Designation: E 1303 – 95 (Reapproved 2000)

Practice for Refractive Index Detectors Used in Liquid Chromatography[1]

This standard is issued under the fixed designation E 1303; 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 practice is intended to describe tests used to evaluate the performance and to list certain descriptive specifications of a refractive index (RI) detector used as the detection component of a liquid chromatographic (LC) system.

1.2 This practice is intended to describe the performance of the detector both independent of the chromatographic system (static conditions, without flowing solvent) and with flowing solvent (dynamic conditions).

1.3 The values stated in SI units are to be regarded as the standard.

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

2. Referenced Documents

2.1 *ASTM Standards:*

E 386 Practice for Data Presentation Relating to High-Resolution Nuclear Magnetic Resonance (NMR) Spectroscopy[2]

3. Significance and Use

3.1 Although it is possible to observe and measure each of several characteristics of a detector under different and unique conditions, it is the intent of this practice that a complete set of detector test results should be obtained under the same operating conditions. It should also be noted that to specify completely a detector's capability, its performance should be measured at several sets of conditions within the useful range of the detector.

3.2 The objective of this practice is to test the detector under specified conditions and in a configuration without an LC column. This is a separation independent test. In certain circumstances it might also be necessary to test the detector in the separation mode with an LC column in the system, and the appropriate concerns are also mentioned. The terms and tests described in this practice are sufficiently general so that they may be adapted for use at whatever conditions may be chosen for other reasons.

4. Noise, Drift, and Flow Sensitivity

4.1 *Descriptions of Terms Specific to This Standard*:

4.1.1 *short term noise*—This noise is the mean amplitude in refractive index units (RIU) for random variations of the detector signal having a frequency of one or more cycles per min. Short term noise limits the smallest signal detectable by an RI detector, limits the precision attainable and sets the lower limit on the dynamic range. This noise corresponds to observed noise of the RI detector only. (The actual noise of the LC system may be larger or smaller than the observed value, depending upon the method of data collection, or signal monitoring of the detector, since observed noise is a function of the frequency, speed of response and the band width of the recorder or other electronic circuit measuring the detector signal.)

4.1.2 *long term noise*—this noise is the maximum amplitude in RIU for random variations of the detector signal with frequencies between 6 and 60 cycles per h (0.1 and 1.0 cycles per min). It represents noise that may be mistaken for a late-eluting peak. This noise corresponds to the observed noise only and may not always be present.

4.1.3 *drift*—the average slope of the long term noise envelope expressed in RIU per hour as measured over a period of 1 h.

4.1.4 *static*—refers to the noise and drift measured under conditions of no flow.

4.1.5 *dynamic*—refers to the noise and drift measured at a flow rate of 1.0 mL/min.

4.1.6 *flow sensitivity*—the rate of change of signal displacement (in RIU) vs flow rate (in mL per min) resulting from step changes in flow rate calculated at 1 mL per min as described in 4.3.12.

4.2 *Test Conditions*:

4.2.1 The same test solvent must be used in both sample and reference cells. The test solvent used and its purity should be specified. Water equilibrated with the laboratory atmosphere containing minimum impurities is the preferred test solvent for measuring noise and drift. Water for this purpose (preferably purified by distillation, deionization or reverse osmosis) should

[1] This practice is under the jurisdiction of ASTM Committee E13 on Molecular Spectroscopy and is the direct responsibility of Subcommittee E13.19 on Chromatography.

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[2] *Annual Book of ASTM Standards*, Vol 03.06.

be drawn, filtered through a 0.45 µm filter and allowed to stand in a loosely covered container for several hours at ambient temperature in the laboratory in which testing is to be carried out. This will ensure complete equilibration of the water with the gases in the laboratory atmosphere.

NOTE 1—It is essentially impossible to maintain a constant RI value of de-gassed water and of very dilute samples in de-gassed water. This is due to the fact that the difference in refractive index between completely de-gassed water and atmosphere-equilibrated water is 1.5×10^{-6}RIU.[3] Thus, small differences in the concentration of dissolved gases between sample and the trapped reference can lead to significant errors in measurement of solutions where the expected difference in RI due to solute is of the order of 10^{-6}RIU or less. Therefore, in order to minimize error in determining samples with small RIU differences between them, atmosphere-equilibrated water (5.2.1) is recommended as the solvent for determining linearity and minimum detectability (Section 5).

4.2.2 The detector should be located at the test site and switched on at least 24 h prior to the start of testing. Some detectors provide an oven to thermostat the optics assembly. The oven should be set at a suitable temperature, following the manufacturer's recommendations, and this temperature should be noted and maintained throughout the test procedures.

4.2.3 Linearity and speed of response of the recorder or other data acquisition device used should be such that it does not distort or otherwise interfere with the performance of the detector.[4] If additional amplifiers are used between the detector and the final readout device, their characteristics should also first be established.

4.3 *Methods of Measurement*:

4.3.1 Connect a 1 m (39.37 in.) length of clean, dry, stainless steel tubing of 0.25 mm (0.009 to 0.01 in.) inside diameter in place of the analytical column. The tubing can be straight, or coiled to minimize the space requirement. The tubing should terminate in standard low dead volume fittings to connect with the detector and to the pump. Commercial chromatographs may already contain some capillary tubing to connect the pump to the injection device. If this is of a similar diameter to that specified, it should be included in the 1.0 m length; if significantly wider, it should be replaced for this test.

4.3.2 Repeatedly rinse the reservoir and chromatographic system, including the detector, with the test solvent prepared as described in 4.2.1, until all previous solvent is removed from the system. Fill the reservoir with the test solvent.

4.3.3 Thoroughly flush the reference cell with the same solvent; keep the reference cell static.

4.3.3.1 It may be necessary to flush both sample and reference cells with an intermediate solvent (such as methanol or acetone), if the solvent previously used in the system is immiscible with the test solvent.

4.3.4 Allow the chromatographic system to stabilize for at least 60 min without flow. The detector range, Note 2, should be set such that the amplitude of short term noise may be easily measured. Ideally, the output should contain no filtering of the signal. If the filtering cannot be turned off, the minimum time constant should be set and noted in the evaluation. Manuals or manufacturers should be consulted to determine if time constant and detector range controls are coupled, and information should be obtained to determine if they can be decoupled for testing. Set the recorder zero to near mid-scale. Record at least 1 h of baseline under these static conditions, during which time the ambient temperature should not change by more than 2°C.

NOTE 2—RI detectors will have one or more controls labeled *attenuation, range, sensitivity*, and *scale factor*. All are used to set the full scale range (in RIU) of an output display device such as a strip chart recorder.

4.3.5 Draw pairs of parallel lines, each between ½to 1 min in length, to form an envelope of all observed random variations over any 15 min period (Fig. 1). Draw the parallel lines in such a way as to minimize the distance between them. Measure the distance perpendicular to the time axis between the parallel lines. Convert this value to RIU (4.2.9). Calculate the mean value over all the segments; this value is the static short term noise.

4.3.6 Now mark the center (center of gravity) of each segment over the 15 min period of the short term noise measurement. Draw a series of parallel lines to these centers, each 10 min in length (Fig. 1), and choose that pair of lines whose distance apart perpendicular to the time axis is greatest. This distance is the static long term noise.

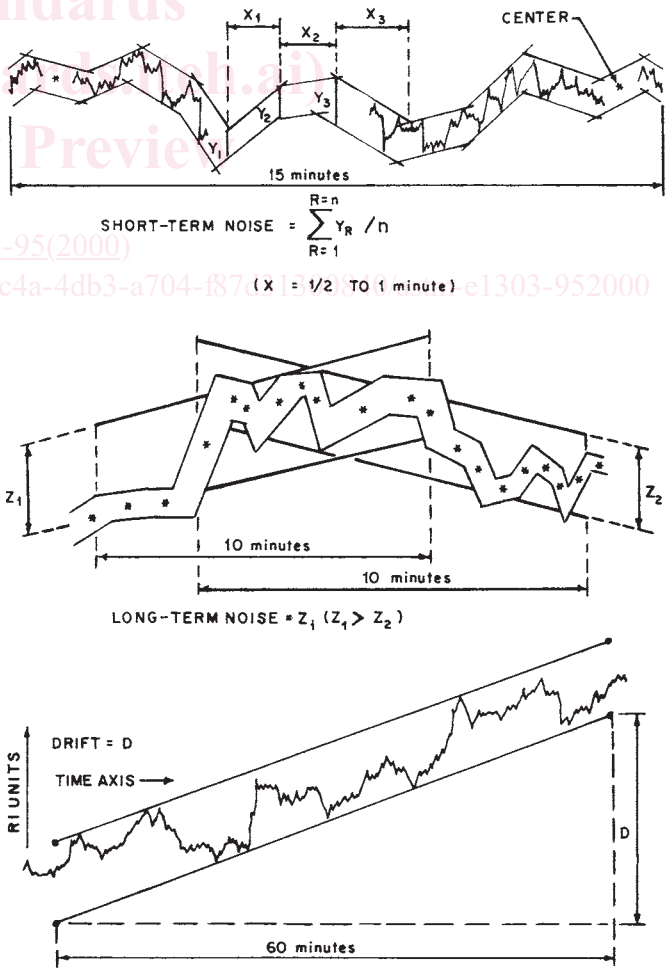

FIG. 1 Examples for the Measurement of Short Term Noise, Long Term Noise and Drift

[3] Munk, M. N., *Liquid Chromatography Detectors*, (T. M. Vickrey, Ed.), Marcel Dekker, New York and Basel, 1983, pp. 165–204.

[4] Bonsall, R. B., "The Chromatography Slave—The Recorder," *Journal of Gas Chromatography*, Vol 2, 1964, pp. 277–284.

4.3.7 Draw the pair of parallel lines, over the 1 h of measurement, that minimizes the distance perpendicular to the time axis between the parallel lines. The slope of either line, measured in RIU/h, is the static drift.

4.3.8 Set the solvent delivery system to a flow rate that has previously been shown to deliver 1.0 mL per min under the same conditions of capillary tubing, solvent, and temperature. Allow at least 15 min to stabilize. Set the recorder zero near mid-scale. Record at least 1 h of baseline under these flowing conditions, during which time the ambient temperature should not change by more than 2°C.

4.3.9 Draw pairs of parallel lines, measure the perpendicular distances and calculate the dynamic short term noise, in the manner described in 4.3.5 for the static short term noise.

4.3.10 Make the measurement for the dynamic long term noise following the procedure outlined in 4.3.6.

4.3.11 Draw the pair of parallel lines as directed in 4.3.7. The slope of this line is the dynamic drift.

4.3.12 Stop the chromatographic flow. Allow at least 15 min for re-equilibration. Set the recorder at about 5 % of full scale and leave the detector range setting at the value used for the noise measurements. Set the solvent delivery system at a flow rate of 0.5 mL per min. Run for 15 min, or more if necessary for re-equilibration, at a slow recorder speed. Increase the flow rate to 1.0 mL per min and record for 15 min or more. Run at 2.0, 4.0 and 8.0 mL per min *if the pressure flow limit of the chromatographic system is not exceeded.* If necessary, adjust the detector range to maintain an on-scale response.

4.3.13 Draw a horizontal line through the plateau produced at each flow rate, after a steady state is reached (Fig. 2). Measure the vertical displacement between these lines, and express in RIU (4.2.9). Plot these values versus flow rate. Draw a smooth curve connecting the points and draw a tangent at 1 mL per min (Fig. 3). Express the slope of the line as the flow sensitivity in RIU min per mL. It is preferred to give the numerical value and show the plot as well.

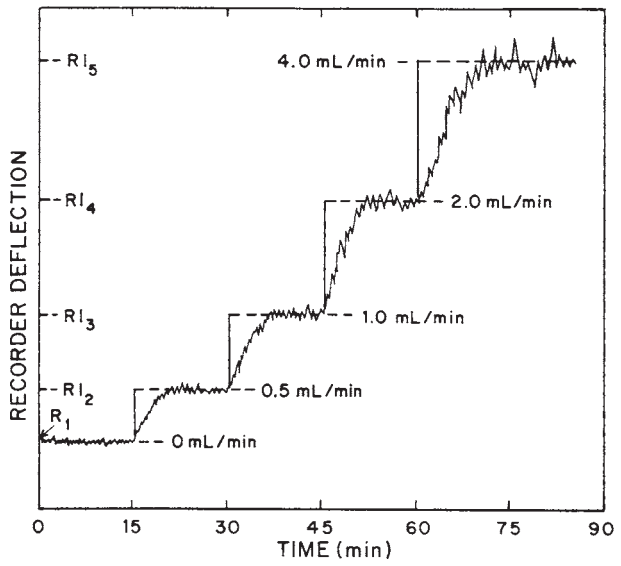

FIG. 2 Example for the Measurement of Flow Sensitivity

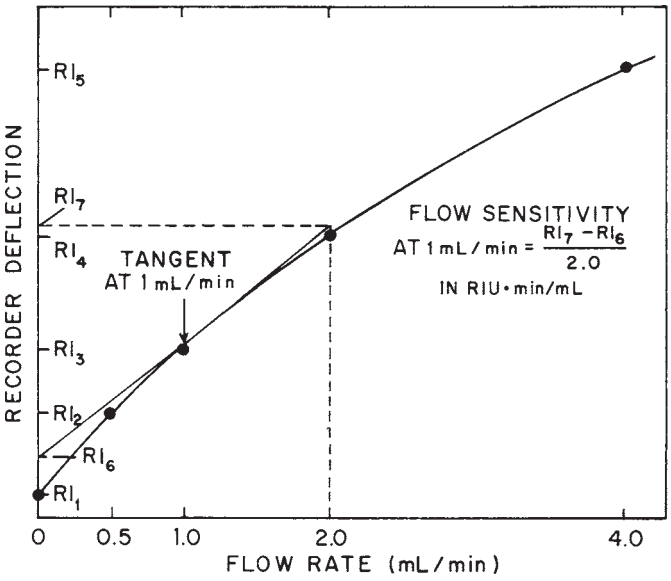

FIG. 3 Example of Plot for Calculation of Flow Sensitivity

5. Minimum Detectability, Linear Range, Dynamic Range, and Calibration

5.1 *Descriptions of Terms Specific to this Standard*:

5.1.1 *minimum detectability*—that concentration of a specific solute in a specific solvent that gives a signal equal to twice the static short-term noise.

5.1.1.1 *Discussion*—The static short-term noise is a measurement of peak-to-peak noise. A statistical approach to noise suggests that a value of three times the rms (root-mean-square) noise would insure that any value outside this range would not be noise with a confidence level of greater than 99 %. Since peak-to-peak noise is approximately five times the rms noise[4,5], the minimum detectability defined in this Practice is a more conservative estimate. Minimum detectability, as defined in this Practice, should not be confused with the limit of detection in an analytical method using a refractive index detector.

5.1.2 *sensitivity (response factor)*—the signal output per unit concentration of the test substance in the test solvent, in accordance with the following relationship:

$$S = R/C \quad (1)$$

where:

S = sensitivity (response factor), RIU·L/g,
R = measured detector response, RIU, and
C = concentration of the test substance in the test solvent g/L.

5.1.3 *linear range*—the range of concentrations of the test substance in the test solvent, over which the sensitivity of the detector is constant to with 5 % as determined from the linearity plot specified in 5.2.13. The linear range may be expressed in three different ways:

[5] Blair, E. J., *Introduction to Chemical Instrumentation*, McGraw-Hill, New York, NY, 1962, Practice and E386.

5.1.3.1 As the ratio of the upper limit of linearity obtained from the linearity plot, and the minimum linear concentration, both measured for the same test substance in the same test solvent as follows:

$$L.R. = C_{max} / C_{min} \quad (2)$$

where:

$L.R.$ = linear range of the detector,
C_{max} = upper limit of linearity obtained from the linearity plot, g/L, and
C_{min} = minimum linear concentration g/L, as defined in 5.2.13.1, the minimum linear concentration should also be stated.

5.1.3.2 By giving the minimum linear concentration and the upper limit of linearity (for example, from 8.72×10^{-3}g/L to 8.72×10^{-1} g/L).

5.1.3.3 By giving the linearity plot itself, with the minimum linear concentration and the upper limit of linearity indicated on the plot.

5.1.4 *Dynamic Range*—That range of concentrations of the test substance in the test solvent, over which an incremental change in concentration produces an incremental change in detector signal. The upper limit is the highest concentration at which a slight further increase in concentration will give an observable increase in detector signal. The dynamic range is the ratio of these upper and lower limits. The dynamic range is larger than or equal to the linear range, but obviously cannot be smaller. The dynamic range may be expressed in three different ways:

5.1.4.1 As the ratio of the upper limit of dynamic range to the minimum detectability. The minimum detectability must also be stated.

5.1.4.2 By giving the minimum detectability and the upper limit of dynamic range (for example from 2.9×10^{-3}g/L to 17.4 g/L).

5.1.4.3 By giving the dynamic plot itself with the minimum detectability indicated on the plot.

5.2 *Method of Measurement*:

5.2.1 Water drawn for the mobile phase and sample dilution (preferably purified by distillation, deionization or reverse osmosis) should be allowed to stand for several hours at the temperature of the room in which the testing is to be carried out. This will ensure complete equilibration of the water with the gases in the laboratory atmosphere (refer to Note 1).

5.2.2 Because a 1×10^{-4}RIU difference is near the middle of the operating range for most refractive index detectors, the solution that gives 1×10^{-4}RIU when measured against water is chosen as the normal solution and defined to have the value of 1.[6] Note the detector range setting at which the normal solution produces a near full scale deflection and term this normal range setting.

5.2.3 Weigh out 43.6 g of glycerin (USP) and dissolve in 1 L of the atmosphere-equilibrated purified water. This stock solution is 50 times the concentration of the normal solution (5.2.2) used for calibration and is assigned a normalized concentration of 50.

5.2.4 Serially dilute the stock solution (5.2.2) to 0.01 relative concentration according to Table 1. Use the stock solution and the diluted solutions for linearity and dynamic range testing.

5.2.5 Because atmosphere-equilibrated water is used as the mobile phase and sample diluent for this procedure, it is advisable to apply a slight back pressure to the sample cell to prevent outgassing in the cell. This may be safely achieved by placing the solvent waste container on a shelf above the detector. Avoid backpressure >690 KPa (100 psi), to prevent cell rupture.

5.2.6 Measure the detector response under static conditions for each of the solutions prepared in 5.2.4. Introduce the solutions conveniently using a liquid chromatography solvent delivery system and an injector equipped with a 5 mL sample loop. (Twenty feet of 1.02-mm (0.04-in.) inside diameter stainless steel tubing has a volume of about 5 mL.) For each measurement, pump atmosphere-equilibrated water through the sample cell until the baseline is stable. Stop the flow and note the position of the baseline on the chart. Load the injector with the test solution and pump the solution into the sample cell. The recorded signal on the chart will change. When the recorded signal for the test solution has stabilized, again stop the flow and note the position of the signal on the chart. Adjust the detector range so that the distance from the water baseline to the test solution signal can be easily measured. Finally, restart the flow to flush the test solution from the sample cell. Repeat this process 3 to 5 times for each test solution. Depending on the configuration of the detector, a second pump may be required to deliver water to the reference cell. As an alternative, fill and flush the sample cell manually using a 10 mL syringe to deliver 5 to 10 mL of solution.

5.2.7 The detector response is the distance in centimetres on the chart from the water baseline to the test solution signal. Calculate an average value for the 3 to 5 replicates for each test solution.

5.2.8 Multiply the measured detector responses by an appropriate scale factor so that all responses correspond to the detector range setting used for the measurement of the response of the normal solution (C = 0.872 g/L). For example, if a setting of 16 is used for the normal solution and lower values

TABLE 1 Concentrations of Test Solutions

Relative Concentration	Actual Concentration (g/L)	Theoretical RI Difference (RIU)
50.0	43.6	5×10^{-3}
20.0	17.4	2×10^{-3}
10.0	8.72	1×10^{-3}
5.0	4.36	5×10^{-4}
2.0	1.74	2×10^{-4}
1.0	8.72×10^{-1}	1×10^{-4}
0.5	4.36×10^{-1}	5×10^{-5}
0.2	1.74×10^{-1}	2×10^{-5}
0.1	8.72×10^{-1}	1×10^{-5}
0.05	4.36×10^{-2}	5×10^{-6}
0.02	1.74×10^{-2}	2×10^{-6}
0.01	8.72×10^{-3}	1×10^{-6}

[6] Scott, R. P. W., "Liquid Chromatography Detectors," 2nd edition, *Journal of Chromatography Library*, Vol 33, Elsevier Scientific Publishing Co., Amsterdam, 1986. This reference is given for general reading.

for detector range correspond to a larger full scale response, then a response measured at a range setting of 2 must be multiplied by 8 to give the scaled response. Similarly, a response measured at a range setting of 64 must be multiplied by ¼to give scaled response.

5.2.9 Calibrate the chart in RIU/cm. Measure the detector responses (5.2.7) for the test solutions of 1.0 and 0.5 relative concentration at a single detector range setting at which both signals are on scale. Given that the refractive index difference from water for a relative concentration of 1 is 10^{-4}RIU, the refractive index difference between the 0.5 and 1.0 concentration solutions will be 5×10^{-5}RIU. Calculate the response per centimetre (calibration factor) by dividing 5×10^{-5}RIU by the difference in the two detector responses (expressed in centimetres).

5.2.10 Convert the detector responses to RIU using the calibration factor determined in 5.2.9. Prepare a Table 2 for the data based on Tables 3-5 that are typical data.

5.2.11 Determine the dynamic range and minimum detectability of the detector as follows:

5.2.11.1 Plot the scaled detector response (Table 2) versus actual concentration on rectilinear graph paper, and draw a smooth curve through the data points as shown in Fig. 4. The upper limit of the dynamic range is the concentration at which the slope of the line becomes zero, or the largest measured concentration if zero slope is not reached. If necessary, use test solutions of higher concentration than those specified in Table 1 if the detector is capable of measuring refractive index changes greater than 5.0×10^{-3}RIU.

5.2.11.2 Determine the minimum detectability (minimum detectable concentration) of the test substance by finding the concentration that would correspond to twice the static short-term noise. Either use the plot prepared in 5.2.11.1 (Fig. 4) or a log-log plot of the data (Fig. 7) for this purpose.

5.2.11.3 Report the dynamic range as specified in 5.1.4.1.

5.2.12 The range of test solutions specified for this test cover a range of refractive index differences 5×10^{-3}RIU to 1×10^{-6}RIU relative to water and is not intended to span the entire dynamic range of all detectors. With the stand-alone detector test procedure described here, small differences in dissolved gas concentration between samples and reference make it difficult to obtain reliable measurements of more dilute test solutions. For this reason, the response data may need to be extrapolated to determine the dynamic range (Fig. 7).

TABLE 2 Test Data, Detector

Concentration (g/L)	Response (cm)	Detector Range	Scaled Response (cm)	Response (RIU)	Sensitivity (RIU·L/g)
43.6	...	...	...	...	...
17.4	...	...	...	...	...
8.72	...	...	...	...	...
4.36	...	...	...	...	...
1.74	...	...	...	...	...
0.872	...	...	...	...	...
0.436	...	...	...	...	...
0.174	...	...	...	...	...
8.72×10^{-2}	...	...	...	...	...
4.36×10^{-2}	...	...	...	...	...
1.74×10^{-2}	...	...	...	...	...
8.72×10^{-3}	...	...	...	...	...

5.2.13 Determine the linear range of the detector as follows:

5.2.13.1 Calculate the sensitivity for each of the test solutions by dividing the scaled detector response (in RIU) by the actual concentration (in g/L). Enter the results in Table 2. Plot the sensitivity versus log (concentration) on semi-log paper as shown in Fig. 10. Draw a smooth line through the data points. Fig. 10 represents some responses that might be found. Calculate $\bar{S}$, the constant value of sensitivity, by a least squares calculation using the data points on the flat portion of the curve in the linearity plot. Give the upper limit of linearity, C_{max}, by the intersection of the line through the data points with the value of $0.95 \times \bar{S}$. The minimum linear concentration, C_{min}, is the greater of the lowest measured concentration or the intersection of the line through the data points with either $0.95 \times \bar{S}$ or $1.05 \times \bar{S}$ (Note 3). Report the linear range as specified in 5.1.3.1.

NOTE 3—If the minimum detectability (see 5.2.11.2) falls below the minimum linear concentration defined above, the linear range cannot be extrapolated to the minimum detectability.

5.2.13.2 If the linearity plot indicates that the detector is non-linear at any of the concentrations used to determine calibration, repeat the calibration using concentrations that fall within the linear range of the detector. If recalibration is necessary, recalculate sensitivities and prepare a new linearity plot.

5.2.14 When minimum detectability determined by this methodology is indicated to be well below 1×10^{-6} RIU and the detector being used in an LC method requires measurements of refractive index differences of 10^{-6}RIU or less, demonstrate the linearity of the method in that region by analyzing suitably diluted standard samples injected on the LC columns used in the analysis. In this instance, make dissolved gases elute as one or more air peaks, that must be separated from peaks of compounds of interest, otherwise a non-linear calibration curve might exist. An example chromatogram is shown in Fig. 13, and operating conditions are noted in the caption. In these situations, prepare calibration curve and linearity plot for injected samples as in Fig. 10.

6. Description of Detector

6.1 Other factors than those previously detailed are important to describe a refractive index detector. These factors are sometimes considered convenience items as they either do not directly affect performance (as described in the previous evaluation section) or do so in an indirect fashion. The factors are listed below, while typical values and units are listed in Tables 6-8.

6.2 *Display Range of Detector*—Identify the highest and lowest settings available at the detector output, expressed in RIU full scale deflection of the recorder specified as standard, so that the designated RIU represents exactly full scale deflection of that recorder, when zero signal is adjusted to recorder zero.

6.3 *Light Source*—Identify the light source that illuminates the sample cell.

6.4 *Cell Volume*—Identify the volume of the cell, where detection takes place, and where mixing occurs.

TABLE 3 Test Data, Detector A[A]

Concentration (g/L)	Response (cm)	Detector Range[B]	Scaled Response (cm)[C]	Response (RIU)[D]	Sensitivity (RIU·L/g)[E]
43.6	16.5	1	528.0	4.26×10^{-3}	9.77×10^{-5}
17.4	14.5	2	232.0	1.87×10^{-3}	1.07×10^{-4}
8.72	14.7	4	118.0	9.52×10^{-4}	1.09×10^{-4}
4.36	14.8	8	59.2	4.77×10^{-4}	1.09×10^{-4}
1.74	11.9	16	23.8	1.92×10^{-4}	1.10×10^{-4}
0.872	12.3	32	12.3	9.92×10^{-5}	1.14×10^{-4}
0.436	12.2	64	6.10	4.92×10^{-5}	1.13×10^{-4}
0.174	9.7	128	2.43	1.96×10^{-5}	1.13×10^{-4}
8.72×10^{-2}	10.3	256	1.29	1.04×10^{-5}	1.19×10^{-4}
4.36×10^{-2}	10.3	512	0.644	5.19×10^{-6}	1.19×10^{-4}
1.74×10^{-2}	8.6	1024	0.269	2.17×10^{-6}	1.25×10^{-4}
8.72×10^{-3}	4.5	1024	0.141	1.14×10^{-6}	1.31×10^{-4}

[A] Detector cell temperature is 37°C.

[B] The values in this column are the actual detector settings used on the specific detector that was used to obtain this data. (1 = maximum RIU full scale, "least sensitive," 1024 = minimum RIU full scale, "most sensitive.") Settings for the various test samples may be different for different manufacturer's detectors.

[C] The "normal range setting" for this detector was 32 (see 4.2.2). Data are responses (cm) calculated for a setting of 32 (for example, for a setting of "8", multiply peak height at that setting by 4, since 32/8 = 4. For 256, use 32/256 = × 1/8).

[D] Data are the scaled responses (cm) multiplied by the calibration factor (RIU/cm) for the "normal range setting." (See 4.2.9.)

[E] Data are the responses (in RIU) divided by the corresponding concentrations (in g/L).

TABLE 4 Test Data, Detector B[A]

Concentration (g/L)	Response (cm)	Detector Range[B]	Scaled Response (cm)[C]	Response (RIU)[D]	Sensitivity (RIU·L/g)[E]
43.6	24.4	128	97.6	6.56×10^{-4}	1.50×10^{-5}
17.4	20.1	128	80.4	5.41×10^{-4}	3.11×10^{-5}
8.72	17.6	128	70.4	4.73×10^{-4}	5.42×10^{-5}
4.36	13.5	128	54.0	3.63×10^{-4}	8.33×10^{-5}
1.74	13.2	64	26.4	1.77×10^{-4}	1.02×10^{-4}
0.872	14.2	32	14.2	9.55×10^{-5}	1.10×10^{-4}
0.436	14.8	16	7.40	4.97×10^{-5}	1.14×10^{-4}
0.174	11.7	8	2.93	1.97×10^{-5}	1.13×10^{-4}
8.72×10^{-2}	12.0	4	1.50	1.01×10^{-5}	1.16×10^{-4}
4.36×10^{-2}	12.1	2	0.756	5.08×10^{-6}	1.17×10^{-4}
1.74×10^{-2}	9.4	1	0.294	1.98×10^{-6}	1.14×10^{-4}
8.72×10^{-3}	4.5	1	0.141	9.48×10^{-7}	1.09×10^{-4}

[A] Detector cell temperature is ambient.

[B] The values in this column are the actual detector settings used on the specific detector that was used to obtain this data. (128 = maximum RIU full scale, "least sensitive," 1 = minimum RIU full scale, "most sensitive.") Settings for the various test samples may be different for different manufacturer's detectors.

[C] The "normal range setting" for this detector was 32 (see 4.2.2). Data are responses (cm) calculated for a setting of 32 (for example, for a setting of "8", multiple peak height at that setting by 1/4, since 8/32 = 1/4. For 128, use 128/32 = × 4).

[D] Data are the scaled responses (cm) multiplied by the calibration factor (RIU/cm) for the "normal range setting." (See 4.2.9.)

[E] Data are the responses (in RIU) divided by the corresponding concentrations (in g/L).

TABLE 5 Test Data, Detector C[A]

Concentration (g/L)	Response (cm)	Detector Range[B]	Scaled Response (cm)[C]	Response (RIU)[D]	Sensitivity (RIU·L/g)[E]
43.6	2.5	32	5.0	3.34×10^{-5}	7.66×10^{-7}
17.4	60.3[F]	32	121.0	8.07×10^{-4}	4.64×10^{-5}
8.72	54.5[F]	32	109.0	7.27×10^{-4}	8.34×10^{-5}
4.36	33.7[F]	32	67.4	4.50×10^{-4}	1.03×10^{-4}
1.74	14.6	32	29.2	1.95×10^{-4}	1.12×10^{-4}
0.872	14.7	16	14.7	9.80×10^{-5}	1.12×10^{-4}
0.436	14.4	8	7.20	4.80×10^{-5}	1.10×10^{-4}
0.174	11.6	4	2.90	1.93×10^{-5}	1.11×10^{-4}
8.72×10^{-2}	12.0	2	1.50	1.00×10^{-5}	1.15×10^{-4}
4.36×10^{-2}	11.1	1	0.697	4.65×10^{-6}	1.07×10^{-4}
1.74×10^{-2}	17.4	1/4	0.272	1.81×10^{-6}	1.04×10^{-4}
8.72×10^{-3}	10.0	1/4	0.128	8.55×10^{-7}	9.80×10^{-5}

[A] Detector cell temperature is 35°C.

[B] The values in this column are the actual detector settings used on the specific detector that was used to obtain this data. (32 = maximum RIU full scale, "least sensitive," 1/4 = minimum RIU full scale, "most sensitive.") Settings for the various test samples may be different for different manufacturer's detectors.

[C] The "normal range setting" for this detector was 16 (see 4.2.2). Data are responses (cm) calculated for a setting of 16 (for example: for a setting of "4", multiply peak height at that setting by 1/4, since 4/16 = 1/4. For 32, use 32/16 = × 2).

[D] Data are the scaled responses (cm) multiplied by the calibration factor (RIU/cm) for the "normal range setting." (See 4.2.9.)

[E] Data are the responses (in RIU) divided by the corresponding concentrations (in g/L).

[F] Recorder full scale response was changed to obtain an on-scale detector response. Measured responses were scaled to 10 mV full scale and entered in this column.