Journal of Pharmaceutical and Biomedical Analysis 149 (2018) 265–270

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

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

Development and validation of a stability-indicating HPLC-UV method for the determination of triamcinolone acetonide and its degradation products in an ointment formulation

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ARTICLE INFO

Article history:
Received 17 August 2017
Received in revised form 20 October 2017
Accepted 7 November 2017
Available online 7 November 2017

Keywords:
Stress testing
Liquid chromatography
Mass spectrometry
Triamcinolone acetonide
Ointment

ABSTRACT

A stability indicating high performance liquid chromatography method has been developed for the determination of triamcinolone acetonide (TCA) and its main degradation products in ointment formulations. The method, based on extensive stress testing using metal salts, azobisisobutyronitrile, acid, base and peroxide, showed that TCA undergoes oxidative degradation. All degradation products were identified using HPLC mass spectrometry. Separation and quantification was achieved using an Altima C18 RP18 HP column (250 × 4.6 mm^2, with 5 μm particles) using a mobile phase consisting of acetonitrile and water buffered at pH 7 using 10 mM phosphate buffer. A gradient mode was operated at a flow rate of 1.5 ml/min and detection was at 241 nm. The method showed linearity for TCA and Impurity C in 0.02–125% of the workload, both square roots of the correlation coefficients were larger than 0.9999. Repeatability and intermediate precision were performed by six consecutive injections of both 1.25% and 125% of the work load for both TCA and Impurity C divided equally over two days. RSD were 0.6% and 0.7% for TCA and 0.5% and 0.1% for Impurity C respectively. Accuracy was determined as well, the average recoveries were 99.5% (±0.1%, n = 3) for TCA and 96.9% (±1.3%, n = 3) for impurity C respectively from spiked ointment samples. The robustness was also evaluated by variations of column (old vs new), mobile phase pH and filter retention. The applicability of the method was evaluated by analysis of a commercial ointment formulation. Interestingly, the extensive stress tests were able to predict all degradation products of TCA in a long term stability ointment sample.

1. Introduction

Triamcinolone acetonide (TCA) is a synthetic glucocorticosteroid with immunosuppressive and anti-inflammatory activity. It binds in the target cell to specific cytosolic glucocorticoid receptors and subsequently interacts with glucocorticoid receptor response elements on DNA thereby altering gene expression [1]. TCA has been used for over fifty years and is still frequently prescribed in the treatment of several skin diseases like eczema and psoriasis. It is used in many cream and ointment formulations, including an ointment that is widely used in the Netherlands: TCA ointment FNA (Formulary of Dutch Pharmacists).

Because of the widespread use of TCA in varying matrices several chromatographic methods for the analysis of the compound have been described [2–9]. These methods are suitable for the determination of the TCA content, but not for the quantification of degradation products. The quantification of degradation products is essential in stability research of pharmaceutical products following the ICH Q2 (R1) guideline [10]. Additionally, the degradation of TCA is poorly described in literature. As a consequence, currently no stability indicating method (SIM) for TCA ointment FNA is available.

To develop and validate a method specificity, stressed samples are essential. According to the ICH Q2 (R1) guideline stressed samples should be created using heat, humidity, acid, base, oxidation and light stress [10]. The vagueness of this guideline leads to a

Abbreviations: ACN, acetonitril; AIBN, azobisisobutyronitrile; FNA, formulary of Dutch pharmacists; PG, propyleneglycol; SIM, stability indicating method; TCA, triamcinolone acetonide.

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https://doi.org/10.1016/j.jpba.2017.11.026
0731-7085/

variety of experience-based approaches which are often not comprehensive in their predictability. The scientific background and practical implementation of adequate stress testing is described extensively elsewhere [11]. Since oxidation is the predominant mechanism of TCA degradation a comprehensive set of oxidative stress testing should be used to attain a more comprehensive prediction of the profile of degradation products [2]. Therefore we incorporated not only a peroxide (e.g. hydrogen peroxide [H_2O_2]), but a radical initiator (e.g. azobisisobutyronitrile [AIBN]) and trace metals (e.g. iron and copper salts) into the set of stress tests [12,13]. All other conditions as mentioned in the ICH-guideline were implemented as well. To show specificity, a four and a half year old ointment sample was used. Degradation product identification was performed in order to assist in method development.

The aim of this study was to develop and validate a HPLC-UV SIM for TCA ointment FNA. In support of this aim TCA degradation products were identified using LC–MS after evaluating the outcome of the set of stress tests.

2. Material and methods

2.1. Reagent and chemicals

HPLC grade acetonitrile (ACN), dichloromethane, methanol (MeOH) and hexane were obtained from Avantor Performance Materials (Center Vally, Pennsylvania, USA). Distilled, deionized water was prepared by an Elga Centra R 60/120 system (Woodridge, Illinois, USA). Copper(II) acetate was obtained from Alfa Aesar (Havehill, Massachusetts, USA). Disodium edetate, hydrogen peroxide (H_2O_2), iron(III) chloride ($FeCl_3$), copper(II) chloride ($CuCl_2$), sodium phosphate monobasic (NaH_2PO_4), sodium phosphate dibasic (Na_2HPO_4) and azobisisobutyronitrile (AIBN) were obtained from Merck (Darmstadt, Germany). Propylene glycol (PG) was obtained from Brenntag (Dordrecht, The Netherlands). 1 M hydrogen chloride (HCl) and 0.01 M sodium hydroxide (NaOH) solutions were prepared on site.

TCA ointment FNA consists of 0.1% TCA, 10% PG, 10% lanolin and 79.9% petrolatum.

2.2. LC–MS analysis

MS was conducted on a Micromass Quattro Ultima TQD system equipped with an electrospray ionization (ESI) source (Waters Chromatography, Etten-Leur, The Netherlands). Masses were scanned from m/z 50–1100, gas flow to 530 l/h, gas temperature to 350 °C and voltage 3 kV. Data was analyzed with Masslynx version 4.0 software. The mobile phase components were ACN and water buffered at pH 6.8 using 12 mM ammonium acetate.

To attain insight in the product specific degradation products, a four and a half year old 0.1% TCA ointment FNA sample that was stored throughout its shelf life at room temperature in aluminum tubes was used. The sample was extracted using the sample preparation method provided in Section 2.6 and analyzed using the settings described above. Mass spectra are included in supplementary data.

2.3. HPLC-UV

Chromatography was conducted on a Shimadzu Prominence-i LC-2030C 3D liquid chromatograph with diode array detector (Kyoto, Japan) and an Altima C18 RP18 HP column (250 × 4.6 mm^2, with 5 μm particles) (Mandel Scientific Company, Ontario, Canada). The flow rate was 1.5 ml/min and UV detection was at 241 nm. Mobile phase components were ACN and water buffered at pH 7 using 10 mM phosphate buffer. Injection volume was 20 μl. Chromatograms were obtained and analyzed with Shimadzu LabSolutions software version 5.5.7. A gradient program was run: 0% ACN from start to 12 min, increased to 32% ACN at 12 min, maintained at 32% until 30 min, increased to 70% at 40 min, decreased to 0% at 42 min and maintained at 0% until 47 min.

2.4. Synthesis of Impurity C

The synthesis of Impurity C (compound 2, Fig. 1) was based upon a method described in literature [14]. Impurity C was synthesized by dissolving 600 mg TCA and 31.5 mg copper(II)acetate in 150 ml MeOH. Air was bubbled through the solution for 60 min. The reaction was quenched by adding 20 ml of 2.5 mg/ml disodium edetate aqueous solution. The solution then was concentrated to 30 ml under cold air and was extracted twice with 200 ml dichloromethane. Finally, the dichloromethane was evaporated under cold air to yield Impurity C.

2.5. Stress testing

Stress testing was performed on 0.5% solutions of TCA in PG. These solutions were exposed to the conditions described in Table 1. Conditions were chosen based on a degradation target of 5–20%. HCl and NaOH were used to simulate acid and base catalyzed degradation. AIBN, H_2O_2 and $FeCl_3$ and $CuCl_2$ were used to simulate radical initiator, peroxide and trace metal mediated oxidation respectively. Light stress was omitted because of irrelevancy as TCA is protected against light in the product by its container.

2.6. Sample preparation

Ointment samples were dispersed in hexane and extracted with ACN and water buffered at pH 7 using 10 mM phosphate buffer (1:1). PG solutions were diluted with ACN or ACN-buffer (1:1). Synthesized Impurity C was dissolved in ACN or ACN-buffer (1:1). TCA references were dissolved in ACN-buffer (1:1).

2.7. Method validation

The method was validated according to the ICH Q2 (R1) guideline. Accuracy, precision (including both repeatability and intermediate precision), specificity, linearity, range and detection and quantification limits (LOD and LOQ) were assessed. Appropriate stressed samples were used for the assessment of specificity and resolution. Stressed samples were appropriate if they showed a degradation between 5 and 20%. Compounds were taken into account if they were present in a concentration of ≥1.0%.

2.7.1. Accuracy

For accuracy a freshly prepared ointment matrix was spiked with TCA and Impurity C in concentrations of 100% and 1% of the work load respectively. Recovery was determined on three consecutive runs.

2.7.2. Precision

For precision repeatability and intermediate precision were performed by using six consecutive injections of both 1.25% and 125% of the work load for both TCA and Impurity C in ACN-buffer (1:1) divided equally over two days.

2.7.3. Specificity

The four and a half year old ointment was used to determine the method specificity by determination of the smallest resolution between any two peaks after sample preparation.