

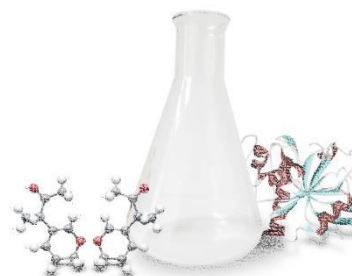
Absolute Configuration by VCD:

Example of (S)-Binaphthol

Introduction

Determination of absolute configuration (AC) of chiral molecules is an important step in any field related to chirality but nowhere is it as critical as in the pharmaceutical industry. The phenomenon of “chiral recognition” – in which the enantiomers of a chiral drug may exhibit differences in biological activity or other processes such as distribution, uptake, and metabolism—makes it a necessity (or requirement) to know the AC not only of the final molecule but as early in the process of development as possible.

Within the past few years it has been conclusively demonstrated that vibrational circular dichroism (VCD) is a reliable method for AC determinations. VCD offers a novel alternative to X-ray crystallography, permitting AC determinations on neat liquids (including oils) and solution-phase samples. VCD requires no derivatization of the sample or growth of a pure single crystal. VCD is defined as the differential absorption of a molecules for left versus right circularly polarized infrared (IR) light during a vibrational transition. VCD combines the structural specificity of vibrational IR absorption spectroscopy with the stereo-chemical sensitivity of a chiroptical spectroscopy such as CD (more recently termed electronic CD or ECD for transitions in visible or UV regions). The absolute stereochemistry is established by comparing the measured VCD spectrum to the results of an *ab initio* quantum chemistry VCD calculation of the same molecule. The calculations are easily carried out in commercial packages such as Gaussian (Gaussian, Inc., Pittsburgh, Pennsylvania).



New USP Chapters <782> & <1782> On Vibrational Circular Dichroism Became Official December 2016

United States Pharmacopeial Convention (USP) and The National Formulary (USP–NF) contain standards for medicines, dosage forms, drug substances, excipients, medical devices, and dietary supplements. Their purpose is to provide leading methods and practices for manufacturers, regulators, researchers and laboratories who are developing, manufacturing, testing, and releasing drug substances and products.¹



In 2013 the United States Pharmacopeial Convention (USP) published a *stimuli* article that discussed VCD as a basis for a General Chapter in the US Pharmacopeial for use in characterization of chiral pharmaceuticals. The article gave insights into how VCD can be used for determination of absolute configuration of chiral pharmaceutical ingredients, and for extending its use to purity analysis, chiral raw material identification and chiral quality control. The stimuli article further demonstrated the usefulness of the VCD technique and how it can address the FDA requirements on proof of AC of the dominant enantiomer and the enantiomeric excess (EE) to a specified level of purity.

On June 1, 2016 USP released a second supplement to USP 39–NF 34 that became official on December 1, 2016. The supplement contains two chapters <782> and <1782> on Vibrational Circular Dichroism (VCD). The chapter <782> spells out in detail various aspects of VCD usage such as qualification of VCD spectrometers, sample measurements, validation and verification of measured spectra. Chapter <1782> further explains with specific examples the instrumentation used, finer aspects on the qualitative and quantitative analysis, comparison between measured and calculated spectra, determination of enantiomeric excess, and concurrent use of VCD for absolute configuration and %EE.

The chapter <1782> also illustrates the benefits of the VCD technique in addressing the real-time chiral measurement for material identification (ID). One of advantages of VCD is “single measurement” of two spectra - VCD spectrum and its associated IR spectrum in the mid-IR or near-IR region. This is in contrast to traditional measurements involving separate IR and optical rotation measurements. The chapter goes on to explain how VCD can be used as a chiral measure for characterization of raw materials for process analytical technology (PAT) during development, synthesis, formulation and final drug product production. The potential of VCD to monitor %EE as a quality control in the pharmaceutical industry is also explained in this chapter.

BioTools' VCD Spectrometer

Vibrational Circular Dichroism (VCD) was first measured in 1974. Twenty-three years later, BioTools commercialized the technology by introducing the first stand-alone VCD spectrometer, the first generation ChiralIR™. It soon became the preferred choice for VCD instrumentation in the pharmaceutical industry, regulatory agencies such as the FDA, and academia. The second generation, the ChiralIR-2X™, introduced patented dual source, all digital signal processing eliminating bulky electronics, 24-48 hour hold time liquid-nitrogen MCT detector, and an upgrade to the revolutionary DualPEM™ (illustrated in Fig. 1) set-up having plug-and-play capability upon delivery and installation.

Figure 1 illustrates a schematic overview of a VCD spectrometer. After preliminary optics, infrared light travels through a photoelastic modulator (PEM1) to produce left and right circularly polarized light. The light then passes through the sample (neat liquid or solution) and an optional second PEM2 making its way to the detector. The VCD signal is extracted via virtual numerical lock-in amplification referenced to the two separate PEM modulator frequencies. Both IR spectra and VCD spectra are measured simultaneously.

The ChiralIR-2X™ spectrometer shown below uses a small, compact benchtop space.

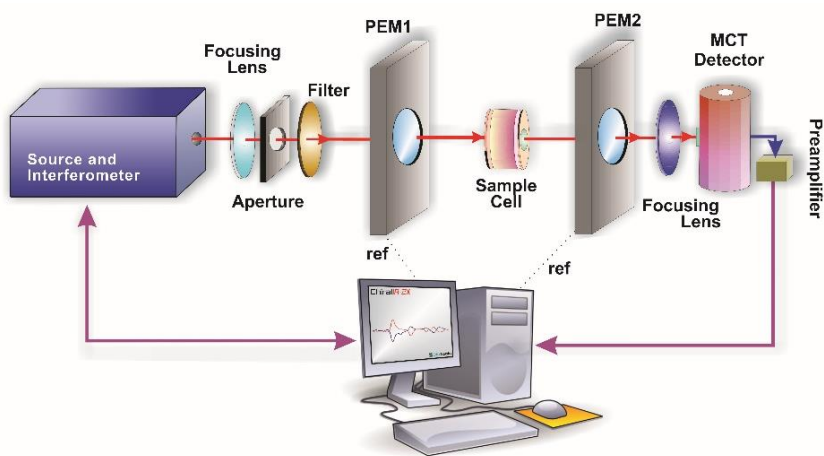


Figure 1: Schematic overview of VCD spectrometer



ChiralIR-2X™ VCD Spectrometer

Sample VCD Spectra

Figure 2 shows the VCD and IR spectra of (+) camphor (red traces) and (-) camphor (blue traces).

The IR spectra overlap completely and are indistinguishable since IR is blind to chirality, whereas the VCD spectra, which display both positive and negative peaks, are equal in intensity but opposite in sign about zero. The VCD intensities are roughly 4 orders of magnitude (10,000 times) smaller than the corresponding IR intensities. Every chiral molecule exhibits VCD spectra. There is no need for a chromophore as required in electronic CD.

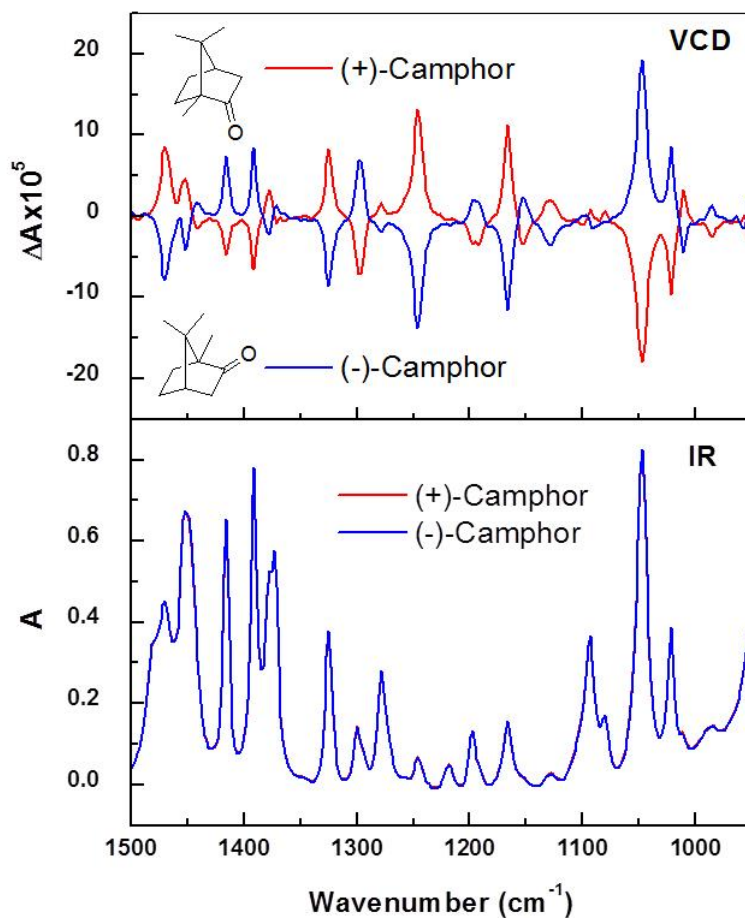


Figure 2: IR (bottom) and VCD (top) spectra of camphor.

Determination of Absolute Configuration

Figures 3 and 4 shows an illustration of the determination of absolute configuration of (-) -binaphthol. The steps involved in determination are as follows:

1. The experimental VCD spectrum is measured using any of the conventional solvents in IR spectroscopy such as CDCl_3 , CCl_4 etc. Typically 5-15 mg of the sample is used, which is recoverable.
2. The VCD of one of the enantiomers is calculated using *ab initio* calculations using ComputeVOA™ / Gaussian09™ or a number of other software packages that are commercially available. The VCD spectrum of the other enantiomer is then obtained by reversing the signs of all the bands or calculating the VCD of the mirror-image structure.
3. The last step is a comparison of the experimental spectrum to the two calculated spectra (Figure 4) to determine the enantiomer that gives the best correlation between the signs and the signal intensities. The confidence level of overlap between two such spectra can be calculated using CompareVOA™ software. In the example in Figure 3, the observed VCD of the (-)-enantiomer matches the calculated spectrum for (S). Thus, the final assignment is (S)-(-)-Binaphthol. The confidence level of assignment is 99% (Figure 5).

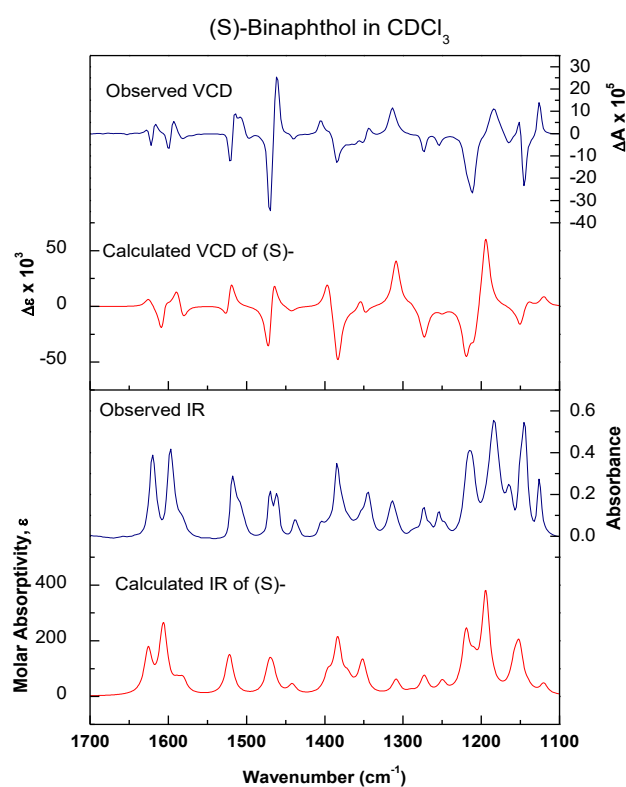
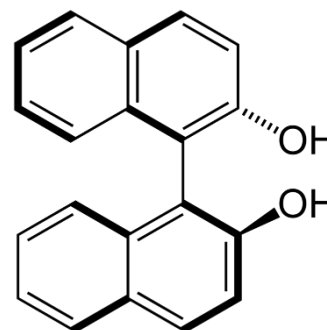


Figure 3: Example of determination of absolute configuration of Binaphthol

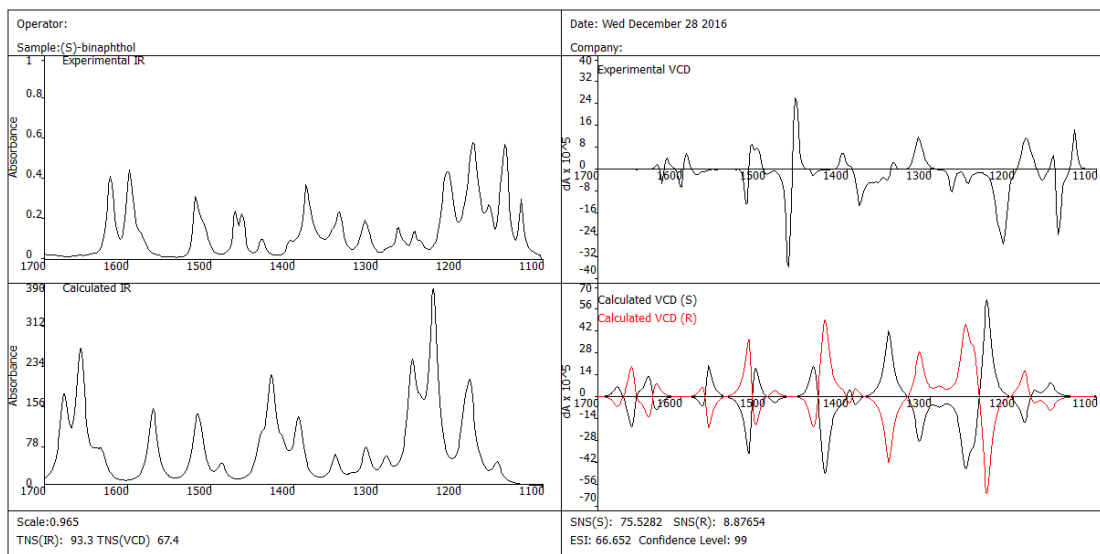


Figure 4:
Comparison of experimental IR (left, top) with calculated IR (left, bottom) and VCD experimental (right, top) with calculated spectra of both enantiomers (right, bottom)

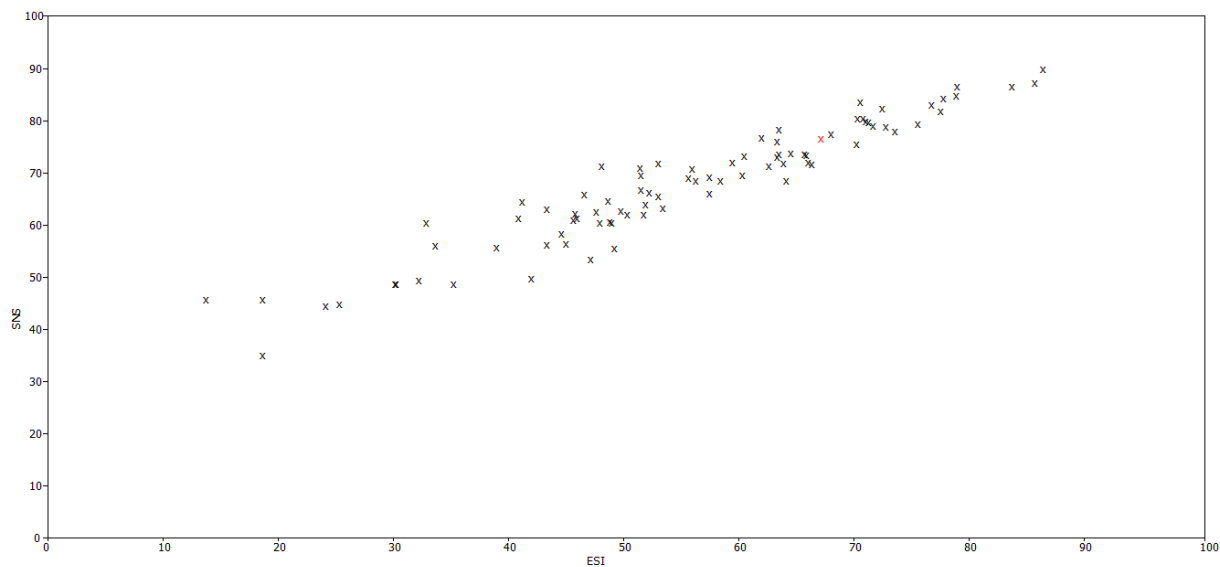


Figure 5:
Statistical plot for the current assignment (red dot) compared to other successful assignments giving confidence level of 99%

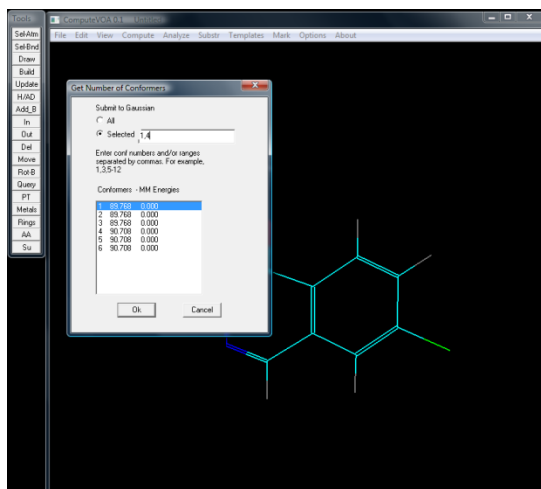
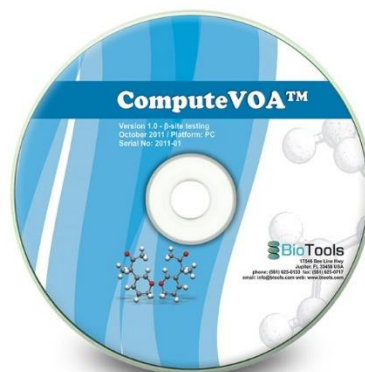
BioTools' VCD Software

ComputeVOA™

An all-in-one software package for the calculation of VCD and ROA spectra.

Combines the following:

- structure building
- extensive conformational search
- easy integration with Gaussian09
- plotting of calculated spectra.



Software screenshot
of ComputeVOA™

ComputeVOA™ is an interactive molecular modeling program designed to automate the process of computing Vibrational Optical Activity. It is designed to be easy to use and has the ability to draw a chiral structure in 3D or import the structure from another structure drawing program such as ChemDraw. The input structure can be optimized using the MMFF94 force field and if the molecule is flexible a conformational search to generate a set of low energy conformers can be carried out. The optimized structure(s) can then be submitted to Gaussian for *ab initio* quantum chemistry calculations of the IR and VCD spectra and the results brought back to ComputeVOA™ for display of the spectra and output of data files that can be used as input for CompareVOA™ for comparison with experimental IR and VCD spectra. The purpose of ComputeVOA™ is to automate the process from structure input to calculated IR and VCD spectra.

Additional Information

References:

1. United States Pharmacopeia Convention (<http://www.usp.org/usp-nf>)

VCD Resources and Products:

- **About VCD**
http://www.btools.com/assets/vcd_information_biotoools.pdf
- **Chiral/IR-2X™ VCD Spectrometer**
<http://www.btools.com/products.html#ir-2x>
- **Compute VOA™ and Compare VOA™**
<http://www.btools.com/products.html#software>
- **Gaussian™ Software**
<http://www.gaussian.com/>

If you have any questions or would like to discuss how VCD can supplement your research, please contact info@btools.com or call 561-625-0133 to speak with one of our scientists.

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