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Short communication

Development, optimization and validation of GC-FID method for determination of levomenthol in topical (gel) formulation

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Introduction

Menthol, more specifically a monoterpenoid, can exist in two enantiomeric forms (+/–), occurring as levomenthol (-) or racemic menthol (±). Levomenthol is the naturally - occurring and main form of menthol and is assigned the (1R,2S,5R) configuration. It is the most abundant optical isomer and is the form used in menthol gel products.

As active organic substance, levomenthol is widely used in the clinical and pharmaceutical practice for its various effects. It is used in various commercially available oral and topical formulations as test masking agent for the treatment of soft - tissue injuries or to relief local pain, rheumatic pain, muscle aches and swellings, sports injuries or treatment for headaches. It also stimulates skin cold receptors, which gives the property of cooling effect and acts as a potent penetration enhancer for drug molecules through the epidermis of the skin due to the ability to disrupt the lipid bilayer of the stratum corneum.

According to several literary data, many methods have been established for the analysis of menthol, including high performance liquid chromatography (HPLC) methods with fluorescence labeling reagents (Lin et al., 2005), refractive index (Shaikh et al., 2010) and polarized photometric detector. Normal-phase HPLC with refractive index detector has also been employed in the analysis of menthol. However, these methods have low sensitivity. Gas chromatography (GC) is considered to be a less expensive and sensitive method for the analysis of menthol and it has been widely employed in the analysis of menthol in food and cosmetics (Savchenko et al., 2018). To the best of our knowledge, there are not many specific analytical methods applicable in actual pharmaceutical samples like topical (gel) formulations.

In order to obtain a valid analytical result, the purpose of this study was to give a completely understandable concept for the implementation of precise, sensitive and fast GC method. The method was validated to fulfill ICH guidelines requirement for various parameters such as specificity, linearity, accuracy, precision and robustness. The objective of validation of an analytical procedure is to demonstrate that the analytical procedure is suitable for the intended purpose (ICH, 2022).

Materials and methods

The analytical standard levomenthol natural (GC purity ≥ 99.0 %) as well as methanol and 1,2-propanediol reagents were purchased from Sigma-Aldrich. All other reagents were of pharmaceutical grade and used as received. Method was developed, optimized and validated in accordance with the recommendations of the relevant guidelines.

GC-FID screening was performed on Agilent GC system equipped with flame ionization detector (FID) and single quadrupole mass spectrometer (MS) with liquid and Headspace autosamplers. Actual chromatographic conditions as well as diluents were established after number of preliminary experiments for selecting the proper method of injection, column and oven temperature. An appropriate capillary column DB-5UI MS (30 m x

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0.25 mm x 0.25 µm) was selected for chromatographic separation using FID detector. The oven temperature programming was applied with initial temperature of 70°C up to 240°C at a rate of 10°C/min. Inlet temperature and detector temperature are fixed at 240°C and 250°C respectively. Standard and sample solutions were prepared in diluent mix of methanol and 1,2-propanediol (85:15) in concentration of 0.15 mg/mL and 3.0 mg/mL and injection volume of 1.0 µL was applied.

Results and discussion

Implementation of the proposed precise and fast GC method enables determination of levomenthol in gel topical formulation. According to the retention times data obtained and well separated peaks in the chromatograms of placebo, standard and sample it was concluded that no peaks were observed at retention time of the levomenthol peak.

Applicability of this method was evaluated by analyzing the samples of gel formulation. The calibration curves of all six marker compounds showed good linear correlation coefficient r^2=0.9986 within the test ranges. Linear regression were obtained between the responses of levomenthol peak related to the six concentrations of standard solutions over the range of 0.06 mg/mL – 0.23 mg/mL. The recovery was evaluated in three levels, calculated by standard addition method and it was found to be between 99.43 % and 101.48 %. Precision was checked at three levels: system repeatability, method precision, using six replicate injections and intermediate precision, using ten replicate injections of sample solutions (RSD was 0.18 % and 0.22 %, first and second day receptivity). The obtained results for precision indicate a good precision of the method. Robustness of the method was measured by making small, deliberate changes to the three chromatographic conditions (column flow ± 1mL/min, injector and detector temperatures ±5°C). The tailing factor and theoretical plates number were evaluated according to the calculated data (tailing factor was below to 1.5 and theoretical plates number were greater than 100 000 for all variable conditions). Observing the effect of these changes on the system suitability parameters show that method was robust.

The essence of system suitability parameters is the concept that the equipment, the samples and the analytical operations constitute a single analytical system, which is amendable to an overall test of the system functions. The benefit of this study is the development of a sensitive, well - characterized GC methodology, suitable for determination and quantification of levomenthol content in gel topical formulation.

Conclusion

During the validation procedure, carried out according to ICH guidelines Q2(R2) specificity, linearity, precision, accuracy and robustness were evaluated and the tested validation parameters were found to be within acceptable limits. The validation procedure shows that the method is suitable for its intended purpose, it is economical labor, rapid, and specific for the assay of the active ingredient levomenthol. Proposed method is used in the routine and formal stability life cycle analysis for topical (gel) formulation.

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