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Hydrazine determination in allopurinol using derivatization and SPE for sample preparation

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ABSTRACT

Hydrazine is a useful building block in the synthesis of organic pharmaceuticals but it is highly toxic so its determination at low ppm range is required.

In this work, hydrazine was determined in allopurinol active pharmaceutical ingredient (API) sample at 2.5 ppm level by using derivatization and solid phase extraction (SPE) followed by reversed phase liquid chromatography (RPLC).

Hydrazine does not contain chromophore part and is not retained in RPLC thus derivatization was necessary for its determination. Benzaldehyde was found to be the most appropriate derivatization reagent so the analyzed solute was benzaldehyde azine, which had adequate UV absorption and could be retained in RPLC. The derivatization reaction was performed in 0.2 M NaOH solution/MeOH = 50/50 (v/v) mixture, which is a proper solvent for allopurinol, too.

Because of the low detection limit, 50 mg sample had to be dissolved in 5 mL solvent. This is a very concentrated solution therefore column overload is expected. Using a C18 SPE for sample preparation allowed to get rid of the huge amount of allopurinol. As allopurinol has a more polar character than benzaldehyde azine, it was easy to wash out from the SPE phase. The benzaldehyde azine can be eluted with a strong solvent and then the eluted sample can be analyzed by RPLC. Limit test validation of the liquid chromatographic method has been performed as well.

This complex but not complicated analysis can be used for the accurate determination of hydrazine in allopurinol API. Furthermore it is applicable for other APIs which are more polar than benzaldehyde azine and soluble in high concentration in the aqueous solvent.

1. Introduction

During the synthesis of pharmaceuticals it occurs that genotoxic ingredients have to be used. In this case the amount of this compound must be limited at ppm level in the final product. This required concentration differs by 5–6 orders of magnitude from the amount of the API.

In order to determine an impurity at ppm level, a huge amount of sample is needed. This high concentration can decrease the efficacy of the analytical method; moreover the overload of the chromatographic system may occur. Therefore during the sample preparation the amount of the API needs to be decreased while the quantity should remain the same for the component to be determined.

Even if huge amount of a component is analyzed but the component has no significant UV absorption or cannot be separated properly from other impurities then appropriate analysis is not feasible. In such cases, derivatization reaction can solve the problem as it may change the solute properties which are then more favourable for its analysis.

The aim of this work was to determine hydrazine content in allopurinol API. Allopurinol is used for the treatment of patients with high uric acid level in blood (hyperuricaemia) since the drug inhibits an enzyme that participates in the formation of uric acid. The hyperuricaemia can result arthritis and in worse case kidney failure. Beside high serum uric acid level increases the risk of high blood pressure and heart failure [1]. Allopurinol drugs are on the market in 100 and 300 mg potency. During application for adults the recommended starting dose is 100 mg once a day. The daily dose can be raised as required (the blood uric acid level is checked in every 1–3 weeks) by 100 mg. The doctor defines the treatment dose based on the seriousness of the illness. In the case of mild hyperuricaemia 100–200 mg, in moderate state 300–600 mg and

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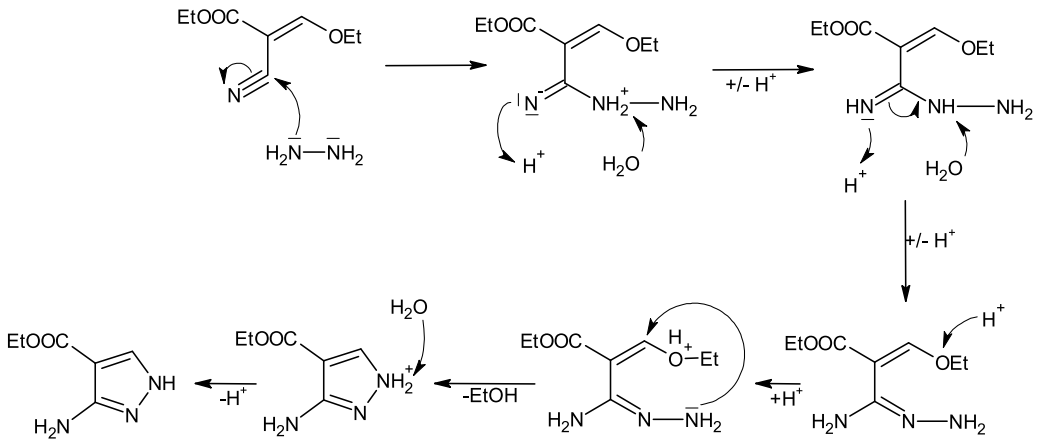

Fig. 1. One of the synthesis routes of allopurinol.

in serious state 700–900 mg is the suggested daily dose. According to the body weight the daily dose of the drug is 2–10 mg per body weight kilograms.

Fig. 1 shows one of the many synthesis routes for the production of allopurinol. The common of the routes is that every of them contain hydrazine as an important building block. Since hydrazine is genotoxic, it is a priority subject to determine its content during the quality control analysis of allopurinol.

Hydrazine is a corrosive and carcinogen substance. According to the European Medicine Agency (EMA) and International Conference on Harmonization (ICH) the maximum daily intake of potential genotoxic impurities for more than 12 months of exposure is 1.5 μg [3]. Considering the daily dose mentioned before, this means that the permitted hydrazine concentration in allopurinol is 2.5 ppm.

The European Pharmacopoeia (Ph.Eur.) contains a method for the determination of hydrazine content in allopurinol [2] but it requires lengthy sample preparation and the nowadays unpopular normal phase liquid chromatography.

The hydrazine molecule does not contain chromophore group so the commonly used UV–vis detector or the highly sensitive fluorescence detector cannot be applied for its direct detection. According to literature, titration [4], electrochemical [5] and spectrophotometric methods [6–8] are recommended for the determination of hydrazine. Furthermore liquid chromatographic method along with derivatization can be found where the samples were taken from biological medium [9] and environment [10].

The permitted concentration mentioned above is very low and accordingly the limit of detection must be fifth or tenth grade lower. According to spectrophotometric and liquid chromatographic literature, this concentration value can be achieved only by derivatization before injecting onto the chromatographic column. Seifart et al. [9] determined hydrazine at low concentration that meets the requirements but in our case the huge amount of matrix resulted in another problem. Several articles were published recently in which the amount of hydrazine was determined in APIs [11–13]. But in these cases the reduction of the amount of API wasn't necessary during the sample preparation. Despite the fact that there is a big difference between the polarity of allopurinol and hydrazine still a huge amount of sample should be injected to the column to attain the low limit of detection. This could cause overloading and formation of new interactions which could destroy the selectivity of the column.

2. Experimental

The quality of the methanol (MeOH) was gradient grade. The quality of benzaldehyde used for the derivatization was reagent grade; the hydrazine sulfate and sodium hydroxide (NaOH) were analytical grade (Merck, Darmstadt, Germany). The allopurinol originates from the synthesis of Egis Pharmaceuticals Plc. Water was prepared freshly using ELGA Purelab system (ELGA, Lane End, UK).

Mettler Toledo analytical and precision balances were used for weighing (Greifensee, Switzerland). Eppendorf automatic pipettes were used for liquid handling (Hamburg, Germany).

For SPE 30 mg/1 mL StrataX and Strata-C18E phases were applied (Phenomenex, Torrance, USA).

Agilent 1260 HPLC system with UV detector was used for the liquid chromatographic measurements (Santa Clara, USA).

MarvinSketch software was applied for the determination of LogD-pH curves (v.6.0.2. ChemAxon, Budapest, Hungary).

The chromatograms were processed with Empower^3 software (Waters, Milford, USA).

3. Results and discussion

3.1. Derivatization reaction

The first and determining step was the implementation of the derivatization reaction, which was based on the method of the Ph.Eur. mentioned before [2].

The reaction according to Fig. 2. took place in 0.2 M NaOH/MeOH = 50/50 (v/v) mixture (later referred as solvent).

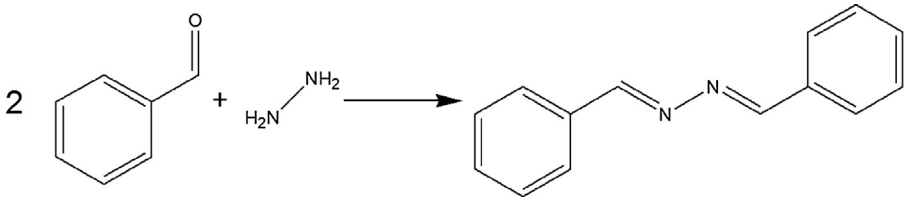

Fig. 2. The derivatization reaction of hydrazine with benzaldehyde.