UV-visible spectroscopy as an alternative to liquid chromatography as analysis method for dissolution testing

Application Note

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Pharmaceutical

Introduction

Dissolution testing is widely used in the pharmaceutical industry to determine quality assurance parameters for batch release. Today's competitive pharmaceutical market demands high efficiency and productivity not only in manufacturing but also in routine analytical processes. In dissolution testing the majority of formulations are already analyzed by UV-visible spectroscopy, but liquid chromatography (LC) is often used for formulations containing interfering excipients or multiple ingredients.

This study demonstrates that identical results can be achieved by replacing the LC method by a faster and more convenient UV-visible method. The two techniques were used in parallel for analysis of the same dissolution process to allow an exact comparison of the results. As examples, single and multi-component pharmaceutical formulations were analyzed and were chosen because LC is the analysis method for both formulations according to United States Pharmacopeia (USP).

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Experimental

The dissolution tests were performed using Distek 2100B, Erweka DT80 and Hanson SR8Plus dissolution baths, which were configured according to apparatus 2, USP paddle method. A software driver supplied by the respective bath manufacturer enabled the baths to be controlled remotely by the Agilent ChemStation.

Tablets for single component analysis contained warfarin (5 mg per tablet) as active ingredient and were dissolved at a stirring speed of 50 rpm. Tablets for multicomponent analysis contained acetaminophen (333 mg) and caffeine (50 mg) and were dissolved with stirring speed of 100 rpm. Water at 37 °C was used as dissolution medium in all experiments.

The UV-visible spectroscopy analyses were performed with an Agilent 8453 dissolution testing system, comprising spectrophotometer and multicell transport as sampling system. Because of the different concentrations of the active ingredients in the tablets, 1 cm flow cells were used for measurement of warfarin whereas 0.1 cm flow cells were used for the measurement of acetaminophen and caffeine.

The liquid chromatography analyses were performed with an Agilent 1100 Series LC—a high performance instrument—comprising vacuum degasser, binary pump, autosampler, thermostatted column compartment and diode array detector.

After each UV-visible measurement cycle, samples for LC analysis were collected directly from the flow cells. These samples were centrifuged at 15000 g and 22 °C for 20 minutes, and the supernatant liquid was transferred to sample vials which were placed in the autosampler. The LC analyses were performed according to USP methods.[1]

The 8-channel multicell transport configuration of the Agilent 8453 dissolution testing system enabled measurement of 6 tablets, blank and control at each measurement cycle. Each vessel was connected to a separate flow cell in a closed loop configuration.

A control with known concentrations was included during all dissolution runs to verify the calibration and to check for system suitability. Initial tests with salicylic acid and prednisone USP calibrator tablets validated the dissolution bath and the UV-visible and LC methodologies.

Results

Single component formulations

The dissolution run of the single component formulation containing warfarin was analyzed by the LC and UV-visible methods in parallel. Diode-array-based UV-visible absorbance detection was used for both methods, which had the advantage of obtaining simultaneously the analytical wavelength at 306 nm and a wavelength range of 380 to 400 nm as internal reference.

Figures 1 and 2 show the dissolution profiles of six of the tablets from the UV-visible and LC analyses respectively. During the run samples from all vessels were analyzed at 0, 5, 12, 20 and 30 minutes and the results displayed as percent-dissolved warfarin.

The dissolution profiles in figure 2 were generated by a spreadsheet application and show the results obtained by LC analysis and data evaluation about two and half hours after the dissolution run.

In contrast the profiles in figure 1 are the results of the UV-visible analyses, which were generated automatically by the dissolution testing software online during the run.

A comparison of the two figures reveals that the dissolution profiles obtained by the UV-visible and LC methods are similar. The profiles of the individual tablets were different, which was confirmed by both analysis methods.

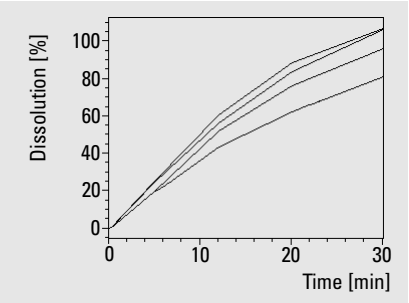

Figure 1
Dissolution profiles from UV-visible analyses

Time	Vessel							Average	StdDev
	1	2	3	4	5	6	7 (Ctrl)		
0	0.00	0.00	0.00	0.00	0.00	0.00	63.56	0	0
5	25.40	18.59	24.57	18.72	16.42	19.81	65.43	20.584	3.591
12	60.69	43.75	56.46	41.99	40.55	51.82	65.34	49.208	8.342
20	88.56	62.49	83.50	61.36	61.59	75.99	65.85	72.247	12.117
30	106.59	81.18	106.30	82.87	78.25	96.03	65.80	91.870	12.830

Table 1
Dissolution results from UV-visible analyses

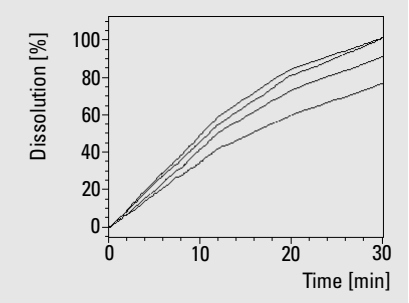

Figure 2
Dissolution profiles from LC analyses

Time	Vessel							Average	StdDev
	1	2	3	4	5	6	7 (Ctrl)		
0	0.00	0.00	0.00	0.00	0.00	0.00	n.d.	0	0
5	25.25	18.08	23.91	18.48	16.52	20.13	64.30	20.393	3.465
12	60.76	43.77	56.63	42.32	40.95	52.36	63.93	49.465	8.285
20	88.06	62.63	83.87	62.00	61.93	76.19	65.06	72.445	11.868
30	106.65	80.86	106.16	82.56	77.60	95.99	64.91	91.637	13.046

Table 2
Dissolution results from LC analyses

For a more detailed comparison, tables 1 and 2 show the dissolution results of the two methods. The values—taken at each time point—represent percent-dissolved warfarin in the six vessels containing the different tablets and in the vessel containing the control. Further, the tables show the average values of the six tablets and the respective standard deviations.

The results in table 2 for the LC analyses were generated by a spreadsheet application, whereas the results in table 1 for the UV-visble analyses were generated automatically with the dissolution testing software directly after each measurement cycle. The dissolution results of both methods were equivalent with a maximum difference of 1.4 % between comparative values. The average values for the six tablets also compare well.

In summary, both analysis methods were applicable for this single component formulation. However, the Agilent dissolution testing system offers more convenient online sampling and online result generation during the dissolution tests.

Multicomponent formulations

An advantage of the LC method is the physical separation of formulation components during analysis. For this reason multicomponent formulations are generally analyzed by LC.

This study demonstrates that accurate analysis of more complex formulations can be achieved using a diode-array-based UV-visible spectrophotometer and a sophisticated multicomponent analysis (MCA) algorithm.

Based on initial tests with standard solutions, a UV-visible analysis method for measuring the dissolution of the multicomponent formulation was development. To improve the quantitative accuracy, first-order derivatives were used instead of absorbance spectra.[2]

A first-order derivative is the rate of change of absorbance with respect to wavelength, see figure 3. Using first-order derivatives ensures correction for minor background absorbance or scattering. By applying derivative spectroscopy in this application example, wavelength ranges could be used for calibration, in which high spectral differences between both compounds were accompanied by best fit of predicted and sample spectra (262–264 nm and 275–278 nm.)

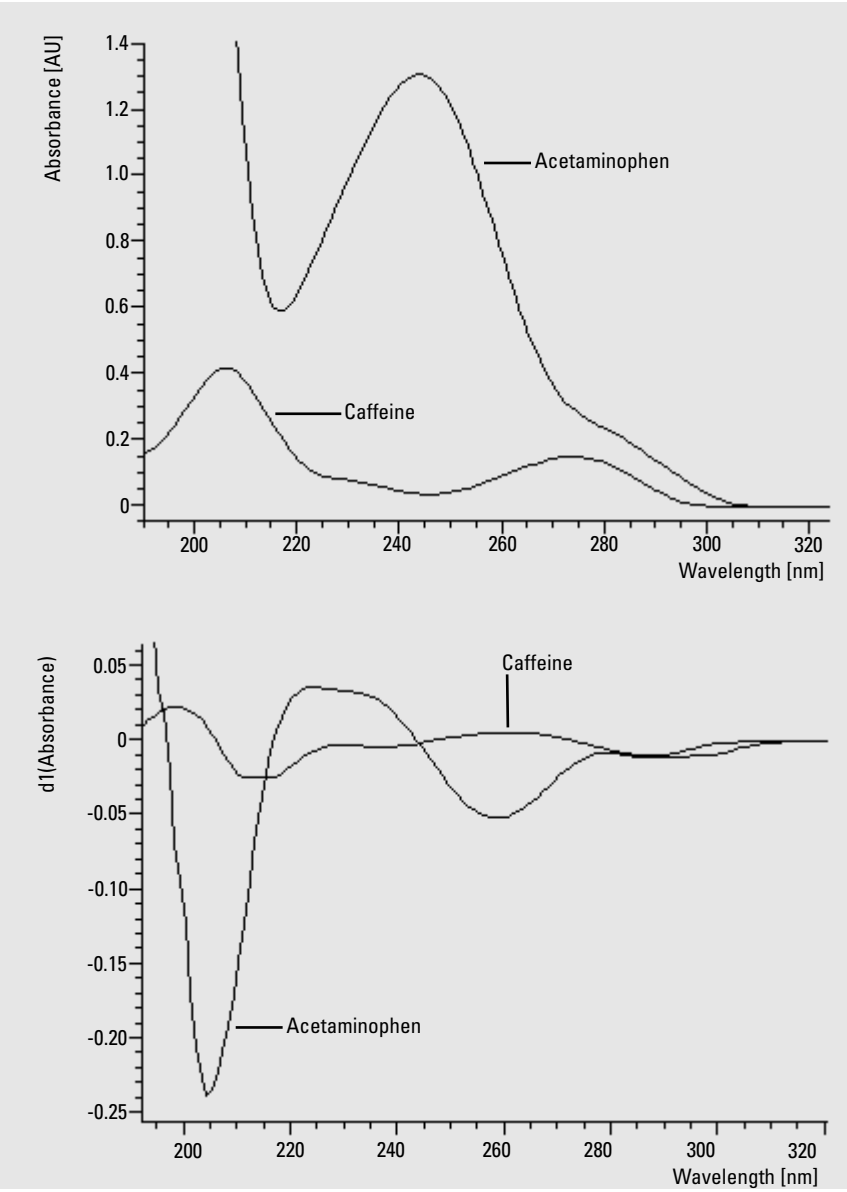

Figure 3
Absorbance spectra (top) and first-order derivatives (bottom)

The Agilent 8453 spectrophotometer's advantages of fast, simultaneous spectral data acquisition and excellent wavelength reproducibility could be exploited, because any wavelength in the entire UV-visible range could be used for data evaluation.

Using a wavelength range instead of a simple, simultaneous-equations method for multicomponent analysis overcame the well-known limitations of traditional MCA.[3]

The UV-visible method was validated by analyzing two series of synthetic mixtures. In each series of mixtures, the concentration of one component was kept at the same level as in the multicomponent formulation, whereas the concentration of the other component was varied from 0 to 150%. Figures 4 and 5 show the results of multicomponent analysis of the synthetic mixtures. The results confirmed the selectivity, accuracy and reproducibility of the analytical method as well as the linearity of the method for different formulation concentrations.

As for SCA, the dissolution of the multicomponent formulation was analyzed in parallel by LC and UV-visible methods. The results are illustrated by table 3 showing fast dissolution of all six tablets. The table shows percent dissolved acetaminophen and caffeine for the six tablets and the control obtained by LC and UV-visible methods. The differences between these results at the three different points in time are also included. The small differences reflect the applicability of both analytical methods for the multicomponent analysis.

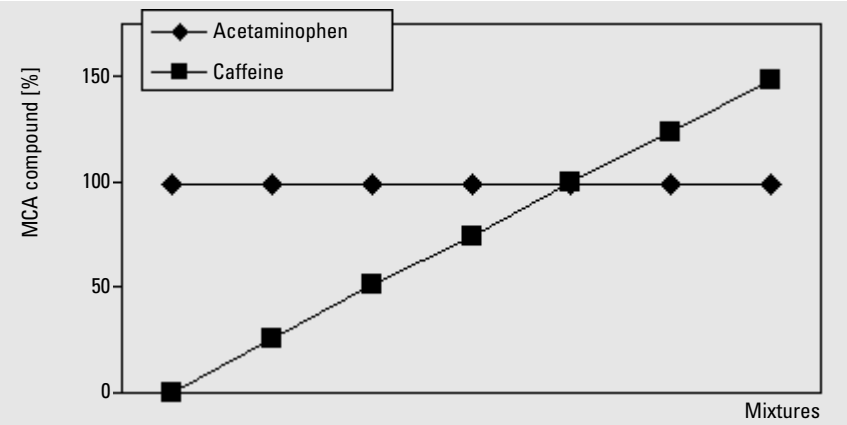

Figure 4
Influence of varying caffeine concentration on acetaminophen analysis

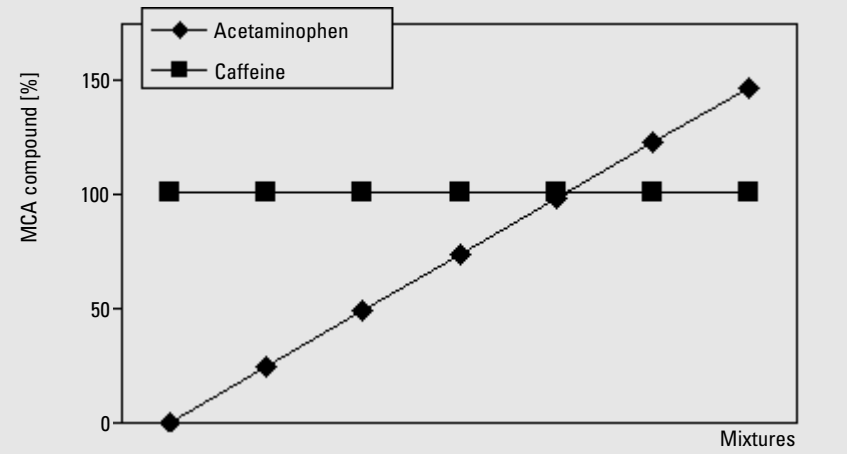

Figure 5
Influence of varying acetaminophen concentration on caffeine analysis

		Acetaminophen [dissolved %]			Caffeine [dissolved %]		
Tablet	Time	LC method	UV method	Difference	LC method	UV method	Difference
1	7	104.14	103.85	0.29	102.61	100.09	2.52
2	7	103.50	104.39	-0.89	102.61	103.90	-1.29
3	7	103.93	104.01	-0.08	105.56	107.64	-2.07
4	7	104.06	104.24	-0.18	104.96	98.28	6.68
5	7	104.14	103.74	0.40	99.71	100.33	-0.63
6	7	103.12	103.71	-0.59	102.12	100.32	1.80
Ctrl	7	58.85	59.56	-0.71	51.60	50.99	0.61
1	20	104.18	104.07	0.10	103.57	101.65	1.92
2	20	104.90	104.52	0.37	105.98	105.18	0.81
3	20	103.42	104.15	-0.72	109.48	108.94	0.54
4	20	104.23	104.25	-0.02	99.73	98.90	0.83
5	20	102.99	103.91	-0.92	101.97	101.39	0.58
6	20	103.28	103.64	-0.36	101.81	100.53	1.28
Ctrl	20	59.38	59.62	-0.25	52.20	50.92	1.28
1	60	104.68	104.33	0.35	102.99	101.57	1.43
2	60	104.84	105.25	-0.41	106.60	107.18	-0.59
3	60	104.07	104.60	-0.53	110.11	109.08	1.03
4	60	104.39	104.45	-0.06	100.25	100.05	0.20
5	60	103.80	104.27	-0.47	102.82	101.80	1.03
6	60	104.91	103.93	0.98	102.21	101.02	1.18
Ctrl	60	59.47	59.82	-0.35	52.49	51.64	0.85

Table 3
Results of LC and UV-visible analyses of the multicomponent formulation

Figure 6 shows the average results from six vessels at different times and the average results of the control measurements for both components, acetaminophen (Acet) and caffeine (Caf). The error bars indicate the standard deviation of each average value. Both analytical methods gave essentially identical results for dissolution of this multicomponent formulation. The small standard deviations of the control values indicate high precision of both methods because the control did not change during the dissolution process.

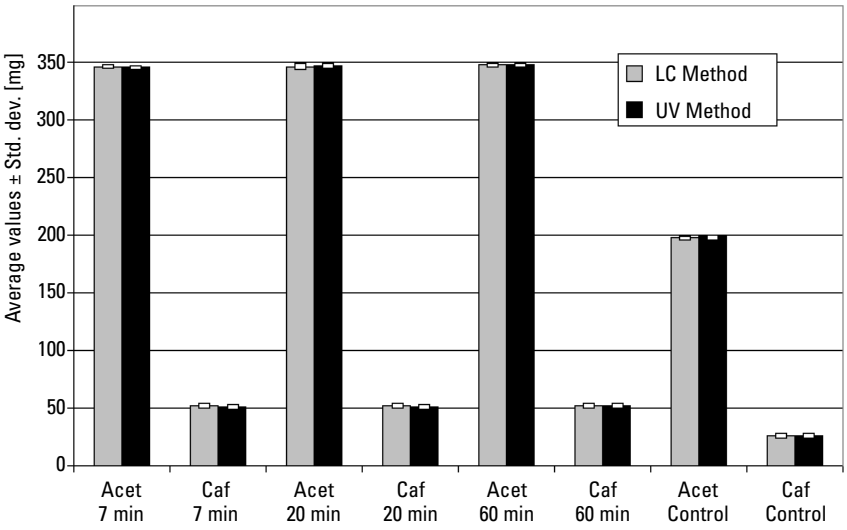

Figure 6
Average results for both components

Summary

A UV-visible spectroscopy analysis method—with the technological advantage of simultaneous spectral data acquisition and the implementation of powerful data analysis algorithms—yielded equally good analysis results compared to a classical LC analysis method for the dissolution of single and multicomponent formulations.

Further, the fast response of the UV-visible technique using the Agilent 8453 dissolution testing system with multicell transport provided for real-time monitoring of the dissolution process. In contrast, the LC method required several additional hours before the complete dissolution result report was available.

The capabilities of the Agilent 8453 dissolution testing system allowed cost savings in investment and running of the instrumentation, without a loss of quality of the results. In addition, the system offered all features for automated measurement, including online monitoring of bath parameters through to report generation and evaluation of release criteria.

Conclusion

Modern diode-array-based UV-visible spectroscopy analysis is applicable for dissolution testing in many cases where LC is the traditional analysis method. The excellent conformity of the results for the application examples examined in this study makes the Agilent 8453 dissolution testing system an ideal tool for quality control and other routine analyses in the pharmaceutical industry.

References

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2. Owen, A. J., "Uses of derivative spectroscopy", *Agilent Technologies Application Note*, **1995**, publication number 5963-3940E

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