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Development and validation of a stability-indicating reversed-phase high performance liquid chromatography method for assay of betamethylepoxide and estimation of its related compounds

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Abstract

Betamethylepoxide (16β-methyl-$\Delta^{1,4}$-pregnadiene-9β-11β-oxide-17α,21-diol-3,20-dione) is a key intermediate for the synthesis of various active pharmaceutical ingredients (APIs) of steroid compounds. A stability-indicating reversed-phase HPLC method for assay of betamethylepoxide and estimation of its related compounds has been developed and validated. This method can accurately quantitate betamethylepoxide in the presence of numerous structurally related compounds (including the α-epimer, known as alphamethylepoxide). This method can also adequately separate most of the impurities from each other and estimate their quantities in betamethylepoxide samples. The stability-indicating capability of this method has been demonstrated by adequate separation of the degradation products from betamethylepoxide in stress degraded and aged stability samples. The HPLC column used in the method was a 5 cm YMC Hydrosphere C_{18} column (4.6 mm I.D.) and the mobile phase consisted of (A) water and (B) acetonitrile:methanol (8:25, v/v).

Keywords: Reversed-phase HPLC; Stability-indicating; Method development; Validation; Steroid intermediate; Betamethylepoxide

1. Introduction

In pharmaceutical industry, the control of the purity of key intermediates is critical to ensure the quality of active pharmaceutical ingredients (APIs) and the final drug products. The impurities in the key intermediates can potentially be carried over throughout the subsequent synthetic steps, or they can undergo similar reactions to form "new impurities" in the final products. The time and effort spent on the identification of the impurities and their sources, or on the toxicity study of the "new impurities" can be saved if the type and amount of impurities in the key intermediates are tightly controlled. Therefore, it is necessary to have an analytical method that can separate the key intermediate from all the potential related compounds (including process related impurities and degradation products), and also separate all the related compounds from each other.

Betamethylepoxide (16β-methyl-$\Delta^{1,4}$-pregnadiene-9β-11β-oxide-17α,21-diol-3,20-dione, see Fig. 1 for structure) is the key intermediate for synthesizing various APIs of steroid compounds such as betamethasone, betamethasone-21-acetate, betamethasone-21-phosphate, betamethasone-21-dipropionate, etc. As indicated in literature, development of a reversed-phase HPLC stability-indicating method for analysis of typical steroid compounds has always been a challenging task [1–3]. One of the major causes of the challenge is the presence of a great number of structurally similar compounds in the samples. In the case of betamethylepoxide, we needed to develop a method that not only has to separate 13 impurities with known structures (listed in Fig. 1), but also must separate impurities with unknown identities and any new degradation products that might form during storage of the samples. The HPLC separation of steroid compounds becomes even more challenging when separation of the epimers of the APIs or key intermediates is necessary for accurate quantitation of the major peak and each individual epimer impurity peak in the samples [4–7]. In the case of betamethylepoxide, the most challenging part of a reversed-phase HPLC analysis for this material

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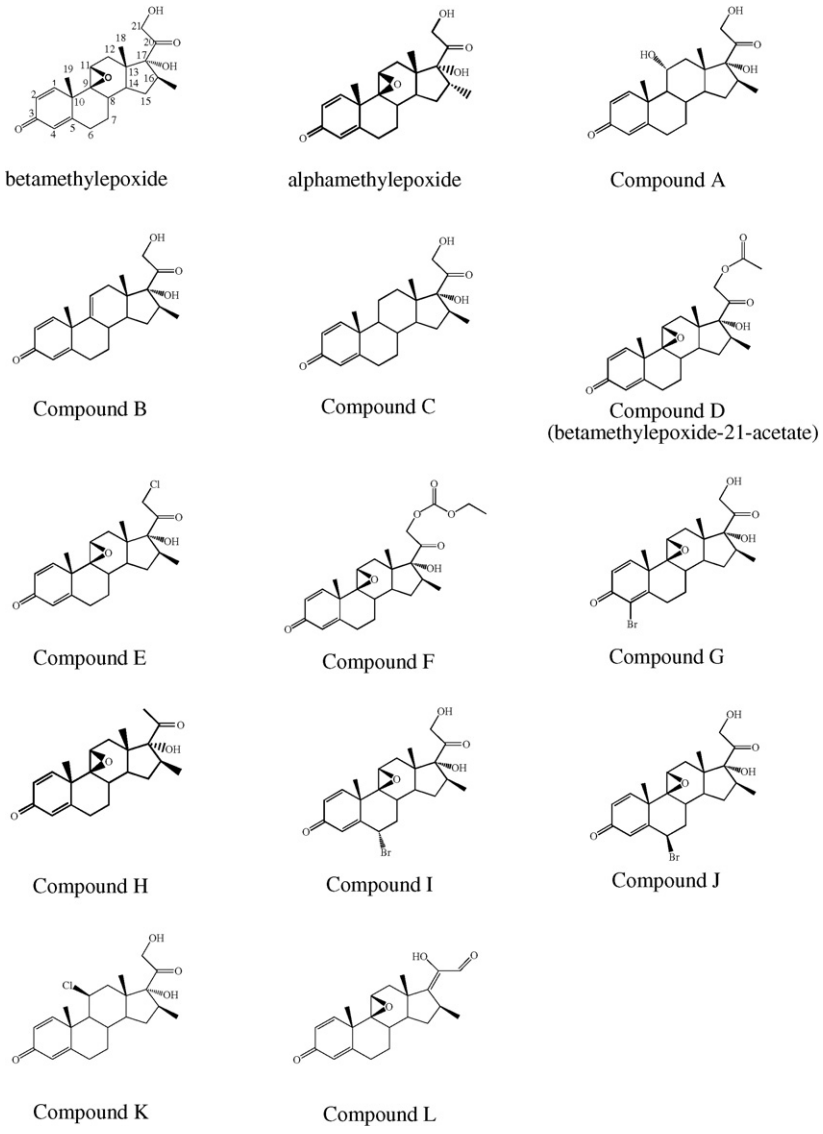

Fig. 1. Chemical structures of betamethylepoxide and some of its related compounds.

is the separation of betamethylepoxide from its α-epimer, i.e., the alphamethylepoxide (16α-methyl-$\Delta^{1,4}$-pregnadiene-9β-11β-oxide-17α,21-diol-3,20-dione, see Fig. 1 for structure). Alphamethylepoxide is the key intermediate for the synthesis of other steroid APIs such as dexamethasone and the esterification compounds of dexamethasone. The β- and α-forms, also known as beta- and dexa-forms, of these compounds have identical chemical structures except that the orientation of the methyl group at the C-16 position is in opposite direction from the plane. Physicochemical characteristics of these two forms of the compounds should be very similar [8,9]. Therefore, it would be difficult to obtain a mobile phase and a stationary phase that would provide adequate differences in thermodynamic parameters (entropy, enthalpy, etc.) between these epimers for a good baseline separation. However, because different isomeric forms of an API may have vastly different physiological effects [10–12], it is preferred that the API of a pharmaceutical product is in one pure form instead of mixed isomers. In our case, accurate quantitation of alphamethylepoxide is deem necessary even when it is present in trace quantity (e.g., about 0.1%) in the betamethylepoxide samples. The epimeric purity of the APIs has to be controlled at the intermediate stage as it can be expected from the similarity in the structures that the epimers will go through the synthesis in a highly similar manner and would result in epimeric impurities in the final APIs.

Previously, some of the steroid molecules that are listed in Fig. 1 had been used to study the retention behavior of the complexes formed between β- or γ-cyclodextrins and these molecules [13]. However, there is neither literature report available on the HPLC analysis of betamethylepoxide and its related compounds, nor on the separation between betamethylepoxide and alphamethylepoxide. In fact, a simple HPLC separation between these two epimers with a resolution factor higher than 3.0 has been achieved in our group. The method development strategy and the method details will be published elsewhere.

In this paper, we describe a reversed-phase HPLC method for the assay of betamethylepoxide and estimation of its related compounds. This method has been demonstrated to be accurate,