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Short communication

Development and validation of an HPLC method for determination of Heparin in Alven® gel

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Introduction

Heparin is an anticoagulant that decreases the clotting ability of blood and helps to prevent the formation of harmful clots that form in blood vessels. Applied topically, heparins are widely used in Europe for the prevention and treatment of the local symptoms associated with peripheral vascular symptoms due to their anticoagulant and antithrombotic effect (Vecchio & Frisinghelli, 2008). Heparin consists of linear chains of mucopolysaccharides called glycosaminoglycans. and exhibits strong acidic properties due to covalently bonded carboxyl groups. In form of Heparin sodium, the hydrogen ions of the sulfate units are partially replaced by sodium ions.

Due to the lack of available analytical methods for determination of heparin in pharmaceutical products, need has arisen for development of simple, reliable and sensitive HPLC method.

The purpose of this study was to develop and validate an HPLC method for quantitative determination of heparin content in Alven gel.

Materials and methods

Materials

Sodium perchlorate monohydrate, Sodium dihydrogen phosphate monohydrate, Ammonium sulphate, Heparin sodium reference standard and water (HPLC grade). Pharmaceutical product used for this research was Alven 300 IU/g gel.

Chromatographic conditions

Analysis was performed on Agilent 1260 Infinity Binary as a part of CDS controlled by Chromeleon software. Optimal chromatography was achieved using a Spherisorb SAX column, 250 x 4.0mm, 5µm. The mobile phase consisted of mobile phase A (buffer solution, pH = 2.5) and mobile phase B containing $NaClO_4xH_2O$ and $NaH_2PO_4xH_2O$. The solvent used is a mixture of 0.2 M $(NH_4)_2SO_4$ and water (HPLC grade), in ratio 50:50 (v/v). The developed method is a gradient method with constant flow of 0.5 mL/min. The detection was performed at 202 nm. The injection volume was 100 µL.

Results and discussion

In this study, the HPLC method for determination of heparin sodium in heparin gel was validated in respect to specificity, linearity, accuracy, precision and robustness.

The selectivity of the method was evaluated by chromatographic analysis of diluent, placebo, standard and sample solution prepared in the working concentration (40 IU/mL) and injected into the chromatograph, to perform screening for possible interfering peaks. The analysis of diluent and placebo chromatograms, showed that no peaks are observed at retention time of the active ingredient Heparin sodium.

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The linearity of the method was determined by a series of standard injections prepared in a concentration range from 20 – 61 IU/mL. The accuracy of the method was evaluated at three different concentration levels from 50% to 150% of the working concentration. Three samples of each concentration level were prepared. The recovery of each sample (%), average value and RSD (%) of the three samples at each concentration level were calculated. The obtained results were in accordance with the acceptance criteria for recovery (90.0-110%) and RSD values were below 5%.

The precision of the system is expressing the degree of proximity between the individual results. System precision was demonstrated by the preparation of a standard solution in the working concentration according to the prescribed analytical procedure and six determinations of the peak areas of heparin. The obtained RSD value was 0.16%. The precision of the method is determined by preparing six sample solutions of a single gel batch. The obtained RSD value was less than 5% (0.9%) indicating the method is precise.

Intermediate precision was verified by preparation of six sample solutions from the same batch of gel, by two analysts on two different days and using different equipment. The obtained Overall RSD value was 1.6%.

In order to prove the robustness of the method, changes were made in the mobile phase flow (± 0.2 mL/min), column temperature (±5^{o}C), $NaClO_4xH_2O$ manufacturer and column serial number. The obtained results indicate that the method is robust in the tested experimental area of the chromatographic conditions.

Conclusion

The results from the validation of the proposed HPLC method for determination of heparin in Alven gel, show that the method is specific, linear, precise, accurate, and robust. The validated method can be applied for content determination of the active ingredient heparin in commercially available batches of Alven gel.

References

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