RESEARCH ARTICLE
Eurasian J Bio Chem Sci, 4(2):44-50, 2021
https://doi.org/10.46239/ejbcs.883492

EJBCS

Eurasian Journal of Biological and Chemical Sciences

Journal homepage: www.dergipark.org.tr/ejbcs

Development of a Liquid Chromatographic Method for Apomorphine Hydrochloride Quantitation in the Active Pharmaceutical Ingredients and in the Dosage Forms

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Received : 19/02/2021
Accepted : 10/09/2021

Abstract: Apomorphine is currently used as a dopamine agonist to treat advanced Parkinson Disease. In the present study, we developed a sensitive, simple, reliable, and robust HPLC method for quality control of apomorphine hydrochloride in bulk drug and pharmaceutical formulations. C18 column was used for separation. Isocratic elution was performed using mobile phase A: 50 mM potassium dihydrogen phosphate solution (pH:3 with ortho-phosphoric acid), B: acetonitrile in the ratio of 85:15 (A: B), and the mobile-phase flow rate was kept at 1 $mL{\cdot}min^{-1}$. Analyses were carried out at 272 nm using a UV detector. A perfect linear relationship between peak-area versus drug concentration in the range of 10-100 $\mu g{\cdot}mL^{-1}$ was observed (r^2, 0.9999). It has been found that the developed method is sensitive (Detection and quantification limits were determined as 1.3 $\mu g{\cdot}mL^{-1}$ and 3.8 $\mu g{\cdot}mL^{-1}$, respectively), precise (RSD<0.9%, for repeatability and <1.2% for intermediate precision, inside appropriate precision ranges), accurate (recovery in various dosage forms, 99.68-100.56%, in a reasonable range, 80-120%), specific and robust (RSD% <0.80, for system suitability parameters). The proposed method for the determination of apomorphine hydrochloride in pharmaceutical formulations has been successfully applied.

Keywords: Apomorophine, HPLC, Pharmaceuticals, Quality control

1. Introduction

Parkinson's disease (PD) is the second most common neurodegenerative disease that affects 1% of the population over the age of 60. This rate reaches up to 3% in the highest age groups. (Tysnes and Storstein, 2017; Poewe et al., 2017). Highly effective treatments for PD are available. Replacement of dopamines by oral levodopa is still the gold standard of many symptomatic treatments. It is balanced only by apomorphine due to its scale of effect on motor symptoms (Kempster et al., 1990). The response to levodopa continues for a long time, but as the disease progresses, motor complications (dyskinesia and other complications) are developed in many patients. (Poewe and Lees and Stern, 1986; Sweet and McDowell, 1975)

Apomorphine's principal role in the treatment of PD is in the regulation of motor problems attributed to levodopa. Apomorphine, derived from the acidification of morphine, is an aporphine alkaloid. Comprises a tetracycline aporphine ring, its structure is responsible for affinity and lipophilicity for dopaminergic receptors. Specifically, the ortho-catechol group is structurally similar to dopamine. (Ernst and Smelik, 1966) It also contains a moiety of piperidine, as most antipsychotic drugs. Apomorphine has been described as an agonist of dopamine, but it varies greatly from other oral agonists of dopamine used in Parkinson's disease. Apomorphine functions as a potent agonist dopamine receptor with a wide range on both D1- and D2- like receptors, due to its catechol moiety (Auffret and Drapier and Vérin, 2018). In contrast, oral dopamine agonists pramipexole and ropinirole predominantly bind to D1, D2, and D3 receptors. (Millan et al., 2002). The action mode of apomorphine is more like that of dopamine or its precursor levodopa. Additionally, apomorphine has antagonistic effects on serotonergic and adrenergic receptors and agonistic effects on serotonergic receptors. (Jenner and Katzenschlager, 2016). Apomorphine has no affinity for opioid receptors unlike its mother compound, morphine. (Le Witt et al., 2009). The main role of

apomorphine in treating PD is in controlling motor problems associated with levodopa.

Apomorphine Hydrochloride (6aβ-Aporphine-10,11-diol hydrochloride hemihydrate) is currently used to treat advanced PD as a dopamine agonist (Figure 1). Arppe first synthesized apomorphine by heating morphine with excess sulfuric acid in 1845 and named it sulfomorphite . Later, Matthiessen and Wright synthesized apomorphine hydrochloride by heating morphine with concentrated hydrochloric acid in 1868. (Arppe, 1845). The compound was called apomorphia to emphasize its origin and distinction from the compound of the mother, morphine. Apomorphine started to attract interest in both veterinary and human medicine after the experiments by Matthiessen and Wright. (Matthiessen and Wright, 1868). Apomorphine has been used in various experiments in humans and animals in the years after its discovery and had several effects which led to its use in various medical fields. According to the experiments carried out in humans and animals by Gee, Hare, Pierce, Siebert, and Harnack, the impacts of apomorphine are associated with activity on the central nervous system. (Taba and Lees and Stern, 2013; Pierce, 1870; Hare, 1912; Gee, 1869; Taba and Lees and Stern, 2013). Most importantly, an emetic reaction was always detected in dogs and humans with parenteral and oral treatment. An adverse effect in the use of apomorphine, emesis has been the drug's primary signal for many decades and has contributed to its application in extracting foreign particles from the esophagus or managing poisoning. In cases of opioid, alcohol, and smoking dependency, this emetic response has been used to induce adverse conditioning by administering the drug with the undesired stimulus. (Raymond, 1963). Oral apomorphine undergoes significant first-pass metabolism resulting in poor bioavailability, and in most trials and tests parenteral delivery of the drug was the favored route of administration. (Hughes and Lees and Stern, 1991; Stibe et al., 1988). Between the beginning of the twentieth and the end of the nineteenth century, the clinical use of apomorphine covered almost every medicinal field. The sedative effects of the drug have been utilized in various psychiatric conditions such as hysteria, mania, anxiety, dementia, schizophrenic excitement, and alcohol-related disorders. (Lemere, 1987). Spontaneous erection in these experiments was observed as an unexpected consequence. This situation led to the commercialization of apomorphine in later years to treat erectile dysfunction. (Mulhall, 2002). De Weill first hypothesized in 1884 that apomorphine may be beneficial in patients with PD. (De Weill, 1884). Many studies supported their findings, which resulted in the acceptance of apomorphine as an alternative therapy to decrease "down" time in advanced PD over the following years.

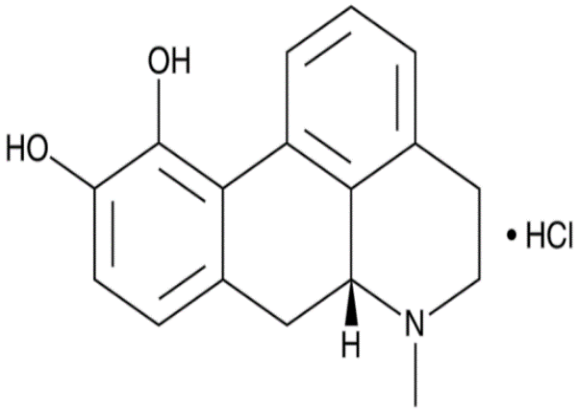

Figure 1. Molecular structure (Apomorphine HCl)

Various analytical methods have been reported for analyzing the apomorphine HCl, including liquid chromatography-electrospray ionization mass spectrometry (Yang, 2006), proton nuclear magnetic resonance spectrometry (Tan, 2016) liquid chromatography . (Ang and Boddy and Liu and Sunderland, 2016), titrimetry (The United States Pharmacopeia; European Pharmacopoeia). These analytical methods (Liquid chromatography-electrospray ionization mass spectrometry, proton nuclear magnetic resonance spectrometry) is more accurate and sensitive, however costly, therefore, limit their utility in the quality control of pharmaceutical formulations. In titrimetric methods, significant quantities of solvent are consumed and these solvents usually adversely impact human and envıronmental health.

In this study, a rapid, simple, accurate, and sensitive HPLC-UV method has been developed. It can precisely and accurately quantify and detect the smallest amount of the drug with a short working time in pharmaceutical formulations. Here, the method has been validated for precision, accuracy, sensitivity, selectivity, and different other validation parameters as per ICH guidelines.

2. Material and Method

2.1. Chemicals

Analytical grade chemicals were used without further purification in this study, Ortho-phosphoric acid (≥85%, Sigma-Aldrich), Potassium dihydrogen phosphate (99.5-100.5%, Sigma-Aldrich), and HPLC-grade acetonitrile (≥99.9%, Sigma-Aldrich) were used. Deionized water was purified using a Milli-Q system (Millipore) with conductivity lower than 18.2 $\mu S \cdot cm^{-1}$. Apomorphine HCl bulk powder and injectable solution (APO-GO, 20 mg 2 mL^{-1}) were obtained from Abdi İbrahim Pharmaceuticals Industry and Trade Co. Ltd. (Istanbul, Turkey).

2.2. Stock standard solution

100 mg pure drug was accurately weighed, dissolved in about 30 mL of deionized water, and transferred to a 100 mL volumetric flask. To get 1 mg mL^{-1} of the stock solution, the volume was completed to 100 mL with deionized water. The resulting stock solution was sonicated and filtered through a 0.45 µm filter. The stock solution was further diluted with deionized water to obtain the required concentration of standard solutions (10-100 $\mu g \cdot mL^{-1}$) before being injected into the system for analysis.

2.3. Sample solution

10 ampoules containing 20 mg 2 mL^{-1} solutions for injection or infusion were broken and their contents were poured into a 50 mL volumetric flask. It was well mixed. 10 mL of this solution was taken and transferred to a 100 mL volumetric flask. The volume is completed with deionized water to obtain a solution containing 1000 $\mu g \cdot mL^{-1}$ and then filtered through a 0.45 µm filter.

2.4. Determination of λmax

Standard solution (40 $\mu g \cdot mL^{-1}$) was subjected to scanning between 200-800 nm on a UV spectrophotometer (Shimadzu UV-1800 spectrophotometer). λ max was examined from the UV spectrum of the standard solution.

2.5. Chromatographic conditions

Chromatographic analysis was performed on a column of ACE C18 (4.6 mm × 150 mm, 5.0 µm). The mobile phase consisted of potassium dihydrogen phosphate 50 mM (pH 3.0) and acetonitrile (85:15, v/v). The mobile phase was filtered and degassed through a 0.45 µm membrane filter before use and then pumped at a flow rate of 1 $mL.min^{-1}$. The column has been thermostated at 30 oC. The run time was 10 min under these conditions.

2.6 Method validation

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

Linearity

Standard calibration has been prepared using six standard solutions within the concentration range of 10-100 $\mu g \cdot mL^{-1}$. In optimized chromatographic conditions, each standard solution was chromatographed for 10 minutes three times. 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). The precision study was performed by injecting six times of standard solution at three different concentrations, 20, 40, and 60 $\mu g \cdot mL^{-1}$ on the same day and three consecutive days.

Specificity/Selectivity

Selectivity is the analytical method's ability to provide an analyte response in the presence of other interventions. The selectivity of the method was tested by comparing the chromatograms obtained for Favipiravir standard, tablet, and blank solutions. The parameters retention time and tailing factor were calculated to prove that the method chosen was specific.

Accuracy

Recovery studies were conducted by the standard addition technique to confirm the accuracy of the developed method. In this method, 80%, 100%, and 120% of three different levels of the pure drug were added to the previously analyzed sample solutions, and favipiravir's recovery was calculated for each concentration.

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 modifications include different flow rates of the mobile phase (± 0.1 mL min-1), acetonitrile ratio in the mobile phase (± 1 %), and column temperatures (± 2 °C). After each change, System suitability parameters were checked by injecting the sample solution into the chromatographic system and the results were compared with those under the original chromatography conditions.

Analysis of marketed formulations

4 mL of the above-prepared sample solution has been transferred into a volumetric flask of 100 mL and filled the mark with deionized water to prepare at the concentration of 40 $\mu g \cdot mL^{-1}$ sample solution. This sample solution was filtered using a 0.45 µm filter and then analyzed.

Stability of standard and sample solution

The stability of the sample and standard solutions has been monitored for 24 hours. For this, standard and sample solutions were injected into the system at 8-hour periods, and the peak area and retention time were evaluated. During the stability study, standard solutions have been stored at ambient temperature (25ºC) and protected from light.

3. Results and Discussion

3.1. Determination of λmax

The wavelength corresponding to maximum absorbance (λmax) was determined as 272 nm from the UV-vis. absorption spectrum of standard solution (Figure 2).

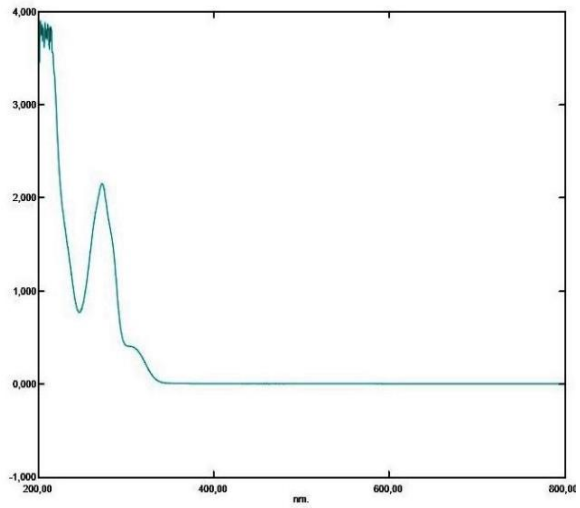

Figure 2. UV-vis. absorption spectrum (Standard solution, 40 µg·mL^{-1})

Method development. Several preliminary studies have been performed to optimize the chromatographic conditions for Apomorphine HCl quantification. Mobile phases consisting of several buffer systems were tried at the beginning of the study, they could not meet the required system parameters. Then only potassium dihydrogen phosphate buffer system was tested without using organic modifiers, long analysis times were obtained. Different acetonitrile solution ratios were investigated to obtain optimum conditions. The acetonitrile ratio was determined as 15% against 50 mM potassium dihydrogen phosphate solution (pH = 3.0) due to the Apomorphine HCl peak being well shaped and symmetrical using this system. Eventually, it was found that the mobile phase consisting of 50 mM potassium dihydrogen phosphate solution (pH: 3.0 with ortho-phosphoric acid) and acetonitrile (85:15, v/v) provided stronger theoretical plates (> 2000) and peak tailing factor (<1.0). Mobile phase running at different flow rates (0.5-1.5 mL·min^{-1}) and containing mixtures of organic solvents and acetate buffers, with ionic strengths and pH ranges were tested. Collectively, the best chromatographic conditions have been achieved using an isocratic mobile phase comprising 50 mM potassium dihydrogen phosphate (pH = 3.0) and acetonitrile (85/15, v/v) at a flow rate of 1.0 mL·min^{-1} on an ACE C18 column (4.6 mm × 150 mm, 5.0 µm). The analysis was conducted at 30°C, which offers a lot of advantages such as good chromatographic peak shape, enhanced column efficiency, and low-column pressure, in addition to being economic. The eluate has been monitored using a UV detector set at 272 nm. Under the chromatographic conditions. Apomorphine HCl was eluted at 4.637 min of retention time. The tablet solution was analyzed for 60 minutes to ensure that no matrix components were remaining in the column for much longer under the specified conditions. However, continuing the analysis after 10 minutes will increase both the analysis time and the cost. Overlapping peaks were not observed to overlap in samples from sample analyses injected into the system consecutively with 10 minutes of analysis time. Due to all these, the analysis time was determined as 10 min.

3.2 Method validation

3.3.1. Linearity

The stock standard solution of Apomorphine HCl has diluted appropriately with deionized water to obtain standard solutions within the concentration range of 10 to 100 µg·mL^{-1}. Each standard solution was injected three times into the HPLC system under the above-mentioned chromatographic working conditions. The linearity of the proposed method has been estimated at 6 concentration levels in the range of 10–100 µg·mL^{-1} (10-20-40-60-80-100) by regression analysis. The calibration curve has been developed by plotting the average peak area versus standard concentration (Figure 3). The correlation coefficient, slope, and intercept of the regression line were determined using the least-squares method. The relation between mean peak area Y(n=3) and concentration, X expressed by the equation Y = a + bX, was linear. Values of the slope, intercept, and correlation coefficient (r) was 56.009, -27.780, and 0.9999, respectively as shown in Table 1. Overlay chromatogram of Apomorphine HCl standard solutions (10-100 µg·mL^{-1}) was demonstrated in Figure 4A.

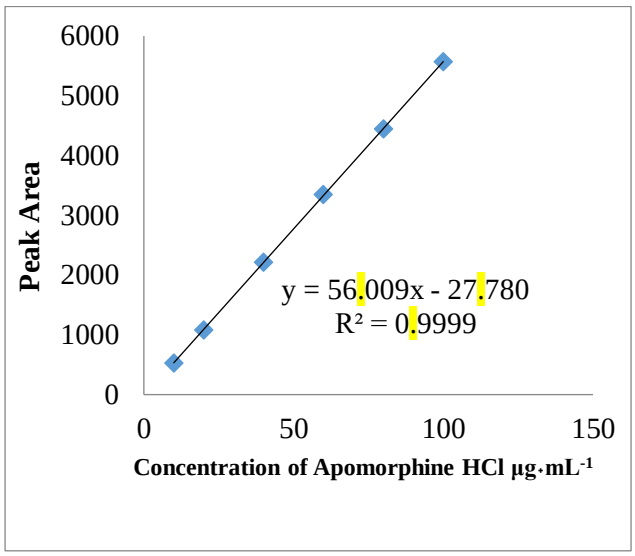

Figure 3. A calibration curve (λ: 272 nm)

Table 1. Statistical data (Calibration curve, Apomorphine HCl)

Parameter	Value
Linearity range (µg·mL^{-1})	10 - 100
Slope	56.009
Intercept	-27.780
Correlation coefficient	0.9999
SE of Intercept	8.6902
SD of Intercept	21.287
LOD/ LOQ (µg mL^{-1})	1.30/3.80

3.3.2. Specificity / Selectivity

The chromatogram of Apomorphine HCl standard solution has been given in Figure 4B. There is only one peak at the retention time of 4.637 min. The chromatogram of the injectable solution has been given in Figure 4C. There is only one peak at the retention time of 4.637 min in this chromatogram. There are no other peaks caused by excipients and additives in this chromatogram. The chromatogram of the mobile phase has also given in Figure

4D. There are no other peaks caused by the contents of the mobile phase in this chromatogram. This indicates that the analytical method is specific. The parameters retention time and tailing factor were calculated to prove that the method chosen has been specific. Retention time, theoretical plate number, and peak tailing factor values were 4.637, 11798, and 0.600, respectively. All of the values were within the accepted level.

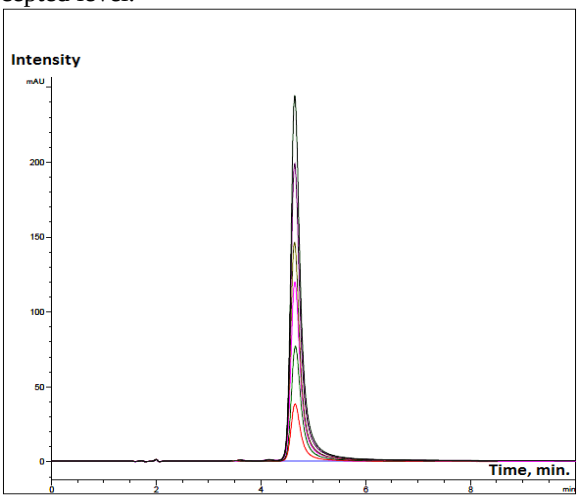

Figure 4. A. Overlay chromatogram (Standard solutions, 10-100 µg·mL^{-1})

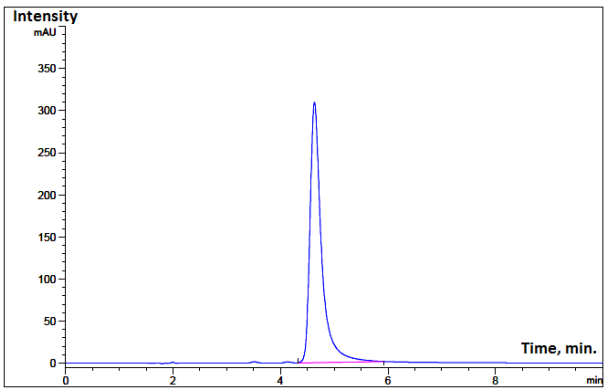

Figure 4. B. Chromatogram (Standard solution, 80 µg·mL^{-1}, λ:272 nm)

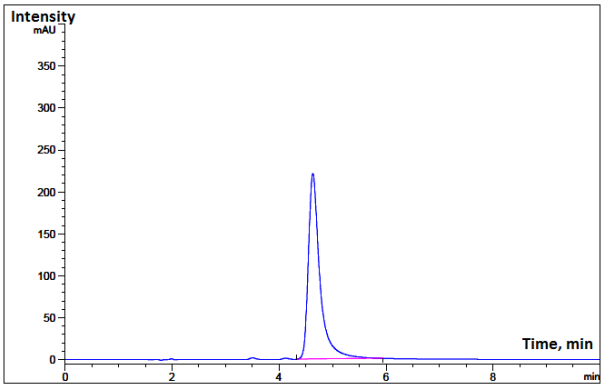

Figure 4. C. Chromatogram (Sample solution, 60 µg·mL^{-1}, λ:272 nm)

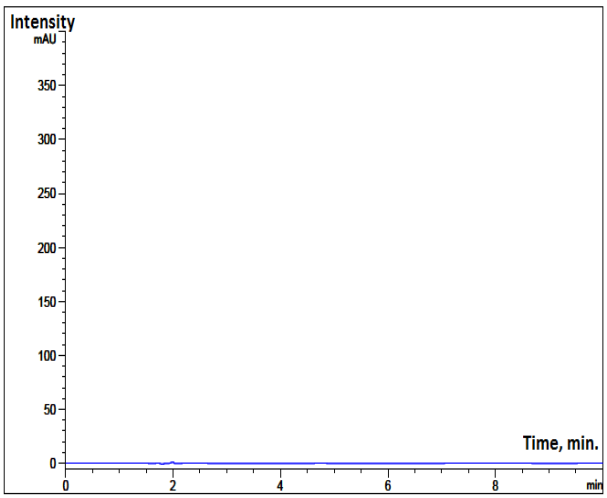

Figure 4. D. Chromatogram (Blank solution, λ:272 nm)

3.3.3. Precision

The precision study was performed by injecting six times of standard solution at three different concentrations, 20, 40, and 60 µg·mL^{-1} on the same day and three consecutive days. The precision data were given in Table 2. All RSD values for retention time and peak area for selected Apomorphine HCl concentrations were less than 0.2 and 1.2 %, respectively. In this case, the method is precise and can be used for our intended purpose.

Table 2. Precision data

Std. Conc. µg·mL^{-1}	**Intraday precision**			**Interday precision**		
	Found conc. (6) µg·mL^{-1}	**Peak area RSD %**	**Retention time RSD %**	**Found conc. (6)** µg·mL^{-1}	**Peak area RSD %**	**Retention time RSD %**
20	20.34	0.819	0.145	19.80	1.105	0.185
40	40.22	0.560	0.095	40.05	0.862	0.123
60	60.08	0.620	0.102	60.18	0.644	0.097

3.3.4. Accuracy Study

A known quantity of standard solution has been added to the sample solutions previously analyzed at three different levels in 50 mL (80%, 100%, and 120%). Concentration was 40 µg·mL^{-1} within the 50 mL. Quantity of apomorphine was 2000 µg. So, 80% level refers to 1600 µg 100% refers to 2000 µg and the last one 120% refers to 2400 µg. The amount recovered for Apomorphine HCl has been calculated for three concentrations. The recovery data were summarized in Table 3. RSD% values for all analyses were less than 0.2% indicating that excipients found in pharmaceutical formulations do not interfere and the analytical method is very accurate.

Table 3. Recovery data **(40 μg·mL^{-1})**

Spiked Level	Amount added (μg·mL^{-1})	Amount recovered (μg·mL^{-1})	Recovery (%)	Average (%)	S.D.	RSD (%)
	32	32.13	100.41			
80%	32	32.24	100.75	100.56	0.174	0.173
	32	32.17	100.53			
	40	39.95	99.88			
100%	40	39.97	99.93	99.93	0.050	0.050
	40	39.99	99.98			
	48	47,83	99.65			
120%	48	47.86	99.71	99.68	0.032	0.032
	48	47.85	99.69			

3.3.7. Robustness

The results showed that the change in flow rate and mobile phase concentration had little effect on the chromatographic attitude of Apomorphine HCl. The small change in the mobile phase flow rate and acetonitrile content have a small impact on the retention time of Apomorphine HCl. The change in the column temperature did not have a significant effect on the method. The results of this study, expressed as RSD%, were presented in Table 4.

Table 4. Robustness data

Condition	Variation	Assay %	SD	RSD %
Mobile phase flow rate (1.00 mL·min^{-1})	0.90 mL min^{-1}	99.65	0.80	0.80
	1.10 mL min^{-1}	99.88	0.75	0.75
Acetonitrile ratio in mobile phase (15 %)	13 %	100.25	0.70	0.70
	17 %	99.90	0.67	0.67
Column temperature (30 °C)	28 °C	99.94	0.50	0.50
	32 °C	100.12	0.45	0.45

Stability of standard and sample solution. The stability of the sample and standard solutions has been monitored for 24 hours. No changes in standard concentrations have been observed over a period of 24 hours. The RSD% for peak area (n = 3) was 0.077% and the value for retention time (n=3) was 0.036% for standard solution. The results have been demonstrated in Table 5. No major changes in inactive ingredient concentration have also been found in the tablet solution.

Table 5. Standard solution stability (40 μg mL−1).

Time, hours	Peak area	Mean	SD	RSD%	Retention time (min)	Mean	SD	RSD %
8	2220.0	2221.30	5.57	0.251	4.636	4.639	0.003	0.115
	2227.4				4.640			
	2216.5				4.641			
16	2217.7	2218.23	3.83	0.173	4.635	4.638	0.003	0.192
	2214.7				4.638			
	2222.3				4.640			
24	2218.2	2219.37	1.32	0.059	4.636	4.637	0.002	0.15
	2219.1				4.635			
	2220.8				4.639			

3.3.8. Application of the method to the marketed tablets

The developed and validated method has been applied successfully for the determination of Apomorphine HCl in pharmaceutical formulations. The result of the assay of the marketed injectable solution of Apomorphine HCl is shown in Table 5. The results obtained are closely related to the amount indicated on the labels of the tablets. This shows that the method for content evaluation is useful.

Table 6. Method application results

Formulation	Label claim (mg)	Amount of drug (mg)	% Assay ± SD
APO-GO	10 mg mL^{-1}	10.03 mg	100.03 ± 0.45

4. Conclusion

Liquid chromatography-electrospray ionization mass spectrometry, proton nuclear magnetic resonance spectrometry-liquid chromatography, and titrimetry are all known methods for apomorphine analysis. These analytical methods are more precise and sensitive, but they are also more expensive, therefore their utility for pharmaceutical formulation quality control is restricted. Titrimetric procedures use a lot of solvents, which are usually bad impression for human and environmental health.

A very quick, cost-effective, precise, and accurate HPLC method for the determination of Apomorphine HCl has been developed and validated in compliance with ICH guidance Q2. Besides the short run time (10 min), retention time (4.637), and flow rate of mobile phase (1 mL·min^{-1}) made the method attractive because these features save analysis time and cost. Sodium acetate, used as a general-purpose buffer, has many interesting properties. The most important of these features are good buffering capacity in the selected pH range, easy availability, low toxicity and cost, and greatly improved separation ability without colon degradation. In short, this method is sensitive, selective, reproducible, and rapid for favipiravir in bulk and tablets.

The accuracy and precision are within reasonable limits, the maximum of quantification is as small as 3.80 $\mu g \cdot mL^{-1}$ and finally, the analytical method is reliable and robust.

Acknowledgments

The author would like to thank Abdi İbrahim Pharmaceuticals Industry and Trade Co. Ltd. (Istanbul, Turkey) for providing a pure drug reference sample and supporting this work.

Authors' contributions:

The article is written by İB and data analyzes were determined by İB. Experiments were done and the last arrangement about article was done by BSA.

References

Tysnes OB, Storstein A 2017 Epidemiology of Parkinson's disease. J Neural Transm. 124(8): 901–905

Poewe W, Seppi K, Tanner CM, et al. 2017. Parkinson disease. Nat Rev Dis Primers. 3: 17013-17018

Kempster PA, Frankel JP, Stern GM, Lees AJ 1990. Comparison of motor response to apomorphine and levodopa in Parkinson's disease. J Neurol Neurosurg Psychiatry. 53(11): 1004–1007

Poewe WH, Lees AJ, Stern GM 1986. Low-dose L-dopa therapy in Parkinson's disease: a 6-year follow-up study. Neurology. 36(11): 1528–1530.

Sweet RD, McDowell FH 1975. Five years' treatment of Parkinson's disease with levodopa. Therapeutic results and survival of 100 patients. Ann Intern Med. 83(4): 456–463.

Ernst AM, Smelik PG. 1966. Site of action of dopamine and apomorphine on compulsive gnawing behavior in rats. Experientia. 22(12): 837–8

Auffret M, Drapier S, Vérin M 2018. Pharmacological insights into the use of apomorphine in parkinson's disease: clinical relevance. Clin Drug Investig. 38(4): 287–312.

Millan MJ, Maiofiss L, Cussac D, Audinot V, Boutin J-A, Newman- Tancredi A 2002. Differential actions of antiparkinson agents at multiple classes of monoaminergic receptor. I. A multivariate analysis of the binding profiles of 14 drugs at 21 native and cloned human receptor subtypes. J Pharmacol Exp Ther. 303(2): 791–804.

Jenner P, Katzenschlager R 2016. Apomorphine pharmacological properties and clinical trials in Parkinson's disease. Park Relat Disord. 33: 13–21.

Le Witt PA, Ondo WG, Van Lunen B, Bottini PB 2009. Open-label study assessment of safety and adverse effects of subcutaneous apomorphine injections in treating 'off' episodes in advanced Parkinson disease. Clin Neuropharmacol. 32(2): 89–93.

Arppe AE 1845. Ueber eine merkwürdige Veränderung des Morphins durch Schwefelsäure. Ann der Chemie und Pharm. 55(1): 96–101.

Matthiessen A, Wright CRA 1868. Researches into the Chemical constitution of the opium bases. Part I. on the action of hydrochloric acid on morphia. Proc R Soc Lond. 17: 455–60

Taba P, Lees A, Stern G. 2013. Erich Harnack (1852-1915) and a short history of apomorphine. Eur Neurol. 69(6): 321–4.

Pierce FM 1870. Notes on Apomorphia. British Medical Jornal. 1(478): 204.

Hare F. 1912. On alcoholism its clinical aspects and treatment. London: JandA Churchill.

Gee S. 1869. On the action of a new organic base, apomorphia. Clin Soc Trans. 2: 166–169

Taba, P., Lees, A., and Stern, G. 2013. Erich Harnack (1852–1915) and a short history of Apomorphine.

Raymond MJ 1963. The treatment of addiction by aversive conditioning with apomorphine. Behav Res Ther. 1(2–4): 287–291

Hughes AJ, Lees AJ, Stern GM 1991. The motor response to sequential apomorphine in parkinsonian fluctuations. J Neurol Neurosurg Psychiatry. 358–360

Stibe CMH, Kempster PA, Lees AJ, Stern GM. 1988. Subcutaneous apomorphine in Parkinsonian on-off oscillations. Lancet. 331(8582): 403-406

Lemere F: 1987. Aversion treatment of alcoholism: some reminiscences. Addiction. 82(3): 257–258

Mulhall JP 2002. Sublingual apomorphine for the treatment of erectile dysfunction. Expert Opinion Investig Drugs. 11(2): 295–302

De Weill E. 1884. l'apomorphine dans certains troubles nerveux. Lyon Med. 47: 411–18

Yang B, Yu Y, Cai L, Deng C, Duan G 2006. Determination of apomorphine in canine plasma by liquid chromatography-electrospray ionization mass spectrometry and its application to a pharmacokinetic study. J. Sep. Sci. 29: 2173–2178

Tan L, Chin SF, Miner VW, Dong L, Gupta S, Fields SM 2016. Determination of apomorphine freebase in sublingual tablets by proton nuclear magnetic resonance spectroscopy. Journal of pharmaceutical and biomedical analysis. 129: 378-382

Ang ZY, Boddy M, Liu Y, Sunderland B 2016. Stability of apomorphine in solutions containing selected antioxidant agents. Drug Des Devel Ther. 10: 3253-65.

Apomorphine Hydrochloride Monographs. The United States Pharmacopeia (USP 32) Volume No. 32(5). 1438-39

Apomorphine Hydrochloride Tablets Monographs. The United States Pharmacopeia (USP 32). 32(5): 1573-1574

Apomorphine hydrochloride Monographs. European Pharmacopoeia (EP 6.0) 1206-1207

ICH. 2005. Q2 (R1), harmonized tripartite guideline, validation of analytical procedures: text and methodology, In Proceedings of the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use.

Center for Drug Evaluation and Research (CDER). 1994. Reviewer Guidance: Validation of Chromatographic Methods. CMC, 3.