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NUMERICAL SPECTROSCOPY: ABSORBANCE INDEX TECHNIQUE AND ALGORITHM FOR QUALITATIVE ANALYSIS IN LIQUID CHROMATOGRAPHY

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SUMMARY

This work was undertaken as part of an effort to provide efficient numerical techniques for utilizing spectroscopic data in high-performance liquid chromatography. Such techniques are necessary because retention time is not sufficient confirmation of peak identity, nor is symmetry proof of peak purity. Several numerical techniques for comparing spectral data exist which range from simple absorbance ratios through spectral deconvolution.

This paper presents a new algorithm called absorbance index which has the calculation simplicity of absorbance ratios but makes more efficient use of the spectral data. The data obtained from this algorithm are used to perform qualitative tasks in liquid chromatographic analysis such as determination of peak identity and peak purity.

INTRODUCTION

Chromatographers are increasingly aware of the importance of spectroscopy to the practice of liquid chromatography. Many papers in the current literature are appearing which present numerical procedures for performing the tasks of:

detection of impurities in an apparently pure chromatographic peak;

confirmation of the identity of a peak when paired with retention time data;

identification of an unknown compound by reference to a user-built library of spectral data;

qualitative and quantitative analysis of a mixed compound peak not separable by high-performance liquid chromatography (HPLC).

Techniques currently being used to perform these tasks include factor analysis in the form of matrix rank analysis[1] and principal components analysis[2], least means squares[3] and absorbance ratios[4,5]. The majority of these techniques involve complex calculations often involving matrix manipulations, and are efficiently performed only with the aid of a computer.

The occasional user who does not have computer capability readily available, however, needs a technique which is suitable for manual calculation. The only technique which we have found in the literature which meets this requirement is the

absorbance ratio, which is obtained through simple division. While attractive from a computational point of view, however, the absorbance ratio technique has a serious limitation for the liquid chromatographer since it requires that the user has some prior knowledge not only of the spectrum of the compound of interest, but also of the spectra of all the compounds that might be present and to which it is to be compared. In the course of investigating this limitation, a new numerical technique, absorbance index, was developed. While related to absorbance ratio, this new technique requires no prior knowledge of the spectra of the compounds to be compared.

EXPERIMENTAL

The spectral data were taken from peaks eluted from the column by stopping the flow and spectrally examining the captive peak. The procedure has been described previously[5]. For our studies, we used corrected absorbance values from the nine wavelengths from 210 nm to 300 nm taken at 10-nm intervals, which is the default program for the Perkin-Elmer LC-75 and Autocontrol detector system and from spectra sampled at 1-nm intervals.

RESULTS AND DISCUSSION

The tasks listed in the Introduction (above) require that we compare the spectrum of a standard to the spectrum of the sample. Thus, to check the purity or homogeneity of a chromatographic peak, the data points of one spectrum are taken on the leading edge and the data points of the other are taken on the trailing edge. For compound confirmation, the data points of the spectrum of the unknown sample are compared to those for the spectrum of the known compound.

In the absorbance index technique, essentially we divide one of the spectra into the other. If the graphed result of this division is a straight horizontal line (constant), then the spectra, and, by inference, the two compounds are the same (Fig. 1A). If the graphed result of this division is not a straight line, then the spectra are different (Fig. 1B). The results can be obtained from as many data points across the spectra as desired. Our experiments indicate, however, that a minimum of nine data points should be used. We call the values which make up this line the absorbance indices (Q). The degree of variation of the line is tested with the discriminator (D), which is the quotient of the largest to the smallest value of Q obtained. For identical compounds, and under ideal conditions, D would be equal to unity. To accomodate instrumental and environmental variations, a value greater than unity is taken as a practical threshold. Normally we set a maximum limit for D at 1.5 for accepting the two spectra as the same if the signal-to-noise ratio is greater than about 10:1.

To put the new method into context, we shall compare the absorbance index technique to the established technique of absorbance ratios. In the absorbance ratio technique, a spectrum is characterized by dividing its absorbance measured at one wavelength by its absorbance measured at some other wavelength. Other spectra can then be compared to this spectrum by measuring the absorbance ratio of the sample spectra at the same two wavelengths. The spectra can be considered identical if the absorbance ratios are the same (within the experimental error of the measuring system). When the sample spectrum is different from the standard spectrum, a pair of
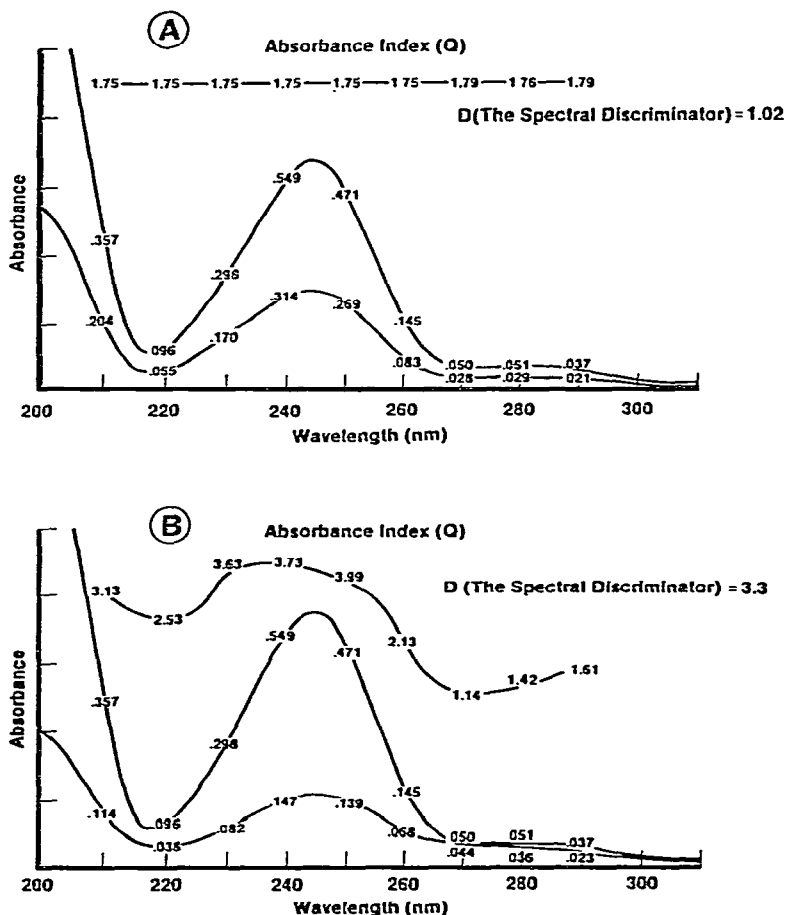
Fig. 1. Comparison of two spectra: A, the same compound at two different concentrations; B, two different compounds at different concentrations.

wavelengths can be found which best indicate this difference. This "best" wavelength pair make the largest quotient when their absorbance ratios are divided. This pair of wavelengths will change depending on the sample spectrum.

An inherent shortcoming of the absorbance ratio technique, however, is that it is always possible to find a sample compound which will give the same ratio as the standard at any given preselected pair of wavelengths. To avoid a false assumption that they are identical, we must perform a second absorbance ratio measurement at a different pair of wavelengths. Even this second measurement may be inconclusive if the difference between the two ratios is within the experimental error of the system. We must, therefore, continue to calculate absorbance ratio measurements at different pairs of wavelengths until a value is obtained which is significantly different from the standard or until no further ratios can be calculated. If none of the ratios are significantly different, then we can make the assumption that the two spectra, and hence, the two compounds are the same.

If we had obtained absorbance measurements at nine wavelengths for both the

standard and the sample, then we could calculate 36 different ratios on both spectra, a total of 72 separate calculations. The best pair of wavelengths would then be those which gave the largest quotient of the respective ratios on the sample and the standard if the two were different.

The calculation of this quotient would require an additional 36 calculations for a total of 108 calculations. This quotient can be expressed as

$$\frac{A_i/A_j}{B_i/B_j}$$

where A_i and A_j are the absorbance values of the standard at wavelengths i and j and B_i and B_j are the absorbance values of the sample at the same two wavelengths. This expression can be rearranged to give

$$\frac{A_i/B_i}{A_j/B_j}$$

which will have its maximum value when the denominator A_j/B_j is a minimum, and when the numerator A_i/B_i is a maximum.

These two expressions are both absorbance index values. By definition, the absorbance index discriminator is the quotient of the maximum and minimum absorbance index obtained. Hence the absorbance index technique will automatically find the best two wavelengths at which to compare the two spectra, if they are different. The absorbance index technique using the same nine wavelengths for both the sample and the standard, only requires ten calculations to obtain information equivalent to that which results from the absorbance ratio technique.

CONCLUSION

The absorbance index technique is a simple, sensitive test for comparison of spectra. The test can be performed manually from discrete absorbance values and the entire process can be accomplished in only a few minutes on peaks eluted by HPLC. The usefulness of this numerical spectroscopy procedure for determining peak purity and for peak confirmation is not only in the simplicity and utility with which the two spectra can be tested for likeness, but also in the consistency and confidence level of the results.

REFERENCES

1 J. C. Sternberg, H. S. Stillo and R. H. Schwendeman, *Anal. Chem.*, 32 (1960) 84.
2 J. M. Halket, *J. Chromatogr.*, 186 (1979) 443.
3 M. J. Milano and K. Y. Kim, *Anal. Chem.*, 49 (1977) 555.
4 R. Yost, J. Stoveken and W. MacLean, *J. Chromatogr.*, 134 (1977) 73.
5 A. F. Poile and R. D. Conlon, *Perkin-Elmer Technical Note No. 78.*