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Simple high-performance liquid chromatographic method for determination of Donepezil HCl in pharmaceutical formulations

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Abstract: Donepezil HCl is a hydrochloride salt of a piperidine derivative acetylcholinesterase inhibitor and, it uses in treatment demantia of Alzheimer's disease. In this study, a sensitive and rapid HPLC-UV method was developed and validated for determination of Donepezil HCl in API and tablet dosage forms. Chromatographic separation was performed using a Ace 5 C18 (5 µm, 250 x 4.6 mm) by using isocratic phosphate buffer at pH:2.0 and acetonitrile (55:45, v/v) mobile phase was used at the rate of 1.2 mL/min. The column temperature was set at 30 °C and the UV detection was recorded at 268 nm. The method was validated with respect to specificity, precision, accuracy, linearity, repeatability and reproducibility parameters in a concentration range of 25-125 µg/mL. The limit of detection (LOD) and limit of quantification (LOQ) were determined as 1.40 and4.20 µg/mL, respectively. The uncertainty budget of the measurement for Donepezil HCl was estiamted as 5.80 % at 95% confidence level (k = 2).

Keywords: Donepezil HCl; HPLC-UV; pharmaceuticals; method validation; measurement uncertainty.

1. Introduction

Alzheimer's disease is a neurodegenerative condition characterized by gradual memory loss and complete dementia following it [1]. Donepezil HCl (DHCl, Table S1) is a piperidine-based drug developed specifically for the treatment of Alzheimer's disease. It belongs to a class of acetylcholinesterase inhibitors having N-benzylpiperidine and an indanone moiety which shows longer and more selective action. It is the second drug approved by FDA for the treatment of mild to moderate Alzheimer's disease [2-3].

A few methods have been developed to determine DHCl, including spectrophotometry [4,5] and voltammetry [6,7] in the literature. However sensitivity of those methods were found as very poor. In addition, more sensitive high-performance liquid chromatography with UV Detection (HPLC-UV) methods for determination of Donepezil in pharmaceutical preparations [8-10] and plasma [11,12] were

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also reported. Nonetheless, most of the above methods have low precision and/or require long time. In further studies, liquid chromatography–mass spectrometry (LC-MS) [13,14], high-performance liquid chromatography with fluorescence detection (HPLC-FD) [15-16] and capillary electrophoresis (CE) [17,18] were reported. However, LC-MS is an expensive tool for routine applications and capillary electrophoresis facility are not available at the pharmaceutical laboratories commonly.

In this study, we aimed to present and validate a robust and efficient HPLC method for quantification of DHCl in drug formulations for the routine quality control activities.

2. Experimental

2.1. Chemicals

All of the chemicals were used without further purification. The DHCl standard (≥98%, Sigma-Aldrich), acetonitrile (≥99.9%, Sigma-Aldrich), potassium dihydrogen phosphate (99.5-100.5%, Sigma-Aldrich), *ortho*-phosphoric acid (≥85%, Sigma-Aldrich) and trimethylamine (≥99.5%, Sigma-Aldrich) were used. A commercial Dozyl film tablet (10 mg, produced by AbdiİbrahimPharmaceutical Company in İstanbul, Turkey) was purchased from a local pharmacy in Uşak. Ultrapure water was obtained using a Milli-Q system (Millipore) with conductivity lower than 0.05 µS/cm.

2.2. Determination of λmax

20 µg/mL standard solution of DHCl in ultrapure water was scanned on a UV spectrophotometer (Shimadzu UV-1800 spectrophotometer). λ_{max} was obtained from the UV spectrum of DHCl (Figure S1).

2.3. Chromatographic Conditions

HPLC experiments were performed by an Agillent Technologies 1260 series HPLC–UV, equipped with an Ace 5 C18 (250 x 4.6 mm, 5 µm) column. The mobile phase was a mixture of 0.05 M phosphate buffer (pH 2.0) and acetonitrile (55:45). For buffer solution: 6.80 g potassium dihydrogen phosphate was accurately weighed. The volume was completed in liters by dissolving in deionized water. Then, 5 mL of triethylamine was added. The pH of the obtained solution was adjusted to 2.3 by adding *ortho*-phosphoric acid. Mobile phase was filtered through a 0.45 µm Millipore Millex-HV filter. Before use, the mobile phase was degassed for 30 min. in an ultrasonic bath. The samples were monitored with UV detection at 268 nm at the flow rate of 1.2 mL/min. Column oven temperature was set to 30 °C and run time of measurement was used as 10 minutes. The mobile phase was used as a diluent for blank preparation and injection volume was 20 µL for all samples.

2.4. Preparation of Standard Solutions

The standard of 50.00 mg DHCl was accurately weighed and transferred to a 100 mL volumetric flask (Class A), and mixed with 35 mL of deionized water, dissolved in an ultrasonic bath until a clear solution was obtained, equilibrated to room temperature (~24 ^{0}C) and, completed to volume with deionized water.

2.5. Preparation of Sample

Ten of tablets were weighed and crushed in mortar and ground into fine powder. Powder samples equal to ~50 mg of DHCl were placed in a 500 mL volumetric flask, diluted with 400 mL of deionized water and dissolved in an ultrasonic bath for 30 minutes. It was equilibrated at room temperature (~24 ^{0}C) and, completed to volume with deionized water. All samples were filtered through a 0.45 µm Millipore Millex-HV filter before injection.

2.6. Method Validation Procedure

DHCl solution, 20 μg/mL in deionized water, was tested by the UV spectrophotometer. λ*max* was obtained from the UV spectrum of DHCl at 268 nm (Figure S1).

The validation study was performed in compliance with the recommendations for validation of the International Conference on Harmonization and the United States Pharmacopoeia [19,20]. Parameters are studied during method qualification as specificity, linearity, accuracy, precision, and suitability of the system, robustness, limit of detection (LOD) and limit of quantification (LOQ).

The method was primarily designed for measuring high drug loads and dissolution tests of drugs. A specific concentration range was selected for the validation procedure based on those considerations. Accordingly, the concentration range for DHCl was selected as 25-125 μg/mL for method validation.

The specificity which is an essential part of the validation of the method has been assessed as follows: Firstly, a collection of this analyte's standard stock solution (DHCl reference material dissolved in ultrapure water) was prepared. Different concentrations of standard solutions were prepared in ultra-pure water from the stock standard solution. Then, the impact of excipients used in the manufacture of the selected marketed dosage forms on the proper assessment of DHCl peaks were screened. After sample analysis, the chromatograms were assessed for peak area and excipient interference at retention periods of DHCl.

The linearity of the analytical method was determined by preparing and injecting standard solutions in the range of 25 to 125 μg/mL of DHCl solution. The calibration curve was formed by drawing the peak area against the concentration of DHCl and the regression equation slope and intersection point were calculated. Each solution was injected six times. Three separate series of calibration standards have been prepared for each calibration interval in order to create linearity.

The accuracy and recovery of method was determined by calculating recoveries by spiking method. Known amount of standard solutions of 10, 50, 150 μg/mL were spiked to known amount sample solutions (50 μg/mL). The amount was estimated by the regression equation of the calibration curve.

The precision was evaluated by repeatability and reproducibility of the method. The intra-day repeatability was assessed by determining the relative standard deviation (RSD %) of the areas obtained from the injection of six replicates of the standard solutions (DHCl, in concentration 50 mg/L) within day. In the evaluation of reproducibility (inter day repeatability), the same standard with concentration 50 μg/mL was injected once a day during a period of six non-consecutive days.

The suitability of the system was assessed by determining the tailing factor, retention factor, sum of theoretical plates, reproducibility of peak areas and retention times. Various method parameters were tested to assess the reliability and robustness of the proposed HPLC method. The self-imposed parameters limits set for these parameters have been inspired by other publications and our experience. We studied parameters as follows: The flow of the mobile phase (± 0.10 mL/min), column temperature (± 5 °C), buffer concentration (± 0.01 M) and pH value (± 0.10) for the mobile phase. It was investigated these parameters by injecting a series of dilutions with three individual standards for lowest, medium and highest concentrations of Donepezil in triplicate. The robustness of the system has been measured by absolute average recovery, RSD (precision) and R^2 of the calibration curves that resulted.

LOD of the drug is a characteristic value for the method's precision in which the corresponding compound is only measurable, whereas LOQ is the lowest concentration with acceptable linearity, accuracy, and certainty. LOD was determined on the based on 3:1 signal-to-noise ratio, while LOQ was determined on 10:1 signal-to-noise ratio.

3. Results and discussion

3.1. Optimization of Method

Several reverse phase HPLC columns (C18 columns) were tested for the optimization study together with mobile phase trials. The shorter columns with the same or fixed period did not allow for a proper peak symmetry and number of plates as achieved in Ace 5 C18 (250 x 4.6 mm, 5 µm) and LiChrospher 100 RP-18 (250 x 4.6 mm, 5 µm) columns. Thus we decided to use 250 mm column length (Ace 5 C18 (250 x 4.6 mm, 5 µm) in validation study as reported in previous studies [8, 9, 11, 12, 16]. After the selection of the chromatographic column, the mobile phase was chosen as a mixture of 0.05 M phosphate buffer (mobile phase A) (pH 2.0) and acetonitrile (mobile phase B) (55:45, v/v). The injection volume and column temperature were determined and, the initial isocratic program was also modified step by step. Finally, chromatographic analysis was conducted on Ace 5 C18 (250 x 4.6 mm, 5 µm), column oven temperature was set to 30 °C, with a flow rate of 1.2 mL/min and a run time of experiment 10 min at 268 nm.

3.2. Specificity

The specificity of the method was evaluated by comparing chromatograms of blank, spike, drug and its combination with excipients. No peak interference was observed between the analytes with blank media, buffer components or excipients of the marketed dosage formulations at the retention time time 3.113 for DHCl (Figure S2).

3.3. Linearity

The linearity of the method was assessed by analyzing standard solutions in the range of 2 5–125 µg/mL of DHCl. Correlation coefficient (r) were ≥0.9999. The linear regression equation was determined as y=25.553x+2.151, where y is the peak area and x is the concentration in µg/mL (Table 1 and Figure S3).

3.4. Recovery, Accuracy and Precision

Recovery experiments were conducted to determine the accuracy of the developed method for the quantification of DHCl. It was determined by spiking of standard solutions of 10, 50, 150 µg/mL to known amount sample solutions (50 µg/mL). RSD % for the DHCl were determined in the range of 0.10-0.31% and the recovery of the method found as 100.0 % (Table S2).

Precision of the applied method was determined by repeating the measurements (n=6) at the declared concentraiton level of the drug on the same day. Intra-day precision (repeatability) and inter-day (intermediate) precision were found to be 1.44% and 3.54%, respectively (Table S3).

Table 1. Summary of validation data of developed HPLC-UV method

RT (min)	Linear range (µg/ mL)	Linear regression equation	R^2	Recovery %	LOD/LOQ (µg/ mL)	U (k=2)
3.113	25.00-125.00	y=25.553x+2.151	0.9999	100.32	1.40/4.20	5.80

3.5. Robustness

The robustness study data show that the linearity, absolute mean recovery and precision of the developed method remain unaffected by small changes in critical method parameters. The corresponding results have been shown in Table S4. Temperature variations, flow rate, ionic strength and pH value did

not affect recovered amount of analyte. The absolute mean drug recovery for all substances was between 99.0% and 101.0% and the level of RSD was less than 1.0%.

The properties of the previously developed HPLC methods and the proposed method are presented in Table 2

3.6. System Suitability

Primary parameters to evaluate system suitability such as symmetry factor, retention factor were evaluated for the lowest (30 μg/mL), medium (60 μg/mL) and highest (120 μg/mL) concentrations of DHCl (Table S5). The method is provided pretty good peak symmetry and, the peaks of the analytes showed consistently low variability in peak areas and retention times. Calibration curve coefficient in this study was above 0.999, which indicates that the method was suitable for samples with simple or rather complex matrices.

3.8. Estimation of Uncertainty

The bottom-up approach was used to estimate the measurement of the uncertainty of the method. The main sources for uncertainty were considered as weighing the sample, calibration curve, recovery reproducibility of the method. Sources and quantification of the uncertainty for the applied method were determined by using EURACHEM/CITAC guide and previous reports on the same title [21-25]. Herein, we summarized the calculation of combined standard measurement uncertainty of the analyte in pharmaceutical formulations in equation 1. The expanded measurement uncertainty was obtained by multiplying the combined standard measurement uncertainty value with 2 (coverage factor) at 95 % confidence level. The main contribution comes from the reproducibility of the method as expected.

$$\frac{u_c(A)}{C_A} = \sqrt{\left(\left(\frac{u}{W_{ss}}\right)^2 + \left(\frac{u\,c_0}{c_0}\right)^2 + u\,(R_M)^2 + u\,(r)^2\right)} \quad (1)$$

where, *uc(A):* Combined standard measurement uncertainty of the analyte
CA: Concentration of the target analyte
u(WSS): Combined standard measurement uncertainty of the sample intake
WSS: Weight of the starting sample
u(c0): Combined standard measurement uncertainty of the calibration curve
c0: Determined concentration of the sample by using the calibration curve
u(Rm): Combined standard measurement uncertainty of recovery
u(r): Standard measurement uncertainty of reproducibility

3.9. Determination of DHCl in the Pharmaceutical Formulations

The approach proposed was applied in the pharmaceutical forms to quantitatively evaluate DHCl. The obtained results were in the agreement with the results specified in the approved labeled content of DHCl (Table 2). The simplicity of procedure, and the short run time make this method suitable for quick and routine analyses. Hence, this method can be adopted effectively for routine quality control measurements in pharma industry.

Table 2. Determination of DHCl in tablets by the proposed methods (n=5)

	Label claim[a] (mg / tablet)	**Mean ± U (k=2)**
Proposed method	10	10.05 ± 0.58

[a]Dozyl Film Tablet® (10 mg)

Table 3. Review of the characteristics of the previously mentioned HPLC methods and the proposed method

Mobile phase	Column	Concentration range	Correlation coefficient (R^2)	Precision (RSD, %)	LOD	Retention time (min)	Application	Reference
Phosphate buffer (0.005 M, pH 3.67) and methanol.	Uptisphere ODB C-18 column (250 x 4.6 mm, 5 µm)	0.35–0.64 mg/mL	0.9980	< 0.3	0.06 µg/mL	6.5	Pharmaceutical formulations and impurities	[8]
Methanol, phosphate buffer 0.02 M and triethylamine (50:50:0.5)	C18 (Microsorb-MV)	10–60 mg/mL	0.9995	0.5	10 µg/mL	9	Pharmaceutical formulations	[9]
Methanol, phosphate buffer (0.02 mol/L) and triethyl amine (pH 3.5) (55:4 :0.5)	C18 STR ODS-II (250 X 4.6 mm, 5 µm)	3–90 ng/mL	0.9987	7.3–7.6	3 ng/mL	8.0	Human plasma	[11]
Hexane,isopropanol and triethylamine (87:12.9:0.1)	Chiralcel OD (Chiral column)	0.05–2 µg/mL	0.9940	< 10	20 ng/mL	12.8	Pharmaceutical formulations and human plasma	[12]
Methanol, phosphate buffer (0.02 mol/L) and triethyl amine (pH 3.5) (55:45:0.5)	C18 Phenyl Hypersil (125 X 4.6 mm, 3 µm)	5–2000 ng/mL	0.9980	< 6.5	2.0 ng/mL	11.4	Pharmaceutical formulations and human plasma	[15]
25 mM citric acid/50 mM Na_2HPO_4 (pH 6.0)-CH_3CN (73:27) containing 3.5 mM sodium 1-octanesulfonate	C30 Develosil Combi-RP-5 (250 X 4.6 mm, 5 µm)	5–500 n mol/L 10–500 n mol/L 1–50 n mol/L	0.9990 1.0000 0.9990	< 9.3	2.5 n mol/L 5.0 n mol/L 0.5 n mol/L	11.6	Rat plasma microdialysate human plasma	[16]
0.05 M phosphate buffer pH 2.0 and acetonitrile (55:45).	C18 Ace 5 (250 X 4.6 mm, 5 µm)	25-125 µg/mL	0.9999	< 0.55	1.40 µg/mL	3.1	Pharmaceutical formulations	this method

As a conclusion, A RP-HPLC method for an effective measurement of DHCl was developed and validated herein. The method is simple, rapid, precise and accurate and it is useful for the routine measurement of DHCl in tablet and other dosage forms.

Author's Contributions

İbrahim Bulduk drafted and prepared the manuscript and Beyza Sultan Aydın performed the experiment and data analysis .There are no ethical issues after the publication of this manuscript.

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