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Removal of Electrophilic Potential Genotoxic Impurities Using Nucleophilic Reactive Resins

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Abstract:

Potential genotoxic impurities (PGI) are chemical compounds that could potentially damage DNA and lead to mutation. Controlling the occurrence of PGIs in active pharmaceutical ingredients (APIs) poses a big challenge for chemists, as levels of these compounds must be reduced well below the amounts required for other types of less toxic impurities. In situations where formation of PGIs cannot be avoided, an ideal solution would allow the complete removal of PGIs after the synthesis is complete, for example, by recrystallization, preparative chromatography or other downstream processing approaches. Some disadvantages of using these approaches are potential high yield loss, high solvent consumption, and additional time and resources required for process development. In this work, we present a simple and rapid approach to remove electrophilic PGIs from APIs. A selected nucleophilic resin can be added to the final API solution to reduce or totally remove the PGI. Esters of methanesulfonic acid (MSA), benzenesulfonic acid (BSA), and *p*-toluenesulfonic acid (pTSA) were used as model electrophilic PGIs. Several nucleophilic resins were screened, and the resins with the highest efficiency of PGI removal were chosen. A recommended procedure is presented for the removal of MSA, BSA, and pTSA esters. The kinetics of PGI removal, resin loading capacity, solvent effects, and API matrix effects are demonstrated.

Introduction

In recent years, global regulatory authorities have paid closer attention to the establishment of a clear guidance on acceptable levels of impurities bearing potential for genotoxicity.[1−3] The European Medicines Agency (EMEA) Committee for Medicinal Products (CHMP) issued a guideline on the limits of genotoxic impurities in 2006.[1,2] The CHMP approach uses the threshold of toxicological concern (TTC) which suggests a limit of 1.5 μg/day for lifetime intake of a genotoxic impurity.[4] Because of varying durations of treatment for different pharmaceutical products, Muller et.al[5] proposed a staged TTC approach to set acceptable daily intake values. These values, ranging from 1.5 μg/day for lifetime intake to 120 μg/day for <1 month intake, are believed to be safe doses and are applicable at any stage of clinical development.

The analysis of potential genotoxic impurities (PGIs) presents some unique challenges to the analytical chemist. Sensitive and robust analytical methods have to be developed to allow monitoring and quantitation of PGIs that may be present at only the parts per million (ppm) level. Although HPLC/UV methods are sometimes suitable for analyzing those PGIs containing strong chromophores,[6,7] HPLC/MS has become more popular due to superior sensitivity and selectivity of the MS detector.[8,9] For volatile PGIs containing poor chromophores, GC/FID[10] and GC/MS[11,12] methods have sometimes been used. The sensitivity and robustness of the analytical method can be greatly affected by matrix interferences. To alleviate such effects, solid-phase and liquid-phase extractions have sometimes been utilized.[13,14] In another study, minimizing matrix interferences was achieved by performing headspace GC/FID analysis.[7]

An additional challenge of PGI analysis stems from the reactive nature of PGIs; the same reactivity that allows these compounds to covalently bind to DNA or proteins can also lead to reaction and degradation during sample preparation or analysis. Consequently, special consideration must be given to sample preparation and storage, as well as selection of analysis conditions and solvents that do not lead to PGI degradation. Such problems can sometimes be addressed by derivatization

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reactions in which the PGI is transformed into a more stable species for more convenient analysis.[15,16]

In general, impurities can be classified into five different classes with respect to their genotoxic potential.[5] Of these, alkyl and aryl sulfonic acid esters are some of the most frequently encountered PGIs in current pharmaceutical manufacturing processes. Alkyl and aryl sulfonic acid esters are classified as class 3 PGI (impurities that are considered as alerting structures and could be linked to genotoxicity on the basis of their structure). In fact, several studies[17−21] confirmed genotoxicity of several alkyl sulfonic acid esters, and Glowienke et.al.[22] reported genotoxicity of other aryl sulfonic acid esters in two *in vitro* studies.

Methanesulfonic acid (MSA), benzenesulfonic acid (BSA), and *p*-toluenesulfonic acid (pTSA) are commonly used in active pharmaceutical ingredient (API) synthetic routes either as counterions to form a salt or as acid catalysts. Esters of these alkyl and aryl sulfonic acids may be formed in the presence of alcoholic solvents such as methanol, ethanol, or isopropanol. As the daily intake of these alkyl and aryl sulfonic acid esters should be limited to <120 μg/day, any synthetic route involving these species must be designed to control the formation of these esters to low ppm levels.

Controlling the formation of PGIs (alkyl and aryl sulfonic acid esters in this case) to low ppm levels poses a big challenge for process chemists. In some cases, alternative synthetic routes must be developed to avoid PGI formation. In other scenarios, purification of the API is preferred to remove the PGI. Ideally, PGI removal can be accomplished by crystallization of the API. Final product crystallization is typically performed in most API syntheses, and the simplest solution to PGI removal is to adjust the crystallization conditions such that PGIs are reduced below target levels. However, not all APIs are crystalline and not all crystallizations are effective in rejecting PGIs. In cases where crystallization is not possible, PGI removal by preparative chromatography can sometimes be employed, despite the high solvent consumption and additional time and cost required for process development.

We have previously reported approaches to the removal of impurities such as utilizing selective adsorbents[23−25] or reactive resins.[26−28] These studies suggested that a similar approach could potentially be used for the removal of reactive PGIs. In this study we investigate the use of nucleophilic reactive resins to remove electrophilic PGIs. Several nucleophilic resins were screened, and the resins with the highest efficiency of PGI removal were selected. The selected nucleophilic resin can be added to the final product stream, allowed to react with the PGI, and then removed by filtration prior to crystallization. A standard screening protocol with different resins is recommended for the different PGIs. The kinetics of PGI removal, resin loading capacity, solvent effects, and API matrix effects are also demonstrated.

Results and Discussion

Figure 1 shows the structures of the various resins used in this study. As shown, all resins have a nuclephilic site (amino group or a thiol group). As opposed to adsorption or chromatographic approaches, the removal of the sulfonic acid esters from solution using nucleophilic resins is based on a chemical reaction between the nucleophilic site of the resin and the electrophilic PGI, as illustrated in Figure 2.

In order to assess the feasibility of this general approach, a model study investigating the treatment of methyl *p*-toluenesulfonate with benzylamine (surrogate for the insoluble resin) soon led to a decrease in methyl *p*-toluenesulfonate with concurrent formation of methyl benzylamine as evidenced by LC/MS (data not shown).

Solutions of methyl, ethyl and isopropyl *p*-toluenesulfonate were treated with different nucleophilic resins for 30 min. Several resins showed extensive removal of methyl *p*-toluenesulfonate, with multiple thiol- or amine-containing resins affording greater than 80% impurity removal under these conditions (Figure 3). These same resins were less effective in removal of the ethyl and isopropyl esters, presumably owing to the increased steric bulk of the ethyl and isopropyl esters versus the methyl ester.[29]

The same screening experiment was performed with methyl, ethyl, and isopropyl bezenesulfonates. Resins showing removal of >50% for methyl ester and >10% for ethyl and isopropyl esters were selected (data not shown). Similarly, the ethyl and isopropyl esters showed lower reactivity relative to that of the methyl ester.

In an analogous fashion, the screening experiment was performed with methyl, ethyl and isopropyl methanesulfonate. These studies showed some removal of methyl mesylate, however, little or no removal of the ethyl or isopropyl mesylate esters was observed, even when pH, temperature, and reaction time were varied.

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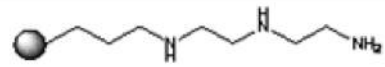
Si-Triamine	
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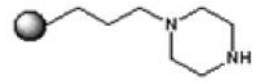
Si-Piperazine	
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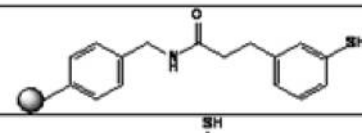
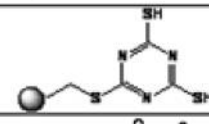
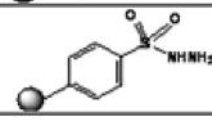
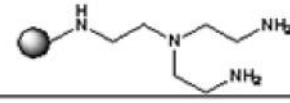
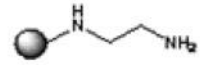
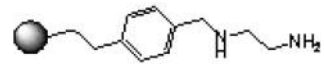
PS-Thiophenol	
Si-TMT	
PS-TS-NHNH2	
Tris(2-aminoethyl)-amine	
Ethylenediamine	
Si-Ethylenediaminobenzyl	

Figure 1. **Structures of the functional groups on the various nucleophilic resins used.**

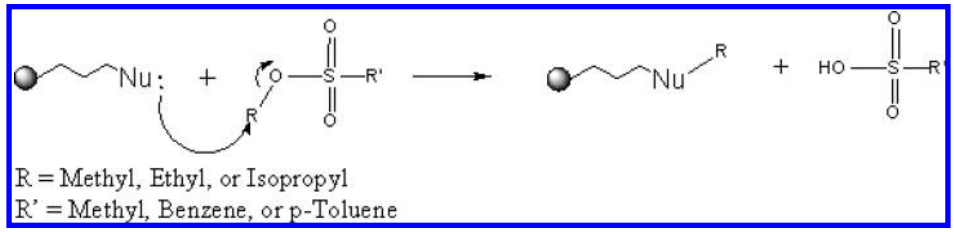


Figure 2. **Proposed mechanism for removal of sulfonate esters using nucleophilic resins.**

The kinetics of the removal of *p*-toluenesulfonate esters using the ethlylene diamine resin was studied at 4, 23, and 40 °C. As shown in Figure 4, the removal of the methyl ester with this resin is favored at elevated temperature, with virtually complete removal of methyl *p*-toluenesulfonate being observed after 90 min treatment at 40 °C.

In contrast to the results seen for the methyl *p*-toluenesulfonate ester, the ethyl and isopropyl esters were less effec-

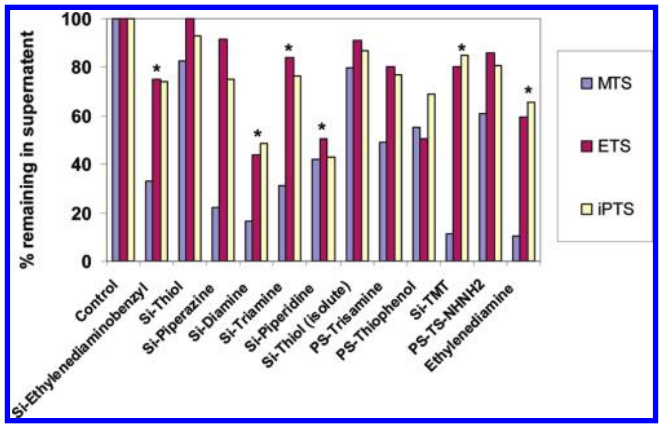


Figure 3. **Screening results for removal of p-TSA methyl (MTS), ethyl (ETS), and isopropyl (iPTS) esters by the different resins. One milliliter of 1 μg/mL pTSA ester solution in MeOH was added to 100 mg of each of the resins listed in Figure 1. The solutions were sonicated for 30 min, filtered through a 0.45 μm filter, and assayed by LC/MS. The resins showing removal of >50% for methyl ester and >15% for ethyl and isopropyl esters were selected (marked with an asterisk).**

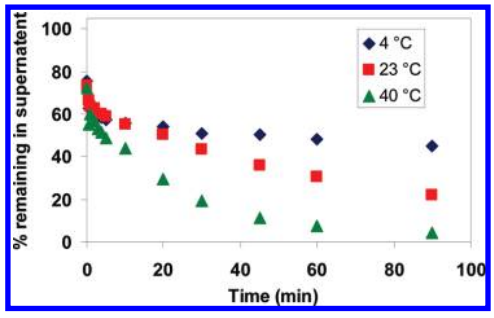


Figure 4. **Kinetic study of pTSA methyl ester at 4, 23, and 40 °C. Fifteen milliliters of 1 μg/mL pTSA methyl ester solution in MeOH were added to 1.5 g of ethylenediamine polymer-bound resin. The solution was stirred at 4, 23, and 40 °C. Aliquots of 1 mL were taken at 0, 0.5, 1, 2, 3, 4, 5, 10, 20, 30, 45, 60, and 90 min. The aliquots were filtered through a 0.45 μm filter, and assayed by LC/MS.**

tively removed. Treatment with ethylenediamine resin, for 90 min at 40 °C led to 96% removal for the methlyl ester, but only 47%, and 31% for the ethyl and isopropyl esters, respectively.

Similar kinetic behavior was observed for the benzenesulfonate esters. Treatment with ethylenediamine resin for 90 min at 40 °C led to 100% removal for the methyl ester, but only 33%, and 31% for the ethyl and isopropyl esters, respectively.

The removal of the methyl methanesulfonate ester was also favored at elevated temperatures, although, as previously mentioned, even prolonged treatment at elevated temperature was ineffective at reducing the levels of either ethyl or isopropyl mesylate. Treatment with ethylenediamine resin for 90 min at 40 °C led to 81% removal for the methyl ester.

Loading capacity is of critical importance for resin selection. A highly reactive and specific resin is of limited value as a tool to rework pharmaceutical batches at scale if it has poor capacity for impurity removal. The extent of impurity reduction observed after 1 h in 1 mL of 1 μg/mL pTSA ester solution was measured while varying the ethylenediamine resin amount from 25−500 mg (Figure 5). As illustrated in the graph, as little

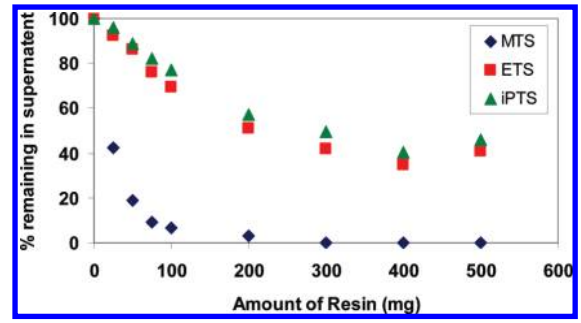


Figure 5. **Loading capacity of ethylenediamine resin. One milliliter of 1 μg/mL pTSA methyl (MTS), ethyl (ETS) and isopropyl (iPTS) ester solution in MeOH was added to 25, 50, 75, 100, 200, 300, 400, and 500 mg of ethylenediamine resin. The solutions, stirred for 1 h at 40 °C, were filtered through a 0.45 μm filter, and assayed by LC/MS.**

as 100 mg of resin is sufficient for near complete removal of the methyl ester under these conditions. More resin is required for removal of the ethyl or isopropyl esters; however, none of the conditions were completely effective in eliminating these PGIs.

The next step was to examine the efficacy of removal of PGI using nucleophilic resins in the presence of an API. Clearly, API compounds that could be reactive with the nucleophilic resin, or could be degraded, racemized, or epimerized by resin treatment could not be cleaned up using this protocol. We, therefore, selected two test APIs meeting these criteria to investigate whether PGI removal using nucleophilic resin treatment would be as effective in the presence of the API. Initially, two different APIs (free form) were spiked with 100 ppm esters (MSA, BSA, and pTSA esters spiked separately) at a resin load of 20:1 (mass of resin:mass of API). The % reduction values observed were comparable to those obtained when no API was present. This result confirms that there is no interference from the excess presence of API on the ability of the nucleophilic resin to remove the PGI. As with the studies on isolated sulfonate esters, the degrees of removal of all sulfonic acid methyl esters, ethyl esters (except ethyl methanesulfonate), and isopropyl esters (except isopropyl methanesulfonate) were approximately 100%, 30%, and 20%, respectively. High API recovery yields were achieved (>90%) for both API treatments. In addition, API impurity profiles of pre- and postresin treatment samples were identical, confirming that no new impurities are being introduced through resin treatment (Figure 6).

It is important to note that this protocol should not be used with APIs containing potentially reactive groups. We did note in our studies that an API intermediate containing an ester functional group afforded poor recovery following treatment with nucleophilic amino resin, presumably owing to loss *via* amide bond formation. Further studies showed that this protocol can be used with APIs that exist as free acids or free bases.

In terms of solvent interference, it is worth mentioning that effective removal of MSA (only methyl ester), BSA, and pTSA esters could be achieved in systems predominantly using any of the following solvents: isopropyl acetate, tetrahydrofuran, toluene, dimethyl formamide, and dimethyl acetamide (Figure 7).

On the basis of these preliminary studies we have found a useful resin screening procedure to consist of treating 1 mL of

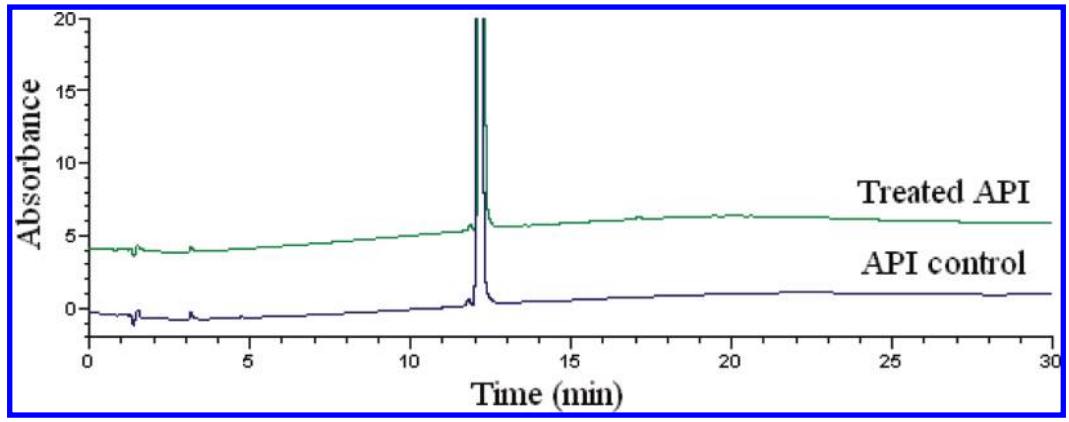


Figure 6. **Ethylenediamine resin treatment removes PGI without introducing new impurities as evidenced by impurity profile assay. Conditions: Xterra RP8 column (4.6 mm × 150 mm, 3.5 μm). The A solvent was 0.1% v/v H_3PO_4 in water, and the B solvent was MeCN. The gradient was 35−85% B in 25 min, then held at 85% B for 5 min. The column temperature was set to 25 °C, and the flow rate was 1.2 mL/min. The injection volume was set at 10 μL, and 220 nm was used as a detection wavelength.**

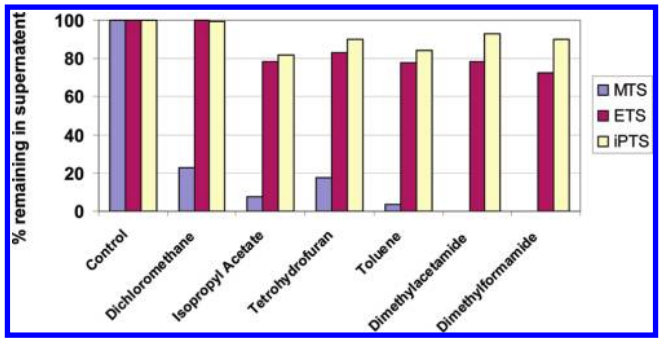


Figure 7. **Removal of p-TSA methyl (MTS), ethyl (ETS), and isopropyl (iPTS) esters from API by treatment with ethylenediamine resin (2:1 resin:API) in different solvents. The solutions, stirred for 1 h at 40 °C, were filtered through a 0.45 μm filter, and assayed by LC/MS.**

100 mg/mL API solution in acetonitrile with 200 mg of resin, with stirring for 1 h at 40 °C. When compared to assays of an untreated control, assays of the supernatant or filtrate using LC/MS for BSA and pTSA esters or GC/MS for MSA methyl ester can be useful in identifying optimal conditions for PGI removal. Our studies have shown the preferred resins for removal of BSA esters to be silica-supported ethylenediaminobenzyl, piperazine, and trimercaptotriazine (TMT) resins, as well as polymer-bound ethylenediamine. For pTSA esters, prefererred resins are silica-supported ethylenediaminobenzyl, diamine, triamine, piperidine, TMT, and polymer-bound ethylenediamine. For methyl methanesulfonate, preferred resins are silica-supported diamine, triamine, TMT, and polymer-bound Tris(2-aminoethyl)amine, trisamine and ethylenediamine.

Finally, it is worth mentioning here that this procedure could be potentially used to purify pharmaceutical compounds contaminated with alkyl and aryl halides (class 3 PGIs). Preliminary studies showed that treatment with Si-TMT resin was very effective and led to 80% removal of benzyl chloride (model aryl halide) from a contaminated API.

Experimental Section

Chemicals and Reagents. HPLC grade acetonitrile (ACN), HPLC grade methanol (MeOH), ammonium acetate, phosphoric acid, methyl methanesulfonate (MMS), ethyl methanesulfonate (EMS), methyl benzenesulfonate (MBS), ethyl benzenesulfonate (EBS), methyl *p*-toluenesulfonate (MTS), ethyl *p*-toluenesulfonate (ETS), ethylenediamine polymer-bound, and tris(2-aminoethyl)amine polymer-bound were purchased from Sigma Aldrich Corp. (St. Louis, MO, U.S.A.). Isopropoyl *p*-toluenesulfonate (iPTS) was purchased from City Chemical LLC (West Haven, CT, U.S.A.). Isopropyl benzenesulfonate (iPBS) and isopropyl methanesulfonate (iPMS) was purchased from Fisher Scientific (Pittsburgh, PA, U.S.A.). SiliaBond Piperidine, SiliaBond Diamine, SiliaBond Triamine, SiliaBond Piperazine, SiliaBond Thiol, SiliaBond Ethylenediaminobenzyl resins were purchased from Silicycle Inc. (Quebec, Quebec, CA). Isolute Si-Thiol, SI-TMT, PS-Thiophenol, PS-Trisamine, PS-$TsNHNH_2$ resins were purchased from Biotage (Charlottesville, VA, U.S.A.). APIs (free bases, free acids, and salt forms) were obtained from the department of Process Research, Merck & Co. Inc. (Rahway, NJ, U.S.A.).

LC/MS. An Agilent 1100 series HPLC/MSD system (Palo Alto, CA, U.S.A.) with an ESI source was used for LC/MS analysis. The separations were performed on a Zorbax Eclipse column (150 mm × 4.6 mm, 5 μm) under isocratic conditions using 90:10 MeOH/ 0.1% w/v ammonium acetate in water. The run time was 5 min, and the column temperature was set to 45 °C. The flow rate was 1.0 mL/min with a 1/20 splitter or 0.5−0.6 mL/min w/o splitter, and the injection volume was set at 5 μL. ESI in positive ion mode was used for the MS detections with SIM at 190 *m*/*z* for methyl benzenesulfonate, 204 *m*/*z* for ethyl benzenesulfonate, 218 *m*/*z* for isopropyl benzenesulfonate, 204 *m*/*z* for methyl *p*-toluenesulfonate, 218 *m*/*z* for ethyl *p*-toluenesulfonate, and 232 *m*/*z* for isopropyl *p*-toluenesulfonate. These *m*/*z* values correspond to $[M + NH_4^+]$.[8] The capillary voltage was tuned to 3 kV, and the drying temperature was set to 350 °C. The nebulizing pressure was set to 35 psi, the drying gas flow rate was 12 L/min, and the fragmenter voltage was 70 mV. Agilent Chemstation (Rev.B.01.03) software was used for instrumental control, data acquisition, and data analysis.

GC/MS. A GC/MS[11] method was developed on an Agilent 6890N GC system coupled with 5973N mass selective detector (Palo Alto, CA, U.S.A.) equipped with an Rxi-5SIL column (20.0 m × 0.18 mm, 0.18 μm film thickness) from Restek (Bellefonte, PA, U.S.A.). After a 1-min hold at 50 °C, the oven

temperature was ramped to 150 at 20 °C/min, then to 250 at 50 °C/min. The injection volume used was 1.0 μL with a 10:1 split, and the inlet temperature was 250 °C. The helium carrier gas pressure was set at 10.19 psi under constant pressure mode. The GC effluent was ionized using electron impact ionization (EI) and mass detection was done by SIM at 80.0, 109.0, 123.0 *m/z* for methyl, ethyl, and isopropyl methanesulfonate, respectively.

Matrix Interference Experiments. Quantities of 1 mL of 10−100 mg/mL API solutions in acetonitrile or methanol were spiked with 100 ppm MSA, BSA, or pTSA esters and added to 200 mg of ethylenediamine resin. The solutions, stirred for 1 h at 40 °C, were filtered through a 0.45 μm filter and assayed by LC/MS (BSA and pTSA esters) or GC/MS (MSA esters). For API recovery yield assays, LC/UV was used after the API solutions were diluted 100−1000×, depending on the initial concentration.

Conclusions

A simple approach to identify reactive resin treatment conditions capable of removing electrophilic sulfonate ester PGIs from contaminated APIs is presented. Removal of methyl sulfonate esters was seen to be quite rapid and effective using a variety of treatment conditions; however, removal of ethyl or isopropyl esters was seen to be slower and less complete. A group of the most effective nucleophilic resins is recommended. An enhanced rate of removal of some PGIs was favored at elevated temperatures. Broad solvent tolerance was observed, with efficient removal of methyl sulfonate PGIs using reactive resins being observed in ACN, IPAc, THF, toluene, DMF and DMAC. Some preliminary data showed the potential of using this approach to remove other electrophilic PGI such as alkyl and aryl halides.

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