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A validated HPLC-UV method for determination of dopamine HCl in injectable solutions

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Abstract: Dopamine is a neurotransmitter that acts in the nervous system as a chemical messenger, which helps specific neurons to interact with each other. A simple, precise, accurate HPLC method has been developed and validated for determination of Dopamine HCl (DOPHCl) in pharmaceutical formulations. Separation was performed on a C18 column (250 x 4.6 mm, 5 µm) with 50 mM potassium dihydrogen phosphate (pH 2.3) as a mobile phase. Mobile phase flow rate was maintained constant at 1 mL min^{-1}. High sensitivity has been achieved with UV detection at 280 nm. Parameters such as interaction of the active pharmaceutical ingredient and excipients, linearity and repeatability were investigated during analytical method development. Retention time was 6.195 min for DOPHCl. The method has been validated for DOPHCl within the range of 20-100 µg mL^{-1} (r^2=0.9998). This method has shown strong reproductibility and recovery within the determined range. This method can be used successfully in pharmaceutical formulations for routine analysis.

Keywords: Dopamine HCl, HPLC, Method, Validation

1. Introduction

Dopamine is a neurotransmitter that acts in the nervous system as a chemical transmitter, which helps specific neurons to interact with each other. It belongs to the family of molecules classified as monoamines, and more precisely to the catecholamine group of chemicals. Dopamine can function either as an excitatory mechanism or as an inhibitory mechanism in the central nervous system depending on the location of dopamine neurons and the receiving characteristics of the next neuron in the chain (Berridge & Robinson, 2018). It plays a significant role in neural functions, including endocrine control, breathing, locomotion, perception and reward (Neve, 2010). In addition, It plays a cirucial role in the function of cardiovascular, renal and hormonal (Hussain and Lokhandwala, 2003; Jose et al., 1998; Ivan et al., 2005; Damase-Michel, Montastruc, 1995). It has been documented that a lack of dopamine containing neurons contribute to neurological disorders, such as schizophrenia and parkinsonism (Guo et al., 2009). Sensitive and precise determination of dopamine is therefore critical not just for diagnosis but also for pathological study. DOPHCl, a hydrochloride salt of dopamine (Figure 1), is widely used in clinical therapies.

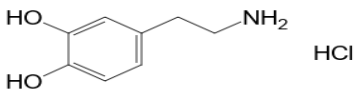


Figure 1. DOPHCl's chemical structure

A variety of techniques have been used for the determination of DOPHCl over the years. Many methods have been documented to date, such as fluorometry, chemiluminescence, high performance liquid chromatography, electrochemical processes, mass spectrometry, capillary electrophoresis for accurate analysis of dopamine. MS detector (Carrera et al., 2007; Zhao&Suo, 2008), UV detector (Liu et al.,2004) fiber optic detector (Silva et al., 2009; Ferreira et al., 2009), electrochemical detector (McKenzie et al., 2002; Cannazza et al., 2005, Sultan et al., 2014; Gupta et al., 2015; Palanisamy et al., 2015; Haldorai et al., 2017) and fluorescent detector (Yoshitake et al., 2004; Fujino et al., 2003) have been widely used in HPLC analysis. Among these methods,

electrochemical methods have attracted attention because of their practicality, sensitivity, accuracy, low cost and portability. However, many of these methods are expensive and are not available in every laboratory. In addition, many of these methods have been developed for dopamine analysis in biological matrices. A simple, fast, precise and accurate method is required for routine determination of DOPHCl in pharmaceutical dosage forms. There are injectable preparations containing DOPHCl at the concentration of 40 mg mL^{-1} and 10 mg mL^{-1} in the Turkish pharmaceutical market. This study introduces the validation of an analytic HPLC method of dopamine hydrochloride determination in injectable preparations.

2. Experimental

2.1. Reagents

All the chemicals used in this study were in the analytical grade and were used without further purification. Dopamine Hydrochloride standard (≥98.5%, Sigma-Aldrich), potassium dihydrogen phosphate (99.5-100.5%, Sigma-Aldrich), ortho-phosphoric acid (≥85%, Sigma-Aldrich) and triethylamine (≥99.5%, Sigma-Aldrich) were used. Deionized water was purified by a Milli-Q system (Millipore) with conductivity lower than 0.05 µS cm^{-1}. A commercial Dopasel ampule (40 mg mL^{-1} produced by Haver Farma Company in İstanbul, Turkey) was purchased from a local pharmacy in Uşak.

2.2. Stock standard solution

Approximately 50 mg of DOPHCl was weighed accurately using an analytical balance (Mettler Toledo ME204T) and transferred into 50 mL of volumetric flask. It has been dissolved by adding ultra pure water and make up the volume with the same solvent to label. The concentration of the prepared stock standard solution is 1000 µg mL^{-1}. Calibration standards of DOPHCl over the concentration range 20–100 µg mL^{-1} were prepared by serial dilution of the standard stock solution on the day, using ultra pure water as a diluent.

2.3. Sample solution

2.5 ml of infusion solution was measured accurately and transferred in to the 100 mL of volumetric flask. Approximately 50 ml of ultra-pure water was added and sonicated to completely dissolve. The volume is filled with the same solvent, up to 100 ml. This solution was filtered through a 0.45 µm pore size membrane filter. Sample solutions (40 µg mL^{-1}) was prepared by diluting with ultra pure water.

2.4. Determination of λmax

Standard solution (40 µg mL^{-1}) was subjected to scanning between 200-400 nm on a UV spectrophotometer (Shimadzu UV-1800 spectrophotometer). λmax was obtained from the UV spectrum of standard solution.

2.5. Chromatographic conditions

The research were performed using an HPLC system (Agilent Technologies® Model 1260 Infinity) which was equipped with a UV-Visible detector. Chemstation software was used to control the instrument and processing of the data. Chromatographic separation was conducted on Inertsil ODS C18 column, (250 x 4.6 mm, 5 µm). Mobile phase consisting of 0.05 M KH_2PO_4 buffer (pH 2.3) was used in an isocratic mode. The mobile phase was filtered through a 0.45 µm pore size membrane filter and sonicated 15 min before use. The flow rate of the mobile phase was held at 1.0 mL min^{-1} and the injection volume was set as 20 µL. This column was kept at the temperature of 30 °C and the UV detection was conducted at 280 nm.

2.6 Method validation

The analytical method validation was performed as per ICH guidelines of Validation of Analytical Procedure: Q2 (R1) (ICH, 2005; Center for Drug Evaluation and Research (CDER), 1994). The validation parameters such as system suitability, linearity, precision, accuracy, specificity, the limit of detection (LOD), the limit of quantification (LOQ), and robustness were addressed.

System suitability: The system suitability parameters (RSD% for retention time, RSD% for peak area, theoretical plates and tailing factor) were investigated after six replicate injections of standard solution (40 µg mL^{-1}) into the HPLC system.

Linearity: Standard calibration has been prepared using five standard solutions within the concentration range of 20-100 µg mL^{-1}. In optimized chromatographic conditions, each standard solution was chromatographed for 10 minutes five times. The least squares linear regression analysis of the average peak area versus concentration data were used to evaluate the linearity of the method.

Precision: Precision was analyzed by calculating variations of the method in intraday (repeatability performed by analyzing standard solution on the same day) and inter-day (repeatability carried out by analyzing standard solution on three different days). These analyses were performed by injecting six times standard solution (100 µg mL^{-1}).

Accuracy: Recovery experiments were conducted by the standard addition technique to confirm the accuracy of the proposed method. In this method, 80%, 100% and 120% of three different levels of pure drug were added to the previously analyzed sample solutions, and DOPHCl recovery was calculated for each concentration.

Selectivity/Specificity: Selectivity is the analytical method's ability to generate an analyte response when external interference is present. The chromatograms of mobile phase solution (blank) and the sample solution were compared with chromatogram of standard solution to evaluate the selectivity of the proposed method. Retention time (Rt) and queuing factor parameters were calculated to demonstrate that chosen method was specific and selective.

LOD and LOQ: These values were determined using the standard error (s) and slope of the regression line (m) as shown in following equations.

$LOD = 3.3 * s/m$

$LOQ = 10 * s/m$

Robustness: A robustness analysis was performed to determine the impact of minor yet systematic differences in chromatographic conditions. The robustness was tested by adjusting minor differences in parameters. The modifications include different concentration of the mobile phase (± 0.01 M), mobile phase flow rates of the mobile phase (± 0.1 mL min^{-1}), pH value of the mobile phase (± 0.10)**,** and column temperatures (± 2 °C). After each change, system suitability parameters were checked by injecting the sample solution into the chromatographic system.

Analysis of marketed formulations (injectable solution)

4 mL of above prepared sample solution was transferred into a volumetric flask of 100 mL and filled the mark with ultra-pure water to prepare the sample solution (approximately at the concentration of 40 µg mL^{-1}). This sample solution was filtered using 0.45 µm pore size membrane filter and then analyzed.

3. Results

3.1. Determination of λmax

The wavelength corresponding to maximum absorbance (λmax) was determined as 280 nm from the UV spectrum of standard solution (Figure 2).

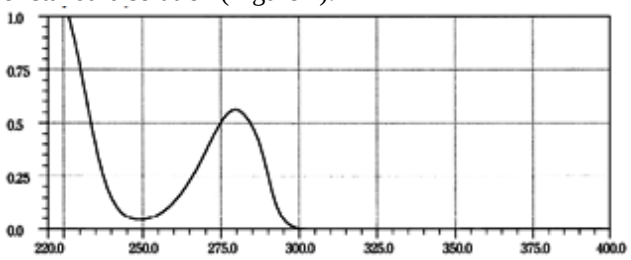


Figure 2. UV spectrum of DOPHCl (20 µg mL-1 standard solution)

3.2. Optimization of Chromatographic Conditions

Several reverse phase HPLC columns (C18 columns) and different mobile phase were tested for the optimization study. The shorter columns did not allow for a proper peak symmetry. Proper peak symmetry was achieved on the Inertsil ODS C18 column (250 x 4.6 mm, 5 µm). Thus, we decided to use the Inertsil ODS C18 column (250 x 4.6 mm, 5 µm) in validation study. After the selection of the chromatographic column, the mobile phase was chosen as a 0.05 M KH2PO4 solution (pH 2.3). The use of chemicals harmful to the environment was avoided in the selection of mobile phase. 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 the Inertsil ODS C18 column (250 x 4.6 mm, 5 µm), column oven temperature was set to 30 °C, with a flow rate of 1.0 mL min^{-1} and a run time is 10 min at 280 nm.

3.3 Method validation

3.3.1. System suitability

Standard solution (100 µg mL^{-1}) was injected six times to the chromatographic system at the same conditions. Symmetry factor, the number of theoretical plates, peak areas and retention times were given in Table 1.

Table 1. System suitability data

System suitability parameters	Standart Solution Concentiration (100 µg mL^{-1})
Symmetry factor	0.903
The number of theoretical plates	6203
Peak areas (% RSD)	0.124
Retention times (% RSD)	0.123

3.3.2. Linearity

The calibration curve for DOPHCl was then plotted for peak-area versus concentration. The least squares linear regression analysis of the peak area versus concentration data was used to evaluate the linearity of this method. The calibration curve has been demonstrated in figure 3 and it's linearity parameters were given in Table 2. The linearity graphs and overlain chromatograme for DOPHCl were shown in figure 3A and 3B.

Table 2. Linearity data (n=5)

Parameter	Value
Retention time (min)	6.106
Linear range (µg mL^{-1})	20 - 100
Regression equation	y = 16.188 x - 18.018
Correlation coefficient(R^2)	0.9999
Slope	16.188
Intercept	-18.018
LOD/ LOQ (µg mL^{-1})	2.00/6.10

3.3.3. Precision

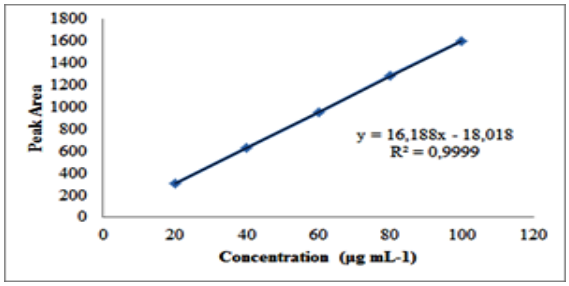
Precision study was performed by injecting six times of standard solution at the concentration of 100 µg mL^{-1}. The precision data were given in Table 3. In any case, the % RSD values < %2 indicate that the method is precise and can be used for our intend.

Table 3. Precision data (n=6)

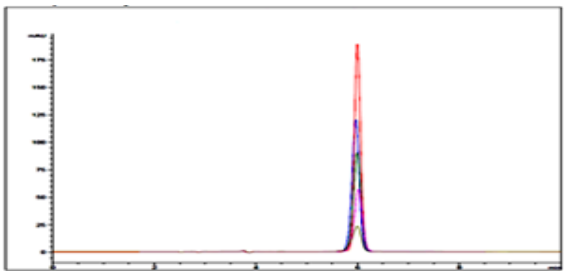
Compound	Intra-day Precision (n=6)		Inter-day Precision (n=6)	
	Mean ± SD	RSD, %	Mean ± SD	RSD, %
DOPHCl	100.05 ± 0.42	0.420	100.20 ± 0.86	0.858

3.3.4. Accuracy

A known quantity of standard solution has been added to the sample solutions previously analyzed at three different levels: 80%, 100% and 120%. The amount recovered for DOPHCl has been calculated for three concentration. The accuracy data were given in Table 4. % RSD values for all analyses were < 2%, indicating that the analytical method is accurate and suitable for our purpose.



3.A



3.B

Figure 3. **A)** Calibration curve for DOPHCl: **B)** Overlay chromatogram of five standard solutions

Table 4. Recovery data (Accuracy)

Spiked Level	Amount added (µg mL^{-1})	Amount recovered (µg mL^{-1})	Recovery (%)	Average (%)	S.D.	RSD (%)
	80	31.97	99,37			
80%	80	31.95	99,42	99,39	0,026	0,027
	80	31.96	99,38			
	100	39.99	99,64			
100%	100	39.98	99,60	99,62	0,020	0,020
	100	39.96	99,62			
	120	117.21	99,88			
120%	120	116.94	99,86	99,86	0,127	0,131
	120	116.96	99,85			

3.3.5. Selectivity / Specificity

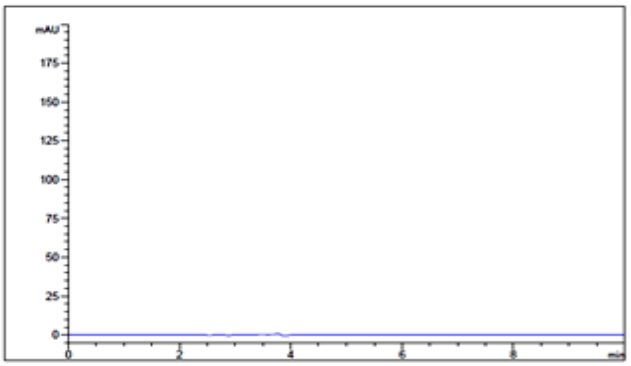
The chromatograms were shown in Figure 4. In the chromatogram of the mobile phase blank, no internally occurring peaks were observed at the retention time of DOPHCl (figure 4A). The retention time of DPHCl in chromatograms of injectable sample solution (figure 4B) and standard solution (figure 4C) are the same. DOPHCl peak did not interfere with the excipients of injectable solutions and mobile phase components, this indicates the selectivity of the proposed method. Parameters of the retention time and the tailing factor were calculated to demonstrate that the method proposed was selective and specific. Retention time and tailing factor parameters have been calculated and shown in Table 1.

3.3.6. LOD and LOQ

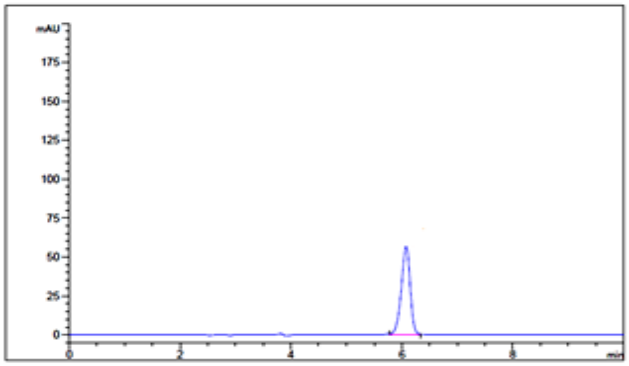
The calculated values have been given in Table 2.

3.3.7. Robustness

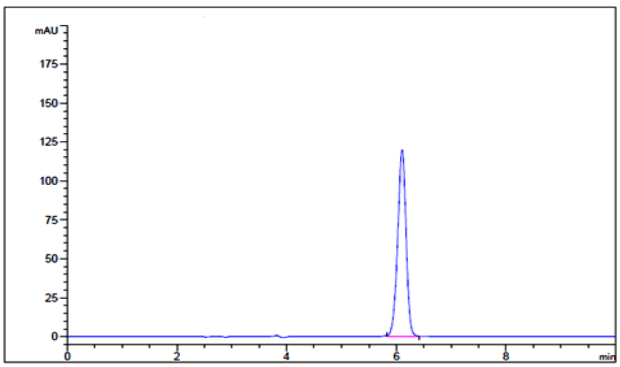
After each change, the sample solution has been injected into the chromatographic system and the system suitability parameters have been checked. The % RSD values were given in Table 5.



4.A



4.B



4.C

Figure 4. A) Chromatogram for blank solution, B) Chromatogram for injectable solution (40 µg mL^{-1}), C) Chromatogram for standard solution (60 µg mL^{-1}):

Table 5. Robustness data

Condition	Variation	Assay %	SD	RSD %
Mobile phase flow rate (1.00 mL min^{-1})	0.90 mL min^{-1}	99.86	0.60	0.60
	1.10 mL min^{-1}	99.94	0.62	0.62
Column temperature (30 ^{0}C)	28 ^{0}C	99.96	0.65	0.65
	32 ^{0}C	100.05	0.66	0.66
Buffer concentration (0.050 M)	0.040 M	100.06	0.60	0.60
	0.060 M	99.94	0.69	0.69
Mobile phase pH value (2.3)	2.20	100.12	0.67	0.67
	2.40	99.96	0.71	0.71

3.3.8. Analysis of marketed formulations (injectable solution)

The amount of DOPHCl in each injected solution was calculated by comparing the test areas with the standard area and found to be 100.05 ± 0.38 for DOPHCl. The results were given in Table 6.

Table 6. Analysis of the marketed formulation (n=3).

Solution	Label Claim ($mg.mL^{-1}$ solution)	Amount of drug ($mg.mL^{-1}$ solution)	% Assay ± SD
Dopasel	40 mg mL^{-1}	40.02	100.05 ± 0.38

4. Discussion & Conclusion

A simple and rapid HPLC method analyzing dopamine hydrochloride was successfully developed. Run time was short (6.1 minutes) and analytical method have outstanding peak shape and acceptable parameters for system suitability. The analytical method was validated in accordance with the ICH Guidelines and was found to be linear, accurate, accurate and specific. The method developed for quantifying dopamine hydrochloride has been successfully applied in an injectable solution. With its excellent performance in terms of specificity and recovery for dopamine hydrochloride in the injectable solution, this method's adaptability to the injectable solution has been proven. Therefore, the method can be used in injectable solution forms for the daily quality control analysis of dopamine hydrochloride. The big advantage of the proposed method is that less time is required and the phosphate buffer used in the mobile phase is compatible with chromatographic system.

Authors' contributions:

BSA: Performed the experiment and made the last arrangements of manuscript.

İB: Analysed the data and wrote the article.

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