Designation: E3297 – 21

Standard Test Method for Lipid Quantitation in Liposomal Formulations Using High Performance Liquid Chromatography (HPLC) with a Charged Aerosol Detector (CAD)[1]

This standard is issued under the fixed designation E3297; 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 is for the separation of lipids in liposomal formulations through high performance liquid chromatography (HPLC) and their quantitation using a mass-flow sensitive charged aerosol detector (CAD).

1.2 This test method is specifically for liposomal formulations containing cholesterol, 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[methoxy(polyethylene glycol)-2000] (DSPE-PEG 2000) and hydrogenated soy L-α-phosphatidylcholine (HSPC).

1.3 This test method is applicable to report the absolute concentrations and ratio of cholesterol, DSPE-PEG 2000, and HSPC in liposomal formulations. Assessment of the stability of the analytes in terms of their degradation profiles as a result of oxidation or hydrolysis is beyond the scope of this test method.

1.4 This test method includes calibration standards preparation, sample preparation, method validation, and sample analysis. This method also contains specifications for instrumentation and the chromatography experimental procedure.

1.5 The detection limit and quantitation limit for the analytes in this test method is in the range of 0.1–2.0 µg/g and 1.0–5.0 µg/g respectively. The analytical measurement range for cholesterol, DSPE-PEG 2000, and HSPC is 5–300 µg/g.

1.6 All observed and calculated values shall conform to the guidelines for significant digits and rounding as established in Practice D6026.

1.7 *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.

1.8 *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.*

1.9 *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

3. Terminology

3.1 *Definitions:*

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

[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.

3.1.1.1 *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. It 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 a component 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 this test method, the charged aerosol detector (CAD) response expressed as current (pA) is plotted against elution time (min). For analysis, the characteristic detector response of the eluting analyte is typically evaluated from the peak area recorded in the chromatogram. This peak area (A) can be expressed mathematically as the integral of detector response for analyte over an elution time interval from t_1 to t_2:

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

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

3.1.13 *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**

3.1.14 *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**).

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

3.1.15.1 *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.

3.1.16 *liposomal formulation, n*—product designed to assist in the delivery of an active pharmaceutical ingredient, either encapsulated or intercalated in a liposome.

3.1.16.1 *Discussion*—Formulated products can contain vesicles having a single lipid bilayer (unilamellar), multiple concentric lipid bilayers (multilamellar) or a mixture of unilamellar and multilamellar vesicles.

3.1.17 *liposome, n*—synthetic vesicle composed of a one or more bilayers formed by amphipathic molecules such as phospholipids that enclose a central aqueous compartment. Adapted from (**4**).

3.1.18 *matrix blank, n*—substance that closely matches the samples being analyzed with regard to matrix components, but has none of the analytes of interest.

3.1.18.1 *Discussion*—Ideally, the matrix blank does not contain the analyte(s) of interest but is subjected to all sample-processing operations including all reagents used to analyze the test samples. The matrix blank is used to determine the absence of significant interference as a result of the matrix, reagents, and equipment used in the analysis.

3.1.19 *matrix effect, n*—influence of one or more components from the sample matrix on the measurement of the analyte concentration or mass.

3.1.19.1 *Discussion*—Matrix effects may be observed as enhanced or suppressed detector responses compared with those produced by simple solvent solutions of the analyte (**5**).

3.1.20 *mobile phase, n*—solvent used to sweep or elute the sample components through the column that may consist of a single component or a mixture of components.

3.1.20.1 *Discussion*—The term eluent is often used for the preferred mobile phase. **E682**

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