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Short communication

Development and validation of HPLC-UV method for quantification of imidurea in shampoo formulation

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Introduction

Imidurea (Imidazolidinyl urea), is an organic compound widely used as a preservative in cosmetic and personal care products. Its primary function is to inhibit the growth of bacteria, yeast, and mold, thereby extending product shelf life and maintaining microbiological stability. Imidurea belongs to the group of formaldehyde-releasing preservatives, which provide effective antimicrobial protection at low concentrations (Lehmann et al., 2006). In shampoos, imidurea is used to prevent microbial contamination during storage and consumer use. Due to its effectiveness and compatibility with various formulation ingredients, it has been a common preservative in hair care products (Lv et al., 2015).

Imidurea is official in USP-NF 2026, but the official monograph lacks a specific high performance liquid chromatography (HPLC) method for assay. The method of analysis for imidurea in the general monography is suitable only for nitrogen content. The goal of this work was to develop and validate a reversed-phase (RP) HPLC method for quantification of imidurea, for routine quality control of a shampoo formulation containing this preservative.

Materials and methods

The reagents that have been used are: acetonitrile, o-phosphoric acid (H_3PO_4) and 1-heptansulfonic acid sodium salt purchased from Thermo Fisher Scientific and VWR Chemicals. All the chemicals used were Ph. Eur grade. Reference standard Imidurea CRM was purchased from Sigma Aldrich. The shampoo, which contains imidurea 2 mg/1 g shampoo as preservative, as well as placebo were obtained from Replek Farm Ltd., Skopje, N. Macedonia. The syringe filters RC, 0,45 μm, were purchased from Agilent Technologies (USA). Chromatographic analyses were performed on a UPLC Shimadzu-PDA NEXERA Lab Solution Systems, with quaternary pump with degasser, autosampler, controller, column oven and PDA detector. Signals were monitored and processed using the Lab solutions software. Additional instrumental equipment was used: analytical balance Mettler Toledo AG285, pH meter Metrohm 827, US bath Branson 3510, and IKA orbital shaker KS 260 basic.

Separation was performed on chromatographic column BDS Hypersil C18 (150 x 4.6 mm, 5 ⌈m). The column temperature was maintained at 40°C. An isocratic elution was used, with flow rate of 1.0 mL/min. The injection volume was 10 μL and detection wavelength was set at 210 nm. Total run

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time is about 20 minutes. A mixture of acetonitrile and 0.005 M 1-heptanesulfonic acid sodium salt, adjusted to pH 5.0 with o-phosphoric acid, in volume ratio 10:90 (%, *v/v*), was used as a mobile phase. A mixture of acetonitrile and 0.005 M 1-heptanesulfonic acid sodium salt, adjusted to pH 5.0 with o-phosphoric acid, in volume ratio 15:85 (%, *v/v*) was used as diluent.

Results and discussion

In order to prevent the release of formaldehyde, as higher pH facilitates decomposition (Engelhardt, H. Klinkner, R., 1985), the samples were prepared in diluent. The chosen diluent and mobile phase compositions improve molecule stability and column retention by reducing its ionization (which is important due to pKa values of imidurea: $pKa_1 \sim -3.3$ and $pKa_2 \sim 7.4$) and the polarity of the molecule with retention time of around 1.6 min, with total run time of 5 minutes.

In this study, the established method was validated according to the International Conference on Harmonization (ICH) Q2(R1) guideline for validation of analytical procedures (ICH, 2005), in respect to selectivity, linearity, precision and accuracy. During selectivity testing, no interference from the formulation excipients was observed. The linearity of the method was proved in five concentration levels, in the range between 80% and 120% of the working concentration of imidurea in the test solution. The results confirmed the linear relationship between peak areas of the examined analytes and the respective concentrations. The results were evaluated by linear regression analysis and following results were obtained: correlation coefficient is 0.9992 and relative standard deviation (RSD) of the response factors for each concentration level is 0.55%. The precision of the RP-HPLC method was evaluated through repeatability (system and method repeatability) and intermediate precision. The system repeatability was evaluated by six determinations of the peaks areas of standard solution and the obtained values for relative standard deviation (RSD) were below 2%. Method repeatability analysis performed on six different test solutions showed that RSD was not more than 2%. The intermediate precision assessed on three consecutive days, by two different analysts, on two different HPLC systems showed that the RSD value obtained from analysis of test solutions is not more than 3%. Accuracy of the method was studied by recovery investigation. The accuracy of the method was tested using nine determinations over three concentration levels in the interval between 80% and 120% of the working concentration of imidurea in the test solution. The obtained recovery values were within the range of 100 ± 2 %. To determine the robustness of the analytical method for simultaneous determination of imidurea the one factor at a time approach was used. The robustness testing showed that the obtained results are not adversely affected by small variations in method parameters.

Conclusion

The developed RP-HPLC-UV method provides a simple, specific, accurate, precise, robust and reproducible quantitative analysis of imidurea in shampoo formulation. The method was validated and proven suitable for its intended use in accordance to (ICH) Q2(R1) guideline, for validation of analytical procedures. This method can be successfully used for fast and economic, routine analysis of imidurea in shampoo formulation, by use of simple sample preparation, short run time and low-cost reagents.

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