Characteristics of artemether loaded PLGA microparticles fabricated by coaxial electrospray: validation of enhanced encapsulation efficiency and bioavailability

Electronic Supporting Information

There are several techniques which can be used for testing the residual solvent. Gas chromatograph–based testing is reliable and is most popular as it is chemically specific for residual solvents [1]. Gas chromatography (GC) has the ability to separate component solvents, thus identifying them even in low limits. Residual solvent analysis by direct injection into a GC is often preferred because of its simplicity and reliability [2]. Acetonitrile is a class 2 solvent in a pharmaceutical products with a permissible limit of 410 ppm [3]. We have used acetonitrile for the preparation of PLGA microparticles. The direct injection method for identifying the concentration of acetonitrile in our samples has been employed [4].

Method

Determination of acetonitrile concentration as a residual solvent in PLGA microparticles by gas chromatography and mass spectroscopy (GC-MS):

A GC instrument (model 7890A, Agilent Technologies) was used equipped with a mass detection; a quadruple mass analyzer (MS) (Agilent Technologies, model 5975C). 7683 autosampler of Agilent Technologies was used with a DB-5MS column (30×0.32 mm i.d) having 0.25 μm film of 5% phenylmethyl siloxane for liquid sample injection was used for residual solvents testing.

A volume of 1 µl of either a standard or sample solution was injected in GC split port, which was maintained at a temperature of 200 °C at a split ratio of 5:1. The constant flow of nitrogen as a carrier gas was maintained at a flow rate of 1.0 mL/min with high purity. The temperature gradient of oven maintained the initial temperature to 35 °C for 3 min, and was then increased at 15 °C/min to 200 °C, then again increased to reach a final temperature of 260 °C and held for 5 min. The total thermal gradient was achieved in 18 min.The ion source temperature was 230 °C, and that of the GC-MS interface was 200 °C. The mass spectrometer was operated in the electron impact mode by full scan detection mode using 70 eV ionization voltages. The scan range was from 20 to 460.

Standard and sample preparation

For standard and sample preparation n-Hexane was selected as diluent**.** Standard solution was prepared by pipetting 1ml of n-hexane in 10 µl acetonitrile. For liquid injection, 100 µl of standard solution was transferred to a 2 ml vial and closed with a Teflon-rubber crimp cap. For sample preparation 10 mg of PLGA microparticles were dissolve in 1 ml n-hexane, which was then vortex in shaking bed for 5 min at room temperature and centrifuged for 10 min at 12000 rpm. After centrifuged 100 µl sample solution was transferred to a 2 ml vial and closed with a Teflon-rubber lined crimp cap.

Results and discussion

Standard solutions and sample solutions of acetonitrile were analyzed using direct injected method by the GC–MS systems. The spectra of the acetonitrile peak were only obtained in the standard and no peak was observed in the samples (Figure S1). The retention time and the mass

spectrum of acetonitrile was obtained at 3.1 min and 41.1 (Figure S2) respectively in the standard.

With this method, we were able to conclude that there was no residual acetonitrile in our PLGA microparticles. The residual acetonitrile allowed was 410 ppm in pharmaceutical products, but there was no trace of acetonitrile that has been detected in the PLGA microparticles. This reveals that the content of acetonitrile is not present in our optimized PLGA microparticles or it is in the undetectable range. This conforms that our PLGA microparticles follow the recommendations of The International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) guideline of residual solvent.

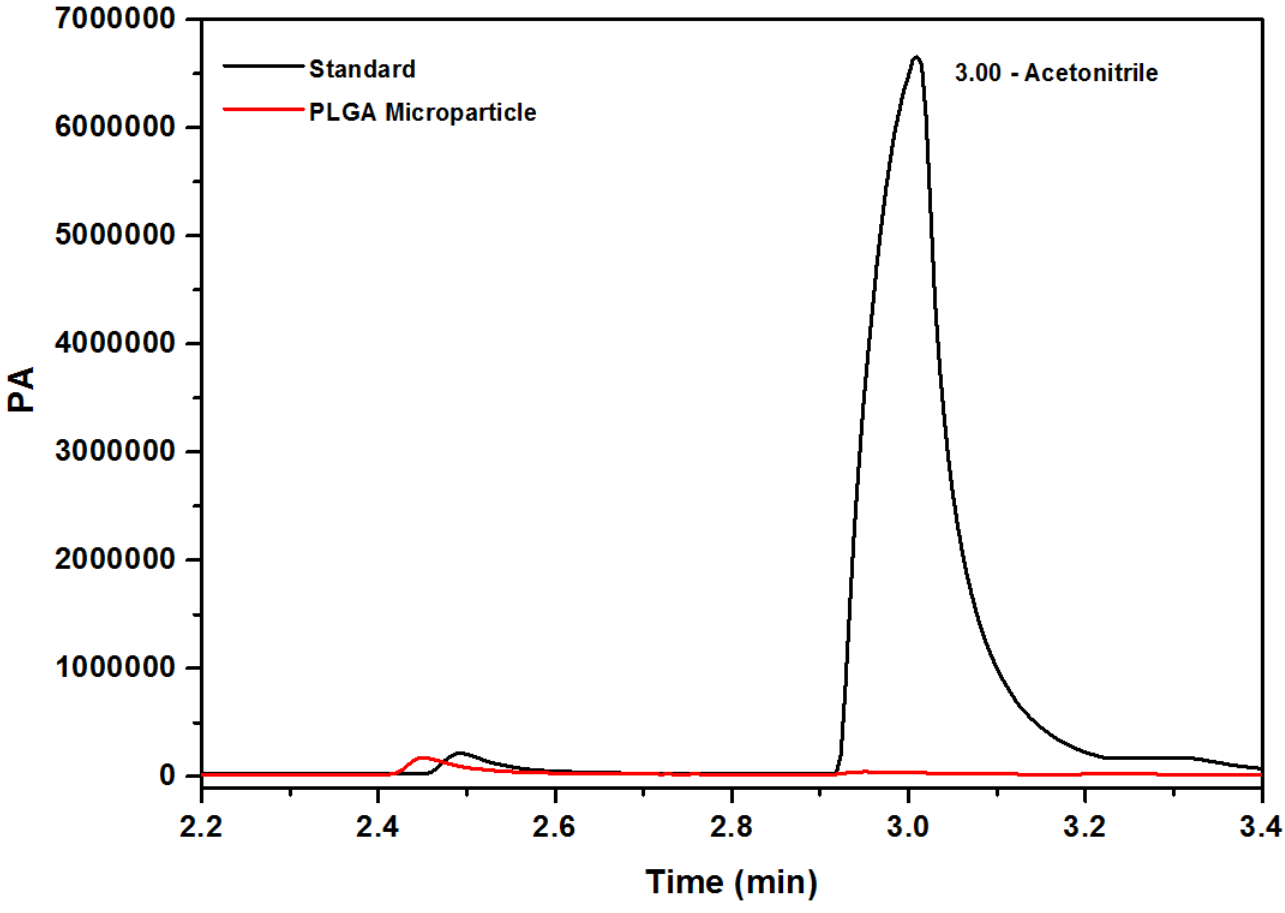

Figure S1: Chromatogram of acetonitrile peak obtained for standard.

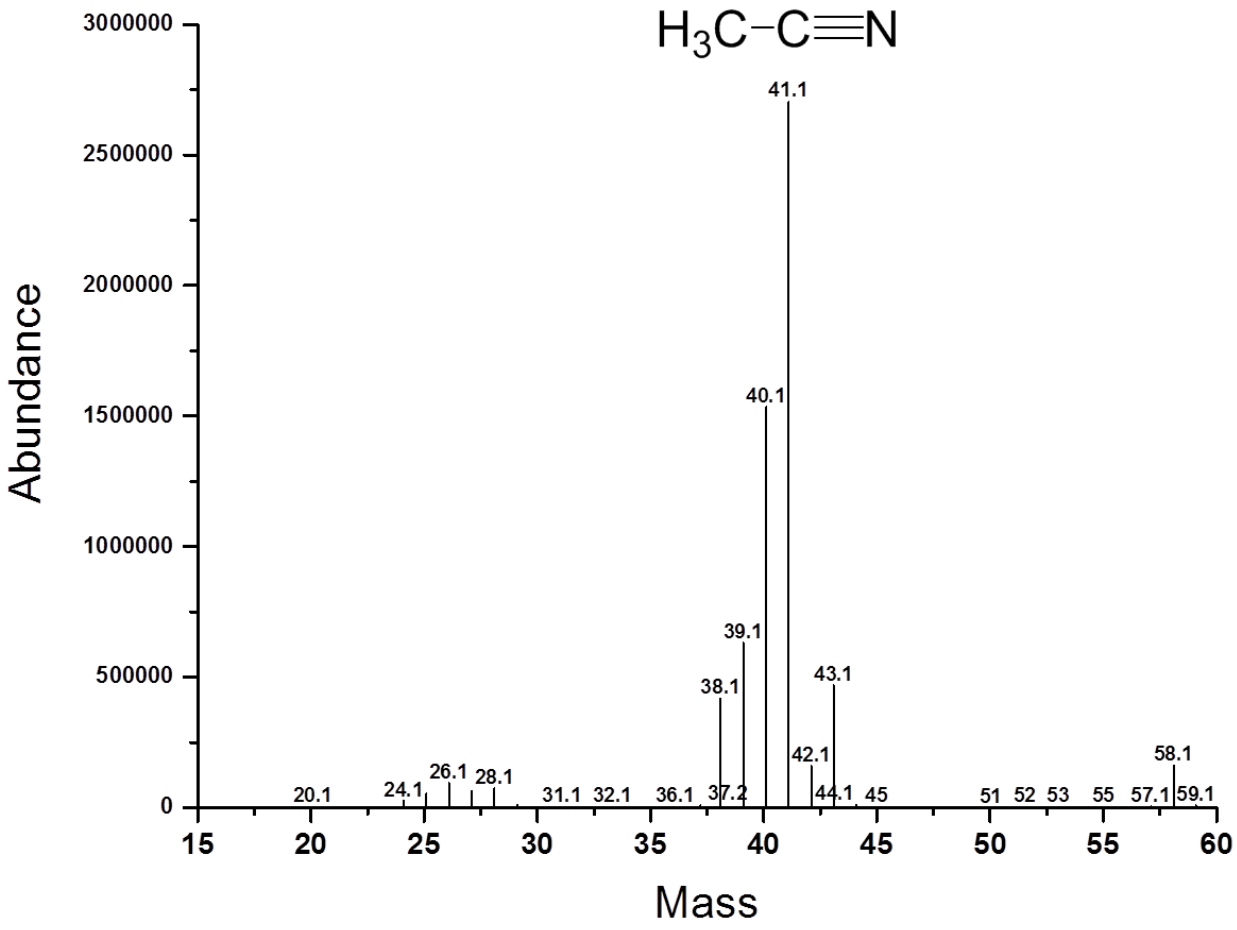

Figure S2: Analysis of residual solvents in PLGA microparticles. Acetonitrile peak was observed at 41.1 in standard.

References:

1. B'Hymer, C., Residual solvent testing: a review of gas-chromatographic and alternative techniques. Pharm Res, 2003. 20(3): p. 337-44.

2. Witschi, C. and E. Doelker, Residual solvents in pharmaceutical products: acceptable limits, influences on physicochemical properties, analytical methods and documented values. European Journal of Pharmaceutics and Biopharmaceutics, 1997. 43(3): p. 215-242.

3. ICH guideline Q3C (R6) on impurities: guideline for residual solvents. European Medicines Agency. 2016.

4. Han, E.-J., A.-H. Chung, and I.-J. Oh, Analysis of residual solvents in poly(lactide-co-glycolide) nanoparticles. Journal of pharmaceutical investigation, 2012. 42(5): p. 251-256.