Reasons for chromatographic peaks to appear double:

1. Too much sample injected.
2. Not enough column equilibration time before the next injection. Ghost peaks….
3. The presence of a sticky substance like DMSO, so that every time a gradient is run a bit more of previous samples are released. DMSO, glycerol, DMF, propylene carbonate, and other high boiling viscous liquids are not UPLC friendly.
4. Trapped air in the flow path that moves when samples pass it.
5. Dirty column entrance. If some material sticks to part of the entrance then some molecules pass through as expected and some are briefly retained before moving on, leading to double peaks. Particles can get stuck unevenly in the entrance. And a burst of very hydrophobic material can be deposited unevenly too. Some calibration standards that work well in gas chromatography gum up UPLC columns. Reversed-phase (C18) separations rely on differences in hydrophobicities.

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