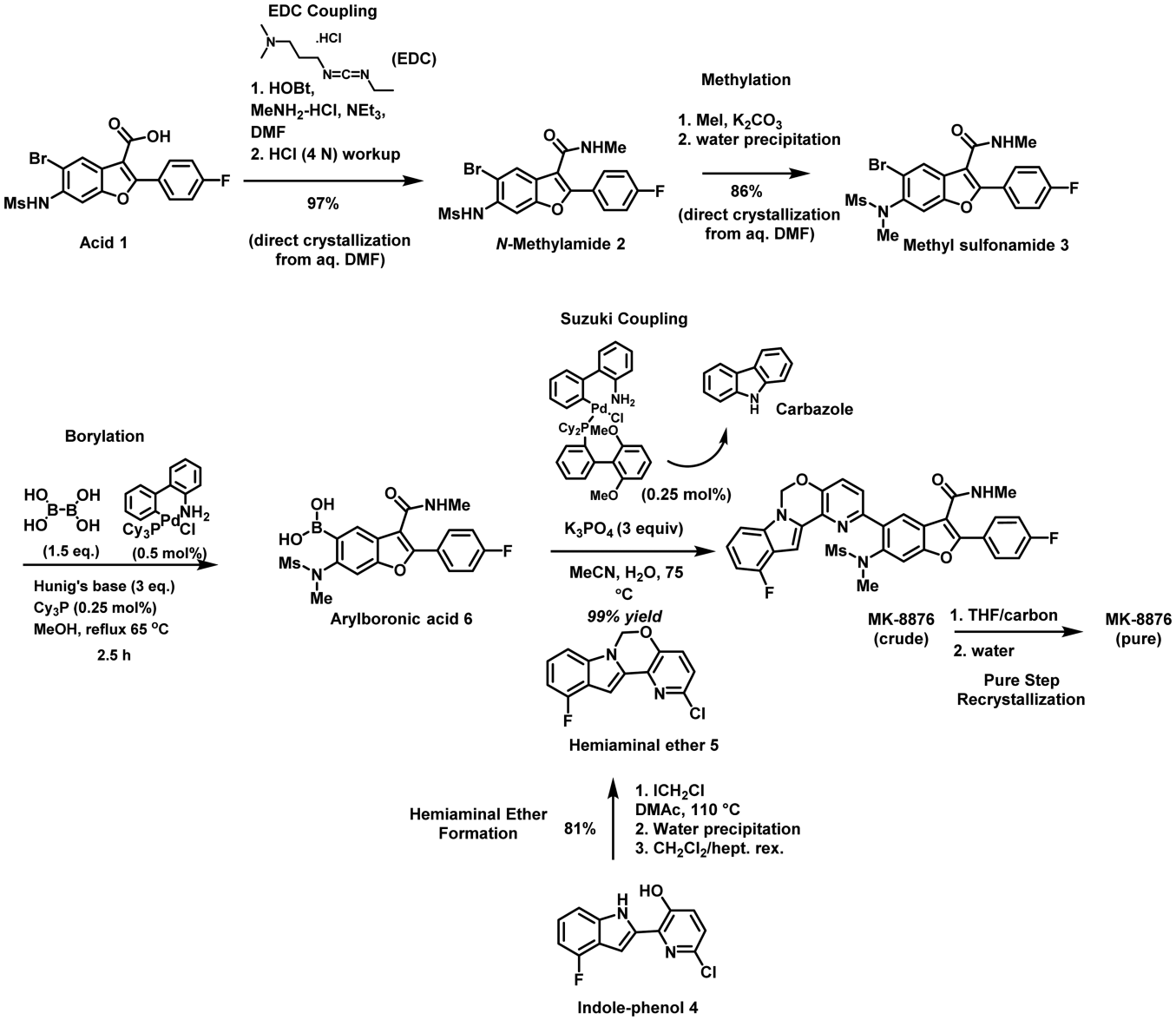
Scheme 1. Final Synthetic Steps towards MK-8876



isolations), which contained <10 ppm EDC.[7] Applying the rationale laid out by Teasdale,[4,5] the high reactivity of EDC (R = 100) together with the observed near quantitative yield support an approximate theoretical maximum of 500 000 ppm (i.e., the 50% excess reagent charge) going into the workup. Aqueous quench (R = 100 for water reactivity) followed by crystallization of the product amide (S = 10) afford a combined purge factor of 1000 for this stage. Reactivity in the ensuing methylation can conservatively be assigned as moderate (R = 10) while reactivity toward the basic aqueous workup/crystallization can afford a larger purge (R = 100 and S = 10). The cumulative purge factor at the isolated methyl sulfonamide is therefore 1×10^7. In the borylation, the reactivity of EDC can be assigned as high (R = 100) because this reaction involves exposure to MeOH at 60 °C. A further purge can be expected during crystallization of the arylboronic acid **6** (S = 10). Overall, this leads to a predicted cumulative purge factor of 1×10^{10}, which is consistent with the analytical observation of <10 ppm in this isolated intermediate.

Methyl Iodide. Methyl iodide (bp 41−43 °C) was used (2.0 equiv) to methylate the methanesulfonyl amide **2**, and there are four isolations after the use of this reagent. For this MI, analytical data was obtained after only two of these isolations at the arylboronic acid intermediate **6**, which contained <10 ppm MeI (LOQ). Given the high yield for the methylation reaction, the approximate maximum remaining MeI going into the workup would be 1,000,000 ppm (i.e., the 100% excess reagent charge). In this case the workup and isolation would have a combined purge factor of 100 (R = 10 for hydrolysis during quench, S = 1 for solubility in water/DMF,[8] V = 10 for volatility during drying at 65 °C under vacuum).

The borylation reaction and isolation of arylboronic acid **6** would have a combined purge factor of 10 000 (R = 100 for reactivity with Hunig's base/MeOH at 65 °C in the presence of Pd(0), S = 1 for an aqueous crystallization, V = 10 for concentration of MeOH solution during workup, and V = 10 for drying of the isolated solid). The cumulative purge factor up to the isolated boronic acid is therefore 1×10^6, which is in

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Table 1. Comparison of Predicted and Measured Purge Factors for the MK-8876 Process

mutagenic impurity	starting amount (ppm)	processing stage	reactivity	solubility	volatility	purge factor/stage	cumulative purge factor	measured purge factor[a]
EDC	1,500,000	EDC coupling	100	1	1	100		
	500,000	EDC workup/ crystallization	100	10	1	1,000		
						stage purge = 1,000	1,000	
		methylation	10	1	1	10		
		methylation workup/ crystallization	100	10	1	1,000		
						stage purge = 10 × 1000 = 10,000	10,000,000	
		borylation	100	1	1	100		
		workup	1	1	1	1		
		boronic acid isolation (MeOH/water)	1	10	1	10		
						stage purge = 100 × 10 = 1,000	1 exp 10	>50,000 (<10 ppm at boronic acid)
methyl iodide	2,000,000	methylation	100	1	1	100		
	1,000,000	methylation workup	10	1	1	10		
		crystallization	1	1	1	1		
		drying	1	1	10	10		
						stage purge = 10 × 10 = 100	100	
		borylation	100	1	1	100		
		workup	1	1	10	10		
		boronic acid isolation (MeOH/water)	1	1	10	10		
						stage purge = 100 × 10 × 10 = 10,000	1,000,000	100,000 (<10 ppm at boronic acid)
chloroiodomethane	1,200,000	HAE formation	100	1	1	100		
	200,000	crude precipitation	1	1	1	1		
		recrystallization	1	10	1	10		
						stage purge = 10	10	
	2,232	Suzuki reaction	10	1	1	10		
		crude API isolation (MeCN/water)	1	10	10	100		
						stage purge = 10 × 100 = 1,000	10,000	20,000 (10 ppm in crude API)
	10	pure API Isolation	1	10	1	10		
						stage purge = 10	100,000	>200,000 (<1 ppm in pure API)
arylboronic acid	1,000,000	Suzuki reaction	100	1	1	100		
		Suzuki workup	1	10	1	10		
		crude API isolation (MeCN/water)	1	10	1	10		
						stage purge = 100 × 10 × 10 = 10,000	10,000	143,000 (7 ppm in crude API)
	7	pure API Isolation	1	3	1	3		
						stage purge = 3	30,000	>1,000,000 (<1 ppm in pure API)
BBA	1,500,000	borylation	100	1	1	100		
	500,000	workup	1	10	1	10		
		crude API isolation (MeCN/water)	1	10	1	10		
						stage purge = 10 × 10 = 100	100	>3333 (<146 ppm in crude API)
	146	pure API Isolation	1	10	1	10		
						stage purge = 10	1,000	>250,000 (<20 ppm in pure API)
carbazole	7,500	Suzuki reaction	1	1	1	1		
		crude API isolation (MeCN/water)	1	10	1	10		
						stage purge = 10	10	
		pure API Isolation	1	10	1	10		
						stage purge = 10	100	>375 (<20 ppm in pure API)

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Table 1. continued

[a]Note that in certain cases (indicated by < ppm value) the actual quantity of the MI may have been lower than stated because the analytical method used was only developed to an appropriate limit of quantitation (LOQ) relative to the TTC.

agreement with the observed analytical value of <10 ppm (LOQ).

Chloroiodomethane. Chloroiodomethane (bp 108 °C) was used (1.2 equiv) to form the cyclic hemiaminal ether (HAE) **5**, and there are three isolations after the use of this reagent. For this MI, analytical data was obtained for the following isolated intermediates: hemiaminal ether **5** (2232 ppm), crude API (10 ppm), and pure API (<1 ppm). In the initial reaction, reactivity toward the substrate can be considered high (R = 100) given the high yield, but the excess reagent charge means that approximately 200 000 ppm can theoretically survive into the workup. The product is directly precipitated by addition of water, and it is anticipated that solubility in the aqueous mother liquor would be rather low (S = 1).

The subsequent recrystallization from CH_2Cl_2/heptane involves a batch concentration under reduced pressure (V = 1 relative to the low boiling CH_2Cl_2) and isolation from an organic solvent with excellent solubility for the MI (S = 10). Hence, with a predicted purge factor of only 10 for this step, it was not surprising to observe 2232 ppm in the isolated chloropyridine intermediate (again, the estimated purge is more conservative than the actual purge here). In the subsequent step (Suzuki cross-coupling under basic conditions) moderate reactivity (R = 10) and good solubility (S = 10)[9] can be assigned for the reaction and isolation stages, respectively.

Analytical data for the crude API indicated 10 ppm of residual chloroiodomethane, suggesting the calculated purge for the Suzuki stage (1000) is slightly high (but still within a factor of 10) relative to the measured stage purge factor (223). For the pure step another purge factor of 10 (S = 10) can be applied, which would predict 1 ppm in the pure API (starting from the 10 ppm in crude API), and indeed, the MI was detectable in the pure API (<1 ppm, LOQ was 1 ppm). In this case, it is noted that, although an individual stage purge prediction (Suzuki stage) was slightly high, the overall cumulative predicted purge factor of 1×10^5 remains conservative with respect to the measured purge of 2×10^5.

Arylboronic Acid 6. Although not alerted by the release version of the DEREK in silico evaluation software available at the time of this project,[10] arylboronic acids were established in the literature as potential MIs,[11] and, as such, are routinely highlighted during internal MI assessments. Arylboronic acid **6** was tested in the Ames assay and delivered a positive result, which therefore mandated strict control of this process intermediate within TTC levels in the API. In the Suzuki cross-coupling, exactly one molar equivalent of arylboronic acid **6** was employed. Reactivity can be assigned as high (R = 100) because the reaction yield is almost quantitative, and there should be good rejection of any unreacted material in the workup/isolation (S = 10).

Although the predicted purge at the crude API stage is only 10 000, the measured purge was much higher (140 000) because only 7 ppm of arylboronic acid was detected analytically. For the pure step, the purge was expected to be minimal (S = 3) due to the moderate solubility in the recrystallization solvent. Combined, this predicts a cumulative purge factor of 30 000 at the API stage. Nevertheless, analytical measurement at the pure API stage indicated <1 ppm of arylboronic acid **6** was present, again supporting the conservative nature of the purge factor calculation.

Bis-Boronic Acid (BBA). Having established that arylboronic acid **6** was Ames positive, concerns were raised around the MI status of the reagent used to prepare this intermediate, namely, bis-boronic acid (BBA). When submitted to the Ames assay, it was determined that BBA was indeed positive and would have to be controlled within the TTC. This finding has significance across the pharmaceutical industry because BBA (and the bis-pinacol ester derivative B_2Pin_2) are widely used in the synthesis of APIs. From an analytical standpoint, detection of this reagent was challenging due to the absence of a chromophore to enable standard LC measurement or reasonable volatility for GC analysis. It was possible to conduct ICP-MS analysis for total boron content; however, the possibility of detecting residual arylboronic acid **6** or simple boric acid (not a MI) could lead to overestimation of BBA content. In practice, the content of **6** was accurately determined by a specific (LC) method and the level of boron arising from **6** was subtracted from the total boron content by ICP-MS.

As shown in the table, the estimated purge factor from end of borylation reaction (50% excess assumed remaining, 500 000 ppm) until crude API was 100 (S = 10 for solubility in basic aqueous cut and S = 10 for solubility during crystallization), which would predict 5000 ppm residual BBA at this stage. Meanwhile, the measured amount of BBA in crude API was <146 ppm, indicating a more favorable purge than predicted. Furthermore, an additional calculated purge of 10 (S = 10) for the pure stage recrystallization would result in a cumulative predicted purge factor of 1000 compared to a measured value of minimum 250 000 (<20 ppm BBA in pure API, down from theoretical 500 000 at the beginning of purge operations).

Carbazole. Carbazole is the known byproduct from activation of the "pre-catalyst", formed via reductive elimination at palladium. Carbazole is a known nonmutagenic carcinogen[12] and was therefore controlled within calculated permissible exposure level (PDE) of 692 μg/day for this API.[13] This MI is generated in both the borylation and the Suzuki cross-coupling steps, and so for the purge factor estimation we considered the worst case scenario where the combined amount is used as the starting point for the calculation. Analytical data at the pure API indicated <20 ppm residual carbazole. The predicted purge factor was 100 (S = 10 for each crystallization of crude and pure API).

■ CONCLUSION

The case study presented here describes the evaluation, detection, and control of potential MIs in a development compound. The primary goal was to compare the analytically determined amounts of the MIs against the values predicted using the purge factor calculation proposed by Teasdale. In Teasdale's original publication it was noted that "the scoring system tends to underpredict the likely purge capacity of a process, which is preferable to overprediction". In the current work it was found that, in all cases, the purge factor calculation does indeed tend to err on the more conservative side as intended. Consequently, this should support future use of the

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purge factor calculation as a way to reduce the analytical burden on API deliveries while avoiding any risk to API quality. Additionally, work within the our toxicology department established that two borylation reagents (B_2Pin_2 and BBA) commonly used in the synthesis of pharmaceuticals are Ames positive and therefore require purging to appropriate sub-TTC levels in GMP APIs intended for human use.

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Notes

The authors declare no competing financial interest.

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(7) In this case the analytical method used had a 10 ppm limit of quantitation (appropriate relative to the TTC) so the actual quantity of EDC present may have been significantly less than 10 ppm.

(8) It could be argued that methyl iodide would have at least some solubility in the water/DMF solvent system employed for the crystallization; however, in keeping with the conservative approach in applying purge factors, it was assumed to have no purge in this instance. It is noted that the ratio of water−DMF in the final mother liquor was >4:1 (i.e., largely aqueous).

(9) In this isolation, the ratio of MeCN−water was >1:1, and so good solubility for chloroiodomethane was observed.

(10) The current version of DEREK has provision for arylboronic acids as alerting structures.

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(13) PDE was calculated as described in Appendix 3 of the ICH Q3C (R5) guideline on residual solvents (ICH Harmonised Tripartite Guideline. Impurities: Guideline for Residual Solvents Q3C(R5), Step 4. 4 February **2011**), using the lowest observed effect level (LOEL) of 166 mg/kg/day for liver tumors in the 2-year oral carcinogenicity study in male B6C3F1 mice (CPDB database), and modifying factors (F1 = 12, F2 = 10, F3 = 1, F4 = 10, and F5 = 10). See also ICH M7 Addendum: http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Multidisciplinary/M7/M7_Addendum_Step_2.pdf.

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