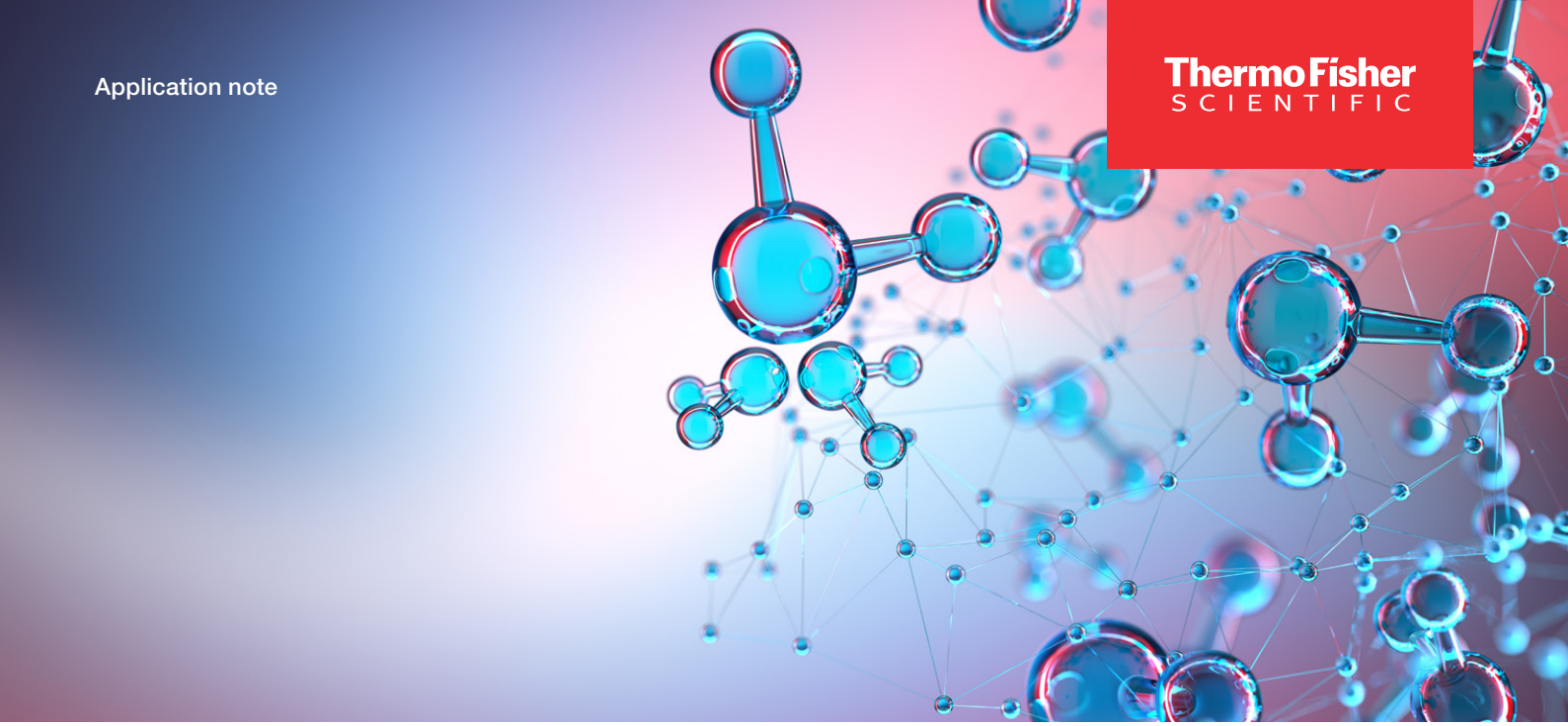
Application note



Mixed vs. Pure: How FTIR can assess enantiomers of amino acid samples

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Thermo Scientific Nicolet Summit X FTIR Spectrometer.

Abstract

Fourier-transform infrared (FTIR) spectroscopy is a valuable tool to rapidly screen analytes for conformity and classification. In this study, a Thermo Scientific™ Nicolet™ Summit™ X FTIR Spectrometer, equipped with an attenuated total reflection (ATR) accessory, was utilized in conjunction with a discriminant analysis classification method to quickly differentiate between enantiopure and racemic amino acids.

Introduction

In the pharmaceutical industry, stereochemistry of a therapeutic agent plays an important role in pharmacokinetic and pharmacodynamic profiles[1–3]. While one enantiomer of a drug compound can produce therapeutic effects, its stereoisomer can yield a reduced or even adverse effect. One well-cited example is Thalidomide, a drug to treat morning sickness that was initially brought to market as a racemate but whose *(S)*-enantiomer was later found to be teratogenic[4,5]. Understanding stereochemistry is therefore of critical importance: pharmaceutical compounds must be delivered in the proper manner, either isolated in specific enantiomeric forms or presented as racemic mixtures, to achieve desired therapeutic effects.

Fourier-transform infrared spectroscopy is a rapid, non-destructive, environmentally friendly, and cost-effective analytical technique. It is widely used in the pharmaceutical industry to verify the final product in a manufacturing process. FTIR provides information about the chemical structure of a sample, with unique structures providing distinct, characteristic spectra. Since stereoisomers, by definition, have the same chemical structure but differ only in spatial arrangements of atoms, FTIR can not distinguish different forms of enantiomers. However, there are reports[6,7] that vibrational spectroscopy, including FTIR and Raman, can distinguish the racemic and enantiomeric forms of chemical compounds due to their differences in crystal symmetry, geometric parameter distinctions, and hydrogen bonding. Herein, we describe a classification method based on FTIR to rapidly differentiate racemic and enantiopure forms of two amino acids.

Experiment

Neat reference samples of the D-, L-, and DL- forms of valine and phenylalanine powders were acquired from Sigma-Aldrich (MO, USA). Their chemical structures are shown in Figure 1. Commercial products labeled as L- and DL-phenylalanine were purchased from online and local retailers. All samples were used as received.

The Nicolet Summit X FTIR Spectrometer equipped with a deuterated-triglycine sulfate (DTGS) detector was used with the Thermo Scientific ™ Everest™ ATR accessory and a diamond ATR crystal, operated with the Thermo Scientific™ OMNIC™ Paradigm Software. Samples were scanned at a spectral resolution of 4 cm^{-1}, with 64 scans co-added for each spectrum. The powder samples were placed directly onto the diamond ATR crystal and pressure was applied using the slip-clutch pressure tower from the Everest ATR accessory. When assessing capsule samples, the capsules were cut, and the powder inside was deposited on the ATR crystal for analysis. A classification model was built using Thermo Scientific™ TQ Analyst™ Software.

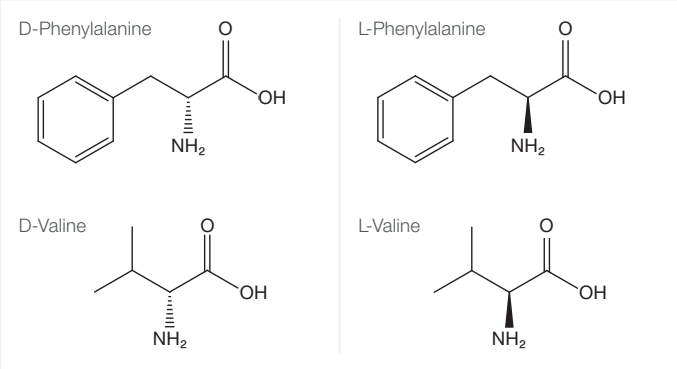

Figure 1. Stereoisomers of Phenylalanine and Valine.

Results and Discussion

Figure 2 shows the FTIR spectra of DL-, D-, and L-valine. It is worthwhile to mention that while 10-16 scans might suffice in many routine FTIR measurements, 64 scans were used in the current study to improve the signal-to-noise ratio (S/N) and thereby facilitate differentiation. As expected, the spectra for the D- and L-valine enantiomers are identical because of their identical chemical structure. However, when comparing the spectra of the enantiopure (D- and L-) and racemic (DL-) forms, clearly identifiable spectral differences are noted (highlighted in red).

The spectral differences between enantiopure and racemic amino acids are further demonstrated using phenylalanine, as shown in Figure 3. Figure 3A shows the comparison of the enantiopure and racemic mixture in the spectral range of 4000-650 cm^{-1}, with differences highlighted in red. Figures 3B and 3C show the zoomed-in spectral regions where spectral features at 1583, 1555, 1035, 1025, and 1003 cm^{-1} are noticeably different between enantiopure and racemic forms.

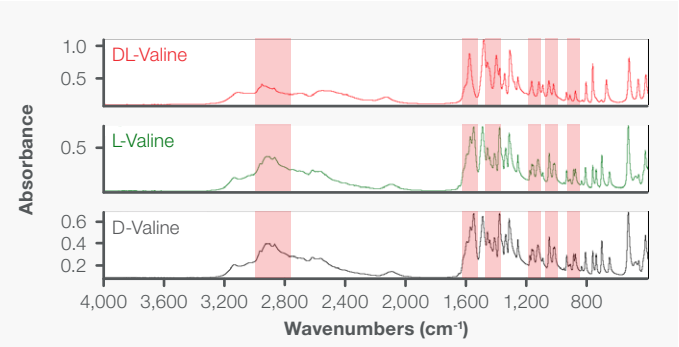

Figure 2. FTIR spectra of DL- (top), L- (middle) and D-valine (bottom).

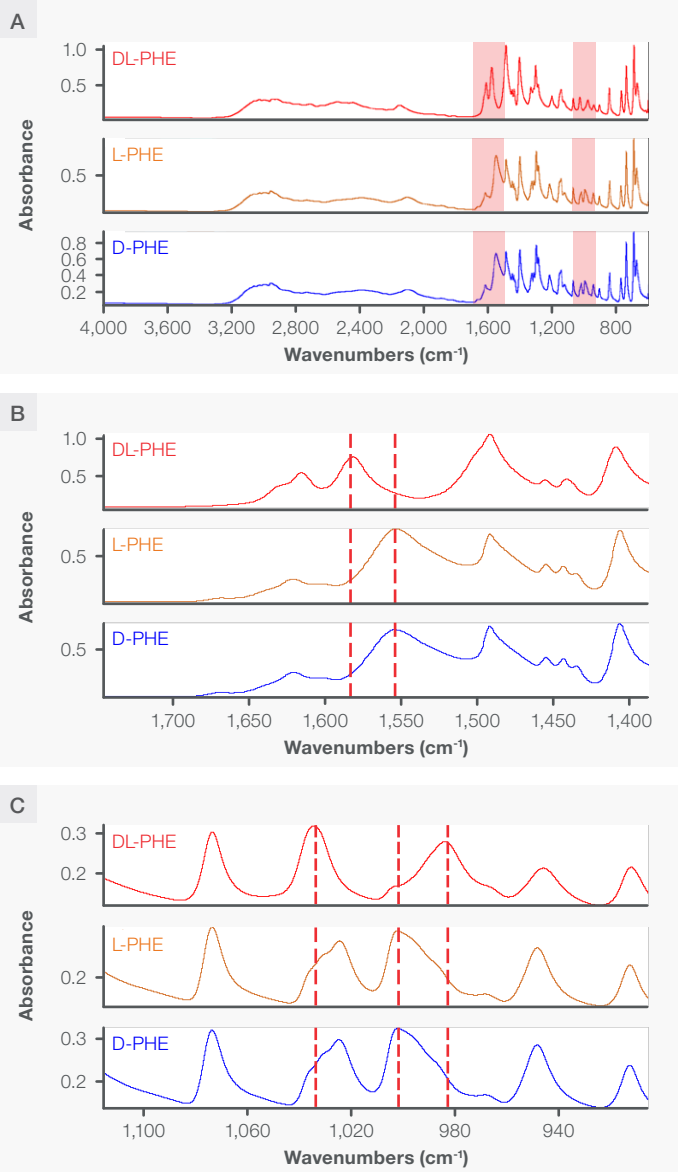

Figure 3. (A) FTIR spectra of DL- (top), L- (middle) and D-phenylalanine (bottom); (B) FTIR spectra between 1700-1500 cm^{-1}; and (C) FTIR spectra between 1200-900 cm^{-1}.

The discriminant analysis (DA) classification technique is often used to screen incoming materials to determine which known material each sample most resembles. A DA classification method was developed using the TQ Analyst software to classify the racemic DL-phenylalanine and enantiopure L-phenylalanine. Ten samples were analyzed from the neat powders of both groups; subsequently, 7 spectra from each group were utilized as calibration, and the remaining 3 were used as validation of the model. The entire spectral range was utilized for the classification model. The developed model was then applied to evaluate the commercial products that are labeled as DL-phenylalanine or L-phenylalanine. Two forms of each class were acquired, with one set being in the powder form and the other in the capsule form. The classification results are summarized in Table 1. Both powder samples were successfully classified into their respective classes. For the capsule samples, however, the two samples were correctly classified into their respective chemical classes, but with a classification warning. The results indicate that, using the DL-phenylalanine capsule as an example, the spectrum of the capsule sample is more similar to the DL-phenylalanine reference spectra than the L-phenylalanine reference spectra used in the model, but there are substantial spectral differences that warrants a warning and further investigation. As can be seen in Figure 4, both capsule samples have additional absorption bands at 2916 and 2849 cm^{-1}, likely arising from the additional ingredients in the product such as vegetable stearates.

Product Label	Best Class	Value	Pass/Fail
DL-phenylalanine powder	DL-phenylalanine	0.25	PASS
L-phenylalanine powder	L-phenylalanine	0.54	PASS
DL-phenylalanine capsule	DL-phenylalanine	1.47	FAIL
L-phenylalanine capsule	L-phenylalanine	1.46	FAIL

Table 1. Discriminant analysis classification of commercial phenylalanine products.

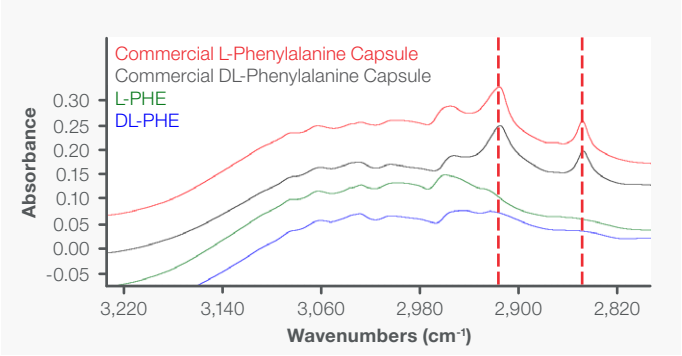

Figure 4. FTIR spectra of phenylalanine capsule samples and neat phenylalanine.

Conclusion

In this note, we have demonstrated that FTIR spectroscopy can be used as a rapid screening, classification, and identification technique to differentiate racemic and enantiopure compounds. The short analysis time, minimal sample preparation, and non-destructive nature make FTIR an attractive option for rapid identification and classification of analytes.

References

1. Mr, I.; Jg, M.; Id, B. Pharmacological Importance of Stereochemical Resolution of Enantiomeric Drugs. *Drug Saf.* **1997**, *17* (3). https://doi.org/10.2165/00002018-199717030-00002.
2. Hutt, A. J.; Tan, S. C. Drug Chirality and Its Clinical Significance. *Drugs* **1996**, *52* (5), 1–12. https://doi.org/10.2165/00003495-199600525-00003.
3. Alkadi, H.; Jbeily, R. Role of Chirality in Drugs: An Overview. *Infect. Disord. Drug Targets* **2018**, *18* (2), 88–95. https://doi.org/10.2174/1871526517666170329123845.
4. Lenz, W. A Short History of Thalidomide Embryopathy. *Teratology* **1988**, *38* (3), 203–215. https://doi.org/10.1002/tera.1420380303.
5. Tokunaga, E.; Yamamoto, T.; Ito, E.; Shibata, N. Understanding the Thalidomide Chirality in Biological Processes by the Self-Disproportionation of Enantiomers. *Sci. Rep.* **2018**, *8* (1), 17131. https://doi.org/10.1038/s41598-018-35457-6.
6. Barańska, H.; Kuduk-Jaworska, J.; Szostak, R.; Romaniewska, A. Vibrational Spectra of Racemic and Enantiomeric Malic Acids. *J. Raman Spectrosc.* **2003**, *34* (1), 68–76. https://doi.org/10.1002/jrs.953.
7. Gosar, A.; Shaikh, T.; Sindwani, A. Fourier Transform Infrared (FTIR) Spectroscopic Analysis of Amino Acid Serine for Its Chiral Identification. *J. Pharm. Qual. Assur. Qual. Control* **2022**, 1–4.