

- **Response Factor**

If an unknown peak is subsequently identified and a corresponding RRF established, the requirements of the investigation and the cleaning limit for that peak may be impacted.

- **Trends**

Trending of unknown peaks investigated should include past incidents (i.e. history) and be included within periodic review for cleaning procedures.

II. Investigative Steps and Possible Causes

The following list is intended to provide a practical sequence of investigative steps to evaluate unknown peaks:

- **Artifacts**

Confirm the unknowns by re-analyzing the sample solution using the same instrument and the same sample vial (if applicable). Also analyze the same sample solution from the original sample submitted from the plant, which has been loaded into a new vial (if applicable). If available, prepare and inject a duplicate swab or rinse sample solution submitted from the same location. Blank swab sample extracts may also be injected. If a pattern emerges suggesting the unknown is an artifact of the analysis, unconfirmed, and/or due to the swabs themselves, a re-clean of the equipment may not be required. If the unknown is considered to be an artifact, it does not require additional investigation of the cleaning procedure.

- **Solvent/Cleaning Agents**

Compare the peak to the solvents and/or cleaning agents from the manufacturing process and/or cleaning process to determine if the unknown peak is a result of solvent or cleaning agent interference. Cleaning evaluation studies would have hopefully uncovered this interaction. For example, toluene and dimethylformamide are solvents that have commonly been observed to cause unknown peaks in chromatography. If an investigation into an unknown peak determines that it is due to an interfering solvent, then future observations of this peak would not require repeated identification of that peak.

- **Degradation**

The peak may also be the result of product degradation or reaction with the cleaning agent. For example, a material containing a carboxylic acid can react with methanol during cleaning to produce an ester. If an investigation into an unknown peak determines that it is due to product degradation, then future observations of this peak would not require repeated identification of that peak. . If the degradation product is likely to be generated during the cleaning procedure, then the analytical method should be updated to include identification of the additional peak.

- **Sampling**