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Alternative Analytical Methods for Quantification of Galantamine in Pharmaceuticals

Farmasötiklerde Galantamin Miktarının Belirlenmesi için Alternatif Analitik Yöntemler

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ÖZET

AMAÇ: Galantaminin yığın halinde ve farmasötik dozaj formunda miktar tayini için, yeni seçici, hızlı, kesin ve doğru spektrofotometrik ve kromatografik prosedürler geliştirildi ve doğrulandı.

GEREÇ VE YÖNTEM: Kromatografik ayırma, bir Agilent Extend C18 (250x4.6 mm, 5 um) kolonu üzerinde gerçekleştirilmiştir. %0.1 TFA içeren ultra saf su ve %0,1 TFA içeren asetonitril (85/15, v/v) 1,0 mL/dk akış hızında mobil faz olarak kullanıldı. Eluent tespiti, bir UV detektörü kullanılarak 288 nm dalga boyunda gerçekleştirilmiştir. Galantamin ise 288 nm dalga boyunda çözeltilerin absorbansı ölçülerek spektrofotometrik teknikle belirlendi.

BULGULAR: Galantamin için Lambert-Beer grafikleri, 5-30 µg/mL konsantrasyon aralığında doğrusal ilişkiler gösterdi. Her iki teknik de Uluslararası Uyumlaştırma Konferansı (ICH) standartlarını karşılamak için istatistiksel olarak değerlendirilmiş ve doğrulanmıştır ve sonuçlar spektrofotometrik ve sıvı kromatografik yöntemlerin doğrusal, hassas, doğru, sağlam ve RSD değerlerinin %1'den düşük olduğunu gösterdi ve geri kazanım yüzdesi standart sınırlar içindeydi (%98-102). Daha sonra bu analitik yöntemlerin istatistiksel bir karşılaştırması yapıldı. Her iki yöntemin sonuçları da %95 güven aralığında ($p<0.05$) birbirlerine göre farklılık göstermedi ve istatistiksel olarak anlamlı değildi.

SONUÇ: Geliştirilen analitik yöntemlerin doğru, oldukça etkili, güvenilir, hızlı, basit olduğu belirlendi ve farmasötik dozaj formunun yanı sıra yığın halindeki galantaminin rutin analizi için başarıyla kullanılabilir. Kalite kontrol analizi ve temizlik doğrulaması sırasında numune analizi için bile kullanılabilirler.

Anahtar Kelimeler: galantamin, HPLC, UV, yöntem, validasyon

ABSTRACT

OBJECTIVE: For the quantitafication of galantamine in bulk and pharmaceutical dosage form, new selective, quick, precise, and accurate spectrophotometric and chromatographic procedures were developed and validated.

MATERIALS AND METHODS: Chromatographic separation was performed on an Agilent Extend C18 (250×4.6 mm, 5 µm) column. Ultrapure water containing 0.1% TFA and acetonitrile containing 0.1% TFA (85/15, v/v) were used as mobile phase at a flow rate of 1.0 mL/min. Eluent detection was performed at a wavelength of 288 nm using a UV detector. On the other hand, galantamine was determined by spectrophotometric technique by measuring the absorbance of the solutions at a wavelength of 288 nm.

RESULTS: Lambert–Beer plots for galantamine showed linear relationships in the concentration range of 5-30 µg/mL. Both techniques have been statistically evaluated and validated in order to meet the standards of the International Conference on Harmonisation (ICH) and the results showed that spectrophotometric and liquid chromatographic methods were linear, precise, accurate, rugged and robust with RSD values less than 1.00%, and the recovery percentage was within standard limits (98-102%). Then a statistical comparison of these analytical methods was performed. The results of both methods showed no difference and not statistically significant with respect to each other in the 95% confidence interval ($p<0.05$).

CONCLUSION: The developed analytical methods were determined to be accurate, highly effective, reliable, fast, simple, and may be employed successfully for routine analysis of galantamine in the bulk as well as pharmaceutical dosage form. They may even be used for quality control analysis and for sample analysis during cleaning validation.

Keywords: galantamine, HPLC, UV, method, validation

INTRODUCTION

Alzheimer's disease now affects more than 42 million individuals worldwide (1). According to certain recent publications, it is seen in approximately 15% of women over the age of 60 worldwide. In addition, it ranks third among the causes of death due to diseases worldwide, after heart disease and cancer.

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Galantamine is an allosteric nicotinic receptor modulator and reversible acetylcholinesterase inhibitor that reduces cognitive and functional deterioration in mild to severe Alzheimer's disease dementia. Although galantamine cannot completely treat this condition, it can help to slow its course. Furthermore, throughout the early to mid-stages of the illness, it can improve symptoms by inhibiting reversible and competitive acetylcholinesterase. It affects just those parts of the brain with low acetylcholine levels (2).

Several analytical techniques for galantamine amount analysis have been reported using techniques such as spectrophotometry (3, 4) capillary zone electrophoresis (5), micellar electrokinetic chromatography (6, 7), high-performance liquid chromatography UV (8, 9), high-performance liquid chromatography photodiode-array radiometric detection (10, 11), ion-exchange chromatography (12, 13), high-performance liquid chromatography tandem mass spectrometry (14-16), and chiral liquid chromatography method (17). The monograph of galantamine hydrobromide is available in both the European (18) and the United States Pharmacopoeia (19). A validated stability studies including structure elucidation and bioactivity assessment of degradation products have been reported for galantamine hydrobromide (20, 21). CE-Electrospray ionization application has been reported for the impurity profile of Galantamine hydrobromide (22).

Analysis procedures require expensive equipment, organic solvents, and significant volumes of specialized reagents. Some of these techniques are somewhat complicated. The analysis process takes a lengthy time.

In our study covers the development and validation of new spectrophotometric and liquid chromatographic methods for galantamine assay, including the determination and identification of galantamine in pharmaceutical products. These analytical methods are precise, accurate, linear, selective, and robust. Therefore, these newly developed and validated methods would be suitable for quality control laboratories for the analysis of galantamine in pharmaceutical products. The analysis of variance (ANOVA) was used to compare the results of these analytical methods. In addition, their reliability was assessed by concentrating on routine quality control analyses.

MATERIAL & METHODS

Reagents

Galantamine Hydrobromide United States Pharmacopeia (USP) reference standard trifluoroacetic acid (≥99.0percentage), methanol (≥99.9percentage), and acetonitrile (≥99.9percentage) was purchased from Sigma-Aldrich Chemie GmbH (St Louis, MO, USA). Reminyl (Cologno, Monzese, Italy) extended-release capsule (8 mg) was bought from the local pharmacy. Ultrapure water was produced using a Merck Millipore water purification system (Bedford, MA, USA).

Analytical instruments and conditions

HPLC analyzes were performed with an Agilent 1260 system (Palo Alto, CA, USA) consisting of a quaternary pump, autosampler, UV detector, and ChemStation software. An Agilent Extend-C18 (4.6 mm × 250 mm, 5.0 µm) column was used, and maintained at 25 ◦C. Chromatographic detection of eluents was performed at 288 nm using a UV detector. The mobile phase consisted of ultrapure water containing 0.1% TFA and acetonitrile (85/15, v/v) containing 0.1% TFA at a flow rate of 1.0 mL/min. The injection volume was 20µl.

Spectrophotometric analyses were performed on a Shimadzu UV 1800 double beam (Shimadzu, Japan) spectrophotometer, with UV-Probe software and 1 cm quartz cuvette. The standard solutions were scanned in the range of 200-800 nm on the UV spectrophotometer to determine the value of λmax and the measurements were obtained against methanol as a blank. The wavelength of 288 nm was selected for the quantitation of galantamine. The absorbance values of the standard solution series at the wavelength of 288 nm were recorded and it was shown that the absorbance values were proportional to the concentration of standard solutions.

Preparation of sample and standard solutions

Galantamine standard solutions: Galantamine hydrobromide reference standard equivalent to 25 mg of galantamine and 20 ml of methanol into a 50 ml volumetric flask and the total volume was completed to 50 ml with methanol. Thus, a stock standard solution was prepared at a concentration of 500 µg/mL. By diluting the standard stock solution with ultrapure water, six standard solutions were prepared in the concentration range of 5-30 µg/mL.

Galantamine sample solutions: 10 capsule contents were emptied and weighed and ground into fine powder by grinding in a mortar. The powder equivalent to 25 mg of galantamine was weighed accurately, taken into a 50 mL of

volumetric flask. 20 mL of methanol was added to dissolve the galantamine, the volume was diluted to 50 mL with methanol when dissolution was complete. Finally, methanol was added up to the marked line, and shaked homogenously for 15 minutes. This supernatant was filtered through membrane filter (0.45 µm pore size). To a 50 mL volumetric flask, 2 mL of transferred this solution and was added methanol to obtain 20 µg/mL of galantamine solution.

Validation

The optimized chromatographic and spectrophotometric methods are fully validated according to the procedures specified in ICH guidelines Q2(R1) for the validation of analytical methods (23, 24). Validation parameters such as sensitivity, linearity, accuracy, robustness, precision, specificity, system suitability tests, stability studies have been investigated.

Linearity: Stock standard solutions containing 500 µg/mL of galantamine in methanol were prepared, in triplicate. Stock standard solutions were diluted with methanol to six different concentrations containing 5, 10, 15, 20, 25 and 30 µg/mL galantamine. Calibration curves were plotted concentration versus peak area for the chromatographic method, and concentration versus absorbance value for the spectrophotometric method. The acquired data were analyzed using the least squares method.

Precision: The intraday precision was assessed by analyzing capsule samples (n = 6) at the test concentration (20 µg mL-1) using spectrophotometric and high-performance liquids chromatographic methods. Similarly, the precision between days was assessed three days in a row (n = 18). Galantamine contents were determined and the relative standard deviations were calculated.

Accuracy: The accuracy of the methods was evaluated at three levels (80, 100, and 120% of the test concentration) with recovery studies. This was done by analyzing a sample of known concentration and comparing the measured value with the "real" value. A well-characterized sample solution (20 µg/mL of galantamine) was used. Samples were prepared in triplicate for each concentration, analyzed by spectrophotometric and high-performance liquids chromatographic methods, and recovery percentages were calculated.

Specificity: A sample solution (20 µg/mL of galantamine) was prepared using the sample preparation procedure and injected into the chromatographic system to assess the possibility of interfering peaks. For spectrophotometric analysis, the UV spectrum of this sample solution was recorded in the wavelength range of 200-400 nm to assess the existence of potentially interfering bands at 288 nm. Furthermore, the UV spectra recorded by a UV detector were used to assess the spectral purity of galantamine peaks in chromatograms produced with sample solutions.

Detection and quantitation limits: Limit of detection (LOD) and limit of quantification (LOQ) were used to evaluate the sensitivity of the chromatographic and spectrophotometric methods. They were calculated separately depending on standard deviation of the slope and intercept of the calibration curve by using the equations (1) and (2), respectively.

$$LOD = 3.3x\sigma/S \quad (1) \qquad || \qquad LOQ = 10x\sigma/S \quad (2)$$

S: *calibration curve slope and σ: standard deviation of y intercept*

Analysis of marketed formulations

Samples of galantamine raw material and Reminyl® capsules were analyzed by the validated chromatographic and spectrophotometric methods. Before analyzing, the capsule contents were weighed and powdered. The sample solutions for the chromatographic and spectrophotometric analyses were prepared as explained in Section 2.3. Galantamine contents were analyzed by both methods. One Way ANOVA Tukey test was used for statistical results and 0.05 was considered significant.

Comparative analysis

For commercial formulations, recovery percentages were compared with the F test and t test using both analytical methods.

Stability of solutions

Over a 24-hour period, the reference standard solutions were evaluated for stability. During the stability research, standard solutions were kept at room temperature (25 °C) and shielded from light.

RESULTS

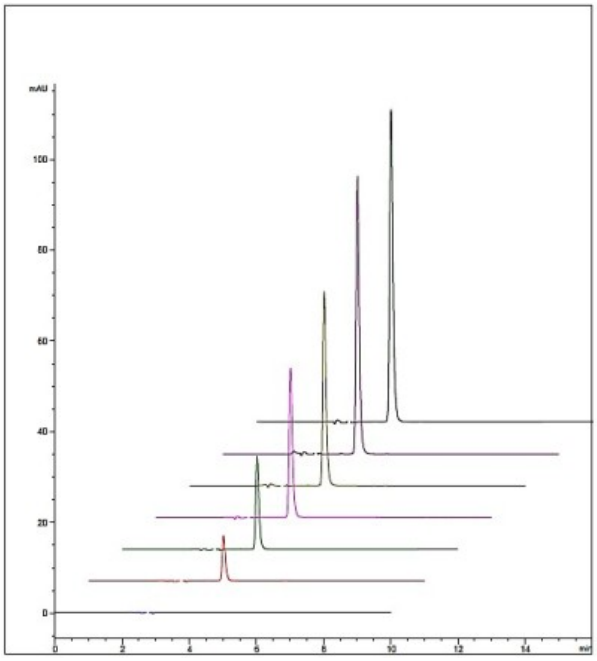
Method development

The tests of mobile phases were carried out with organic solvents, ultrapure water and different pH range at different flow rates (0.8–1.5 mL min-1). The best

chromatographic conditions were achieved using an isocratic mobile phase comprising ultra-pure water containing 0.1% TFA (pH=1.5)-acetonitrile containing 0.1% TFA (85/15, v/v) at a flow rate of 1.0 µg/mL on an Agilent Extend-C18 (4.6 mm × 250 mm, 5.0 µm) that was kept at 25°C.

Eluent was measured using a UV detector at a wavelength of 288 nm. In these chromatographic conditions, the retention time for galantamine was determined to be 4.04 minutes as demonstrated in the chromatogram of Fig. 1.

Figure 1: *Overlay chromatogram obtained for galantamine standard solutions (5-30 µg/mL)*



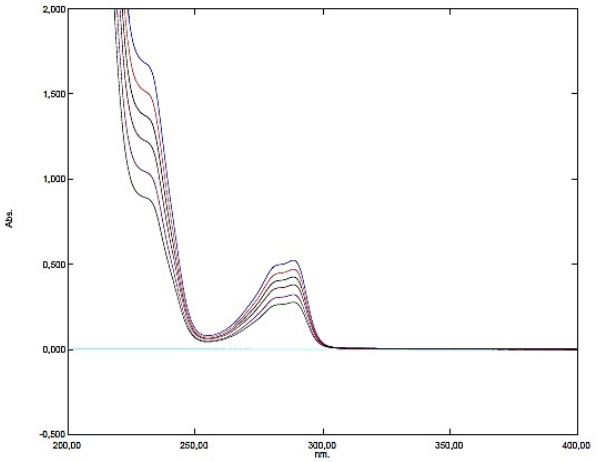
The UV spectrum of in the range of 200-400 nm was evaluated (Fig. 2). Galantmaine has shown sufficient molar absorptivity at a wavelength of 288 nm. The wavelength of 288 nm showed higher selectivity with respect to possible interfering compounds in the samples. For all these reasons, a wavelength of 288 nm was chosen for detection.

Analytical validation

A linear relationship was determined between galantamine concentrations and the responses of both UV and HPLC methods. Regression data analysis are given in Table 1. For both methods, high regression coefficient (r2) values were obtained (>0.999). There was no significant deviation in linearity in the analysis range.

The precision data obtained from the analytical methods are presented in Table 2. R.S.D.% values were less than 2.0 for both analytical methods. This provides good sensitivity in both methods.

Figure 2: *Overlay spectrum of galantamine standard solutions (5-30 µg/mL)*



Accuracy was investigated by means of the standard addition method. The results of the recovery test are presented in Table 2. Both analytical methods exhibited average recoveries close to 100% and showed sufficient accuracy.

Table 1: *The linearity data obtained for galantamine by both methods*

Regression parameters	HPLC	UV
Number of points	6	6
Concentration range (µg mL^{-1})	5-30	5-30
Relative standard error (%)	1.2155	0.0026
Slope ± standard error	14.4340±0.1300	0.0098±0.0003
Intercept ± standard error	-3.2667±0.080	0.2279±0.0040
Regression coefficient (r^2)	0.9999	0.9993

Table 2: *Validation parameters of the developed methods*

Parameters	HPLC	UV
Intra-day precision, (R.S.D. %, *n* = 6)	0.34	0.45
Inter-day precision, (R.S.D. %, *n* = 18)	0.56	0.63
Accuracy, (mean recovery, %, *n* = 9)	99.78	99.25
LOD (µg mL^{-1}) / LOQ (µg mL^{-1})	0.70/2.10	2.10/6.50

Analysis of marketed formulations

The validated chromatographic and spectrophotometric methods were applied to the analysis of galantamine in raw material and Reminyl tablets (Table 3). The ANOVA test revealed that there was no statistically significant difference at a confidence level of 0.05 between the results obtained for the raw material and tablet samples from the different methods. Tukey's multiple comparison test showed that the means obtained by HPLC and UV for raw material analysis

were statistically equivalent ($p < 0.05$). Tukey test for analysis of tablets revealed statistical equivalence ($p < 0.05$) between HPLC and UV averages.

Table 3: *Galantamine contents in raw material and capsule sample (n=6)*

Samples	Galantamine content (%) ± SD	
	HPLC	UV
Raw material	99.82±0.32	99.57±0.46
Capsule (8 mg)	8.02±0.03	7.98±0.05
F-test F calculation/Ftable	0.32/0.54	
t-test t calculation/ttable	1.45/2.78	

SD: Standard Deviation

Statistical comparison of methods

Both methods were statistically compared using the F-test and the t-test. Statistical analyzes have shown that there is no significant difference between the values obtained from the analyzes performed by both methods.

F-value and t-value were calculated and these values were found to be lower than the table values of both methods at the 95% confidence interval. It is clear that both of the proposed methods are applicable for the quantification of galantamine in dosage forms. Table 3 shows the statistical comparison results of both methods.

Stability of standard solutions

The stability of the reference standard solutions over a 24-hour period was examined. Standard solutions were injected into the HPLC system for 8 hours and peak area and the retention time were recorded (Table 4).

Table 4. *Standard solution stability (n=3, 20 µg mL^{-1})*

Time period hours	Peak area	Average Peak area	SD	RSD (%)	Retention time min.	Average Retention time min.	SD	RSD (%)
0	286.5	286.5	0.2	0.053	4.006	4.010	0.005	0.118
	286.3				4.008			
	286.6				4.015			
24	286.3	286.1	0.3	0.092	4.016	4.014	0.005	0.118
	285.8				4.009			
	286.2				4.018			
48	286.4	286.3	0.3	0.107	4.016	4.014	0.006	0.146
	286.6				4.007			
	286.0				4.018			

R.S.D percentages at the end of the 48-hour period were determined as 0.146% for the retention time and 0.107% for the peak area. No significant change was observed in the active metabolite concentration in the standard solution.

DISCUSSION

Within the scope of the chromatographic method development studies for the quantification of galantamine, several preliminary studies have been carried out to optimize the conditions. Various chromatographic methods have been performed to optimize galantamine quantification. In the study, firstly, only ultrapure water was tested and long analysis times were obtained. Optimal conditions were provided by investigating different acetonitrile ratios. Containing 0.1% TFA ultrapure water and acetonitrile (85/15, v/v) were used as the mobile phase to obtain a good galantamine peak symmetry and shape. Finally, this mobile phase provides more powerful theoretical plates (>7.000) and a peak queuing factor (<1.0). Analyzes at 25C° were not only economical, but also had advantages in terms of low column pressure, column efficiency and good chromatographic peak shape (3-6, 8).

To ensure results, tablet samples were analyzed for 60 minutes and no matrix components remained in the column much longer in the specified conditions. However, continuing the analysis for more than 10 minutes will increase the analysis time and cost. No overlapping peaks were observed in the samples injected into the system for analysis for consecutive 10-minute periods. Therefore, the analysis time was determined as 10 minutes.

In the studies of assessing the specificity of the HPLC method, peak purities of more than 99.0% for galantamine were obtained on chromatograms of sample solutions. This showed that other compounds did not elute together with the main peak. No interfering peaks were observed in the retention time of galantamine in the chromatogram obtained with a mixture of tablet excipients (20-22).

No absorption bands were detected at 288 nm in the spectrum obtained with a mixture of tablet excipients in methanol for the UV method. Therefore, the method was shown to be selective for quantifying galantamine at this wavelength.

The LOD and LOQ values for the chromatographic method were calculated as 0.70 and 2.10 µg mL-1, respectively. In spectrophotometric analysis, the absorbance value of galantamine standard solution at a concentration of 0.70 µg mL-1 at a wavelength of 288 nm was measured as 0.2348. Therefore, this concentration is set as the detection limit. The absorbance value of galantamine standard solution at a concentration of 2.10 µg mL-1 at a wavelength of 288 nm was measured as 0.2485. Therefore, this concentration is set as the quantitation limit.

The validation parameters of both analytical methods are presented in Table 2. According to the results obtained, it is proved that the HPLC method is a more sensitive method that allows determining the amount of galantamine at concentrations about four times lower than the UV method.

Potential interactions in raw material studies were not identified in any of the evaluated methods, despite the fact that spectrophotometric analysis assessed degradation products or related chemicals with comparable chemical structures. It was observed that the chromatographic method is the most sensitive and selective method. This method can be successfully applied for the determination of the amount of galantamine. However, the time and expense of analysis cannot be overlooked. The spectrophotometric approach is clearly more cost-effective and needs less analytical time while also being simple to apply. Because galantamine is such a widely used anticholinesterase drug, it is critical to develop and validate simple and reliable procedures to verify the quality of raw materials and pharmaceutical formulations on the market today.

CONCLUSION

The UV spectrophotometric method has advantages over the LC chromatographic method because the UV spectrophotometric method generally does not require detailed processes and procedures as in the LC chromatographic method. UV spectrophotometric method is more economical and consumes less time than LC chromatographic method. However, statistical comparison of both methods shows that the LC chromatographic method is more precise and accurate than the UV spectrophotometric method. HPLC and UV spectrophotometric methods have shown that they are both adequate methods to determine the amount of galantamine in raw materials, tablets and injectable solutions, and the most reliable results have been obtained. No interfering peaks were observed during the retention time of galantamine in the chromatographic method and no interfering absorption bands were observed at 288 nm in the spectrophotometric method. Because HPLC and UV are simple and rapid methods, they can be successfully applied to quality control analysis to quantify and determine the amount of galantamine in marketed formulations. Our study is complementary to the lack of information in the literature to make a comparative evaluation of HPLC and UV spectrophotometric methods in galantamine.

Etik: Bu çalışmada etik kurul onayı gerekmez.

No ethical approval required.

Yazar katkı durumu; Çalışmanın konsepti; NT, İB, dizaynı; NT, İB, Literatür taraması; NT, İB, verilerin toplanması ve işlenmesi; NT, İB, istatistik; NT, İB, yazım aşaması; NT, İB,

Author contribution status; The concept of the study; NT, İB, design; NT, İB, literature review; NT, İB, collecting and processing data; NT, İB, statistics; NT, İB, writing phase; NT, İB,

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