Extended Abstract

Simultaneous Determination of Silymarin and Glibenclamide by HPLC–ESI–MS Technique: Method Development and Validation [†]

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(1) Background: The aim of the study was to develop and validate a HPLC–ESI–MS method to simultaneously determine silymarin (Sil) and glibenclamide (Gly) in aqueous solutions, from chitosan-based microparticles. **(2) Methods:** Sil and Gly, in different concentrations, were loaded into chitosan microparticles using the ionic gelation method [1]. Briefly, the drugs were dissolved in the minimum volume (0.5 mL) of proper solvent and this solution was then added to 3 mL of 1% chitosan acetic acid solution. The mixture was stirred at room temperature for 2 h and then dropped through a syringe needle into 20 mL of 2% sodium tripolyphosphate (TPP) solution. After 12 h of stirring at room temperature, the beads formed were separated from the TPP solution, 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 MilliQ water (A) and acetonitrile (B) applied in gradient (% B: 0’–25; 5’–55; 9’–70; 12’–30; 15’–25). The diode-array detector DAD separation was monitored at 230, 280, 298, 300 nm, 0.1 mL/min of the elute was directed to ESI/Q-TOF MS, operated at an ionization voltage of –4000 V, 325 °C, with an ion scan value of 50–1000 m/z in negative ion mode. The method was validated using recommended parameters [3] and Sil:Gly (1:1) standard solutions. **(3) Results:** By using the M8 method, the Sil_{RT} was registered at 5.41 min, Sil_B at 5.66 min, and Gly at 10.54 min. 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 the _{RT} for the sample and standard have the same value, and the blank solution proved no interference. The linearity of the answer function is absolute for Sil_A ($R^2 = 1$), and almost absolute for Sil_B ($R^2 = 0.9998$), and Gly ($R^2 = 0.9991$). S/N values for all compounds at all studied concentrations maintained similar values. For Sil_A, we obtained an LOD = 0.285 mg/mL and LOQ = 0.95 mg/mL; for Sil_B we obtained an LOD = 0.045 mg/mL and LOQ = 0.15 mg/mL; for Gly we obtained an LOD = 0.038 mg/mL and LOQ = 1.275 mg/mL. **(4) Conclusion:** We developed a high resolution HPLC–ESI–MS method to simultaneously determine Sil and Gly in a concentration range of 0.025–1 mg/mL.

Supplementary Materials: The supplementary file is available online at <https://www.mdpi.com/article/10.3390/IECP2020-08703>.

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Conflicts of Interest: The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

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