

Development and validation of a RP-HPLC method for simultaneous determination of terbutaline sulfate, guaifenesin, bromhexine hydrochloride and sodium benzoate in a syrup formulation

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Introduction

Terbutaline sulfate is a selective beta-2 adrenergic agonist used as a bronchodilator, used to treat wheezing and shortness of breath from lung problems (e.g. asthma, chronic obstructive pulmonary disease, bronchitis and emphysema).

Bromhexine hydrochloride is mucolytic, which helps clear chest congestion, thus contributes to a secretomotoric effect. It also has antioxidant properties.

Guaifenesin is an expectorant, working by thinning and loosening mucus in the airways, cleaning congestion, and making breathing easier. It is used to treat coughs and congestion caused by the common cold, bronchitis, and other breathing illnesses (McCrory et al., 2013).

Sodium benzoate is used as a preservative in the syrup formulation.

There are a number of methods available for determination of terbutaline sulfate, bromhexine hydrochloride and guaifenesin individually (Ph. Eur., BP, USP), but only a few methods for simultaneous determination of a combination of them in a syrup formulation.

The aim of our work was to develop and validate a simple and rapid reversed-phase high performance liquid chromatography (RP-HPLC) method for simultaneous estimation of the active substances, terbutaline sulfate, guaifenesin, bromhexine hydrochloride and the preservative sodium benzoate, in a cough syrup formulation.

Materials and methods

The reagents that have been used are: ammonium dihydrogen phosphate ($\text{NH}_4\text{H}_2\text{PO}_4$) and 85% *o*-phosphoric acid (H_3PO_4) purchased from Sigma Aldrich, USA, methanol and acetonitrile procured from Merck, Darmstadt, Germany, and the demineralized water was "in house" prepared with conductivity of 0.05 $\mu\text{S}/\text{cm}$. The terbutaline sulfate reference standard, bromhexine HCl CRM, guaifenesin CRM and sodium benzoate analytical standard, were purchased from Sigma-Aldrich, USA, and the syrup formulation was obtained from Replek Farm Ltd., Skopje, N. Macedonia. The syringe filters Nylon and RC, 0,45 μm , were purchased from Agilent Technologies (USA).

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Instruments that have been used are: UPLC Shimadzu Nexera XR system with LPG quaternary pump with degasser, autosampler, controller and PDA detector and column oven, controlled by Lab Solutions software, version 5.97.; analytical balance Mettler Toledo AG285; pH-metter Metrohm 827 pH Lab; and IKA orbital shaker KS 260 basic.

The separation was accomplished using Inertsil RP8 250 mm × 4.6 mm, 5 µm column from GL Sciences, Tokyo, Japan.

Results and discussion

The chromatographic separation of all three active substances and the preservative (all with significant differences in their concentrations, polarities, solubility, UV absorbing spectra and molar absorption coefficients) was carried out on a GL Sciences Inertsil RP8 250 mm × 4.6 mm, 5 µm column, under isocratic conditions, with mobile phase consisting of 20% v/v methanol, 20% v/v acetonitrile and 60% v/v 20 mM NH₄H₂PO₄ pH 2.5, with flow rate was 1.2 mL/min, detection wavelength at 220 nm, column temperature of 30 °C and injection volume of 5 µL.

The isocratic elution of the analytes was achieved in 12 min, with retention time of terbutaline sulfate, guaifenesin, sodium benzoate and bromhexine HCl on 2.3, 4.6, 7.6 and 9.7 minutes, respectively. All four chromatographic peaks are well separated between each other, to the baseline. The obtained values for number of theoretical chromatographic plates for terbutaline sulfate, guaifenesin, sodium benzoate and bromhexine HCl, were 4025, 10201, 12641 and 10151, respectively.

The established method was validated according to the International Conference on Harmonization (ICH) Q2(R1) guideline for validation of analytical procedures. During selectivity testing, no interference from the formulation excipients was observed. The linearity of the method was proved in five concentration levels, for each substance of interest and the following results were obtained by regression analysis: correlation coefficient >0.9990 and relative standard deviation of the response factors for each concentration level <2%, in all cases. The precision of the system and of the method were also evaluated and the obtained relative standard deviation of the responses was less or equal

to 2%, in both cases, for each substance. Accuracy of the method was studied by recovery investigation. The obtained recovery values were within the range of 100±2%, for each substance. The robustness testing of the method, showed that the obtained results are not adversely affected by small variations in method parameters.

Conclusion

The developed RP-HPLC method enables simultaneous, fast and accurate determination of the three active substances, terbutaline sulfate, guaifenesin and bromhexine HCl, and the preservative, sodium benzoate, all with different physico-chemical properties, in syrup formulation. The method was validated and proved as suitable for its intended use. The proposed method, using simple sample preparation and low cost reagents, provides reproducible quantification of all substances of interest and can be successfully used for routine analysis of cough syrups containing this combination of active substances.

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