Доклади на Българската академия на науките
Comptes rendus de l'Académie bulgare des Sciences
Tome 76, No 2, 2023

CHEMISTRY
Analytical chemistry

HPLC DETERMINATION OF BIPERIDEN IN SOLID DOSAGE FORMS

Trifon Milchev Angelov#, Biljana Slaveva Krastanova*, Alexandar Svilenov Pashev, Maya Chavdarova Jotova

Received on September 6, 2022
Presented by B. Petrunov, Member of BAS, on December 20, 2022

Abstract

A rapid, new analytical method, based on liquid chromatography with UV detection, has been developed and utilised for the determination of biperiden hydrochloride in solid dosage forms. The chromatographic separation was performed on a conventional C_{18} reversed phase column with a mobile phase composed of acetonitrile-buffer mixture. Ultraviolet detection was carried out at 205 nm. A rapid extraction procedure using ultrasonic bath obtained good extraction yield values for the analyte ($\geq$ 98.4%). The method was developed specifically for routine analysis of biperiden in solid dosage forms in the applicable concentration range. The linearity and range of the method investigated for the assay of biperiden is between 2–6 µg mL^{-1}. The method is selective and sufficiently precise. Thus, the method developed is suitable for routine analysis of biperiden.

Key words: biperiden, HPLC-UV, assay, method development

Introduction. Biperiden is used to treat parkinsonism. It is a muscarinic receptor antagonist. Biperiden is also used in the treatment of arteriosclerotic and postencephalitic parkinsonism, and alleviates extrapyramidal symptoms induced by phenothiazine derivatives and reserpine.

Being a weak peripheral anticholinergic agent, biperiden has, therefore, some antispasmodic, antisecretory and mydriatic effects, and has a nicotinolytic activity [1,2].

#Corresponding author.
DOI:10.7546/CRABS.2023.02.02

The parenteral biperiden rapidly takes under control the akinesia, the akathisia, dyskinetic tremors, rigour, oculogyric crisis and spasmodic torticollis; profuse sweating is markedly reduced or eliminated [1].

Biperiden is a white odourless crystalline powder, which is freely soluble in chloroform, sparingly soluble in alcohol and practically insoluble in water [3]. Usually, the hydrochloric salt of biperiden is used because of its better solubility. Biperiden is a heterocyclic chiral compound (Fig. 1).

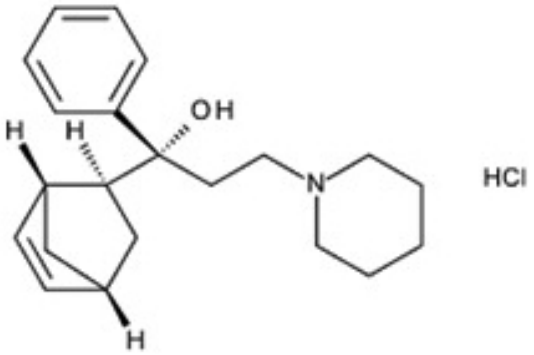


Fig. 1. Chemical structure of the molecule of biperiden hydrochloride

The assay of biperiden hydrochloride in drug formulation is very important for the determination of the correct dosage of the drug and, respectively, for guaranteeing its therapeutic effect. In scientific literature, different analytical techniques for the assay of biperiden are described: UV-Vis spectrophotometry, HPLC and GC [3–6]. As most applicable and useful analytical method, the UV-Vis method described in USP and used up to 2017 has been established. The compendial (USP) methods of assay of biperiden in bulk form involve the use of titrimetric and spectrophotometric procedures, respectively. In comparison to other analytical techniques like HPLC and GC, UV-Vis spectrophotometry has a lower selectivity, and its derivatisation procedure is very complicated and time-consuming. This method was in use for a very long time as the official method of FDA because of its reliability and especially because of the instrument's simplicity in comparison to the HPLC and GC methods. Even the long derivatisation procedure was acceptable, because of the very good repeatability and reproducibility of this UV-Vis analytical method. Among the advantages of the other two techniques – HPLC and GC – are their selectivity (the possibility for simultaneous determination of biperiden in the presence of closely related compounds) and the absence of the time-consuming derivatisation procedure.

There are only several HPLC methods described in scientific literature for the determination of biperiden in drugs [5,7] and in blood plasma [8], and some other methods with techniques related to chromatography [9]. The reason of the

small number of HPLC methods is because of the bad UV absorption of the molecule of biperiden and its hard retention at the condition of reversed phase high performance liquid chromatography at acidic pH of the mobile phase using conventional C_8 or C_{18} columns.

As the pH is specified for the aqueous part of the mobile phase, usually after adding the organic solvent, the pH of the entire mobile phase very often changes. This is the reason why the pH of the complete mobile phase is specified after all the ingredients are mixed. The retention time of the molecules of the investigated compounds strongly depends on their structure and physical properties, but the retention time is also a function of the pH of the mobile phase, especially for ionisable compounds.

As a function of the pH of the mobile phase, the retention time of the investigated compounds depends on their own pKa value.

Even at insignificant changes of the pH of the mobile phase, the change of the retention time for strongly pH-dependent compounds can be remarkable. The strong requirement for the stability of the pH of the mobile phase in HPLC analysis is the reason why well-buffered systems are used. If the pH of the mobile phase is close to the pKa value of the active substances in the studied samples, dissociated and undissociated forms of the investigated compound exist in the sample solution, and usually the peaks of both (dissociated and undissociated) forms of the compound can appear simultaneously in the chromatogram.

As more of the molecules of active pharmaceutical ingredients are molecules with a large non-polar part in the molecule structure, the retention time of such compounds does not depend only on the pH of the environment but the hydrophobic interaction also plays a significant role of the usually non-polar carbon chain of the molecule with the non-polar stationary phase (C_{18} or C_8) of the chromatographic column. In such cases, the retention has mixed-mode mechanism. It is a compromise between the change in the retention according to the change in the polarisation of the molecule, which depends on the dissociation due to the pH and the pKa value of the compound and the hydrophobic "normal" reversed phase retention, respectively.

The purpose of the current work is to develop an easy and quick HPLC method for the assay of biperiden in solid dosage forms and to replace the long and time-consuming USP method. The HPLC methods described in scientific literature use ion-pair reagents or strong acids and the described chromatographic conditions are very hard and unfriendly to the conventional chromatographic columns. When using ion-pair reagents, because of the very long and difficult cleaning procedure, very often the chromatographic column is dedicated especially to analysis with a specific ion-pair reagent and the column is not used for analytical procedures that do not utilise such reagents. The achievement of the developed method will be to make the chromatographic conditions friendlier to the column and to avoid the use of ion-pair reagents which will prolong the column's life.

Experimental. ***Chemicals and reagents.*** Methanol gradient grade, sodium dihydrogen phosphate and H_3PO_4 from Honeywell, sodium perchlorate monohydrate from Merck. Biperiden HCl Working Standard obtained from Sriam Labs, India.

Instruments, experimental conditions and solution preparations.
Chromatographic conditions for the determination of assay:
Apparatus: HPLC Shimadzu Nexera – I LC-2040C Plus
Column: Kromasil 100 C_8 5 µm, 100 × 4.6 mm
Flow: 1.0 ml/min
Column temperature: 25 °C
Wavelength: 205 nm
Chromatography time: 10 min
Injection volume: 20 µl
Mobile phase A: Buffer with pH 2.5
Mobile phase B: Methanol
Preparation of the solutions:
Buffer with pH 2.5: Dissolve 6.0 g sodium dihydrogen phosphate + 2.0 g sodium perchlorate monohydrate in 1000 ml purified water, adjust the pH to 2.5 with H_3PO_4. Filter the buffer through membrane filter with pore size 0.45 µm.
Mobile phase: Phase A:Phase B (40:60)
Solvent: Water, acetonitrile, dissolution medium
Retention time: Biperiden HCl ∼ 4.2 min
Standard solution: Weigh 20.0 mg Biperiden HCl Working Standard and transfer it quantitatively to a volumetric flask of 50.0 ml. Add 12.5 ml purified water and 20.0 ml acetonitrile. Shake on ultrasound for 15 min. After cooling, dilute to the volume with acetonitrile. Filter through membrane filter with pore size 0.45 µm Nylon, throw out the first 4–5 ml of the filtrate.
Dilute 1.0 ml of the solution in a volumetric flask of 100.0 ml to the volume with purified water.
$C_{\text{Biperiden HCl}} = 0.004$ mg/ml.
Sample preparation: Weigh 20 tablets for average mass. From the crushed mixture, weigh an amount equivalent to 2 mg Biperiden HCl in a volumetric flask of 50.0 ml, add 12.5 ml purified water and 20.0 ml acetonitrile. Shake on ultrasound for 20 min. After cooling, dilute to the volume with acetonitrile. Filter through membrane filter with pore size 0.45 µm Nylon, throw out the first 4–5 ml of the filtrate.
Dilute 1.0 ml of the solution in a volumetric flask of 10.0 ml with purified water to the volume.
$C_{\text{Biperiden HCl}} = 0.004$ mg/ml.

Results and discussion. ***Method optimisation and selection of chromatographic conditions.*** In choosing the best chromatographic conditions to obtain the best shape, symmetry and theoretical plates of the peak of the analysed

Table 1

Changes of chromatographic conditions

Tests				
1. Chromatographic columns	Kromasil 100 C_8 5 µm, 100 × 4.6 mm			Restek Pinnacle DB C_8 5 µm, 150 × 4.6 mm
2. Small changes in the flow (±10.0%)	0.9 ml/min	1.0 ml/min	1.1 ml/min	1.0 ml/min
Theoretical plates/meter	8295	7803	7398	7242
Tailing factor	1.28	1.26	1.27	1.30
3. Small changes in mobile phase	57:43	60:40	63:37	60:40
Theoretical plates/meter	8286	7803	7285	7242
Tailing factor	1.27	1.26	1.27	1.30
4. Changes in pH of mobile phase ±0.2	2.3	2.5	2.7	2.5
Theoretical plates/meter	6993	7803	6242	7242
Tailing factor	1.25	1.26	1.22	1.30

compound, we tried different columns, flow rates and ratios of the composites of the mobile phase. The results are presented in Table 1.

The first step of the development of chromatographic conditions is to set a suitable pH range. Phosphate buffer was selected as suitable for use in a wide range of the acidic diapason.

As the molecule of biperiden is pH dependent, it can have full or partial protonation and can obtain respectively full or partial positive charge which affects its retention, depending on the pH of the environment (mobile phase). The charged molecules have bad retention of the conventional C_8 and C_{18} columns, so to improve it usually ion-pair reagents are used [4]. As our aim is to avoid utilisation of an ion-pair reagent, the other option is to use compounds which neutralise the charge of the molecule of biperiden and increase its retention time (chaotropic agents) [6]. Usually ion-pair reagents provide bigger retention to the analytes, as they have a long tail which interacts strongly with C_8 or C_{18} ligands from the RP column surface. Because of this strong interaction, it is very difficult to clean ion-pair reagents from the column. The difference between chaotropic agents and ion-pair reagents is that the molecules of chaotropic agents have only charge but in general are non-polar, while the molecules of the ion-pair reagents have simultaneously polarity and charge. When the molecule of the chaotropic agent approaches the molecule of biperiden which is charged in acidic pH, this will neutralise the positive charge of the molecule of biperiden. Thus, its retention will increase, so the analyte will be detected in a more appropriate retention time and the results will be more reproducible and more reliable. If we choose the suitable concentration of the chaotropic agent, we can adjust the retention time in the chromatogram far enough from the death time of the analytical column and

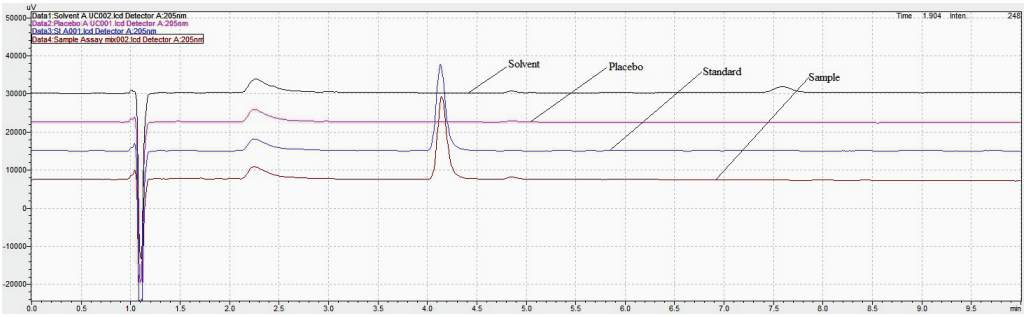


Fig. 2. Solvent, placebo, standard and sample

avoid overlapping peaks, if such exist in the chromatogram. As a chaotropic agent in the current method, sodium perchlorate is used. The advantages of the proposed chromatographic conditions are that avoiding the use of ion-pair reagents and strong acids in the mobile phase, we can prolong the life of the conventional analytical columns used for the assay of drugs containing biperiden.

Method validation. The developed method was validated according to selectivity, linearity and recovery.

Selectivity. The selectivity is proved by the investigation of the solutions of placebo, mobile phase, solvent, reference and test solutions (Fig. 2).

On the chromatograms obtained from the solutions of placebo, mobile phase and solvent, no peaks with the same retention time as the main peak (Biperiden HCl) are found. There are no peaks which can impact the method.

Linearity. The linearity of the method for the determination of assay of biperiden HCl in drug oral solution formulations is set from 50% to 150% of the target concentration. The calculated correlation coefficient is very close to the ideal value. The excellent correlation expressed as R^2 is 0.9997 (Fig. 3).

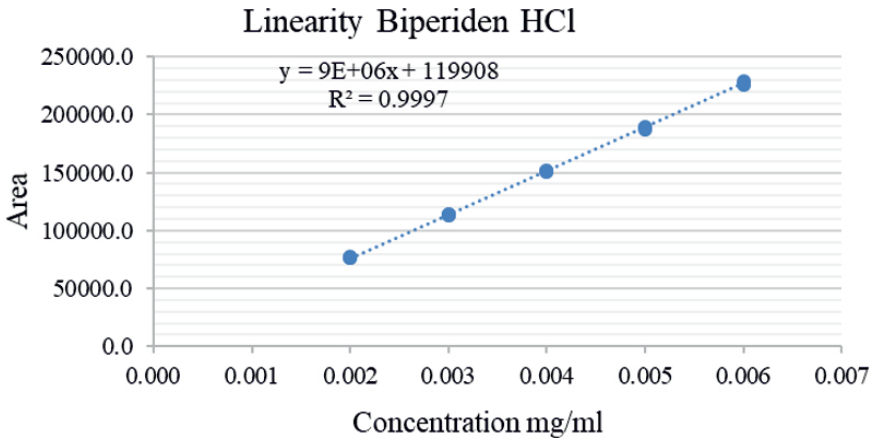


Fig. 3. Linearity of Biperiden HCl

Accuracy. The accuracy of the method was assessed by determining the recovery of Biperiden HCl in a test solution at the following concentrations: 0.0032 mg/ml; 0.0040 mg/ml, and 0.0048 mg/ml, and confirmed by quantitative determination of Biperiden HCl in model mixtures in quantities from 80%, 100% and 120% from its target concentration. Every concentration level is average measurement of three determinations. The mean value of the recovery is 99.13% and RSD of 0.693%.

By changing the different parameters of the analytical procedure shown in Table 1, we can not only obtain the best chromatographic conditions, but also prove the robustness of the analytical method.

Conclusion. The method for the determination of biperiden in solid dosage forms described in the current work is quick, reliable and simple in comparison with UV spectrophotometric methods. The UV methods are time-consuming and with complicated derivatisation procedures.

In comparison to other HPLC methods for the analysis of biperiden, the current method is safer for the analytical column and quicker because of its chromatographic conditions. As it avoids strong acids in the composition of the mobile phase, this method is less harmful for the stationary phase. Moreover, by avoiding the use of ion-pair reagents, the equilibration time of the column dramatically decreases.

REFERENCES

[1] Drugbank Online. Biperiden. `https://go.drugbank.com/drugs/DB00810` (accessed June 10, 2022).

[2] Kostelnik A., A. Cegan, M. Pohanka (2017) Anti-Parkinson drug biperiden inhibits enzyme acetylcholinesterase, BioMed Res. Int., 1–5, DOI: `https://doi.org/10.1155/2017/2532764`.

[3] United States Pharmacopeial Convention (2011) Official Monographs, Biperiden Hydrochloride Tablets. In: USP35 NF30, Rockville, 2363–2364.

[4] Issa F. A. A., S. M. EL-Helbawy, A. M. Abed EL-Magied (2012) Simultaneous determination of biperiden and chlorphenoxamine in various dosage forms by spectrophotometry, Anal. Chem.: Indian J., **11**(2), 49–55.

[5] Mohammadi A., A. Mehramizi, F. A. Moghaddam, L. E. Jabarian, M. Pourfarzib et al. (2007) Development and validation of a stability-indicating high performance liquid chromatographic (HPLC) assay for biperiden in bulk form and pharmaceutical dosage forms, J. Chromatogr. B, **854**(1–2), 152–157.

[6] Capacio B. R., S. T. Caro, J. R. Smith, C. E. Byers (2002) The determination of biperiden in plasma using gas chromatography mass spectrometry: pharmacokinetics after intramuscular administration to guinea pigs, Biomed. Chromatogr., **16**(1), 47–55, DOI: `https://doi.org/10.1002/bmc.113`.

[7] Lotfy H. M., E. M. Abdel-Moety, E. G. Nouman (2014) Stability indicating HPLC-determination of biperiden hydrochloride in presence of its oxidative and acid

degradation products, Anal. Chem.: Indian J., **14**(3), 106–114.

[8] Hadidi K. A. (2004) Development of a screening method for the most commonly abused anticholinergic drugs in Jordan; trihexyphenidyl, procyclidine and biperiden, Legal Medicine, **6**(4), 233–241, DOI: https://doi.org/10.1016/j.legalmed.2004.06.001.

[9] Mandrioli R., A. Musenga, S. S. Lasaponara, M. A. Saracino, S. Fanali et al. (2006) Enantioseparation and quality control of biperiden in pharmaceutical formulations by capillary electrophoresis, Analytica Chimica Acta, **560**(1–2), 57–63, DOI: https://doi.org/10.1016/j.aca.2005.12.027.

Faculty of Pharmacy
Medical University of Pleven
1 St. Kliment Ohridski St
5800 Pleven, Bulgaria
e-mails: t.angelov-mupleven@outlook.com
alexandar.pashev@gmail.com
m_jotova@mu-pleven.bg

* *Tchaikapharma High Quality Medicines Inc.*
1 G. M. Dimitrov Blvd
1172 Sofia, Bulgaria
e-mail:
b.Krastanova.hq@tchaikapharma.com