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# Standard Test Method for Lipid Quantitation in Liposomal Formulations Using Ultra-High-Performance Liquid Chromatography (UHPLC) with Triple Quadrupole Mass Spectrometry (TQMS)[1]

This standard is issued under the fixed designation E3324; 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 the determination of lipid components in liposomal formulations, which includes sample solubilization in methanol followed by separation of the analytes using ultra-high-performance liquid chromatography (UHPLC) and detection with tandem mass-spectrometry (MS/MS). This test method adheres to multiple reaction monitoring (MRM) mass spectrometry on a triple quadrupole mass spectrometer (TQMS).

1.2 This test method is specific 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 of cholesterol, DSPE-PEG 2000, and HSPC and their ratio (DSPE-PEG 2000: HSPC: cholesterol) in liposomal formulations. Assessment of the stability of the analytes in terms of their degradation as a result of oxidation or hydrolysis is beyond the scope of this test method.

1.4 This test method includes calibration and standardization, sample preparation, UHPLC-TQMS instrumentation, potential interferences, method validation with acceptance criteria, sample analysis, and data reporting.

1.5 The detection limits for cholesterol, DSPE-PEG 2000, and HSPC using this test method are 5.3, 0.5, and 0.5 ng/g, respectively. In addition, the quantitation limits for cholesterol, DSPE-PEG 2000, and HSPC are 10.6, 0.8, and 0.5 ng/g, respectively.

1.6 This test method is intended for concentration ranges of 8-1600 ng/g for cholesterol, and of 2-400 ng/g for DSPE-PEG 2000 and HSPC.

1.7 All observed and calculated values shall conform to the guidelines for significant digits and rounding 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

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

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

[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.

2.2 *Federal Standard:*

21 CFR 211.194(a)(2) Code of Federal Regulations Title 21, Food and Drug administration, Department of Health and Human Services, Drugs: Current Good Manufacturing Practice for Finished Pharmaceuticals, Laboratory Records[3]

## 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 component and a common systematic error or bias component. **E177**

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

3.1.3 *analytical instrument qualification, n*—collection of documented evidence that an instrument performs suitably for its intended purpose (**1**).[4]

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

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

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

3.1.5.1 *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.

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

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

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

3.1.9 *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 *electrospray ionization, ESI, n*—sensitive ionization technique in which a solvent spray is formed by the application of a high-voltage potential held between a stainless-steel capillary containing a solution and the instrument orifice and the axial flow of a nebulizing gas (typically nitrogen).

3.1.10.1 *Discussion*—Solvent droplets from the spray evaporate in the ion source of the mass spectrometer releasing ions to the gas phase for analysis in the mass spectrometer (**2**).

3.1.11 *fragment ion, n*—product ion that results from the collision-induced dissociation of a preselected precursor ion (**3**).

3.1.12 *internal standard, ISTD, n*—chemical substance that is added in a known amount to calibration standards and samples with unknown concentrations to correct for analyte loss during sample preparation, ion suppression effects, source fouling, or matrix effects during analysis (**2**).

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 or test samples 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.

3.1.15 *ionization efficiency, n*—ratio of the number of ions generated to the number of molecules introduced into the ion source of a mass spectrometer (**3**).

3.1.16 *ion suppression, n*—matrix effect in liquid chromatography-mass spectrometry that results in a diminished analytical signal independent of the sensitivity or selectivity of the mass analyzer (**2**).

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

3.1.17.1 *Discussion*—The limit of detection is usually expressed as the concentration of the analyte in the sample (**4**). Different approaches to determine detection limits are possible that are based on the visual evaluation, signal-to-noise ratio, or standard deviation of the response and the slope. In the case of instrumental analytical procedures, a common approach is to compare the measured signals from samples having a known low concentration of analyte with that of blank samples. The minimum concentration at which the analyte can be reliably detected is established. Typically, acceptable signal-to-noise ratio is 3:1 (**4, 5**).

3.1.18 *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**).

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

3.1.19.1 *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.

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

3.1.20.1 *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] Available from www.govinfo.gov.

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