

HPLC

for Pharmaceutical Scientists

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HPLC FOR PHARMACEUTICAL SCIENTISTS

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PREFACE
CONTENTS

PART I

1 Introduction

Yuri

1.1

1.2

1.3

1.4

1.5

1.6

1.7

variety of methods could be used such as GC and HPLC. To ensure the quality of the key raw materials, the level and type of impurities present in these materials need to be determined and appropriate specifications need to be set. These can be determined by running a small-scale synthesis (i.e., front run) with the key raw material; and if an intermediate with an acceptable purity is obtained in the next step of the synthesis, the level and type of impurities present in the key raw materials can be considered acceptable. Note that the impurities may carry forward in the downstream chemistry and/or may react to form new synthetic by-products. If these synthetic by-products are carried forward to the final API, these impurities must be qualified in the appropriate toxicological studies if they are above a certain level (also, any potential genotoxic impurities must be identified and well-controlled with sensitive analytical methods). Different lots from the same manufacturer and/or lots of key raw materials from different manufacturers are usually tested.

In the following example, the importance of determining the quality of the key raw material is highlighted. Trimethoprim (TMP) (2-4-diamino-5-(3,4,5-trimethoxybenzyl)pyrimidine) is an antibacterial, folic acid antagonist that is usually used with sulfamethoxazole as a treatment for urinary tract infections. Shown in Figure 8-1 is one synthetic scheme of TMP [1]. The starting material that is used is 3,4,5-trimethoxy benzaldehyde (a key raw material). Depending on the quality of this key raw material, the impurities from this starting material may further react in the subsequent synthetic steps (downstream chemistry) at the benzaldehyde functionality to produce undesired synthetic by-products. TMP manufactured by five different manufacturers from three countries of origin were analyzed by the same HPLC chromatographic method; two impurities, Impurity 1 and Impurity 2, were found in multiple lots of TMP, and the levels depended upon the country of origin (Figure 8-2). [2].

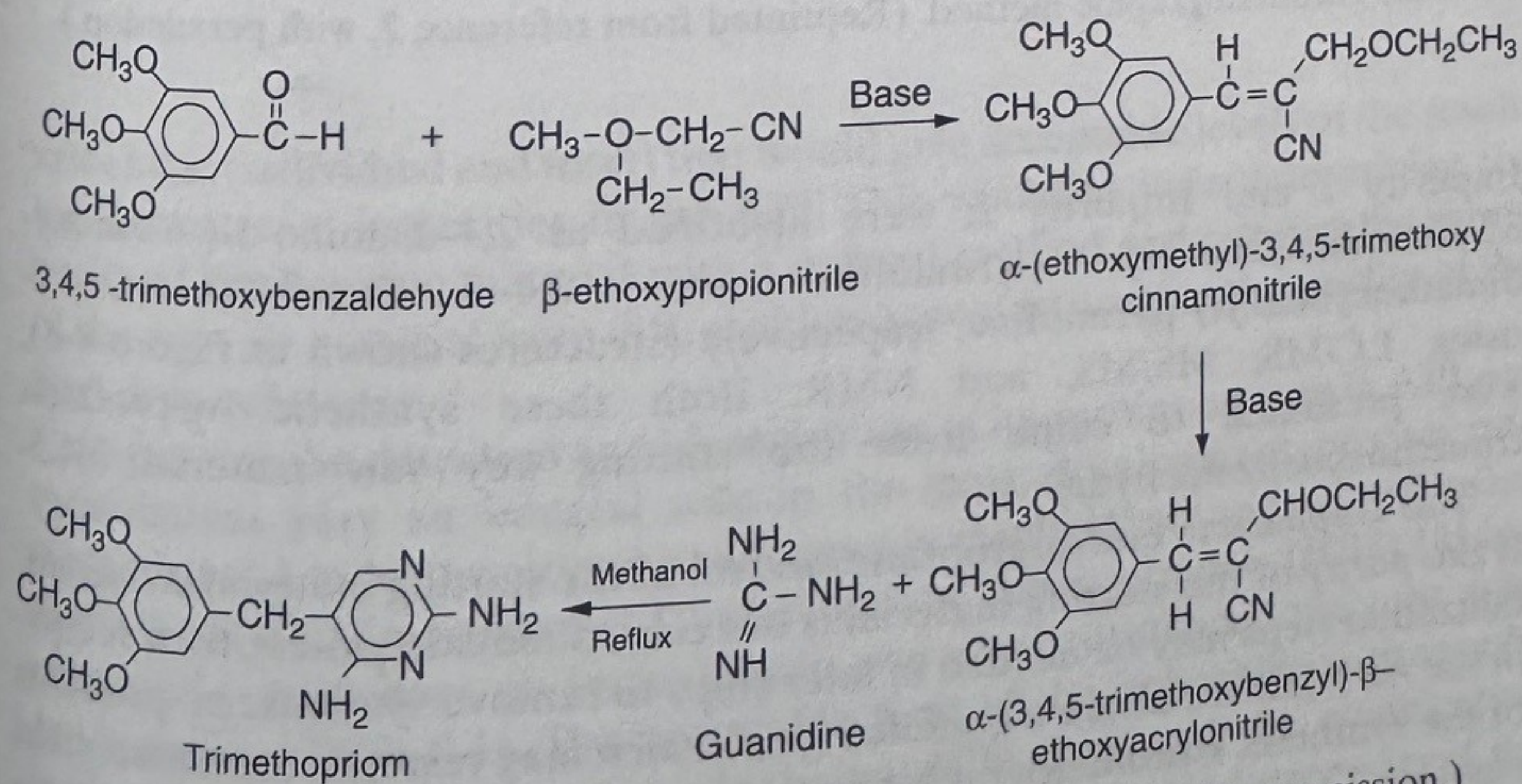


Figure 8-1. Synthesis of TMP. (Reprinted from reference 1, with permission.)

the synthesis of an HIV drug candidate [39]. A second application was for catalyst screening [39].

14.7 IMPURITY IDENTIFICATION

A critical aspect of drug development is the control and identification of impurities in the active pharmaceutical ingredient. Regulatory requirements dictate the control of impurities and the identification, where possible, of impurities that exceed 0.1% [40–42]. The identification of these impurities provides information toward their potential toxicity. Impurities in the drug substance can originate from incomplete reactions, over-reactions, and side reactions and can also originate from impurities in starting materials, reaction solvents, catalysts, or residual solvents. Impurities may also occur as a result of degradation. Common degradation pathways are hydrolysis, dehydration, oxidation, dimerization, or a combination thereof. Delineation between process related and degradation impurities is not always possible. Impurities may be organic or inorganic in nature. Inorganic impurities may originate from the counterion if the drug substance is in salt form, from reaction vessels, filters, adsorbents, and tubing, and also from catalysts and inorganic reagents used in the synthesis. Control of impurities that appear in the drug substance is not only undertaken at the final isolation step but also through control of impurities in raw materials, intermediates, and solvents and at critical synthetic steps. Whenever possible, the level of impurities originating from the starting materials should be limited by in-process controls rather than at the final step. Chromatography as a consequence of its selectivity and precision is an excellent tool for the monitoring of impurities.

The identification of process-related impurities can ultimately lead to elucidation of their mechanistic pathways. The first step in this process entails determination of structures for the impurities using tools such as NMR or LC-MS. With an elucidated structure, one can then attempt to propose a mechanism for its formation and to identify the synthetic steps that lead to its formation. This information can then be utilized to manipulate synthetic conditions so as to minimize its formation or develop efficient recrystallization procedures to reduce impurity level while still maintaining acceptable yield.

The retention times of impurities found in a drug substance impurity profile should be first compared to those of known process-related impurities, intermediates, and starting materials which could have been carried forward to the final product. Where facile, as a first step, the process chemist can synthesize some of the potential impurities on a small scale. Potential impurities include the penultimate intermediates, products of over-, under-, and side reactions, and degradation products. Analogs may have also been synthesized during the drug discovery stage for screening that can also be potential impurities. These isolated impurities can then be correlated to impurities that appear in