Quantitative analysis of complex multi-component mixtures

Application Note

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Introduction

Many analytical methods are designed to determine quantitatively a specific component in a mixture. These analyses are often only possible after time consuming sample preparation involving each component of interest being extracted, separated, chemically transformed or even purified. Methods may also require different measuring parameters for each component in a mixture and the simultaneous analysis of complex multi-component mixtures is not possible. There is therefore the need for a simple, fast and robust method which does not require extensive sample pretreatment, particularly in industries that need continuous process control.

There are often strict rules and regulations about the safety and quality of manufactured products such as in the pharmaceutical industry. There is a continuing interest in developing assays which can be used to test the final products accurately and reliably while reducing the cost of materials and time for testing.

The Cary multi-component software provides the accuracy and convenience such methodologies require. Individual components can be simultaneously measured without the need for separation and extraction.

The software contains a powerful algorithm which allows up to 10 individual components in a mixture to be measured quantitatively.

This at-work examines how the software can be be used to calculate concentrations using common multi-component examples and demonstrates the accuracy that can be achieved for different types of mixtures.

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The following multi-component systems are used:

- Vitamin B group using B1, B2 and B6
- Antihistamines using pseudoephedrine, dextromethorphan and triprolidine
- Other pharmaceutical compounds often found together such as salicylic acid, salicylamide, caffeine and 4-acetoamidophenol.

Equipment

- Cary 1/3 4/5 spectrophotometer
- Volumetric flasks
- Weighing boats
- Analytical weighing machine

Software

- Base system
- Multicomponent software:
  For Cary 1/3
  For Cary 4/5

Results

System 1: Vitamins B1, B2, B6

1. Vitamins B1, B2, B6 from Sigma
2. M HCl

The vitamins are stable in acidic solutions and thus 0.1 M HCl was used to dissolve them into both standards and mixtures. A baseline was collected after instrument zero at a region where none of the 3 vitamins give an absorbance value. The results calculated by the software for this system and the expected values are shown in Tables 1 and 2.

The %differences between the 2 values, (i.e. (calculated-expected)/calculated*100) increases slightly as the concentrations of individual components decrease. This is as expected but the overall results are still in close proximity to expected values. The accuracy of the Kalman filter algorithm is demonstrated by these results. The optimal conditions for these vitamins were found to be a Kalman interval of 1 nm, Bin error of 0.002% and a Bin size of 12 over the wavelength range of 225 to 500 nm. The S/N ratio 5000 was adequate to give the accuracy shown in Tables 1 and 2. If further reduction in photometric noise is needed by the analyst, S/N ratio can be further increased. In Cary 1/3, the photometric noise can be adjusted accordingly by using the SAT function. Figure 1 shows the scans for the standards. Figures 2 and 3 show examples of 2 and 3 component mixtures.

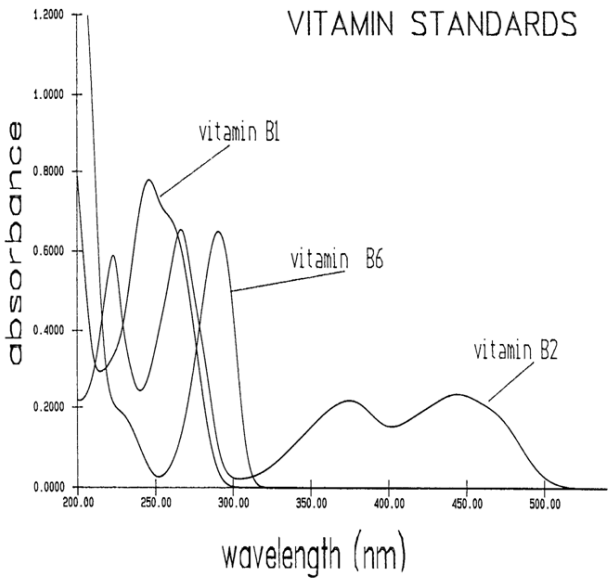

Figure 1. Vitamin Standards

Table 1. Two component mixture (system 1)

Kalman interval: 1 nm

Bin error: 0.002%

Bin size: 12

From: 225 to 500 nm

Type of Solution	Component name (Vitamins)	Conc (mg/L) measured	Expected Theoretical Conc. (mg/L)	% diff.
Standards	B2	6.79121	6.795	0.06
	B6	15.27476	15.277	0.01
Mix 2	B2	6.84323	6.795	-0.71
	B6	8.22429	8.333	1.30

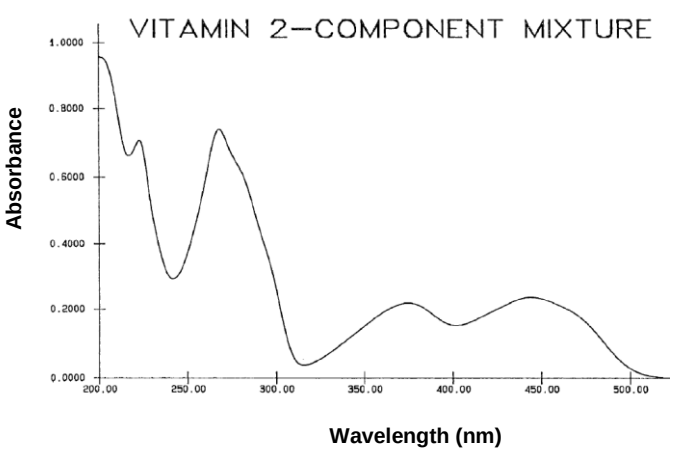

Figure 2. 2-component vitamin mixture

Table 2. Three component mixtures (system 1)

Kalman Interval: 1 nm

Bin Error: 0.002%

Bin size: 12

From: 225 to 500 nm

Type of Solution	Component name (Vitamins)	Conc (mg/L) measured	Expected Theoretical Conc. (mg/L)	% diff.
	B1	9.57610	9.580	0.04
Standards	B2	6.79121	6.795	0.06
	B6	15.27476	15.277	0.01
	B1	6.86946	6.843	-0.39
Mix 1	B2	6.88951	6.795	-1.39
	B6	8.31621	8.333	0.20
	B1	6.94829	6.843	-1.54
Mix 2	B2	2.28238	2.265	-0.77
	B6	6.95105	6.940	-0.16
	B1	3.49227	3.422	-2.05
Mix 3	B2	2.31322	2.265	-2.13
	B6	3.52853	3.47	-1.69
	B1	6.91029	6.843	-0.98
Mix 4	B2	1.19294	1.33	5.29
	B6	6.97136	6.940	-0.45

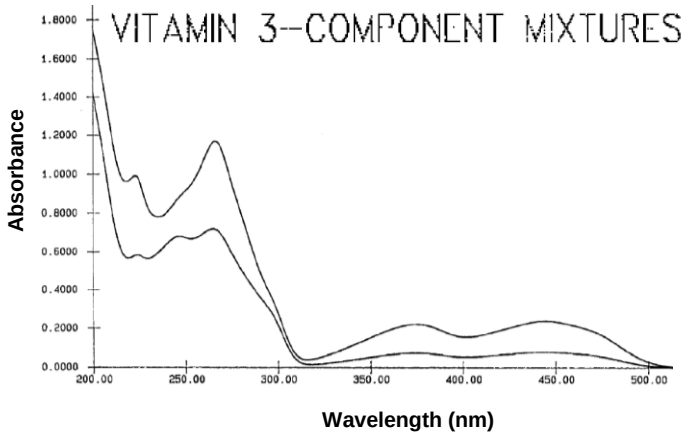

Figure 3. 3-component vitamin mixtures (system 1)

System 2: Antihistamines

1. Pseudoephedrine, Triprolidine, Dextromethorphan (pure grade).
2. 0.1 M HCl

A baseline was obtained as in system 1 with 0.1 M HCl over the wavelength range of analysis to remove interferences from the solvent. SAT used was 3 secs on a Cary 1/3. The results obtained from the software calculations are compared with expected concentrations in Table 3.

Figure 4 shows scans for standards as well as 2 examples of 3 component mixtures.

Table 3. Three component mixtures (system 2)

Kalman interval: 0.5 nm

Bin error: 0.05%

Bin Size: 10

From: 248 to 325 nm

Type of Solution	Component name (anti-histamines)	Conc (mg/L) measured	Expected Theoretical Conc. (mg/L)	% diff.
	pseudo-epinephrine	482.3057	483.400	0.2
Standards	triprolidine	19.22452	19.296	0.4
	dextro-`methorphan	167.20940	167.400	0.1
	pseudo-epinephrine	116.82610	120.850	3.3
Mix 1	triprolidine	4.83044	4.824	0.1
	dextro-`methorphan	41.57935	41.850	0.3
	pseudo-epinephrine	92.77284	96.600	3.9
Mix 2	triprolidine	3.74599	3.860	2.8
	dextro-`methorphan	33.31929	33.400	0.3

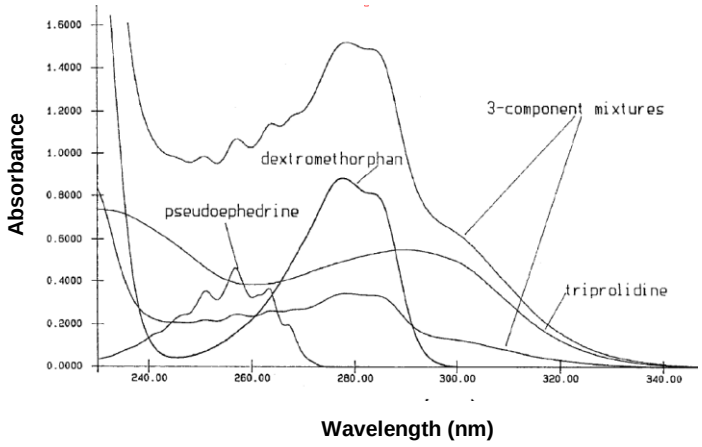

Figure 4. Multi-component and standards scans of antihistamines

System 3: Pharmaceutical compounds

1. Salicylic acid, salicylamide, caffiene, 4-acetoamidophenol from Sigma (AR grade).
2. Ammonium phosphate (pH 9.8)

These compounds are often found in commercial preparations such as 'headache tablets'. This system is an example of a 4 component mixture. The baseline was collected with the phosphate buffer before the standards and mixture were read. The results and conditions for this system are as shown in Table 4.

Table 4. Four component mixtures (system 3)

Kalman interval: 2.0 nm

Bin error: 0.01%

Bin Size: 10

From: 227 to 375 nm

Type of Solution	Component name (pharma-ceuticals)	Conc (mg/L)	Expected Theoretical Conc. (mg/L)	% diff.
	salicylic acid	50.42220	50.500	-0.2
	salicylamide	14.52486	14.532	-0.05
Standards	caffiene	16.34892	16.368	-0.1
	4-aceto-amidopheno	11.84186	11.856	-0.1
	salicylic acid	10.60374	10.648	0.4
	salicylamide	9.90178	9.688	2.2
Mix 4	caffiene	16.44481	16.368	0.5
	4-aceto-amidopheno	11.78894	11.856	0.6

Figure 5 shows the spectral scans for the standards and the mixture example.

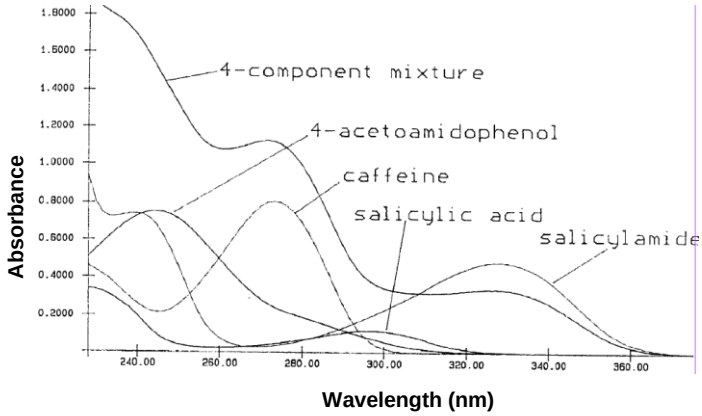

Figure 5. Multi-component and standards scans of some pharmaceutical compounds

Discussion

The 3 multi-component systems show that calculated concentration values obtained from a complex system of up to 4 components can be as accurate as from a simple 2 component mixture despite overlapping absorbance peaks if optimum conditions are selected.

The results tabulated in Tables 1,2,3 and 4 show that the multi-component calculations made were accurate for the respective spectral regions of analyses. Figures 4 and 5 are examples of mixtures with overlapping absorbance peaks.

The Kalman filter used by the software gives concentration values for these pharmaceutical mixtures comparable to expected values. The filter is shown to be robust yet sensitive to initial estimations and only a few iterations are required to obtain the best results for a mixture system. However, initial studies should be made about the dynamics of the mixture system. The multi-component package like any calibration method gives optimum results if the following conditions are met:

1. The absorbance characteristics of the components in the mixture follow Beer's law.
2. The components do not interact chemically in a mixture so as to affect the absorption spectrum.
3. All measurements are taken under the same conditions such as the same optical cell, the same signal averaging time (SAT) and data interval (DI).
4. Individual standards should be available in pure form to allow for calibration.

The longest SAT and the smallest DI which gives an acceptable collection time should be used on the Cary.

For accurate calculations, the standards and samples should be collected using the same conditions and time, especially if solutions are chemically unstable. Solvent effects should be corrected for by means of a baseline collected over the same spectral region.

Samples and standards should be capped during measurements to prevent evaporation especially if volatile organic solvents are used.

The comparison between alternative calculation methods have distinguished the Kalman filter multi-component method as particularly useful because it can show extra robustness to unexpected or missing components [2,3] and when the number of components increase[1]. Since the Kalman algorithm's accuracy depends on the amount of photometric noise and peak separation of the standards, the more points used for calculation, the better the estimates for results.

Applications of the software are widespread, for example, in areas of routine analytical work or in quality control. If all the conditions are met and instrumental parameters optimized, sample concentrations can be calculated typically with less than 1-2 % error. The accuracy may deteriorate when measuring very low concentrations in multi-component mixtures such as in mix 4, Table 2.

The validity of the chosen parameters may be confirmed by re-measuring the standards as samples and checking the accuracy of the results. Additional checks possible are by the function 'synthesize' (which compares the actual mixture spectrum collected with a synthetic spectrum calculated by the software). This software allows additional error checking and will also give the calculated error values.

The software also allows both fast on-line analyses (as it performs calculations immediately after each sample is collected), as well as post sample collections (using either stored or stored mixture data files). This is found to be a useful feature for both routine and automated measurements of samples.

Conclusion

Three multicomponent systems were analyzed to determine the individual component concentrations. These calculated concentrations are compared with expected concentrations. The % differences between these values show that the multicomponent software can be used to quantitatively measure complex mixtures.

References

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