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Impact of metal-induced degradation on the determination of pharmaceutical compound purity and a strategy for mitigation

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ABSTRACT

Case studies are presented demonstrating how exposure to traces of transition metals such as copper and/or iron during sample preparation or analysis can impact the accuracy of purity analysis of pharmaceuticals. Some compounds, such as phenols and indoles, react with metals in the presence of oxygen to produce metal-induced oxidative decomposition products. Compounds susceptible to metal-induced decomposition can degrade following preparation for purity analysis leading to falsely high impurity results. Our work has shown even metals at levels below 0.1 ppm can negatively impact susceptible compounds. Falsely low results are also possible when the impurities themselves react with metals and degrade prior to analysis. Traces of metals in the HPLC mobile phase can lead to chromatographic artifacts, affecting the reproducibility of purity results. To understand and mitigate the impact of metal induced decomposition, a proactive strategy is presented. The pharmaceutical would first be tested for reactivity with specific transition metals in the sample solvent/diluents and in the HPLC mobile phase. If found to be reactive, alternative sample diluents and/or mobile phases with less reactive solvents or addition of a metal chelator would be explored. If unsuccessful, glassware cleaning or sample solution refrigeration could be investigated. By employing this strategy during method development, robust purity methods would be delivered to the quality control laboratories, preventing future problems from potential sporadic contamination of glassware with metals.

1. Introduction

Throughout the development life cycle of a drug substance, analytical methods are an essential part of the control strategy for the molecule. These methods must be sufficiently robust to ensure consistent, accurate results. Most commonly, impurities are determined using chromatographic methods which require sample preparation prior to analysis. This sample preparation typically involves dissolving a portion of the sample in diluent. Guidance from CDER indicates that data should be available to demonstrate solution stability under normal laboratory conditions for the duration of the test procedure and recommends at least 24 h [1]. There are several conditions that may affect the solution stability of either the drug substance or potential impurities, including pH of the diluent, light exposure and temperature. In addition, many organic compounds are susceptible to metal catalyzed oxidative reactions [2]. When this degradation occurs during the analysis of these compounds (i.e., degradation as an artifact of the analysis), inaccurate results may be generated. It is the purpose of this paper to explore sources of potential metal contamination, discuss development of analytical methods for compounds which are susceptible to metal-catalyzed degradation in solution and provide a strategy for building robustness into analytical impurity methods when metal-induced degradation is possible. This strategy is exemplified for two susceptible compounds under development in our laboratories, arzoxifene hydrochloride, a phenol-containing compound, and enzastaurin hydrochloride, an indole-containing compound.

During early method development, the stability of the drug substance in solution is examined under various conditions. If the potential exists for the drug substance, or impurities in the drug substance, to degrade in the presence of trace metals, the method developer needs to include a strategy to ensure solution stability in the event of metal contamination. Stress testing, including examination of susceptibility to metal-induced oxidation, is an important tool to predict the degradation of pharmaceutical compounds [3]. A study examining the stress testing strategies of twenty pharmaceutical companies indicated that only three companies perform stress testing using copper (CuII) and iron (FeIII) [4].

As per ICH guidelines for impurities [5,6], there are three thresholds listed for impurities. For the drug substance, unless the impurity is unusually toxic or if the maximum daily dose is >2 g/day,

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these thresholds are typically 0.05%, 0.10%, and 0.15%, for reporting, identification, and qualification, respectively. Therefore, unqualified or unknown impurities due to solution degradation that exceed 0.05% in the drug substance may cause concern for quality control laboratories. If these impurities are above 0.10% or 0.15%, a laboratory investigation would be triggered, potentially resulting in rejection of the batch. For the drug product, there are comparable guidelines and considerations.

In our experience, the presence of metal contaminants in chromatographic solutions is typically discovered accidentally, due to the generation of aberrant results. This requires costly and time-consuming investigations to determine the assignable cause. The purpose of this paper is to review previous issues and recommend a proactive approach to identify and handle metal-induced contamination. Two examples of known metal degradation issues are presented and a proactive approach to eliminate the impact of metal-induced degradation early in the development of analytical methods is described, eliminating the need for costly laboratory investigations and method re-development.

2. Experimental

2.1. Purity method for arzoxifene hydrochloride drug substance

An Agilent 1100 series HPLC instrument was used for analysis of the purity of arzoxifene hydrochloride (phenol containing) drug substance. The method employed a 15 cm × 4.6 mm i.d., 3.5-μm particle size, Zorbax® SB C18 column. The operating conditions were as follows: 20 μL sample injection, 2.5 mL/min flow rate, 315 nm UV detection and column oven temperature at 40 °C. Aqueous buffer, approximately 35 mM total phosphate (sodium salt), adjusted to pH 2.5, was used as the weak mobile phase (reservoir A). The strong mobile phase was a blend of acetonitrile (reservoir B) and methanol (reservoir C). The gradient profile was as follows: Hold at 55.0/23.4/21.6 A/B/C for 1.4 min, adjust linearly to 20.0/41.6/38.4 A/B/C over 11.2 min, return to initial conditions over 0.4 min, and re-equilibrate for 3.2 min. Samples were prepared at a concentration of 0.5 mg/mL in a sample diluent equivalent to the initial mobile phase composition, stored protected from light, and were compared to an external standard of the main component prepared at 0.0005 mg/mL in sample diluent. Solvents and reagents were of suitable purity for use in HPLC analysis. Water purified with a Milli-Q system (Millipore, New Bedford, MA, USA) or HPLC grade bottled water was used.

2.2. Purity method for enzastaurin hydrochloride drug substance

An Agilent 1100 series HPLC instrument was used for analysis of the purity of enzastaurin hydrochloride (indole containing) drug substance. The method employed a 15 cm × 4.6 mm i.d., 3.5-μm particle size, Zorbax® Bonus RP column. The operating conditions were as follows: 20 μL sample injection, 1.0 mL/min flow rate, 255 nm UV detection and column oven temperature at 30 °C. The weak aqueous mobile phase (reservoir A) was a 0.1% blend of trifluoroacetic acid in water. The strong mobile phase (reservoir B) was a 0.1% blend of trifluoroacetic acid in acetonitrile. The gradient profile was as follows: Hold at 75/25 A/B for 6.0 min, adjust linearly to 40/60 A/B over 30.0 min, hold at these conditions for 1.0 min, return to initial conditions over 0.1 min, and re-equilibrate for 7.9 min. Samples were prepared at a concentration of 1.0 mg/mL in a sample diluent composition of 75/25/0.05 water/acetonitrile/trifluoroacetic acid and were compared to an external standard of the main component prepared at 0.001 mg/mL in the same sample diluent. Solvents and reagents were of suitable purity for use in HPLC analysis. Water purified with a Milli-Q system (Millipore, New Bedford, MA, USA) or HPLC grade bottled water was used.

2.3. Preparation of metal solutions

Sample diluent containing copper (II) ion at various levels was prepared by dissolving copper (II) sulfate, reagent grade, in a portion of the appropriate purity method sample diluent. For the evaluation of the impact of copper in the mobile phase, copper (II) sulfate was dissolved in the aqueous buffer used for the gradient analysis. Iron (II) solutions were prepared in a similar fashion from iron (III) nitrate nonahydrate, reagent grade.

2.4. ICP analysis

Analysis for trace levels of iron and copper were performed using inductively coupled plasma-mass spectrometry (ICP/MS) using a Thermo-Electron X-Series II ICP/MS instrument. Concentrations of the metals were determined by testing the laboratory sample preparations used in the purity analysis. These sample solutions were further diluted in 50/50 (v/v) acetonitrile/2% nitric acid and compared versus standard curves over the range of 1–100 ppb. Standards were prepared from 1000 ppm aqueous (5% nitric acid) stock solutions of the metals (obtained from VHG Labs, Inc., Manchester, New Hampshire) and diluted in 50/50 (v/v) acetonitrile/2% nitric acid.

3. Results and discussion

3.1. Source of metal contamination

There are multiple potential sources for the metal contamination of sample solutions including reagents and glassware. While there is the potential that the sample itself may contain trace metals or that trace metals may be present in the reagents used to prepare the diluent, it is the experience of the authors that trace metals were most typically introduced into sample solutions from glassware used in the analysis.

Various experiments have been performed to understand the source of the metal contamination. Typical test samples were prepared with sample diluent in volumetric flasks, allowed to stand for several hours, and subsequently tested for metals by ICP/MS. New glassware was compared with glassware that had been used and washed in a large-scale commercial glassware washer (dishwasher). Different sized volumetric flasks were tested in order to assess potential patterns in metal levels. Results for these samples were compared to the diluent and its individual components analyzed separately. From this experiment, the laboratory glassware, and in particular the volumetric flasks used to prepare the samples, was found to be a major source of residual metals (see Fig. 1).

The results shown in Fig. 1 indicate that glassware washed by an automatic dishwasher typically contained the highest level of trace metals compared to new “out of the box” glassware. The source of the copper was believed to be the use of potable, non-deionized, water during automated glassware washing, with incomplete removal by the deionized water rinse cycle. As shown in Fig. 1, there was a significant amount of variability in the amount of copper present from sample to sample, even though all levels were relatively low (below 200 ppb). Iron levels in the glassware were lower than copper, less variable from sample to sample, and did not appear to be significantly different from levels present in new volumetric flasks. Based on this information, copper contamination was viewed as a higher risk to impact chromatography. One potential remediation strategy for copper contamination would be to develop robust, well-controlled dishwashing procedures, which

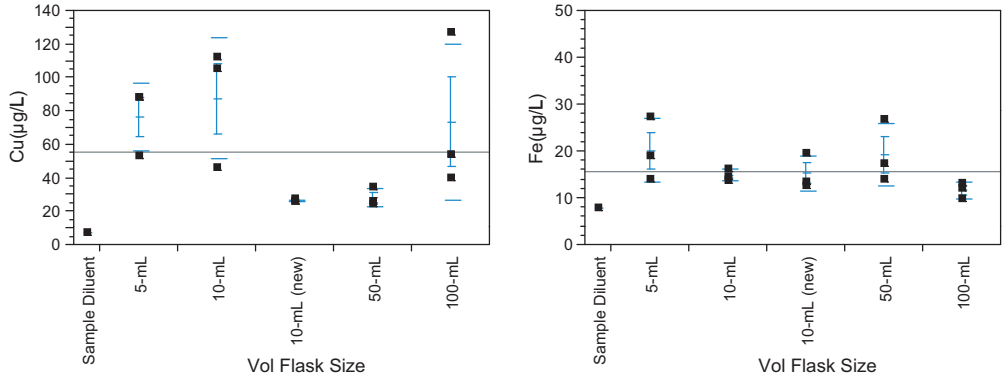

Fig. 1. ICP/MS results for copper and iron in test solutions prepared in new and washed volumetric flasks of different sizes. Individual points plotted with standard deviation lines and mean error bars versus the overall mean (solid line).

may include a significant distilled water rinse. While this is a desirable approach, it may not be the most robust strategy considering that methods that may be executed in multiple laboratories with different dishwashing machines and practices. Even relatively low levels of metals were found to induce oxidative decomposition in the compounds studied.

3.2. Case studies

3.2.1. Arzoxifene hydrochloride drug substance (a phenol)

During validation studies for the purity method for arzoxifene hydrochloride drug substance (Fig. 2), the stability of the standard and sample solutions was investigated. Sample solutions prepared in the initial mobile phase were not stable, showing evidence of dimerization to form compound 467739 (see Fig. 2). When freshly prepared test solutions of these samples were analyzed, the compound 467739 peak was not observed, indicating that this compound was an artifact of the analysis. The cause for this was discovered to be traces of residual copper in the laboratory glassware. As shown in Fig. 1, samples of arzoxifene hydrochloride can contain as much as 120 μg/L copper when prepared in typical glassware. When samples were spiked with about 1 ppm (1000 μg/L) copper (II), the amount of dimer product that was formed exceeded the ICH specification limit for unspecified impurities (0.15%) in less than 2 h (see Fig. 3). Samples spiked with iron (III) also showed an increase in compound 467739, but to a lesser extent than for copper (II). Since typical iron levels in glassware were lower than copper, and iron appeared to be less reactive, a strategy for the elimination of copper-induced degradation was pursued for arzoxifene hydrochloride.

To better understand the mechanism for the formation of compound 467739, a survey of the literature was performed. The use of copper-containing catalysts to polymerize phenols is well documented in the literature [7–10]. The mechanism for the dimerization reaction has been established as (1) coordination of a copper-amine complex with the phenol, (2) electron transfer to form a phenolate radical, and (3) reaction of the radical with a second phenol molecule to form a dimer [11]. Brussee et al. reported the dimerization of 2-napthol using copper as a catalyst [12]. Brussee found that the reaction did not take place unless an amine was present to activate the copper (II). Tsuruya et al. described the use of a copper (II)-acetonitrile complex to polymerize phenols [13,14]. Based upon this, the following mechanism has been proposed (Fig. 4).

A number of experiments were conducted to assess the mechanism and possible ways to manage or control the reaction creating the artifact. First, acetonitrile was removed from the sample diluent. Instead of the initial mobile phase (buffer/methanol/acetonitrile), a buffer/methanol mixture was utilized. Use of the buffer/methanol mixture as the sample diluent was found to eliminate the formation of compound 467739 in test solutions spiked with 5 ppm copper. As an alternative approach, the effectiveness of pre-cleaning the volumetric glassware was investigated. Use of acidic aqueous solutions was found to be successful in removing the trace metals from the glassware, thus eliminating the dimer artifact peak. Addition of 0.1 mM ethylenediaminetetraacetic acid (EDTA), disodium salt, as a metal chelator to the buffer/acetonitrile/methanol sample diluent was also effective in eliminating the artifact. However, use of EDTA introduced a large un-retained peak in the chromatogram. The use of the buffer/methanol mixture as the sample diluent was selected as the most robust approach, since it had no impact on the chromatography, and would not require careful glassware pretreatment.

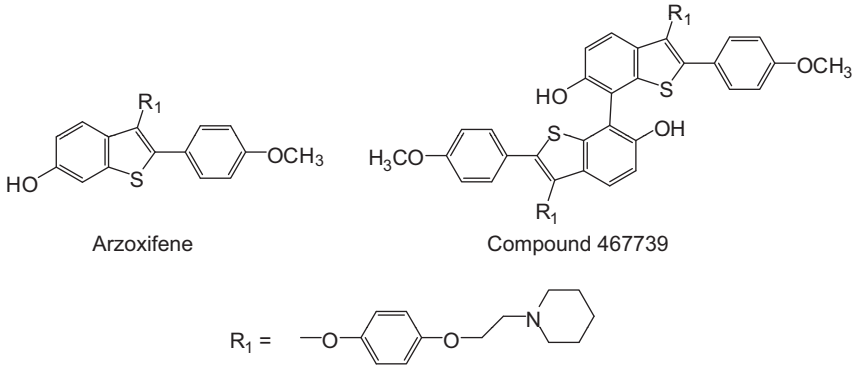

Fig. 2. Chemical structures for phenol-containing drug substance (arzoxifene hydrochloride) and its dimer (compound 467739).

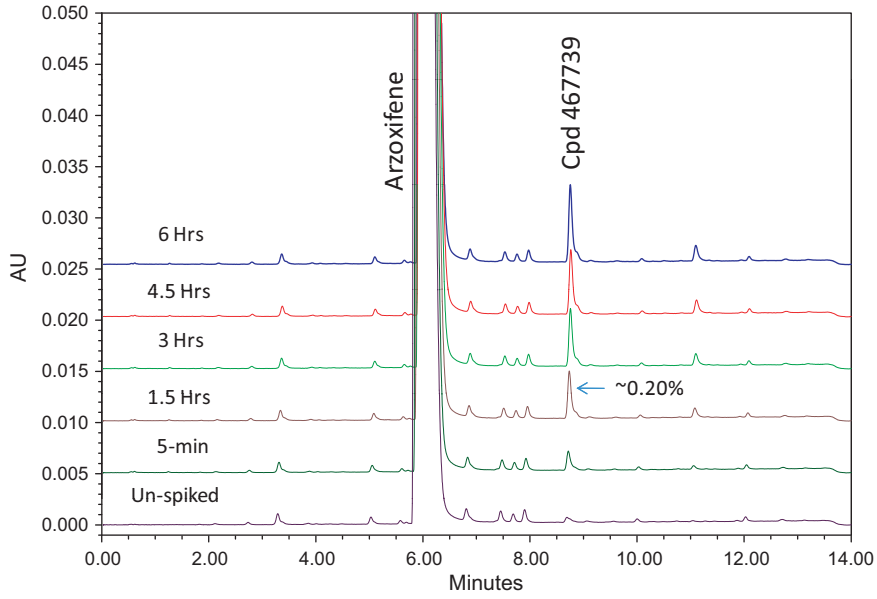

Fig. 3. Overlay of chromatograms for a sample of arzoxifene hydrochloride spiked with 1 ppm copper (II) and analyzed over time while held at room temperature.

In addition to investigating the impact of the presence of trace metals in sample diluent, the impact of trace metals in the HPLC mobile phase was investigated. Approximately 10 ppm copper (II) was added to the buffer solution used as mobile phase A, and a set of samples was analyzed following the standard gradient. Fig. 5 shows an overlay of a drug substance sample containing a representative set of impurities, analyzed with and without copper in the mobile phase. When copper is present in the mobile phase, a large rise in the baseline following the elution of the main peak is observed for concentrated samples. The baseline drops just following the retention time for compound 467739. This disturbance appears to be due to on-column degradation of arzoxifene to produce compound 467739. In addition to the baseline disturbance, a decreased response for one of the impurities was observed. This illustrates that traces of metals in the mobile phase can impact not only the main compound, but also any impurities present in the samples that are susceptible to metal-induced decomposition.

While the introduction of copper (II) into the mobile phase at a 10 ppm level had an impact on the chromatography, it is important to note that this level is one or two orders of magnitude higher than levels the authors have observed as a result of volumetric glassware contamination. Over a period of several years, the method has been in use in multiple laboratories, without any evidence of on-column degradation due to trace metals. Therefore, the probability that trace metals in the mobile phase would cause issues for the arzoxifene hydrochloride purity method was considered low, and no further remediation such as the addition of EDTA to the mobile phase was pursued.

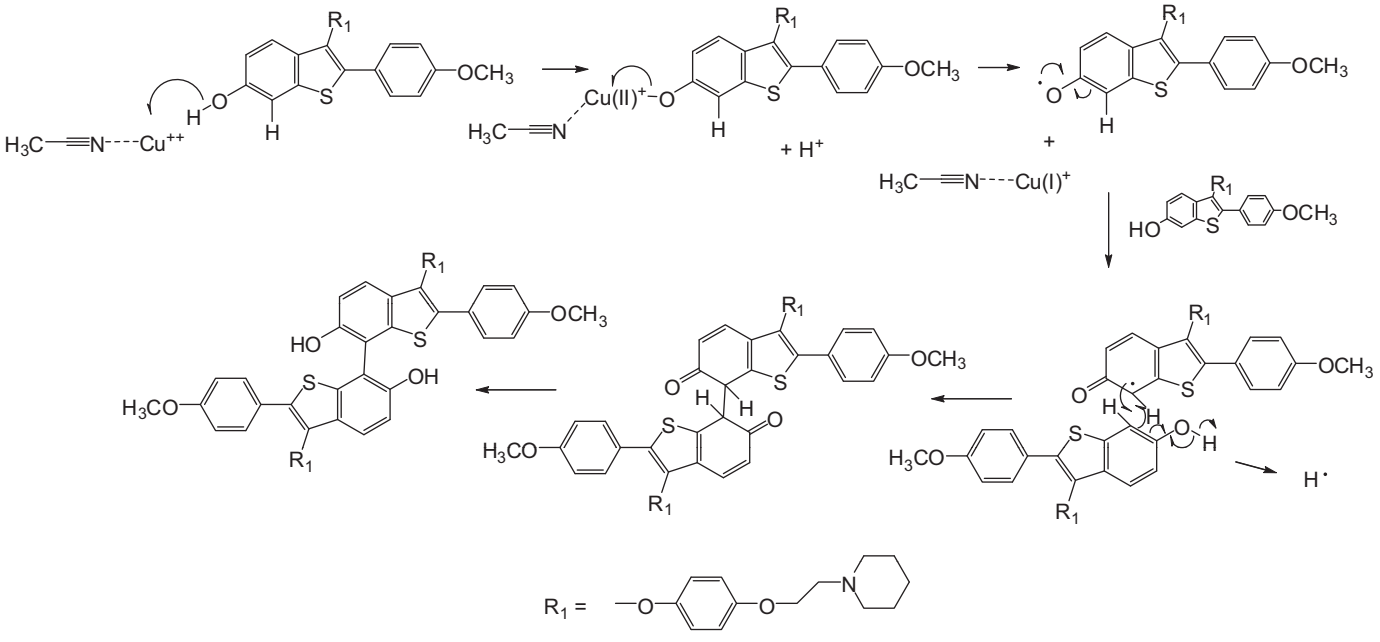

Fig. 4. Mechanism of the reaction of arzoxifene with copper to produce compound 467739. Note that the copper (II) is proposed to be complexed with acetonitrile (structure unknown) but is depicted here without coordination for simplicity.

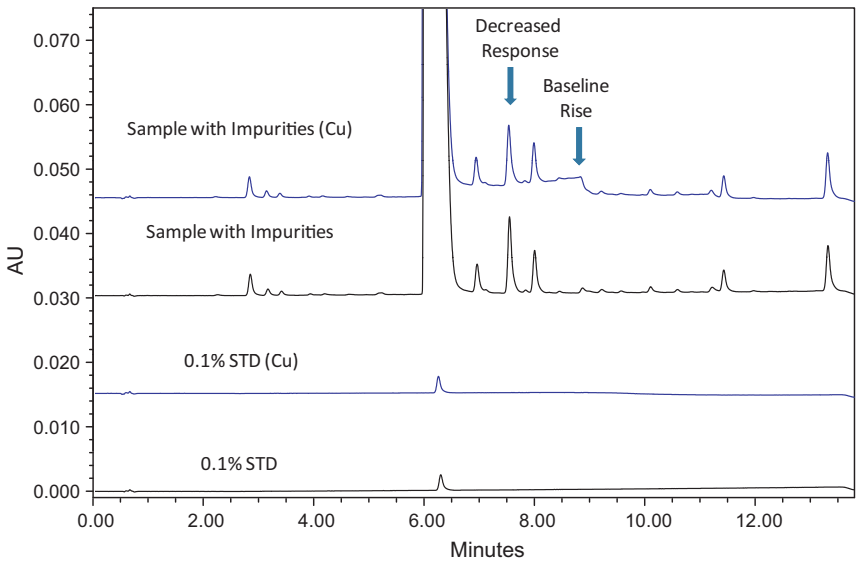

Fig. 5. Overlay of chromatograms for a sample and standard of arzoxifene hydrochloride analyzed with 10 ppm copper (II) added to the aqueous mobile phase buffer (Cu) and analyzed without copper added.

3.2.2. *Enzastaurin hydrochloride drug substance*

Occasionally elevated levels of an impurity were observed during purity analysis of enzastaurin hydrochloride drug substance. The impurity retention time correlated with a process impurity (structure not shown), but observed levels were inconsistent with process knowledge. In each case, analysis of a fresh sample preparation revealed much lower levels of this impurity, and subsequent investigations indicated that the artifactually high levels of compound 2579539 were due to metal-induced degradation due to traces of residual iron in laboratory glassware (see Fig. 6). When samples were spiked with about 1 ppm (1000 μg/L) iron (III), the amount of impurity grew to a level of 0.24% over 35 h and exceeded the 0.15% ICH qualification threshold in just a few hours. Samples spiked with about 1 ppm (1000 μg/L) copper (II) showed only a small increase in compound 2579539, demonstrating it was much less reactive, and therefore further investigations focused on the impact of iron on oxidation of enzastaurin hydrochloride.

Oxidation of indole to 2-oxindolinone (2-oxindole) by hydrogen peroxide with chloroperoxidase has been reported by Hartmann and co-workers [15]. 2-Oxindole was identified by Capdevielle and Maumy as an intermediate in the oxidation of indole by copper (I) chloride and oxygen in dry acetonitrile [16]. Oxidation at the 2-position was also observed by Kawaguchi-Murakami et al. in an oxidative stress-degradation study for a pyrrole-containing pharmaceutical [17]. These examples demonstrate the susceptibility of the 2-position of the indole group toward oxidation.

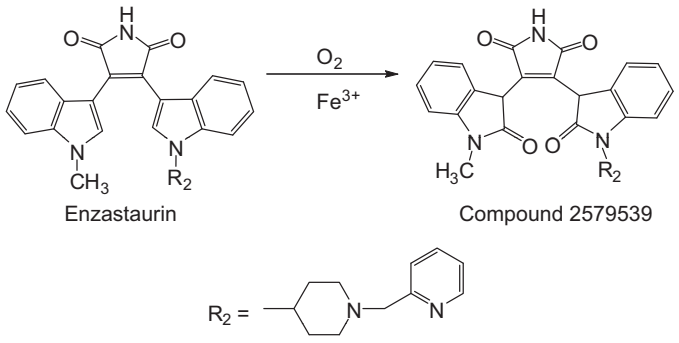

Fig. 6. Structures for, and mechanism of, the reaction of enzastaurin with iron to produce oxidative degradation product, compound 2579539.

The purity test sample solution uses acetonitrile as the primary diluent (buffer/acetonitrile), so alternate solvents were screened to investigate the impact on solution stability. Methanol and dimethylformamide were used in place of acetonitrile, but this was found to be ineffective at inhibiting the formation of compound 2579539 in test solutions spiked with 1 ppm iron. The effectiveness of pre-cleaning the volumetric glassware was also investigated. As with arzoxifene hydrochloride drug substance, the use of acidic aqueous solutions to pre-clean glassware was successful in removing trace metals and eliminating the formation of the oxidation impurity. Addition of EDTA to the buffer/acetonitrile sample diluent inhibited formation of the oxidation impurity, but added a large un-retained peak in the chromatogram (Fig. 7). Use of 5 °C (refrigerated) autosampler temperature was also evaluated as a control. While lower temperature did not prevent formation of the oxidation impurity, the rate and extent of formation was significantly lowered. When samples were spiked with about 1 ppm iron (III) and refrigerated, the impurity remained less than the 0.15% ICH qualification threshold for more than 35 h (Fig. 8). Use of 5 °C autosampler and pre-cleaning volumetric glassware were selected to mitigate the metal-induced degradation.

In addition to investigating impurity growth with iron (III) present in the diluent, the impact of iron in the mobile phase was assessed with respect to the chromatography. Fig. 9 compares the profiles of a sample that was not exposed to iron to samples that were exposed to iron via spiking at approximately 1 ppm in the diluent and 10 ppm in the mobile phase. Exposure to significant levels of iron in the mobile phase increased baseline artifacts and led to the formation of an oxidative degradation product (structure not shown) that eluted on the tail of the main peak. It did not, however, lead to the formation of compound 2579539, which was seen when iron was present in the diluent. Clearly higher iron levels in the mobile phase can impact chromatography, however, as with arzoxifene hydrochloride, the method has been in use in multiple laboratories without the observation of these negative effects. Therefore addition of EDTA to the mobile phase was not implemented.

3.3. *Strategy for mitigation of metal-induced degradation*

Based on the authors' experiences outlined in the previous sections, the process depicted in the flow chart (Fig. 10) was devised

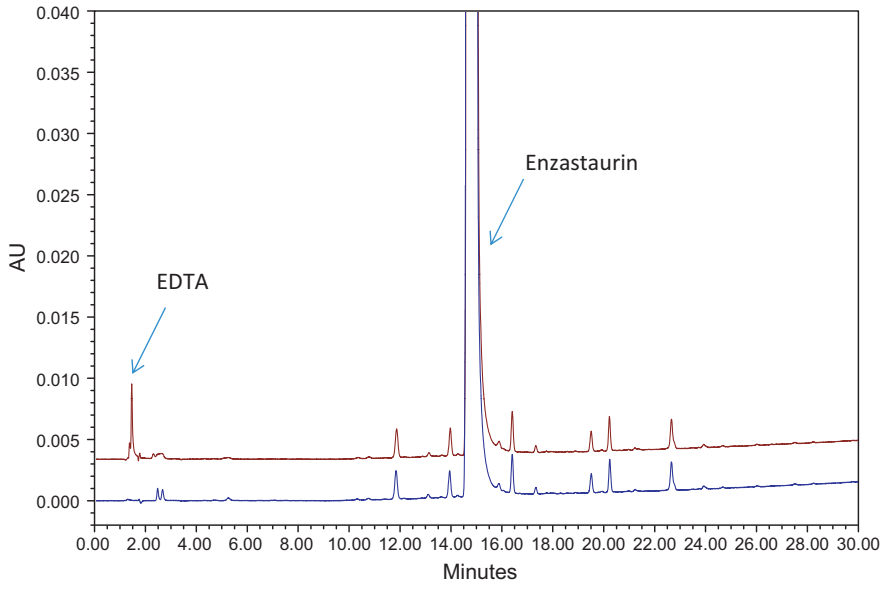

Fig. 7. Overlay of chromatograms for a sample of enzastaurin hydrochloride prepared with (top) and without (bottom) 0.1 mM EDTA added to sample diluent.

to proactively mitigate any metal-induced degradation issues with methods early in method development.

3.3.1. Assessment of metal-induced degradation susceptibility

Initially, the compound of interest should be evaluated for metal-induced degradation susceptibility by spiking the sample solution with approximately 1 ppm iron (III) and copper (II), annotated as step 1 on the flow chart (Fig. 10). Forced degradation studies performed on the drug substance using metals provide important information about its susceptibility to metal-induced oxidation. However, it would still be important to assess the compound under the specific conditions (i.e., organic solvent and pH) of the purity method, and by the evaluation of impurity-rich samples, the impurities themselves would be assessed in addition to the drug substance. Spiking the metals at 1 ppm can produce levels higher than would typically be expected via contamination and provides a worst-case assessment in order to ensure that a response is not missed. After allowing the solution to set for several hours to age, the resulting spiked solution would be analyzed and evaluated for new impurities that are present at significant levels (>0.05%) as well as for impurities that were initially present, but are now absent/reduced. If no significant changes to the chromatogram are noted relative to an unspiked sample, it suggests the compound and related impurities are not susceptible to metal-induced degradation at levels needing to be controlled. In this case, no further evaluation or change to the method would be necessary; however, if the compound or related impurities underwent metal-induced degradation, additional efforts could be undertaken to evaluate method modifications to eliminate or minimize the impact. The flow chart is set-up to evaluate the most impactful changes first to yield a robust method. Once a suitable solution has been found, no other modifications would need to be assessed, unless redundant controls are desired. It is noted that trace levels of metals can be present in the glassware used to prepare sample diluent as well as the mobile phase, thus the impact of metals in both solutions should be evaluated if the compound is found to be susceptible to metal induced degradation. The evaluation of spiking metals into the mobile phase is annotated as #6 on the flow chart.

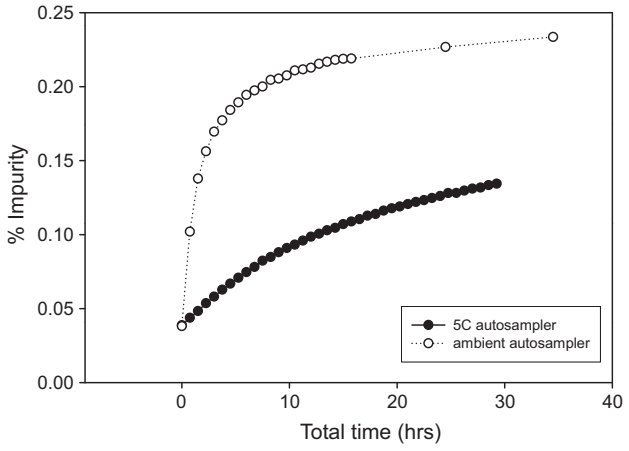

Fig. 8. Compound 2579539 formation resulting from a sample spiked with 1 ppm iron (III) and stored at room temperature versus 5 °C.

3.3.2. Assessment of diluent composition

Screening of alternate solvents and diluent compositions, annotated as #2 on the flow chart, should be evaluated first. A change here may limit the growth of degradation impurities, yielding a simple and straightforward solution that yields robustness, as was the case for arzoxifene hydrochloride.

To assess whether an alternative solvent is effective in inhibiting the degradation, the sample solution should be spiked with a low level of iron or copper and the impurity profile monitored as a function of time. If no significant changes in impurity levels are observed (e.g., additional degradation peaks or decreased response of existing impurities) over several hours, the solvent is suitable and no further assessment would be required. If substitution of alternate solvents does not minimize degradation or causes other undesired chromatographic issues (e.g., peak distortion due to sample solvent mismatch with the mobile phase), the addition of EDTA as an additive is suggested as the next step to be evaluated (#3 on the flow chart).

3.3.3. Assessment with EDTA

EDTA is a well-known chelator of metals; therefore inclusion in the diluent or eluent can in many cases either dramatically reduce or eliminate metal catalyzed oxidation. However, while artifact peaks due to metal-induced degradation often are minimized or eliminated, the presence of EDTA in the diluent may produce a

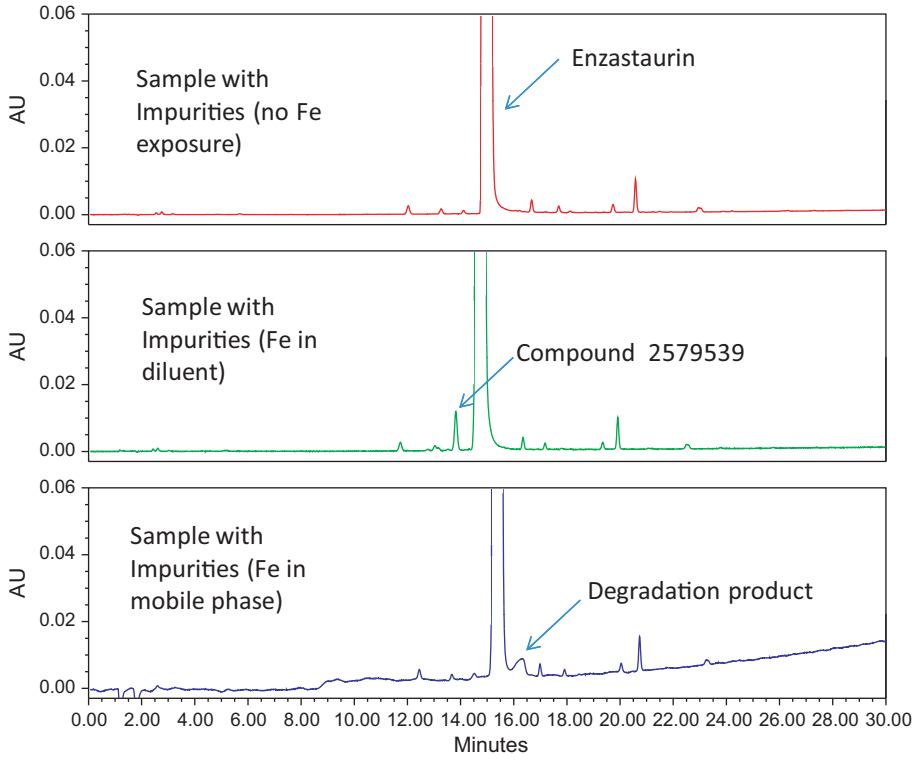

Fig. 9. Overlay of chromatograms for a sample of enzastaurin hydrochloride not exposed to iron (top), spiked with 10 ppm iron (III) in the diluent (middle) and spiked with 10 ppm iron (III) in the aqueous mobile phase buffer (bottom).

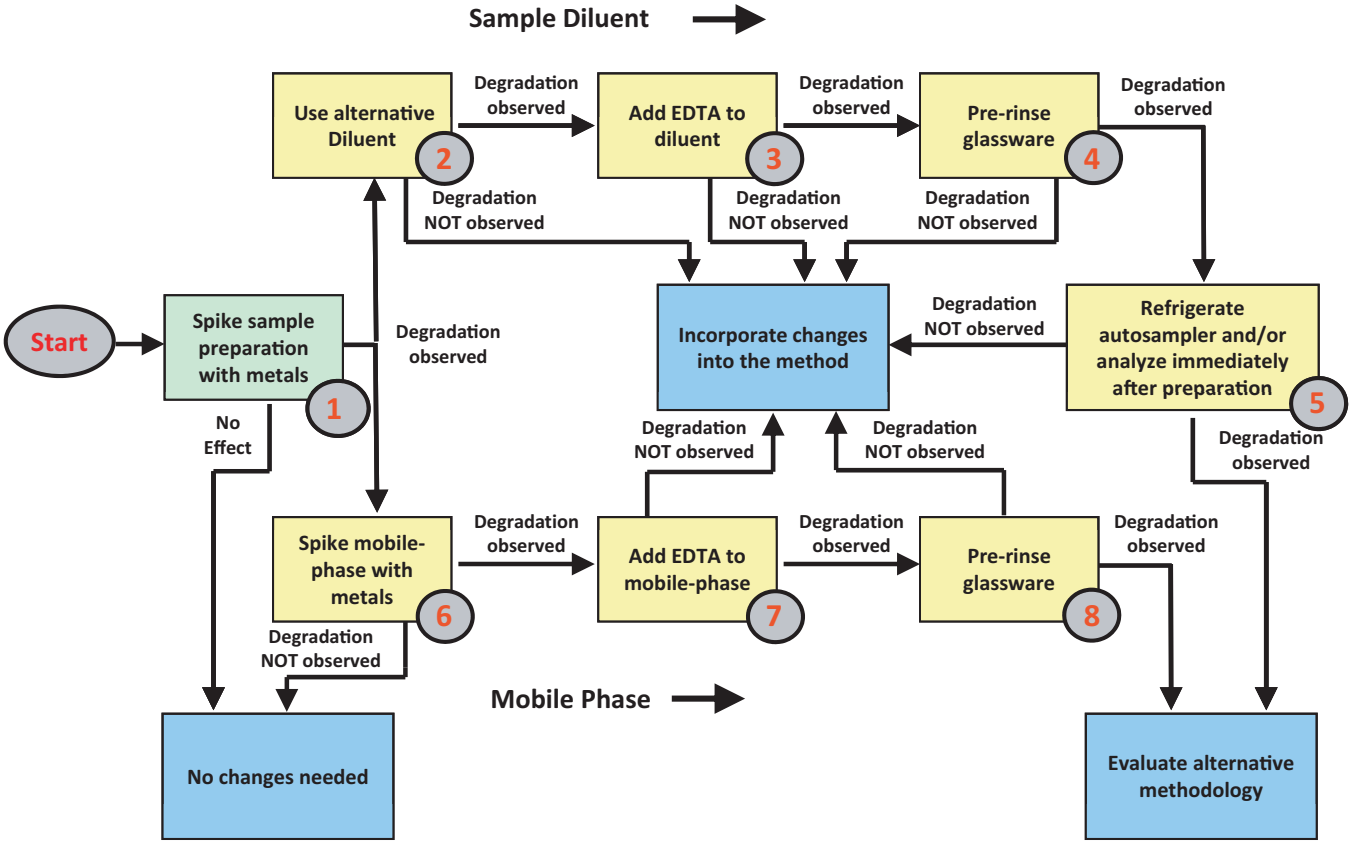

Fig. 10. Flow chart depicting the process to investigate and mitigate the impact of metal induced degradation for pharmaceutical test methods during development.

new peak near the void volume and can be a potential interferent for compounds that are not well retained. To evaluate the effect of EDTA (annotated as #3 and #7 on the flow chart), EDTA can be added to the diluent (or eluent) at a concentration of about 0.1 mM. After spiking in 1 ppm iron (III) and copper (II), the absence of new degradation impurities growing in over several hours may confirm the effectiveness of EDTA chelation. Verification that the EDTA peak does not interfere with analytes of interest should also be carried out. Should the addition of EDTA not provide an adequate solution, glassware treatment (pre-rinsing) can be an effective solution to mitigate trace metal contamination from the glassware.

3.3.4. Impact of glassware pre-rinsing

Rinsing glassware that is used for sample preparation or mobile phase preparation prior to use may effectively remove trace metal residue (annotated as #4 and #8 on the flow chart). In some cases rinsing with the sample diluent is sufficient, particularly if it is an acidic aqueous solution. For arzoxifene hydrochloride, the sample diluent had a high organic solvent content, and was not as effective at removing trace metals as a dilute acid wash. Rinsing with the aqueous phosphate buffer alone was effective in removing the metal residue, and had the advantage of not adding to the potential for contamination by introducing a new acidic reagent. For enzastaurin hydrochloride, the diluent was primarily an acidic aqueous solution and was effective in removing metal residue.

3.3.5. Impact of sample refrigeration and immediate analysis

A simple control can be implemented by storing samples at cool temperatures after preparation (annotated as #5 on the flow chart). The metal-induced degradation kinetics are typically fast, so the diluted samples should be placed directly into refrigerated storage right after preparation for the most effective control. This includes placing volumetric flasks containing sample preparations into a refrigerator, and vials waiting for injection on a cooled autosampler. While this control is not likely to completely inhibit the degradation, the reaction will occur more slowly, allowing for extended solution stability storage time. This control may be used as a primary control or in conjunction with other controls as a secondary control. Alternatively the samples could be immediately analyzed after preparation. The "dilute and shoot" approach does not require solution stability since the solution is injected right after dilution, minimizing the amount of time for degradation to occur. While this is an effective resolution to avoid degradation, it is labor intensive since the autosampler cannot be stocked to automatically run solutions.

4. Conclusion

Through the case studies presented, we have demonstrated how traces of transition metals in laboratory glassware can impact the accuracy of the results for analysis of the purity of pharmaceuticals. Two pharmaceutical compounds, a phenol and an indole, have been found to react with metals in the presence of oxygen to produce metal-induced oxidative decomposition (or reaction) products. Laboratory glassware may contain part per billion levels of copper and/or iron which can impact susceptible compounds by producing new impurities as artifacts of sample test solution instability, leading to falsely high impurity results. Falsely low results can also be produced by the degradation of susceptible impurities in test solutions. The authors have also shown that traces of metals in the HPLC mobile phase can lead to chromatographic artifacts, affecting the reproducibility of purity results.

As an approach to understand and proactively mitigate the impact of metal-induced decomposition, we have presented a logical strategy which includes first testing the pharmaceutical for susceptibility in the sample diluent and in the mobile phases used in the HPLC method. If the compound is found to be reactive with trace metals, alternative sample diluent with less reactive solvent, or addition of a metal chelator, is explored. If this is unsuccessful in providing a robust method, a glassware cleaning procedure is provided, and if needed, sample solution refrigeration is employed. In parallel, the impact of traces of metals in the mobile phase is examined for susceptible compounds. Any significant impact may be mitigated by the addition of a chelator to the mobile phase, or removal of the metals by glassware cleaning.

By routine application of this strategy, robust purity methods can be developed to provide accurate assessment of the quality of pharmaceutical compounds. Since trace metal contamination of glassware can be sporadic and intermittent, implementation of this approach early in the method development process can prevent future problems in quality control testing laboratories.

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