Designation: E3323 – 22

Standard Test Method for Lipid Quantitation in Liposomal Formulations Using High Performance Liquid Chromatography (HPLC) with an Evaporative Light-Scattering Detector (ELSD)[1]

This standard is issued under the fixed designation E3323; the number immediately following the designation indicates the year of original adoption or, in the case of revision, the year of last revision. A number in parentheses indicates the year of last reapproval. A superscript epsilon (ε) indicates an editorial change since the last revision or reapproval.

1. Scope

1.1 This test method describes an analytical technique to quantify lipid components that are often present in liposomal formulations as major components.

1.2 This test method uses high performance liquid chromatography (HPLC) to separate lipids in liposomal formulations and evaporative light-scattering detection (ELSD) to quantify the individual components.

1.3 This test method quantifies three major organic components in liposomal formulations: cholesterol, 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[methoxy(polyethylene glycol)-2000] (DSPE-PEG 2000), and hydrogenated soy L-α-phosphatidylcholine (HSPC).

1.4 This test method can estimate the absolute concentration of cholesterol, DSPE-PEG 2000, and HSPC and their ratio (DSPE-PEG 2000: HSPC: cholesterol) in liposomal formulations.

1.5 This test method describes preparation of calibration standards and samples, HPLC and ELSD instrumentation, method development and method validation, sample analysis, and data reporting.

1.6 The detection limits and quantitation limits for the analytes (lipid components) in this test method are in the range of 2 µg⁄g to 4 µg/g and 7 µg⁄g to 10 µg/g, respectively. The analytical measurement ranges for cholesterol, DSPE-PEG 2000, and HSPC are 10 µg⁄g to 165 µg/g, 10 µg⁄g to 300 µg/g, and 10 µg⁄g to 200 µg/g, respectively.

1.7 Significant digits and rounding of all reported values have been performed according to the guidelines as established in Practice D6026.

1.8 *Units*—The values stated in SI units are to be regarded as the standard. Where appropriate, c.g.s units in addition to SI units are included in this standard.

1.9 *This standard does not purport to address all of the safety concerns, if any, associated with its use. It is the responsibility of the user of this standard to establish appropriate safety, health, and environmental practices and determine the applicability of regulatory limitations prior to use.*

1.10 *This international standard was developed in accordance with internationally recognized principles on standardization established in the Decision on Principles for the Development of International Standards, Guides and Recommendations issued by the World Trade Organization Technical Barriers to Trade (TBT) Committee.*

2. Referenced Documents

2.1 *ASTM Standards:*[2]

D1193 Specification for Reagent Water
D1356 Terminology Relating to Sampling and Analysis of Atmospheres
D6026 Practice for Using Significant Digits and Data Records in Geotechnical Data
D7439 Test Method for Determination of Elements in Airborne Particulate Matter by Inductively Coupled Plasma–Mass Spectrometry
E131 Terminology Relating to Molecular Spectroscopy
E177 Practice for Use of the Terms Precision and Bias in ASTM Test Methods
E456 Terminology Relating to Quality and Statistics
E682 Practice for Liquid Chromatography Terms and Relationships
E2490 Guide for Measurement of Particle Size Distribution of Nanomaterials in Suspension by Photon Correlation Spectroscopy (PCS)
E3025 Guide for Tiered Approach to Detection and Characterization of Silver Nanomaterials in Textiles
E3080 Practice for Regression Analysis with a Single Predictor Variable

[1] This test method is under the jurisdiction of ASTM Committee E56 on Nanotechnology and is the direct responsibility of Subcommittee E56.08 on Nano-Enabled Medical Products.

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[2] For referenced ASTM standards, visit the ASTM website, www.astm.org, or contact ASTM Customer Service at service@astm.org. For *Annual Book of ASTM Standards* volume information, refer to the standard's Document Summary page on the ASTM website.

3. Terminology

3.1 *Definitions:*

3.1.1 *accuracy, n*—closeness of agreement between a test result and an accepted reference value.

3.1.1.1 *Discussion*—The term accuracy, when applied to a set of results, involves a combination of random components and a common systematic error or bias component. **E177**

3.1.2 *aerosol, n*—suspension of solid particles or liquid droplets or both in a gaseous medium. **D1356**

3.1.3 *analyte, n*—chemical constituent of interest in an analytical procedure. **E3025**

3.1.4 *analytical instrument qualification, n*—collection of documented evidence that an instrument performs suitably for its intended purpose (**1**).[3]

3.1.5 *baseline noise, n*—combination of high-frequency signal fluctuations and low-frequency signal drift that affect baseline stability.

3.1.5.1 *Discussion*—These signal fluctuations can originate from line-voltage fluctuations, shot noise (Poisson noise) from electronic circuits, improper solvent degassing, temperature instability, and other nonequilibrium effects. Noise is representative of detector response that is not related to responses from analytes or matrix interferences.

3.1.6 *calibration curve, n*—relationship between measured response values and analytical concentrations of a standard or reference material. **D7439**

3.1.6.1 *Discussion*—A set of calibration standards are used to construct a calibration curve, and the concentration of analyte present in an unknown sample can be determined by comparing the detector response with the calibration curve.

3.1.7 *calibration standards, n*—set of solutions with known analyte concentration used to construct calibration curves.

3.1.8 *carryover effect, n*—systematic error that is derived from the preceding sample injection being introduced into the next sample affecting accurate quantitation.

3.1.9 *cholesterol, n*—steroidal organic compound that stabilizes the lipid bilayer in liposomal formulations.

3.1.10 *chromatogram, n*—graphical presentation of detector response plotted as a function of elution time or effluent volume as the sample components elute from the column and reach the detector.

3.1.10.1 *Discussion*—In the case of evaporative light-scattering detection (ELSD), the detector response is often expressed as voltage (mV) over a range of elution time (t), where voltage is a function of the intensity of light scattered by nonvolatile particles inside the optical chamber. For analysis, the characteristic response of ELSD for an eluting analyte is typically evaluated from the peak area under the curve that is recorded in the chromatogram. This peak area (A) can be expressed mathematically as an integral of detector response for analytes over an elution time interval from t_1 to t_2:

$$A(t) = \int_{t_1}^{t_2} (S)dt \tag{1}$$

Where $A(t)$ and $S(t)$ = Peak area and the instantaneous detector's response at time, t, respectively (**2**).

3.1.11 *coefficient of determination, n*—statistical measure of the linear relationship between X and Y calculated by:

$$r^2 = \frac{\left(\sum_{i=1}^{n} X_i Y_i\right)^2}{\left(\sum_{i=1}^{n} X_i\right)\left(\sum_{i=1}^{n} Y_i\right)} \tag{2}$$

Where n = number of observations. **E131**

3.1.12 *evaporation, n*—process by which an element or compound transitions from its liquid state to its gaseous state.

3.1.12.1 *Discussion*—In the ELSD technique, only the solvent and volatile buffer components of the HPLC-effluent are removed during the evaporation process and less-volatile or nonvolatile analytes are left behind as dried particles. This process is also called "desolvation". The evaporation process depends on various factors including gas pressure, flow rate of the carrier gas, nature of the solvents, and temperature of the evaporation tube. It is important to choose the appropriate mobile phase components that are volatile; nonvolatile buffers are not compatible with this test method.

3.1.13 *intermediate precision, n*—closeness of agreement between test results obtained under specified intermediate precision conditions. **E177**

3.1.14 *intermediate precision conditions, n*—conditions under which test results are obtained with the same test method using test units taken at random from a single quantity of material that is as nearly homogeneous as possible and with changing conditions such as operator, measuring equipment, location within the laboratory, and time. **E177**

3.1.15 *limit of detection, n*—least amount of analyte in a sample that can be detected but not necessarily quantitated under the stated experimental conditions.

3.1.15.1 *Discussion*—The limit of detection is usually expressed as the concentration of the analyte in the test sample.

3.1.16 *limit of quantitation, n*—least amount of analyte in a sample that can be quantitatively determined with suitable precision and accuracy.

3.1.16.1 *Discussion*—The limit of quantitation is usually expressed as the concentration of the analyte in the test sample.

3.1.17 *linearity, n*—ability of the analytical method (within a certain range) to obtain test results that are directly proportional to the concentration (amount) of the analyte in the sample (**3**).

3.1.17.1 *Discussion*—To establish response linearity, a minimum of six analyte concentrations are recommended. Regression analysis by the method of least squares (r^2) provides a mathematical estimate of the degree of linearity.

3.1.18 *lipids, n*—diverse group of organic compounds that are soluble in organic solvents but are insoluble in water.

3.1.18.1 *Discussion*—In this test method, lipids refer to cholesterol, DSPE-PEG 2000, and HSPC. The chemical structures of these three lipids are presented in Appendix X1.

[3] The boldface numbers in parentheses refer to a list of references at the end of this standard.