Removal of Acrolein from Active Pharmaceutical Ingredients Using Aldehyde Scavengers

Rustem Kecili,^{†,‡} David Nivhede,[†] Johan Billing,[†] Mats Leeman,[†] Börje Sellergren,[‡] and Ecevit Yilmaz^{*,†}

[†]MIP Technologies AB, a Subsidiary of Biotage AB, Box 737, 22007 Lund, Sweden [‡]INFU, Universität Dortmund, Otto Hahn Strasse 6, 44221 Dortmund, Germany

ABSTRACT: A variety of chemical compounds, intermediates, and reagents are used during the process of synthesizing active pharmaceutical ingredients (APIs). Some of these chemicals, intermediates, and reagents, as well as byproducts of synthetic processes, can have toxic properties and be present as impurities at low levels in the API or final drug formulation. If present at high concentrations, the toxic impurities could cause adverse health effects in humans. This paper describes a simple and rapid approach for selective removal of acrolein from APIs using iodixanol as a model API. Several scavengers were tested, and the resins which showed highest binding efficiency and selectivity were chosen for further evaluations. The kinetics of acrolein scavenging in the presence of the API iodixanol and the scavenging capacity of resins were demonstrated in this paper. The most complete scavenging is obtained with PS-NH₂ which removes 97.8% of acrolein without any substantial removal of the API during 20 min of reaction time.

INTRODUCTION

Pharmaceutical genotoxic impurities (GTIs) may induce genetic mutations, chromosomal breaks, or chromosomal rearrangements, and have the potential to cause cancer in human.¹⁻³ Therefore, exposure to even low levels of such impurities present in the final active pharmaceutical ingredient (API) may be of significant toxicological concern.^{4–6} The analysis of these impurities in APIs has received increased attention,⁷⁻¹¹ and guidelines were recently issued.¹² During production of active pharmaceutical ingredients (APIs), reactive intermediates, catalyst, acids, or bases are often used. These compounds or their derivatives can potentially end up at trace levels in final pharmaceutical substances. The Viracept (nelfinavir mesylate) contamination incident is an example of a case that demonstrates potential dangers of genotoxic impurities in a pharmaceutical compound. In June 2007, excess levels of genotoxic sulfonate ester (ethyl mesylate) were detected in the API of Viracept produced by Roche, and this led to the global recall of Viracept from all European Union (EU) markets.¹³

It is important for process chemists to explore possible opportunities to avoid the use and generation of these genotoxic materials in the manufacturing process. Since a very large number of solutes are used or tested in drug synthesis and development, no list of target solutes is available, but rather a list of "structural alert functionalities" is used.^{8,14} This list includes sulfonates (e.g., ethyl methane sulfonate), alkylhalides, arylamines, epoxides, etc.

The presence of genotoxic impurities in pharmaceutical formulations is a serious issue for the pharmaceutical industry. Therefore, the control of these genotoxic impurities (GTI) during the development of pharmaceutical compounds is a growing concern in the pharmaceutical industry. The European Medicines Agency (EMEA) issued a guideline for GTI limits in June 2006. According to the guideline, a threshold of toxicological concern (TTC) value of 1.5 μ g/day intake of

GTI is considered to be associated with an acceptable risk in the absence of other data. $^{12}\,$

One of the potentially genotoxic impurities based on the structural alerts is acrolein. Acrolein is an α,β -unsaturated aldehyde that is used as a building block in the production of pharmaceuticals¹⁵ and the highly reactive C=C double bond and C=O carbonyl group moieties in the conjugated C=C-C=O system are responsible for its electrophilicity. The available toxicology studies for acrolein have recently been summarized in a report by the U.S. Department of Health and Human Services.¹⁶ Acrolein irritates the gastrointestinal organs, but despite the fact that it readily reacts with biological nucleophiles, no clear carcinogenic effects related to oral intake have been found, and a reasonable target limit in pharmaceuticals may therefore be the ICHQ3A (R2) qualification threshold of toxicological concern of 0.05% for APIs with a maximum daily dose (MDD) of 2 g/day.¹⁷

Many APIs under development today are produced by multistep processes requiring purification techniques such as crystallization, chromatography, and other downstream processing approaches. Such processes include several steps which often result in loss of API and therefore increase the total cost of the final product. The use of adsorbents and reactive scavengers for the removal of undesired impurities from pharmaceutical compounds is a well-known approach;^{18–23} another approach using membranes for removal of GTIs from active pharmaceutical ingredients was recently reported.²⁴

This paper presents the acrolein scavenging performance of polystyrene and silica based aldehyde scavengers in organic media in the presence of the API iodixanol. The kinetics of genotoxic impurity removal and binding capacity of scavengers is also demonstrated. It should be noted that iodixanol is used

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as a model compound and we are not aware of any actual contamination issues involving this API.

Figure 1 shows the structures of acrolein (GTI) and iodixanol (API).



Figure 1. Structure of acrolein (GTI) and iodixanol (API).

RESULTS AND DISCUSSION

Analysis of Acrolein. The analysis of low-molecular weight aldehydes, which are highly volatile and polar substances, is difficult to accomplish by direct RP-HPLC methods. This is first due to the fact that many such carbonyl compounds have no chromophores and, thus, are not detectable by UV methods. Due to the reactive carbonyl group present in those lowmolecular weight aldehydes and ketones, those can easily be derivatized with, for example, 2,4-dinitrophenylhydrazine (DNPH) in order to impart a chromophore. In this particular case, DNPH reacts in an acidic environment with the carbonyl group of acrolein to form stable hydrazones (DNPH-carbonyls) which can be separated by RP-HPLC on a C18 column and are easily detected by UV. In Scheme 1, the derivatization reaction of DNPH with an aldehyde or ketone is shown.



A recent publication examined the potential use of such resins to remove genotoxic impurities from APIs²² where the successful removal of methyl sulfonate esters was reported. However, related ethyl and isopropyl esters were only partially removed. Nevertheless, the authors concluded that the use of such resins showed some potential and suggested that this could be extended to the other classes of genotoxic compounds.

The various scavenger resins that were evaluated in this study were based either on cross-linked polystyrene-divinylbenzene or on porous silica.

The majority of the polymeric scavenger resins are only slightly cross-linked; i.e. the content of divinylbenzene is around 1–2%. Only one candidate of the scavenger resins has a higher degree of cross-linking. More specifically, the following scavenger resins have a low degree of cross-linking: PS-amine with aminomethyl groups as the scavenging moiety; PStrisamine modified with tris-(2-aminoethyl)amine groups as multifunctional amine groups; PS-tosylhydrazine modified with sulphonyl hydrazine groups on its surface as a reactive scavenging functionality; MP-trisamine is a scavenger resin that has a high degree of cross-linking and has been functionalized with difunctional amino groups.

The following scavenging materials are based on silica: Sitosyl hydrazine is a silica-supported equivalent of ptoluenesulfonyl hydrazine; Si-trisamine is a silica-bound tris(2-aminoethyl)amine, and Si-triamine is a silica-bound tri(2-aminoethyl)amine.²⁵

In Figure 2, the schematic structures of the scavengers used in this work are shown.



Figure 2. Structures of scavengers used in this study.

We wanted to evaluate the impact of batch mode and flowthrough mode on the scavenging of acrolein in the presence of iodixanol. This would fundamentally determine the way in which the scavenger could potentially be used in a real process.

To begin with, the outcome of flow-through scavenging experiments of acrolein in the presence of iodixanol using modified polystyrene- and silica-based scavengers is summarized in Figure 3. Trisamine-modified polystyrene showed 74% acrolein removal with only very little loss of iodixanol. However, trisamine-modified silica and triamine-modified silica showed 71.4% and 66.8% acrolein removal, respectively, with extensive removal of iodixanol. This nonspecific binding can be expected to be adsorption to acidic silanol groups on the surface of silica.

In the flow-through mode, the short interaction time between analyte and resin reduces the effectiveness of scavenging. For the next step, a batch-scavenging mode recommended by the supplier of the scavengers was carried

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Figure 3. Results of flow-through scavenging of acrolein in the presence of iodixanol. A quantity of 75 mg of each scavenger was packed in 1 mL SPE cartridges, and after loading of 1 mL of a solution of 5 μ g/mL acrolein and 2 mg/mL iodixanol in EtOH, 100 μ L of each of the collected aliquots of the sample was derivatized by DNPH and assayed by HPLC.

out to increase the interaction time and efficiency of scavenging acrolein.

Batch-scavenging experiments of acrolein in the presence of iodixanol using modified polystyrene- and silica-based scavengers are shown in Figure 4. Most of the scavengers showed



Figure 4. Results of batch scavenging of acrolein in the presence of iodixanol. A quantity of 75 mg of each scavenger was placed in a HPLC vial, and after the addition of 1 mL of a solution of 5 μ g/mL acrolein and 2 mg/mL iodixanol in EtOH, each mixture was shaken for 30 min, and an aliquot of 100 μ L was derivatized by DNPH and assayed by HPLC.

extensive removal of acrolein. Amine-, multiple amine-, and hydrazine-modified polystyrene scavengers led to higher than 90% acrolein removal with only very little loss of iodixanol, whereas silica-based scavengers, on the other hand, resulted in extensive losses of iodixanol. The less cross-linked polymerbound scavengers display a far better selectivity than the silicabased scavengers and also the macro-porous highly cross-linked scavengers. Therefore, only the polymer-bound scavengers based on the less cross-linked polymers were used for further evaluation. Apparently, under the conditions tested, iodixanol exhibits a certain affinity for both the silanol surface and the macro-porous surface of the polymer. Other APIs may behave differently, and thus, other scavengers may display a more advantageous selectivity profile than the currently chosen scavengers.

Next, the kinetics of the removal of acrolein using $PS-NH_2$, $PS-TS-NHNH_2$ and PS-trisamine scavengers was studied in order to determine the reaction duration required for the effective scavenging step.

As shown in Figure 5, PS-trisamine binds acrolein very rapidly and removes over 80% within 2 min. After 20 min, no scavenger exhibited any significant changes for acrolein binding. The most effective scavenging is obtained with PS-NH₂ which removes up to 97.8% of acrolein and only 2.0% of iodixanol. If a process can allow scavenging times of 20 min or more, then all of the tested scavengers demonstrate a considerably high level



Figure 5. Results of kinetic study for acrolein scavenging. One milliliter of a solution of 5 μ g/mL acrolein and 2 mg/mL iodixanol in EtOH was added to 75 mg of each scavenger. The solution was stirred, and an aliquot of 100 μ L of each mixture was taken at 0, 0.5, 1, 2, 5, 10, and 20 min and derivatized by DNPH and assayed by HPLC.

of acrolein removal. However, if the process interaction time is very limited, then PS-trisamine is the scavenger that shows the fastest scavenging kinetics—already after 2 min \sim 90% of acrolein is removed.

Loading capacity is an important parameter for the resin selection and for production process economics, and we have therefore conducted loading capacity determinations of the polymer-based resins (shown in Figure 6).



Figure 6. Results of loading capacity of scavengers. One milliliter of a solution of 5 μ g/mL acrolein and 2 mg/mL iodixanol in EtOH was added to 25, 50, 75, 100, and 200 mg of scavengers. The solutions stirred for 30 min, and aliquots of 100 μ L were derivatized by DNPH and assayed by HPLC.

As can be seen in Figure 6, at least 75 mg of scavenger is needed for removal of a large amount of acrolein in the solution. Increasing the amount of scavengers gives no further improvement in acrolein removal. On the basis of the assumption that 75 mg of scavenger affords close to complete removal of acrolein, the capacity of the scavengers can be estimated to be 64.8 μ g/g for PS-NH₂, 60 μ g/g for PS-TS-NHNH₂, and 56 μ g/g for PS-trisamine under these conditions. Tosylhydrazine-modified reactive resins have been used for removal of an aldehyde impurity in a previously reported study,²³ but it is important to consider that leaching of hydrazine and hydrazine derivatives would be a concern with this type of resin. The present work shows that PS-NH₂ and PS-trisamine resins are equally well suited for removal of acrolein without this potential problem.

If we take the model system above with 5 μ g/mL acrolein, then we can transfer the capacity to larger volumes. A 1 L API solution with 5 mg/L acrolein (5 ppm) would require 75 g of amine modified polystyrene scavenger to reduce the concentration from 5 ppm down to 0.15 ppm. The initial concentrations correspond to an impure API with 0.25% of acrolein; to reduce this level to the ICH - Q3A limit of 0.05% (80% reduction), ~50 g of scavenger would be sufficient.

EXPERIMENTAL SECTION

Materials. Acrolein, 2,4-dinitrophenylhydrazine (DNPH), formic acid, iodixanol, and also HPLC-grade solvents were purchased from Sigma-Aldrich (Steinheim, Germany). Scavengers were from Biotage GB Limited (Cardiff, UK).

All distilled water used was purified using an ultrapure water system from Elga (High Wycombe, UK)

Preparation of Stock Solutions. Stock solutions (1 mg/ mL) of acrolein (genotoxic impurity) and iodixanol (API) were prepared in EtOH and stored in glass flasks in the freezer.

Derivatization of Acrolein with DNPH. To perform derivatization of acrolein with DNPH, a procedure adapted from literature²⁶ was followed. Briefly, DNPH (50 mg) was dissolved in 20 mL of acetonitrile and acidified with 0.4 mL of formic acid. The DNPH solution (12 mM) was stable for 1 week when stored at 4 °C. Derivatization of acrolein was performed by mixing 100 μ L of the sample with 100 μ L of the derivatizing agent and incubating at room temperature for 1 h.

HPLC Analysis. HPLC experiments were carried out on a Shimadzu LC10 AD equipped with a PDA detector (SPD-M10A) and an autosampler (SIL-HT_A). The column was Supelco Ascentis Express C18 ($2.7 \mu m$, 50 mm × 4.6 mm).

Gradient elution was performed with ultrapure water (mobile phase A) and acetonitrile (mobile phase B).

The gradient was started with water/acetonitrile 60:40 (v/v), then a linear gradient elution up to 55% acetonitrile within 2 min that was raised to 100% acetonitrile in another 2 min. Then the gradient was decreased to 40% acetonitrile in 1 min. The final composition was maintained for 3 min before reequilibrating the column with the initial mobile phase (water/acetonitrile 60:40 (v/v)). Flow rate was 0.6 mL/min, detection wavelengths were 370 nm (for acrolein) and 254 nm (for iodixanol), and the injection volume was 10 μ L.

Flow-through Scavenging Procedure for Acrolein. Flow-through scavenging experiments of acrolein in the presence of iodixanol were carried out using a scavenger resin packed in solid-phase extraction (SPE) cartridges. The resins were manually packed as 75 mg amounts in 1 mL SPE cartridges. The flow-through scavenging procedure for acrolein and iodixanol is described below.

Flow-through Scavenging Procedure.

Conditioning

1 mL of MeOH - let equilibrate for 2 min

1 mL of EtOH - let equilibrate for 2 min

Loading

1 mL of 5 μ g/mL acrolein and 2 mg/mL iodixanol in EtOH - let equilibrate for 5 min

Batch-Scavenging Procedure for Acrolein. Each scavenger (75 mg) was placed in HPLC vials; after the addition of 1 mL of a solution of 5 μ g/mL acrolein and 2 mg/mL iodixanol in EtOH, the mixtures were shaken for 30 min, derivatized by DNPH, and assayed by HPLC-UV.

Capacity of Scavengers. Scavengers were put into HPLC vials with varying amounts of resins (10, 25, 50, 75, 100, and 200 mg) and 1 mL of a solution of 5 μ g/mL acrolein and 2 mg/mL iodixanol in EtOH was added to each vial, stirred for 30 min, derivatized by DNPH, and then assayed by HPLC-UV.

CONCLUSIONS

In the present study, we have evaluated a collection of commercially available scavenging resins for the removal of the genotoxic compound, acrolein. We have found that, for our model API process solution system, scavenging of acrolein was seen to be quite fast and effective using both polymer- and silica-based scavengers. However, less cross-linked polymerbased scavengers display a more advantageous selectivity profile than silica-based scavengers and scavengers with highly crosslinked polymers; as we have observed, those scavengers displayed an undesired high level of nonspecific binding to the API.

In essence, we have described an easy and simple procedure for effective removal of acrolein from contaminated API solutions using iodixanol as a model compound. The most effective and selective scavenging is obtained with PS-amine which removes up to 97.8% of acrolein and only 2.0% of iodixanol within 20 min using a batch-mode extraction procedure. However, a comprehensive genotoxicological risk assessment on aldehyde impurities in pharmaceutical formulations should be carried out before using this or any other type of cleanup, as impurities with the same structural alerts may have similar modes of action and need to be considered jointly.

AUTHOR INFORMATION

Corresponding Author

*E-mail: ecevit.yilmaz@biotage.com. Telephone: +46 (0) 46 102 611. Fax: +46 (0) 46 163901.

Notes

The authors declare no competing financial interest.

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