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Standard Test Method for Partition Coefficient (N-Octanol/Water) Estimation by Liquid Chromatography[1]

This standard is issued under the fixed designation E 1147; 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 a procedure for the estimation of the log of the octanol/water partition coefficient (log K_{ow}) of chemicals over the range from 0 to 8.

1.2 This test method uses an empirically derived equation to relate the octanol/water partition coefficient to an experimentally determined retention time on a liquid chromatographic column.

1.3 This test method has been designed to estimate log K_{ow}values for both non-ionizable and ionizable compounds. This is accomplished by buffering the liquid chromatographic solvent at a pH that will force the test compound into either the non-ionized or ionized form.

1.4 This test method requires some knowledge of the detector response to the chemical being tested.

1.5 *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 safety and health practices and determine the applicability of regulatory limitations prior to use.*

2. Referenced Documents

2.1 *ASTM Standards:*

D 1193 Specification for Reagent Water[2]

E 200 Practice for Preparation, Standardization, and Storage of Standard Solutions for Chemical Analysis[3]

E 682 Practice for Liquid Chromatography Terms and Relationships[4]

E 1022 Practice for Conducting Bioconcentration Tests with Fishes and Saltwater Bivalve Molluscs[5]

3. Terminology

3.1 *Definitions:*

3.1.1 *octanol/water partition coefficient (K_{ow})*—the equilibrium ratio of the molar concentrations of a chemical in n-octanol and water, in dilute solution. K_{ow} is a constant for a specific chemical at a given temperature. Since K_{ow} is the ratio of two molar concentrations, it is a dimensionless quantity. K_{ow} is often reported as log K_{ow}.

3.1.2 *retention time (t_R, t_o)*—the reference compound or test chemical retention time (t_R) is the time from sample injection to maximum concentration (peak height) of eluted reference compound or test chemical. The internal standard retention time (t_o) is the time from sample injection to the maximum concentration (peak height) of the eluted internal standard.

4. Summary of Test Method

4.1 This test method is based on the work of Veith et al **(1).**[6] Another similar test method is available from OECD **(2)**.

4.2 The test substance (solute) is injected onto a liquid chromatograph column containing a solid-phase support onto which a commercially available long-chain hydrocarbon (for example C8 or C18) has been bonded. Chemicals injected onto such a column move along it by partitioning between the mobile phase and the stationary hydrocarbon phase. A methanol/water solvent system is typically used to elute the solute which is subsequently analyzed using an ultraviolet/visible absorption detector, refractive index detector, electrochemical detector, or other appropriate detector. If the test substance is not amenable to detection by the available LC detectors, the analyst may collect fractions of the column effluent and analyze for the test substance using gas chromatography, liquid scintillation, or other appropriate technique.

4.3 The K_{ow} of the test compound is estimated from a linear regression equation developed from a plot of log ($t_R - t_o$) versus log K_{ow}, using data determined in a calibration step that involves injecting into the chromatograph a mixture of reference chemicals.

4.4 A calibration graph of log ($t_R - t_o$) versus log K_{ow} is developed for a number of reference compounds (typically between 5 and 10) which are structurally similar to the test chemical. Lists of values of measured log K_{ow} are available for many chemicals **(3, 4, 5).** If data on the partition coefficients of structurally related compounds are not available, a more general calibration graph must be developed using other reference compounds. This is a less accurate approach than that

[1] This test method is under the jurisdiction of ASTM Committee E-47 on Biological Effects and Environmental Fate and is the direct responsibility of Subcommittee E47.06 on Environmental Fate of Chemical Substances.

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[2] *Annual Book of ASTM Standards*, Vol 11.01.

[3] *Annual Book of ASTM Standards*, Vol 15.05.

[4] *Annual Book of ASTM Standards*, Vol 14.01.

[5] *Annual Book of ASTM Standards*, Vol 11.05.

[6] The boldface numbers in parentheses refer to the list of references at the end of this test method.

using partition coefficient values for related compounds.

5. Significance and Use

5.1 The octanol/water partition coefficient has been shown to correlate with the tendency of a chemical to partition into and bioconcentrate in the lipid tissues of fish and other animals **(6).** Since 1974, K_{ow} has been used as an indicator of the bioconcentration potential in aquatic and other living organisms. However, Mackay, et al, have described some of the problems associated with interpreting octanol/water partition coefficient data for high molecular weight chemicals **(7).** The numerical value of the octanol/water partition coefficient is one factor to be considered in determining whether to conduct bioconcentration studies. For more information on bioconcentration studies, see Practice E 1022.

5.2 The octanol/water partition coefficient has been proposed by Hansch to relate chemical structure with biological activity **(8).**

5.3 Karickhoff et al **(9)** showed a relationship between $K_{ow}$and the sorption of organic compounds on the organic matter of soils and sediments.

5.4 K_{ow} is an important value in estimating the environmental partitioning of an organic chemical in the environment.

5.5 K_{ow} values may also be obtained by the direct measurement of the chemical in equilibrated n-octanol and water **(10)** or by estimation using a substituent constant method **(11).** The direct measurement method can be difficult to perform, especially if emulsions are formed, and there often is considerable delay before equilibrium conditions are established. However, development of a dynamic coupled column liquid chromatographic technique **(12)** for determining the water solubility of organic chemicals led to adoption of the generator column features of that method for more rapid establishment of equilibrium between octanol and water and the use of the technique in measuring K_{ow} **(13).** The direct measurement method also requires the use of a pure test chemical. The substituent constant method for estimating K_{ow} requires knowledge of the chemical structure and the fragment constants for each substituent group. The data base for fragment constants is incomplete and, under some conditions, there may be large deviations from the ideal contribution of fragment constants for some constituent groups.

5.6 The liquid chromatographic method for estimating K_{ow} provides a rapid technique that does not require either purification of the test substance or complete identification of its structure, unless impurities cause unresolved peaks or difficulties in the identification of peaks.

5.7 This test method is not applicable to strong acids and bases, metal complexes, or surface active agents.

6. Apparatus

6.1 *Liquid Chromatograph Equipped With a Pump*, capable of operating against a pressure of about 875 psi, with a high-pressure stopflow injector and an appropriate recorder.

6.2 *Column Types*, a commercial microparticulate reverse phase packing or ready-packed column to which octadecylsilane or other suitable stationary phase is bonded.

6.3 *Detector*, appropriate for the chemical under evaluation, such as a variable wavelength ultraviolet/visible absorption detector, refractive index detector, or other suitable detector.

7. Reagents and Materials

7.1 *Purity of Reagents*—Reagent grade chemicals shall be used in all tests. Unless otherwise indicated, it is intended that all reagents conform to the specifications of the Committee on Analytical Reagents of the American Chemical Society where such specifications are available.[7] Other grades may be used, provided it is first ascertained that the reagent is of sufficiently high purity to permit its use without lessening the accuracy of the determination.

7.2 *Purity of Water*—Unless otherwise indicated, references to water shall be understood to mean reagent water as defined by Type II of Specification D 1193.

7.3 All reagents must be of the same purity for both the calibration solutions and solutions of unknowns. Methanol and buffer chemicals should be reagent grade or better, as defined in Practice E 200.

7.4 The eluting solvent is typically a solution of 85 parts methanol and 15 parts water (v/v). This is a typical starting point and the solvent may be varied to improve chromatographic separation.

7.5 The eluting solvent may be buffered to force the test compound into either an ionized or non-ionized form. The proper selection of a buffer may be important in obtaining a desired ionic form for certain chemicals. Typically the solvent is buffered within the operating range of the column, which is usually between 2 and 8. However, since pH values less than 5 do not normally occur in natural waters, the significance of making measurements below pH 5 is questionable.

7.6 An internal standard is used to provide a reference retention time against which the reference or test chemical's retention time can be normalized. For test chemicals that have a low K_{ow}, an internal standard which will not be significantly retained by the column, such as the dipotassium salt of 2,5-dihydroxy-p-benzene disulfonic acid, is recommended. For test chemicals having a high K_{ow}, acetanilide is recommended for use as the internal standard.

7.6.1 An internal standard using 2,5-dihydroxy-p-benzene disulfonic acid dipotassium salt may be prepared by dissolving 0.5 to 1.0 g of the compound in 100 mL of distilled water.

7.6.2 An internal standard using acetanilide may be prepared by dissolving 200 mg of the compound in 100 mL of methanol. Acetanilide may also be added directly to the calibration solution of reference compounds.

7.7 A calibration solution is prepared with 200 mg/L of each of 5 to 10 reference compounds plus an internal standard in a solvent such as methanol or other eluent-miscible solvents such as acetonitrile, acetone, or THF. Reference compounds should be selected that are structurally similar to the test chemical(s) and span the range of expected sample K_{ow}'s. If a water-soluble internal standard is used, it is recommended that the reference compounds be prepared first in the organic solvent and then

[7] "Reagent Chemicals, American Chemical Society Specifications," Am. Chemical Soc., Washington, DC. For suggestions on the testing of reagents not listed by the American Chemical Society, see "Reagent Chemicals and Standards," by Joseph Rosin, D. Van Nostrand Co., Inc., New York, NY, and the "United States Pharmacopeia."

add the aqueous-based internal standard at one tenth the volume of the organic solvent. If the calibration solution becomes turbid upon the addition of multiple reference compounds or the internal standard, insolubility of one or more of the compounds is suggested. If upon centrifugation the turbid calibration solution does not become clear, a new calibration solution at lower reference compound concentrations should be prepared. Alternatively, separate calibration solutions of a single reference compound and the internal standard may be prepared.

7.8 A test compound solution is prepared in the same manner as the calibration solution.

7.9 The concentration of the reference compounds and the test chemical is not critical but must be sufficient to give a response of at least 2.5 × the noise level of the detector being used and not so concentrated as to overload the column.

8. Sampling

8.1 Any sample can be used that contains the chemical or chemicals for which K_{ow} is to be estimated, providing the test chemical is soluble in the eluting solvent, the chemical(s) are present at a sufficient concentration to be detected, and that other sample components do not interfere with the chromatography.

9. Calibration

9.1 After conditioning the column with the eluting solvent, inject 20µ L of the calibration solution onto the column. Elute the reference compounds using eluting solvent or suitably buffered eluting solvent. A20-µL injection of the calibration solution should give an adequate recorder response for calibration purposes. However, both the solution concentration and the amount injected may be increased or decreased without affecting retention times since t_R is independent of concentration with dilute solutions.

9.2 Adjust the mobile phase composition, mobile phase flow rate, or column length, if necessary, to achieve adequate resolution.

9.3 Determine the normalized retention times, $t_R - t_o$, for each reference compound.

9.4 Construct a plot of log $(t_R - t_o)$ versus known log K_{ow} for the reference compounds.

9.5 Perform the calibration step with each set of unknowns, so that possible changes in column performance may be identified and compensated for in the calculations.

9.6 Additional information on using liquid chromatography equipment can be found in Practice E 682.

10. Procedure

10.1 Determinations are made at ambient temperatures with no more than 2°C difference between runs of reference compounds and unknowns.

10.2 Immediately following column calibration, inject 20 µL of the test solution onto the column. Elute the test chemical(s) using the same eluting solvent used for the reference compounds.

10.3 Determine the normalized retention time, $t_R - t_o$, for each unknown.

11. Calculation of Results

11.1 Using the plot of log $(t_R - t_o)$ versus log K_{ow} for the reference compounds, compute the linear regression equation of the form $\log K_{ow} = a \log (t_R - t_o) + b$, where a and b are the slope and intercept, respectively.

11.2 From the standard curve or regression equation, calculate an estimated log K_{ow} for the test compound corresponding to the measured log $(t_R - t_o)$.

12. Report

12.1 Report the standard curve of log $(t_R - t_o)$ versus log $K_{ow}$for each buffered or unbuffered eluent, or report the regression equation in the form of $\log K_{ow} = a \log (t_R - t_o) + b$.

12.2 Report the estimated log K_{ow} for each test chemical for each buffered and unbuffered eluent, as determined from the standard curve or regression equation.

12.3 Provide a description of, or reference for, the liquid chromatography, mobile phase, column and detector(s) used.

12.4 Describe the test and reference compounds and their purity.

12.5 Report the pH and temperature at which each determination was made.

13. Precision and Bias

13.1 The precision and bias have not been determined for this test method. However, the partition coefficient can usually be estimated to within 1 log unit of the shake-flask value. Typical correlations can be found in the literature **(14, 15, 16).** Higher accuracy may be achieved when calibration plots are based on structurally related compounds **(17).**

REFERENCES

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