# The Separation of Popular Cold, Sinus, and Allergy Medications using TSK-GEL ODS-140HTP Columns

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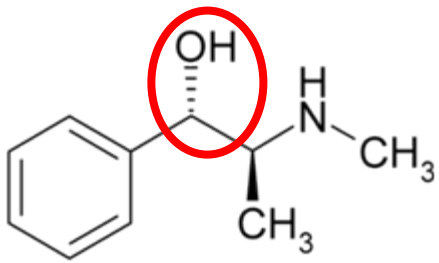
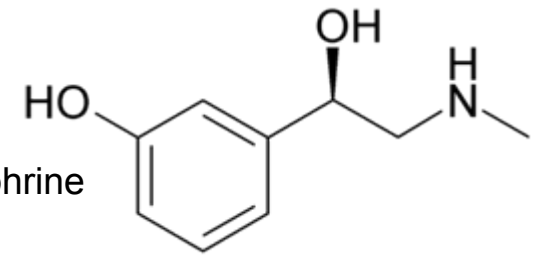
# Introduction

- Since the USA Patriot Act was enacted, many pharmaceutical companies have reformulated their over the counter (OTC) drug products with phenylephrine (a nasal decongestant) as a substitute for pseudoephedrine.
- Phenylephrine comes as a tablet, a liquid, or a dissolving strip to take orally – all as a treatment for cold symptoms.
- In addition to phenylephrine, most pharmaceutical formulations for common cold and sinus medications often contain multiple active ingredients to treat different types of symptoms along with numerous excipients.
- From an analytical perspective, the challenge is to develop chromatographic conditions that allow high throughput, quantitative analysis of a variety of excipients that vary widely in hydrophobic properties.

Pseudoephedrine




Phenylephrine



# Introduction Continued

- We used a TSKgel ODS-140HTP, 2.3µm, 2.1mm ID x 5cm reversed phase column to address the need to revalidate the test methods for new phenylephrine formulations with the overall goal to reduce retention time of the APIs (active pharmaceutical ingredients).
- Six different drug standards were selected covering a wide range of hydrophobicities, all of which are commonly used as APIs in many cold and sinus medicines.
- We report for the first time the separation of these six drug standards within 4 minutes using a conventional HPLC system.
- We also report the analysis of four OTC drugs: Children's Tylenol®, Q-dryl®, Pediatric Robitussin® and Excedrin®.
- High throughput separation of the active pharmaceutical ingredients using a conventional HPLC with an upper pressure limit of 41.4MPa is valuable in method development for the identification of drugs utilizing LC/MS .

# Material and Methods

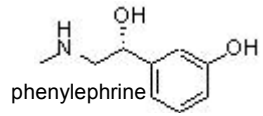
- High purity Sigma brand chemicals were used for the preparation of stock solutions of phenylephrine, acetaminophen, doxylamine succinate, chlorpheniramine maleate, dextromethorphan hydrobromide and diphenhydramine hydrochloride in 50% methanol (HPLC grade from Fisher).

- To avoid solvent mismatch at the time of injection, the working standards were prepared by diluting the stock standards in mobile phase A. Similarly, Children's Tylenol, Q-dryl, Pediatric Robitussin, and Excedrin were also diluted in mobile phase A without any other pre-column treatment prior to injection.

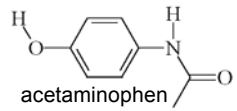
- The final concentration of each drug standard was 0.8µg/mL in the drug cocktail. The standards and samples were filtered through a 0.45µm membrane prior to injection.

# Material and Methods Continued

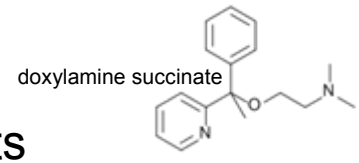
- **phenylephrine HCl** (CAS# 61-76-7, FW=203.67)
  Nasal decongestant



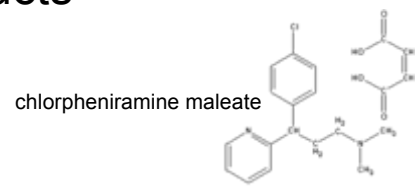

- **acetaminophen** (CAS#103-90-2, FW=151.17)
  Analgesic, antipyretic used in pain and fever reducing products



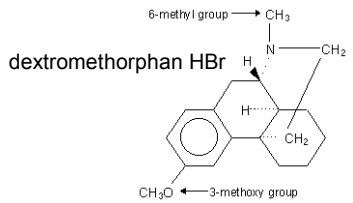

- **doxylamine succinate** (CAS#562-10-7, FW=388.5)
  Antihistamine/sedative used as a sleep aid in many OTC products



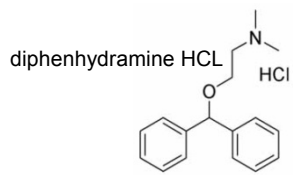

- **chlorpheniramine maleate** (CAS#113-92-8, FW=390.5)
  Antihistamine used in many cold & sinus products




- **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



# Materials and Methods: Chromatographic Conditions

**LC System:** HP-1100 HPLC with Chemstation (ver B.03.01)

**Column:** TSKgel ODS-140HTP, 2.3µm, 2.1mm ID x 5cm

**Mobile Phase:** A: water with 0.15% TFA;
B: 100% ACN with 0.15% TFA

**Temperature:** 50˚C

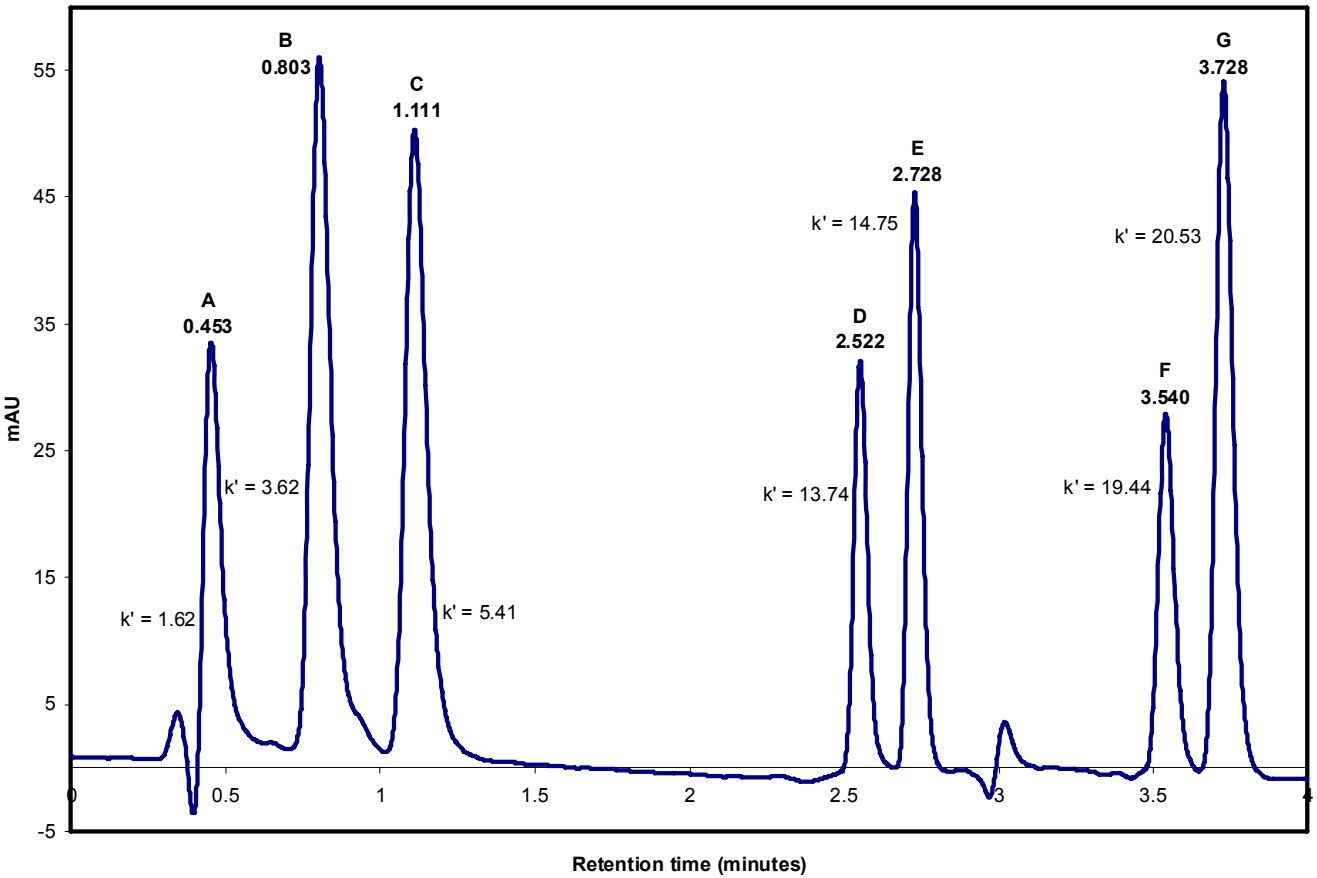
**Injection volume:** 10µL

**Detection, Flow rate and Mode:** as mentioned in respective chromatograms

# Properties of TSK-GEL ODS-140HTP Reversed Phase Column

| | |
|---|---|
| **Pore size (silica):** | **140Å** |
| **Endcapped:** | **yes** |
| **Particle size:** | **2.3µm** |
| **pH stability:** | **2.0 - 7.5** |
| **Functional group:** | **C18 (polymeric bonding chemistry)** |
| **% carbon** | **8%** |
| **Column dimensions:** | **2.1mm ID x 5cm, 2.1mm ID x 10cm** |

# Figure 1: Analysis of Six Cold and Sinus Drug Standards using TSKgel ODS-140HTP, 2.3µm, 2.1mm ID x 5cm Column




| Gradient: | | |
|---|---|---|
| **Time (min)** | **% B** | **Flow (mL/min)** |
| 0 | 1.0 | 0.6 |
| 1.4 | 2.0 | 0.6 |
| 1.5 | 24.0 | 1.4 |
| 2.1 | | 1.4 |
| 2.2 | | 0.8 |
| 4.0 | 25.0 | 0.8 |
| 4.1 | 1.0 | 0.6 |
| **Detection:** UV@215nm | | |

**Key to Chromatograms (peak labels)**

**A -- Maleate**
**B -- Phenylephrine HCl**
**C -- Acetaminophen**
**D -- Doxylamine succinate**
**E -- Chlorpheniramine**
**F -- Dextromethorphan HBr**
**G -- Diphenhydramine HCl**

**Theoretical plate numbers: 15,000 for peaks D, E, F and G (gradient)**

**Pressure range: 12.5MPa – 16.3MPa**

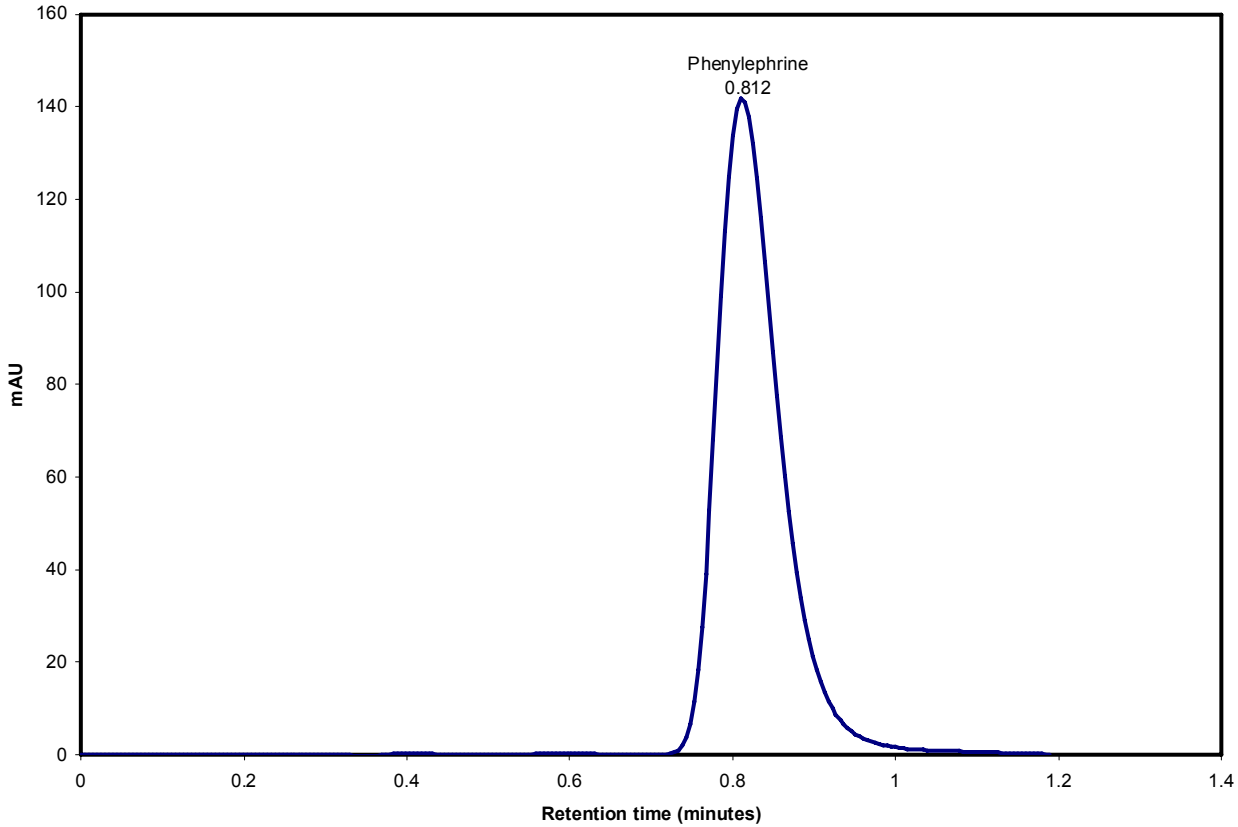
# Table 1: Calculation of % RSD in Analysis of Cold and Sinus Drugs Standards over 13 Consecutive Injections

| Retention Time (min) Compound | | | | | | | |
|---|---|---|---|---|---|---|---|
| **Run #** | **A** | **B** | **C** | **D** | **E** | **F** | **G** |
| 1 | 0.476 | 0.863 | 1.204 | 2.566 | 2.735 | 3.543 | 3.748 |
| 2 | 0.472 | 0.830 | 1.149 | 2.543 | 2.723 | 3.625 | 3.848 |
| 3 | 0.423 | 0.707 | 0.971 | 2.526 | 2.696 | 3.533 | 3.741 |
| 4 | 0.424 | 0.752 | 1.049 | 2.519 | 2.685 | 3.521 | 3.722 |
| 5 | 0.427 | 0.773 | 1.082 | 2.513 | 2.679 | 3.523 | 3.732 |
| 6 | 0.459 | 0.755 | 1.020 | 2.533 | 2.706 | 3.545 | 3.731 |
| 7 | 0.437 | 0.717 | 0.963 | 2.537 | 2.695 | 3.491 | 3.673 |
| 8 | 0.445 | 0.788 | 1.095 | 2.546 | 2.699 | 3.464 | 3.655 |
| 9 | 0.438 | 0.771 | 1.071 | 2.554 | 2.727 | 3.556 | 3.76 |
| 10 | 0.451 | 0.786 | 1.090 | 2.523 | 2.692 | 3.539 | 3.746 |
| 11 | 0.485 | 0.928 | 1.319 | 2.566 | 2.731 | 3.544 | 3.73 |
| 12 | 0.476 | 0.859 | 1.199 | 2.537 | 2.706 | 3.578 | 3.783 |
| 13 | 0.473 | 0.840 | 1.164 | 2.522 | 2.689 | 3.544 | 3.757 |
| Average | 0.841 | 1.481 | 2.054 | 4.712 | 5.023 | 6.572 | 6.947 |
| Std Dev | 0.022 | 0.063 | 0.100 | 0.017 | 0.018 | 0.039 | 0.047 |
| **%RSD** | **2.625** | **4.271** | **4.876** | **0.365** | **0.368** | **0.587** | **0.678** |

A = Maleate B = Phenylephrine C = Acetaminophen D = Doxylamine

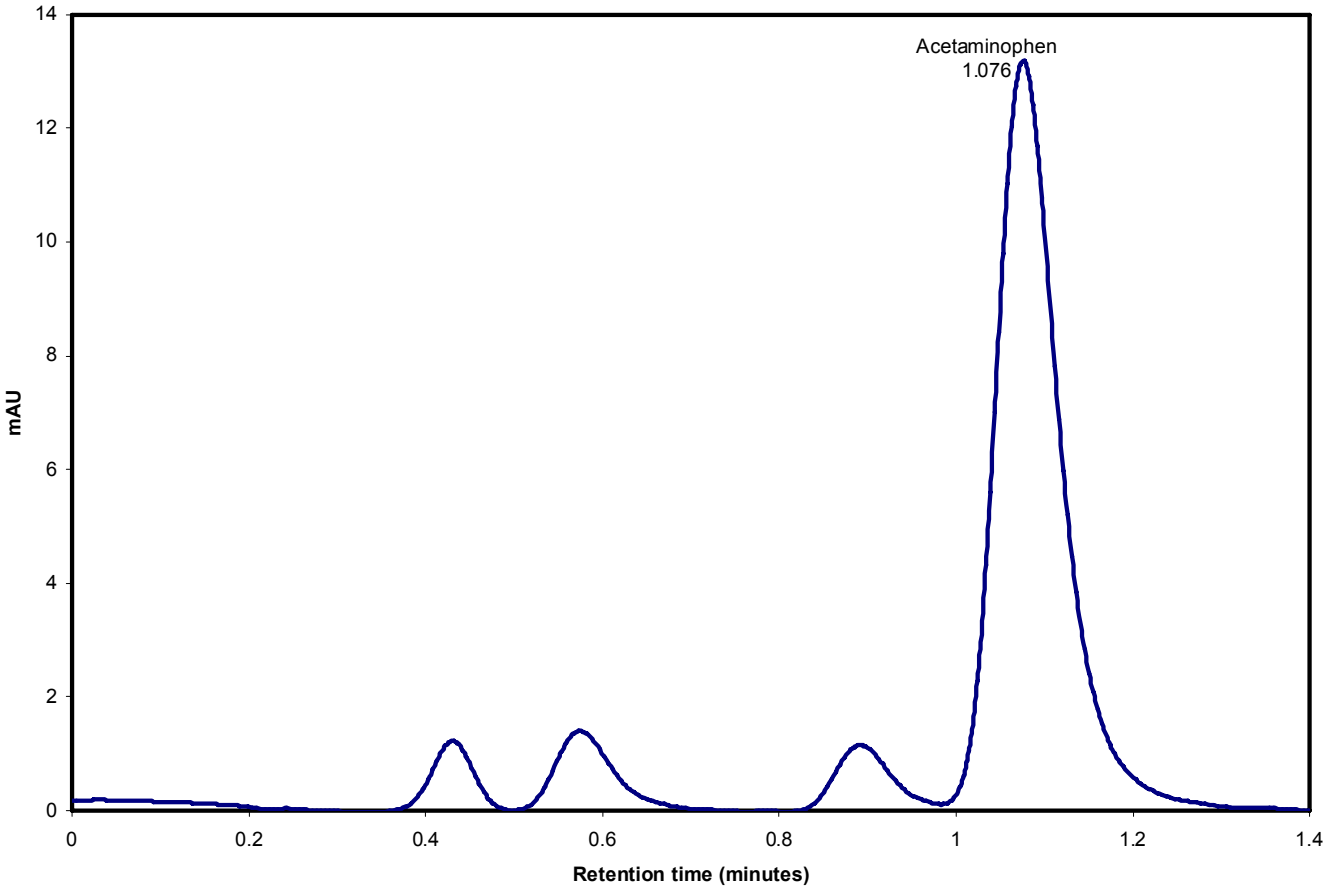
E = Chlorpheniramine F = Dextromethorphan G = Diphenhydramine

# Figure 2: Analysis of Cold and Sinus Drug Standard Phenylephrine using a TSKgel ODS-140HTP, 2.3µm, 2.1mm ID x 5cm Column



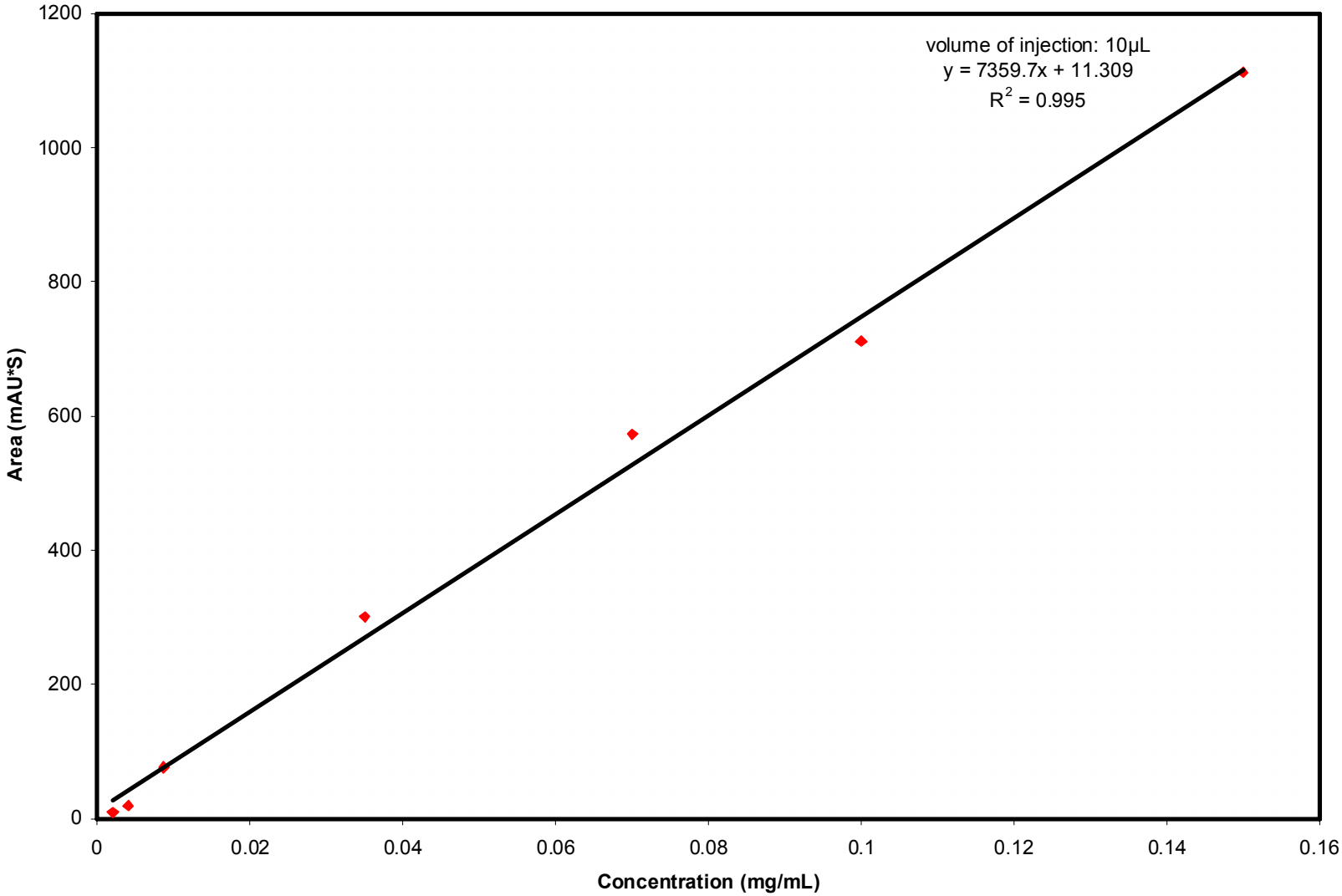

**Mobile Phase:** 1% ACN with 0.15% TFA
**Flow rate:** 0.6mL/min
**Detection:** UV@275nm
**Pressure range:** 12.0 – 12.6MPa

# Figure 3: Analysis of Children's Tylenol with Acetaminophen using a TSKgel ODS-140HTP, 2.3µm, 2.1mm ID x 5cm Column




| | |
|---|---|
| **Mobile Phase:** | **1% ACN with 0.15% TFA** |
| **Flow rate:** | **0.6mL/min** |
| **Detection:** | **UV@275nm** |
| **Pressure range:** | **12.0 – 12.6MPa** |
| **%RSD:** | **0.77** |

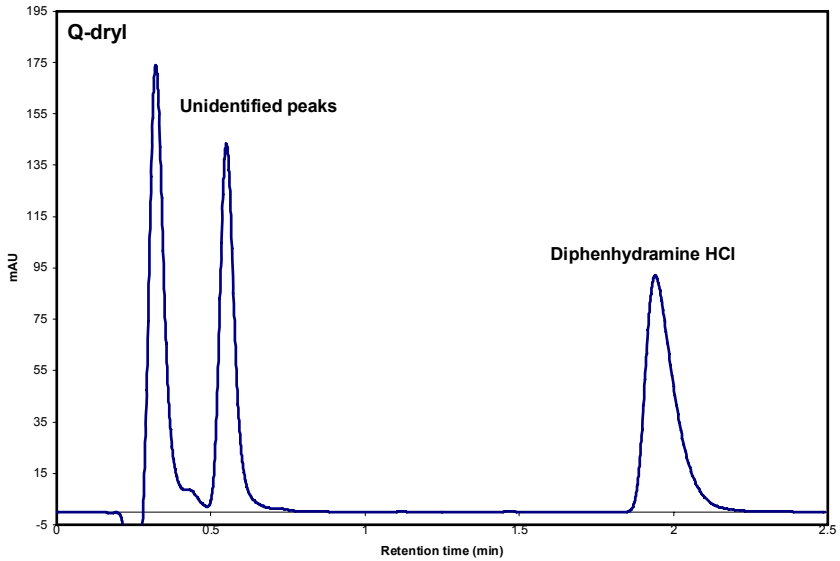
# Figure 4: Calibration Curve of Acetaminophen – Linearity Check

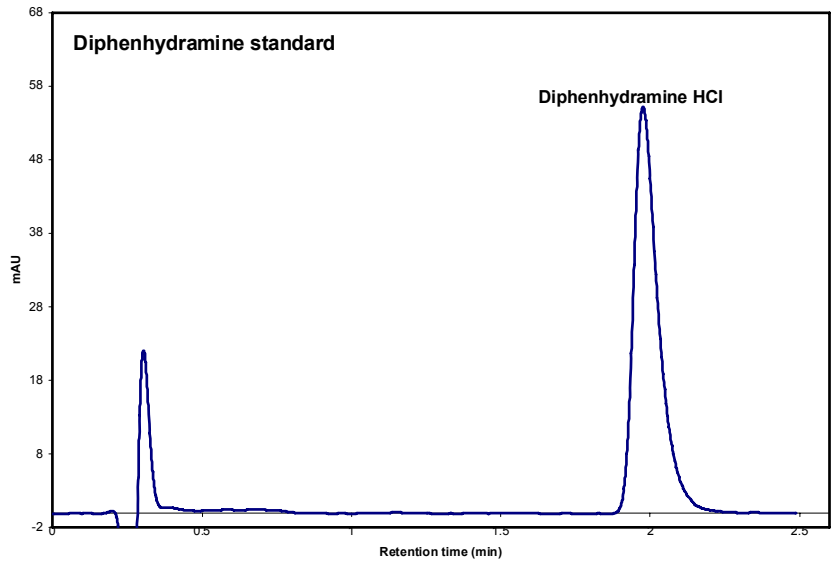


# Table 2: Calculation of % RSD in Analysis of Phenylephrine Standard and Acetaminophen (Children's Tylenol)

| phenylephrine | | acetaminophen (Children's Tylenol) | |
|---|---|---|---|
| Run # | RT (min) | Run # | RT (min) |
| 1 | 0.812 | 1 | 1.061 |
| 2 | 0.806 | 2 | 1.072 |
| 3 | 0.790 | 3 | 1.076 |
| 4 | 0.802 | 4 | 1.064 |
| 5 | 0.803 | 5 | 1.081 |
| Average | 0.803 | Average | 1.071 |
| Std Dev | 0.008 | Std Dev | 0.008 |
| **%RSD** | **1.057** | **%RSD** | **0.774** |

# Figure 5: Analysis of Q-dryl containing Diphenhydramine HCl using TSKgel ODS-140HTP, 2.3µm, 2.1mm ID x 5cm Column



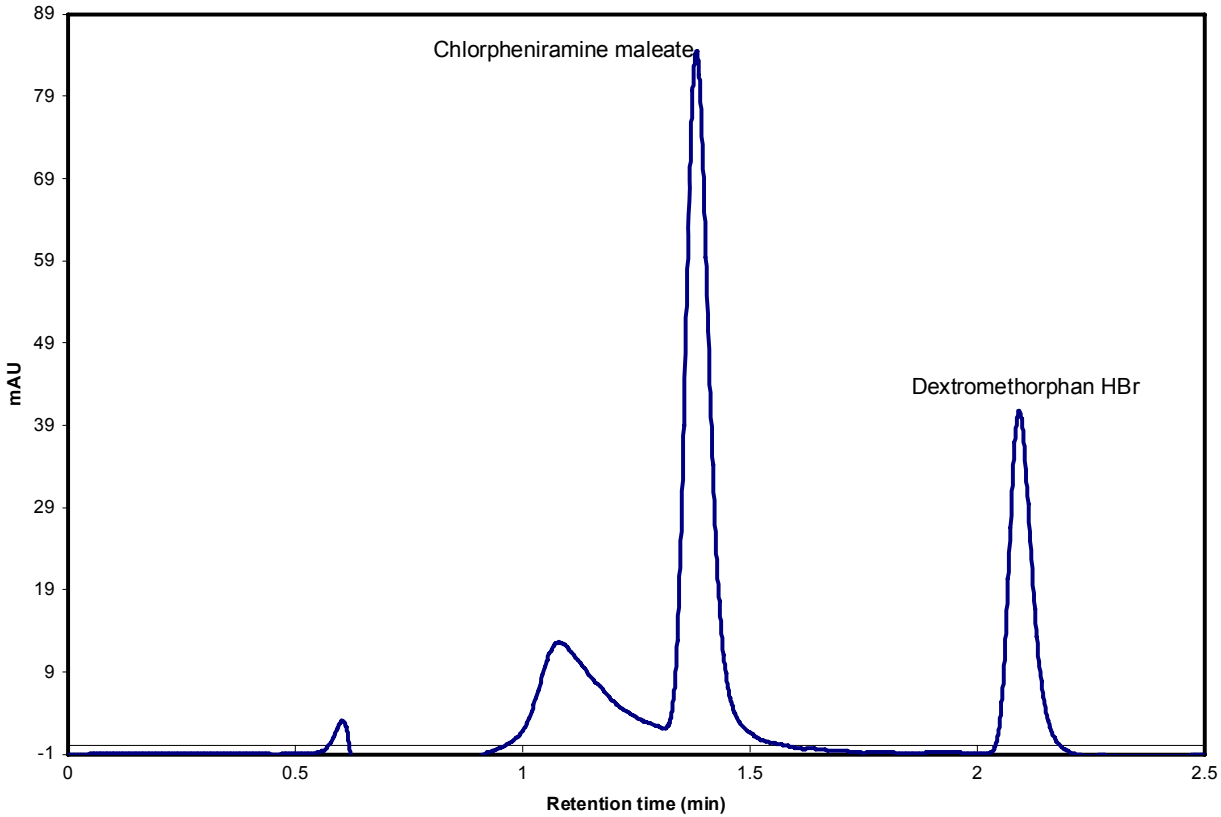




**Mobile Phase: 20% ACN with 0.15% TFA**

**Flow rate: 0.8mL/min**

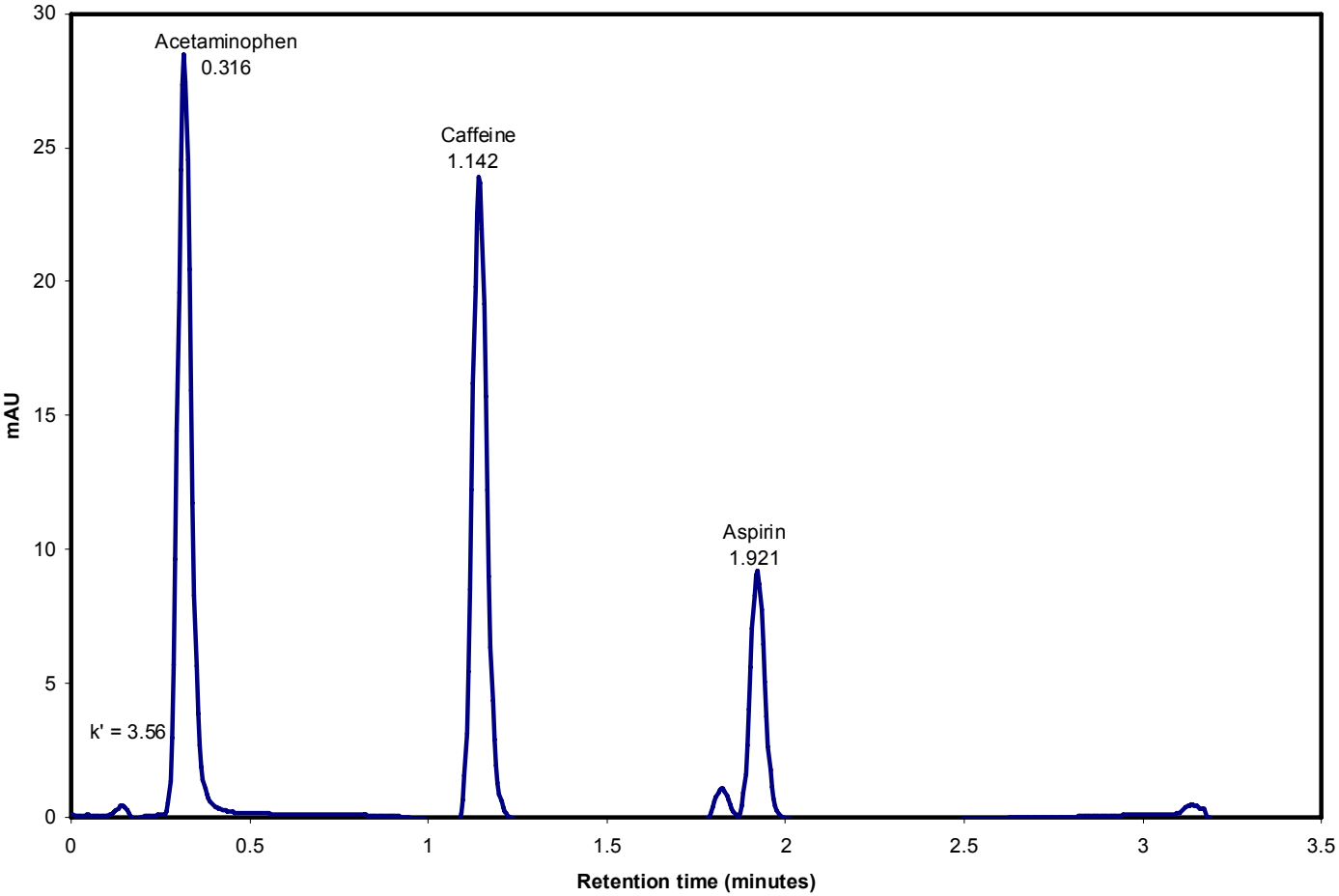
**Detection: UV@215nm**

# Figure 6: Analysis of Pediatric Robitussin containing Chlorpheniramine Maleate and Dextromethorphan HBr using TSKgel ODS-140HTP, 2.3µm, 2.1mm ID x 5cm Column




| Gradient: | | |
|---|---|---|
| **Time (min)** | **%B** | **Flow (mL/min)** |
| 0 | 20.0 | 0.3 |
| 1.2 | 20.0 | 0.3 |
| 1.3 | 20.0 | 1.2 |
| 3.0 | 20.0 | 0.3 |
| 3.1 | 20.0 | 0.6 |
| **Detection:** UV@215nm | | |

# Figure 7: Analysis of Excedrin containing Acetaminophen, Aspirin and Caffeine using TSKgel ODS-140HTP, 2.3µm, 2.1mm ID x 5cm Column




**Gradient:**

| Time (min) | %B | Flow (mL/min) |
|---|---|---|
| 0 | 1.0 | 1.5 |
| 3.0 | 30.0 | 1.5 |

**Detection:** UV@215nm

**# of Theoretical Plates:**
Caffeine: 3,610
Aspirin: 10,714

**Pressure range:** 32.5 –35.0MPa

# Table 3: Calculation of % RSD in Analysis of Excedrin

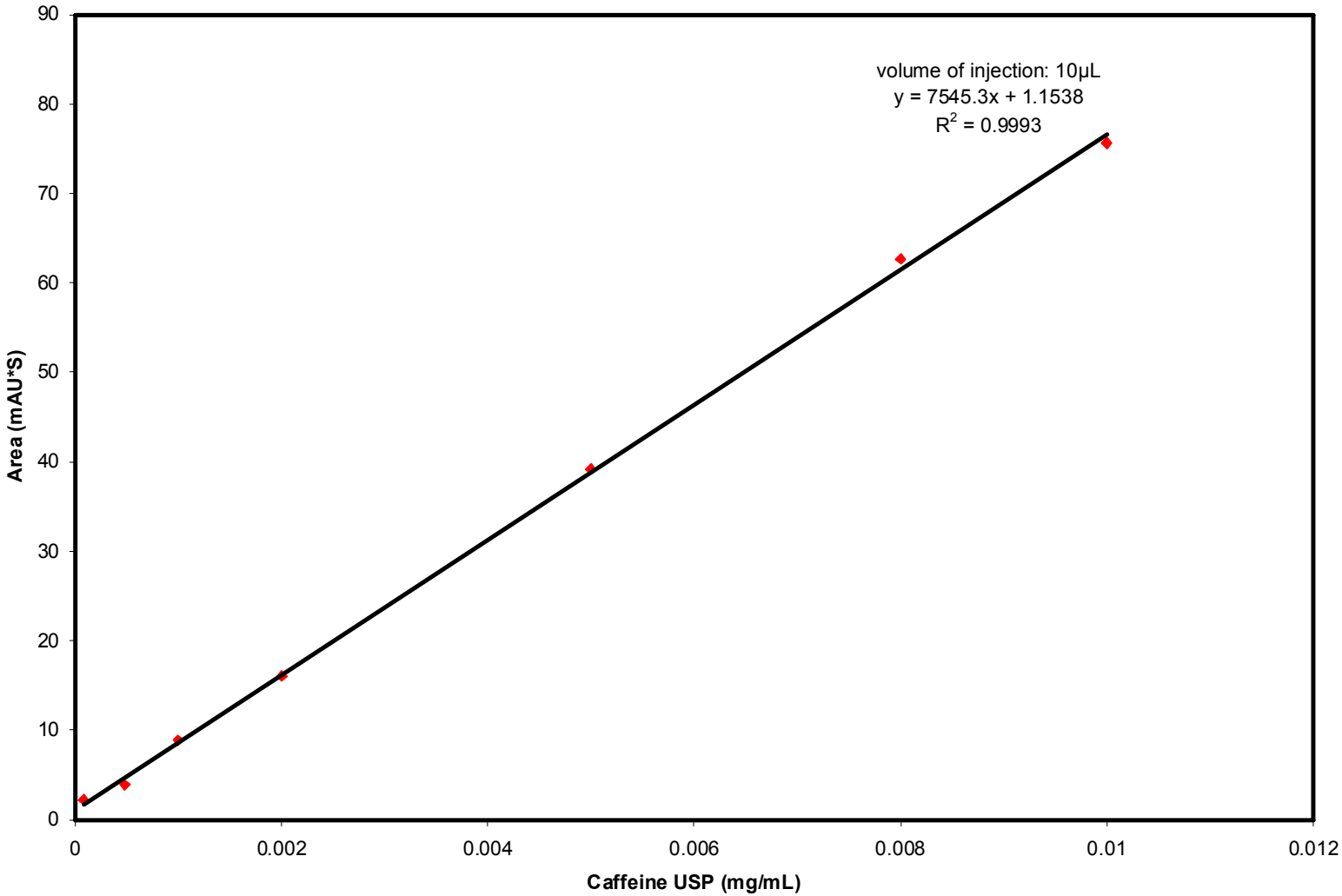
**A: %RSD of peak retention times of three API's over 5 replicate injections**

| Run # | Acetaminophen | Caffeine | Aspirin |
|---|---|---|---|
| 1 | 0.316 | 1.142 | 1.921 |
| 2 | 0.320 | 1.146 | 1.919 |
| 3 | 0.324 | 1.151 | 1.926 |
| 4 | 0.318 | 1.147 | 1.923 |
| 5 | 0.317 | 1.138 | 1.921 |
| Average | 0.319 | 1.144 | 1.922 |
| Std Dev | 0.003 | 0.005 | 0.003 |
| **%RSD** | **0.991** | **0.434** | **0.138** |

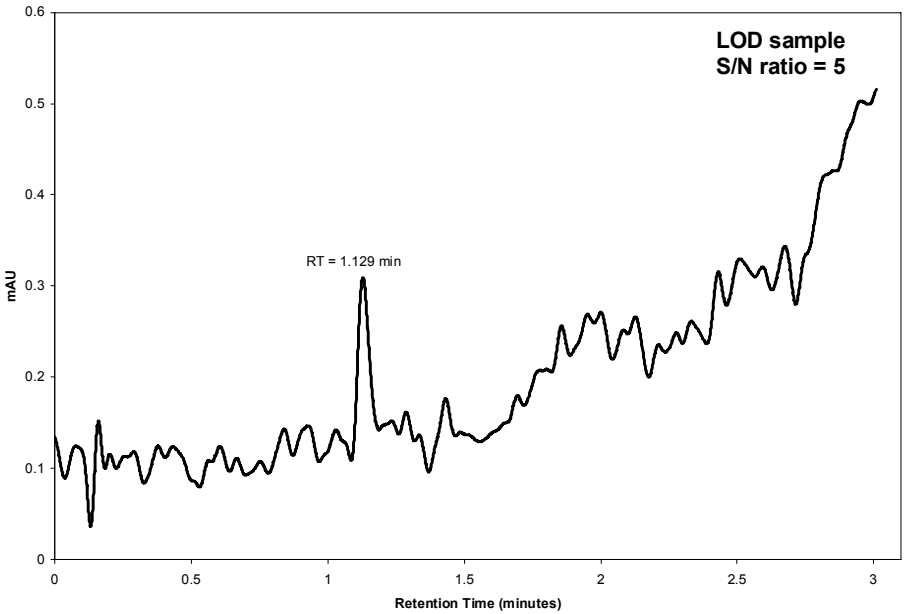
**B: %RSD of peak parameters of caffeine over 5 replicate injections**

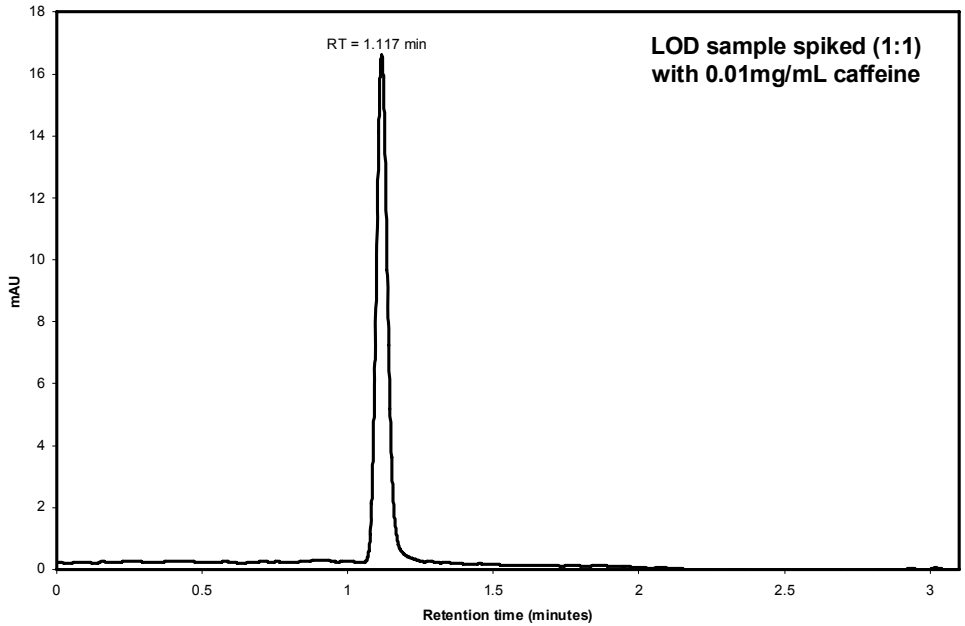
| Run # | RT (min) | k' | (Area) | Symmetry | Plates |
|---|---|---|---|---|---|
| 1 | 1.142 | 15.49 | 69.33 | 0.87 | 3610 |
| 2 | 1.146 | 15.54 | 70.69 | 0.87 | 3708 |
| 3 | 1.151 | 15.62 | 70.77 | 0.87 | 3744 |
| 4 | 1.147 | 15.55 | 70.32 | 0.86 | 3725 |
| 5 | 1.138 | 15.43 | 70.03 | 0.87 | 3561 |
| Average | 1.145 | 15.526 | 70.228 | 0.868 | 3669.6 |
| Std Dev | 0.005 | 0.071 | 0.583 | 0.004 | 79.726 |
| **%RSD** | **0.434** | **0.457** | **0.831** | **0.515** | **2.173** |

# Figure 8: Calibration of Caffeine, Detection of Linearity



# Figure 9: Determination of Limit of Detection (LOD) and Limit of Quantitation (LOQ) of Caffeine (USP)



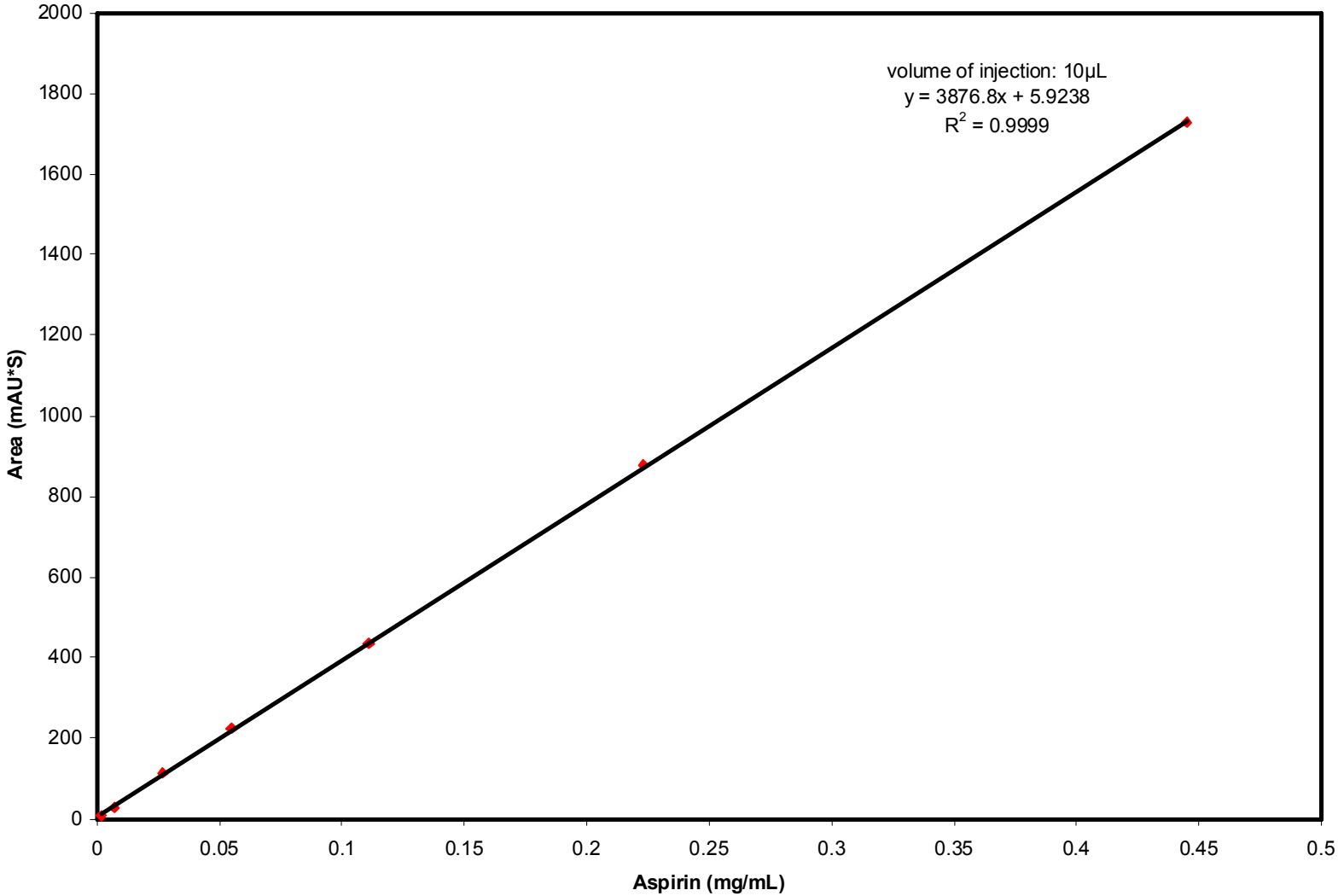




- **The lowest limit of caffeine was estimated to be in ppb level, i.e. 80ng/mL, eluting at 1.13 minutes with a signal to noise ratio of 5.**
- **The identity of the LOD peak of caffeine was further established by spiking the LOD sample with 0.01mg/mL caffeine in 1:1 ratio.**
- **The spiked sample yielded a single peak at the same retention time of 1.12 minute.**
- **The LOQ was found to be 0.48µg/mL with a signal to noise ratio of 10:1.**
- **System suitability test of caffeine USP at the LOQ concentration yielded 0.33% RSD in peak area calculation for 5 replicate injections.**

# Analysis of Caffeine

- Intra-day and inter-day precision in determining the caffeine USP peak retention time always yielded <1% RSD (data not shown here).
- Inter-day precision in retention time and symmetry of caffeine USP peak yielded 0.70 and 0.94% RSD values respectively (data not shown here).
- Theoretical plate value of caffeine yielded an average value of 3103 with a 2% RSD over a span of three months.

# Figure 10: Calibration of Aspirin, Detection of Linearity



# Results and Discussions

- In this study, it is clearly demonstrated that a TSKgel ODS-140HTP, 2.3µm, 2.1mm ID x 5 cm column can successfully be employed using a conventional HPLC system with operational pressure limit of 41.4MPa to obtain a simple, fast and reliable separation of a wide variety of drugs used in common cold and sinus OTC medicines with remarkable reduction in overall run time.

- Six cold and sinus drug standards: phenylephrine (B), acetaminophen (C), doxylamine succinate (D), chlorpheniramine maleate (E), dextromethorphan HBr (F) and diphenhydramine HCl (G) with wide difference in hydrophobicities could be separated as sharp peaks with good resolution within 3.8 minutes using a TSKgel ODS-140HTP column.

- The two drug substances diphenhydramine and dextromethorphan have very similar and strong hydrophobic properties with a tendency to co-elute or elute with considerable overlap. They were separated with a resolution of 1.9.

- The retention time of diphenhydramine is considerably lower than that reported in literature.

- The capacity factor of the early eluting peak of acetaminophen (C) was 3.62 showing considerable retention within the column.

# Results and Discussions Continued

- The maleate(A) peak, which was not quantified, has a capacity factor of 1.62
- The number of theoretical plates for peaks D, E, F and G was 15,000 under gradient conditions.
- The goal of most HPLC methods is to achieve baseline separation with a resolution of 1.5 or more for all key analytes – which was achieved in this study.
- Active ingredients of OTC drugs were separated without any interference from their excipients.
- All of the active ingredients of Excedrin, i.e. acetaminophen, caffeine and aspirin were separated within 2 minutes as sharp and symmetrical peaks.
- The method was also validated in terms of system suitability and linearity of caffeine USP, aspirin and acetaminophen standards.
- The limit of detection (LOD) and limit of quantitation (LOQ) of caffeine USP were determined.
- Theoretical plate value of caffeine yielded 2% RSD over a span of three months.

# Results and Discussions Continued

- The caffeine content in Excedrin tablet was estimated from its peak area under the same chromatographic conditions yielding 107.75 % correlation with the label claim (data not shown here).
- The estimation was well within the generally acceptable USP range of 80 – 120% content uniformity for tablets (coated and uncoated) unless otherwise specified in a USP monograph.
- Decreased run times help reduce the amount of solvent waste as well analysis cost, which is particularly important during this time of acetonitrile shortage.

# Conclusion

- A simple, fast and reliable high throughput separation of pharmaceutical ingredients using conventional HPLC with an operational pressure limit of 41.4MPa will be useful in identification of drugs using LC/MS applications.
- Further study using the TSKgel ODS-140HTP, 2.3µm column for LC/MS analyses is in progress.