

DEVELOPMENT AND VALIDATION OF A NEW METHOD BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY FOR THE QUANTITATIVE ANALYSIS OF MAGNOLOL LOADED IN SILICA PARTICULATE SYSTEMS

ALINA ȘTEFANACHE¹, LĂCRĂMIOARA OCHIUZ^{1*}, MARIA IGNAT², ANDREEA CREȚEANU¹, GLADIOLA ȚÂNTARU¹

¹"Grigore T. Popa" University of Medicine and Pharmacy Iași, 16 Universității Street, 700115, Iași, Romania

²"Al. I. Cuza" University, Faculty of Chemistry, 11 Carol I Bvd., 700506, Iași, Romania

*corresponding author: ochiuzd@yahoo.com

Manuscript received: September 2015

Abstract

In order to analyse magnolol (MG) in modern pharmaceutical forms, we developed and validated a new high performance liquid chromatography (HPLC) method with UV detection. We optimized the working conditions for the chromatographic system and recorded the chromatograms at the wavelength of 290 nm. The method was validated by the determination of the following parameters: method *repeatability* and *intermediate precision* for the concentration of 0.5 mg/mL for which we obtained RSD values of ≤ 2.5 for peak area, retention time and concentration, method *linearity* in the concentration range 0.025 - 1.5 mg/mL; *calibration curve equation* for MG ($y = 492.05x + 6.5518$) ($R^2 = 0.9985$); *detection limit* of 0.00988 mg/mL and *limit of quantification* of 0.02993 mg / mL; method *robustness* for MG concentration of 0.5 to 1 mg/mL; *accuracy* of method, for which the achieved recovery rate was in the range 98.42 - 103.83%. The method was tested by the determination of MG loaded in nanoporous silica. Results showed a 96.57% degree of retrieval and confirm the hypothesis that this method can be used for the quantitative determination of MG loaded in silicate matrices as modified release formulations.

Rezumat

În vederea analizei magnololului (MG) din forme farmaceutice moderne a fost elaborată și validată o nouă metodă de determinare cantitativă a MG prin cromatografie de lichide de înaltă performanță (HPLC) cu detecție UV. Au fost optimizate condițiile de lucru pentru sistemul cromatografic și au fost înregistrate cromatogramele la lungimea de undă de 290 nm. Metoda a fost validată prin determinarea următorilor parametri: *repetabilitatea* și *precizia intermediară* a metodei pentru concentrația de 0,5 mg/mL, când am obținut valori ale $RSD \leq 2,5$ pentru aria *peak*-ului, timp de retenție și concentrație, *liniaritatea metodei* în domeniul de concentrație 0,025 - 1,5 mg/mL; *ecuația dreptei de calibrare* pentru MG ($y = 492,05x + 6,5518$) ($R^2 = 0,9985$); *limita de detecție* 0,00988 mg/mL și *limita de cuantificare* 0,02993 mg/mL; *robustețea* metodei pentru concentrația de 0,5 și 1 mg/mL MG; *acuratețea* metodei pentru care s-a obținut grad de recuperare cuprin în intervalul 98,42 - 103,83%. Metoda a fost testată prin determinarea MG încărcat în silice nanoporoasă. Rezultatul obținut a evidențiat un grad de regăsire de 96,57% și confirmă ipoteza privind utilizare a acestei metode pentru determinarea cantitativă a MG încărcat în matrici silicate ca forme farmaceutice cu cedare modificată.

Keywords: magnolol (MG), quantitative determination, silica, chromatography

Introduction

Magnolol (MG) is a polyphenolic compound (Figure 1) extracted from the root and bark of magnolia - *Magnolia* spp. (*M. obovata* and *M. officinalis*) [1].

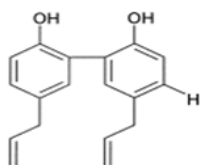


Figure 1.

Chemical structure of magnolol

MG has multiple pharmacological activities, among which anti-inflammatory, antioxidant, antidiabetic, and antidepressant effects [2-4]. Its oral administration has the highest compliance in patients. MG has a low oral bioavailability, of only 5%, which is determined, on one hand, by its reduced absorption in the gastro-intestinal tract, and, on the other hand, by extended metabolism through the hepatic first-pass effect [2]. In order to develop modified release oral therapeutic systems, we chose to encapsulate MG in nanoporous silicates which can host different drug substances [5-7].

In the literature we have identified various methods for the quantitative determination of MG, such as capillary electrophoresis, spectrophotometry, HPLC with UV detection, ion pairs, electrochemical or mass spectroscopy. They have varying degrees of sensitivity [8-12].

The objective of this paper was to develop and validate a new HPLC method for the quantitative determination of MG in nanoporous silicate matrices as systems able to develop new modified release formulations for the oral administration of MG.

Materials and Methods

Materials

Tetraethyl orthosilicate (TEOS, 98%), cetyltrimethylammonium bromide (CTAB, 99%) and NaOH were purchased from Merck. Ethanol (99%), aqueous ammonia (25%), and hydrochloric acid (32%) were purchased from Chemical Company LDT (Romania).

Aparatus and reagents

Liquid Chromatograph Thermo Fisher Scientific, UltiMate 3000, equipped with UV-VIS Diode Array Detector; chromatography column Teknochroma Tracer Excel 120 OSDB C18, 150 mm x 4.6 mm, particle size 5 µm. Methanol (MeOH), chromatographic purity (Merck, Germany); glacial acetic acid (Merck, Germany); trifluoroacetic acid (TFA) (Merck, Germany); acetonitrile (ACN), chromatographic purity (Merck, Germany); *magnolol* - reference substance, purity 99.80% (Sigma-Aldrich, Germany) *magnolol*, purity 98% (Zhejiang Sanmen Hengkang China Pharmaceutical Co. Ltd.); double distilled water: Millipore, conductivity – 0.01 µS/cm.

Methods

Chromatographic conditions and method validation

Identification of peaks

The working conditions were: separation column TRACER EXCEL 120 ODSB (Teknokroma Analitica SA., Spain), 4.6 x 150 mm, 5 µm; column temperature - 30°C; injection of a sample volume of 20 µL; mobile phase used - 0.1% TFA in H₂O (solvent A) and methanol (solvent B), in a ratio of 20:80 (v/v). We carried out an isocratic elution for 12 minutes, and the pump flow rate was 1 mL/min.

Chromatograms' recording was made at the wavelength of 290 nm. In order to identify the peak corresponding to MG, we injected two solutions of different concentrations - 0.5 mg/mL and 1 mg/mL, respectively.

Determination of repeatability and intermediate precision

For the study of the accuracy of the chromatographic system we prepared a solution of MG (concentration - 0.5 mg/mL), and measured signal height, half-width, asymmetry, number of theoretical plates and

resolution compared to the signal of an unrestrained compound. To assess *repeatability*, we performed 10 successive injections and calculated the standard deviation and relative standard deviation of the peak areas, the retention times and the concentration.

Determination of linearity

The linearity range studied was from 0.025 to 1.5 mg/mL MG. Each level of concentration was injected in triplicate and we calculated the mean value, which was later used in plotting the calibration curve, and calculation of the standard deviation and the relative standard deviation.

Determination of detection and quantification limits

To determine the quantification and detection limits, calibration curves were drawn in the concentration range 0.00625 – 0.1 mg/mL MG. Standard deviation and calibration curve slope were also calculated. The limits of detection and quantification were calculated using the following formulas:

$$LOD = \frac{3.3 \times \sigma}{b} \quad (1)$$

$$LOQ = \frac{10 \times \sigma}{b} \quad (2)$$

where: σ – residual relative standard deviation; b – slope calculated for the range under consideration.

Determination of the robustness of the method

To assess the robustness of the method, we injected two solutions for each compound, in various concentrations and conditions (0.5 mg/mL and 1 mg/mL, respectively). These concentrations were successively injected under injection rate variation (0.9 - 1.1 mg/mL) and operating temperature variation (28 - 32°C).

Determination of the accuracy of the method

The accuracy of the analysis method was estimated by determining the degree of recovery, by using three solutions with three different concentrations: 0.1 mg/mL, 0.5 mg/mL and 1 mg/mL.

Silicate matrices were synthesized by modified Stöber method [13, 14], according to the following working steps: (a) preparation of the mixture TEOS:CTAB:ammonium hydroxide solution 25% = 1:1:2; (b) ultrasonication for 2 minutes; (c) washing with water, filtration and drying (24 h, 60°C); (d) calcination at 550°C for 4 hours.

Inclusion of drug molecules in silica matrices

MG immobilization in silica matrices was achieved by its adsorption from a solution containing 1 mg/mL MG, under vigorous magnetic stirring, for 4 h. The resulted MG-loaded silica particles were recovered by vacuum filtration. The percentage of MG loading in the matrix (35.13%) was calculated by gravimetric analysis using the previously published method [15]. The quantitative determination of the MG included in the silica matrix was attained by dispersing 300 mg of silica loaded in 100 mL of methanol and stirring at 600 rpm/10 min. Subsequently, 2 mL of the solution was diluted to

20 mL with the mobile phase. Determinations were performed in triplicate and the results presented are the mean of the values obtained.

Results and Discussion

The data obtained in the method optimization stage confirm that by using the mobile phase of 0.1% TFA in H₂O:MeOH (A:B), in a ratio of 20:80%, at

a temperature of 30°C and a flow rate of 1 mL/min, the separation is effective for MG and meets the specifications of international guidelines with respect to peak asymmetry. Moreover, following the injections of the mobile phase and solvent, the absence of peaks interfering with the peaks of interest was noted (Figure 2).

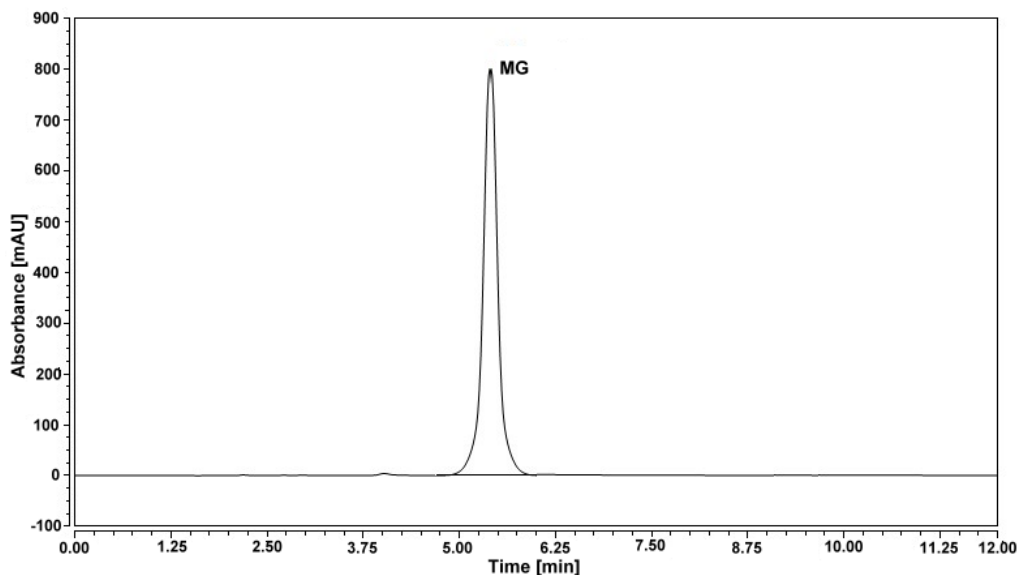


Figure 2.

Chromatogram of the magnolol reference substance

The retention time was 5.39 min and the peak area was 247.7372 mAU*min for a concentration of 0.5 mg/mL MG and 609.8394 mAU*min for 1 mg/mL MG, respectively. System accuracy evaluation revealed the following results: retention time = 5.397 min; signal height = 799.04 mAU; signal half-width = 0.205 min; asymmetry = 1.11; number of theoretical

plates = 3822; purity index (PPI) = 295.4 nm. A chromatographic system is precise when $RSD \leq 2.5$ [16-18].

Accordingly, the obtained data concerning both retention time and area confirm the compatibility and accuracy of the system (Table I).

Table I

Determined chromatographic parameters related to repeatability

Sample	Magnolol		
	Retention time (min.)	Area (mAU*min.)	Quantity (mg/mL)
1	5.383	251.5269	0.497866274
2	5.383	253.0609	0.500983843
3	5.383	254.0409	0.502975511
4	5.383	253.1663	0.501198049
5	5.387	253.6087	0.502097145
6	5.383	253.005	0.500870237
7	5.383	254.0251	0.502943400
8	5.383	254.0252	0.502943603
9	5.377	253.8179	0.502522305
10	5.380	254.1786	0.503255360
SD	0.00254951	0.804858649	0.001635725
RSD	0.047366647	0.317566692	0.325993934

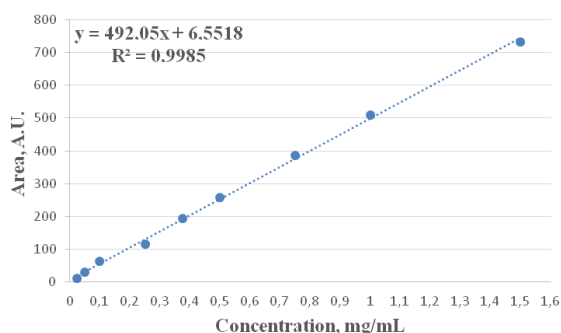
The obtained results confirm the linearity of the method in the range of the tested concentration (0.025 - 1.5 mg/mL MG) (Table II). Each

concentration level was assessed in triplicate. The mean value was calculated and used subsequently in drawing the calibration curve (Figure 3).

Table II

Determined chromatographic parameters adequate to linearity

Conc. of MG (mg/mL)		0.025	0.05	0.1	0.25	0.375	0.5	0.75	1	1.5
Area (mAU*min.)	Series 1	10.9349	30.5444	62.5236	115.8474	191.9478	257.3348	386.9400	508.3981	733.3924
	Series 2	10.8504	30.6641	62.2851	115.9313	191.7209	257.0284	385.7402	508.6213	731.5526
	Series 3	10.7435	31.1444	62.3569	115.9938	195.0082	257.4804	386.2993	510.5923	731.4546
Average		10.84293	30.7843	62.38853	115.9242	192.8923	257.2812	386.3265	509.2039	732.1332
SD		0.095918	0.317547	0.122356	0.07346	1.835932	0.230718	0.600362	1.207558	1.0916
RSD		0.884615	1.031522	0.19612	0.063369	0.951791	0.089675	0.155403	0.237146	0.149098

**Figure 3.**

Calibration curve for the magnolol

In the concentration range 0.00625 - 0.1 mg/mL, the method has a corresponding sensitivity and exhibits

a quantification limit of 0.002993 mg/mL and a detection limit of 0.00988 mg/mL (Table III).

Table III

Quantification and detection limit of the method

Conc. of MG (mg/mL)	0.00625	0.0125	0.025	0.05	0.1	Standard deviation (σ)	LOD (mg/mL)	LOQ (mg/mL)
Area (mAU*min.)	2.1237	5.4886	10.944	29.376	57.414	1.79431	0.00988	0.002993

Data for determining method robustness and accuracy show that the method of MG determination is

reliable and manifests constant validity (Table IV and Table V).

Table IV

Robustness evaluation of the separation method for MG

Parameters			Result		
Concentration MG (mg/mL)	Flow (mL/min)	Temperature (°C)	Retention time (min)	Area (mAU*min)	Quantity (mg/mL)
0.5	1.1	30	4.897	230.9259	0.4559
1	1.1	30	4.893	452.4205	0.9061
0.5	1	28	5.510	249.9597	0.4946
1	1	28	5.497	501.0643	1.0050
0.5	1	32	5.273	253.5710	0.5020
1	1	32	5.260	498.6737	1.0001

Table V

Accuracy evaluation of the analysis method for MG

MG Concentration (mg/mL)	Area (mAU*min)	Recovery (%)	SD	RSD
0.1	57.64	103.831	1.046	1.0176
	56.61	101.740		
	57.37	102.860		
0.5	251.33	99.497	0.587	0.5948
	248.99	98.543		
	248.70	98.425		
1	500.26	1.003	0.273	0.2719

The method developed and validated was used for the quantitative determination of MG loaded in

silicate matrices. The results obtained showed a recovery of 96.57% (Table VI).

Table VI

Quantitative determination of MG in silicate matrix

Sample	Theoretical concentration (mg/mL)	Statistic results	Peak area (mAU*min)	Actual MG concentration (mg/mL)	Recovery
MG-silica particles	0.1053		58.162	0.1013	96.20 %
			58.563	0.1020	96.86 %
			58.449	0.1018	96.67 %
		Average	58.391	0.1017	96.57 %
		SD	0.20663	3.6055	0.3397
		RSD	0.08025	1.8690	1.1036

Conclusions

In this study we developed and validated a new HPLC method determination for magnolol with UV detection. The working conditions optimized for the chromatographic system involved the utilization of the mobile phase consisting in a sol. 0.1% TFA in H₂O (solvent A) and methanol (solvent B), in a ratio of 20:80 (v/v), working temperature - 30°C in column TRACER EXCEL 120 ODSB (Teknokroma Analytics SA., Spain), 4.6 x 150 mm, 5 µm, injection of a sample volume of 20 µL and chromatogram recording at a wavelength of 290 nm. The method was validated by determining the following parameters: method *repeatability* and *intermediate precision* for a concentration of 0.5 mg/mL, when we obtained RSD values ≤ 2.5 for peak area, retention time and concentration; *method linearity* in the concentration range of 0.025 - 1.5 mg/mL, *equation of curve calibration* $y = 492.05x + 6.5518$ ($R^2 = 0.9985$); *detection limit* - 0.00988 mg/mL and *quantification limit* - 0.02993 mg/mL, method *robustness* was determined by injecting MG solutions (concentrations 0.5 and 1 mg/mL) under various flow and temperature conditions. The obtained data reveal that the method for the quantitative determination of MG is reliable. The recovery degree was in the range of 98.42 to 103.83%. The method was applied for the quantitative determination of MG loaded in silicate matrices. Results showed a retrieval degree of 96.57% and confirm the initial hypothesis that this method can be used for the quantitative determination of MG loaded in silicate matrices as modified release formulations. In conclusion, the new method for the HPLC determination of MG is simple, easy to apply, sensitive, linear, accurate, precise, and can be successfully used in *in vitro* dissolution tests for the quantitative evaluation of MG in silica-based particulate systems.

Acknowledgements

This research was supported by the Internal Research Program (**Project No. 30889/2014**) funded by the University of Medicine and Pharmacy “Grigore T. Popa” of Iasi, Romania.

References

- Chen Y.H., Liu P.L., Magnolol: A multifunctional compound isolated from the Chinese medicinal plant *Magnolia officinalis*. *Eur. J. Integrat. Med.*, 2011; 3: 317.
- Lee Y.J., Lee Y.M., Lee C.K., Jung J.K., Han S.B., Hong J.T., Therapeutic applications of compounds in *Magnolia* family. *Pharmacol. & Therap.*, 2011; 130(2): 157-176.
- Wang J.J., Zhan R., Liang Ji-chao., Chen Y., Anti-diabetic and anti-oxidative effects of Honokiol on diabetic rats induced by high-fat diet and Streptozocin. *Chinese Herbal Medicines*, 2014; 6: 42-46.
- Wang L., Waltenberger B., Pferschy-Wenzig E.M., Blunder M., Liu X., Malainer C., Blazevic T., Schwaiger S., Rollinger J.M., Heiss E.H., Schuster D., Kopp B., Bauer R., Stuppner H., Dirsch V.M., Atanasov A.G., Natural product agonists of peroxisome proliferator-activated receptor gamma (PPAR γ): a review. *Biochem. Pharmacol.*, 2014; 92(1): 73-89.
- Ochiuz L., Peptu C.A., Grigoras A., Grigoras C., Applications of mesoporous silica in hosting and controlled release of sodium alendronate. 15th International Multidisciplinary Scientific GeoConferences SGEM., 2015, In Extensio Proceeding: 2015; 3-10.
- Tam D., Ashley C.E., Xue M., Carnes E.C., Zink J.I., Brinker C.J., Mesoporous silica nanoparticle nano-carriers: Biofunctionality and biocompatibility. *Acc. Chem. Res.*, 2013; 46: 792-801.
- Burcea Dragomiroiu G.T.A., Cimpoiesu A., Ginghină O., Baloesu C., Bârca M., Popa D.E., Ciobanu A.M., Anuța V. The Development and Validation of a Rapid HPLC Method for Determination of Piroxicam. *Farmacia*, 2015; 63(1): 123-131.
- Bao Z.J., Dai L., Miao Z.T., Ma Z.G., Ding Z.T., Determination of the dissociation constants of magnolol and honokiol by UV spectrophotometry. *Nat. Sci.*, 2004; 26: 66-69.
- Wu Y.T., Lin L.C., Tsai T.H., Simultaneous determination of honokiol and magnolol in *Magnolia*

- officinalis* by liquid chromatography with tandem mass spectrometric detection. *Biomed. Chromatogr.*, 2006; 20: 1076-1081.
10. Kotani A., Kojima S., Hakamata H., Determination of Honokiol and Magnolol by micro HPLC with electrochemical detection and its application to the distribution analysis in branches and leaves of *Magnolia obovata*. *Chem. Pharm. Bull.*, 2005; 53: 319-322.
11. Tang W., Wan M., Zhu Z., Chen G., Huang X., Simultaneous determination of eight major bioactive compounds in Dachengqi Tang (DT) by high-performance liquid chromatography. *Chim. Med.*, 2008; 3: 1-6.
12. Jiang Y., Vaysse J., Gilard V., Balayssac S., Déjean S., Malet-Martino M., Barbin Y., Quality Assessment of Commercial *Magnoliae Officinalis* Cortex by ¹H-NMR-based Metabolomics and HPLC Methods. *Phytochem. Anal.*, 2012; 23: 387-395.
13. Lu J., Liong M., Li Z.X., Zink J.J., Tamanoi F., Biocompatibility, Biodistribution and Drug-Delivery Efficiency of Mesoporous Silica Nanoparticles for Cancer Therapy in Animals. *Small*, 2010; 6: 1794.
14. Vallet-Regi M., Balas F., Arcos D., Mesoporous Materials for Drug Delivery. *Angew. Chem. Int. Ed.*, 2007; 46: 7548.
15. Stefanache A., Tomoiaga A.M., Peptu C.A., Spac A., Ochiuz L., Development of prolonged-release systems with magnolol based on silica SBA-15. 15th International Multidisciplinary Scientific GeoConferences SGEM, 2015, In Extensio Proceeding: 2015; 53-60.
16. ICH Topic Q2B. Validation of Analytical Procedures (CPMP/ICH/281/95). London: The European Agency for the Evaluation of Medicinal Products, 1996.
17. Pavalache G., Dorneanu V., Popescu A., Validation and Application of a New DAD-HPLC Method for Determination of Loratadine from Pharmaceuticals. *Farmacia*, 2015; 63(3): 366-370.
18. Bosinceanu A., Paduraru O.M., Vasile C., Popovici I., Tantar G., Ochiuz L., Validation of a new HPLC method used for determination of amiodarone from the complex with hydroxypropyl-beta-cyclodextrin and from commercial tablets. *Farmacia*, 2013; 61(5): 856-864.