APPLICATION NOTE

UHPLC

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

Introduction

The common cold is a frequent upper respiratory tract infection caused by a number of different types of viruses. Common cold affects billions of people worldwide every year; its typical symptoms include a runny nose, nasal congestion and sneezing. Colds can also cause sore throat, cough and headache. Common cold viruses do not respond to antibiotics and there are no known cures. Although the symptoms are normally resolved within ten days, they can cause a great deal of discomfort. Fortunately, these symptoms can be alleviated by the use of over-the-counter medicines. These cold remedies invariably include acetaminophen (a pain reliever and fever reducer), a cough suppressant (antitussive) and a nasal decongestant. Commonly used antitussive and nasal decongestant are dextromethorphan HBr and phenylephrine HCL. Dextromethorphan temporarily relieves cough by decreasing activity in the part of the brain that causes the coughing. Phenylephrine relieves nasal discomfort and sinus congestion by reducing the swelling of blood vessels in the nasal passages. Since they don't treat the underlying cause of the illness, cold medicines do not necessarily speed the recovery.

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Because the common cold is the most frequent human infectious disease, over-the-counter cold medicines are widely misused, causing serious health problems. Phenylephrine can cause hypertension and people with heart problems are advised against taking it; dextromethorphan can have hallucinogenic effects when the dosage exceeds the maximum recommended. Even more concerning is the potential fatal liver failure that can be caused by the usage of acetaminophen at doses that exceed the four gram daily limit.

The misuse of cold remedies can be prevented by appropriate medical indications and accurate label claims. To that end, the U.S. Food and Drug Administration and the pharmaceutical industry have made it a standard procedure to routinely test drug products to ensure the accuracy of the amount of active ingredients. This application note presents a method for the simultaneous analysis of acetaminophen, dextro-metorphan and phenylephrine (Figure 1). Method conditions and precision are presented. A cold medicine tablet is analyzed and the type and amount of active ingredient are confirmed.

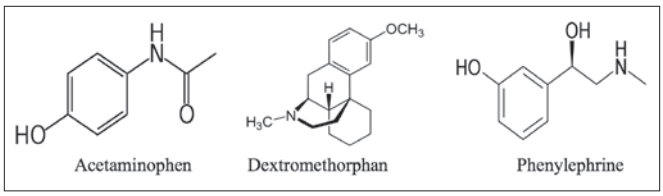

Figure 1. Molecular structure of drug substances analyzed.

Experimental

A working standard with 0.3 mg/mL of acetaminophen, 0.1 mg/mL of phenylephrine HCL and dextromethorphan HBr was prepared by transfer of net weight into 100 mL volumetric flask, to which about 3 mL of methanol was added followed by two min. sonication. The solution was then diluted with 75 mL of 0.1% acetic acid (diluent) and sonicated for five min.; the solution was left to return to room temperature and brought to volume with diluent. Precision was evaluated with five injections of the working standard. A preparation of 0.1 mg/mL of phenylephrine HCL (preparation 1) was made by dropping two drug tablets with a label claim of 325/10/5 mg (acetaminophen/dextro-methorphan HBr/phenylephrine HCL) into a 100 mL vol. flask and proceeding as with the standard solution preparation. A second solution of 0.1 mg/mL of dextromethorphan HBr (preparation 2) was prepared by transferring 5.0 mL of the preceding solution into 10 mL vol. flask and to volume with diluent. A third sample of 0.3 mg/mL of acetaminophen (preparation 3) was prepared by pipetting 2.0 mL of preparation 1 into 50 mL volumetric flask and to volume with diluent. 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, 50 x 2.1 mm column with 2.7 µm superficially porous particle.

***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 2 µL; injector wash and carrier: water
PDA Detector:	Scanned from 190 – 700 nm, recording setting 275 nm Reference 400 nm, bandwidth 10 nm
UHPLC Column:	PerkinElmer Brownlee SPP C-18, 50 x 2.1 mm, 2.7 µm at 55 °C Cat. No. N9308402
Mobile Phase:	A: 20 mM Sodium Acetate B: Acetonitrile

Time (min)	Flow rate (mL/min)	B %	Curve
0.8	0.8	10	1
1	0.8	10-80	1
1.2	0.8	80	1

Three minutes equilibration after injection.

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

Results and discussion

The column temperature was set at 55 °C, and the flow rate at 0.8 mL/min. The pressure stabilized at about 5300 PSI and the three peaks eluted within 3 min. The resolution and sensitivity were outstanding (Figure 2, Page 3). Chromatograms from the three sample preparations: pheneylephrine prep., dextromethorphan prep., and acetaminophen prep. are shown in Figures 3, 4 and 5 (Page 3). In addition to the identification of peaks by retention time, as it is typically done in liquid chromatography, from the spectra obtained from the standard solution, a spectral library was created (Figure 6, Page 3) and used for peak identification confirmation in the sample, adding another level of confidence in the result.

The method has excellent precision with values ranging from 0.60 to 0.90 % RSD. Details of the method performance and results of the sample tested are presented in Table 2 (Page 3).

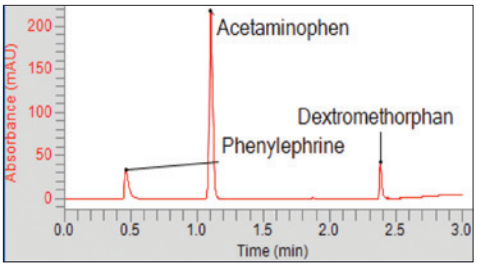

Figure 2. Analysis of the standard solution.

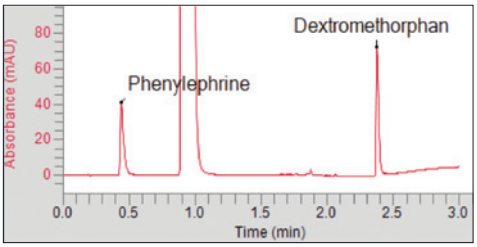

Figure 3. Tablet analysis for phenylephrine.

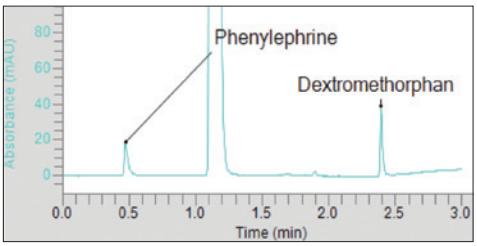

Figure 4. Tablet analysis for dextromethorphan.

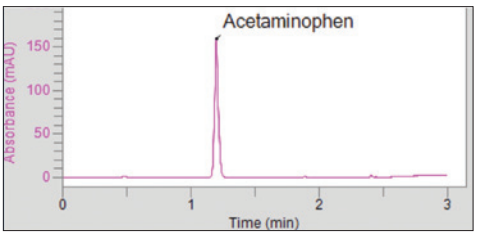

Figure 5. Tablet analysis for acetaminophen.

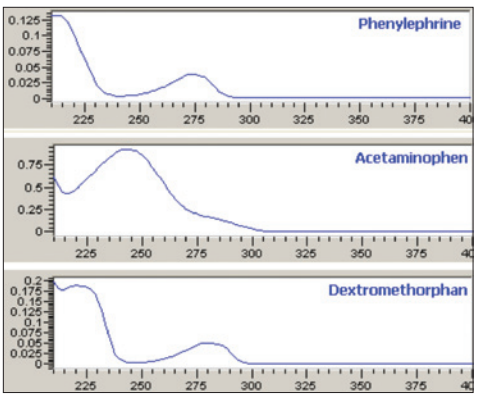

Figure 6. UV spectra from the standard solution run.

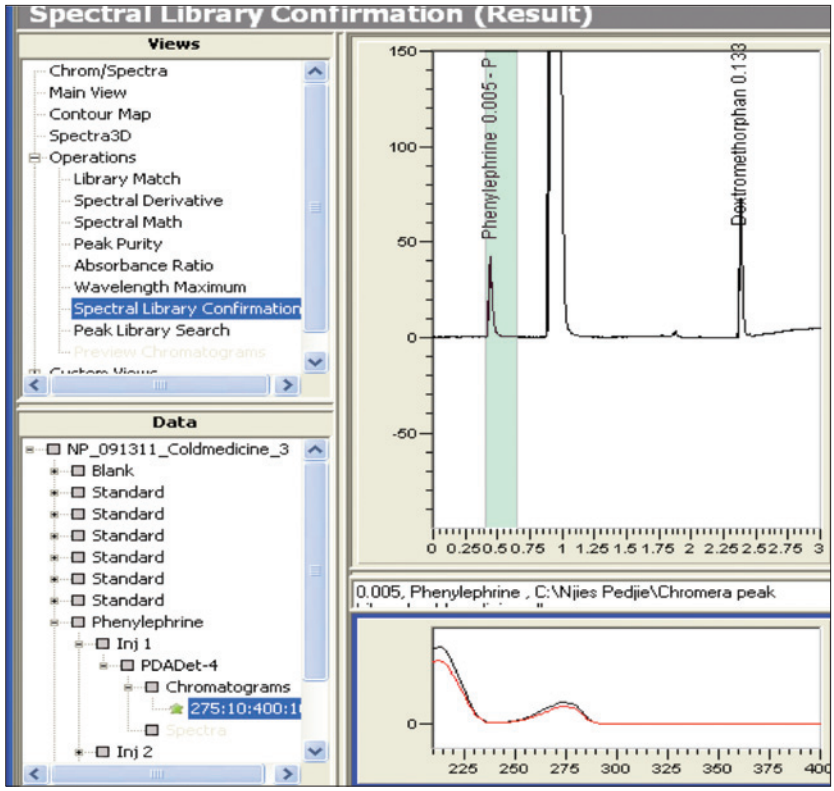

Figure 7. Peak identification in a solution of a cold medicine using the spectral library.

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

	%RSD (n=5)	Tablets (mg/tablets)	% of Label Claim
Acetaminophen	0.86	307	94.5
Dextromethorphan HBr	0.60	10	100.7
Phenylephrine HCL	0.90	5	106.4
Average	0.78	NA	100.5

Conclusion

PerkinElmer's Flexar FX-15 UHPLC system and the PerkinElmer Brownlee SPP C-18, 50 x 2.1 mm with 2.7 µm superficially porous particle resolved all three drug substance peaks within 3 min. The method was shown to be precise with an average % RSD of 0.78. The drug product tested has 307/10/5 mg of acetaminophen/dextromethorphan HBr/phenylephrine HCL per tablet for a label claim of 325/10/5 mg. These are well within the limit of not less than 90.0% and not more than 110.0% for each of the labeled amounts as specified in the corresponding USP monogram. Furthermore, instead of three different methods as called for by the USP monograms, the separation was achieved using a single method.

References

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Note: this application note is subject to change without prior notice.

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