Gas Chromatographic Methods

Robinson (59) described a gas chromatographic method for measuring residual water in freeze-dried smallpox vaccine in 1972. The method was developed to optimize quality control of a tissue culture smallpox vaccine. Water is extracted from the sample with benzene and determined by gas chromatography with thermal conductivity (hot wire) detection and columns packed with Chromosorb 102.

Moisture Evolution Analyzer

The TA Instruments MEA has been adapted for use for determining residual moisture in freeze-dried allergenic extracts. Jewell et al. (60) applied the method to several allergenic extracts including freeze-dried mold, ragweed, and house dust allergenic extracts. All determinations were performed at low humidity in a controlled humidity dry box using phosphorus pentoxide as desiccant. In the MEA, the sample is heated in a controlled oven. The moisture driven off by the heat applied is carried by a dry nitrogen purge gas to an electrolytic cell where it reacts with phosphorus pentoxide. The current required to regenerate the phosphorous pentoxide is converted to micrograms of water and is identified as the amount of water evolved from the sample.

OTHER METHODOLOGY

A tritium isotope technique and an NIR technique have been reported in the literature but have not been formally approved for use of moisture methods for freeze-dried biological products.

Tritium Isotope

In 1973, Kassai and Sikos (61) described the determination of moisture content in freeze-dried products by tritium isotope. This method involves adding tritium oxide to 5 to 10 ampoules of the dissolved product in experimental lots containing 2000 to 8000 ampoules prior to lyophilization.

After lyophilization, the dry material is assayed for radioactivity using liquid scintillation counting and the water content is calculated. A comparison was made between the tritium method and the gravimetric method. The sensitivity of the tritium method was stated to be 1 μg of water; it measures the water content of 1 mg of dry material with an accuracy of ±0.1%. It was noted that small amounts of tritium were found in the nontest ampoules after lyophilization. In addition, tritium oxide was used to measure the exchange of water molecules between stopper and material. Kassai and Sikos stated that "the product distributed into the vial takes up water from the stopper until a state of equilibrium ensues."

NIR Spectroscopy

Last and Prebble (62) developed an NIR method for the determination of moisture in an experimental freeze-dried injection product. NIR spectra were collected through the bases of unopened product vials using a horizontal instrument accessory. The samples in these vials were then used for Karl Fischer analysis to generate a standard curve for the analysis. The NIR data must be correlated with an accepted residual moisture technique to yield a meaningful