APPLICATION NOTE

UHPLC

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Analysis of Drug Substances in Headache Medicines with the PerkinElmer Flexar FX-15 System Equipped with a PDA Detector

Introduction

Acetaminophen and aspirin are the drugs of choice used to relieve the symptoms of common headache. Acetaminophen, which is also called paracetamol, is widely used as a pain reliever (analgesic) and fever reducer (antipyretic). Because of its fast onset (eleven minutes after intake), acetaminophen is very effective. However, every year its misuse (dose exceeding the daily adult limit of four grams) can cause fatal liver damage. In fact, acetaminophen toxicity is the main cause of acute liver failure and accounts for most drug overdoses in the United States. The other common active ingredient in headache medicines is acetylsalicylic acid (aspirin), which is an analgesic, antipyretic and anti-inflammatory drug. Despite its usefulness, aspirin has a harmful side effect. For many people it can cause or exacerbate gastrointestinal ulcers by destroying the mucosal lining. This is a major setback for a medicine that is otherwise very inexpensive and can also be used for its antiplatelet effect to prevent heart attack and stroke. In formulations specially designed to treat common headaches (tension headache), acetaminophen and aspirin are often combined with caffeine. In these formulations, caffeine not only increases the effectiveness of the two drugs but it also stimulates the central nervous system and temporarily wards off tiredness.

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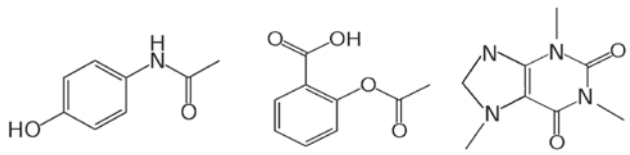
The hepatotoxicity of acetaminophen combined with the risk of stomach bleeding linked to aspirin are serious health problems that can be prevented by accurate label claims and proper medical indications. That is why in the pharmaceutical industry, routine assay testing to ensure the accuracy of the amount of active ingredients is a standard procedure and also a part of the Food and Drug Administration requirements.

This application note presents a modified USP method for the analysis of aspirin, acetaminophen and caffeine (Figure 1) using a superficially porous particle column and a PerkinElmer® UHPLC platform. The changes to the USP method are intended to increase the throughput and reduce the solvent consumption. Method conditions and performance are presented. Two headache medicines, one brand name and one generic are tested and the amount of the active ingredients determined.



Acetaminophen Aspirin Caffeine

Figure 1. Molecular structure of drug substances analyzed.

Experimental

A stock standard with 0.25 mg/mL of acetaminophen and aspirin and 0.065 mg/mL of caffeine were prepared by dilution of net weight with mobile phase, a one min. vortexing was followed by two to three min. sonication. For the working standard, 4.0 mL of the stock solution and 0.3 mL of internal standard (6 mg/mL of benzoic acid in methanol) were transferred into a 10 mL volumetric flask and brought to volume with mobile phase to obtain a solution of 0.1 mg/mL of acetaminophen and aspirin, 0.026 mg/mL of caffeine and 0.18 mg/mL of internal standard. Repeatability was evaluated with seven injections of the working standard. Linearity was determined across the range of 0.1 µg/mL to 100.0 µg/mL. Tablets from a brand name form of the drug, and capsules from a generic form of the drug, both with labeled amount of 250/250/65 mg (acetaminophen/aspirin/caffeine), were prepared by transferring a net weight equivalent to a tablet or capsule into individual 100 mL volumetric flask. Both of the preparations were diluted with mobile phase, followed by a minute vortexing and 15 min. of sonication. 2.0 mL of each preceding solution and 1.5 mL of internal standard were transferred into separated 50 mL volumetric flasks and brought to volume with mobile phase. Samples were thoroughly mixed and filtered through a 0.2 µm nylon membrane prior to testing.

A PerkinElmer Flexar™ FX-15 UHPLC system fitted with a Flexar FX PDA photodiode array detector was the platform for this experiment. The separation was achieved using a PerkinElmer Brownlee SPP C-18, 2.7 µm, 100 x 2.1 mm column. The run time was 4.4 minutes with a back pressure of 4000 PSI (276 bar).

***Table 1.* Detailed UHPLC system and chromatographic conditions.**

Autosampler:	Flexar™ FX UHPLC
Setting:	50 µL Loop and 15 µL needle volume, partial loop mode 350 µL mixer volume Injection: 10 µ, 2 µL; injector wash: water
HPLC Column:	PerkinElmer Brownlee Analytical C18, 100 x 4.6 mm, 5 µm Part No. N9303512 at 45 °C
Mobile Phase:	B: 69:28:3 water/methanol/acetic acid 1.0 mL/min, 12 min.
UHPLC Column:	PerkinElmer Brownlee SPP C18, 100 x 2.1 mm, 2.7 µm Part No. N9308404 at 55 °C
Mobile Phase:	B: 69:28:3 water/methanol/acetic acid 0.25 mL/min, 4.4 min.
PDA Detector:	Scanned from 190 – 700 nm, recording setting 275 nm Alternate setting to 275 nm: Optimized wavelengths

Time (min.)	Wavelength (nm)
0-1.3	245
1.3-2.5	275
2.5-4.4	232

Software:	Chromera® Version 3.0
Sampling Rate:	5 pts/sec

Results and Discussion

Initially, the USP method was implemented as it is with a conventional C18, 100 x 4.6 mm, 5 µm particle size HPLC column with 1.0 mL/min flow rate and 45 °C; all the peaks eluted within 12 minutes (Figure 2). By using a shorter column with smaller particle size (PerkinElmer Brownlee SPP C18 100 x 2.1 mm, 2.7 µm) at 55 °C, and by using the mobile phase as diluent instead of the mixture of methanol and glacial acetic acid (95:5) called for by the USP method, the pressure stabilized at about 4000 PSI and the run time was dramatically reduced from 12 minutes to 4.4 minutes. Moreover, the resolution and sensitivity were significantly improved (Figure 3). Prior to running the sample, from one injection of the standard, the maximum wavelength for each peak was determined and the wavelength recording setting

was optimized, resulting in the improvement of the UV absorbance up to fivefold (see Figures 4 and 5). From the standard solution chromatogram, a spectral library was created (Figure 6), and was later used for peak identification confirmation in the samples (Figures 7 and 8).

In addition to about a threefold reduction in chromatographic run time, the flow rate was reduced to 0.25 mL/min. from 1.0 mL/min. Thus, 63% reduction in testing time and 90% reduction in solvent usage was achieved by moving to a UHPLC method, resulting in far less solvent disposed of as dangerous waste. Overall, by moving a traditional HPLC method to a UHPLC method, the cost of labor and chemicals are significantly reduced, providing for a greener laboratory operation.

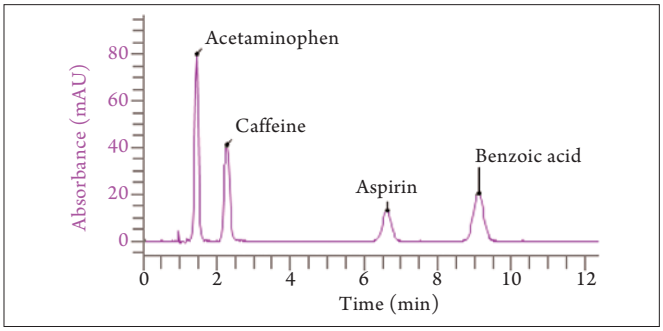


Figure 2. Chromatogram from the analysis of the standards using a conventional C18 column.

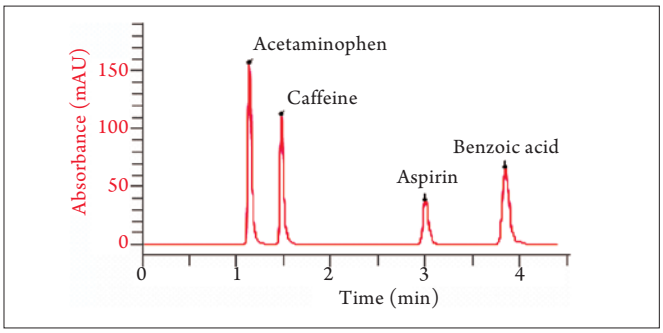


Figure 3. Chromatogram from the analysis of the standards using a superficially porous particle C18 column.

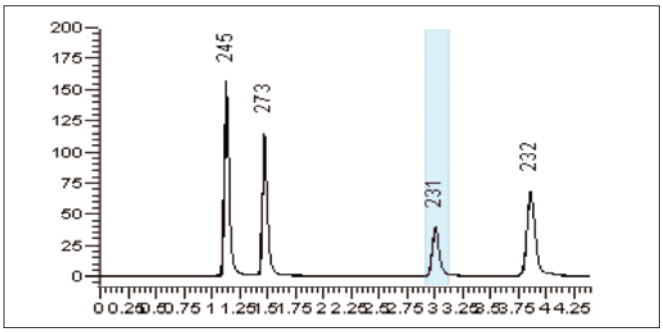


Figure 4. Chromatogram from the analysis of the standards showing the maximum absorbance for each peak.

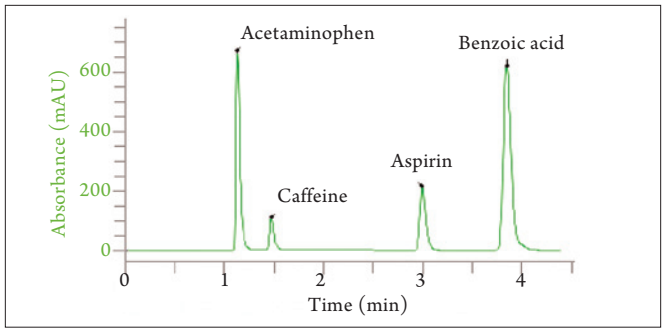


Figure 5. Chromatogram from the analysis of the standards with wavelengths setting optimized.

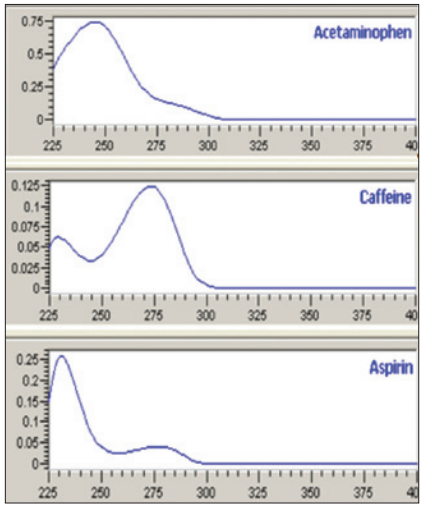


Figure 6. UV Spectra from the standard solution run.

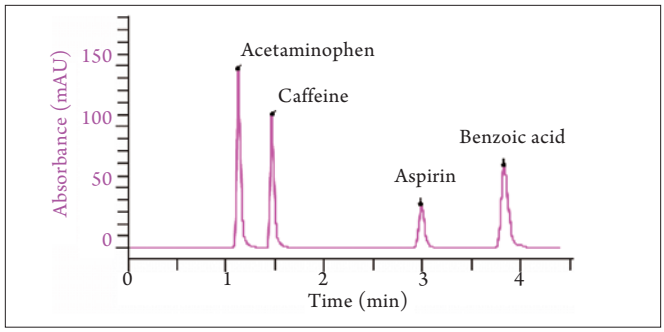


Figure 7. Peaks of drug substances in a generic headache medicine.

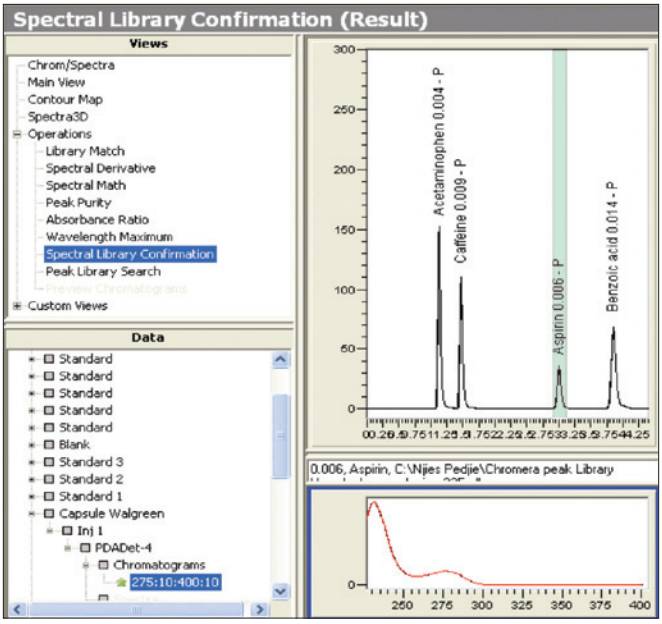


Figure 8. Peak identification of a generic headache medicine using the spectral library.

Excellent method performance was achieved. The linearity of the analysis shows R-squared values of 0.9999 or 1 and precisions values ranging from 0.58% to 0.98% RSD. Details of the method performance and results of the samples tested are presented in Table 2.

***Table 2.* Precision, linearity and amount in samples.**

	%RSD (n=7)	Linearity r^2	Range (µg/mL)	Generic	Brand name
Acetaminophen	0.76	1	0.60-100	100.5%	96.9%
Caffeine	0.58	1	0.16-26	98.2%	97.6%
Aspirin	0.98	0.9999	1.25 -100	93.3%	93.8%
Average	0.77	1	NA	97.3	96.1

NA = not applicable

Conclusion

The application of UHPLC to the analysis of acetaminophen, aspirin and caffeine in headache medicine resulted in a 63% reduction in run time, as well as a 90% reduction in solvent usage. The PerkinElmer Flexar FX-15 UHPLC system and PerkinElmer Brownlee SPP C-18, 2.7 µm, 100 x 2.1 mm resolved all three drug substances. The method was shown to be linear with R-squared ≥ 0.9999 and precise with %RSD ≤ 0.98. The brand name drug product has 243/235/64 mg per tablet of acetaminophen/aspirin/caffeine, and the generic form of the drug has 275/233/64 mg; these are both well within the limit of not less than 90.0% and not more than 110.0% of the labeled amounts as specified in the USP monogram. The Chromera software offers many data acquisition and processing features, including spectral library creation, absorbance maximum for each peak, which are powerful tools for interrogating the information content of a 3D photodiode array chromatogram. The spectral library creation and search function allowed the storage of standard peaks spectra that were later used for peak identification confirmation in the samples.

References

1. Larson, A.M., Polson, J., Fontana, R.J., *et al,* December 2005. "Acetaminophen-induced acute liver failure: results of a United States multicenter, prospective study." Hepatology 42 (6): 1364–72.

2. USP 34. Official USP Monogram; Acetaminophen, Aspirin, and Caffeine Tablets; May 1, 2011, pages 1725, 1726.

3. Drugs.com prescription drug information and side effects. Retrieved August 12, 2011.

4. National Center for Biotechnology Information (NCBI), PubMed Health http://www.ncbi.nlm.nih.gov/pubmedhealth/PMH0000521/

5. U.S. Preventive Services Task Force; "Aspirin for the Prevention of Cardiovascular Disease: U.S. Preventive Services Task Force Recommendation Statement;" Annals of Internal Medicine; March 17, 2011.

Note: This application note can be changed without prior notice.

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