Original Article

Investigation of HPLC behaviour and system suitability estimation for combination Galantamine Hydrobromide and Pymadine

Dobrina Tsvetkova*, Stefka Ivanova

Medical University-Sofia, Faculty of Pharmacy, Department of Pharmaceutical Chemistry, 2 Dunav Str., Sofia 1000, Bulgaria.

Correspondence: Dobrina Tsvetkova, Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Medical University-Sofia, Dunav str. N: 2, 1000, Sofia, Bulgaria. E mail: dobrinka30@mail.bg

ABSTRACT

The aim of the current study was the investigation of HPLC behaviour, separation and system suitability for the combination of Galantamine hydrobromide/Pymadine in model mixtures, in accordance with the new trend of multi-target therapy of Alzheimer's disease by combining acetylcholinesterase inhibitor with its potential synergist. The system suitability test for the simultaneous determination of the components was carried out by the following criteria: 1) retention times in the analysis of 6 model mixtures; 2) the change of values for separation degrees in minor changes of the ratio of mobile phase components and mobile phase flow rates. The system suitability was confirmed by the lack of the statistically significant differences between the values of parameter retention time t_R [min.]: t_R = 3.179 (Galantamine hydrobromide), t_R = 5.272 (Pymadine). In changing the ratio of mobile phase components, the data for separation degree were in the range of 1.76 ÷ 1.83, and the symmetry factor (T) was in the range of 0.94 ÷ 1 (Galantamine hydrobromide), 0.96 ÷ 1.03 (Pymadine). Upon varying the mobile phase velocity, T values varied between 0.94 ÷ 1 (Galantamine hydrobromide) and 0.95 ÷ 1.06 (Pymadine). The system suitability was confirmed by the fact that minor changes in the ratio of the mobile phase components or in the mobile phase flow rate didn't decrease the degree of separation.

Keywords: HPLC, Galantamine hydrobromide, Pymadine, system suitability

Introduction

Alzheimer is a chronic progressive neurodegenerative disease [1] in which the amyloid plaques, neurofibrillary tangles and oxidative stress lead to cholinergic neurons loss in brain, and cause a disorder of higher cortical functions and cognitive impairements [2]. Free-radical processes damage biomarker molecules: proteins, lipids, nucleic acids (DNA, RNA) leading to nerve cell damage and apoptosis, and they are one of the causes of pathogenetic changes [3, 4].

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The conventional treatment strategy for Alzheimer's disease has been an application of acethylcholinesterase inhibitors, like natural alkaloid Galantamine (Fig. 1.) [5]. A potent inhibitory activity against acetylcholinesterase possess extracts of *Ocimum sanctum* [6] and *Cinnamon zeylanicum* [7].

Galantamine leads to the enhancement of concentration in Alzheimer [8] with cerebrovascular disease, vascular dementia and ischemia [9] due to its antioxidant effects [10, 11], decreases neurodegeneration, and protects against β-amyloid toxicity, due to its action as a reversible acethylcholinesterase inhibitor, and an allosteric nicotinic receptors modulator [5], and improves memory alone, and in combination with 4-aminopyridine (Pymadine)(Fig. 1.) [12]: Nivalin P [13]. 4-aminopyridine is a drug for symptomatic multiple sclerosis treatment, and increases the acetylcholine release [14].

A new trend in Alzheimer's disease treatment has been the combination of Galantamine/Pymadine due to the potential synergy of their pharmacological effects [14].

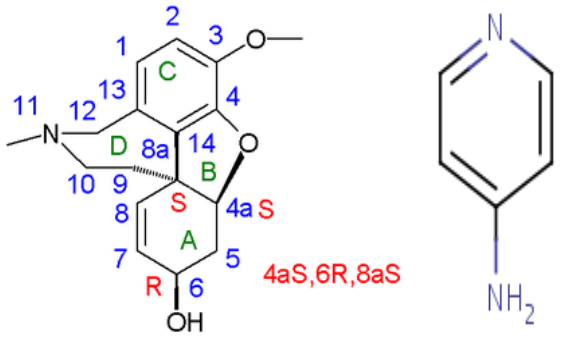


Figure 1. Chemical structures of Galantamine hydrobromide and 4-aminopyridine.

HPLC methods are often applied for analysis of different drugs: Amlodipine besylate [15], Lansoprazole and Aspirin [16], Pantoprazole and Mosapride [17], Olmesartan Medoxomil, Atorvastatin calcium [18], and Cefadroxil Monohydrate [19].

For analysis of Galantamine hydrobromide substance, HPLC-methods with mass-detection [20] and UV-detection [20-23] at $\lambda = 225$ nm [21] and $\lambda = 289$ nm [23] have been developed.

4-aminopyridine in plasma has been analysed by HPLC [24], gas chornatography [25], and capillary electrophoresis [26].

The aim of the current study was the investigation of HPLC behaviour, separation and system suitability in combination of Galantamine hydrobromide/Pymadine in model mixtures.

Materials

I. Pharmacopoeial purity compounds were investigated including: Galantamine hydrobromide (Sopharma, N: 10796132); 4-aminopyridine.

II. Reagents with pharmacopoeal purity were studied including: disodium hydrogen phosphate (99.5 %) (Merck, N: K28661174105).

III. Solvents with pharmacopoeial purity were investigated including: acetonitrile (99.9 %) (Sigma Aldrich, N: SZBD 150 SV UN 1648), methanol (99.9 %) (Sigma Aldrich, N: SZBD 063AV UN 1230), distilled water.

Methods

HPLC-method was used for simultaneous determination of Galantamine hydrobromide and Pymadine in model mixtures.

I. Equippment

HPLC system Shimadzu LC-10 Advp Liquid Chromatograph, fixed-length wavelength SPD 10 AVP UV-VIS detector were used.

II. Chromatographic conditions.

The chromatographic conditions applied were: isocratic mode, stationary phase: column RP C_{18} ODC Spherisorb (250 mm × 4.6 mm × 5 мm), column temperature: 25 °C, mobile phase: 50 mM disodium hydrogen phosphate: acetonitrile = 80:20 v/v, flow rate: 1.5 ml/min., UV-detection at л = 280 nm, injection volume: 20 µl. The column was equilibrated for 30 min with degased mobile phase.

III. Preparation of model mixtures of reference substances: Galantamine hydrobromide and 4-aminopyridine

6 model mixtures were prepared at the following manner: from reference substances an accurately messured quantities: 0.1 g Galantamine hydrobromide and 0.05 g 4-aminopyridine were dissolved in destilled water in 100.0 ml volumetric flasks.

Results and Discussion

I. Selectivity

In the same manner like model mixtures, a blank solution without Galantamine hydrobromide and Pymadine was prepared for the estimatiton of analytical parameter selectivity, which was proved by the fact that in chromatograms of blank solutions, there were not observed peaks with retention time, corresponded to retention times of Galantamine hydrobromide and Pymadine.

II. Investigation of chromatographic behavior of Galantamine hydrobromide and Pymadine in different chromatographic systems

For the selection of a chromatographic system for separation of mixture components, the chromatographic behavior of Galantamine hydrobromide and Pymadine was investigated with isocratic HPLC-method, stationary phase: 250 mm× 4.6 mm × 5 µm and other different chromatographic conditions.

Results were summarized on Table 1. that inducates: C – concentration, t_R – retention time, A – peak area, H – peak hight.

Table 1. Chromatographic behavior of Galantamine hydrobromide and Pymadine at different chromatographic systems.

C [g/ml]		t_R [min.]	A	H
methanol:acetonitrile:water = 70:20:10v/v; 1 ml/min.; 25 °C; λ = 288 nm				
Galantamine hydrobromide	1.10^{-4}	3.374	325950	8847
Pymadine	1.10^{-3}	3.085	5949137	127165
Galantaminehydrobromide	5.10^{-3}	3.301	22848681	478908

Pymadine	5.10^{-3}			
methanol:acetonitrile = 70:30 v/v; 1 ml/min.; 25 °C; λ = 282 nm				
Galantamine hydrobromide	1.10^{-3}	2.903	2501296	74015
Pymadine	1.10^{-3}	2.736	14352632	391237
Galantamine hydrobromide Pymadine	1.10^{-3} 1.10^{-3}	2.991	13184587	305740
methanol:acetonitrile = 10:90 v/v;1 ml/min.; 25 °C; λ = 288 nm				
Galantamine hydrobromide	1.10^{-3}	3.092	1388930	35093
Pymadine	5.10^{-4}	2.673	1536717	42872
Galantamine hydrobromide Pymadine	$2.5.10^{-4}$ 5.10^{-4}	2.987	861811	21067
50mM disodium hydrogenphosphate:acetonitrile = 50:50 v/v; 1 ml/min.; 25 °C; л= 280 nm				
Galantamine hydrobromide	1.10^{-3}	2.737	23302359	768816
Pymadine	5.10^{-4}	2.721	9976943	312689
Galantamine hydrobromide Pymadine	$2.5.10^{-4}$ 5.10^{-4}	2.729	14807814	473382
Galantamine hydrobromide Pymadine	2.10^{-3} 1.10^{-3}	2.774	17044039	5412379

The results included in Table 1., showed that the retention times of Galantamine hydrobromide and Pymadine were close, and that only one peak was obtained in analyzing the model mixture, therefore these systems weren't suitable for separation of the components.

Table 2. presents the data for: t_R – retention time, A – peak area, H – peak height, N – number of theoretical plates and Rs – degree of separation for isocratic HPLC, and chromatographic systems, by which two peaks were obtained in the analysis ofthe model mixtiures with Galantamine hydrobromide and Pymadine.

Table 2. Chromatographic parameters of Galantamine hydrobromide and Pymadine at different chromatographic systems.

Parameters	t_R [min.]	A	H	N	Rs
methanol:acetonitrile:water = 70:20:10v/v;1 ml/min.;25 °C; λ = 220 nm					
Pymadine	3.504	2010320	58877	215	0.92
Galantamine hydrobromide	4.697	6015356	141670	183	
methanol:water = 50:50 v/v; 1 ml/min.; 25 °C;λ =288 nm					
Pymadine	3.245	1029411	43111	889	0.44
Galantamine hydrobromide	3.461	2188596	113306	484	
methanol:water = 95:5 v/v; 1 ml/min.; 25 °C;λ =288 nm					
Pymadine	3.469	913993	36690	361	0.1
Galantamine hydrobromide	3.617	905668	37048	400	
50mM disodium hydrogenphosphate: acetonitrile= 80:20 v/v; 1 ml/min.;25 °C; л= 244 nm					
Pymadine	3.071	8011684	235963	196	0.53
Galantamine hydrobromide	3.890	3578087	104476	165	
50 mM disodium hydrogenphosphate:methanol = 80:20 v/v;1 ml/min.; 25 °C; л= 280 nm					
Pymadine	3.307	483035	15498	369	0.89
Galantamine hydrobromide	3.917	252838	8177	256	
50 mM disodium hydrogenphosphate:acetonitrile = 90:10 v/v; 1 ml/min.;25 °C;л= 280 nm					
Pymadine	3.550	844190	22462	144	0.59
Galantamine hydrobromide	4.498	1176047	28695	104	

The values for parameter (Rs) given in Table 2. were lower than 1, demonstrating that these systems didn't achieve separation of Galantamine hydrobromide and Pymadine.

For the separation and simultaneously identification and determination of the components of the model mixture of Galantamine hydrobromide and Pymadine, HPLC is suitable with isocratic mode of operation on the chromatographic system: stationary phase; column RP of (250 mm× 4.6 mm × 5 μm); column temperature of 25 °C; mobile phase of 50 mM disodium hydrogenphosphate:acetonitrile = 80:20 v/v; flow rate of 1 ml/min.; and UV-detection at λ = 280 nm.

III. System suitability test

The suitability test was eatimated on the basis of ICH requirements and criteria [27, 28]. The system suitability test for

the simultaneous determination of Galantamine hydrobromide and Pymadine was carried out by following criteria: 1) retention times in the analysis of 6 model mixtures; 2) change of degree of separation (Rs) in minor change of the ratio of mobile phase components, and of mobile phase flow rate.

The results for retention times t_R [min.] for Galantamine hydrobromide and Pymadine in model mixtures have been presented in Table 3.

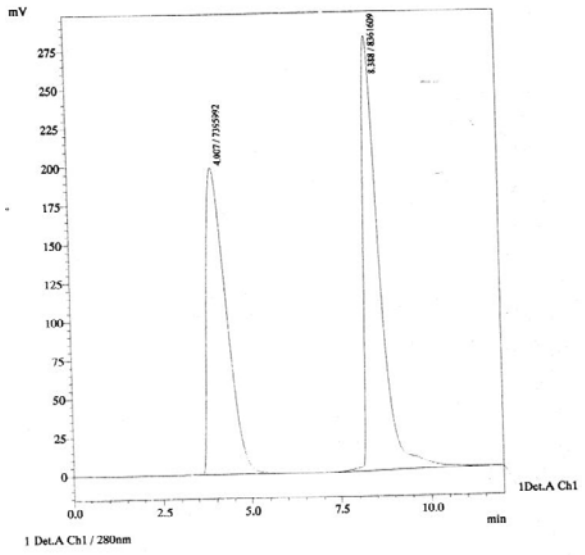
Table 3. Retention times t_R [min] for Galantamine hydrobromide and Pymadine in model mixtures.

N: Model mixture	Galantamine hydrobromide	Pymadine
	t_R [min.]	t_R [min.]
1.	3.188	5.223
2.	3.180	5.218
3.	3.175	5.231
4.	3.173	5.522
5.	3.181	5.238
6.	3.175	5.198
$\bar{X} \pm$ SD	3.179 ÷ 0.006	5.272 ÷ 0.12
RSD [%]	0.19	2.28

The system suitability was confirmed by the lack of a statistically significant difference between the values of the chromatographic parameter retention time for the components in the analysis of 6 samples: t_R = 3.179 (Galantamine hydrobromide), t_R = 5.272 (Pymadine) (Table 3.).

The chromatograms of model mixtures obtained with HPLC system for the minor change of the ratio of mobile phase components, and the minor change of mobile phaseflow rate are illustrated in Fig. 2. (80:20 v/v), Fig 3. (77:23 v/v), Fig.4. (83:17 v/v).

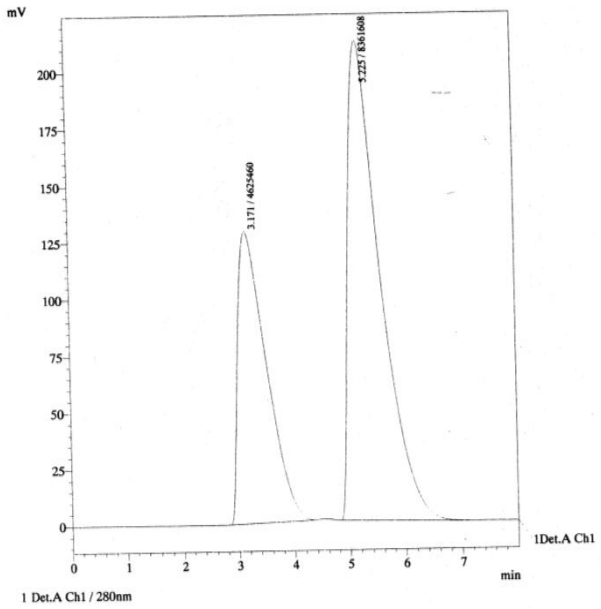
0.8 ml/min.



Detector A Ch1 280 nm

Peak#	Ret. Time	Area	Height	Area%	Height%
1	4.007	7395992	198703	46.936	41.377
2	8.388	8361609	281525	53.064	58.623
Total		1557602	480228	100.000	100.000

1.2 ml/min.



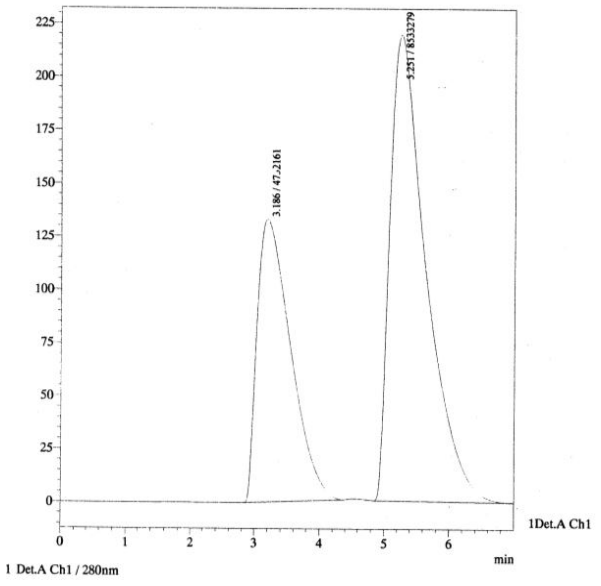
Peak Table

Detector A Ch1 280 nm

Peak#	Ret. Time	Area	Height	Area%	Height%
1	3.171	4625460	129061	35.616	37.849
2	5.225	8361608	211925	64.384	62.151
Total		12987069	340986	100.000	100.000

Figure 2. Chromatograms of solutions containing Galantamine hydrobromide and Pymadine in system: 80:20 v/v = 50 mM disodium hydrogenphosphate: acetonitrile; 25 °C; λ = 280 nm.

1 ml/min.

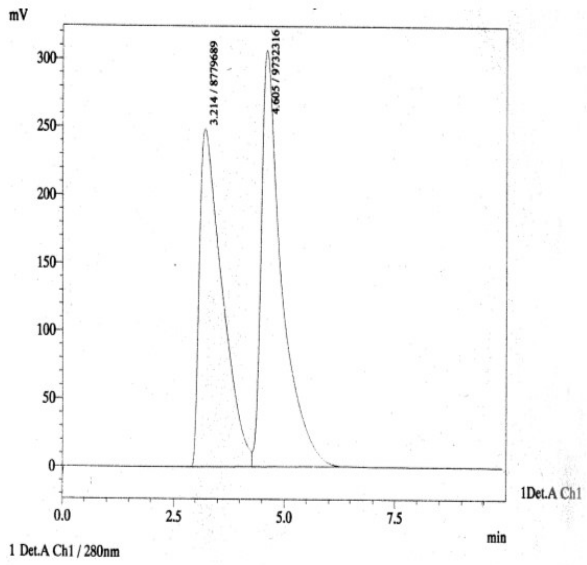


Peak Table

Detector A Ch1 280 nm

Peak#	Ret. Time	Area	Height	Area%	Height%
1	3.186	4752161	132904	35.770	37.767
2	5.251	8533279	218996	64.230	62.233
Total		13285440	351900	100.000	100.000

1.2 ml/min.



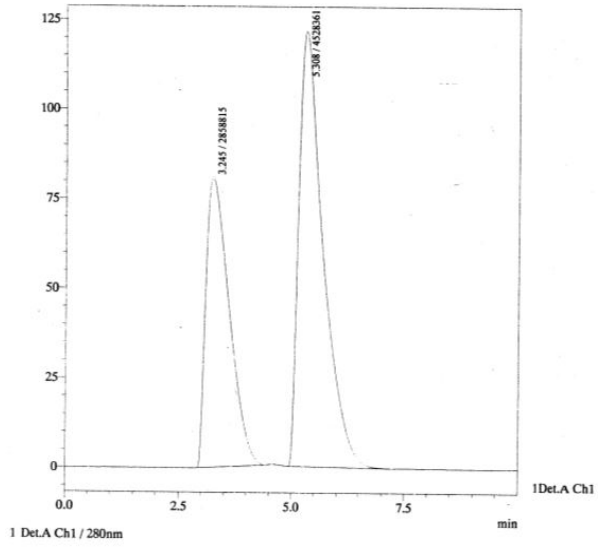
Peak Table

Detector A Ch1 280nm

Peak#	Ret. Time	Area	Height	Area%	Height%
1	3.214	8779689	248809	47.427	44.789
2	4.605	9732316	306700	52.573	55.211
Total		18512005	555509	100.000	100.000

Figure 3. Chromatograms of solutions containing Galantamine hydrobromide and Pymadine insystem: 77:23 v/v = 50 mM disodium hydrogenphosphate: acetonitrile; 25 °C; λ = 280 nm

0.8 ml/min.

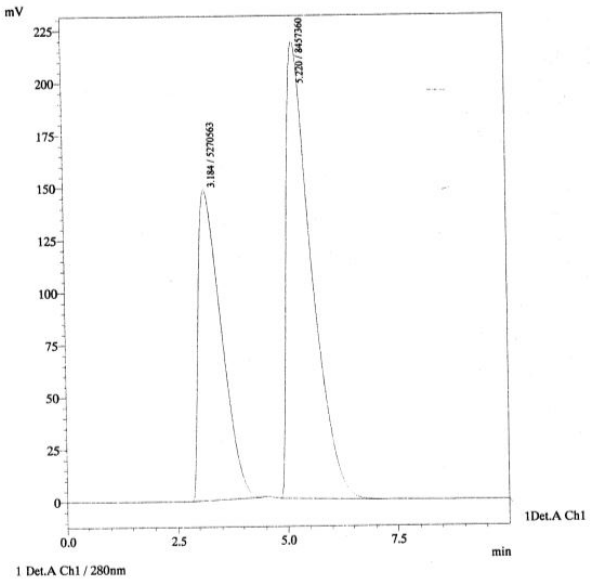


Peak Table

Detector A Ch1 280 nm

Peak#	Ret. Time	Area	Height	Area%	Height%
1	3.245	2858815	80981	38.700	40.001
2	5.308	4528361	121468	61.300	59.999
Total		7387176	202449	100.000	100.000

1 ml/min.



Peak Table

Detector A Ch1 280nm

Peak#	Ret. Time	Area	Height	Area%	Height%
1	3.184	5270563	148308	38.393	40.465
2	5.220	8457360	218200	61.607	59.535
Total		13727923	366508	100.000	100.000

Figure 4. Chromatograms of solutions containing Galantamine hydrobromide andPymadine in system: 83:17 v/v = 50 mM disodium hydrogenphosphate : acetonitrile; 25 °C; λ = 280 nm.

Results for system suitability test were shown in Table 4., where:

N/m –number of the theoretical plates at the peak width of baseline and column length 1 m

T – tailing factor

f – distance from the beginning of the peak to the intersection of peak height with

peak width at 5% of the peak height

As – asymmetry factor

S – symmetry factor

Table 4. HPLC system suitability for Galantamine hydrobromide/Pymadine mixtures.

Parameter	t_R [min.]	A [AU]	H [AU]	N/m	Rs	$T = \frac{W\,0.05}{2.\,f}$	$As = \frac{A\,0.1}{B\,0.1}$	$s = \frac{N\,0.1}{N\,0.5}$
80:20 v/v = 50 mM disodium hydrogenphosphate:acetonitrile;1 ml/min.; 25 °C: Л = 280 nm								
Pymadine	3.188	5493395	151586	464		1	0.92	0.92

Galantamine hydrobromide	5.223	10078460	246787	1272	1.77	1	1	1.16
80:20 v/v = 50 mM disodium hydrogenphosphate:acetonitrile; 0.8 ml/min.; 25 °C; л = 280 nm								
Pymadine	4.007	7395992	198703	696		0.95	1	1.05
Galantamine hydrobromide	8.388	8361609	281525	3892	2.8	0.94	1	1.11
80:20 v/v = 50 mM disodium hydrogenphosphate:acetonitrile; 1.2 ml/min.;25 °C; л = 280 nm								
Pymadine	3.171	4625460	129061	680		1	1	1.51
Galantamine hydrobromide	5.225	8361608	211925	1516	2.06	1	0.93	1.35
77:23 v/v = 50 mM disodium hydrogenphosphate:acetonitrile; 1 ml/min.; 25 °C; л = 280 nm								
Pymadine	3.186	4752161	132904	576		1.03	1	1.22
Galantamine hydrobromide	5.251	8533279	218996	1360	1.83	0.94	1	1.08
77:23 v/v = 50 mM disodium hydrogenphosphate:acetonitrile; 1.2 ml/min.; 25 °C; л = 280 nm								
Pymadine	3.214	8779689	248809	828		1.06	0.8	1.51
Galantamine hydrobromide	4.605	9732316	306700	1376	1.6	1	1	1.05
83:17 v/v = 50 mM disodium hydrogenphosphate:acetonitrile; 0.8 ml/min.; 25 °C; л = 280 nm								
Pymadine	3.245	2858815	80981	628		1	1	1.67
Galantamine hydrobromide	5.308	4528361	121468	1684	2.0	1	0.91	1.31
83:17 v/v = 50 mM disodium hydrogenphosphate:acetonitrile; 1 ml/min.; 25 °C; л = 280 nm								
Pymadine	3.184	5270563	148308	576		0.96	1	1.67
Galantamine hydrobromide	5.220	8457360	218200	1272	1.76	0.96	1	1.16

In changing the ratio of mobile phase components, the separation degree data were in the range of 1.76 ÷ 1.83, and the symmetry factor (T) was in range of 0.94 ÷ 1 (Galantamine hydrobromide), 0.96 ÷ 1.03 (Pymadine). Upon varying the mobile phase velocity, T values varied between 0.94 ÷ 1 (Galantamine hydrobromide) and 0.95 ÷ 1.06 (Pymadine).

Conclusion

For the separation, simultaneous identification and determination of Galantamine hydrobromide and Pymadine in model mixture, isocratic HPLC was Suitable with RP (250 mm × 4.6 mm ×5 μm); column temperature of 25 °C; mobile phase of 50 mM disodium hydrogenphosphate:acetonitrile = 80:20 v/v; flow rate of 1 ml/min.; and UV-detection at λ = 280 nm. The system stability was confirmed by the fact that the minor changes in the ratio of mobile phase components or in mobile phase flow rate did not decrease the degree of separation.

Conflicts of Interests

All authors had none to declare.

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