

Stability indicating HPLC-determination of biperiden hydrochloride in presence of its oxidative and acid degradation products

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ABSTRACT

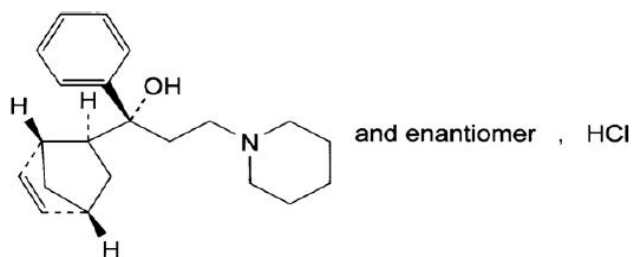
A RP-HPLC method for stability indicating determination of biperiden hydrochloride (BIPER) in presence of its oxidative and /or acid degradation products, was developed, optimized, validated and applied either in tablets dosage form or in raw materials. The separation could be achieved on a reversed phase column [Nucleodure C₁₈ (5µm, 25 cm x 4.6 mm i.d.)] isocratically by using a mixture of 0.2% 0.1 N perchloric acid in 0.01M sodium perchlorate solution and acetonitrile in a ratio (50 : 50% v/v) as the mobile phase with UV-detection at 210 nm. Significant linearity was observed in the ranges of 8-100 µg mL⁻¹. Statistical evaluation of the results was obtained by adopting the proposed method and those of reference ones has been undertaken by applying the student *t*-testing, *F*-ratio calculation and by one-way ANOVA assessment. © 2014 Trade Science Inc. - INDIA

KEYWORDS

Biperiden;
Nucleodure;
Stability indicating;
RP-HPLC.

INTRODUCTION

Biperiden hydrochloride, (1*RS*)-1-[(1*RS*,2*SR*,4*RS*)-Bicyclo[2.2.1]hept-5-en-2-yl]-1-phenyl-3-(piperidin-1-yl)propan-1-ol hydrochloride, is used as an anticholinergic drug^[6].



C₂₁H₂₉NO. HCl 347.9

Figure 1

BP-2008^[6] biperiden hydrochloride can be deter-

mined potentiometrically in pure form by using 0.1M alcoholic potassium hydroxide as a titrant.

USP-31^[8] determines biperiden hydrochloride in pure form by chemical titration with 0.1N perchloric acid using crystal violet indicator, and colorimetrically using phosphate buffer-bromocresol purple solution in tablets dosage form.

The first problem that was facing the analysis of BIPER in quality control was the inability of the present official methods to test the stability and detect the degradation products of biperiden hydrochloride (BIPER) either in raw materials or in pharmaceutical preparations.

The second problem was in absence of available data about the degradation pathways and degradation products of biperiden hydrochloride which help in desciding the suitable precautions which should be applied during its storage and manufacturing steps.

The aim of this work was:

- Developing a simple and reliable stability indicating high performance liquid chromatographic method for the determination of biperiden hydrochloride in presence of its oxidative and acid degradation products for its analysis in raw materials and pharmaceutical preparations.
- Preparation, isolation and identification of the expected degradation products experimentally.
- Establishment of kinetic studies for the degradation pathways.

EXPERIMENTAL

Chemicals and reagents

- Sodium perchlorate, E.Merck, Germany.
- Acetonitrile (HPLC grade), E.Merck, Germany.
- Methanol (HPLC grade), E.Merck, Germany.
- Perchloric acid (Analytical grade), E.Merck, Germany.
- Hydrogen peroxide (Analytical grade), E.Merck, Germany.
- Hydrochloric acid (Analytical grade), E.Merck, Germany.
- ULtraPure deionized water was obtained from 'Elix[®]-5' Millipore Water Purification System (WPS), Millipore GmbH, Schwalbach-Germany.

Samples

Pure reference samples

Standard substances were kindly supplied by the Arab Drug Co.-Cairo-Egypt.

- Biperiden hydrochloride BN: BPH040907 was assayed by BP-2008 method⁽¹⁾ and its purity was found to be (99.64 ± 0.50) %.

Market dosage formulations:

- Akinitone[®] tablets is labelled to contain 2 mg biperiden hydrochloride in each tablet, manufactured by Arab Drug Co., BN: 510091 and 410017.
- Achtinone[®] tablets is labelled to contain 2 mg biperiden hydrochloride in each tablet, manufactured by Arab Drug Co., BN: 810082 and 710202.

Apparatus and experimental conditions

Liquid chromatograph consisted of an isocratic

pump (Agilent Model-G1310A), a variable wavelength UV-detector (Agilent 1100 Series, Model-G1314A), with a Rheodyne injector (Model-7725, CA-USA)-equipped with 20- μ l injector loop- Agilent Technologies, Inc. (Santa Clara, CA-USA). Stationary phase: Nucleosil C₁₈ analytical column (10 μ m, 15 cm \times 4.6 mm, *i.d.*), Alltech (USA). Mobile phase composed of 20 mM NaH₂PO₄ solution and CH₃OH (30:70, v/v) was running isocratically at 1.5 mL min⁻¹. The mobile phase was filtered through a 0.45- μ m millipore membrane and was degassed for about 15 minutes in an ultrasonic bath prior to use. The rate of flow was controlled at 1.5 mL min⁻¹, isocratically at ambient temperature (\sim 25 °C) with UV-detection at 240 nm. The samples were filtered also through a 0.45- μ m membrane filter.

- Gas chromatograph- mass spectrometer: Shimadzu QP1000 EX (Kyoto, Japan).

Preparation of the degradation products of biperiden hydrochloride

a. Oxidative degradation products

10 mg of pure biperiden hydrochloride was accurately weighed into 50 mL conical flask, dissolved in about 25 mL methanol, the methanolic solution was subjected to oxidation by mixing with 20 mL of 10% hydrogen peroxide solution in methanol with heating at 40°C water bath, 1 mL was taken every 30 minutes, evaporated near dryness, diluted with 10 mL methanol then the solution was tested for complete oxidative degradation using the proposed HPLC method. It was found that no peak appeared at retention time 5.04 min. at which the intact biperiden hydrochloride peak appears, complete oxidative degradation was achieved after 6 hours.

b. Acid degradation products

10 mg of pure biperiden hydrochloride was accurately weighed into 50 mL volumetric flask, dissolved in about 25-mL methanol, the methanolic solution was subjected to acid degradation by mixing with 20 mL of 1N hydrochloric acid with heating at 40°C water bath 1mL was taken every 10 minutes, evaporated near dryness, diluted with 10 mL methanol then the solution was tested for complete acid degradation using the proposed HPLC method. It was found that no peak appeared at retention time 5.04 min. at which the intact biperiden hydrochloride peak appears, complete acid degrada-

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tion reaction was achieved after 2 hours which followed by neutralization with 1 N NaOH and filtration.

- After complete oxidative and acid degradation, degradation products were subjected to mass spectral analysis for subsequent identification. Good and interpretable results were obtained (*Scheme 4-5*).

The assignment of the degradation products were based on the comparison of mass spectral data for the separated compounds with that of the intact. *Figure. 41-42*.

Stock standard solutions

All standard solutions are stable for three days if kept in the refrigerator ($\sim 5^{\circ}\text{C}$).

- Stock solution of biperiden hydrochloride (0.4 mg mL^{-1}) in methanol: 20 mg of pure biperiden hydrochloride was accurately weighed into 50 mL calibrated volumetric flask, dissolved in about 25 mL methanol and the volume was completed to the mark with methanol.
- Stock solution of oxidative degradation products: the same procedures detailed in III-4.2.2.5. were applied and after the degradation was completed, appropriate dilutions were made in order to obtain concentration of 0.2 mg.mL^{-1} .
- Stock solution of acid degradation products: the same procedures detailed in III-4.2.2.5. were applied and after the degradation was completed, appropriate dilutions were made in order to obtain concentration of 0.2 mg.mL^{-1} .

and their mixtures were prepared by careful complete dissolution of accurately weighed aliquots of the substance(s) in calculated volumes of methanol.

Calibration

Aliquot volumes of biperiden hydrochloride (0.4 mg mL^{-1}) in methanol, were transferred in to a series of 100 mL volumetric flasks, such they cover the concentration range of $8\text{--}100\text{ }\mu\text{g mL}^{-1}$. $20\text{ }\mu\text{L}$ were injected in triplicates to liquid chromatograph. Relative peak area values (peak area of biperiden hydrochloride to that of external standard $20\text{ }\mu\text{g mL}^{-1}$) were then plotted against the corresponding concentrations of biperiden hydrochloride to obtain the calibration graph. To reach good equilibria, the analysis was usually performed not before passing $\sim 50\text{--}60\text{ mL}$ of the mobile phase, just for

conditioning and pre-washing of the stationary phase.

The limit of detection (LOD) and limit of quantification (LOQ) were fixed at 3 and 10 times, respectively. The precision of this method expressed by measuring its repeatability and reproducibility. The repeatability has been estimated by analyzing the concentration of BIPER (six replicates), the reproducibility has been estimated by analyzing the same concentration of the five components at three successive days. The precision of the analytical procedure was expressed by the relative standard deviation percentage (RSD%).

Analysis of laboratory prepared mixtures

Laboratory prepared mixtures containing different ratios of BIPER and its oxidative and acid degradation products were prepared, as detailed in TABLE 2, and the mixtures were chromatographed as under the calibration curves starting from: "Sample volumes each of $20\text{ }\mu\text{L}$ were injected ...". The concentration intact BIPER was calculated from its corresponding regression equation.

Analysis of pharmaceutical dosage forms

The average weight of a tablet was determined by weighing not less than 20 tablets. Tablets were pulverized and an aliquot was transferred into 50 mL measuring flasks and was shaken with 25 mL methanol, diluted with methanol to the volume to get the required concentrations. Each tablet's extract was chromatographed as described under the preparation of calibration curves starting from: "Sample volumes each of $20\text{ }\mu\text{L}$ were injected ...". The concentration of BIPER was calculated from its corresponding regression equation.

RESULTS AND DISCUSSION

Method optimization

A simple and reliable stability indicating high performance liquid chromatographic method was developed for determination of biperiden hydrochloride in presence of its oxidative and acid degradation products for its analysis in raw materials and finished pharmaceutical products. Official methods (BP and USP) which based on titration or spectrophotometry are non stability indicating methods and unable to detect the

degradation products of biperiden hydrochloride. By applying the proposed HPLC method on many batches of raw materials, it was found that some of them were suffering from the presence of degradation products and their potencies was found to be less than the minimum permitted limits which affects the efficiency and shelf-life of the finished product. Oxidative and acid degradation of biperiden hydrochloride were carried out as mentioned in the procedures. Mass spectra for the oxidative degradation product were characterized by its molecular ion peak at m/z 218, and for the two acid degradation products at m/z 218 and m/z 279 respectively.

Method optimization

Several trials have been carried out to obtain satisfactory separation between intact biperiden hydrochloride and its oxidative and acid degradation products.

Choice of mobile phase

The trials involved the use of different mobile phases with different flow rates and ratios. The mobile phase of choice was found to be 0.2% 0.1 N perchloric acid in 0.01 M sodium perchlorate solution and acetonitrile in a ratio (50 : 50 v/v) with isocratic elution. It was found that addition of 0.2% 1N perchloric acid increases the sharpness and accelerate the elution of BIPER peak, increasing the concentration of perchloric acid leads to bad resolution between the intact BIPER peak and its degradation products peak. Increasing the ratio of acetonitrile leads to bad resolution between peaks, and its decrease leads to broadening and delaying in all peaks.

Choice of stationary phase

Different stationary phases C_8 and C_{18} with different dimensions and particle sizes were used, it was found that C_{18} column with particle size $5\mu\text{m}$ gave the optimum resolution, while the use of C_8 column failed in this separation. 25 cm was found to be the optimum column length and when it was substituted with shorter length C_{18} column ($5\mu\text{m}$, 15 cm x 4.6 mm, id.) it gave bad resolution between peaks.

Choice of detector wavelengths

The choice of wavelength was optimised at 210 nm at which good absorbances in methanol was

achieved.

Validation

System suitability and final assay condition

System suitability parameters calculated under the optimized experimental conditions. The retention time values of the separated peaks together with other chromatographic parameters are collected in TABLE 1. The table describes the calculated resolution values (R_s) as well as selectivity factor (α) which ensures complete or 100% separation of the components under investigation. The Tailing factor of drug peak also revealed linear isotherm peak elution without tailing. On applying the described experimental optimized HPLC-conditions, excellent separation of BIPER and its oxidative and acid degradation products was established (Figures 2-4).

TABLE 1 : System suitability parameters

Parameter	Biperiden hydrochloride
Retention time(t_R) minutes	5.035
Capacity factor (k')	3.58
Resolution (R_s)	5.04
Selectivity factor (α)	4.03
Tailing factor*	1.04
Theoretical plates (column efficiency)	10182

a Reference values ; $R_s > 0.8$; $T = 1$, for a typical symmetrical peak; $\alpha > 1$; $K = 1 - 10$ are acceptable; Theoretical plate = The higher the value, the more the column efficiency

Linearity

The linearity was evaluated by determining the standard solutions of intact biperiden hydrochloride in different ranges. Calibration curve was constructed relating the relative peak areas to the corresponding concentrations.

Linear relationship was obtained; the regression equation was computed between the relative peak areas (peak areas of BIPER to that of external standard $20\mu\text{g mL}^{-1}$) versus the corresponding concentrations. Tab. 3

Accuracy and precision

Accuracy of the results was calculated by % recovery of pure samples of intact drug analyzed by the proposed method (TABLE 2). The percentage recoveries biperiden hydrochloride in laboratory prepared mixtures of them were determined (TABLE 2). One-

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way ANOVA was used to compare the results of the proposed method and a reference one, where it was concluded that there is no statistically significant differ-

ences between them. The inter- & intra-day accuracy and precision of the proposed method were also determined (TABLE 3). The relative standard deviation expressed in percentage (RSD%) of the assay results was used to assess the method precision.

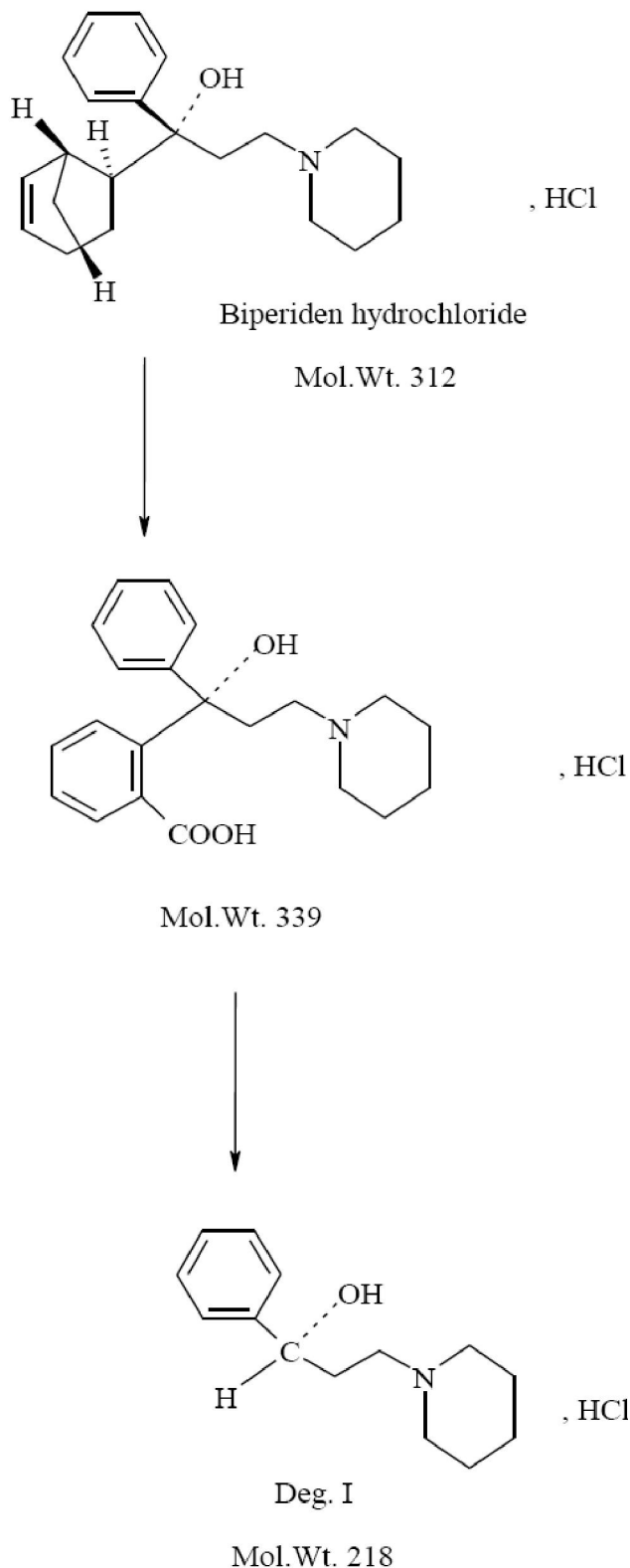


Figure 2 : Oxidative degradation pathway of biperiden hydrochloride.

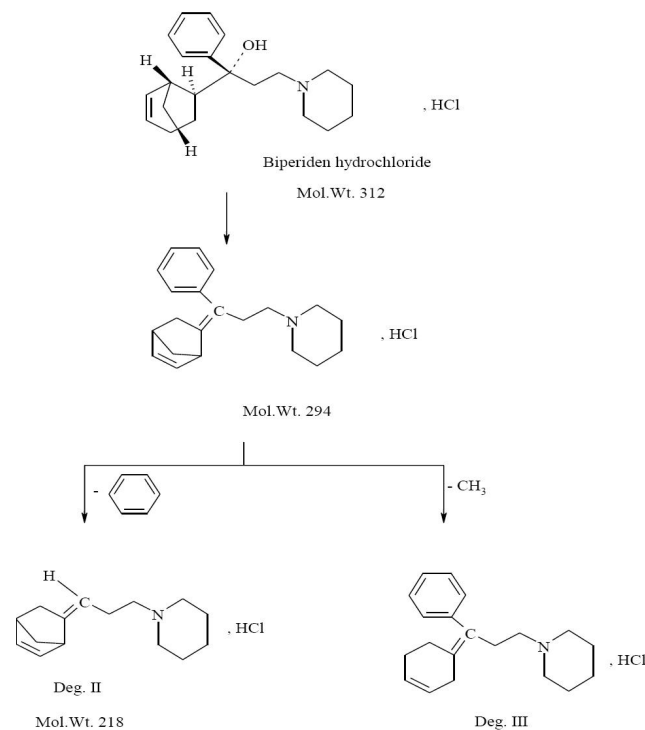


Figure 3 : Acid degradation pathway of biperiden hydrochloride

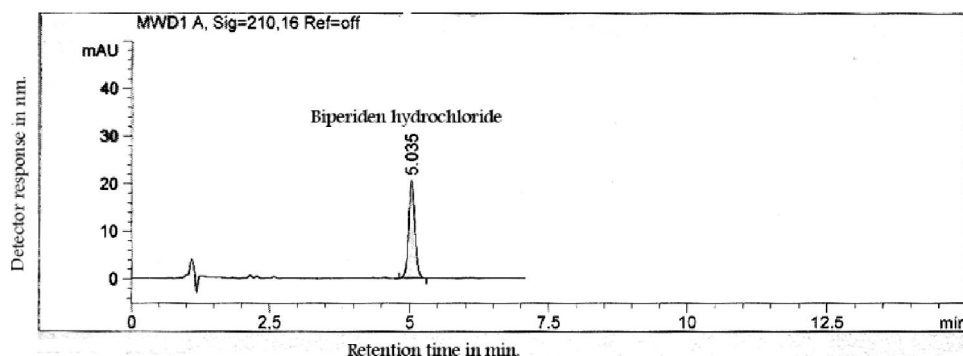
TABLE 2 : Summary of the validation parameters of the proposed HPLC method

Parameters	Biperiden hydrochloride
<u>Linearity</u>	
Slope	0.0491
Intercept	+0.032
Correlation coefficient (r)	0.9999
Range ($\mu\text{g/mL}$)	8 - 100
Accuracy (Mean \pm RSD) %	99.71 \pm 0.807
<u>Precision (RSD%)</u>	
Repeatability*	0.316 - 0.225
Intermediate precision**	0.721 - 0.503
Limit of detection ($\mu\text{g/mL}$)	2.152
Limit of quantitation ($\mu\text{g/mL}$)	6.521

* The intraday (n = 3), average of three different concentrations repeated three times within the day.; ** The interday (n = 3), average of three different concentrations repeated three times in three successive days

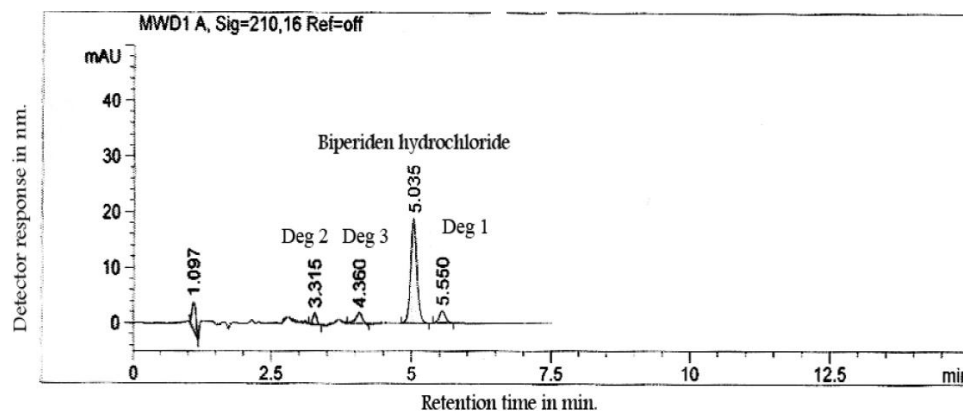
Specificity

Specificity is the ability of the analytical method to



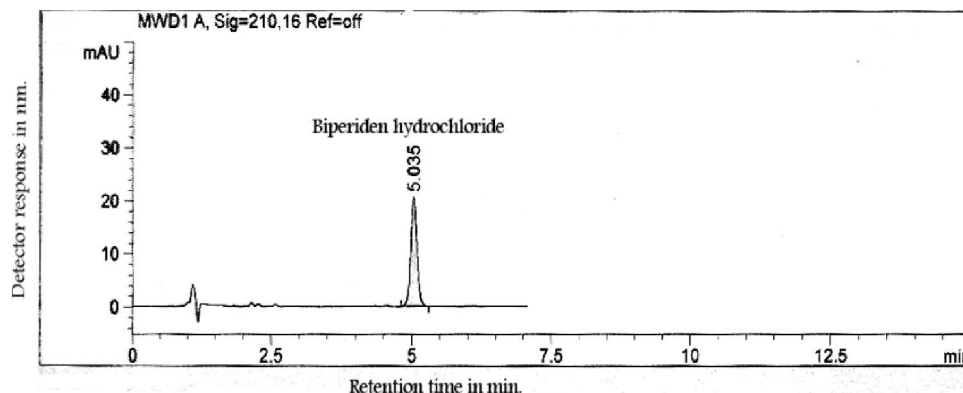
HPLC chromatogram of standard solution of intact biperiden hydrochloride in methanol ($40\mu\text{g.mL}^{-1}$).

Figure 4 : Liquid chromatographic separation of analgin (1.59 min.), caffeine (2.389 min.) and ergotamine tartarate (4.68 min.) from Amigrain™ tablets (by following the specified chromatographic conditions).



HPLC chromatogram of laboratory prepared mixture containing intact biperiden hydrochloride ($40\mu\text{g.mL}^{-1}$), oxidative degradation products (Deg1) and acid degradation products (Deg 2 & Deg 3) in methanol.

Figure 5 : chemical structures of the five components



HPLC chromatogram of Akinitone® tablets containing $40\mu\text{g.mL}^{-1}$ biperiden hydrochloride in methanol

Figure 6 : Liquid chromatographic separation of analgin (1.59 min.), paracetamol (1.94 min.), caffeine (2.39 min), domperidone (3.64 min.) and ergotamine tartarate (4.69 min.), as illustrated under the specified chromatographic condition.

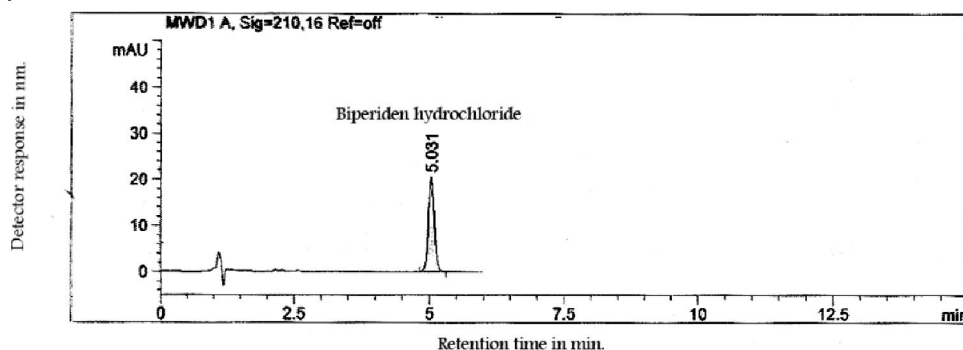
measure the analyte response in the presence of interferences (degradation products, related substances, excipients, etc.). In application of the proposed methods to pharmaceutical formulation no interference from the tablet's excipients appeared. Hence the proposed method is able to determine the named drug selectively in their pharmaceutical formulations. Standard addition

technique (SAT) has been also applied to assess the accuracy and specificity of the proposed method (TABLES 4).

Robustness

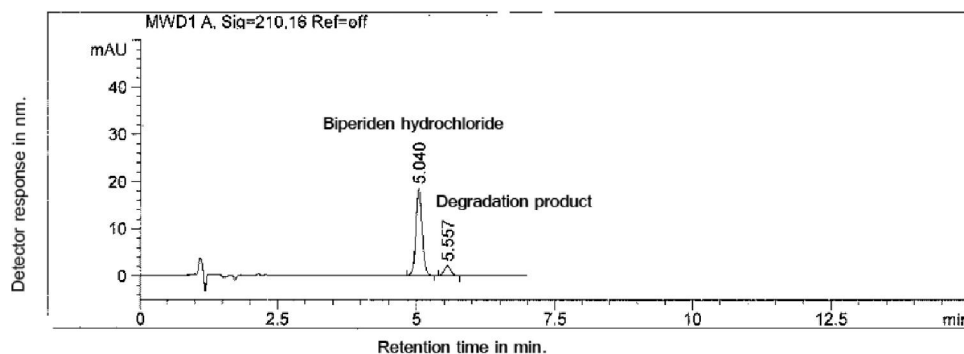
The robustness of a method is its ability to remain unaffected by small changes in parameters. In HPLC

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HPLC chromatogram of Achitnone® tablets containing 40 µg.mL⁻¹ biperiden hydrochloride in methanol.

Figure 7 : Liquid chromatographic separation of paracetamol (1.93 min.), caffeine (2.38 min.), domperidone (3.63 min.) and ergotamine tartarate (4.66 min.) from No-migrain® tablets (see the specified chromatographic conditions).



HPLC chromatogram of Achitnone® tablets containing 35.80 µg.mL⁻¹ intact biperiden hydrochloride in methanol and shows the presence of oxidative degradation products

TABLE 3 : Analysis of laboratory prepared mixtures of biperiden hydrochloride and its oxidative and acid degradation products by the proposed HPLC method

Mix. No.	Deg. %	Intact µg.mL ⁻¹	Deg. µg.mL ⁻¹	Recovery % of intact
1	20	32	8	100.06
2	40	24	16	100.13
3	60	16	24	99.82
4	80	8	32	100.25

Deg.: oxidative or acid degradation products.; % Deg.: % of oxidative and/or acid degradation products added relative to the intact.

method, small changes in proportions of different components, by up to ±0.5 % mainly of the organic part of the mobile phase, in addition to the ionic strength of the o-phosphate salt component, which did not affect the good separation of the components. Only very minor changes in both of the resolution values (R_s) and the selectivity factors (α).

Limit of detection and limit of quantification

According to ICH recommendations the approach based on SD-values of the responses and the corre-

TABLE 4 : Comparison between the analysis of biperiden hydrochloride in pharmaceutical dosage forms (Akinitone® and Achitnone® tablets) by the proposed HPLC method and by the official method

	Proposed HPLC method	Official method ⁸
Akinitone® tablets Batch No. 510091	99.32 ± 0.317	99.16 ± 0.704
Akinitone® tablets Batch No. 410117	96.16 ± 0.205	99.44 ± 0.821
Achitnone® tablets Batch No. 810082	100.93 ± 0.156	100.61 ± 0.668
Achitnone® tablets Batch No. 710202	89.49 ± 0.418	98.14 ± 0.702

*Manufacturer follows the USP-31 official method as described in the literature review for each compound

sponding slopes, the detection and quantitation limits were determined. The theoretical values were assessed

TABLE 5 : Comparison between the analysis of biperiden hydrochloride in different raw material samples by the proposed HPLC method and by the official method

	Proposed HPLC method	Official method ⁸
Batch No. BPH050907	91.13 ± 0.263	100.62 ± 0.274
Batch No. BPH071007	79.83 ± 0.306	99.32 ± 0.322
Batch No. BPH040907	99.71 ± 0.218	99.64 ± 0.501

*Manufacturer follow the USP-31 official method as described in the literature review for each compound

TABLE 6 : Statistical analysis of the results obtained by the proposed HPLC method and the official method for biperiden hydrochloride in pure form

	Proposed HPLC method	Official method ⁸
Mean	99.71	99.64
SD	0.804	0.499
RSD%	0.807	0.501
Variance	0.646	0.249
n	7	6
Student's t-test	0.185 (2.201)**	-
F-Value	2.594 (4.95)**	-

** The figures in parenthesis are the corresponding tabulated values at P = 0.05

practically as they are given in TABLE 2.

Stability

Analyzing commercial samples kept at room temperatures (~22±0.5 °C) on the laboratory bench or in the refrigerator (~5°C) for two weeks has been carried out. The results were found to be not deviated from those in cases of the laboratory prepared mixtures.

CONCLUSION

The goal of this work was achieved by quantification of biperiden hydrochloride by HPLC method in the presence of its oxidative and acid degradation products.

The proposed HPLC method is suitable for the analysis of biperiden hydrochloride either in raw material or in its pharmaceutical preparations such as Akinitone[®] and Achtinone tablets.

This method is very helpful in establishment of ki-

netic studies for improvement of biperiden hydrochloride stability in tablets dosage form.

The obtained results were statistically compared with those obtained by reference ones using one-way ANOVA-testing, from which it is concluded that there is no significant difference between both sides. The suggested method can be successfully applied to the analysis of the cited components single, combined in laboratory prepared mixtures and in the pharmaceutical preparations. The validity of the proposed method is further assessed by applying the standard addition technique.

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