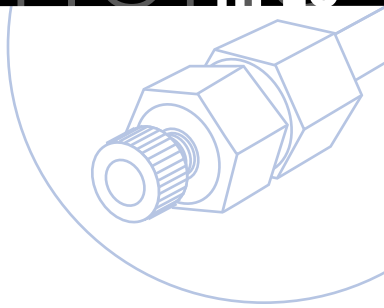

Isocratic Separation of Acetaminophen, Phenylephrine, Chlorpheneramine and Dextromethorphan on Gemini® C18

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Introduction

Over-the-Counter (OTC) cold remedies are widely used by the population at large and in 2006 alone, about four billion dollars worth of these medications were sold[1]. These compositions are complex mixtures, each component being effective for a specific symptom. One such popular remedy is a quaternary mixture, comprising phenylephrine hydrochloride (decongestant), acetaminophen (paracetamol, analgesic/antipyretic), chlorpheneramine maleate (antihistamine) and dextromethorphan hydrobromide (antitussive). The structures of these drugs are shown in **Figure 1**.

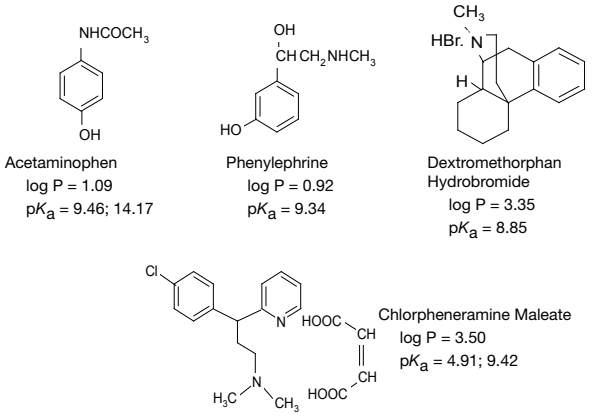

Figure 1. Structures and log P/pK_a values of the four ingredients of a cold remedy

This mixture is a conglomeration of neutral, acidic and basic compounds, together with the anions chloride and bromide. They also contain excipients (non-active ingredients) such as tapioca, glycerol, coloring agent, soft soap and water. As pointed out by Palabiyik and Onur in a recent publication[2], most of the published methods for quantitative analysis of these drugs deal with individual components or some combinations of them, but very few describe determination of all of them simultaneously. In their studies, Palabiyik and Onur used an ACE® C18 column (250 x 4.6 mm) (Advanced Chromatography Technologies, UK) and employed a mobile phase consisting of acetonitrile and perchlorate (0.01 M, pH 3.0) with a linear gradient from 5 % to 60 % organic in 8 min at a flow rate of 1.4 mL/min. In this note, we describe an isocratic method for the simultaneous determination of the four component cold remedy that can be used for high-throughput screening. For comparison, a gradient protocol and a two-step flow gradient under isocratic conditions are also explored.

Experimental Conditions

Individual components of the cold remedy mix were obtained from Sigma (St. Louis, Mo). All solvents used were HPLC grade from Fisher. Gemini C18 (150 x 4.6 mm, 5 µm) HPLC columns were from Phenomenex. An Agilent 1100 instrument equipped with a diode array detector, injector, degasser and a quaternary pump, and ChemStation™ (Rev. Version B. 02.01) was used for HPLC studies. Stock solutions of each analyte were prepared by dissolving about 1.0 mg in 1.0 mL of 5:95 acetonitrile/phosphate buffer (10 mM, pH 2.5). A mix of these analytes was prepared by adding 50 µL of the stock solution of each analyte to 800 µL of the same 5:95 binary solvent. For injection, 100 µL of this mix was diluted to 1.0 mL with the buffer and 30 µL of this diluted solution was injected.

For isocratic runs on the Gemini 150 x 4.6 mm column, a mobile phase of 60:24:16 phosphate buffer (10 mM, pH 2.5) /methanol/ acetonitrile was used at 1.0 mL/min with dual wavelength (215 nm and 280 nm) detection.

For the gradient run, the Gemini 150 x 4.6 mm column was used and a starting mobile phase of 5:95 acetonitrile/phosphate buffer (10 mM, pH 2.5) at a flow rate of 1 mL/min with the organic increasing to 60 % in 15 min and held for 2 min at this concentration. For the flow gradient, a mobile phase of 40:60 methanol/phosphate buffer (10 mM, pH 5.0) was used, with a flow rate of 0.8 mL/min from 0 to 3.9 min and then at 1.4 mL/min to 20 min. Detection wavelengths of 215 nm and 280 nm were used.

The log P and pK_a values reported in **Figure 1** were calculated using ChemAxon's MarvinSketch software.

Results and Discussion

Figure 2 shows a representative chromatogram on the Gemini C18 column (150 x 4.6 mm) under isocratic conditions. The entire run is completed in about 6.2 min. In addition to the well-resolved peaks for the four active ingredients of the cold remedy mix, three additional peaks are obtained, which correspond to maleic acid (from the maleate counter ion of chlorpheneramine), the chloride ion (counter ion from phenylephrine hydrochloride) and bromide ion (counter ion from dextromethorphan hydrobromide). Even these counter ion peaks are also well resolved in the chromatogram. An interesting feature is the earlier elution of the

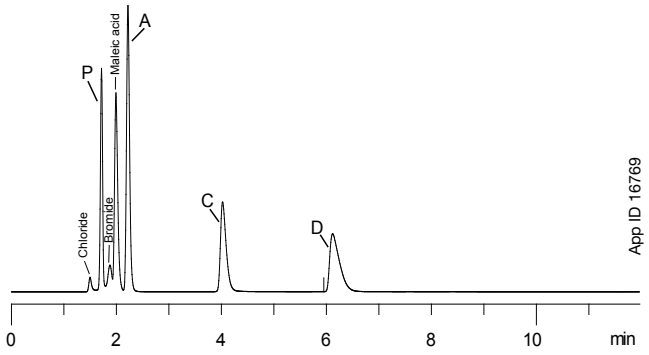

Figure 2: Isocratic elution of the quaternary cold remedy mix on Gemini C18 (150 x 4.6 mm, 5 µm) using 60:24:16 phosphate buffer (pH 2.5)/methanol/acetonitrile at 1.0 mL/min, detection at 215 nm (elution order: chloride ion, phenylephrine, bromide ion, maleic acid, acetaminophen, chlorpheneramine and dexotromethorphan)

more hydrophobic chlorpheneramine in comparison with the slightly less hydrophobic dextromethorphan (see **Figure 1** for log P values). This is attributable to the fact that the chlorpheneramine molecule has two basic nitrogens which can be protonated, while the dextromethorphan has only one protonatable basic site. Since protonation makes the molecules considerably more polar, chlorpheneramine elutes earlier than dextromethorphan. Phenylephrine and acetaminophen follow elution order in consonance with their hydrophobicities. The maleic acid peak does not show up in the chromatogram using 280 nm detection wavelength.

The gradient run employing % acetonitrile change from 5 to 60 % is shown in **Figure 3**. The entire run is complete in about 10 min. The acetaminophen is retained considerably longer under gradient conditions, but apart from this feature, there is no additional advantage in using these conditions over the isocratic run.

The gradient run data on the Gemini C18 (150 x 4.6 mm) column is shown in **Figure 4**. Under these conditions, a single run takes about 16 min, but overall the mobile phase is the same (isocratic). We have determined that a change of flow affects the peak area of the early eluting peaks only and does not alter the area of the late eluting peaks. So, if this method is to be chosen, the flow should not be changed until the polar components (which elute early under RP-HPLC conditions) have eluted from the column.

Marin and Barbas[3] investigated the retention of cold remedy ingredients (excluding dextromethorphan) on five HPLC stationary phases with 20:80 acetonitrile/phosphate buffer (pH 2.5 or 4.6 or 7.0). Of particular interest to this note is their observation that on Discovery® C18 (Sigma-Aldrich Biotechnology, St. Louis, MO), the retention of acetaminophen and phenylephrine does not change with pH, while that of chlorpheneramine increases with increase in pH. We studied an isocratic elution on the Gemini C18 (150 x 4.6 mm) column, using a mobile phase of 50:50 methanol/ phosphate buffer, pH 5.0 at 1.0 mL/min. The chromatogram presented in **Figure 5** shows that a shift from pH 2.5 (**Figure 2**) to 5.0 does not increase retention, assuming that the organic strength of the mobile phase is roughly the same in both.

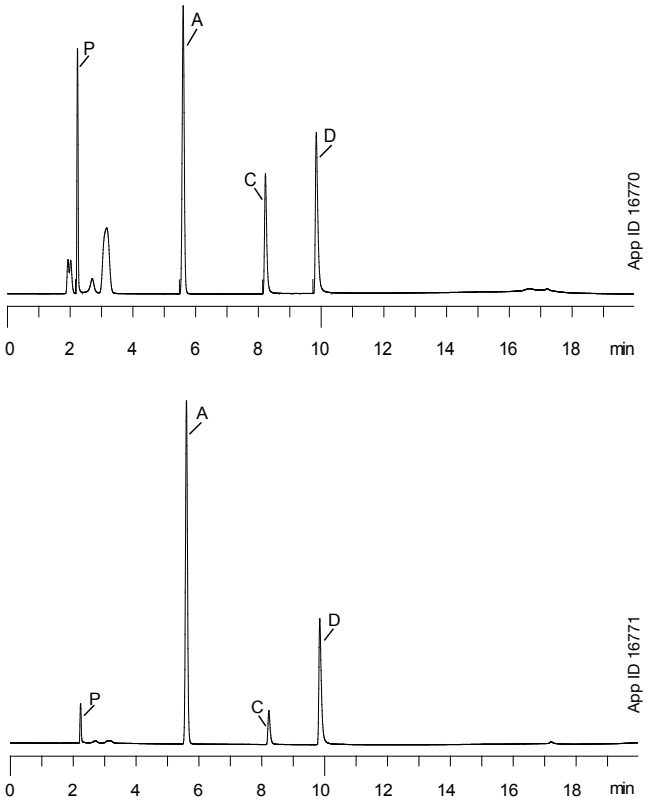

Figure 3: Gradient elution of the quaternary cold remedy mix on Gemini C18 (150 x 4.6 mm, 5 µm) with 5:95 acetonitrile/ phosphate buffer (pH 2.5) with gradient from 5 % to 60 % acetonitrile in 20 min, 1.0 mL/min; detection: 215 nm (top) and 280 nm (bottom) (elution order: phenylephrine=P; acetaminophen=A; chlorpheneramine=C; and dextromethorphan=D)

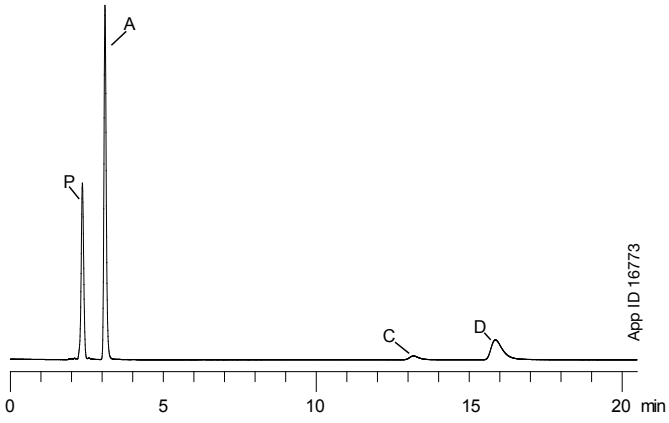

Figure 4: Gradient run on Gemini C18 (150 x 4.6 mm, 5 µm), with 40:60 methanol/ phosphate buffer (pH 5.0); 0.8 mL/min from 0 to 3.9 min, then 1.2 mL/min, detection at 280 nm (elution order: phenylephrine=P; acetaminophen=A; chlorpheneramine=C; and dextromethorphan=D)

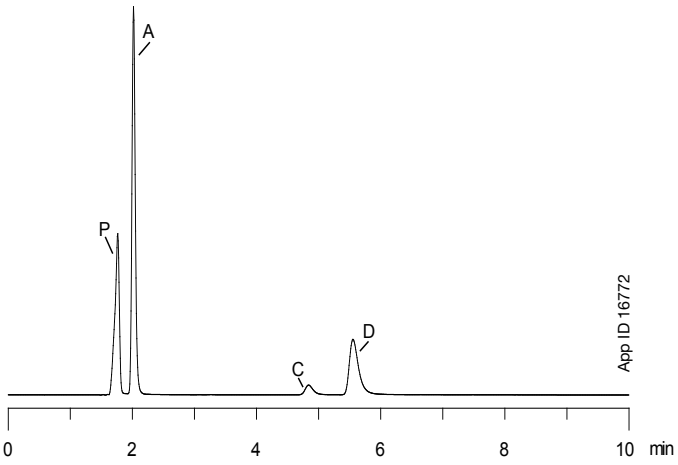

Figure 5: Isocratic run on Gemini C18 (150 x 4.6 mm, 5 µm) with 50:50 methanol/ phosphate buffer (pH 5.0); flow rate 1.0 mL/min; detection at 280 nm (elution order: phenylephrine=P; acetaminophen=A; chlorpheneramine=C; and dextromethorphan=D)

Conclusion

The results obtained demonstrate that Gemini C18 is suitable for separation of complex cold remedy mixtures under isocratic conditions and satisfies high-throughput requirements.

References

1. **Drug Stores News, Web Edition, March 2007**.
2. I.M. Palabiyik and F. Onur, **Chromatographia Suppliment,** 2007, **66**, S93-S96.
3. A. Marin and C. Barbas, **J. Pharm. Biomed. Anal.,** 2006, **40**, 262-270.

ORDERING INFORMATION

Part No.	Description
00F-4435-E0-TN	Gemini 5 µm C18, 150 x 4.6 mm
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