

Quantification of Galantamine in *Sternbergia* Species by High Performance Liquid Chromatography

Sternbergia Türlerinin Yüksek Basınçlı Sıvı Kromatografisi ile Galantamin İçeriklerinin Araştırılması

INTRODUCTION

Galantamine is approved by FDA for the treatment of mild to moderate Alzheimer's disease.¹ Razadyne® (formerly Reminyl®) and Nivalin® are licensed drugs of galantamine, currently available in the market.² This drug inhibits acetylcholinesterase enzyme reversibly and act as allosteric modulator of the nicotinic cholinergic receptor. Interaction potentiates cholinergic nicotinic neurotransmission by modulating ion channel activity in the presence of acetylcholine.¹⁻⁴ This drug provides the requisite cholinergic stimulation without producing desensitization. Furthermore, galantamine appears to be more powerful elevator of frontal cortical dopamine levels compared to other cholinesterase inhibitors such as donepezil.¹ Galantamine exerted neuroprotection on neuronal cell cultures subjected to oxidative stress or amyloid beta ($A\beta$) stress. Neuroprotection in rat hippocampal slices subjected to oxygen and glucose deprivation followed by a reoxygenation period was also demonstrated by galantamine. Galantamine also act as a neuroprotective agent *in vivo* model of global cerebral ischemia, even when given after the ischemic insult.⁵⁻⁶

Galantamine was firstly isolated from snowdrop, *Galanthus woronowii*. Generally Amaryllidaceae plants including *Narcissus*, *Galanthus*, *Lycoris* and *Leucojum* species are used for extraction of galantamine. *Leucojum aestivum* is known as the main source of this compound. *Narcissus* species also contain galantamine in varying amounts from trace amounts to as much as 2.5% of dry weight. Synthetic methods for production of galantamine has been developed however due to high cost, plants are still main sources for galantamine production.⁷

Galantamine content of Amaryllidaceae plants were investigated by different high performance liquid chromatography (HPLC) methods.⁷ Isocratic solvent system consisting of acetonitrile:methanol:water (containing 7.5 mM triethanolamine, pH 6.9) mixture as mobile phase was used for detecting of galantamine on RP-C8 column in *L. aestivum*.⁸ In another study which was conducted on *L. aestivum*, acetonitrile:methanol:buffer pH 4.5 (10:10:80) mixture was used for elution on RP-C18 column to determine galantamine amount.⁹ Lubbe et al. also reported HPLC analysis of galantamine in *Narcissus pseudonarcissus* on C18 column

using 10% (v/v) acetonitrile in water containing 0.1% TFA as mobile phase.¹⁰ *Galanthus elwesii* was also analyzed for its galantamine content by using the mobile phase comprised TFA:water:ACN (0.01:90:10) mixture on RP-C18 column.¹¹ Petruczynik et al. analyzed galantamine on RP-C18 column with mobile phase containing 5% MeCN, 20% acetate buffer at pH and 0.025 mL⁻¹ diethylamine as well as on SCX column using 8% MeCN and phosphate buffer at pH 2.5 mixture as mobile phase in *L. aestivum*, *L. vernalis* var. *carpathicum*, *G. nivalis*, *Zephyranthes rosea*, *Clivia minata*.¹²

Sternbergia Waldst. & Kit. (Amaryllidaceae) genus is represented by eight species and they are widely distributed from East Mediterranean to Caucasia. In Turkey six taxa of this genus grows naturally.¹³ *Sternbergia* species are well-known due to their alkaloid contents i.e. lycorine and galantamine with interesting pharmacological properties.¹⁴⁻¹⁵ Alkaloids including lycorine, homolycorine, haemanthidine, haemanthamine, 6 α - and 6 β -hydroxy-haemanthamine and tazettine have been isolated from *Sternbergia* species.¹⁴⁻¹⁸ It has been reported that *Sternbergia* species contain especially crinine-type and lycorine-type Amaryllidaceae alkaloids.¹³

In order to investigate new sources for galantamine, *Sternbergia* species were investigated by using HPLC in current study. *S. lutea*, *S. sicula*, *S. fischeriana*, *S. clusiana* and *S. colchiciflora* which were collected from different locations of Anatolia were analyzed using HPLC. Isocratic system was developed and used for HPLC analysis. Galantamine which was isolated from *S. fischeriana* bulbs previously was used for quantification of *S. lutea*, *S. sicula*, *S. fischeriana*, *S. clusiana* and *S. colchiciflora* for their galantamine contents.

EXPERIMENTAL

Plant Materials

Sternbergia species were collected from different parts of Anatolia as shown in Table 1. Voucher specimens are kept at the Herbarium of Ankara University, Faculty of Pharmacy with their herbarium numbers (Table 1).

Isolation of Galantamine

Galantamine was isolated from *S. fischeriana* bulbs. The dried bulbs (500 g) were extracted with ethanol (5 L) by percolation. Ethanolic extract was filtered and concentrated under vacuum at 50 °C by evaporation. The pH of the extract was adjusted to 3 by addition of HCl % 5. After filtration CHCl₃ was used for liquid-liquid extraction. Chloroform part was

concentrated under vacuum by evaporation to obtain extract A (13.7538 g) which contain lycorine and tazettine. Remaining asidic-water part was extracted with CHCl_3 after addition of alkali solution (NH_4OH 25% to obtain pH 8). Concentrated chloroform part gave extract B (1.9712 g). The extract B was separated by chromatotron on alumium oxide GF Gypsum (Merck 1092) plates. Elution was performed with CHCl_3 :MeOH (9:1) mixture. Fraction 1-4 was subjected to further separation by preparative TLC on precoated TLC sheets (Merck 5744) eluting with CHCl_3 :MeOH (85:15) to obtain galantamine (5.04 mg).¹⁹ Structure of the isolated compound was eluciadated by ^1H - NMR and comparison these data by literature.¹⁹⁻²³

Galantamine: ^1H NMR (CDCl_3 , 400 MHz, δ , ppm, J/Hz): 6.63 (1H, d, $J= 8$ Hz, H-12); 6.53 (1H, d, $J= 8$ Hz, H-11); 6.02 (1H, d, $J= 10.3$ Hz, H-4); 6.00 (1H, d, $J= 10.3$ Hz, H-3); 4.47 (1H, brs, H-16); 4.10 (1H, m, H-2 α); 4.06 (1H, d, $J= 15.2$ Hz, H-9 β); 3.98 (1H, d, $J= 15.2$ Hz, H-9 α); 3.05 (1H, m, H-7 α); 2.95 (1H, m, H-7 β); 2.56 (1H, m, H-1 α), 2.45 (1H, m, H-1 β); 1.50 (1H, m, H-6 α); 1.47 (1H, m, H- β), 3.72 (s, O- CH_3), 2.51 (s, N- CH_3).

HPLC Analysis

HPLC analyses were carried out using Agilent LC 1100 model chromatograph (Agilent Technologies, Inc., California, USA). The Diod-Array Detector (DAD) was set at wave length, 292 nm, and peak areas were integrated automatically by computer using Agilent software. The chromatograms were plotted and processed by using the above-mentioned software. Separation was carried out using a Supelcosil LC-18 column (250 \times 4.6 mm i.d.; 5 μm ; Supelco, Belleforte, PA, USA). The mobile phase was made up of ammonium carbonate (Laboratory BDH Reagent, England) water solution (purified water was obtained by using Milli-QPlus System (Millipore Corp., Molsheim, France) and acetonitrile (HPLC grade 99.93 % purity, Sigma-Aldrich 270717) (85:15 v/v) applied at a flow rate of 1 mL/min, column temperature 24 $^\circ\text{C}$, and 20 μL portions were injected into the liquid chromatography.

Preparation of standard and sample solutions

Standard stock solution was prepared as 1 mg/mL. Galantamine was weighed in 10 mL volumetric flask and dissolved in as prepared by dissolving 10 mg of galantamine in 10 mL of 1% H_2SO_4 . Different concentration levels (0.025 mg/mL, 0.05 mg/mL, 0.075 mg/mL, 0.1 mg/mL, 0.2 mg/mL, 0.3 mg/mL, 0.4 mg/mL) were prepared by diluting the stock solution.

Sample solutions were prepared by extraction of dried and powdered bulbs (10 g) of each plant with 1% H_2SO_4 by rinsing at room temperature for 7 days. Extraction procedure was

controlled by Mayer's reagent to be sure all alkaloids were extracted. Each extract was filtered through a 0.45 mm membrane filter and adjusted to a final volume of 500 mL with acidic solution.

Limit of detection and quantification

Limit of detection (LOD) and limit of quantification (LOQ) were established at a signal to noise ratio (S/N) of 3 and 10 respectively. LOD and LOQ concentrations were experimentally verified by six injections of galantamine. The precision of the method (intra-day variations of replicate determinations) was checked by injecting galantamine nine times at the LOQ level.

RESULTS AND DISCUSSION

In present study *Sternbergia* species were investigated for their galantamine contents. Previous studies reported that galantamine was isolated from *S. lutea*, *S. sicula*, *S. fischeriana*, *S. clusiana* and *S. colchiciflora*. However any *Sternbergia* species growing in Turkey has been reported that do not contain galantamine. In addition galantamine has not been determined any *Sternbergia* species growing in Turkey.^{13,24} This study has led to the isolation of this compound from the bulbs of *S. fischeriana* collected from Antakya-Yayladağ province. Additionally, current study describes the development of a method for identifying and quantifying of galantamine in *Sternbergia* species. Good separation and determination of this compound was achieved using mobile phase consisting ammonium carbonate and acetonitrile (85:15 v/v) on a Supelcosil LC-18 column (250 × 4.6 mm x 5 μm) at the wavelength 292 nm as shown in Fig. 1 and Fig. 2.

LOD and LOQ values were determined as 7.5 μg and 25 μg respectively. Table 2 shows the wavelength measured, the calculated calibration curve, the LOD and LOQ results for this compound. The precision of the method expressed as the RSD % at the LOQ level, was % for galantamine.

Presence of galantamine in *S. lutea* ssp. *lutea*, *S. lutea* ssp. *sicula*, *S. candida*, *S. fischeriana* and *S. clusiana* was analyzed quantitative and qualitative by HPLC. Current study results, as shown in Table 3, have revealed that all plant samples contain galantamine and the highest content was determined in *S. lutea* ssp. *sicula* (0.0165±0.0002% dw) followed by *S. lutea* ssp. *lutea* (0.0100±0.0005% dw). According to the previous studies *Galanthus woronowii* and *Leucojum aestivum* contain 0.003-0.506% and 0.0028-0.2104% galantamine respectively.²⁵⁻²⁶ The galantamine content ranged from 0.05 to 0.36 mg/g dw in the bulbs of *G. nivalis* and from 0.3 to 0.033 mg/g dw in the bulbs of *N. tazetta* samples which were collected from

different locations of Iran. According to the results geographical regions and cultural practices affected the chemical composition of the plants. The chemical variations can be attributed to environmental factors.⁷ *L. aestivum* which were collected during different periods of vegetation, were analyzed for their galantamine contents and the amounts were determined as 0.13 and 0.14 % respectively for the plant in bloom and fructification respectively.⁹ According to the Petruczynik et al. different extraction procedures such as maceration, extraction in ultrasonic bath and extraction in ultrasonic bath following maceration allowed to yield different amounts of galantamine. Galantamine content in *L. aestivum* was determined as 0.0196 mg/mL, 0.0273 mg/mL and 0.0949 mg/mL respectively which were applied to mentioned extraction procedures. Ultrasonic bath following maceration induced relatively in high amount of galantamine extraction. In the same study the highest amount of galantamine was determined in *L. aestivum* roots with 2.3524 mg/g dw followed by leaves with 1.6611 mg/g dw. *Zephyranthes rosea* bulbs and *Clivia minata* leaves as well as roots were found to contain galantamine as 0.8384 mg/g dw and 0.1489 mg/g dw, 0.0284 mg/g dw. All parts of the *G. nivalis* galantamine content varied from 0.0003 mg/g dw to 0.0178 mg/g dw.¹² *G. elwesii* samples collected from two different location from Turkey, İzmir and Karaburun, contain 0.026 % and 0.007 % galantamine respectively.¹¹ Amount of galantamine has also been varied from 2.36 mg/g dw to 3.32 mg/g dw in the *N. pseudonarcissus* bulbs which were collected from Netherland.¹⁰ According to our results galantamine content of the *Sternbergia* species was lower than *L. aestivum* when compared. Current study results have been revealed that *Sternbergia* species are not valuable sources for galantamine extraction. Differences in galantamine content of all investigated species could be explained by the existence of chemotype. Furthermore a number of factors such as temperature, season, stages of maturity, geographical origin, climatic conditions, soil can affect the phytochemical content of plants.²⁷⁻
²⁸ The plants, cultivated with different conditions, exhibit an alteration in the quantity of phytochemicals and therefore display varied therapeutic effects.²⁹⁻³⁰

CONCLUSION

Present study is the first report of galantamine isolation from *Sternbergia* species growing in Turkey. An HPLC method was developed for identification and quantification of galantamine in genus *Sternbergia*. The presence of galantamine could be related to growing conditions such as temperature, season, climatic conditions, soil or stages of maturity as well as geographical origin. Chemotype of the mentioned species could be also the reason of galantamine presence in *Sternbergia* species. Therefore further studies will be planned to

investigate *Strenbergia* species collected from different locations of Turkey for their galantamine contents.

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Table 1. Plant materials, collected places and herbarium numbers

Species	Herbarium numbers	Collection sites
<i>Sternbergia candida</i> Mathew & T. Baytop	AEF 23794	Mugla-Fethiye
<i>Sternbergia clusiana</i> (Ker Gawl.) Ker Gawl. Ex Sprengel	AEF 23697	Kahramanmaras-Goksun
<i>Sternbergia fischeriana</i> (Herbert) Rupr.	AEF 23793	Antakya-Yayladag
<i>Sternbergia lutea</i> ssp. <i>lutea</i> Walts. A. Kit.	AEF 23694	Izmir-Torbali
<i>Sternbergia lutea</i> ssp. <i>sicula</i> Tineo ex Guss.	AEF 23695	Mugla-Marmaris

Table 2. Linearity results, Limit of Quantification (LOQ), Limit of Detection (LOD)

Compound	λ	Equation	r^2	Slope %RSD	Intercept %RSD	LOQ (μg)	LOD (μg)
Galantamine	292	Y=118484.33X + 448.2	0.995	2.0594	4.4493	25	7.5

Table 3. Galantamine contents of *Sternbergia* species

Species	Galantamine % (n=3, mean \pm SD)
<i>S. candida</i>	0.0092 \pm 0.0005
<i>S. clusiana</i>	0.0077 \pm 0.0001
<i>S. fischeriana</i>	0.0069 \pm 0.0006
<i>S. lutea</i> ssp. <i>lutea</i>	0.0100 \pm 0.0005
<i>S. lutea</i> ssp. <i>sicula</i>	0.0165 \pm 0.0002

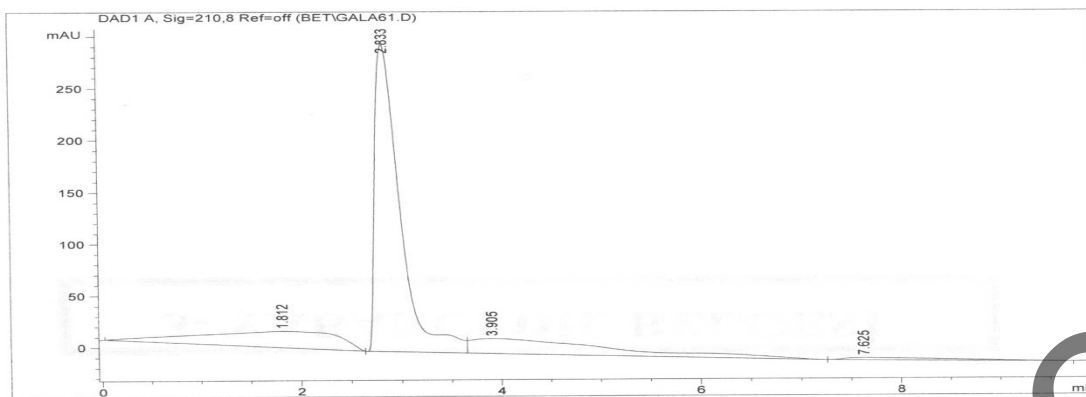


Fig. 1. HPLC chromatogram of galantamine

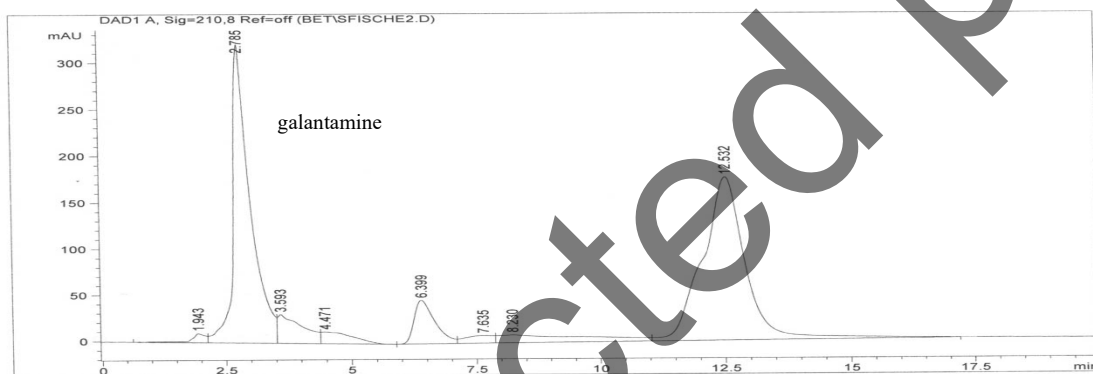
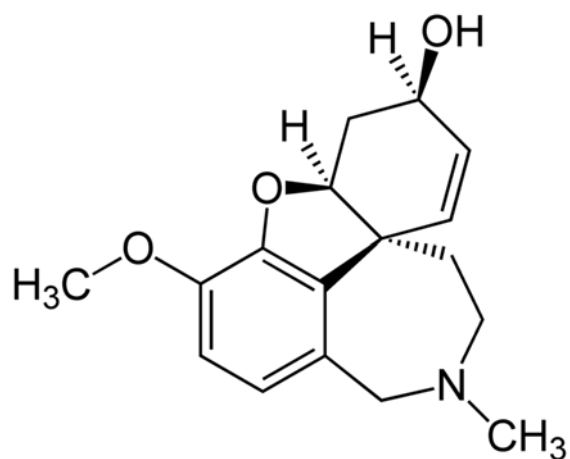


Fig. 2. HPLC chromatogram of *S. fischeriana*

Figure Captions

Fig. 3 Structure of the galantamine



Uncorrected proof