High performance thin layer chromatography (HPTLC) analysis of test lot RK-3-28-1-ES (ethanolic extract) showed a band corresponding to the retention factor (Rf) of L-ephedrine ( (-)ephedrine) standard (Rf ~0.25) (HPTLC Figure 1, lanes 1 and 2), a marker of *E. sinica*. Because testing laboratory’s *E. sinica* reference materials did not produce any bands in this analysis, a second HPTLC analysis was conducted. Second HPTLC analysis showed number of bands for the test lot sample at Rfs similar to those of testing laboratory’s reference materials for *E. sinica* (HPTLC Figure 2, lanes 1-3). Few additional bands (Rfs ~ 0.2-0.3 and ~0.45) were detected in the test lot sample (HPTLC Figure 2, lane 1) which were not clearly visible in testing laboratory’s *E. sinica* reference materials (HPTLC Figure 2, lanes 2-3), likely due to variability of constituents and/or concentrations in botanicals due to myriad of factors (e.g., growing/harvesting conditions, extract preparation). Although these regions in the test lot (HPTLC Figure 2, lane 1) matched with those seen in testing laboratory’s *E. americana* reference material (HPTLC Figure 2, lanes 4-5), L-ephedrine was absent in *E. americana* reference material (HPTLC Figure 2, lanes 4-5), but was present in test lot (HPTLC Figure 2, lane 1, Rf ~ 0.4).

DNA barcoding was conducted using the raw material (JT-1031) used to prepare ethanolic extract RK-3-28-1-ES along with reference materials for other *Ephedra* species. ITS sequences of samples were compared to available sequences from NCBI. The smaller part of the ITS region (i.e. the analyzed ITS2 region) of JT-1031 and NCNPR 589 matches with that of both *E. intermedia* and *E. sinica* but not *E. americana* (DNA Barcoding). Based on this sequence comparison, the test sample JT\_1031 can be either *E. sinica or E. intermedia.*

The test lot RK-3-28-1-ES was further characterized using liquid chromatography coupled with ultraviolet (UV), charged aerosol (CAD) and high resolution mass spectrometry detection (HRMS) (UPLC-UV-CAD/HRMS) (DNA Barcoding). The analysis confirmed the presence of known *E. sinica* markers in test lot sample at anticipated concentrations (DNA Barcoding).

When all data are considered, the test lot RK-3-28-1-ES contains significant features of *E. sinica* including anticipated marker constituents and concentrations.