The Separation of Popular OTC Cold, Sinus, Allergy and Sleep Medications using TSK-GEL ODS-100V and TSK-GEL ODS-100Z Columns

Bruce Kempf, Atis Chakrabarti,

Shigeru Nakatani, and J. Kevin O'Donnell

Tosoh Bioscience LLC

www. tosohbioscience.com

Abstract

   Recently the FDA made changes to the regulation of drugs containing the popular decongestant pseudoephedrine. These changes resulted in many pharmaceutical companies reformulating their products with phenylephrine as a substitute.

   To address the need to reformulate and revalidate these test methods, Tosoh Bioscience LLC developed application data using the newly introduced, highly adaptable TSK-GEL ODS-100V and TSK-GEL ODS-100Z reversed phase columns to separate phenylephrine from some of the most common combinations of cold and sinus medications on the market today. We compared the separations of phenylephrine, acetaminophen, doxylamine, chlorpheniramine, dextromethorphan, and diphenhydramine on both the 3μm and 5μm 4.6mm X 15cm TSK-GEL ODS-100V columns as well as on the 5μm 4.6mm X 15cm TSKgel ODS-100Z column.

   Samples compounds were adsorbed at 96% mobile phase A (0.15% TFA in water) and 4% mobile phase B (0.02% TFA in 75% ACN and 25% methanol) and eluted during a 15 minute linear gradient to 40% mobile phase A and 60% mobile phase B. We were able to achieve sharp peaks that exhibited excellent separation and high theoretical plates on all three columns tested.

Methods

- A mixture of six common cold, sinus and analgesic medications were separated in this study utilizing commercially available ODS columns.

- The analytes were high-purity Sigma-brand standards which were individually tested to determine their respective retention times. All analytes were then mixed into a single ~16μg/mL solution.

- Initial experimental conditions were identified by modifying a published method. The gradient and instrument conditions were extensively altered to allow diphenhydramine to separate from dextromethorphan, which are similar in their hydrophobic properties.

- After determining that the TSKgel ODS-100V, 4.6mm X 15cm, 3μm column gave superior performance for the cold mixture, several additional analytes were evaluated on this column and shown to separate well.

- The separation of popular cold, sinus, allergy, and sleep OTC medicines using a TSKgel ODS-140HTP, 2.3μm, 2.1mm × 10cm column is also shown.

Phenylephrine HCl (CAS# 61-76-7, FW=203.67): Nasal decongestant that is currently replacing pseudoephedrine in many cold & sinus products due to recent restrictions by the FDA

Acetaminophen (CAS#103-90-2, FW=151.17): Analgesic, antipyretic used in pain and fever reducing products

Doxylamine succinate (CAS#562-10-7, FW=388.5): Antihistamine/sedative used as a sleep aid in many OTC products

Chlorpheniramine maleate (CAS#113-92-8, FW=390.5): Antihistamine used in many cold & sinus products

Dextromethorphan HBr (CAS#6700-34-1, FW=370.3): Cough suppressant

Diphenhydramine HCl (CAS#147-24-0, FW=291.82): Antihistamine/sedative used for allergies and sleep

All standards were purchased from Sigma.

Common OTC products evaluated: Excedrine®, Robitussin® DM, Histenol-Forte®II, pseudoephedrine.

Stock solutions for each of the commercial products, depending on dosage form were prepared as follows: one tablet or 5mL was placed into a 100mL volumetric flask and diluted to volume with (50:50) methanol/water and mixed until dissolved. An aliquot of each stock solution (Excedrin 1.5mL), (Robitussin DM 8.0mL), (Histenol Forte-II 10.0mL) and (pseudoephedrine 15.0mL) was filtered through a 0.45µm syringe filter and diluted to 25mL with the (50:50) methanol/water diluent.

Table 1: Column Properties

This table shows the properties and specifications of the analytical columns tested in the current study.

	TSKgel ODS-100V	TSKgel ODS-100Z	TSKgel ODS-140HTP	Supplier-1	Supplier-2	Supplier-3
Carbon Content	15%	20%	8%	20%	18%	8%
Endcapped	Yes	Yes	Yes	Yes	Yes	Yes
Particle Size (µm)	3 and 5.5	5.5	2.3	3.5	3.0	3.5
Pore Size (Å)	100	100	140	100	100	95
Preferred Sample Type	Polar	Hydrophobic	Hydrophobic	Not Specified	Not Specified	Not Specified
Bonded Phase Structure	Monolayer	Monolayer	Polymeric	Not Specified	Not Specified	Not Specified
Specific Surface Area (m²/g)	450	450	100	352	426	160
*Asymmetry Factor **USP Tailing Factor	0.90 - 1.15 *(Naphthalene)	0.90 - 1.15 *(Naphthalene)	0.90 - 1.3	1.04 **(Acenaphthene)	1.01 **(Naphthalene)	1.03 **(Toluene)
Theoretical Plates	>14,000 (Naphthalene)	>14,000 (Naphthalene)	>14,000	18,997 (Acenaphthene)	20,041 (Naphthalene)	24,888 (Toluene)

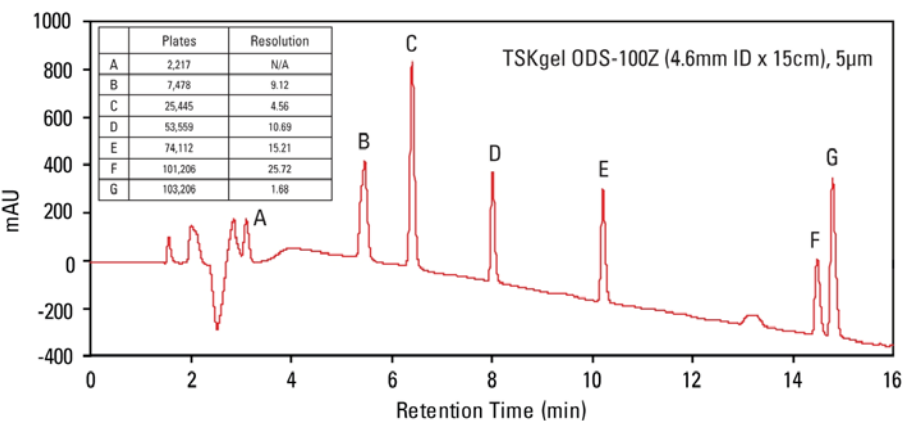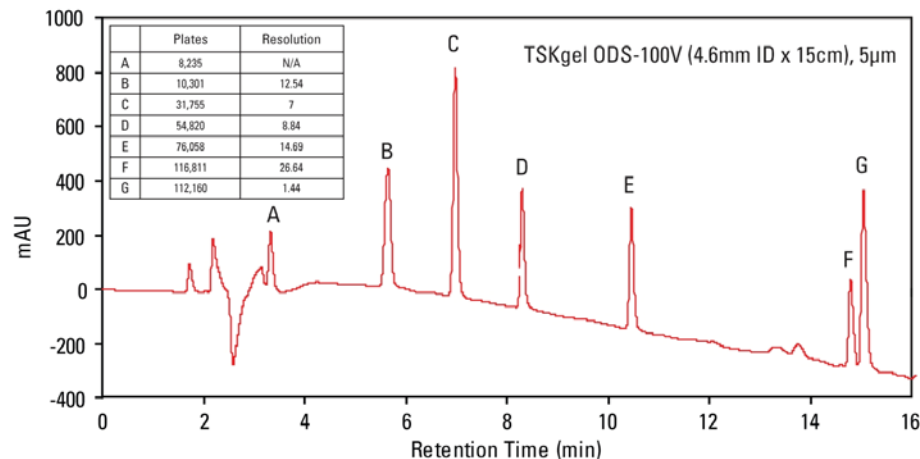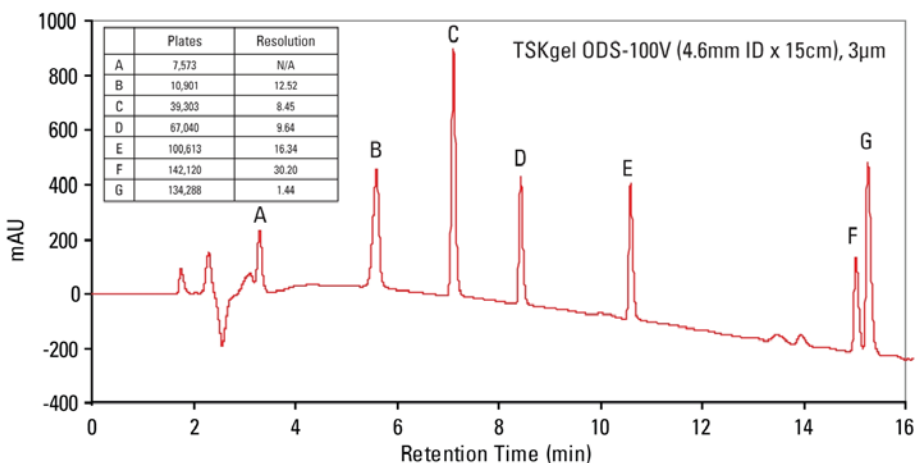# Figure 1: Initial Test Method on the TSKgel ODS-100V, 3µm Column
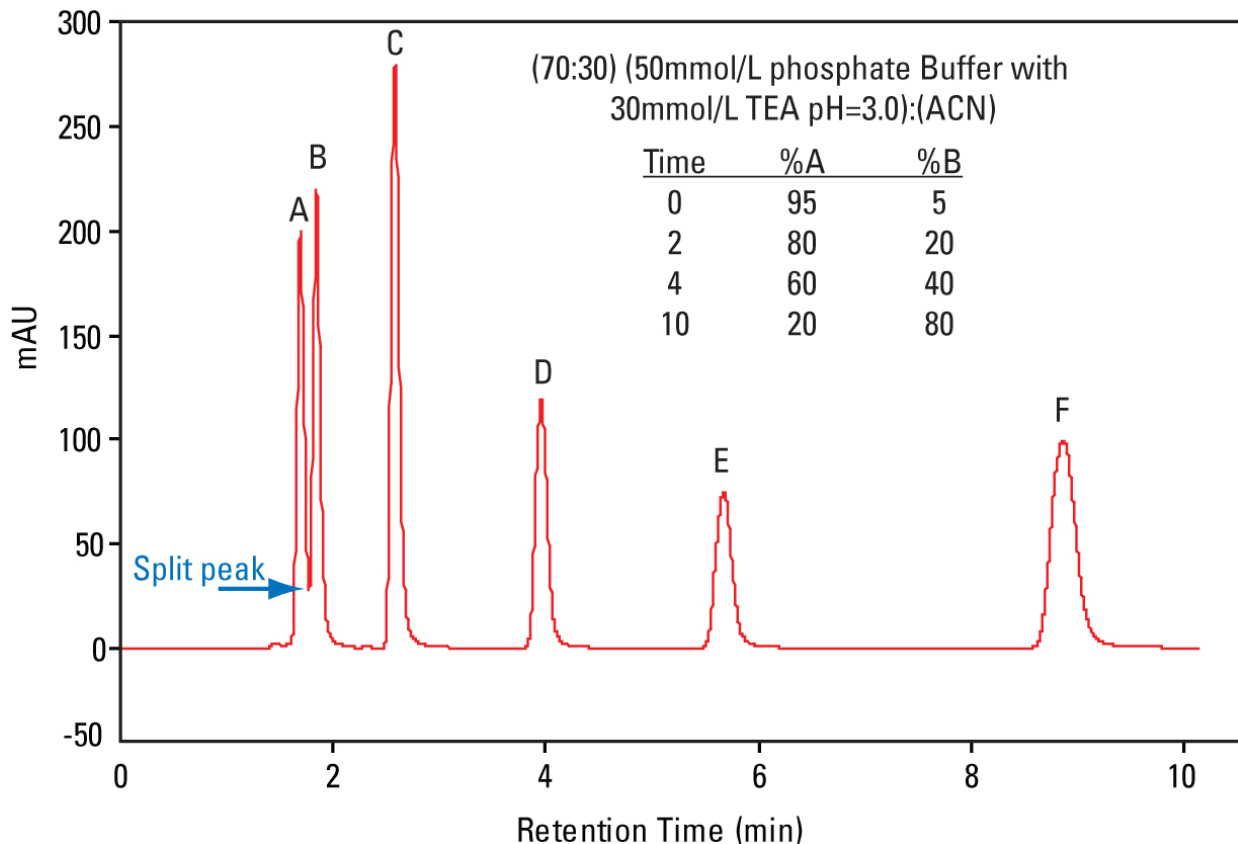
Key to Chromatograms (peak labels)

A -- Maleate

B -- Phenylephrine HCl

C -- Acetaminophen

D -- Doxylamine succinate

E -- Chlorpheniramine

F -- Dextromethorphan HBr

G -- Diphenhydramine HCl

Initial method development was performed using a (70:30) (50mmol/L potassium phosphate buffer with 30mmol/L TEA pH=3.0):ACN. Difficulty was encountered getting the maleate salt peak to separate from the chlorpheniramine maleate compound. This mobile phase was eventually abandoned because adequate separation could not be achieved by altering the gradient. The above chromatogram was the most optimal result we could achieve with this mobile phase composition. NOTE: Dextromethorphan was not included in this mixture.

Figure 2: The Separation of Cold Mixture on the TSK-GEL Columns

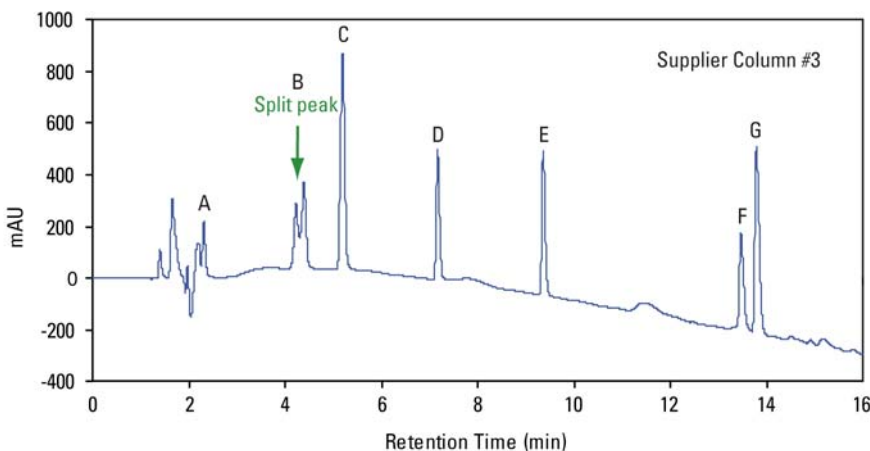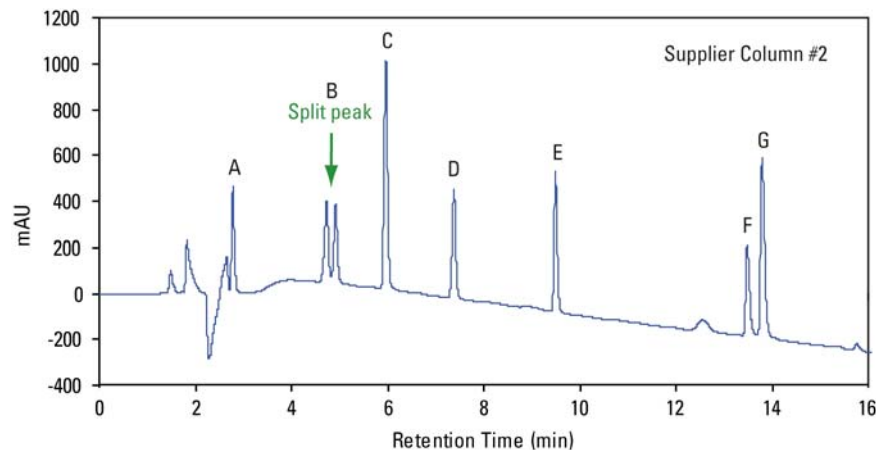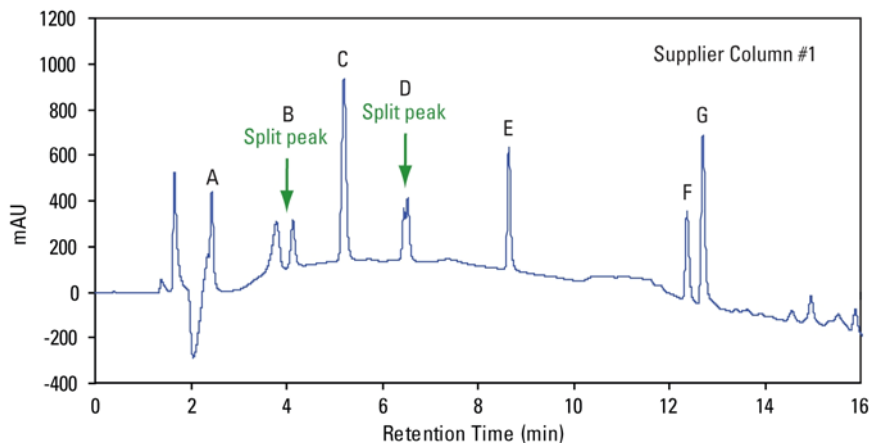**Key to Chromatograms (peak labels)**

A – Maleate B -- Phenylephrine HCl C – Acetaminophen D -- Doxylamine succinate

E – Chlorpheniramine F -- Dextromethorphan HBr G -- Diphenhydramine HCl

Instrument:	HP-1100 series Chemstation
Detection:	UV@210 nm
Column Temp:	40°C
Flow rate	1.0mL/min
Injection Volume:	20µl
Run Time:	17minutes
Mobile Phase A:	0.15% TFA in water
Mobile Phase B:	0.02% TFA in: (75:25) ACN:Methanol
Sample/Std Diluent:	(50:50) Methanol:Water
Gradient:	0min (96%A, 4%B) 15min (40%A, 60%B) 17min (40%A, 60%B)

This figure shows the chromatographic separation of a cold mixture containing six common components. The TSKgel ODS-100V, 3µm column had slightly better resolution than the TSKgel ODS-100V, 5µm or the more hydrophobic TSKgel ODS-100Z column.

Key to Chromatograms (peak labels)

A – Maleate B -- Phenylephrine HCl C – Acetaminophen D -- Doxylamine succinate

E – Chlorpheniramine F -- Dextromethorphan HBr G -- Diphenhydramine HCl

Instrument:	HP-1100 series Chemstation
Detection:	UV@210 nm
Column Temp:	40°C
Flow rate	1.0mL/min
Injection Volume:	20μl
Run Time:	17minutes
Mobile Phase A:	0.15% TFA in water
Mobile Phase B:	0.02% TFA in: (75:25) ACN:Methanol
Sample/Std Diluent:	(50:50) Methanol:Water
Gradient:	0min (96%A, 4%B) 15min (40%A, 60%B) 17min (40%A, 60%B)

This figure shows three commercially available ODS columns of the same dimensions separating the same cold mixture with the same method and conditions. It can be seen that all of the three columns were unable to produce a single phenylephrine peak but instead produced two smaller peaks that have approximately half the area as the single peak produced on the TSK-GEL columns. Also, one of the columns exhibited peak-splitting on the doxylamine peak.

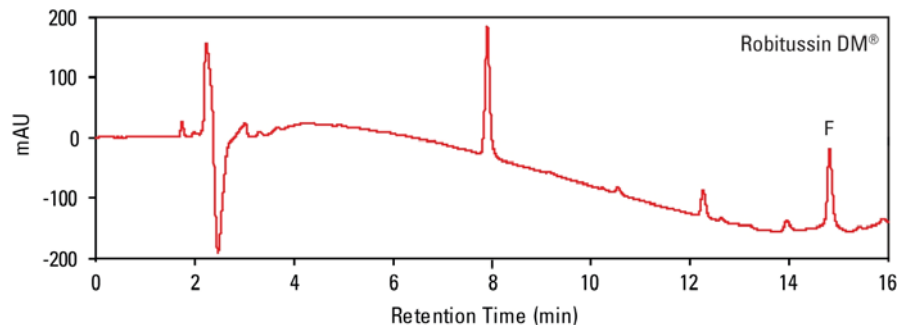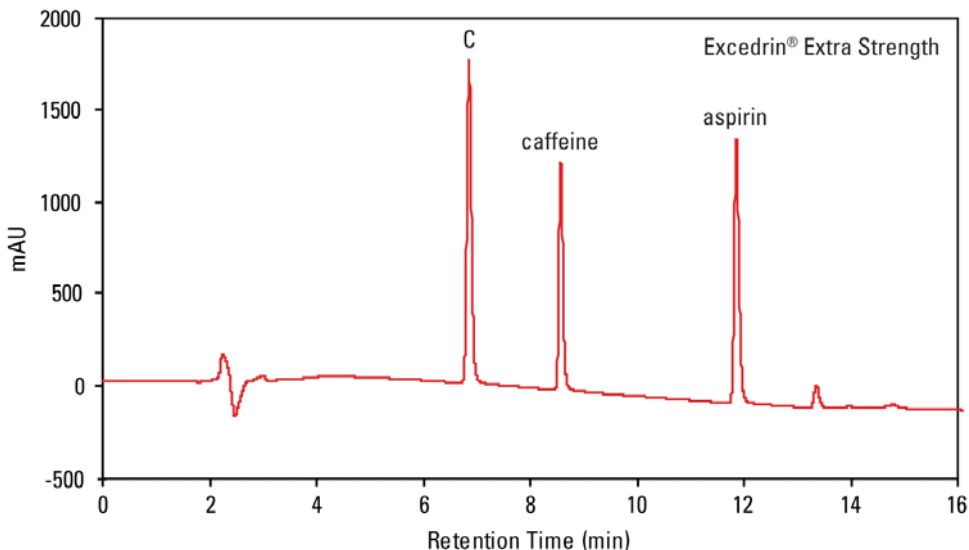# Table 2: Retention Time* Comparison of Cold Mixture on Each 4.6mm ID x 15cm Column

This table shows a summary of the retention time results for each component tested on the columns used in the study.

	TSKgel ODS-100V 4.6 mmID x 15cm, 3μm	TSKgel ODS-100V 4.6 mmID x 15cm, 5μm	TSKgel ODS-100Z 4.6 mmID x 15cm, 5μm	Supplier-1 4.6 mmID x 15cm, 3.5μm	Supplier-2 4.6 mmID x 15cm, 3μm	Supplier-3 4.6 mmID x 15cm, 3.6μm
Maleate	3.30	3.33	3.10	2.44	2.78	2.31
Phenylephrine	5.58	5.64	5.48	3.79 & 4.13 (Split)	4.72 & 4.92 (Split)	4.22 & 4.39 (Split)
APAP	7.11	6.98	6.41	5.20	5.96	5.19
Doxylamine	8.43	8.30	8.01	6.53 (Split)	7.38	7.17
Chlorpheniramine	10.59	10.46	10.21	8.64	9.49	9.36
Dextromethorphan	15.03	14.80	14.48	12.37	13.48	13.47
Diphenhydramine	15.26	15.05	14.79	12.70	13.79	13.79

*Retention time is reported in minutes.

Figure 4: Common OTC Products on a TSKgel ODS-100V, 3μm Column
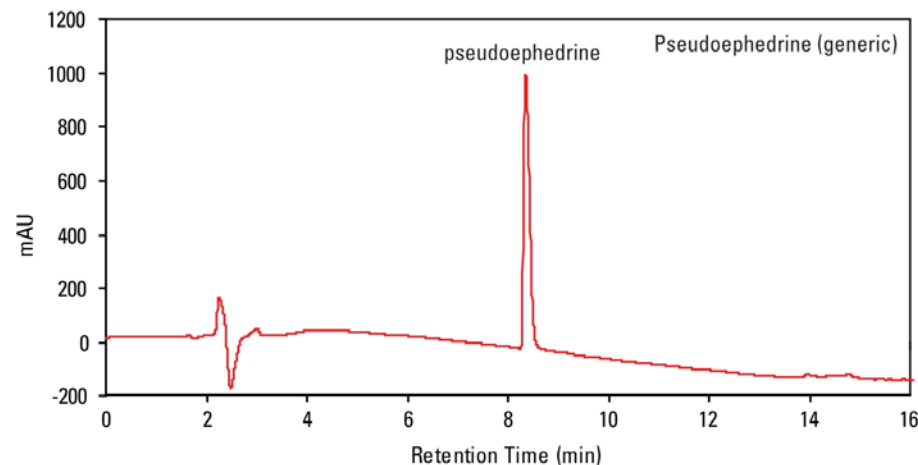

Key to Chromatograms (peak labels)
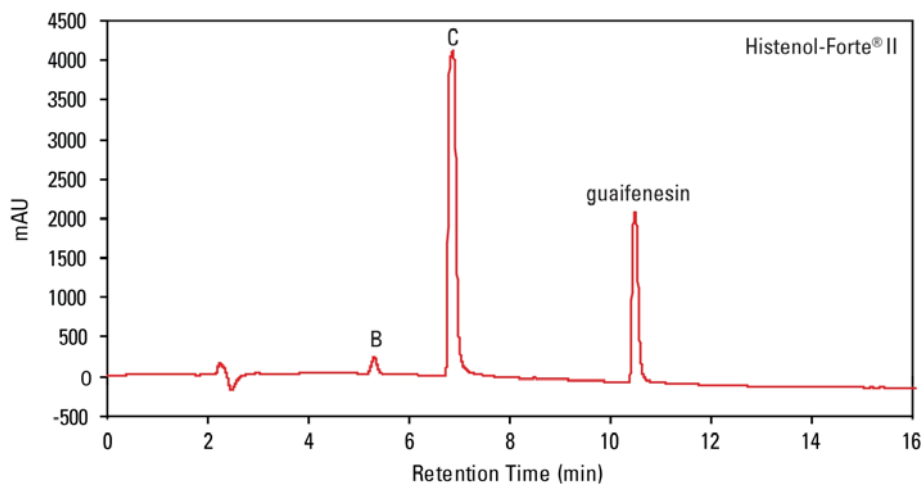
A – Maleate B -- Phenylephrine HCl

C – Acetaminophen D -- Doxylamine succinate

E – Chlorpheniramine F -- Dextromethorphan HBr

G -- Diphenhydramine HCl

Instrument:	HP-1100 series Chemstation
Detection:	UV@210 nm
Column Temp:	40°C
Flow rate	1.0mL/min
Injection Volume:	20μl
Run Time:	17minutes
Mobile Phase A:	0.15% TFA in water
Mobile Phase B:	0.02% TFA in: (75:25) ACN:Methanol
Sample/Std Diluent:	(50:50) Methanol:Water
Gradient:	0min (96%A, 4%B) 15min (40%A, 60%B) 17min (40%A, 60%B)

This figure shows two common over-the-counter cold, sinus and analgesic products nicely separated on a TSKgel ODS-100V, 3μm column.

Histenol-Forte® II

Pseudoephedrine (generic)

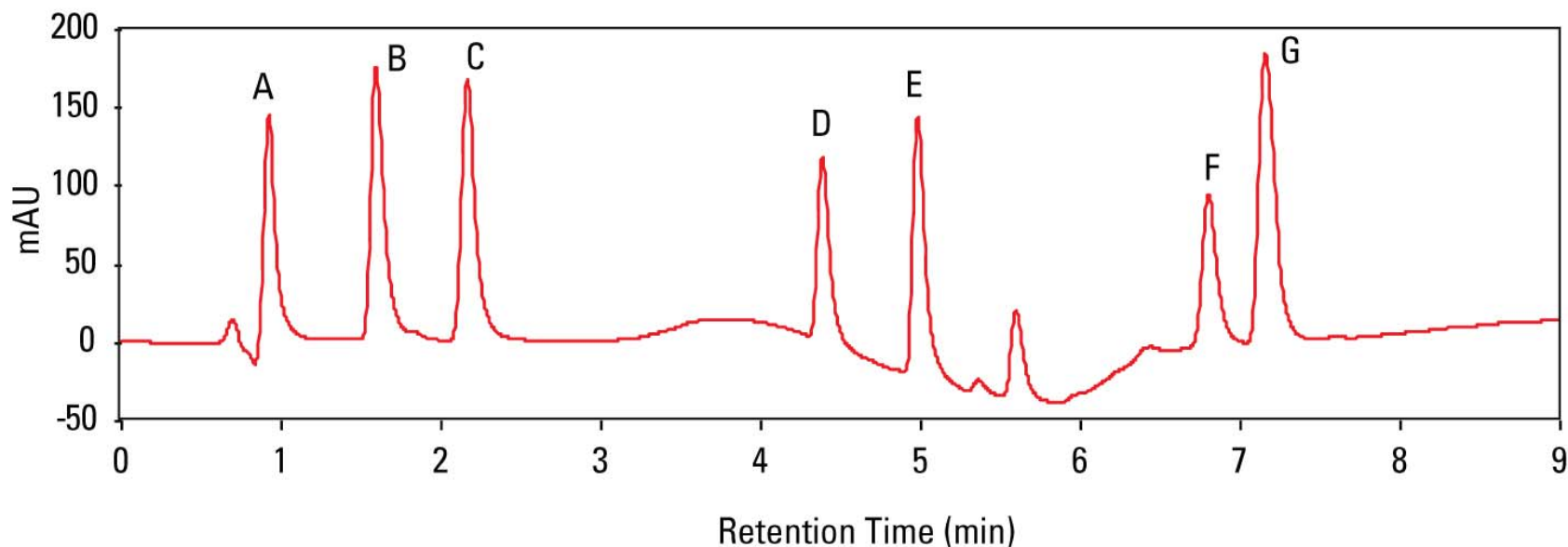**Key to Chromatograms (peak labels)**

A – Maleate B -- Phenylephrine HCl

C – Acetaminophen D -- Doxylamine succinate

E – Chlorpheniramine F -- Dextromethorphan HBr

G -- Diphenhydramine HCl

Instrument: HP-1100 series Chemstation
Detection: UV@210 nm
Column Temp: 40°C
Flow rate   1.0mL/min
Injection Volume: 20µl
Run Time: 17minutes
Mobile Phase A: 0.15% TFA in water
Mobile Phase B: 0.02% TFA in: (75:25) ACN:Methanol
Sample/Std Diluent: (50:50) Methanol:Water
Gradient: 0min (96%A, 4%B) 15min (40%A, 60%B)
                        17min (40%A, 60%B)

This figure shows two common over-the-counter cold, sinus and analgesic products nicely separated on a TSKgel ODS-100V, 3µm column.

Key to Chromatograms (peak labels)

A – Maleate B -- Phenylephrine HCl

C – Acetaminophen D -- Doxylamine succinate

E – Chlorpheniramine F -- Dextromethorphan HBr
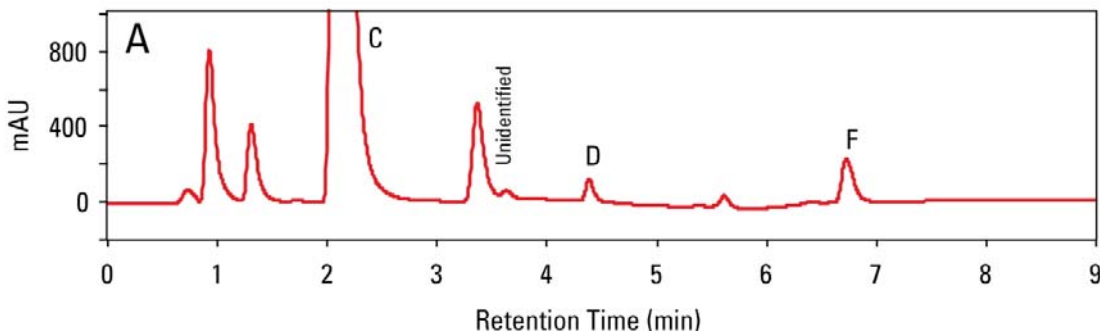
G -- Diphenhydramine HCl

Instrument: HP-1100 series Chemstation
Detection: UV@215 nm
Column Temp: 40°C
Flow rate: 0.4mL/min
Injection Volume: 10μl (of 1/5 dilution of previous sample)
Run Time: 9 minutes
Mobile Phase A: 0.15% TFA in water.
Mobile Phase B: 0.02% TFA in: (75:25) ACN:Methanol
Sample/Std Diluent: mobile phase A
Gradient: 0min (96%A, 4%B) 2min (65%A, 35%B) 4min (65%A, 35%B) 9min (40%A, 60%B)
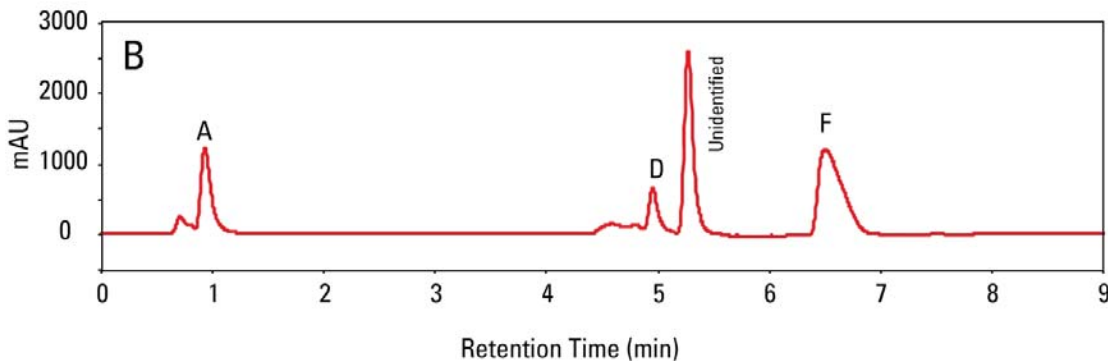   12min (40%A, 60%B) 12.1min (96%A, 4%B) 15min (96%A, 4%B)

Vicks MyQuil®: Each 15 ml contains: Acetaminophen 500 mg, Dextromethorphan 15 mg, Doxylamine Succinate 6.25 mg

Key to Chromatograms (peak labels)

A – Maleate B -- Phenylephrine HCl

C – Acetaminophen D -- Doxylamine succinate

E – Chlorpheniramine F -- Dextromethorphan HBr

G -- Diphenhydramine HCl

Pediatric Robitussin®: Each 5 ml contains: Chlorpheniramine Maleate 1.0 mg, Dextromethorphan HBr 7.5 mg
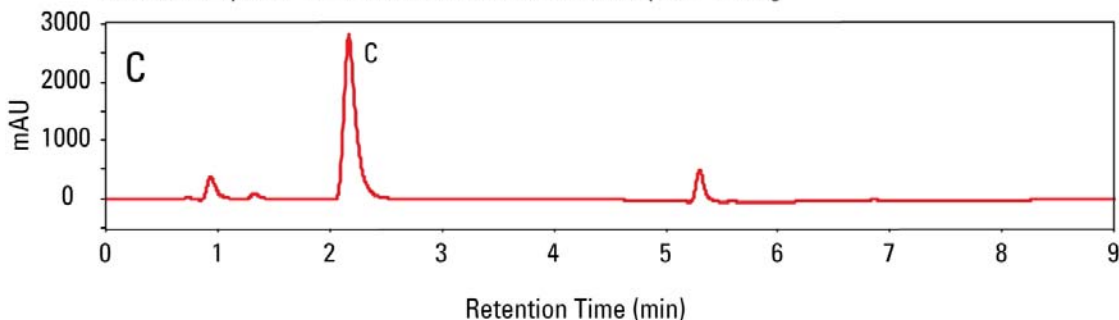

Instrument:	HP-1100 series Chemstation
Detection:	UV@215 nm
Column Temp:	40°C
Flow rate:	0.4mL/min
Injection Volume:	10μl (of 1/5 dilution of previous sample)
Run Time:	9 minutes
Mobile Phase A:	0.15% TFA in water.
Mobile Phase B:	0.02% TFA in: (75:25) ACN:Methanol
Sample/Std Diluent:	mobile phase A
Gradient:	0min (96%A, 4%B) 2min (65%A, 35%B) 4min (65%A, 35%B) 9min (40%A, 60%B) 12min (40%A, 60%B) 12.1min (96%A, 4%B) 15min (96%A, 4%B)

Stock solutions for each of the commercial products were prepared by diluting the liquid medicine or dissolving the tablet in water containing 0.1 % TFA to a suitable concentration of ~ 100 - 300μg/ml, depending on the nature of drug composition.

Children's Tylenol®: Each 5 ml contains: Acetaminophen 160 mg

Key to Chromatograms (peak labels)

A – Maleate B -- Phenylephrine HCl

C – Acetaminophen D -- Doxylamine succinate

E – Chlorpheniramine F -- Dextromethorphan HBr

G -- Diphenhydramine HCl

Tylenol ®: Each 5 ml contains: Acetaminophen 500 mg

Instrument:	HP-1100 series Chemstation
Detection:	UV@215 nm
Column Temp:	40°C
Flow rate:	0.4mL/min
Injection Volume:	10µl (of 1/5 dilution of previous sample)
Run Time:	9 minutes
Mobile Phase A:	0.15% TFA in water.
Mobile Phase B:	0.02% TFA in: (75:25) ACN:Methanol
Sample/Std Diluent:	mobile phase A
Gradient:	0min (96%A, 4%B) 2min (65%A, 35%B) 4min (65%A, 35%B) 9min (40%A, 60%B) 12min (40%A, 60%B) 12.1min (96%A, 4%B) 15min (96%A, 4%B)

Stock solutions for each of the commercial products were prepared by diluting the liquid medicine or dissolving the tablet in water containing 0.1 % TFA to a suitable concentration of ~ 100 - 300µg/ml, depending on the nature of drug composition.

Figure 6 Con't: Analysis of Cold and Sinus Drug OTC Medicine using TSKgel ODS-140HTP, 2.3μm Column
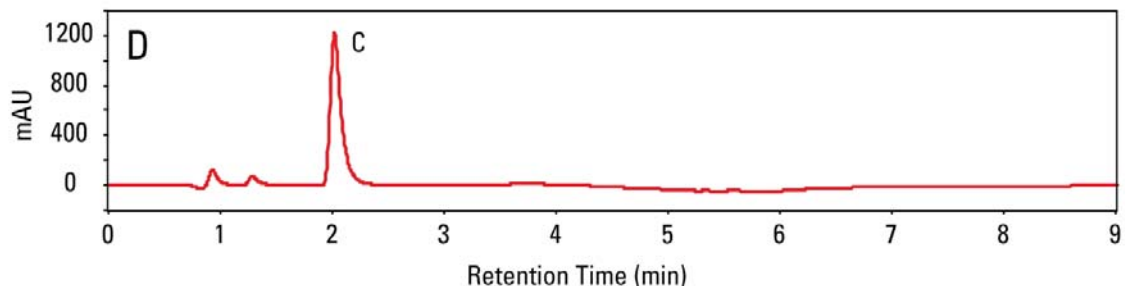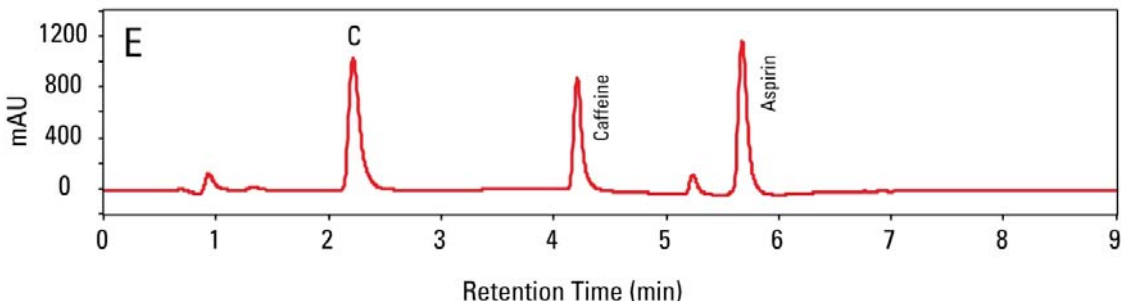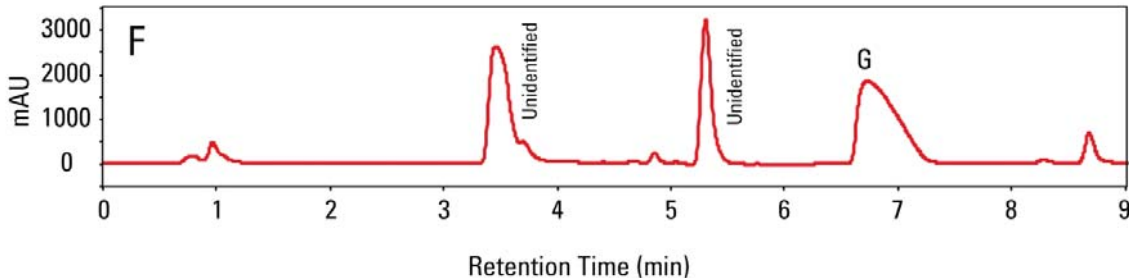
Excedrine®: Each Tablet contains: Acetaminophen 250 mg, Aspirin 250 mg, Caffeine 65 mg

Key to Chromatograms (peak labels)

A – Maleate B -- Phenylephrine HCl

C – Acetaminophen D -- Doxylamine succinate

E – Chlorpheniramine F -- Dextromethorphan HBr

G -- Diphenhydramine HCl

Q-Dryl®: Each 5 ml contains: Diphenhydramine Hydrochloride 12.5 mg

Instrument:	HP-1100 series Chemstation
Detection:	UV @ 215 nm
Column Temp:	40°C
Flow rate:	0.4mL/min
Injection Volume:	10μl (of 1/5 dilution of previous sample)
Run Time:	9 minutes
Mobile Phase A:	0.15% TFA in water.
Mobile Phase B:	0.02% TFA in: (75:25) ACN:Methanol
Sample/Std Diluent:	mobile phase A
Gradient:	0min (96%A, 4%B) 2min (65%A, 35%B)
	4min (65%A, 35%B) 9min (40%A, 60%B)
	12min (40%A, 60%B) 12.1min (96%A, 4%B)
	15min (96%A, 4%B)

Stock solutions for each of the commercial products were prepared by diluting the liquid medicine or dissolving the tablet in water containing 0.1 % TFA to a suitable concentration of ~ 100 - 300μg/ml, depending on the nature of drug composition.

EAS 2008, Somerset, NJ, November 19

15

Conclusions

1) The TSKgel ODS-100V 3μm column performed better than TSKgel ODS-100Z and other commercially available columns for the separation of all six cold products.

(2) The three TSK-GEL ODS columns produced a single component peak for the analysis of phenylephrine as well as doxylamine, both of which had difficulty on some of the competitive columns.

(3) The TSKgel ODS-100V 3μm column provided sharp peaks.

(4) All compounds were resolved by TSK-GEL ODS-100V and ODS-100Z columns in less than 17 minutes.

(5) The TSKgel ODS-140HTP, 2.3μm column can be effectively used in conventional HPLC for the quick and high-throughput separation of cold standards and a number of OTC medicines.

(6) Sharp peaks and considerably low retention times of less than 8 minutes were achieved by a TSKgel ODS-140HTP column.