Supplementary material

Silymarin nanoparticles through emulsion solvent evaporation method for oral delivery with high antioxidant activities, bioavailability, and absorption in the liver

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Optimization study details

Effect of drug concentration

The effect of drug concentration on the MPS is shown in Fig.2 (a). Increasing the SM solution concentration from 0.5 mg/ml to 13 mg/ml decreased the MPS of emulsion of 239.6 nm to 40.2 nm. However, the MPS increased to about 94.3 nm at the concentrations of 15 mg/ml. Higher concentrations make SMNs grow and aggregate. According to the production efficiency, the optimum concentration of SM solution was selected to be 15 mg/ml.

The effect of the proportion of water to organic phase

The second factor was volume ratio of water to the organic phase. The ratios were examined to be within the range from 6:1 to 38:1. From Fig.2 (b), it can be clearly seen that with the increasing volume ratio of water to organic phase, the MPS of SMNs increased from 50.3 nm to 111.4 nm firstly and feeble decreased subsequently. Finally, the ratio of 6:1 was selected as the optimal proportion of water to organic phase to use in subsequent tests after the water and organic phase were tested to form a stable emulsion system.

The effect of the surfactants

Poloxamer 188, a high macromolecule non-ionic surfactant, was chosen as the surfactant during emulsification. The third factor was concentration of poloxamer 188 in the water phase. Fig. 2 (c) showed the effects of the concentration of surfactant on MPS. When the amount of poloxamer188 increased from 0.5 mg/ml to 4.5 mg/ml, the MPS of SM nanoemulsion decreased from 238.7 to12.5 nm. Finally, considering the production efficiency, the optimum concentration of poloxamer 188 was selected to be 0.5 mg/ml.

The effect of homogenate speeds and time

The already established optimal SM concentration (15 mg/ml), optimal poloxamer188 concentration (0.5 mg/ml) and volume ratio of water to organic phase (6:1) were used in this part. From the Fig. 2 (d), when the homogenate speeds increased from 3000 rpm to 5000 rpm, the MPS decreased from 251.1 to 102.9 nm, followed by a significant increase of MPS from 119.3 nm to 299.7 nm when the homogenate speeds up to 6000 rpm until 11000 rpm. So a higher homogenate speeds in the low speed range is helpful to emulsification, and higher homogenate speeds increased the energy of the emulsion which break the stability of the droplets, resulting in a larger particle size. Thus, it was determined that it was not useful to homogenize at a high speed. Therefore, considering the production efficiency, the optimum homogenate speed was selected to be 3000 rpm.

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The effect of homogenate time was as shown in the Fig. 2 (e). When the homogenate time increased from 0.5 min to 15 min, the MPS of SMNs increased from 143.1 nm to 253.7 nm. Thus, longer homogenizing time was not helpful, as they negatively influenced the reaching particles of a smaller size. A longer homogenizing time increased the energy of the emulsion which breaks the stability of the droplets, resulting in a larger particle size. We determined that 0.5 min was enough for our experiments. Therefore, the optimum homogenate time was determined to be 0.5 min.

The effect of homogenization pressure and times

Homogenization can ensure a smaller particle size and a more uniform droplet (19). A sample was prepared under the optimum conditions as described, SM solution concentration of 15 mg/ml, a ratio volume of 6:1 of water to organic phase, and a homogenization speed of 3000 rpm for 0.5 minute to examine the influence of homogenization pressures and the times at each pressure on particle size. First of all, we examined homogenization pressures within the range from 300 to 1100 bar as it was shown in Fig. 2 (f). When the homogenization pressure increased from 300 bar to 600 bar, the MPS decreased from 82.7 to 47.9 nm, followed by a significant increase of MPS from 52.2 nm to 178.2 nm when the homogenization pressure up to 700 bar until bar. We determined that the greater homogenization pressure is helpful to nanoemulsion in the low pressure range; the stable nanoemulsion droplets would be obtained. As the homogenization pressure was performed increased, the temperature of the system increased leading to the higher energy of the emulsion that breaks the stability of the droplets and homogenizer plays a role to shear, resulting in a larger particle size. Thus, 600 bar was selected as the optimal homogenization pressure.

The effect of homogenization times was as shown in the Fig. 2 (g). At first, the MPS of SM nanoemulsion decreased from 137 nm to 45.9 nm with the increment of homogenization times. Because high-pressure homogenization process reduced and uniformed SM nanoemulsion particle size. However, the size of nanoparticles increased with the number of homogenization times increased from 7 to 11 times. Because the limited emulsifier could not be effectively adsorbed to the particle surfaces, so it lead to reduce the emulsification, aggregate the droplets and increase the particle size. Ultimately, we chose 6 times as the optimal number of homogenization times.

The effect of the proportion of silymarin to mannitol

Mannitol is widely used as a lyoprotectant which play an active role in protecting drug nanoparticles from denaturation during freeze-dried process. A sample was prepared under the optimal conditions as described. A certain amount of SMNs after the freeze-dried process was completely dissolved in water to examine the effects of the proportion of SM to mannitol from 1:1 to 1:17 on MPS. From Fig. 2 (h), Increasing in the ratio range of 1:1 to 1:7 decreased the MPS from 289.2 nm to 107.1 nm. However, there was a gradual stabilization state at ratios from 1:9 to 1:17. Thus, the ratio of 1:7 was selected as the optimal proportion of SM to mannitol during the freeze-dried process.

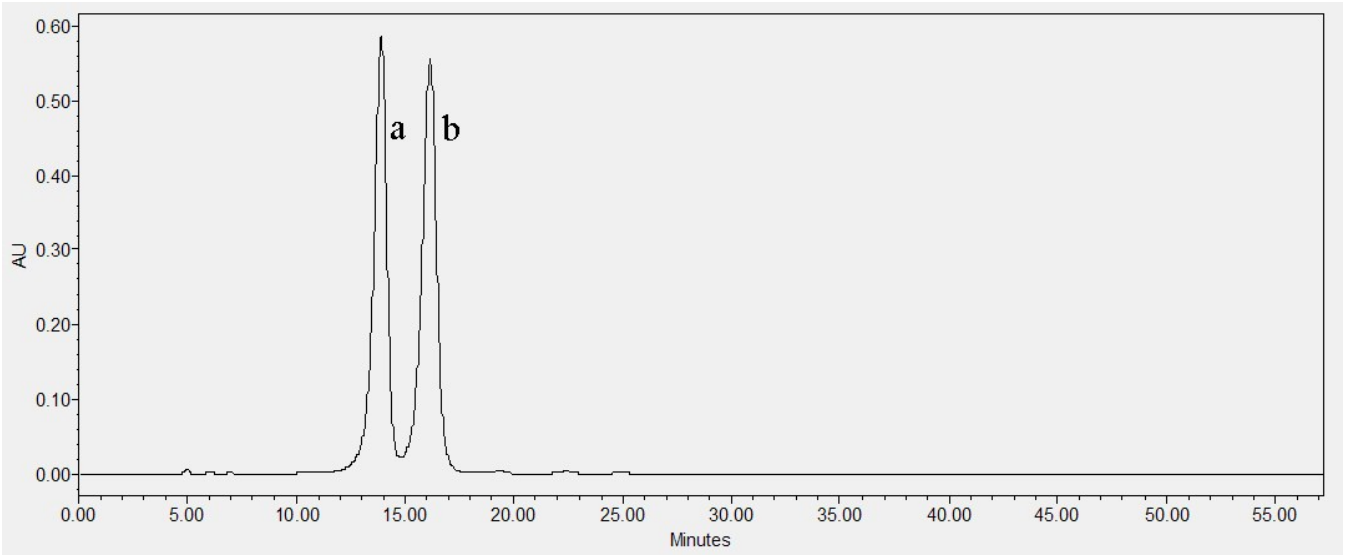

Fig. S1. HPLC figure of (a) silybin and (b) isosilybin

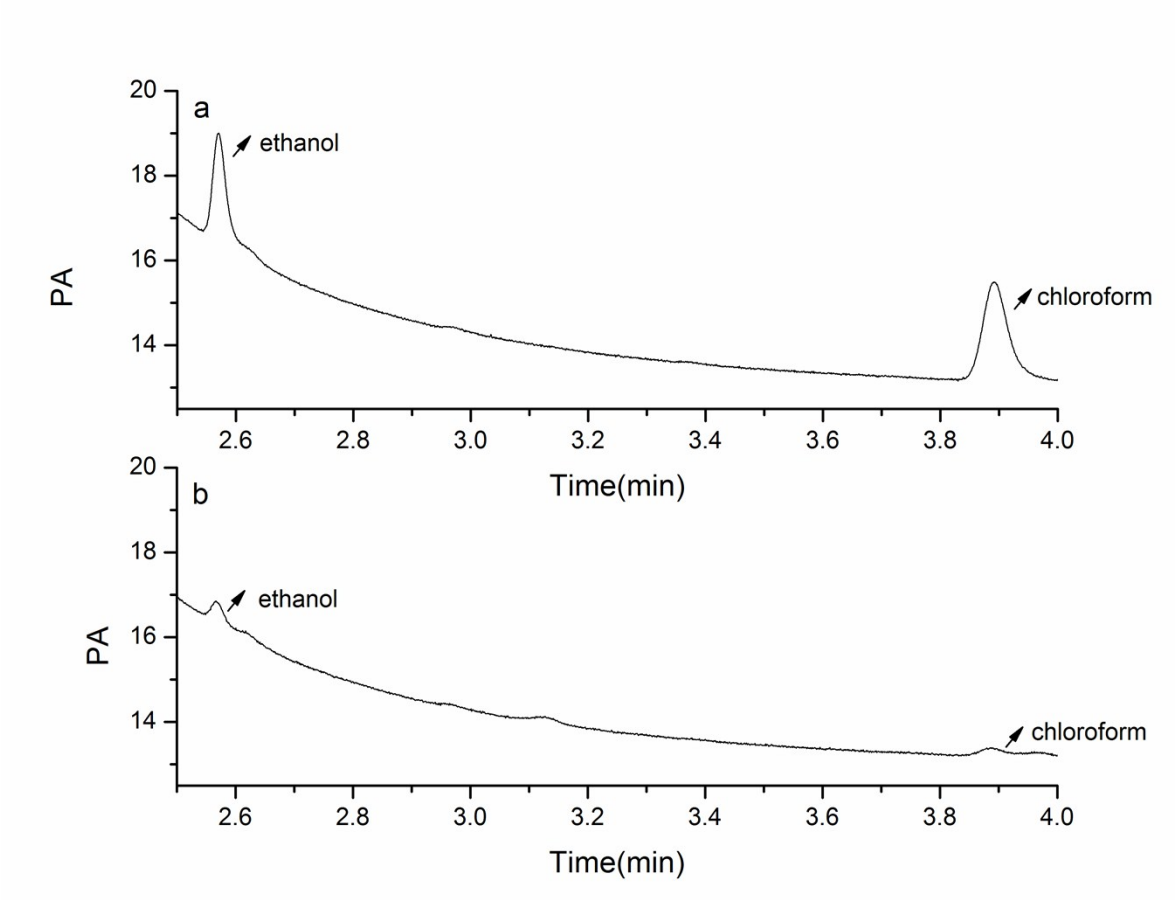

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Fig. S2 Gas chromatograms of (a) ethanol and chloroform standard solution and (b) SMNs

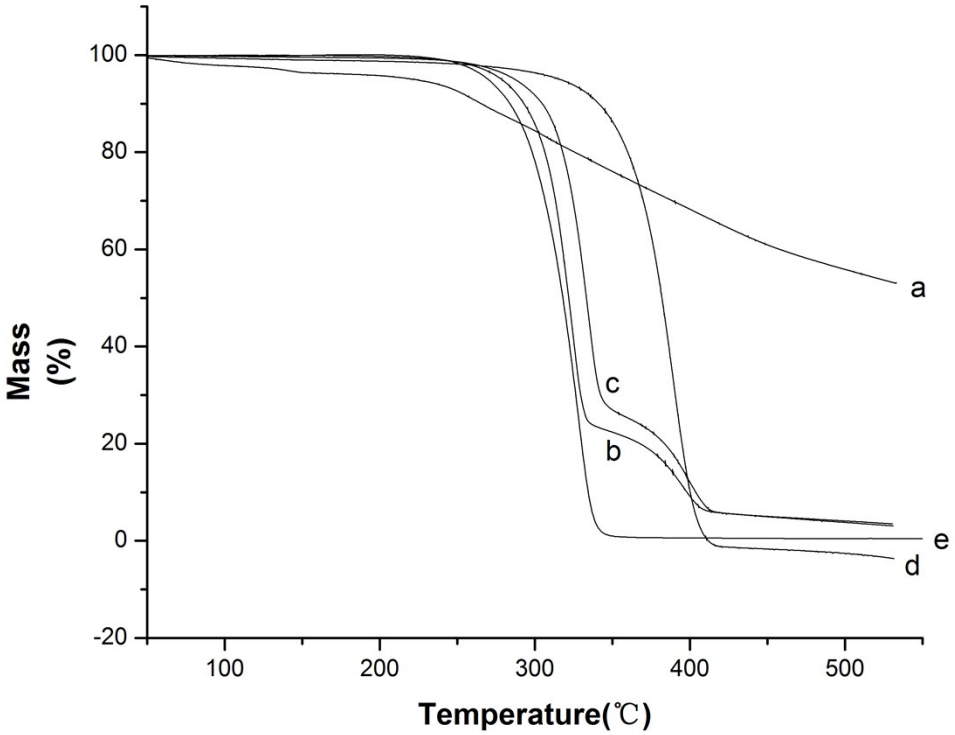

Fig. S3. TGA results of (a) raw SM; (b) SMNs; (c) physical mixture of SM, poloxamer 188 and mannitol; (d) poloxamer 188; (e) mannitol