UNIVERSITATEA DE MEDICINĂ ȘI FARMACIE
GRIGORE T. POPA IAȘI

SIMULTANEUS DETERMINATION OF SILYMARIN AND GLIBENCALMIDE BY HPLC-ESI-MS TEHNIQUE; METHOD DEVELOPMENT AND VALIDATION

*Iustina-Mihaela Condurache (1), Anca-Roxana Petrovici (2) *, Mariana Pinteala(2), Lenuta Profire(3)*

[1] Department of Biomedical Sciences, "Grigore T. Popa" University of Medicine and Pharmacyof Iasi, 700115 Iasi, Romania; mihaela-iustina.condurache@umfiasi.ro

[2]Centre of Advanced Research in Bionanoconjugates and Biopolymers, "Petru Poni" Institute of Macromolecular Chemistry, 41A Grigore Ghica Voda Alley, 700487, Iasi, Romania; petrovici.anca@icmpp.ro

[3] Department of Pharmaceutical Chemistry, "Grigore T. Popa" University of Medicine and Pharmacyof Iasi, 700115 Iasi, Romania; lenuta.profire@umfiasi.ro

* Correspondence: petrovici.anca@icmpp.ro; Tel.: +40 740 673 523 ; +40-332 880 050

Introduction

➢In recent years, in the therapy of type 2 diabetes, various therapeutic strategies have been used to ensure adequate glycemic control and at the same time to improve the chronic complications induced by this condition.

➢The aim of the study was to develop and validate a HPLC-ESI-MS method to determine simultaneously silymarin (Sil) and glibenclamide (Gly) in aqueous solutions, from chitosan-based microparticles.

Materials and methode

➢ Sil and Gly, in different concentrations, were loaded into chitosan microparticles using ionic gelation method [1].

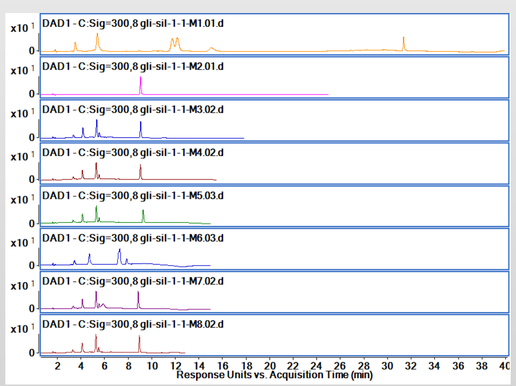
➢ Briefly, the drugs were dissolved in the minimum volume (0.5 mL) of proper solvent and then was added into 3 mL of 1.2% chitosan acetic acid solution. The mixture was stirred at room temperature for 2 h and then was dropped through a syringe needle into 20 mL of 2% TPP solution. After 12 h of stirring at room temperature, the formed beads were separated from the TPP solution and washed with distilled water and then dried at room temperature [2].

➢ For identification and quantification of the loaded drugs, a HPLC-ESI-MS method using an Agilent 1200 Series HPLC system coupled to an Agilent 6520 accurate-mass quadrupole time-of-flight (Q-TOF) mass spectrometer, was developed. The separation was made on a Hypersil C18 column with 0.1% formic acid in MiliQ water (A) and acetonitrile (B) applied in gradient (% B: 0'-25; 5'-55; 9'-70; 12'-30; 15'-25). The DAD separation was monitored at 230, 280, 298, 300 nm, 0.1 mL/min of elute was directed to ESI/Q-TOF MS, operated at an ionization voltage of -4000 V, 325 °C, with ions' scan 50-1000 m/z in negative ion mode. The method was validated using recommended parameters [3] and Sil:Gly (1:1) standard solutions.

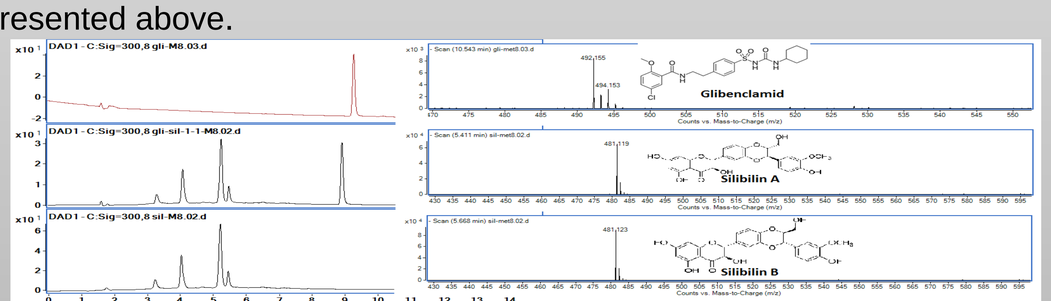
Results and discutions

➢ By using M8 method, the SilA Rt was registered at 5.41 min, SilB at 5.66 min and Gly at 10.54 min.

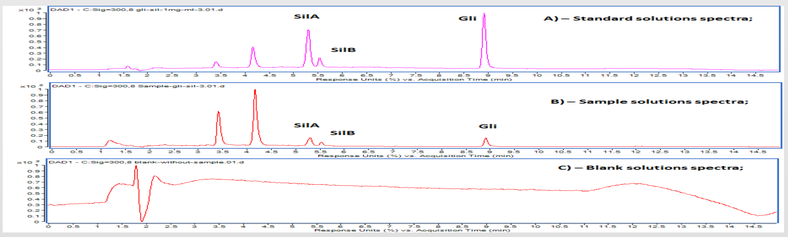
Method	Mobile Phase	Gradient B (%)	Flow (mL/min)	Method run time (min)
M1	Acetonitrile	0'- 25; 12'- 27; 22'- 30; 26'- 45; 31'- 70; 37'- 75; 40'- 25;	1 mL/min	45
M2	Acetonitrile	0'- 25; 5'- 45; 10'- 55; 15'- 75; 20'- 25; 25'- 25;	1 mL/min	25
M3	Acetonitrile	0'- 25; 5'- 55; 10'- 70; 20'- 25;	1 mL/min	20
M4	Acetonitrile	0'- 25; 5'- 55; 10'- 70; 12'- 45; 15'- 25;	1 mL/min	15
M5	Acetonitrile	0'- 25; 5'- 55; 10'- 60; 12'- 30; 15'- 25;	1 mL/min	15
M6	Acetonitrile	0'- 25; 5'- 35; 8'- 60; 10'- 30; 15'- 25;	1 mL/min	15
M7	Acetonitrile	0'- 25; 5'- 55; 8'- 70; 10'- 30; 15'- 25;	1 mL/min	15
M8	Acetonitrile	0'- 25; 5'- 55; 9'- 70; 12'- 30; 15'- 25;	1 mL/min	15



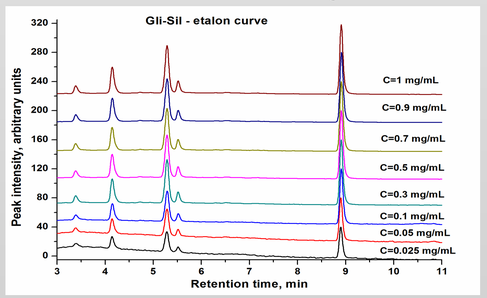
➢ The loaded drugs were identified using MS-MS spectra and m/z characteristics for all compounds were found in the higher intensity for Rt presented above.

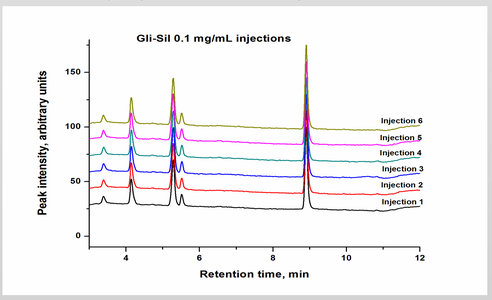


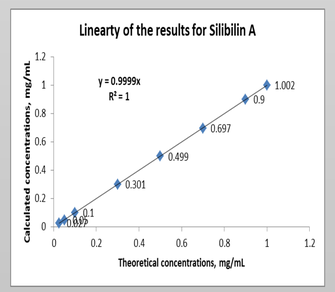
➢ The selectivity and precision of the methods are absolute because Rt for sample and standard have the same value, and blank solution proved no interference.

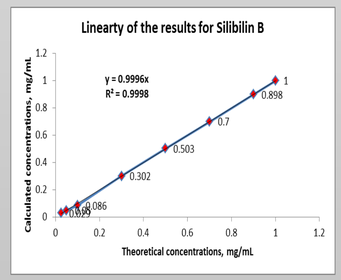


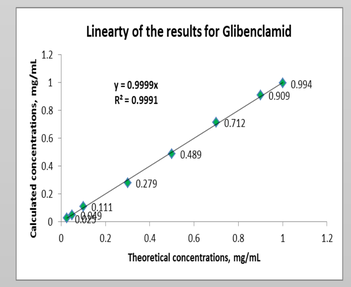
➢ The linearity of the answer function is absolute for SilA (R^2=1), and almost absolute for SilB (R^2=0.9998), and Gly (R^2=0.9991).











➢S/N values for all compounds at all studied concentrations maintain similar values.

➢ For SilA we obtained a LOD=0.285 mg/mL and LOQ=0.95 mg/mL; for SilB we obtained a LOD=0.045 mg/mL and LOQ=0.15 mg/mL; and for Gly we obtained a LOD=0.038 mg/mL and LOQ=1.275 mg/mL.

Conclusions

➢ We developed a high resolution HPLC-ESI-MS method to determine simultaneusly Sil and Gly in a concentration range of 0.025-1 mg/mL.

References

1. Constantin, S.M.; Buron, F.; Routier, S.; Vasincu, I.M.; Apotrosoaei, M.; Lupașcu, F.; Confederat, L.; Tuchiluș, C.; Constantin, M.T.; Sava, A.; Profile, L. Formulation and characterization of new polymeric systems based on chitosan and xanthine derivatives with thiazolidine-4-one scaffold. *Materials* **2019**, *12*, 1–20, doi:10.3390/ma12040558.

2. Avram, I.; Lupascu, F.G.; Confederat, L.; Constantin, S.M.; Stan, C.I.; Profire, L. Chitosan microparticles loaded with antidiabetic drugs—Preparation and characterization. *Farmacia* **2017**, *65*, 443–448.

3. Nageswara Rao, T., Validation of Analytical Methods in *Calibration and Validation of Analytical Methods - A Sampling of Current Approaches*, Stauffer, M.T., Edited by Intechopen, **2018**, pp. 131-141 DOI: 10.5772/intechopen.72087. http://dx.doi.org/10.5772/intechopen.72087

Aknoweldgements

This research activity was financially supported by AUF-IFA 2019-2020, contract no. 28/2019 and by a grant of the Romanian Ministery of Research and Innovation, CCCDI – UEFISCDI, project number PN-III-P1-1.2-PCCDI-2017-0697/13PCCDI/2018, within PNCDI III.