

〈782〉 VIBRATIONAL CIRCULAR DICHROISM SPECTROSCOPY

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1. INTRODUCTION

Vibrational circular dichroism (VCD) is a form of chiroptical spectroscopy for which chiral molecules have non-zero spectra with intensities that are identical for mirror-image pairs (enantiomers) of chiral molecules but with signs that are opposite with respect to zero intensity. For a chiral molecular substance, two important properties are 1) absolute configuration (AC), which indicates which of two mirror-image forms of the molecule is present; and 2) enantiomeric excess (EE, also called enantiomeric purity), the fractional excess of one enantiomer of a chiral molecule over its mirror-image enantiomer. The principal chapter in *USP–NF* that addresses molecular chirality is *Optical Rotation* 〈781〉. Optical rotation (OR) and electronic circular dichroism (ECD) are the traditional forms of chiroptical spectroscopy, whereas VCD is a newer form of chiroptical spectroscopy that contains more molecular structure information and recently has been adopted more broadly for use in the pharmaceutical industry.

In this chapter, the required methodology for the use of VCD is described in terms of instrument qualification; sign and intensity calibration; procedures, validation, and verification of VCD measurement; calculation; and statistical analysis as needed for the determination of AC and EE of chiral pharmaceutical products. VCD is the extension of ECD from the region of electronic transitions in the UV and visible regions of the spectrum to vibrational transitions in the infrared (IR) region.

VCD is defined as:

$$\Delta A = A_L - A_R$$

- ΔA = difference in the IR absorbance
 A_L = sample absorbance for left circularly polarized (LCP) radiation
 A_R = sample absorbance for right circularly polarized (RCP) radiation

Unpolarized IR intensity is defined as the average of LCP and RCP intensities:

$$A = (A_L + A_R)/2$$

- A = IR absorbance
 A_L = sample absorbance for left circularly polarized (LCP) radiation
 A_R = sample absorbance for right circularly polarized (RCP) radiation

The IR intensities of enantiomers are identical, whereas enantiomers have equal and opposite-signed VCD intensities.

For discussion of the theory and principles of VCD measurements, as well as a more detailed explanation of the application of VCD to problems of pharmaceutical interest, see *Vibrational Circular Dichroism Spectroscopy—Theory and Practice* 〈1782〉.

2. QUALIFICATION OF VCD SPECTROMETERS

This section on qualification is divided into three subsections: 2.1 *Installation Qualification* (IQ), 2.2 *Operational Qualification* (OQ), and 2.3 *Performance Qualification* (PQ) of a Fourier transform (FT)-VCD instrument. Although a VCD instrument can be based on a scanning dispersive spectrometer, no such VCD spectrometers are commercially available, and hence they will not be considered. Additional details on the qualification of instruments can be found in *Analytical Instrument Qualification* 〈1058〉.

2.1 Installation Qualification

The IQ requirements provide evidence that the hardware and software are properly installed in the desired location. An FT-VCD instrument is a sensitive, circular-polarization-difference, FT-infrared (FT-IR) spectrometer. Its installation requires a stable bench-top surface, access to dry air or nitrogen for purging of water vapor, and standard electrical outlets. Evidence for IQ involves ensuring that the IR interferometer is scanning, that its photoelastic modulator(s) [PEM(s)] is operating, and that

the electronics hardware is interacting with both the FT-IR main bench and the PEM. The final evidence for IQ is that the software display for acquisition of a VCD spectrum is ready for operator use.

2.2 Operational Qualification

In OQ, an instrument's performance is characterized using a qualification standard of known spectral properties to verify that the system operates within target specifications. The purpose of OQ is to demonstrate that instrument performance is suitable. OQ is a check of the key operational parameters and should be performed after installation and after repairs and/or maintenance.

The OQ tests described in the following sections are typical examples only. Other tests and samples can be used to establish specifications for OQ. Instrument vendors often have samples and test parameters available as part of the IQ/OQ package. The acceptance criteria provided in this section are applicable for general use, whereas specifications for particular instruments and applications may vary, depending on analytical methods used and desired accuracy of the final result.

CHARACTERIZING INSTRUMENT PERFORMANCE

FT-VCD spectrometers measure simultaneously the VCD spectrum and the parent IR spectrum. The VCD spectrum is obtained from a VCD interferogram arising from the combined high-frequency PEM polarization modulation and the Fourier frequencies, whereas the parent IR spectrum arises from the conventional FT-IR interferogram based only on the instrument's Fourier frequencies. Part of the instrument performance is from the underlying FT-IR operation, and the remaining part of the instrument performance is from those components that are uniquely associated with the FT-VCD operation. The combined FT-IR and FT-VCD criteria are wavenumber accuracy and absorbance linearity, whereas those uniquely associated with the VCD operation are VCD sign and intensity calibration, empty-beam baseline accuracy, sample-associated baseline deviations and artifact levels, baseline stability and spectral reproducibility, and signal-to-noise ratio. As described in (1782), two standard samples are commonly used for specifying VCD performance. For sign and intensity calibration, neat α -pinene is used as a validation standard, whereas for baseline characteristics, a solution of camphor in carbon tetrachloride (CCl_4) is used as the qualification standard. VCD spectra presented in (1782) serve as examples of performance characteristics of typical spectra for VCD sign and intensity calibration, baseline stability, and signal-to-noise ratio.

WAVENUMBER ACCURACY

The wavenumber accuracy of FT-VCD instruments is based on the corresponding wavenumber accuracy of the parent FT-IR operation. This accuracy is determined by fringe counting of the visible laser co-operating with the FT-IR interferometer. The frequency calibration is accurate to within the spectral resolution of the instrument and requires no calibration standard. The accuracy of wavenumber frequency should be checked by measuring the IR spectrum of a 35- μm -thick polystyrene film, as described in the *USP-NF* chapter on mid-IR spectroscopy, *Mid-Infrared Spectroscopy* (854), with additional information in *Mid-Infrared Spectroscopy—Theory and Practice* (1854). Because the FT-VCD and FT-IR spectra are simultaneously measured by the action of the same interferometer, the wavenumber accuracy of the VCD spectrum is the same as that of the FT-IR operation of the instrument.

LINEARITY AND STABILITY

Commercial FT-IR instruments have excellent linearity, provided that the infrared detector remains unsaturated. FT-IR spectra are particularly prone to detector saturation, because the intensity of the entire spectrum is present simultaneously at the detector. Detector saturation can be avoided by the use of a long-wavelength pass filter or appropriate adjustment of the gain of the preamplifier to ensure that the maximum of the FT-IR interferogram lies within the range of preamplifier voltage capability. The hallmark of the onset of nonlinearity is distortion of the FT-IR interferogram that can lead to negative FT-IR absorbance values at the endpoint frequency of the spectral coverage, which is clearly incorrect for an absorbing sample. Aperture reduction, insertion of screens, or neutral density filters can be used to avoid detector saturation.

These same considerations apply to the linearity of FT-VCD measurements. The linearity of VCD is more sensitive to the onset of detector saturation than is the parent IR spectrum. VCD spectra are unstable and distorted when the parent FT-IR spectrum has any saturation, and in some cases linearity in the VCD is not restored until the FT-IR intensity is well below saturation. Saturation can be tested by varying the throughput of the instrument by changing the sample path length and making sure that all of the points across the spectrum are changed by the same factor, i.e., there are no relative shape changes in the spectrum. These relative shape changes should be less than the apparent noise level or, at most, 2% (1.00 ± 0.02), depending on the accuracy needed for the VCD measurements.

PHASE CORRECTION, SIGN, AND INTENSITY CALIBRATION

The raw VCD spectrum is measured as the ratio of the FT of the VCD interferogram to that of the FT of the IR interferogram. Both of these interferograms must be phase-corrected before performing the FT. The phase correction for the IR is straightforward, but the phase correction of the VCD interferogram requires transfer of a phase file from a VCD interferogram representing only positive intensities. Most commercial FT-VCD instruments provide such phase files for the measurement of VCD.

Next, the measured raw VCD spectrum must be calibrated such that VCD spectra with the correct signs and intensities are produced. The signs can be determined by measuring the qualification standard of (–)-camphor or (+)-camphor in carbon tetrachloride solution and comparing it to a previously published standard VCD spectrum of this molecule, such as that provided in (1782). Either all of the signs agree, in which case the sign is correctly set, or all of the signs are opposite, in which

case the phase of the VCD synchronous detection (lock-in amplifier or numerical processing) must be changed by 180° to reverse the signs of all VCD bands, positive-to-negative and negative-to-positive.

Calibration of VCD intensities is performed by dividing the raw VCD by a calibration curve representing the magnitude of unit VCD intensity for the VCD spectrometer. The shape of the calibration curve follows a first-order Bessel function, which in turn depends on the settings of the PEM generating LCP and RCP radiation. The maximum of the calibration curve corresponds to the maximum wavenumber value set on the PEM controller. The calibration curve for a given PEM setting can be obtained from the crossing points of curves generated by placing a multiple-wave plate followed by a polarizer in place of the sample and measuring the VCD spectrum in the usual manner. Commercial VCD instruments provide such curves, and the correct calibration curve must be used for each wavenumber setting of the PEM. For an example of a calibration curve for the PEM setting at 1200 cm^{-1} , see (1782).

After calibration of the intensities of the VCD instrument, a measurement of a 0.9 M solution of (+)-*R*-camphor in carbon tetrachloride measured in a $100\text{-}\mu\text{m}$ path-length cell should be approximately 2×10^{-4} . After baseline subtraction, described below and in (1782), this difference should be a positive peak ΔA value of $+1 \times 10^{-4}$ absorbance units at 1240 cm^{-1} and a negative ΔA peak value of -1×10^{-4} at 1040 cm^{-1} .

EMPTY BEAM BASELINE CHARACTERISTICS

To avoid significant levels of baseline deviations from the absorbance features of a sample, the empty beam baseline for a VCD instrument should be as close as possible to the true zero of the measurement. A minimum standard for FT-VCD spectrometers is to have empty beam baselines sufficiently close to zero so that deviations from true zero are $<25\%$ of the span of maximum positive-to-negative absorbance intensity of a 0.9 M solution of (+)-*R*-camphor in carbon tetrachloride, measured in a $100\text{-}\mu\text{m}$ path-length cell. This magnitude, noted above, is approximately 2×10^{-4} as determined from a positive peak value of $+1 \times 10^{-4}$ at 1240 cm^{-1} and a negative peak value of -1×10^{-4} at 1040 cm^{-1} . The value of 25% of this intensity range is approximately 5×10^{-5} . VCD empty beam baseline deviations (maximum to minimum), over the mid-IR range from 900 to 1800 cm^{-1} , should not exceed $\pm 5 \times 10^{-4}$ and should not deviate more than $+5 \times 10^{-4}$ or -5×10^{-4} from the zero of VCD intensity over this wavenumber range.

SAMPLE BASELINES AND ABSORPTION ARTIFACTS

An important test for a VCD measurement is the VCD spectrum of a racemic sample relative to that of the baseline of the empty instrument or the VCD spectrum of a nonchiral solvent, such as carbon tetrachloride. Because of possible baseline changes due to the sample cell, it is preferable to compare the VCD of the solvent with that of a solution of the racemic sample of the chiral molecule in the same solvent. Both should have zero VCD, but the racemic solution may have baseline artifacts (offsets) at the location of the absorption bands. Absorption bands change the index of refraction of the sample and can cause baseline deviations that are particular to the sample molecule. If such absorption artifacts are present, the only way to recover the artifact-free VCD spectrum is by subtraction of the racemic mixture (equal quantities of both enantiomers) from that of the measured VCD spectrum. Alternatively, subtraction of the VCD spectrum of the opposite enantiomer is divided by 2, because the VCD intensity doubles if the VCD spectrum of one enantiomer is subtracted from that of the other. The qualification standard used for measuring the VCD baseline with an absorbing sample present is racemic camphor in carbon tetrachloride solution versus the VCD spectrum of neat carbon tetrachloride in the range $900\text{--}1800\text{ cm}^{-1}$, where there are only absorption bands from camphor and none for carbon tetrachloride. Both samples should have zero VCD and a baseline that is featureless with respect to the camphor absorption bands.

SIGNAL-TO-NOISE RATIO

The signal-to-noise ratio of a VCD measurement can be determined from the noise curve generated by subtracting one-half of the FT scans of a measurement from the other half, thus cancelling the signal and exposing the noise level. The noise level varies across the spectrum and increases when the light level reaching the detector decreases, either due to the overall throughput of the instrument in a particular spectral region or directly within the absorption bands of the sample. The level of noise in the noise curve can be reduced by increasing the measurement time of the spectrum. The noise level is inversely proportional to the square root of the measurement time. This is illustrated with a particular example in *Figures 9 and 10* of (1782).

Smoothing a VCD spectrum without increasing the measurement time will reduce the size of the peak-to-peak noise excursions but will not reduce the overall accuracy of a VCD measurement, because the VCD baseline, and hence the VCD intensity relative to the zero baseline, will undergo the same level of variations, although the VCD spectrum appears to have less noise after smoothing. To know the true level of the noise and hence the uncertainty level of a VCD spectrum, all smoothing functions, if any, should be turned off. Noise can legitimately be reduced by lowering the spectral resolution. In evaluating the noise level of the VCD measurement, the resolution and interferogram apodization must be specified.

2.3 Performance Qualification

PQ determines if the instrument is capable of meeting the user's requirements for all the parameters that may affect the quality of the measurement. Depending on typical use, the specifications for PQ may be different from the manufacturer's OQ specifications; however, for general use across a range of typical chiral molecules and sampling conditions, the specifications for OQ will be the same as those needed for PQ. For validated methods, specific PQ tests, also known as system suitability tests, can be used in lieu of PQ requirements.

Specific procedures, acceptance criteria, and time intervals for characterizing performance depend on the instrument and intended application. Demonstrating stable instrument performance over extended periods of time provides some assurance that reliable measurements can be taken from test sample spectra.

Particular long-term stability tests that can be carried out for PQ evaluation beyond those carried out for IQ or OQ are as follows. It is recommended that a 60-min measurement of a 0.9 M solution of (+)-*R*-camphor in carbon tetrachloride, measured in a 100- μ m path-length cell, be performed between different applications of the VCD spectrometer to ensure that the signs, intensities, and signal-to-noise ratio of the VCD spectrum established as a qualification test during OQ are maintained. This is particularly important if there has been an interruption in power or a long hiatus between measurements.

If averaging over long periods of time, such as overnight, it is important that the stability of both the VCD baseline and the sample VCD spectrum are stable over time and that the signal-to-noise ratio improves as expected with the square-root of measurement time or number of interferometer scans, as illustrated by the example in (1782). In general, stability should be maintained to within the noise level of the VCD measurement. Because VCD intensities are generally three to four orders of magnitude smaller than the parent IR intensity, the instrument stability for the FT-IR performance of the instrument is typically larger over time relative to the noise level of the VCD spectrum.

3. PROCEDURE

The measurement of the VCD spectrum of a sample follows the same general rules as the measurement of an IR spectrum as for mid-IR spectroscopy described in (854).

3.1 Sample

A VCD spectrum can be measured in all types of standard IR transmission cells, either for neat liquids or solutions. For solids, such as in potassium bromide (KBr) pellets, mulls, or thin films, it is recommended that additional steps be taken to ensure that artifacts arising from sample linear birefringence are not present as described in (1782) for advanced instrumentation methods, such as dual PEM modulation or rotating sample cells.

Because of the importance of noise control in a VCD measurement, the absorbance of a sample should not exceed $A = 1.0$ in the region of measurement interest. For absorbance >1.0 , insufficient radiation is present at the detector, and noise dominates the VCD spectrum. The optimum level of absorbance relative to the sample reference (empty beam or solvent) is approximately 0.4. One should adjust the path length and/or concentration of the sample such that the average absorbance is approximately 0.4–0.5 over the region of interest, with limits of 0.1–1.0. For samples with a wide range of absorbance, more than one measurement can be carried out with differing average absorbance values to ensure that a VCD spectrum of adequate quality is obtained over the entire range of interest.

To perform a solution-state VCD measurement, first find a solvent that dissolves an adequate amount of solute to achieve a desired absorbance level. Solvents without CH bonds are the most desirable for this purpose; these include carbon tetrachloride, deuterated chloroform (CDCl_3), or deuterated dimethyl sulfoxide ($\text{DMSO}-d_6$). The IR cell should have windows that are transparent in the mid-IR region, with generally low levels of linear birefringence. Commonly used materials are barium fluoride (BaF_2), which has good transmission to 800 cm^{-1} , and calcium fluoride (CaF_2), which is used if coverage to only $1100\text{--}1200\text{ cm}^{-1}$ is needed, such as for aqueous solutions.

3.2 Standard

To ensure that the analyst can use an IR cell for a transmission measurement of a solution, it is recommended that a standard OQ measurement of a 0.9 M solution of (+)-*R*-camphor in carbon tetrachloride using a 100- μ m barium fluoride cell be performed. For different choices of concentration or path length, one can use the linear dependence of these quantities on the measured ΔA to ensure that an accurate measurement with the correct signs and intensities has been achieved.

3.3 Analysis

VCD spectra, once measured, can be analyzed in a variety of ways, depending on the intended application. The principal application of VCD in the pharmaceutical industry is the determination of absolute configuration (AC) in conjunction with a VCD calculation using a quantum chemistry computation software package. A second application is the determination of EE of a sample, either for the purpose of determining the EE of a sample or for monitoring the EE of one or more species during the course of the chemical reaction involving chiral species. Achievement of the highest level of accuracy in the determination of EE requires the use of a chemometric software package that uses the entire range of the VCD spectrum, as opposed to a single band or wavenumber frequency. Other applications of VCD are its use with a standard database of VCD and IR spectra to simultaneously ensure the correct identity, AC, and EE of a chiral substance, for example as a chiral raw material identification. VCD can also be used to specify the AC and EE of a drug product in the presence of either achiral or chiral excipients, such as carbohydrates. These applications are described in more detail in (1782).

4. VALIDATION AND VERIFICATION

4.1 Validation

The objective of VCD procedure validation, as with the validation of any analytical process, is to demonstrate that the measurement is suitable for its intended purpose. The application of VCD spectroscopy is somewhat different from that of conventional vibrational spectroscopic techniques because it is not a primary technique. Rather, it is the polarization difference spectroscopy of the parent IR spectroscopy that has been used by itself for decades as a primary identification and quantitation technique as described for mid-IR spectroscopy in (854). The differential VCD spectrum supplements the parent IR spectrum

with a new set of intensities at each position of an IR absorption band. The new set of intensities then reveals stereospecific properties of the sample molecules that must be chiral to have a non-zero VCD spectrum. There are two primary properties of a sample of a chiral molecule: one is the AC of the dominant chiral species (dominant enantiomer), and the second property is the EE of the dominant enantiomer over its lesser (mirror-image) enantiomer. In addition, the VCD spectrum carries additional information about the solution-state conformation, or conformational distribution, of the molecule that may be available in only lesser detail, if any, using the parent IR spectrum alone. Applications that require validation for the use of VCD spectroscopy also exist for the combined use of the measurement of the VCD of a substance for which there exists a reference spectrum with known AC and EE. Any subsequent measurement of the VCD spectrum, together with the simultaneous measurement of its parent IR spectrum, confirms in one measurement the molecular identity of the sample as well as the AC and EE. Such applications are useful, for example, for chiral raw-material identification of AC and EE, for which there is currently no established methodology for a single measurement. VCD can also confirm, in a single measurement, the AC and EE of a final drug product, including the chirality (if any) of the excipients.

Before validation of VCD for AC determination, an overall instrument validation should be carried out by the measurement of the VCD spectrum of neat (–)-*S*- α -pinene or (+)-*R*- α -pinene using a 75- μ m cell. The calibrated, baseline-corrected VCD spectrum should yield a positive peak maximum of ΔA equal to approximately $+5 \times 10^{-4}$ absorbance units at 1220 cm^{-1} and a negative peak value of approximately -4×10^{-4} at 1130 cm^{-1} . The noise level should be comparable to that established for the instrument during the OQ phase of instrumental qualification described above. Because the VCD spectrum of α -pinene is relatively large, sufficient signal-to-noise ratio is available with a 20-min collection to verify that the instrument is operating with correct VCD intensities, signs, and noise levels for the applications of VCD described below.

Validation for AC determination by VCD can be achieved by the successful comparison of the measured VCD spectrum of the validation standard, neat (–)-*S*- α -pinene, to its quantum-chemistry-calculated VCD spectrum. Success is achieved by measuring the baseline-corrected VCD spectrum with sufficient signal-to-noise ratio that the signs, frequencies, and relative intensities of the major VCD bands can be compared clearly to the corresponding VCD signs, frequencies, and relative intensities of the calculated VCD spectrum. If the signs, frequencies, and relative intensities agree, then the AC of the measured sample of (–)- α -pinene is the same as that for the model of *S*- α -pinene that has been constructed for the calculation of this standard molecule. If the frequencies and relative VCD intensities are opposite after instrument sign calibration, then the AC of the sample is opposite (the mirror image) of the enantiomer used for the calculation. A successful comparison requires pretreatment of the VCD data for baseline subtraction and intensity calibration. It also requires a computational procedure that is known to be accurate in calculating VCD spectra. Finally, a program should be used to impartially assess the degree of agreement between measured and calculated VCD spectra such that one relies on more than an analyst's visual assessment of the agreement, or lack of agreement, between a measured spectrum and a calculated spectrum. An example of this procedure can be found in (1782) as well as in the references cited in (1782).

Validation of VCD spectroscopy for the determination of EE is achieved by plotting the EE predicted by VCD versus the EE for a sample of neat (–)-*S*- α -pinene for a series of samples with differing values of %EE over the range from 100% EE to 0% EE. The EE predicted by VCD involves measurement of the VCD spectra at a chosen spectral resolution and time of collection to achieve a desired level of signal-to-noise ratio, followed by pretreatment of the measured VCD spectra for baseline subtraction. The prediction of EE could be based on a size of a single VCD band for the samples with differing VCD, normalized for constant IR intensity; but more generally and with better accuracy, the entire range of VCD bands across the spectrum is used for each spectrum by means of a chemometrics software package. Validation is accompanied by a measure of the %EE accuracy through a cross-correlation analysis, yielding a root mean square (RMS). An example of such a determination of %EE by VCD for neat (–)-*S*- α -pinene is provided, along with literature references, in (1782).

More generally, validation is required when a method is intended for use as an alternative to the official procedure for testing an official article. The objective is to demonstrate that the measurement of VCD is suitable for its intended purpose; for examples of various types of measurements, see *Validation of Compendial Procedures* (1225). For %EE determination, creation of a linear plot of actual versus VCD-predicted values of %EE establishes that the magnitude of IR-normalized VCD spectra, as evaluated with chemometrics, exhibits sufficient linearity, range, accuracy, specificity, precision, detection limit, quantitation limit, and robustness with respect to the detection of %EE.

Chapter (1225) provides definitions and general guidance on analytical procedures validation without indicating specific validation criteria for each characteristic. The intention of the following sections is to provide the user with specific validation criteria that represent the minimum expectations for VCD technology. For each particular application, tighter criteria may be needed to demonstrate suitability for the intended use.

ACCURACY

Accuracy can be determined by the measurement of a VCD spectrum of the established validation standard of neat (–)- α -pinene or (+)- α -pinene, as presented, for example, in (1782), and verifying that the measured signs and intensities of the VCD spectrum agree with the standard VCD spectrum to within the noise level of the measurement. In particular, the VCD spectrum should yield a positive peak maximum of ΔA equal to approximately $+5 \times 10^{-4}$ absorbance units at 1220 cm^{-1} and a negative peak value of approximately -4×10^{-4} at 1130 cm^{-1} . This validation ensures that the VCD spectrometer is accurately calibrated and has the correct signs for the VCD bands.

4.2 Precision

REPEATABILITY

Validation criteria: Measurement of VCD spectra of the validation standard sample, neat (+)- α -pinene or (–)- α -pinene, in 20-min blocks with variations in the VCD spectra at or less than the noise level of each 20-min block that is approximately ΔA equal to or less than 1×10^{-5}

INTERMEDIATE PRECISION

The effect of random events on the analytical precision of the validation criteria of VCD measurement should be established. Typical variables include performing the analysis on different days, using different instrumentation, or having the method performed by two or more analysts. As a minimum, any combination of at least two of these factors totaling six experiments will provide an estimation of intermediate precision. (For example, this could be two analysts on each of 3 days, or two analysts on two sets of equipment on 2 days for each analyst–equipment combination.)

Validation criteria: The *Validation criteria* are the same as those for *Repeatability*.

SPECIFICITY

Specificity is not a required metric for the determination of AC. For the measurement of EE for more than one chiral species in a single set of measurements, the analytical procedure, including chemometric analysis, must be able to unequivocally assess the EE of chiral species in the presence of components that may be expected to be present, i.e., solvent and other nonchiral species.

Validation criteria: The *Validation criteria* are demonstrated by meeting the *Accuracy* requirement.

QUANTITATION LIMIT

For the determination of AC, there is no quantitation limit (QL). The QL for EE can be determined by carrying out a determination of quantitation of %EE for the VCD validation standard of neat α -pinene for a series of EE values, performing a chemometric analysis of VCD-predicted EE values and performing a determination of RMS error, as illustrated, for example, in (1782).

Validation criteria: The analytical procedure should be capable of determining the analyte precisely and accurately at a level equivalent to approximately 1% EE for neat α -pinene.

LINEARITY

A linear relationship between the analyte concentration and response should be demonstrated by preparing NLT 5 standard solutions at concentrations encompassing the anticipated concentration of the test solution. The standard curve should then be evaluated using appropriate statistical methods, such as a least-squares regression, for the area of peak height of a selected VCD band, or for the entire spectrum, using chemometric analysis such as partial least squares. For experiments that do not yield a linear relationship between analyte concentration and response, appropriate statistical methods must be applied to describe the analytical response.

Validation criteria: The *Validation criteria* find the correlation coefficient above 0.95 for a linear plot of actual EE versus VCD-predicted EE.

ROBUSTNESS

The reliability of an analytical measurement should be demonstrated by deliberate changes to experimental parameters, such as changes in spectral resolution, concentration, or path length for measurement of VCD.

4.3 Verification

U.S. Current Good Manufacturing Practices regulations [21 CFR 211.194(a)(2)] indicate that users of the analytical procedures, as described in *USP–NF*, are not required to validate these procedures if provided in a monograph. Instead, they must simply verify their suitability under actual conditions of use.

The objective of procedure verification is to demonstrate that the procedure, as prescribed in a specific monograph, can be executed by the analysts with suitable accuracy, specificity, and precision using the instruments, and sample solutions of chiral molecules available. If the verification of the compendial procedure by following the monograph is not successful, the procedure may not be suitable for use with the article under test (for additional information, see *Verification of Compendial Procedures* (1226)). It may be necessary to develop and validate an alternative procedure as allowed in *General Notices*, 6.30 *Alternative and Harmonized Methods and Procedures*.

Verification of compendial methods should, at a minimum, include the execution of the validation parameters for specificity, accuracy, precision, and limit of quantitation, when appropriate, as indicated in 4.1 *Validation*.