Definitions: “Purity” and “Assay”

**Purity (HPLC)** – purity by HPLC (High Performance Liquid Chromatography) is determined by measuring the area of the peak that corresponds to the compound of interest. With regard to antibiotics, we generally interested in the API or active pharmaceutical ingredient which is often the largest peak displayed on the chromatograph.

**Assay** – assay refers to either a microbial bioassay or a chromatography based purity assay where a compound of unknown activity or purity is compared to a reference standard with precisely determined bioactivity or purity. The assay specifications listed on a certificate of analysis generally do not specify the type of assay and are reported as “Assay (as is)” or “Assay (on dried basis).”

- *Bio-assay* results are determined by testing a compound of unknown potency against certain microorganisms and recording how effective the compound was at inhibiting microbial growth and comparing this result with the potency of the reference standard.
- *Purity assay* results are determined by comparing HPLC peak responses of the compound to be tested with those obtained from the appropriate reference standards.

**Key notes:**

- A product specification titled “purity” generally refers to the purity of the product itself and is not compared to a reference standard. Purity by HPLC is reported as a percentage.
- Assay units are either reported as “µg/mg,” “U/mg,” “IU/mg,” or “%.” It should be noted, “µg” and “IU” and “U,” and “%” are arbitrary units of activity/potency and do not correspond to accepted units of weight. For example, an assay value of 980 µg/mg does not suggest that for each milligram, .98 mg is active or that 98% of the material is active. It should be noted that these units are not interchangeable and one unit cannot be converted to another.
- Assay results labeled “as is” are calculated with water content. Assay results labeled “on dried basis” or “anhydrous” are calculated without water content. To convert from “on dried basis” to “as is,” use the following formula:
  
  \[ Y \times (1-X) \]
  
  \[ Y = \text{Assay value on dried basis} \]
  
  \[ X = \text{Loss on drying} \]

- Historically, reference standards were thought to be pure compounds and were assigned a potency value of 1,000 µg/mg. Advances in manufacturing and purification led to antibiotics of higher potency and purity than the original reference standards. It is therefore possible to obtain and report potency and purity results that exceed 1,000 µg/mg.
- USP assay methods: [click here](#)
- EP assay methods: [click here](#)
- For more information on the specific assay for each product, search for the products’ EP or USP monograph.

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