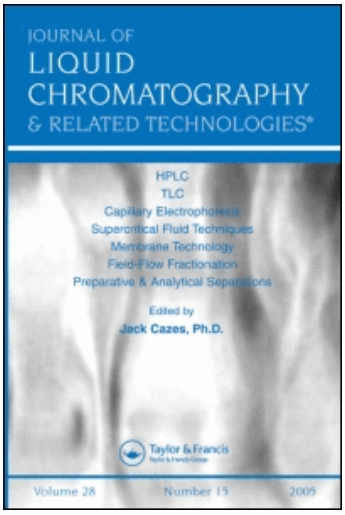

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Selective Removal of a Pharmaceutical Process Impurity Using a Reactive Resin

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ABSTRACT

A low-level (0.5%), but troublesome, aldehyde impurity in a pharmaceutical product was conveniently removed from an existing process stream by employing a reactive, polystyrene-based sulfonylhydrazine resin. Selection of this resin resulted from screening a number of adsorbents and reactive resins using a high throughput LC–MS approach. The sulfonylhydrazine resin was able to quickly remove an impurity from an existing, highly concentrated, product stream in DMF at a level of 20 mg resin for each gram of product. The material obtained from such treatment showed a greatly improved impurity profile, with an 85% reduction in the level of the aldehyde impurity and without introduction of additional impurities.

Key Words: Reactive resin; Scavenger resin; Selective adsorbents; Adsorbent screening; LC-MS screening; Flow injection analysis.

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INTRODUCTION

The rigorous purity requirements for pharmaceutical products mean that levels of trace impurities, including residual starting materials, must be kept to an absolute minimum. Pharmaceutical manufacturing processes often rely on crystallization as a purification technique, although problems may sometimes result from impurities that are not easily rejected in product recrystallization. In such cases alternative purification strategies must be employed. The use of adsorbents and reactive resins for the selective removal of unwanted impurities from pharmaceutical process streams is a well known approach[1–4] that is gaining renewed popularity as new materials and experimental approaches become available.[5–7] We have recently developed analytical screening techniques that expedite the selection of an appropriate solid phase material for a given purification task.[8–13] These high-throughput approaches make possible the rapid identification and implementation of solutions to emergent purification problems, thereby potentially reducing the time required in bringing a new drug to market.

We recently became involved with a pharmaceutical candidate under investigation having the generalized structure **1**. During the course of development of this program, we became alerted to the presence of a troublesome aldehyde impurity **2** that was not rejected during the crystallization of the final product as the besylate salt, but was carried forward as a contaminant at a level of 0.5 area %. The impurity originated as residual starting material carrying over from the preceding step. Owing to the poor solubility of the free base and other considerations, removal of the aldehyde impurity needed to be effected in a highly concentrated DMF solution of the product as the benzenesulfonic acid salt.

In an extension of our previous work on the development and use of selective adsorbents,[13] we evaluated a number of commercial materials for selective adsorption of the aldehyde impurity. We also evaluated a number of commercial reactive resins, reasoning that the differential

Figure 1. Structures of product **1** and impurity **2** investigated in the study.

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reactivity of aldehyde, **2**, and product, **1**, could provide a basis for separation.

EXPERIMENTAL

General Methods and Materials

Adsorbent evaluation was carried out in glass, deep-well, 96-well microplates (Zinsser Analytic, Frankfurt, Germany), with liquid transfers to polypropylene (Costar, Corning Lifesciences, Acton, MA) or glass (Zinsser Analytic, Frankfurt, Germany) standard 96-well microplates, being carried out using 8× or 12× multichannel pipetters (Eppendorf, Hamburg, Germany). Compound **1** was available from previous studies and contained **2** as an impurity at a level of 0.5 area %.

A variety of commercial process adsorbents and reactive resins were investigated in the initial screens, with aminopropyl silica (amino-3-silica, Silicycle, Quebec, Canada) and polystyrene-based tosylhydrazine resin (PS-TsNHNH$_2$, Argonaut Technologies, Inc., Foster City, CA) showing sufficient performance to warrant further study.

LC–MS Assay

Flow injection LC–MS analysis was carried out using an 1100 MSD HPLC–MS system (Agilent Technologies, Palo Alto, CA) operating in the electrospray ionization mode. The column was an Extend C18 (4.6 × 50 mm, Agilent Technologies, Palo Alto, CA) thermostated at 25°C. Mobile phase was 70% acetonitrile/water, containing 2 mM formic acid/ammonium formate at pH 3.5. Flow rate was 1.2 mL/min, sample injection size was 1 μL, and run time was 1 min. The MSD detector was operated in positive ion mode with selected ion monitoring at the masses corresponding to M + 1 for product ($m/z = 588$) and aldehyde impurity ($m/z = 354$), with optimized settings for detection of product and impurity of Frag 120 and Vcap 2000.

Initial Screening of Resins for Impurity Removal

A variety of commercially available process adsorbents and reactive resins (about 10 mg each) are placed into the individual wells of a glass 96-well deep-well microplate. A solution of crude product **1** as the besylate salt (300 μL of an 0.1 mg/mL solution in DMF) is added to each well and the plate is shaken for 15 min. After settling, a 100 μL

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aliquot of the supernatant solution from each well is transferred using a multiplex pipetter to a daughter microplate. The daughter plate is analyzed by flow injection analysis LC–MS (ESI) with selected ion monitoring for the diagnostic M + 1 ions of product and impurity. Key to identity of resins: A = no resin; B = DMF only; C = Ecosorb C981; D = Ecosorb C933; E = Ecosorb C962; F = Ecosorb C905; G = Ecosorb C902; H = Ecosorb C908; I = Silica; J = Argonaut Ts-$NHNH_2$; K = Darco G60; L = SiliCycle Diol; M = SiliCycle Cyclohexyldiol; N = SiliCycle Cyano; O = Silicycle Amino.

Microplate Evaluation of Resin Loading and Reaction Timecourse

Weighed amounts of resin are added to the individual wells of a glass deep-well 96-well microplate. A 300 mg/mL solution of **1** as the besylate salt is prepared by adding DMF to 4.8 g of **1** and 1.2 g of benzene sulfonic acid to a to total volume of 20 mL. A 300 μL portion of this solution is added to each of the resin-containing wells and to one control well containing no resin. The plate is shaken for 15 min, and the resin allowed to settle. A 10 μL aliquot of the supernatant solutions of the control well, and each of the wells corresponding to the 15 min timepoints, is transferred to a daughter plate containing 1 mL of DMF in each of the receiving wells. The plate is shaken for a few seconds, then a 30 μL aliquot from each well is transferred to a granddaughter plate containing 1 mL of DMF in each of the receiving wells, thereby, effecting an overall 3300× dilution of the resin screening solution. Similar treatment is done at each designated timepoint. Analysis of the granddaughter plate by LC–MS using selected ion monitoring for product **1** and impurity **2** (Fig. 1) and comparison with control (no resin) provides a gauge of the kinetics of impurity removal.

Sample Cleanup Using Sulfonylhydrazine Resin

Removal of the aldehyde impurity using the reactive polystyrene-based sulfonylhydrazine resin was investigated on a multigram scale. The sulfonylhydrazine resin was pre-washed by slurrying 70 mg resin in 5 mL DMF at ambient temperature for 20 min, then decanting the supernatant solution. Following a second washing and decanting, 12 mL of DMF, 3.0 g of **1** as the free base and 0.85 g of benzenesulfonic acid were added to the washed resin. The slurry was allowed to age for one hour at ambient temperature with stirring. Resin was removed by vacuum filtration to afford a clear yellow solution. The resin was rinsed with 3 mL of DMF and the rinse added to the reaction solution. The product was crystallized by addition of 63 mL of

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isopropyl acetate at ambient temperature, followed by cooling to 5°C and aging 12 hours. The resulting crystals were isolated by vacuum filtration and washed with 2*15 mL of 95 : 5 IPAc : DMF. HPLC analysis on the isolated product revealed 86% removal of the aldehyde impurity. No impurity rejection was observed without resin treatment. The mother liquor losses were similar to the process without resin treatment and no new impurities were seen by HPLC.

RESULTS AND DISCUSSION

Initial screening to identify the most selective adsorbents is typically carried out at high dilution, with subsequent experiments focusing on solvent optimization and determination of loading capacity. In this study we were limited to implementing a solution with the existing product stream in DMF containing benzene sulfonic acid, rendering evaluation of other solvent systems unnecessary. Considering the structures of the product and impurity, the task of selective adsorption of impurity **2** in the presence of product **1** from a DMF solution seemed a somewhat daunting challenge. Nevertheless, we selected a representative group of commercial process for preliminary screening.

The fact that only impurity **2** contains an aldehyde functional group suggested that separation based on differential reactivity might be a reasonable approach. We considered approaches based on the formation of imine, acetal, and hydrazone derivatives as illustrated in Fig. 2 and included samples of two diol silicas, one amino silica, and one sulfonylhydrazine resin in our group of materials for initial screening. In contrast to adsorption approaches, which typically reach equilibrium quickly, reaction with solid phases can sometimes be sluggish, meaning that the kinetics of the solid phase reaction becomes an important consideration in resin evaluation.

Resin evaluation follows a relatively straightforward approach originally developed for screening chiral stationary phase libraries.[8] Resins for evaluation (typically about 10 mg each) are placed into the wells of a 96-well glass microplate, and a dilute solution (typically about 300 μL at a concentration below 10^{-4} M) of the compound mixture to be evaluated is added. The plate is then shaken for 15 min and the resins are allowed to settle. Adsorption of a given component by the stationary phase results in decreased concentration of that component in the supernatant solution. Analysis of the concentration of the two (or more) components in the supernatant solution and comparison with a control solution containing no added adsorbent, allows both the extent and selectivity of adsorption to be determined. In cases where the desired product and the impurity differ in mass and can be detected, ESI-MS sometimes provides a rapid and useful method for determining adsorbent selectivity. In the present instance, we were able to develop a fast 1 min LC–MS assay using

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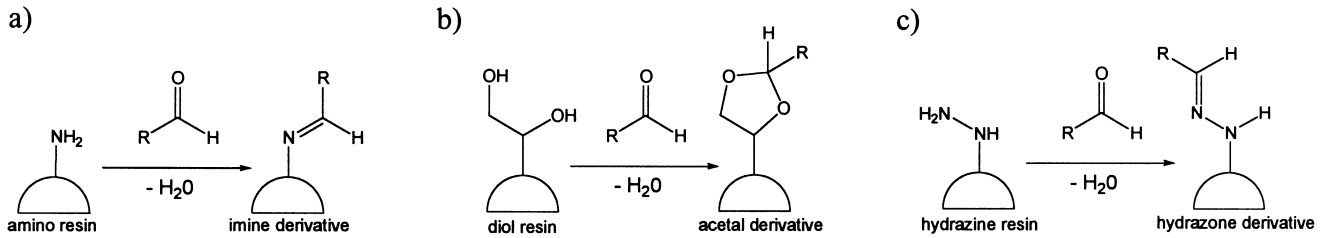

Figure 2. Three strategies for covalent removal of aldehyde impurity, (a) imine formation using amino resin, (b) acetal formation using diol resin, (c) hydrazone formation using sulfonylhydrazine resin.

selected ion monitoring, to follow both the product **1** and the impurity **2** as their M + 1 ions (588 and 354 amu, respectively).

The results of initial resin screening in DMF (Fig. 3) reveal that there is generally no adsorption of either product or impurity with most materials, although encouraging results are seen for resin J, a commercial (Argonaut Technologies) polystyrene-based sulfonylhydrazine resin.[14–16] We noted only low levels of impurity removal with the amine and diol resins, although it is possible that improved results could have been obtained by employing dehydrating conditions. The poor performance of the adsorbents was not entirely surprising, given the considerable solvating power of DMF and the larger size and functional group distribution of the product.

Further investigation of resin capacity and reaction time showed that the polystyrene-based sulfonylhydrazine resin possesses outstanding properties for impurity removal. The rate of reaction is very rapid at room temperature. As shown in Fig. 4, as little as 2 mg of the sulfonylhydrazine resin and a reaction time of only 15 min are sufficient to effectively remove impurity **2** from 300 μL of a 300 mg/mL DMF solution of the besylate salt containing 0.5% impurity. These results suggest that nearly all of the hydrazine moeities (2.6 mmol/g) on the resin are participating in impurity capture, indicating that only 20 g of resin would be required for cleanup of one kilogram of product.

Removal of the aldehyde impurity using the reactive polystyrene-based sulfonylhydrazine resin was investigated on a multigram scale. The sulfonylhydrazine resin was pre-washed by slurrying 70 mg resin in 5 mL DMF

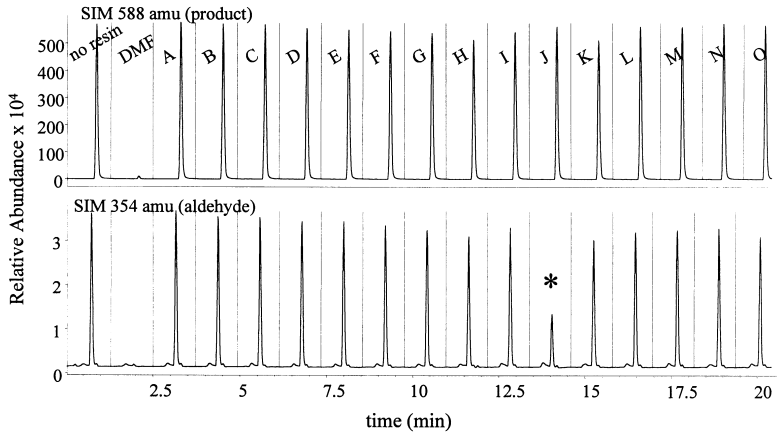

Figure 3. Flow injection analysis showing initial screening of a group of 15 commercial resins and process adsorbents for selective removal of aldehyde impurity. Conditions and key to resin identity as described in Materials and Methods section.

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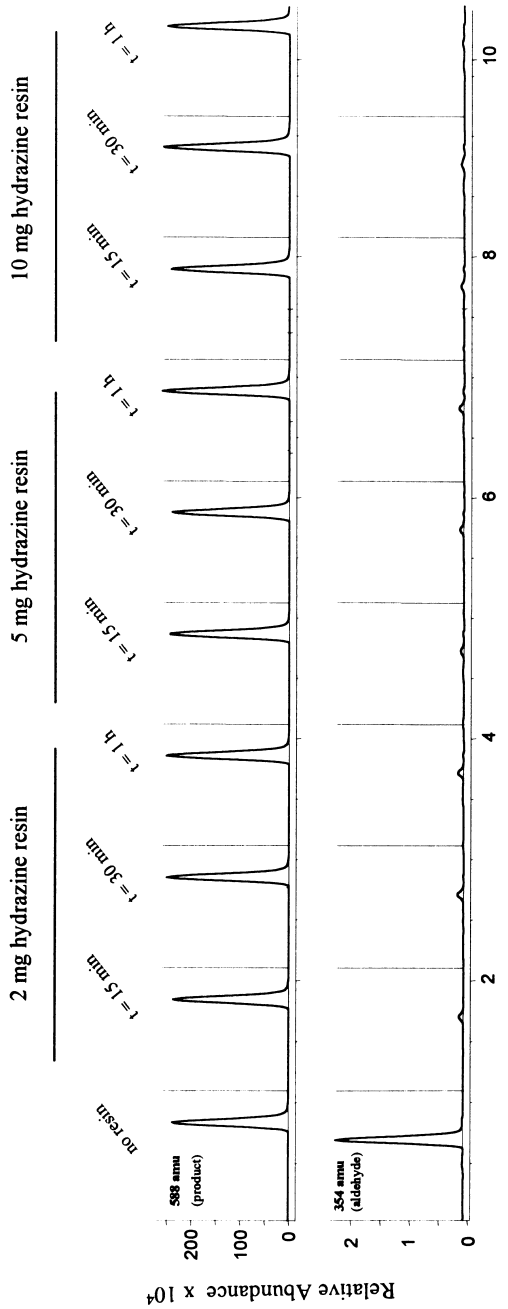

Figure 4. Selective removal of aldehyde impurity, **2**, using polystyrene-based sulfonylhydrazine resin. Conditions as described in Sec. 2.4.

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at ambient temperature for 20 min, then decanting the supernatant solution. Following a second washing and decanting, 12 mL of DMF, 3.0 g of **1** as the free base, and 0.85 g of benzenesulfonic acid were added to the washed resin. The slurry was allowed to age for one hour at ambient temperature with stirring. Resin was removed by vacuum filtration to afford a clear yellow solution. The resin was rinsed with 3 mL of DMF and the rinse added to the reaction solution. The product was crystallized by addition of 63 mL of isopropyl acetate at ambient temperature, followed by cooling to 5°C, and aging for 12 hours. The resulting crystals were isolated by vacuum filtration and washed with 2∗15 mL of 95 : 5 IPAc : DMF. HPLC analysis on the isolated product revealed 86% removal of the aldehyde impurity. No impurity rejection was observed without resin treatment. The mother liquor losses were similar to the process without resin treatment and no new impurities were seen by HPLC.

CONCLUSION

The use of a high-throughput resin evaluation technique allowed rapid identification of a workable solution to a challenging purification problem in pharmaceutical process development. The sulfonylhydrazine resin that was identified provides a rapid, efficient, and economical solution to an otherwise difficult purification problem.

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