Journal of Chromatography A, 1339 (2014) 65–72

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

# Fast identification of selective resins for removal of genotoxic aminopyridine impurities via screening of molecularly imprinted polymer libraries

Rustem Kecili[a,*], Johan Billing[a], David Nivhede[a], Börje Sellergren[b], Anthony Rees[c], Ecevit Yilmaz[a]

[a] MIP Technologies AB, A subsidiary of Biotage AB, Box 737, Lund 22007, Sweden
[b] Malmö University, Faculty of Health and Society, Department of Biomedical Sciences, Malmö 20506, Sweden
[c] Biotage AB, Box 8, Uppsala 75103, Sweden

ARTICLE INFO

*Article history:*
Received 9 December 2013
Received in revised form 24 February 2014
Accepted 25 February 2014
Available online 4 March 2014

*Keywords:*
Active pharmaceutical ingredients
Aminopyridines
Genotoxic impurities
Molecularly imprinted polymers

ABSTRACT

This study describes the identification and evaluation of molecularly imprinted polymers (MIPs) for the selective removal of potentially genotoxic aminopyridine impurities from pharmaceuticals. Screening experiments were performed using existing MIP resin libraries to identify resins selective towards those impurities in the presence of model pharmaceutical compounds. A hit resin with a considerable imprinting effect was found in the screening and upon further investigation, the resin was found to show a broad selectivity towards five different aminopyridines in the presence of the two model active pharmaceutical ingredients (APIs) piroxicam and tenoxicam.

© 2014 Elsevier B.V. All rights reserved.

## 1. Introduction

Molecularly imprinted polymers (MIPs) are a type of selective cross-linked resins that can display high affinity and selectivity towards a single molecule or a family of related molecules. MIPs are usually prepared by polymerizing a functional monomer and a cross-linker in the presence of a template molecule that forms a complex with the functional monomer so that a polymer with cavities complementary to the template is formed. Conceptually, MIPs are easy to prepare, stable and capable of selective molecular recognition [1–4]. MIPs are sometimes called artificial antibodies and a commonly found statement in the scientific literature is that MIPs are highly selective towards their imprinted target compounds and can distinguish between molecules with only very small chemical differences. However, MIPs may also display selectivity towards other compounds that have related structures, a phenomenon known as cross-reactivity. As an extension of this phenomenon, MIPs sometimes may also recognize whole families of compounds that share a common feature [5,6], a property we term sub-site selectivity [7].

When MIPs are prepared using the target compound as template, leakage of trace amount of template molecule (so-called bleeding) from the MIP may occur at low levels under harsh conditions. This leakage is considered by some to be one of the main drawbacks of MIPs, especially in the quantification of trace amounts of compounds. In such applications, it is not desirable to use the target compound as the template and instead an elegant solution has been developed that utilizes the cross-reactivity of MIPs through using a similar compound as a 'dummy template' in the imprinting process instead of the target compound itself. In an early study following this route, Matsui, Fujiwara and Takeuchi succeeded in preparing MIPs with a selectivity towards triazine pesticides by using a related, non-pesticide compound, namely trialkylmelamine, as the template [8]. A related example where the bleeding problem was successfully circumvented by using a related drug derivative as the template was presented by Andersson, Paprica and Arvidsson [9].

The utilization of the inherent cross-reactivity of MIPs and their ability to recognize molecules that share common features with the template molecule has also been reported by many others. For example, Haupt, Mayes and Mosbach [10] have described a 2,4-dichlorophenoxyacetic acid imprinted polymer that displayed an extended cross-reactivity profile. Experiments showed that other compounds with the phenoxyacetic acid moiety, such as the fluorescent compound 7-carboxymethoxy-4-methylcoumarine, could

* Correspondence author. Tel.: +90 535 4674262; fax: +90 222 3204910.
*E-mail address:* rkecili@anadolu.edu.tr (R. Kecili).

http://dx.doi.org/10.1016/j.chroma.2014.02.073
0021-9673/© 2014 Elsevier B.V. All rights reserved.

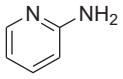
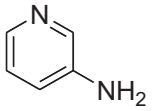
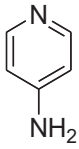
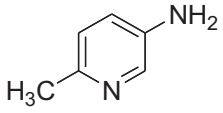
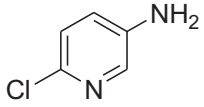
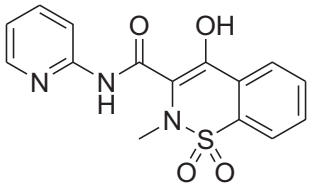
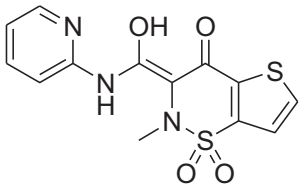
compete with the binding of the target. Interestingly, there are also examples reported where a somewhat unexpected cross-reactivity between a studied analyte and the template molecule was observed. When Martin, Wilson and Wilson [11] imprinted the beta antagonist propranolol, they found that this MIP also displayed selectivity and an imprinting effect towards tamoxifen, a compound used in the treatment of breast cancer. Further, Wulff and Schauhoff observed cross-reactivity in a covalently imprinted polymer. A chiral, D-galactose imprinted polymer that recognized D-galactose over L-galactose was found to also show an inverse chiral selectivity towards L-fructose over D-fructose [12]. The cross-reactivity of MIPs is also successfully utilized in a commercial MIP for trace analysis of chloramphenicol in food samples [13] where the template used contains a related chemical moiety but belongs to a different compound class.

Pharmaceutical genotoxic impurities (GTIs) are compounds that may be found as impurities in active pharmaceutical ingredients (APIs) as a result of the synthetic process used in the production of the API. GTIs have the potential to cause genetic mutations, chromosomal breaks and chromosomal rearrangements and may be carcinogenic in humans [14,15]. Thus, any level of human exposure to such GTIs due to their presence in an API may present a significant problem [16,17]. The removal of GTIs from APIs is therefore an important purification challenge for the pharmaceutical industry. Where possible, the undesired GTIs may be removed from pharmaceuticals using common unit operations such as crystallization, extraction, chromatography, precipitation or distillation. Some of these methods may result in loss of API and hence in an increase of cost of the final product. In addition to the traditional methods, API purification has been described using adsorbents [18,19], reactive scavengers [20–22], organic solvent nanofiltration [23], MIPs [24–26] and MIP-membrane composites [27].

Aminopyridines are widely used starting materials in the production of pharmaceutical compounds and may potentially be present as genotoxic impurities at trace levels in APIs [28,29]. For example, 2-aminopyridine is a precursor in the production of piroxicam and may also arise as a degradation impurity in the final product [30]. The present study involves five different aminopyridines and two model APIs piroxicam and tenoxicam. Piroxicam and tenoxicam are non-steroidal anti-inflammatory drugs commonly used for the treatment of diseases such as rheumatoid arthritis, osteoarthritis and musculoskeletal disorders [31,32]. The chemical structures of the aminopyridine GTIs and the model APIs are shown in Table 1.

At MIP Technologies (a subsidiary of Biotage AB), a large library of MIP resins is available in-house for screening and in this work the library was screened to rapidly identify a resin with high selectivity towards aminopyridines. All resin particles in the library are in the SPE size range with an average particle size of 50–65 μm. The resins are designed for retention of small and medium sized molecules, such as drugs, pesticides, short peptides and other similar molecules and hence have pore sizes of around 50 Å to 150 Å. All the polymers in the library are highly cross-linked (>40%), have low swelling and are pressure-stable in columns. The polymers have been treated with numerous washing steps to obtain the required low levels of leachables.

**Table 1**
Structure of aminopyridine GTIs and the model APIs.

| GTI/API | Structure |
|---|---|
| 2-Aminopyridine |  |
| 3-Aminopyridine |  |
| 4-Aminopyridine |  |
| 5-Amino-2-methylpyridine |  |
| 5-Amino-2-chloropyridine |  |
| Piroxicam |  |
| Tenoxicam |  |

## 2. Materials and methods

### 2.1. Materials

2-Aminopyridine, 3-aminopyridine, 4-aminopyridine, 5-amino-2-methylpyridine, 5-amino-2-chloropyridine, piroxicam, tenoxicam, Amberlite CG-50 and also HPLC-grade solvents were purchased from Sigma-Aldrich (Steinheim, Germany). Distilled water was purified using an ultra-pure water system from Elga (High Wycombe, UK).

### 2.2. LC–MS/MS analysis

The analysis of the aminopyridines and their corresponding APIs was carried out using a Shimadzu HPLC instrument consisting of two HPLC pumps (LC-20AD), a vacuum degasser (DGU-20$A_3$) and an autosampler (SIL-20AC) connected to an Applied Biosystems mass spectrometer (API3200) with an electro spray ionization interface. The analytical column was a Supelco Discovery HSF5 (5.0 μm, 150 × 4.0 mm). Gradient elution was performed with 10 mM ammonium formate buffer ($NH_4FA$) pH 6.5 (mobile phase A) and acetonitrile (MeCN) (mobile phase B). The gradient was a linear gradient from A:B 85:15 (v/v) to 50% B within 1 min, the mobile phase was then kept at that level for 3 min and then increased to 90% B within 1 min and kept at that level for 1 min. Then, the composition was decreased to 15% B within 0.1 min and maintained there for 1.5 min to re-equilibrate the column with the initial mobile phase. The total run time was 8.0 min. The flow rate was 0.5 mL/min and the injection volume was 10 μL.

The turbo ion-spray source was used in positive mode ($ESI^+$) for all analytes with the following settings: electrospray capillary voltage, 4500 V; ion source temperature, 400 °C; curtain gas ($N_2$), 15 psi; collision gas ($N_2$), 8 psi. Data was acquired through multiple

Download English Version:

https://daneshyari.com/en/article/7613229

Download Persian Version:

https://daneshyari.com/article/7613229

Daneshyari.com