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*Short communication*

# Development and validation of a RP-HPLC method for simultaneous determination of Benzydamine hydrochloride and Cetylpyridinium chloride in oral spray solution

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## Introduction

Benzydamine hydrochloride is a non-steroidal anti-inflammatory drug with local anesthetic and analgesic properties providing both rapid and extended pain relief, as well as a significant anti-inflammatory treatment for the painful inflammatory conditions of the mouth and throat (Sugiarto et al., 2020).

Cetylpyridinium chloride is a quaternary pyridinium antiseptic with bactericidal activity against Gram-positive and, at higher concentration, some Gram-negative bacteria. It is use as lozenges, gels or solutions for the treatment of minor infections of the mouth and throat (Steyer et al., 2021).

There are a number of methods available for determination of Benzydamine hydrochloride and Cetylpyridinium chloride, individually (Ph.Eur., BP, USP), but only a few methods for simultaneous determination of a combination of them in final dosage forms.

The aim of this study was to develop simple, fast and efficient reverse phase high performance liquid chromatography (RP-HPLC) method for simultaneous determination of Benzydamine hydrochloride and Cetylpyridinium chloride in final dosage form preparations and validate this method in accordance with current International Conference on Harmonization (ICH) Analytical Method Validation requirements.

## Materials and methods

*Materials*

The reagents used for RP-HPLC determination of Benzydamine hydrochloride and Cetylpyridinium chloride are: potassium dihydrogen phosphate ($KH_2PO_4$), 85 % *o*-phosphoric acid ($H_3PO_4$) and triethylamine ($(C_2H_5)_3N$), purchased from Fisher Scientific by Thermo Fischer Scientific (UK) and Carlo Erba (Italy), as well as methanol and acetonitrile procured from Carlo Erba. The demineralized water was obtained "in house" by use of Simplicity UV System, with conductivity of 0.05 µS/cm. The Benzydamine hydrochloride reference substance was purchased from MHRA, London, United Kingdom and Cetylpyridinium chloride reference standard, was purchased as the United States Pharmacopeia standard. The RC (regenerated cellulose) 0,45 µm syringe filters, were purchased from Agilent Technologies (USA).

The oral spray solution containing these two active substances was obtained from Replek Farm Ltd., Skopje, N. Macedonia.

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–meter Metrohm 827 pH Lab; and IKA orbital shaker KS 260 basic.

As to HPLC column, it is used InertSustain C8 4.6 mm x 100 mm, 5 µm, purchased from GL Sciences Inc. (Japan).

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*Method*

The optimal chromatographic separation of both active substances was performed by using an analytical column of InertSustain C8 4.6 mm x 100 mm, 5 µm. The active substances were eluted by a mobile phase consisted of 35% [25 mM $KH_2PO_4$ containing 0.1% (v/v%) *o*-phosphoric acid (85% *o*-$H_3PO_4$)] which pH value was adjust to pH 3.0 with triethylamine, 5% methanol and 60% acetonitrile, under isocratic conditions, at a flow rate of 1.1 mL/min, detection wavelength at 257 nm, column temperature of 35 °C and injection volume of 5 µL.

## Results and discussion

These two active substances are very different in their hydrophobicity and interaction affinity towards alkyl chain based bonded phase columns, C8 and C18. Use of shorter, 100 mm column, in combination with properly selected mobile phase in isocratic mode of elution, enables analysis with decent run time.

The isocratic elution of the analytes was achieved in seven minutes, with retention time of Cetylpyridinium chloride and Benzydamine hydrochloride on 1.2 minutes and 5.1 minutes, respectively. Both chromatographic peaks are well separated between each other, to the baseline. The obtained values for number of theoretical chromatographic plates per meter for Benzydamine hydrochloride and Cetylpyridinium chloride, were 18605 and 35579, respectively.

The established HPLC method was validated in accordance to the International Conference on Harmonization (ICH) Q2(R1) guideline for validation of analytical procedures. The method was tested for selectivity, linearity, precision and accuracy. During selectivity testing, no interference from the formulation excipients was observed. Linearity for Benzydamine hydrochloride and Cetylpyridinium chloride was studied in five concentration levels, for each substance, in the concentration range of 0.015 - 0.045 mg/mL for Benzydamine hydrochloride and 0.05 - 0.15 mg/mL for Cetylpyridinium chloride. The obtained results for linearity, show that the method is linear for determined compounds. The correlation coefficient is more than 0.9990 for both compounds and the relative standard deviation of the response factors for each concentration level is < 2%, in all cases. The precision of the system and method precision were also evaluated and the obtained relative standard deviation of the responses was less or equal to 2%, in both cases, for each substance. Accurancy of the method was studied by recovery investigation. The obtained recovery values were within the range of 100 ± 2%, for each substance.

Our further focus of research that is in progress, is check of applicability of less hydrophobic or more polar interaction-based chromatography mechanism and columns, like phenyl, cyanopropyl, HILIC, DIOL, hexafluoro phenyl etc. These research activities for new more polar interaction-based separation of these two active substances are still in progress.

## Conclusion

The developed analytical method for simultaneous determination of Benzydamine hydrochloride and Cetylpyrdinium chloride in a final dosage form by HPLC was found to be rapid, simple, reliable and requires low cost reagents. The method was validated and proved as suitable for its intended use. The proposed method, could be successfully used for routine analysis in quality control laboratories for simultaneous determination of Benzydamine hydrochloride and Cetylpyridinium chloride in combined pharmaceutical dosage forms.

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