application note

Multi-Component Analysis of a Vitamin B Mixture by UV-Visible Spectrometry.

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Introduction

The analysis of a number of components in a sample or mixture is of importance in a number of industries such as pharmaceutical, food, dye and paint manufacturing. Quite often these industries are subject to strict rules and regulations regarding safety and quality during product manufacture. There is considerable interest in developing methods to test the final products accurately and reliably whilst reducing the time and cost of testing.

Many analyses of multi-component systems involve time-consuming sample preparation, as each component of interest needs to be extracted, separated, chemically transformed and purified. Alternatively, they may require different methods of analysis for each component. Often the simultaneous analysis of complex multi-component mixtures is not possible. However, multi-component analysis by UV-Visible spectrometry can be a cheaper and faster alternative to these methods, whilst still providing a high level of accuracy.

A UV-Visible multi-component analysis mathematically separates each component of the sample. This requires the measurement of spectra for known standards of all the components which are present at any significant level in the sample. The mathematical method attempts to minimize the sum of the squares of the residual spectrum. This is done by constructing a matrix of the cross-products of each pair of standard scans, and then solving this with respect to the cross-product of the standard scans with the sample scan. The final step is to calculate the actual sample component concentrations from the known concentrations in each standard.

Accurate multi-component determination by UV-Visible spectrometry requires that the following conditions are met:

1. All components of the mixture can be identified, and absorb within the wavelength range of the instrument.
2. The absorbances of the components in the mixture follow Beer's law.
3. There is some degree of spectral difference between the components. The greater the similarity between the spectra of the individual components the more difficult the analysis.
4. The spectrum.of the mixture is the sum of components, i.e., the components must not interact to cause photometric or wavelength shifts.
5. There should be no interaction between components and the solvent.
6. Very large or very small absorbances should be avoided.
7. There should be no absorbances in the analytical wavelength region due to impurities.

If any of these assumptions do not hold, then the multi-component analysis is invalid.

In this study, a specific software application program for GBC double beam UV-Visible spectrometers was used to calculate concentrations in a Vitamin B group mixture consisting of Vitamin B_1 (Thiamine HCl), Vitamin B_2 (Riboflavine) and Vitamin B_6 (Pyridoxine HCl).

Experimental

Reagents/Materials

Stock solutions of vitamins B_1 (1000 mg/L), B_2 (50 mg/L) and B_6 (1000 mg/L) were prepared by dissolving weighed amounts in 100 mL of 1% hydrochloric acid. The stock solutions were used for preparing calibration standards and sample mixtures. The concentrations of the standards and six sample mixtures are given in Table 1. The concentrations of the standards were selected to approximate the concentrations of the components in commercial mixtures.

Table 1. Standard and sample mixture concentrations.

Type of solution	Vitamin B_1 (mg/L)	Vitamin B_2 (mg/L)	Vitamin B_6 (mg/L)
Standard 1	10.0	0.0	0.0
Standard 2	0.0	10.0	0.0
Standard 3	0.0	0.0	10.0
Sample 1	10.0	0.0	0.0
Sample 2	0.0	10.0	0.0
Sample 3	0.0	0.0	10.0
Sample 4	10.0	10.0	10.0
Sample 5	5.0	5.0	5.0
Sample 6	10.0	10.0	1.0
Sample 7	10.0	1.0	10.0
Sample 8	1.0	10.0	10.0

A GBC 914 double beam UV/Visible spectrometer running Multi-component .application software was used in this study. The instrument was fitted with an auto-sipper and automatic sample changer.

The auto-sipper is a peristaltic pump-based system and the auto sample changer includes a carousel which holds 10 standards and 60 samples, with separate positions for rinse and blank solutions. The sipper and automatic sample changer are computer controlled, with programmable measurement time, delay time, rinse time, flow speed, flow timing and flow direction.

A 10 mm path length quartz micro flow cell with 4 mm path width was used with the sipper/autosampler. The Multi-component software allows full spectral data acquisition, display of graphical and tabular results, and storage and recall of methods and data. Components may be determined from pure or mixed standards using whole scans, a selected scan range or selected wavelengths from the wavelength scans. Derivatives (up to the 4th order) of the collected scan may also be used for component determination.

A baseline was recorded on 1% hydrochloric acid, and the spectra of the standards and mixtures measured over the wavelength range 210 nm to 550 nm.

The instrument operating parameters are given in Table 2 and the sipper/autosampler parameters are given in Table 3. A 2-second rinse with 1% hydrochloric acid was programmed between samples and a 0.4-second air slug was programmed to help prevent cross-contamination.

Table 2. Instrument operating parameters.

Upper Wavelength	550 nm
Lower Wavelength	210 nm
Scan Speed	500 nm/min
Wavelength Step	0.21 nm
Slit Width	2 nm
Beam Mode	Double Beam
Lamp Change Wavelength	350 nm
D2 Lamp On	When Necessary

Table 3. Sipper/autosampler operating parameters

Sipper Fill Time	6 s
Sipper Empty Time	0 s
Read Delay Time	2 s
Rinse Time	2 s
Air Slug	0.4 s

Results

The spectra of the standards are shown in Figure 1.

All three components can be quantified, as there is a large degree of spectral difference between the components. Vitamin B_2 is the only component that absorbs in the region 300 to 500 nm and has a strong absorption band at 266 nm. Vitamin B_6 has a strong absorption band at 290 nm and Vitamin B_1 has a strong absorption band at 245 nm.

Figure 2 shows the wavelength scans for samples 4 and 7 as representative examples.

The multi-component analysis of the Vitamin B system was optimized by limiting the calculation range to between 225 nm and 500 nm. In the region above 500 nm there is no absorbance from any component, and in the region below 225 nm the spectra of the components have little structure. The results of the multi-component analysis using this reduced wavelength range are shown in Table 4.

The simplest way to validate the selected method of calculation is to re-rneasure the standards as samples and check the accuracy of results. As can be seen in Table 4, the comparison of the calculated and expected results for the standards re-measured as samples (Samples 1 to 3) shows agreement to within 1.5%, thus validating the selected data collection and calculation parameters.

The results in Table 4 for samples 4 to 8 show that the multi-component calculations are accurate for the chosen calculation parameters (compare with Table 1). For samples with relatively high concentrations of a component the calculated results are within 5% of the expected results. However, the % difference between the expected and calculated values increases slightly as the concentrations of individual components decreases. Generally, sample concentrations can be calculated to within 2–5%.

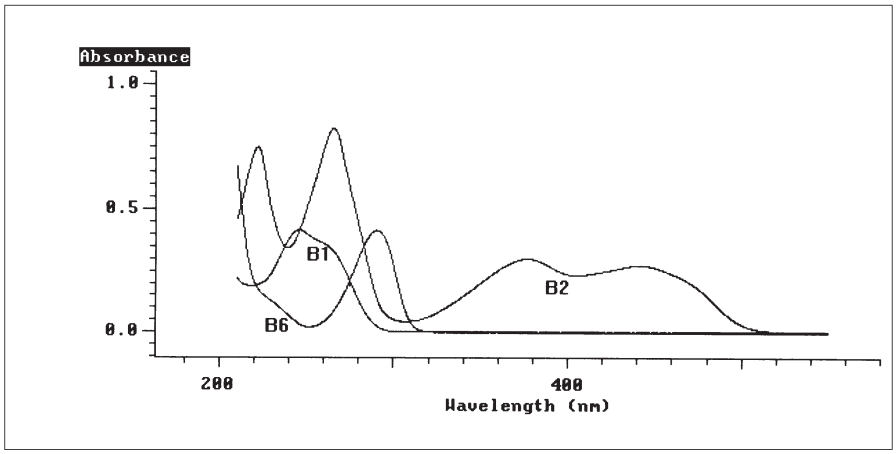

Fig. 1. Standard scans

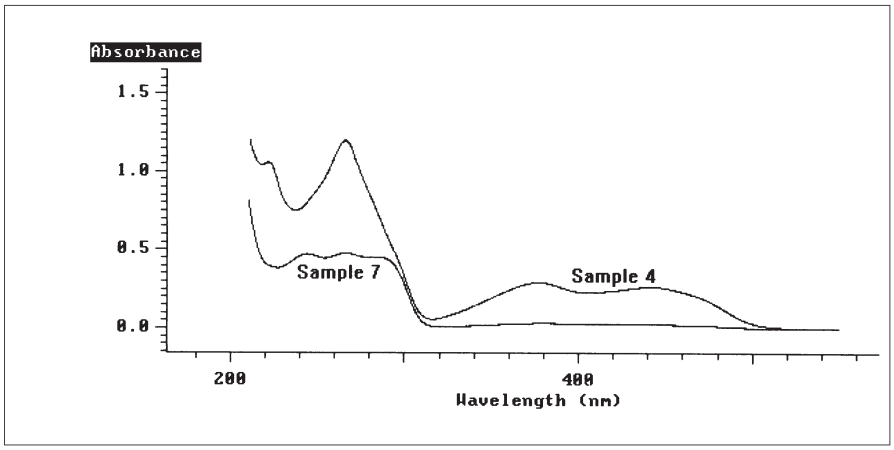

Fig. 2. Sample mixyure scans, (samples 4 and 7)

Table 4. Printout of multi-component analysis using wavelength range 225 to 500 nm.

GBC 914 Multi-Component V1.5			
12:27:56 pm 10 Jun 1993			
Method: VITAMIN B	8-Jun-1993 11:41:21 am		
Upper	550.0 nm		
Lower	210.0 nm		
Scan Speed	500 nm/min		
Wavelength Step	0.2 nm		
Slit Width	2 nm		
Beam Mode	Double beam		
Lamp Change wavelength	350.0 nm		
D2 Lamp On	When Necessary		
Components determined using scan range			
Calculation Upper W/L	500.0 nm		
Calculation Lower W/L	225.0 nm		
Smoothing Wavelength steps	1		
Standard Type	Pure Components		
Component	Conc. mg/L		
Vitamin B_1	10.0		
Vitamin B_2	10.0		
Vitamin B_6	10.0		
Sample	Component Conc. mg/L		
1	10.10	0.02	0.04
2	0.06	9.83	0.00
3	0.01	0.05	10.04
4	9.52	9.78	9.96
5	5.17	4.81	5.25
6	9.93	10.13	1.13
7	9.60	0.98	9.64
8	0.87	10.07	10.24

Conclusions

A GBC 914 UV-Visible spectrorneter/sipper/autosampler system running Multi-component software has been used to quantitatively determine the components of a Vitamin B group mixture. The GBC 914/916/918/920 range of double beam UV-Visible spectrorneters combined with suitable accessories provides a simple and versatile means for the determination of individual component concentrations in a rnulti-component system. The % differences between the calculated and expected values show that the Multi-component software can be used to quantitatively measure complex mixtures.

The application of this software is not limited to determining multi-component mixtures of vitamins. It can be used in a variety of applications provided that the seven conditions given in the introduction are met, and instrument and calculation parameters are optimized for a given application.