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Adsorbent Screening Using Microplate Spectroscopy for Selective Removal of Colored Impurities from Active Pharmaceutical Intermediates

Christopher J. Welch,* William R. Leonard, Derek W. Henderson, Benjamin Dorner, Karla Glaser Childers, John Y. L. Chung, Frederick W. Hartner, Jennifer Albaneze-Walker, and Peter Sajonz*

Separation and Analysis Technologies, Process Research, Merck & Co., Inc. Rahway, New Jersey 07065, U.S.A.

Abstract:

A screening approach for identifying adsorbents and conditions for selective removal of colored impurities from solutions of pharmaceutical intermediates is described. In this method, a variety of process adsorbents are evaluated using a combination of HPLC or LC−MS and 96-well microplate UV–vis spectroscopy. Several representative examples are shown that illustrate the use of the technique for the selective removal of colored impurities from pharmaceutical development candidates or related intermediates.

Introduction

Removal of colored impurities by adsorption is a time-honored technique with a long and interesting history.[1] Charcoal has been used to remove colored impurities in the commercial production of sugar for nearly two centuries, and the use of adsorbents for decolorization is a key component of modern industrial chemistry, especially in the dyes and wastewater treatment fields. In organic chemistry, treatment of a solution with "decolorizing carbon" as a polishing step prior to crystallization is a technique learned by every organic chemist in introductory organic laboratory courses. In pharmaceutical manufacturing, removal of colored impurities by adsorption is similarly important, as a high value is placed on the production of colorless active pharmaceutical intermediates (APIs).

We have described a number of related approaches for the rapid screening of adsorbents to enable the removal of specific types of impurities from pharmaceutical intermediates, including discrete chemical impurities,[2] residual reactive starting materials,[3,4] and residual metal species.[5] In these studies, we utilized high-throughput analysis techniques, such as flow injection analysis LC−MS, to enable rapid screening of a number of different adsorbents. In contrast to other impurity problems, analyzing the removal of colored impurities can be somewhat problematic, as the offending compounds are sometimes of complex or oligomeric structure and consequently often not easily observed by analytical methods such as HPLC or NMR. Process chemists typically investigate color removal problems using either the naked eye or cuvette-based UV–vis spectrometry to measure the progress of color removal. In this report, we describe the use of a 96-well microplate UV–vis spectrometer, the "plate reader" commonly used in biotechnology research, to support adsorbent screening.

Experimental Section

Chemicals. The sample mixtures that were investigated are proprietary intermediates of active pharmaceutical ingredients in preclinical development at Merck Research Laboratories (Rahway, NJ, USA). Methanol, ethyl acetate and acetonitrile (HPLC grade) were obtained from EMD Chemicals (Gibbstown, NJ, USA). Isopropyl acetate and HPLC grade water were obtained from Sigma-Aldrich (St.Louis, MO, USA).

Adsorbents. Alumina was obtained from ICN Biomedicals GmbH (Eschwege, Germany), Silica gel from Eka-Kromasil (Bohus, Sweden), Amberlite XAD-16 from Aldrich Chemical Co. (Milwaukee, WI, USA), HP20 and HP25 from Mitsubishi Chemical Corporation (Tokyo, Japan), Ecosorb GL-961, C902, C905, C908, C933, C941 and C943 from Graver Technologies (Newark, DE, USA), Darco KBG, Darco KBB, Darco G60 from Norit Americas Inc. (Atlanta, GA, USA), SN-Bio, MP-TMT, tris amine and TPP polymer from Biotage (Biotage, Charlottesville, VA, USA), Nuchar TAC, RGC, 300, 1600, and Aquaguard from MeadWestvaco (Covington, VA, USA), PL-DETA and PL-BnSH from Varian (Palo Alto, CA, USA), Smopex 105 and 111 from Johnson Matthey (Deptford, NJ, USA), Thiol-3 from Silicycle Chemical Division (Quebec, Canada), Reillex 402 polymer from Reilly (Indianapolis, IN, USA), CalgonADP from Calgon Carbon Corporation (Pittsburgh, PA, USA).

Procedure for Adsorbent Screening. Adsorbent screening was carried out by using polypropylene vials containing 50 mg of adsorbent. Typically, 1 mL of process solution was added to these vials at 50 mg/mL to give a product/adsorbent loading ratio of 1:1 or at other concentrations depending on the process stream. The vials were then agitated for 30 min, followed by centrifugation at 10,000 rpm for 5–10 min using a tabletop microcentrifuge (Eppendorf, Westbury, NY, USA).

A Spectromax M5 Microplate Specrophotometer (Molecular Devices, Sunnyvale, CA, USA) was used for the measurement of UV spectra to evaluate color removal. Quartz 96-well flat-bottomed microplates (Hellma, Plainview, NY, USA) were used for these studies, although inexpensive plastic microplates are

* To whom correspondence should be addressed. Email: christopher_welch@merck.com; peter_sajonz@merck.com.

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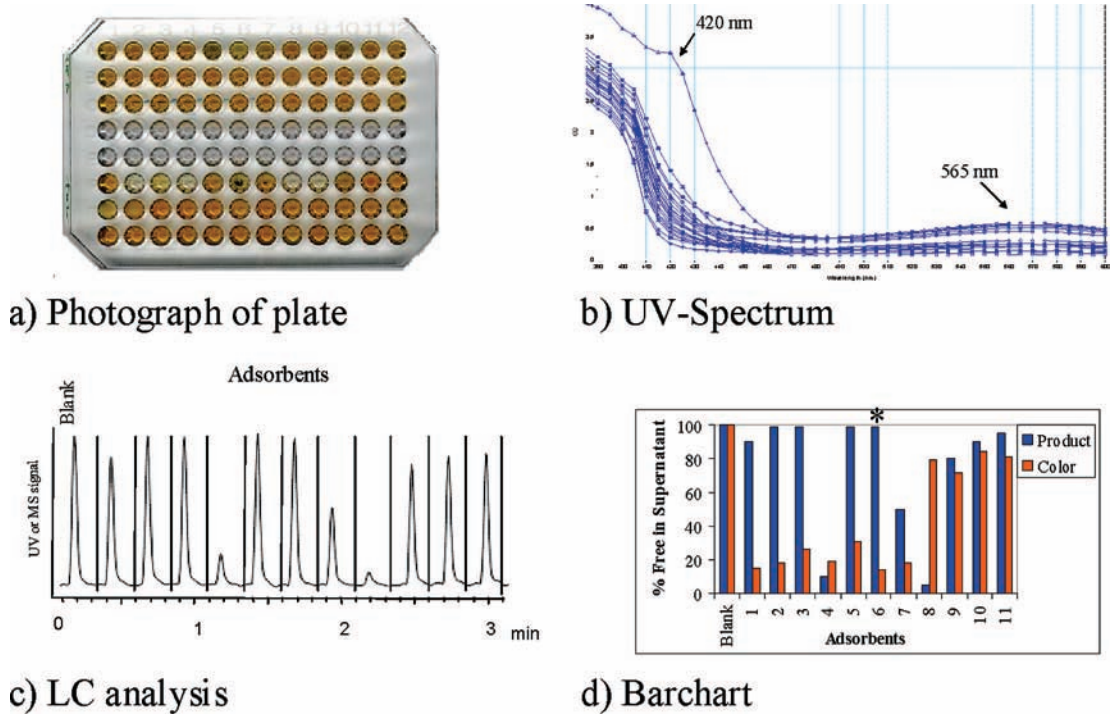

Figure 1. **General approach for adsorbent screening. Samples treated with a variety of process adsorbents are allowed to equilibrate and centrifuged, and then supernatant solutions (a) are analyzed using a UV–vis microplate spectrometer (b) to quantitate color removal. Product loss is then analyzed, typically using flow injection analysis HPLC or HPLC−MS (c). The fraction of product and impurity removal for each of the treatments is then represented graphically (d), facilitating the selection of the optimal adsorbent and treatment conditions.**

also suitable for monitoring impurity removal at higher wavelengths and when solvent compatibility allows. Product recovery in the supernatant was typically carried out by HPLC analysis or HPLC−MS flow injection analysis.

HPLC Analysis. An Agilent 1100 system with diode array UV–visible detection was used to carry out HPLC analysis (Agilent Technologies, Palo Alto, CA, USA). The system was also equipped with an Agilent 1100 MSD detector. Mass spectroscopy analysis was generally accomplished in positive ion mode using a pH 3.5 acetonitrile/water eluent buffered with 0.2 mM ammonium formate.

Results and Discussion

Undesired impurities may be removed from pharmaceutical intermediates via selective adsorption, provided that a resin or adsorbent with suitable selectivity and capacity can be identified. We have previously described a useful microplate-based screening approach that enables rapid evaluation of a number of different adsorbents and treatment conditions.[2] A modification of this protocol is used in the present study to search for appropriate adsorbents and conditions for selective removal of colored impurities in several recent examples from these laboratories.

The general procedure for the selection of a suitable adsorbent for the removal of colored impurities is illustrated in Figure 1. A solution containing the desired component along with the colored impurity is divided into a number of small tubes, vials, or wells, each of which is treated with a small amount of a process adsorbent. Following equilibration, the samples are centrifuged, and the supernatant solutions are analyzed for loss of color using a UV–vis microplate spectrometer (plate reader). The UV–vis plate reader can measure the absorbance at a single wavelength of an entire quartz 96-well microplate (Figure 1a) in 1 min or less and can even be used to rapidly monitor full UV spectra (Figure 1b). Next, the supernatant solutions are examined for the presence of the desired component, typically using HPLC flow injection analysis with UV or MS detection (Figure 1c), with comparison to an untreated control solution. The extents of product loss and color removal are then compiled into a bar graph to provide an easy graphical comparison of the efficacy of each adsorbent treatment (Figure 1d). In some cases, the preferred treatments are easily recognized, the ideal case being virtually no product loss combined with almost complete color removal. However, in some cases, it is not readily apparent which treatment is best, and calculation of the selectivity factor (α), which reflects the ratio of the equilibrium constants for adsorption of impurity versus desired component, may be useful. The selectivity factor, α, can be calculated using the equation

$$\alpha = \frac{1 - x_{\text{imp}}}{x_{\text{imp}}} \Big/ \frac{1 - x_{\text{prod}}}{x_{\text{prod}}} \qquad (1)$$

where x_{imp} and x_{prod} are the color and product fractions that remain in the supernatant after adsorbent treatment.[6] As we have previously pointed out, care should be taken when calculating selectivity in cases where adsorption is near 0 or 100%, as small

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errors in measurement at these extremes can result in significant variation in the calculated result. The whole procedure of selecting a suitable adsorbent for color removal is usually quite fast, taking 1−2 h in most cases.

We present several examples of the use of this adsorbent treatment screening approach in successfully developing color removal treatments for kilogram-scale syntheses of preclinical candidates in the pharmaceutical process research environment.

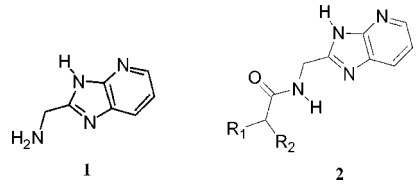

Figure 2. **Formation of product 2, plagued by the presence of difficult to remove dark-colored impurities arising from intermediate 1.**

In the first example (Figure 2), synthesis of the target compound, **2**, involves condensation of a complex intermediate with heterocycle **1**, the synthesis of which is plagued by the formation of dark-colored impurities that are difficult to remove by conventional treatments. Carrying colored **1** forward in the reaction affords product **2**, also contaminated with difficult to remove dark-colored impurities. At this point, we turned to adsorbent screening to try to identify a suitable treatment for color removal. Removal of color from either product **2** or intermediate **1** would meet the needs of the project; thus both were screened.

Our investigations focused first on the "downstream" coupled product, **2**, which was a brown-colored solution in methanol (20 mg/mL). The analysis of color was performed on a UV–vis spectrophotometer using a wavelength of 405 nm, with analysis of **1** carried out by HPLC. Initial screening of a group of adsorbents in methanol solution afforded the results illustrated in Figure 3.

A visual examination of the colors of the individual wells of the microplate shows that several treatments are useful in removing color.This can also readily be seen from the bar graph, which permits a more exact comparison of the treatments. A number of adsorbents, particularly the carbon-based adsorbents, such as Ecosorb 943 and Ecosorb 933, are effective in reducing color. However, product loss is also substantial, making these treatments poorly suited for practical color removal.

We next investigated heterocyclic intermediate **1**, which was a brown solution in 80% MeOH/water (20 mg/mL). In this case, screening of adsorbents for color removal monitored at 490 nm afforded the results shown in Figure 4. Several adsorbents, notably Darco KB and Ecosorb 933, afford substantial color removal with only minimal loss of product. The selectivity for both of these treatments was excellent ($\alpha > 50$).

Based on these results, a strategy for removal of color by treatment of intermediate **1** was clearly identified and implemented in the first kilogram-scale synthesis of **2** to support preclinical evaluations. Later, the treatment was employed on a larger scale to support 10- and 90-kg synthesis campaigns. Darco KB and Darco G60 in methanol/water at a 1:1 loading of adsorbent/intermediate were used on these larger scales.

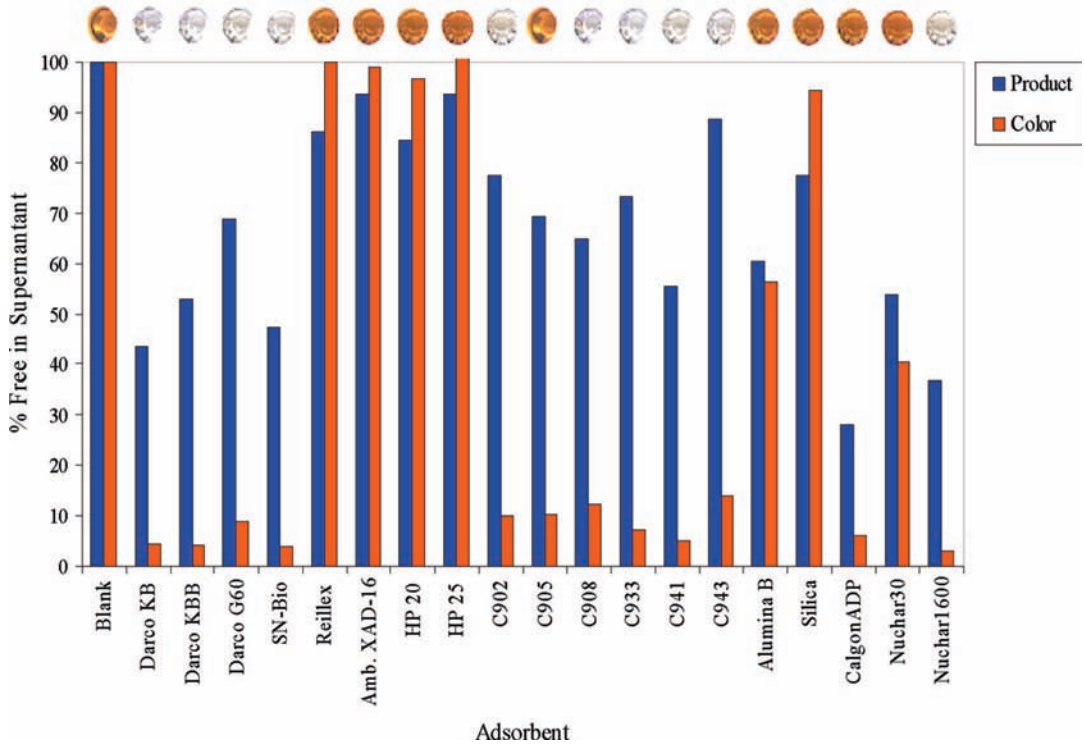

Figure 3. **Selective removal of color from a solution of "downstream" coupled product, 2. Photographs of supernatant solutions from treated wells clearly show effective color removal in some instances, although the bar graph of color removal as monitored by UV–vis and product loss as monitored by HPLC indicate substantial product loss, making these treatments poorly suited for color removal. Adsorbent screening was carried out at 50% (w/w) loading in methanol with a concentration of 2 at 20 mg/mL.**

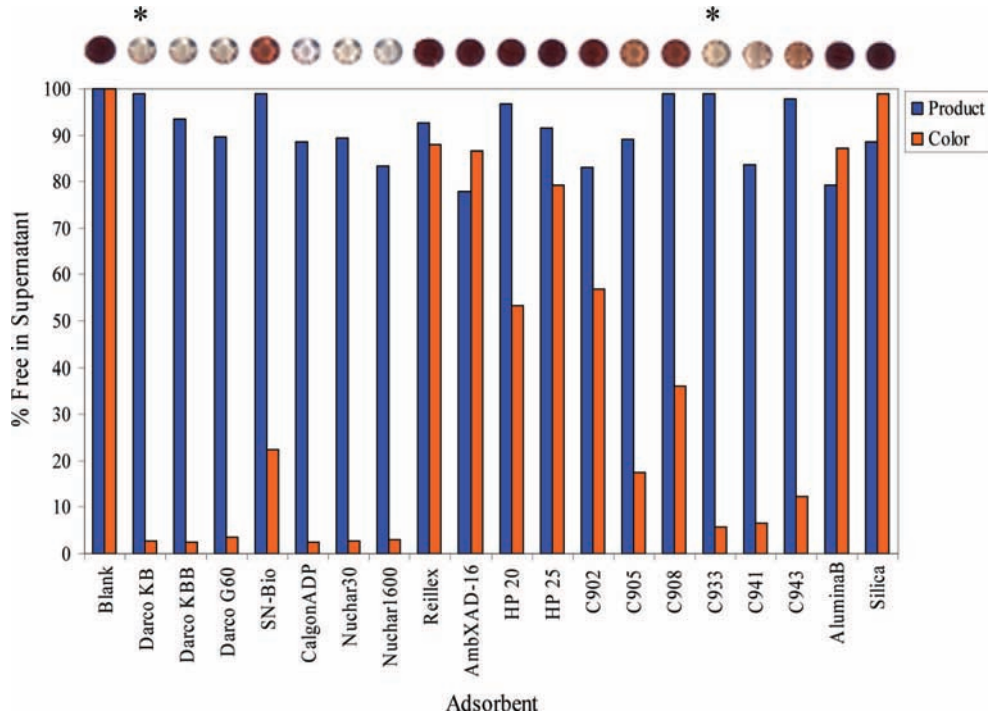

Figure 4. **Selective removal of color from a solution of heterocyclic intermediate 1. Photographs of supernatant solutions from treated wells show effective color removal in several instances, with the bar graph of color removal as monitored by UV–vis at 490 nm and product loss as monitored by HPLC revealing treatments with Darco KB (*) and Ecosorb C943 (*) being the best. Adsorbent screening was carried out at 50% (w/w) loading using a solution of 1 in 80% MeOH/water at 20 mg/mL.**

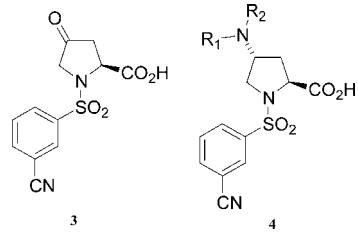

Figure 5. **Intermediates containing ruthenium and golden color impurities.**

Adsorbent-based removal of color can be strongly influenced by solvent, and a particular treatment can often be fine-tuned by changes in solvent, temperature, or other parameters. However, we often have found that when investigating the removal of color or other impurities, it may be prudent to initially focus the investigation on actual process streams. In this way, adsorbent treatments can often be identified that can readily be plugged into existing processes without the need for solvent switches or other additional changes.

The next example (Figure 5) involves the removal of color that arises from the use of an organometallic catalyst in a process. Problems relating to removal of metal impurities are becoming increasingly common as organometallic catalysts become more frequently used in pharmaceutical processes. In this example, preparation of ketone, **3**, involved the use of a ruthenium catalyst. The resulting ruthenium impurities and attendant golden color were difficult to remove from **3**. Color and ruthenium impurities were also not easily removed when carried on to the reductive amination product, **4**, the next intermediate in the synthetic sequence. Again, we elected to perform adsorbent screening on both intermediates as actual process streams coming from the synthesis.

We first investigated the ketone intermediate **3**, which contained ruthenium at 120 ppm and showed a golden yellow color. Adsorbent screening of a 77 mg/mL stream of **3** in MeCN/EtOAc afforded the results shown in Figure 6. Product loss was monitored using FIA−LC−MS, and color removal was monitored by UV–vis at 400 nm. Several treatments were effective in removing color without substantial product loss, most notably Ecosorb C908, where color was reduced by 89%, with only negligible product loss (∼1%). ICP−MS analysis confirmed that the Ru impurity level in the treated solution correlated well with color removal, suggesting a ruthenium species as the colored impurity.

We also investigated color removal from the reductive amination product, **4** (21 mg/mL in EtOAc stream, 119% w/w load), with color removal monitored at 590 nm and product loss by HPLC (Figure 7). In this example, several adsorbents that effectively removed color were identified, but all also showed appreciable product loss as well.

Consequently, color removal was carried out on intermediate **3** in the first kilogram-scale preparation of the candidate.

In another example the removal of a green color of unknown origin from **5** (Figure 8), an API, was required.[7]

The analysis of color using a UV–vis microplate spectrophotometer indicated significant high wavelength absorbance, including a discrete band centered at 630 nm (Figure 8b). Adsorbent screening was conducted on solutions of **5** at 66 mg/mL in IPAc using a set of 19 different readily available process adsorbents at 50% (w/w) load. The analysis of product loss in the resin screens was performed using HPLC, while disappearance of color was monitored using a UV–vis plate reader.

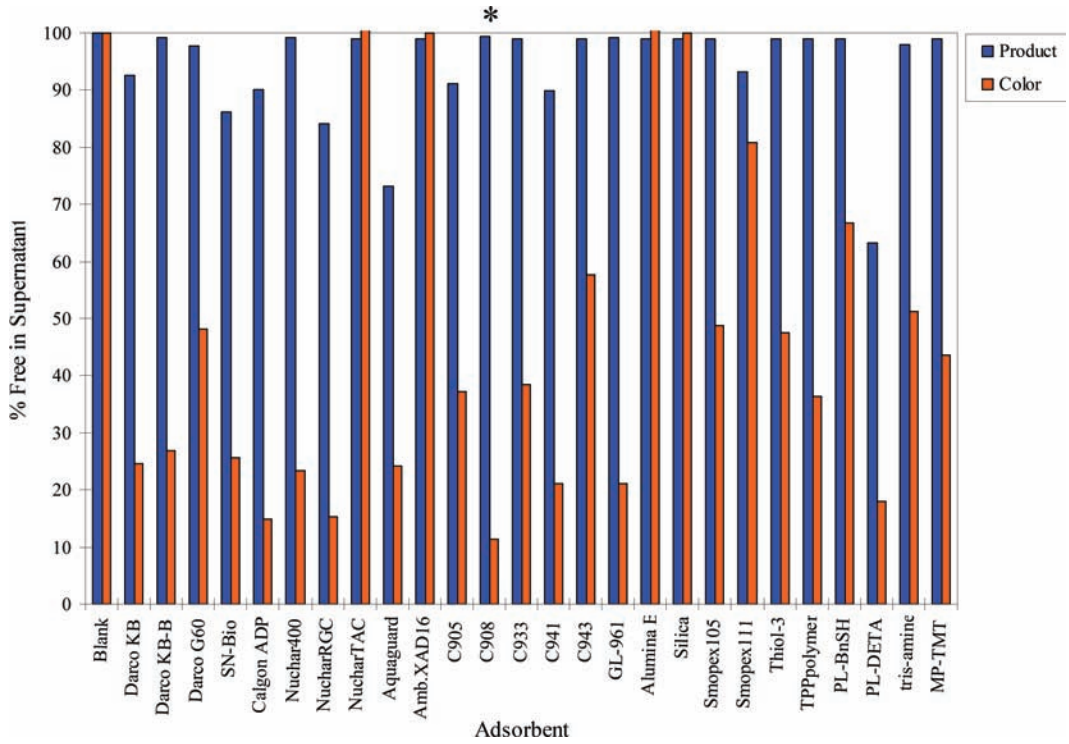

Figure 6. **Selective removal of color from process streams of compound 3. Adsorbent screening was carried out at 50% (w/w) loading in acetonitrile/ethyl acetate at 77 mg/mL. Product loss was monitored using LC−MS with flow injection mode, and color removal was monitored by UV–vis at 400 nm.**

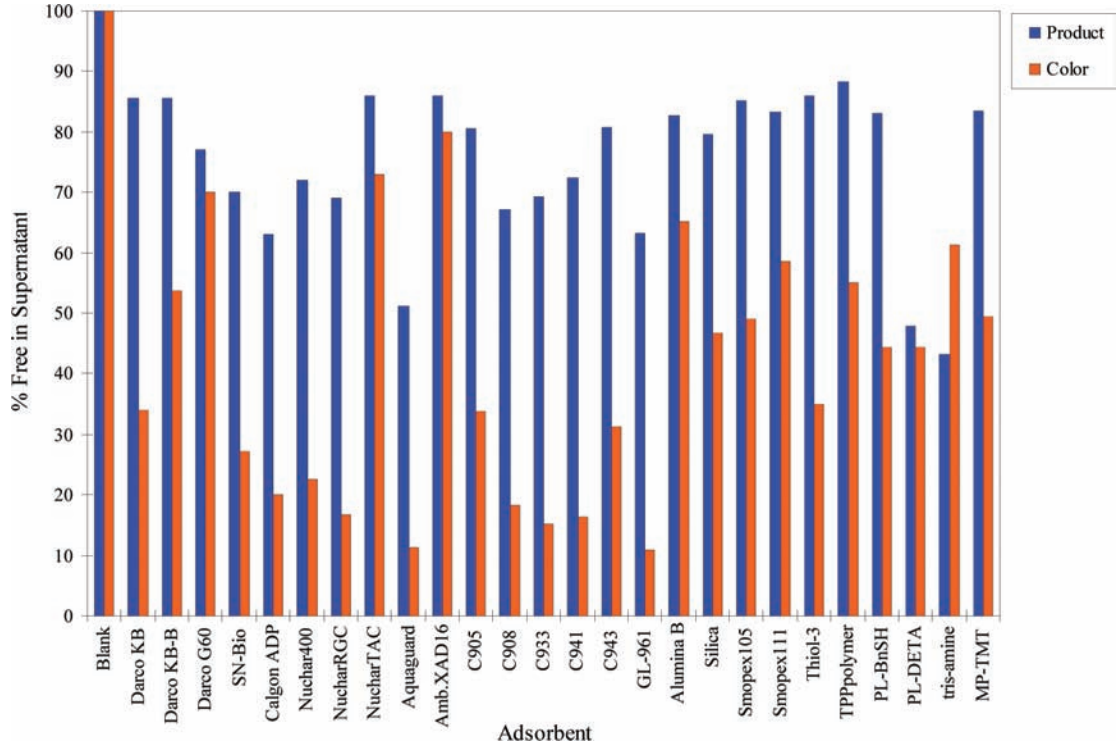

Figure 7. **Selective removal of color from process streams of compound 4. Adsorbent screening was carried out at 119% w/w loading with a concentration of 21 mg/mL in EtOAc stream. Color removal monitored at 590 nm and product loss by HPLC.**

Ecosorb 933 (Graver) and silica proved most effective, reducing color (630 nm) by 83–84%. Recovery of **5** from these treatments was 99%.

In another example, removal of a colored impurity from intermediate **6** was investigated using both microplate UV–vis spectroscopy and the more traditional HPLC approach (Figure 9).

Crude **6** was a yellow solution in methanol. Adsorbent screening was conducted on solutions of **6** at 100 mg/mL in methanol using a set of 20 different readily available process

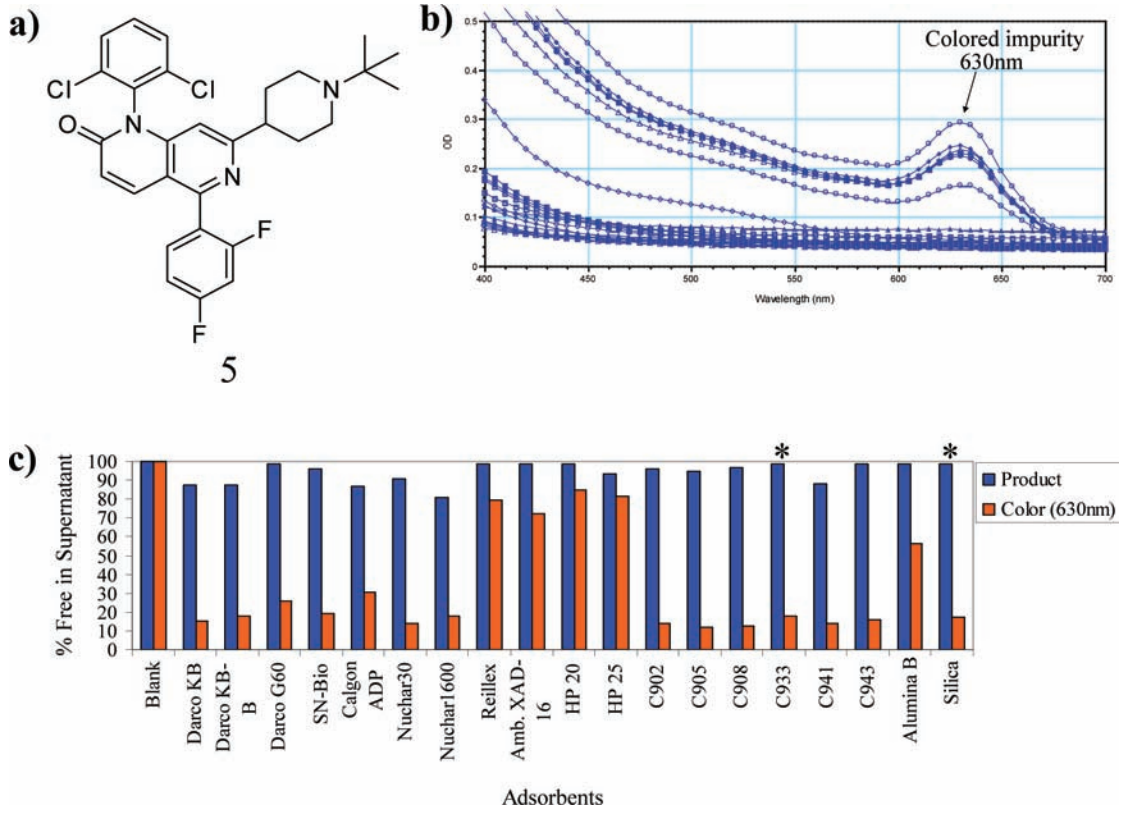

Figure 8. **(a) Structure of colored API 5 that should be colorless. (b) Spectra showing supernatants of 5 after adsorbent treatments. (c) Selective removal of color from process streams of compound 5. Adsorbent screening was carried out on solutions of 5 at 66 mg/mL in IPAc using a set of 19 different readily available process adsorbents at 50% (w/w) load. The analysis of product loss in the resin screens was performed using HPLC, while disappearance of color was monitored using UV at 630 nm.**

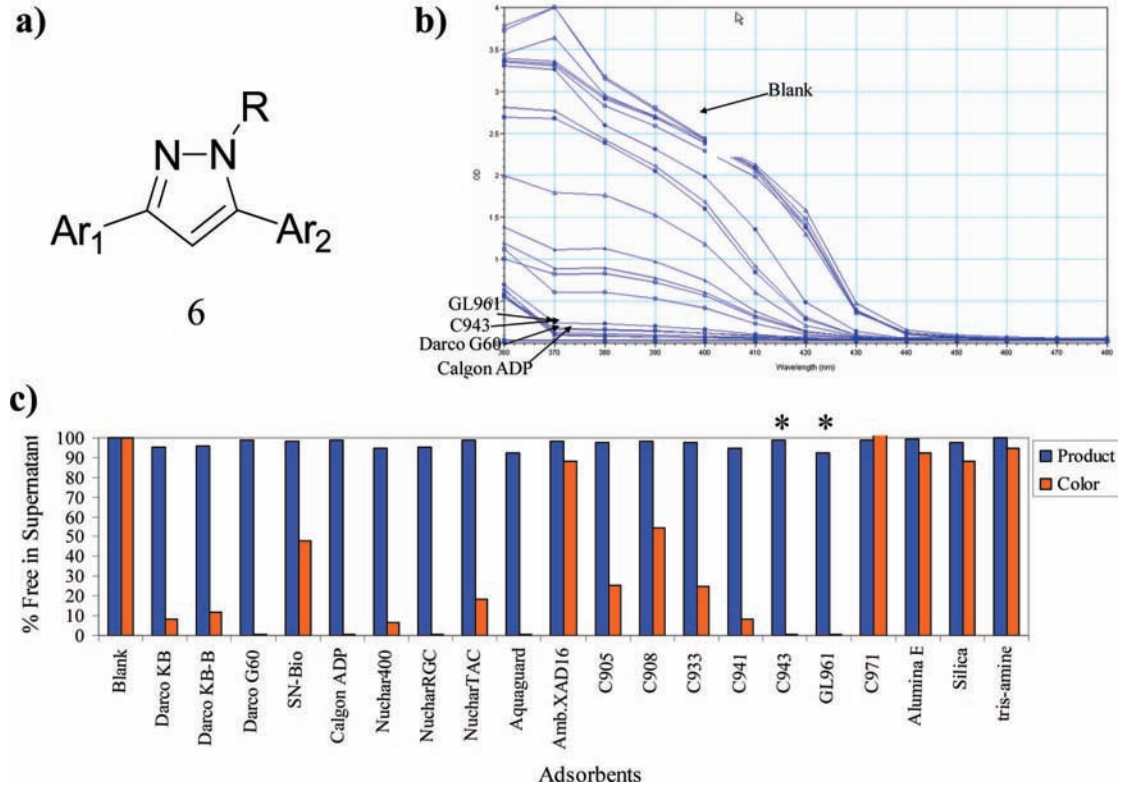

Figure 9. **(a) Intermediate containing yellow color impurities. (b) Spectra showing supernatants of 6 after resin treatments. (c) Selective removal of color from process streams of compound 6. Adsorbent screening was carried out on solutions of 6 at 100 mg/mL in methanol using a set of 20 different readily available process adsorbents at 50% (w/w) load. The analysis of color was performed using a UV–vis spectrophotometer, with spectral analysis from 360 to 450 nm. The analysis of product loss and impurity removal in the adsorbent screens was also monitored using an HPLC assay that resolved the colored impurity as a discrete peak.**

adsorbents at 50% (w/w) load. The analysis of color was performed using a UV–vis spectrophotometer, with spectral analysis from 360 to 450 nm. The analysis of product loss and impurity removal in the adsorbent screens was also monitored using an HPLC assay that resolved the colored impurity as a discrete peak. Ecosorb C-943 (Graver) and Ecosorb GL-961 proved most effective, reducing color by >98%, with recovery of **6** between 92% and 99%.

Conclusions

The examples shown in this study illustrate the power of a simple and a cost-effective approach for screening adsorbents for the removal of colored impurities from pharmaceutical intermediates. This approach greatly speeds and facilitates the task of selecting the best adsorbent and treatment conditions from among countless choices. Key to the success of this approach is the use of the UV–vis microplate spectrophotometer, which greatly facilitates the rapid monitoring of the removal of color, even when discrete colored impurities are not easily identified and tracked. Finally, several examples illustrate the strategic advantage of screening different intermediates in a synthetic sequence to increase the chances of finding an effective color removal treatment.

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