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Rapid Analysis of Residual Palladium in Pharmaceutical Development Using a Catalysis-Based Fluorometric Method

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ABSTRACT: Measurement of residual metals in pharmaceutical intermediates is routinely performed using inductively coupled plasma-optical emission spectroscopy (ICP-OES) or inductively coupled plasma-mass spectrometry (ICP-MS). However, these techniques suffer from drawbacks that limit their utility in pharmaceutical process development, including the requirement for expensive instrumentation, complex sample preparation, slow turnaround time, limited sample throughput, and the difficulty of performing the required measurements on the 'spot' within pilot plants or manufacturing environments. We investigate the use of a fast and inexpensive high-throughput approach for detection of residual palladium (Pd), based on the Pd-catalyzed Tsuji–Trost deallylation of an allylic ether substrate to produce a highly fluorescent product. We demonstrate the effectiveness of this fluorescence assay for accurate quantitation of Pd levels in a variety of 'real world' samples, including mixed oxidation-state samples containing strong Pd ligands.

INTRODUCTION

Recent years have seen a dramatic upsurge in the use of palladium catalysts in the synthesis of active pharmaceutical ingredients (APIs).[1] Concurrently, a number of methodologies have been developed for removing residual palladium from APIs.[2] Typically, process development for a palladium-removal step involves the evaluation of a number of different metal-removing conditions, producing dozens or even hundreds of samples that are analyzed by inductively coupled plasma-mass spectrometry (ICP-MS) or inductively coupled plasma-optical emission spectroscopy (ICP-OES). While undeniably effective, this general approach has several significant limitations. First and foremost, the technique requires expensive instrumentation and complex sample preparation, meaning that samples are typically handed off to specialist groups, rather than being analyzed immediately in the laboratories where the samples are generated. Sample handoff can also lead to significant delay, especially for those researchers in smaller companies without ICP-MS or ICP-OES resources, where samples are often shipped to a contract analytical laboratory.

The idea of a simple and easy to use fluorometric chemosensor or chemodosimeter to monitor metal removal 'on the spot' in a laboratory or pilot-plant environment has long been recognized as a preferred solution to this problem;[3] however, until recently no Pd chemosensors or chemodosimeters were available. Several years ago, Koide and co-workers reported the development of a fluorogenic Pd chemodosimeter based on the Pd-catalyzed Tsuji–Trost deallylation of allyl Pittsburgh Green ether (APE) to produce a highly fluorescent product (Figure 1).[4] The group successfully applied an assay based on this reaction to the quantitation of palladium in a variety of samples, including synthetic samples and druglike compounds spiked with palladium. Despite these examples, the utility of this approach for successfully monitoring the removal of residual palladium impurities in routine pharmaceutical process research remained an open question. Indeed, early attempts to apply this approach at Merck were disappointing, with considerable variation between reported Pd values and the actual values as determined by ICP-MS (unpublished results). The fact that this assay proved challenging is perhaps not surprising, as Pd residues in crude API samples are often present as a mixture of different oxidation states, only some of which are effective in catalyzing APE deallylation. Furthermore, in addition to residual Pd, these reaction residues often contain strong ligands specifically designed for chelating and enhancing the reactivity of palladium—which might be expected to influence the ability of palladium to catalyze the APE deallylation reaction. Finally, some of the most difficult problems encountered in the removal of Pd from pharmaceutical intermediates involve compounds that are themselves very good ligands for Pd, a property that might be expected to alter 'typical' APE deallylation rates.

In this study, we investigate the application of the method described in Figure 1 to the detection of Pd in pharmaceutical process research samples and describe conditions that render this simple and user-friendly approach suitable for the measurement of residual Pd levels in 'real world' samples from pharmaceutical process research studies.

RESULTS AND DISCUSSION

Preliminary investigations using palladium standard solutions in the absence of any interfering compounds confirmed the ability of the APE method to measure palladium concentration at low levels (Figure 2). We also investigated the effectiveness of using a single-point fluorescence measurement after defined incubation times, finding that with these conditions, a single read following a 10 min incubation at 45 °C permitted an acceptable linear range for detection between 1 and 30 ppb.

We next studied 'real world' samples of pharmaceutical intermediates containing various levels of residual palladium.

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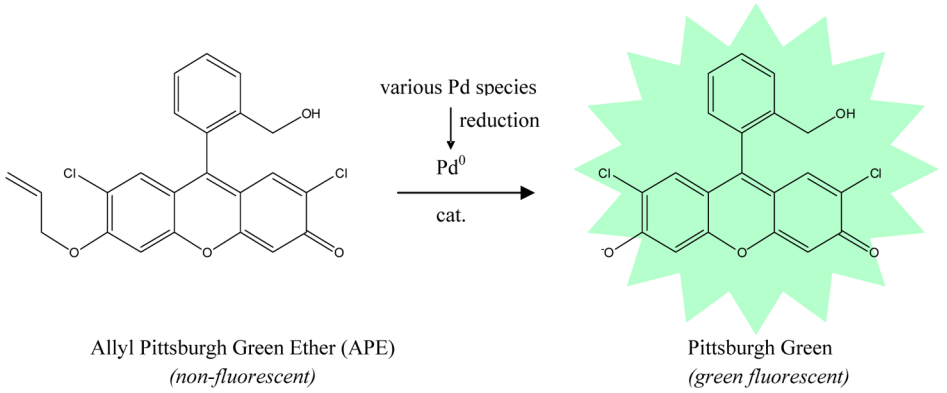

Figure 1. Method developed by the Koide group for fluorometric palladium determination and based on the Pd^0-catalyzed deallylation of allyl Pittsburgh Green Ether (APE).[4].

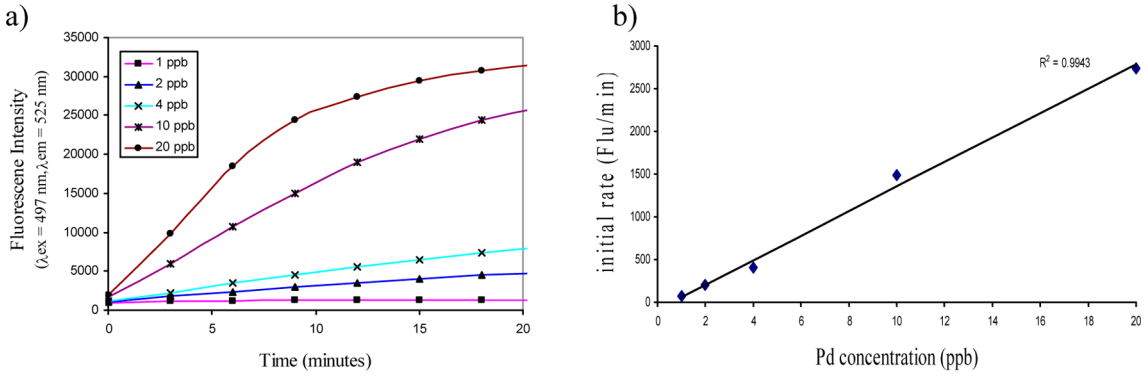

Figure 2. Rate of appearance of fluorescence provides an accurate measure of palladium concentration in a set of palladium standards. Conditions: palladium standard solutions (30 μL, 0–400 ppb in 1% HNO_3) were treated with a 1.8 mM solution of tri-2-furyl phosphine in DMSO, then diluted with concentrated phosphate buffer, pH 7. Allyl Pittsburgh Green ether (APE) was then added, and the fluorescence signal at 525 nm was monitored over time while incubating at 45 °C.

The samples included a number of intermediates where palladium removal had been problematic for various reasons. Earlier attempts to apply the APE method to samples of this type were somewhat disappointing; however, the most recently developed procedure[4d] from the Koide laboratory, which employs a sodium borohydride pretreatment to drive conversion of palladium to the desired Pd^0 species, is considerably better, as illustrated in Figure 3.

With the sodium borohydride treatment, the fluorimetric palladium measurements generally showed about 60–100% of the value determined by ICP-MS. While not ideal, such a moderately accurate correlation could potentially be useful, especially for tracking the relative amount of residual Pd in a number of scavenger-treated samples. The fact that certain samples exhibit lower palladium levels by the fluorescence method as compared with ICP-MS is perhaps not surprising, as many of these samples were from particularly challenging palladium removal problems where the intermediates themselves possess strong coordinating ligands that can potentially sequester palladium. Another challenge is that some intermediates may themselves fluoresce or act to quench fluorescence, thereby interfering with the accurate estimation of Pd concentration. Koide previously reported that a sample pretreatment involving digestion with strong acid could be useful in such cases, presumably by degradation and decomposition of the interfering substances.[4d]

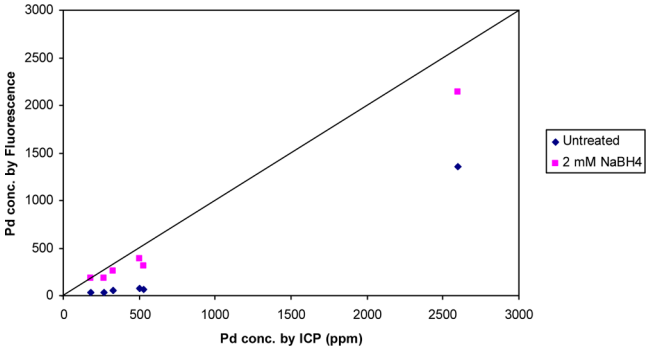

Figure 3. Sample pretreatment with $NaBH_4$ significantly improves the accuracy of the APE method for measuring residual palladium in a variety of pharmaceutical process intermediates. Conditions are as for Figure 2 with/without pretreatment with 2 mM $NaBH_4$.

The influence of a variety of different acid pretreatments on the accuracy of the fluorescence method for Pd determination is illustrated in Figure 4. All acid pretreatments significantly improve accuracy, with the *aqua regia* pretreatment showing the best performance overall. The resulting range of accuracy from 80 to 110% is less than ideal, but the general ability of the method to provide results that are reasonably close to the actual values offers some promise that the technique may become a useful laboratory method.

Although effective, sample pretreatment with *aqua regia* is potentially problematic, as this reagent is extremely hazardous.[5] The risk of *aqua regia* pretreatment for fluorimetric palladium

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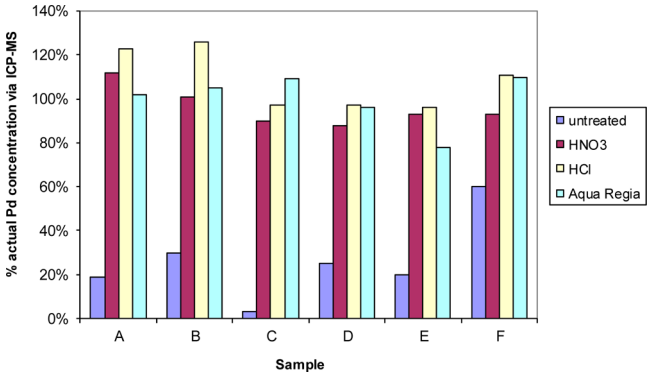

Figure 4. Sample pretreatment with various strong acids to disrupt and destroy strong palladium ligands that may interfere with the Pd-catalyzed deallylation of APE. Conditions: Samples were dissolved in 3:1 DMSO/H_2O (untreated) or concentrated acid to afford 1.0 mg/mL solutions, which were incubated at room temperature for 1h, then diluted 10-fold with 3:1 DMSO/H_2O and analyzed using the conditions described in Figure 2.

determination in the general laboratory should be weighed against the value of the improved accuracy for palladium measurement, which is fairly modest in many instances.

Having thus settled on a generic pretreatment protocol involving initial incubation with *aqua regia* followed by sodium borohydride reduction, we next examined a number of pharmaceutical intermediates representing routine palladium contamination problems. The results, summarized in Figure 5, show that the fluorometric method for detection of palladium is reasonably accurate, certainly good enough for monitoring routine palladium impurity remediation studies.

We next investigated the utility of the fluorescence method for on-the-spot monitoring of palladium removal in pharmaceutical process development studies. We previously reported the use of screening kits containing a variety of different adsorbents (Figure 6a) for the rapid identification of optimal conditions for removal

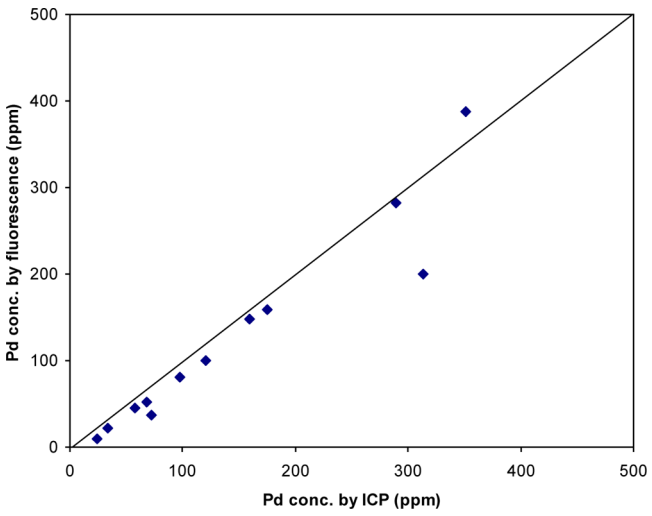

Figure 5. A generic pretreatment protocol involving initial incubation with *aqua regia* followed by sodium borohydride reduction provides reasonably good accuracy for the detection of residual palladium in a variety of routine pharmaceutical process intermediates. Conditions: Samples were diluted *aqua regia* to a target concentration of 1 mg/mL, incubated for 1 h at room temperature, diluted 10-fold with 3:1 DMSO/H_2O, and analyzed using the conditions described in Figure 2.

of metal impurities,[2a] an approach that requires close coordination with ICP-MS specialists to allow 'same day' problem solving. In contrast, the fluorescence method would enable rapid determination of palladium concentrations, in the same laboratory where the process development studies are being carried out. Figure 6b shows a microplate from such a screening study with 24 different adsorbents. Interestingly, gross differences in the samples can be observed with the naked eye, even without UV irradiation, although the use of a fluorescence plate reader (Figure 6c) quickly shows (read time <1 min) the most effective palladium removal treatments to be wells A4, B6, and D4.

In an effort to further improve user friendliness, we investigated the premixing of reagents required in the assay. We initially hoped that all reagents could be combined into a single cocktail solution, but soon found that the inclusion of $NaBH_4$ led to degradation of the APE substrate over time. We ultimately settled on the cocktail solution shown in Figure 7, which remains functional at room temperature for 24 h, although we suggest that the solution be stored at −20 °C when not used. The use of the reagent cocktail greatly simplifies the application of the fluorescence method for the estimation of Pd impurity levels in process chemistry samples. Following sample preparation, a 20 μL sample aliquot is added to 1.96 mL of the reagent cocktail, followed by 20 μL of a $NaBH_4$ solution. The sample is then incubated, with the appearance of fluorescence or color being monitored by several possible methods. In many cases, simple visual observation and comparison with standards is sufficient to detect the color of the Pittsburgh Green dye produced in the reaction, an approach that can be augmented by the use of a hand-held UV lamp. Quantitation using fluorescence measurement is a more sensitive approach, with the use of relatively commonplace fluorescence plate readers allowing for readily accessible high-throughput analysis options. For laboratories without access to fluorometers, measurement of the increase of UV−vis absorbance at 515 nm provides another way for quantifying color production.

An example showing the utility of the reagent cocktail approach is illustrated in Figure 8. A series of adsorbent treatments for removal of residual palladium was monitored using conventional ICP-MS, as well as the APE method with the reagent cocktail as depicted in Figure 7. The results obtained by monitoring fluorescence were very close to those provided by ICP-MS, with the selection of the 'top two' adsorbent treatments (A4, B6) being identical in both cases. However, reliance on UV measurement can be seen to be considerably more problematic, leading to the correct identification of only one of the two best adsorbent treatments (A4) while completely missing the other (B6) and also leading to the false identification of a 'red herring' (A2) that could potentially lead to considerable lost time in process development. Some caution may therefore be warranted in using UV−vis detection for quantitating Pd levels, with fluorescence clearly being preferred.

A rapid and convenient method for measuring residual palladium in the process chemistry laboratory is a considerable improvement over previous approaches requiring specialized equipment and personnel. At the outset of our studies, we believed that an ability to estimate palladium concentration using a simple reagent cocktail and a fluorescence plate reader could represent an important breakthrough for process chemists performing remediation of palladium impurity problems, an expectation that seems borne out by our findings. We were delighted to also find that naked eye observations could be used

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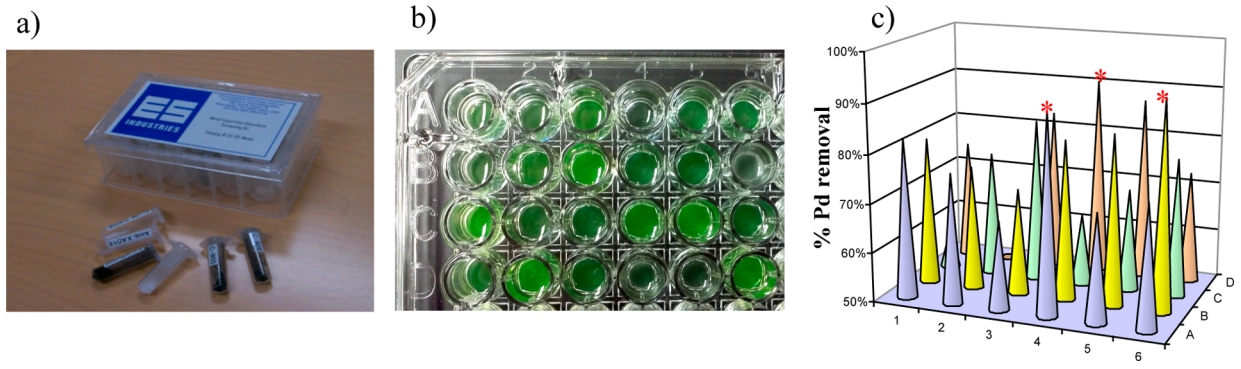

Figure 6. Combining adsorbent screening for Pd removal with high-throughput fluorescence Pd detection. (a) Screening kits[4a] containing a variety of commercial adsorbents are exposed to a solution of the Pd-containing intermediate (b) Aliquots of supernatant solutions from screening kits are evaluated for Pd content using the fluorescence method, as described in Figure 5. Note: Gross differences in Pd concentration are readily apparent to the naked eye. (c) Measurement of fluorescence intensity using a fluorescence plate reader provides rapid (~1 min) analysis compared to the traditional use of specialized ICP-MS laboratory. * indicates the most effective Pd removal conditions.

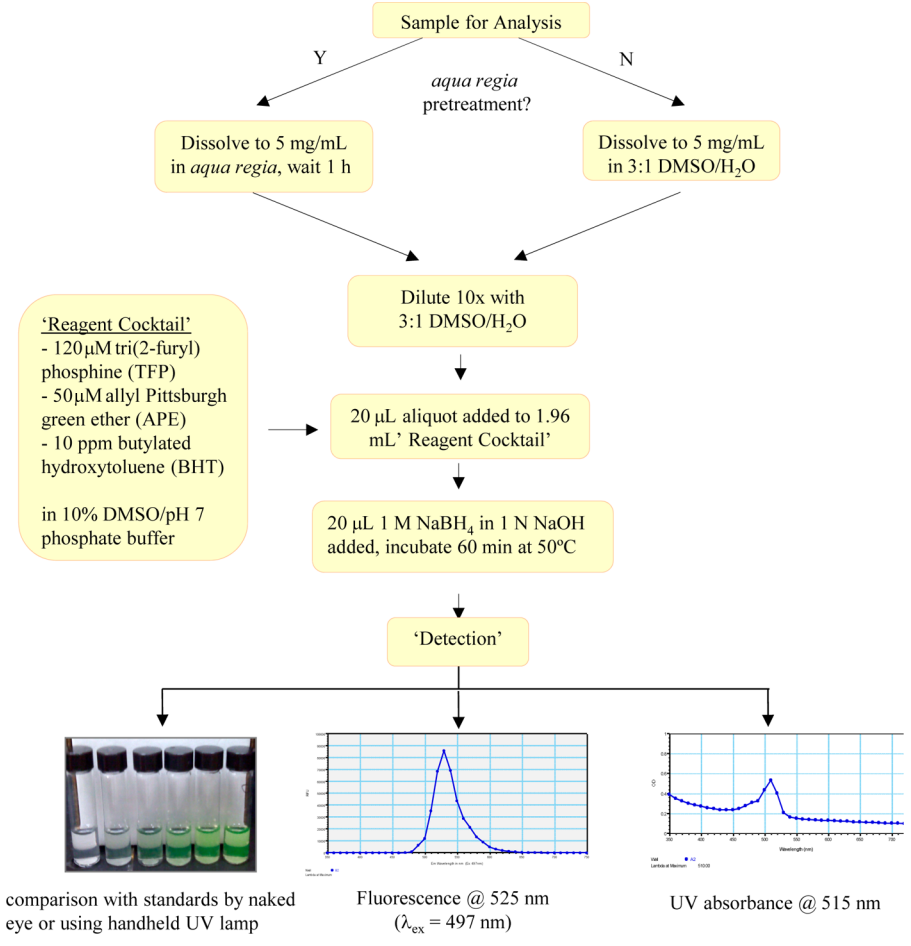

Figure 7. Simplified testing procedure using a premixed reagent solution allows for simplified application of the fluorescence method for palladium detection. Quantitation of palladium can be performed using either visual examination or measurement of fluorescence or UV−vis absorbance.

to assess palladium concentration, making the technique simpler and even more amenable to widespread utilization. Further improvements can be envisioned, potentially leading to even simpler 'test strips' or other forms of self-performing assays that could be used 'on the spot' for rapid readout of palladium concentration in the process chemistry laboratory. Overall, this example provides a nice illustration of how preferred platform solutions to complex problems in process chemistry tend to become faster and simpler over time.[6]

CONCLUSION

The utility of the catalysis-based fluorometric method for the detection of palladium, developed by the Koide group, was evaluated for use in pharmaceutical process chemistry investigations, where the advantage of quick turnaround measurements not requiring specialized instrumentation suggested a potential for on-the-spot palladium measurements to support laboratory or pilot-plant investigations of palladium removal.

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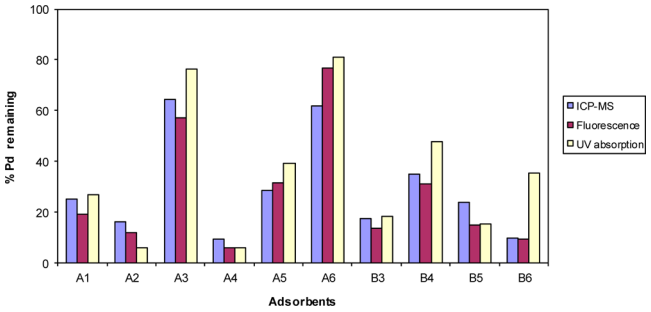

Figure 8. Case study showing the application of the reagent cocktail approach illustrated in Figure 7 to monitoring the efficacy of palladium removal from a pharmaceutical intermediate by adsorbent treatment. Measurement of residual palladium using either ICP-MS or the Koide method with fluorescence detection led to the correct identification of the two most effective treatments. In contrast, the use of UV–vis detection for quantifying palladium was less effective.

Excellent sensitivity and linearity were found with palladium standards, and a reasonably good (80–110% of actual) ability to quantify palladium in the presence of pharmaceutical intermediates was observed for samples in which appropriate pretreatments with *aqua regia* and $NaBH_4$ were performed. The linearity was significantly expanded compared to our previous studies. This is the first report that the use of $NaBH_4$ improves the signal recovery. Finally, a streamlined assay procedure utilizing a predispensed reagent cocktail that is stable for a day at room temperature (and for months in the freezer) is described.

EXPERIMENTAL SECTION

Reagents. Allyl Pittsburgh Green ether (APE) was prepared according to the published procedures.[4a] A palladium standard solution was purchased from VWR and used as received. $NaBH_4$ was purchased from VWR and used as received. Tri(2-furyl)phosphine (TFP) was purchased from TCI and used as received. Trace metal-grade HNO_3 and trace metal-grade HCl was purchased from Thermo Fisher and used as received. DMSO was purchased from J.T. Baker and used as received. A concentrated pH 7 buffer solution ([phosphate] = 1.23 M) was purchased from Thermo Fisher and used as received.

A solution of TFP in DMSO was freshly prepared before each experiment. A solution of $NaBH_4$ (2.5 M in 10 N NaOH or 1 M in 1 N NaOH) was freshly prepared weekly and stored at ambient temperature. A solution of $NaBH_4$ (30 mM) was freshly prepared before each experiment by diluting the 2.5 M solution with purified water.

Fluorescence Spectroscopy. Fluorescence spectra were recorded in a black, round-bottom, 96-well plates on a Spectra Max M5 spectrometer (Molecular Devices, Sunnyvale, CA) under the control of a Windows-based PC running software pro V5. The samples were excited at λ = 497 nm, and the emission intensity was collected at λ = 525 nm. All spectra were corrected for emission intensity by using the manufacturer-supplied photomultiplier curves.

Metal Analysis by ICP-MS. The samples were either diluted or suspended directly in concentrated acid or evaporated with a rotary evaporator first and then redissolved in concentrated acid for ICP-MS analysis. Depending on the concentration range of the element, either the Perkin-Elmer Elan 6000 quadrupole ICP-MS spectrometer (Perkin-Elmer, Norwalk, CT) or the Thermo Finnigan Element 2 high-resolution ICP-MS spectrometer (Finnigan, Bremen, Germany) was used for the analysis.

Analysis of Palladium Standards: Figure 2. A solution of TFP (200 μL, 1.8 mM in DMSO) was mixed with a palladium standard solution (30 μL, 0–100 ppb in 1% HNO_3). A concentrated phosphate buffer (pH 7, 2.57 mL, [phosphate] = 1.23 M) was added. The resulting mixture was incubated at 45 °C for 30 min. $NaBH_4$ (100 μL, 30 mM in 0.12 N NaOH) and allyl Pittsburgh Green ether (APE, 100 μL, 375 μM in DMSO) were then added in sequence. A 200 μL aliquot of the resulting solution was then transferred to a black round-bottom 96-well plate, placed into the SpectraMax M5 fluorescence plate reader at 45 °C., and the appearance of fluorescence at 525 nm was monitored as a function of time (λ_{em} = 497 nm).

$NaBH_4$ pretreatment on Pd contaminated API samples – Figure 3. A concentrated phosphate buffer (pH 7, 2.57 mL, [phosphate] = 1.23 M) was mixed with a pretreated solution of palladium contaminated API (30 μL, API 0.1 mg mL^{-1} in 10% acid). A solution of TFP (200 μL, 1.8 mM in DMSO) was added. The resulting mixture was incubated at 45 °C for 30 min. $NaBH_4$ (100 μL, 0 or 2 mM in 0.12 N NaOH) and allyl Pittsburgh Green ether (APE) (100 μL, 375 μM in DMSO) were added in sequence. The resulting samples were incubated for 1 h at 45 °C. Following incubation, 200 μL of the solution was transferred to a black, round-bottom, 96-well plate, and fluorescence was measured.

Effect of Acid Pretreatment on Pd-Contaminated Samples of Pharmaceutical Intermediates: Figure 4. Ten milligrams of each palladium-contaminated pharmaceutical intermediate was dissolved in 10 mL of pure acid and incubated at ambient temperature for 1 h. The pretreated samples were then diluted 10-fold using 3:1 DMSO/water and analyzed as above.

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Notes

The authors declare no competing financial interest.

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