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Development and validation of a novel stability-indicating HPLC method for the simultaneous assay of betamethasone-17-valerate, fusidic acid, potassium sorbate, methylparaben and propylparaben in a topical cream preparation

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ABSTRACT

A novel stability-indicating reversed phase high performance liquid chromatographic (RP-HPLC) method for the simultaneous assay of betamethasone-17-valerate, fusidic acid and potassium sorbate as well as methyl- and propylparaben in a topical cream preparation has been developed. A 100 mm × 3.0 mm ID. Ascentis Express C18 column maintained at 30 °C and UV detection at 240 nm were used. A gradient programme was employed at a flow-rate of 0.75 ml/min. Mobile phase A comprised of an 83:17 (v/v) mixture of acetonitrile and methanol and mobile phase B of a 10 g/l solution of 85% phosphoric acid in purified water. The method has been validated according to current International Conference on Harmonisation (ICH) guidelines and applied during formulation development and stability studies. The procedure has been shown to be stability-indicating for the topical cream.

1. Introduction

Inflamed skin conditions, such as eczema and dermatitis, can be further complicated by bacterial infections. For this reason, a topical cream preparation containing the active pharmaceutical ingredients (APIs) betamethasone-17-valerate and fusidic acid as well as the preservative compounds potassium sorbate, methylparaben and propylparaben (Fig. 1) has been developed.

Betamethasone-17-valerate (BV) is a potent corticosteroid with anti-inflammatory properties applied topically for the treatment of a variety of skin conditions, including eczema, atopic dermatitis and psoriasis [1] where it helps to relieve associated symptoms such as oedema and itching. Fusidic acid (FA) is a bacteriostatic antibiotic with steroid structure first isolated from the fermentation broth of *Fusidium coccineum* by Godtfredsen et al. at the laboratories of Leo Pharmaceutical Products in 1962 [2]. It is used for the treatment of Gram-positive bacterial infections, particularly those caused by *Staphylococcus* species. It has regained popularity in recent years due to its effectiveness in the treatment of methicillin-resistant *Staphylococcus aureus* (MRSA) [3,4].

Potassium sorbate (PS) and both methyl- and propylparaben (MP, PP) are antimicrobial substances, found in many pharmaceutical and cosmetic preparations, which are effective against fungi and Gram-positive bacteria [5]. Their function is to ensure that microbiological purity is maintained throughout the shelf-life of the product.

According to current guidelines [6,7], APIs and preservatives in finished pharmaceutical products must be quantified as a general quality control requirement during both release and shelf-life testing. This is in order to demonstrate an acceptable level of quality throughout the life-cycle of the pharmaceutical product. Initially, the stability of trial batches must be determined during the formulation development phase, necessitating the employment of a stability-indicating analytical procedure. In the past, it was common in analytical laboratories to have several individual HPLC procedures for the separate analysis of APIs and preservatives in the same finished formulation. In recent times, however, there appears to be a trend towards developing a single procedure for the analysis of all components in a single run. Examples include Shaikh et al. [8], who developed a procedure for the simultaneous quantitation of chlorocresol, mometasone furoate and fusidic acid in a topical

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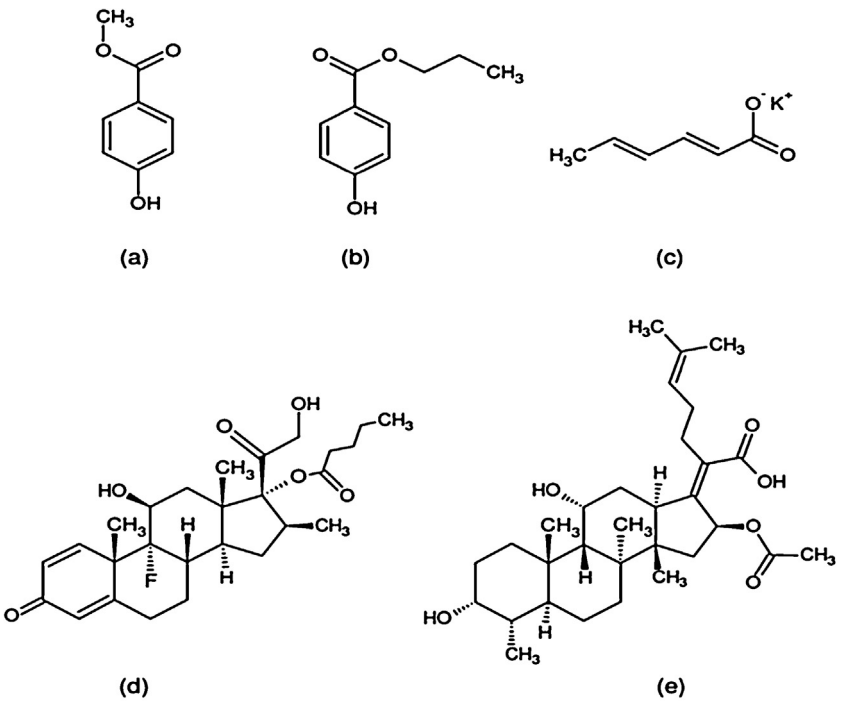

Fig. 1. Chemical structures: MP (a), PP (b), PS (c), BV (d) and FA (e).

cream with a run time of 8 min, and Goswami et al. [9], who developed a procedure for the analysis of halometasone, fusidic acid, methylparaben and propylparaben in a single run. This trend has most likely been driven by advances in analytical technology, such as UPLC and more efficient, more selective analytical columns, as well as environmental awareness issues and cost efficiency.

From the outset the aim of the current analytical development was to employ a single method for the analysis of all compounds. However, none of the analytical methods reported in the literature are suitable for the simultaneous analysis of the 5 components of interest in a single chromatographic run. The majority of reported methods are only suitable for the analysis of a maximum of 1 or 2 of the compounds of interest [10,11] and are often based on poorly selective procedures such as UV–vis spectrophotometry [12] or atomic absorption spectrometry [13] which can no longer be considered state-of-the-art and/or stability-indicating. Consequently, it was necessary to develop a novel procedure for the analysis of all five components. The simultaneous analysis with the aid of a single HPLC run would allow for a much more cost-effective and less time-consuming analysis of cream samples.

In this paper the development of a novel stability-indicating RP-HPLC method for the simultaneous assay of fusidic acid, betamethasone-17-valerate and potassium sorbate, as well as methyl- and propylparaben in a topical cream preparation is reported. The method has been validated according to current International Conference on Harmonisation (ICH) guidelines and applied to development formulations and finished product samples.

2. Materials and methods

2.1. Reagents

Fusidic acid hemihydrate and betamethasone-17-valerate were purchased from OJSC Biosintez (Penza, Russia) and Crystal Pharma (Valladolid, Spain), respectively. Potassium sorbate was obtained from Merck (Darmstadt, Germany) and both methyl- and propylparaben were purchased from Clariant (Pontypridd, UK). All compounds were of Ph. Eur. or cosmetic grade.

Gradient grade methanol and acetonitrile were purchased from VWR International GmbH (Darmstadt, Germany). Purified water was obtained from the in-house purification system at mibe GmbH Arzneimittel (Brehna, Germany). Phosphoric acid (85%, analysis grade) was purchased from Merck (Darmstadt, Germany). All cream samples were provided by mibe GmbH Arzneimittel.

2.2. Instrumentation

Shimadzu Prominence HPLC Systems (Shimadzu, Japan) were used for method development and validation. The HPLC systems were equipped with a binary pump (LC-20AD), a temperature-controlled auto-sampler (SIL-20AC$_{HT}$), a temperature-controlled column compartment (CTO-20AC) as well as an on-line degasser (DGU-20A$_5$) and a photo-diode-array detector (SPD-M20A). The software used was SHIMADZU LC solution version 1.24 SP1.

2.3. Chromatographic conditions and sample preparation

The HPLC column used was the Ascentis Express (Fused-Core®), C18, 100 × 3.0 mm ID, with 2.7 μm particles (Supelco®, Bellefonte, USA) and a suitable guard-column. Mobile phase A comprised of an 83:17 (v/v) mixture of acetonitrile and methanol and mobile phase B of a 10 g/l mixture of phosphoric acid (85%) in purified water. A gradient elution programme was employed (Table 1) with a mobile phase flow-rate of 0.75 ml/min. The column-oven and auto-sampler temperatures were set at 30 °C and 25 °C, respectively. The detection wavelength was 240 nm and an injection volume of 10 μl was used.

Samples were prepared by weighing approximately 1.00 g of cream into a 50.0 ml volumetric flask, adding 30 ml of acetonitrile