Application Note

Biopharma/Pharma

Agilent

Trusted Answers

# Modernizing LC Methods for USP Phenylephrine HCl and Pramoxine HCl in OTC Products


**Authors**

Monique Paré Speirs, PhD
and Megan K. Bean
Rhyz Analytical Labs

Rongjie FU
Agilent Technologies
(Shanghai) Co., Ltd, China



## Abstract

Phenylephrine HCl and pramoxine HCl are active pharmaceutical ingredients (APIs) used independently or combined in a broad range of multisymptom relief over the counter (OTC) products. These APIs are generally tested separately using different extraction techniques, HPLC methods with outdated column technologies, and LC-damaging salt buffers. Combining the separate techniques into a single extraction and acquisition method with LC-friendly mobile phases and smaller modernized particle technology is critical to improve the efficiency and sustainability of product quality control (QC) testing. An LC method to determine both compounds from a single, simple sample extraction was developed. This method was performed with a gradient of 0.05% trifluoroacetic acid (TFA) and methanol in lieu of traditional salt buffers. The analysis was conducted on an Agilent 1260 Infinity II LC system with an Agilent InfinityLab Poroshell 120 EC-C8 (3.0 × 150 mm, 2.7 µm) column and diode array detection at 224 nm.

## Introduction

Phenylephrine HCl is used as an API in decongestants, ophthalmic formulations, and vasoconstrictor skin applications. Pramoxine HCl is used in local anesthetic topical treatments for minor skin irritations. No published literature was found with applications where both phenylephrine HCl and pramoxine HCl are analyzed in the same LC acquisition run.

The United States Pharmacopeia (USP) or other outdated LC methodologies based on classic HPLC column technologies and LC-destructive buffer salts are generally applied to determine pramoxine or phenylephrine in OTC drugs.[1–8] Each compound is tested separately, making the overall process unnecessarily inefficient for samples containing both compounds, such as multisymptom relief topical treatments. To achieve suitable chromatography for each compound, two different sample preparations and two different system calibrations and HPLC acquisition runs are required per sample since separately prepared buffers containing unfavorable levels of salts are used in the mobile phases. Since different types of buffers are required for each method, the salt from the first buffer must be flushed from the LC system before equilibrating with the second mobile phase to test the second compound. Alternatively, two LC systems can be dedicated to testing the two different compounds. Significant flushing is required to remove salts postanalysis and transfer the systems and columns into safer conditions for standby.

The mobile phases used for traditional test applications impart detrimental effects on process efficiency and LC system fitness. The USP monograph for pramoxine HCl cream uses dibasic potassium phosphate salt buffer (pH 7.5) as the aqueous phase and acetonitrile as the organic phase.[1] No USP monograph for phenylephrine HCl in cream was found in the USP Access Point. However, the USP monographs for the phenylephrine HCl assay (for raw material testing)[2] or the phenylephrine HCl in nasal solution (representative of a traditional QC test method of a finished product)[3] use sodium 1-octane sulfonate buffer (pH 3) as the aqueous phase. Additionally, either acetonitrile (for the assay) or methanol (for the nasal solution) is used as the organic phase.

Given that each buffer requires a pH adjustment and vacuum filtration before use, mobile phase preparation is time-consuming. The octane sulfonate buffer is most problematic as it foams while flowing from the mobile phase bottle through the inlet filter. The foam introduces air into the pump, often leading to a loss in backpressure that cannot be recovered without vigorous purging with water or replacing the purge valve frit or pump seal. A rapid solution is to remove the inlet filter, but this is not recommended as it increases the risk of particles entering and clogging the LC and/or column. When used in combination with organic solvents in binary mobile phase programs, 1-octanesulfonic acid salt is highly subject to precipitation and is extremely difficult to wash off the column and system postanalysis. If the method is run for successive acquisitions over the course of two days or more, backflushing is often necessary to wash precipitated salts off the stationary phase and restore column performance. Guard or analytical columns often need replacing to restore chromatographic performance after prolonged exposure to octane sulfonate salts.

There is an increasing demand to transfer methods from traditional HPLC columns, mobile phases, and LC acquisition methods to greener, smaller, more efficient, and modernized particle technologies with LC-friendly mobile phases.[9,10] Classic HPLC columns are characterized by large particle sizes (3 to 5 µm) and column dimensions (generally, 4.6 × 250 mm). Among the limitations of these columns are high flow rates (typically 1 to 2 mL/min) and long acquisition run times (often > 15 minutes), which decrease laboratory throughput and increase solvent consumption. The revised guidelines in USP General Chapter <621> have been updated to facilitate growing industry needs, enabling transfers of traditional HPLC to modern UHPLC methodologies.[9,10] This revision motivates method development efforts to improve current inefficient and outdated OTC test methods, including those presented in this application note.[9,10]

Based on a literature search, this application note presents the first method for the determination of both phenylephrine HCl and pramoxine HCl in a single liquid chromatography diode array detector (LC-DAD) acquisition run. This method is performed with 0.05% trifluoroacetic acid (TFA) in lieu of buffer salts in the mobile phase, eliminating the challenges that standard phenylephrine HCl and pramoxine HCl assays pose on LC system fitness. Furthermore, separation and detection of both compounds are achieved in a single injection with newer column technology (InfinityLab Poroshell 120 EC-C8 column, 3.0 × 150 mm, 2.7 µm), lower flow rate (0.6 mL/min), and a reasonable run time (15 minutes). This method significantly improves laboratory efficiency and reduces solvent consumption relative to previous applications.

Product-specific method validations are used to demonstrate that a newly developed method is suitable for the intended purpose.[11,12] In this application note, validation experiments were conducted to evaluate key method performance characteristics for the determination of phenylephrine HCl (0.22% w/w) and pramoxine HCl (1% pramoxine HCl) in a representative multisymptom relief cream. These characteristics included specificity, system suitability, linearity, system precision, method precision, intermediate precision, accuracy, and robustness.[11,12] Matrix extension testing of other commercially available OTC products was also conducted, including 1% pramoxine HCl itch relief lotion and 0.033% phenylephrine HCl cough syrups.

# Experimental

## Chemicals, standards, and samples

The chemical properties of phenylephrine HCl and pramoxine HCl are shown in Table 1. The solutions required for standards, mobile phases, and sample preparations are shown in Tables 2 and 3. The USP reference standards used for system suitability and calibration are shown in Table 4.

**Table 1.** Chemical properties of phenylephrine HCl and pramoxine HCl.

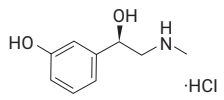
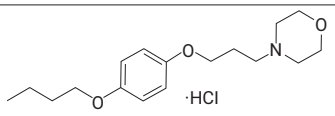
| Compound | Structure |
|---|---|
| Name: Phenylephrine HCl<br>CAS No: 61-76-7<br>Molecular Formula: $C_9H_{13}NO_2$•HCl<br>Molecular Weight (g/mol): 203.67 |  |
| Name: Pramoxine HCl<br>CAS No: 637-58-1<br>Molecular Formula: $C_{17}H_{27}NO_3$•HCl<br>Molecular Weight (g/mol): 329.86 |  |

**Table 2.** Reagent information.

| List Reagent | Grade |
|---|---|
| Water | HPLC grade (0.2 µm filtered) |
| Methanol | HPLC grade |
| Trifluoroacetic Acid (TFA) | ReagentPlus, 99% |

**Table 3.** Mobile phase and diluent.

| Solution | Function |
|---|---|
| 0.05% TFA in HPLC Grade Water | Mobile phase A and Diluent A |
| 0.05% TFA in HPLC Grade Methanol | Mobile phase B and Diluent B |

**Table 4.** Reference standards.

| Standard Analyte | Source | Lot Number | Purity (%) |
|---|---|---|---|
| Phenylephrine HCl | USP | R113V0 (Current) | 99.9 |
| Pramoxine HCl | USP | J0K379 (Current) | 99.8 |

## Standard preparation and calibration

phenylephrine HCl and pramoxine HCl standards were accurately weighed and diluted in Diluent A:Diluent B (70:30). Calibration curves were generated by plotting the responses against the corresponding concentrations of five calibration levels ranging from 0.01 mg/mL phenylephrine HCl + 0.02 mg/mL pramoxine HCl (Level 1) to 0.2 mg/mL phenylephrine HCl + 0.4 mg/mL pramoxine HCl (Level 5).

## Sample preparation for LC analysis

Samples were mixed thoroughly to homogenize them before weighing them out. For LC analysis, 200 mg of the multisymptom relief cream containing 1% pramoxine HCl and 0.22% phenylephrine HCl or 200 mg of anti-itch lotion containing 1% pramoxine HCl were accurately weighed into 15 mL centrifuge tubes. Additionally, 1.5 g of cough syrup samples containing 0.033% phenylephrine HCl were accurately weighed into 15 mL centrifuge tubes.

The tubes were filled to the 8.0 mL mark with Diluent A and vortexed until the contents were well homogenized. The tubes were then filled to the 12 mL mark with Diluent B, vortexed, sonicated for 10 minutes at 60 °C with intermittent mixing, and filtered through a 0.2 µm nylon syringe filter.

## Sample preparation for accuracy

The multisymptom relief cream placebo blank was mixed thoroughly to homogenize before weighing it out, and 200 mg portions were accurately weighed in sextuplicate into 15 mL centrifuge tubes. Placebos were spiked with low, mid, or high concentration levels (two replicates per level) of standard solution covering the analytical range. Spiked samples were then extracted using the methods of sample preparation for LC analysis described earlier.

## Sample preparation for forced degradation

For forced degradation analysis, 1 mL of the multisymptom relief cream sample and placebo blank were transferred in triplicate to 2 mL centrifuge tubes. The following were respectively added to the tubes: 0.1 mL of 1 N HCl, 0.1 mL of 1 N NaOH, or 0.1 mL of 30% $H_2O_2$. The samples were mixed well, incubated at room temperature for 30 minutes, then extracted using the methods of sample preparation for LC analysis described earlier.

### LC conditions and gradient program

Analysis was conducted using a 1260 Infinity II LC system with DAD detection. Tables 5 and 6 detail the conditions and gradient program used.

**Table 5.** LC conditions.

| Sampler | Agilent 1260 Inifinity II Vialsampler |
|---|---|
| Injection Volume | 1 µL |
| Column Compartment | Agilent 1260 Inifinity II multicolumn thermostat (MCT) |
| Column | Agilent InfinityLab Poroshell 120 EC-C8 column, 3.0 × 150 mm, 2.7 µm (p/n 693975-306T) |
| Column Temperature | 50 °C |
| Pump | Agilent 1260 Infinity II quaternary pump |
| Flow Rate | 0.6 mL/min |
| Run Time | 15 min |
| Detector | Agilent 1290 Infinity diode array detector at 224.0 nm |
| Integrator | Agilent OpenLab software version 2.7 |

**Table 6.** LC gradient program.

| Time (min) | Mobile Phase A (%) (0.05% TFA in Water) | Mobile Phase B (%) (0.05% TFA in Methanol) |
|---|---|---|
| 0.00 | 93.0 | 7.0 |
| 1.00 | 93.0 | 7.0 |
| 4.00 | 80.0 | 20 |
| 10.00 | 5.0 | 95.0 |
| 11.00 | 5.0 | 95.0 |
| 11.01 | 93.0 | 7.0 |

### Acceptance criteria

Product-specific method validations are used to determine if the LC system and method are fit for the intended QC assay.[11,12] The test results generated from a representative sampling of the product must be accurate, reliable, and consistent.[11,12] The results of each method performance test are compiled and compared to relevant acceptance criteria, which vary depending on multiple factors, including analyte concentration, product type, analytical system/method variability, and laboratory requirements.[11,12,14] Generic acceptance criteria appropriate for the samples, system, and method used for this application are implemented throughout the discussion for exemplary purposes. Note that acceptance criteria may be adjusted by future users based on laboratory regulations and sound scientific judgment of the validation data as needed.[11,12,14]

## Results and discussion

### System suitability and specificity

**Overview:** The standard solution and multi-system relief cream sample were prepared and injected per this method. Representative standard and sample chromatograms are shown in Figures 1 and 2. The chromatograms were used to determine if the system and method were suitable for the identification and chromatographic analysis of phenylephrine HCl and pramoxine HCl in the extracted cream sample.

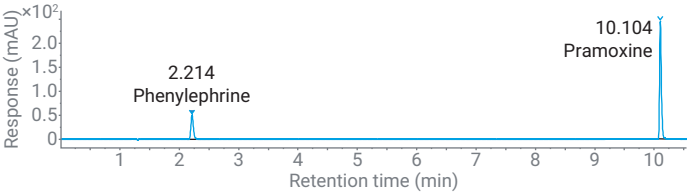



**Figure 1.** Standard chromatogram.

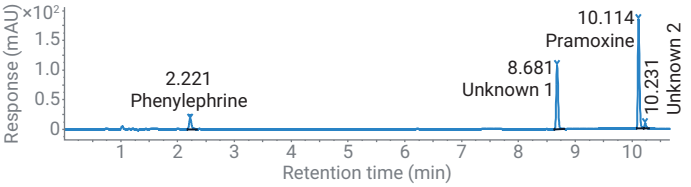



**Figure 2.** Chromatogram of the multisymptom relief cream sample.

**Specificity:** Specificity is the ability to explicitly identify the analytes of interest in the presence of expected components such as other formula ingredients (collectively referred to as the sample matrix) or degradation products.[11,12] Excipients must not interfere with the responses of the analytes of interest.[11,12] Identification tests were performed to confirm the identities of the analytes in the cream sample matrix. The retention times (RTs) of the peaks identified as phenylephrine HCl and pramoxine HCl in the sample were compared to those of the standard. The RT comparison is expressed as the RT ratio ($RT_{Sample}/RT_{Standard}$). The RTs of phenylephrine HCl and pramoxine HCl in the sample chromatogram were consistent with the corresponding standards (RT Ratios = 1.00).

UV spectra (100 to 400 nm) were extracted from the apexes of the analyte peaks in both the standard and sample (Figures 3 and 4). The standard phenylephrine HCl spectra and standard pramoxine HCl spectra were assigned as the reference spectra. Agilent OpenLab software was used to compare the sample UV spectra to the respective reference spectra. The software calculated a UV confirmation match factor, which indicates the degree of spectral similarity between the sample and the respective reference in Table 8. The UV confirmation match factors were ≥ 997.54, meeting the level recommended by the OpenLab CDS Data Analysis Reference Guide (> 990).[13]

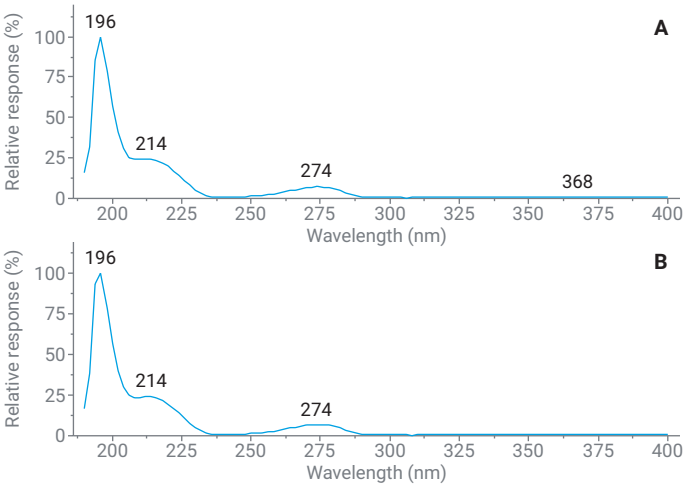



**Figure 3.** Extracted UV spectrum of the phenylephrine HCl peak in (A) the standard chromatogram and (B) the multisymptom relief cream sample chromatogram.

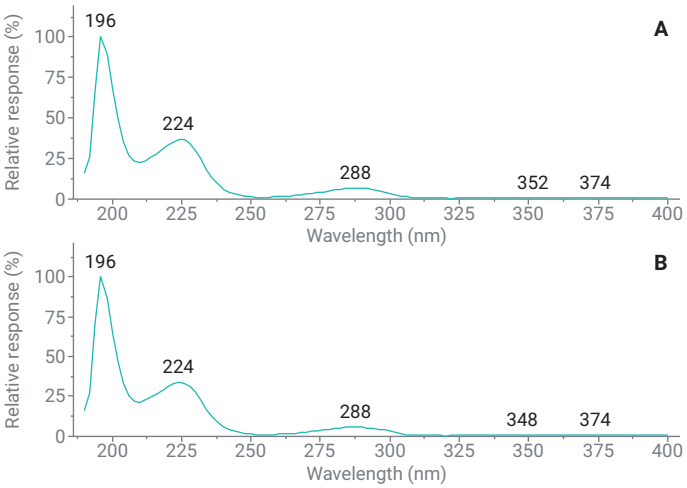



**Figure 4.** Extracted UV spectrum of the pramoxine HCl peak in (A) the standard chromatogram and (B) the multisymptom relief cream sample chromatogram.

Purity is another important component of LC-DAD validations as it demonstrates that the analyte peak response is not attributable to other components, that is, coeluting impurities. OpenLab software was used to determine if the peaks identified as phenylephrine HCl and pramoxine HCl in the sample contained coeluting impurities, based on the comparison of extracted spectra (190 to 400 nm) recorded during peak elution. After applying a baseline correction, the spectrum at the maximum wavelength was compared to all significant spectra within the peak. The software then calculated a purity match factor, which represents the degree of similarity between the spectra shown in Tables 7 and 8. The peak purity match factors were ≥ 998.80, meeting the level recommended by the OpenLab CDS Data Analysis Reference Guide (> 990).[13] The system and method were therefore deemed suitable for the identification of phenylephrine HCl and pramoxine HCl in the sample matrix.

To further investigate method specificity, the multisymptom relief cream samples and placebos were treated with degradatory agents (acid, base, or peroxide), prepared, and injected per the method. No phenylephrine HCl, pramoxine HCl, or other potential coeluting matrix interferences to the analytes of interest were detected in the placebos. The RTs and UV spectra of phenylephrine HCl and pramoxine HCl in the multisymptom relief cream samples treated with degradatory agents were consistent with the standards (RT ratios: 1.00 to 1.01, UV confirmation match ≥ 994.87). Furthermore, no significant coeluting impurities were found (peak purity match ≥ 999.21), indicating that method specificity was acceptable (Tables 9 to 11).

**Table 7.** System suitability and specificity—standard solution.

| Analyte | RT (min) | USP Resolution (R) | USP Tailing (T) | USP Plates (N) | UV Purity* | UV Confirmation Match Factor* |
|---|---|---|---|---|---|---|
| Phenylephrine HCl | 2.214 | NA** | 1.25 | 13,490 | 998.12 | NA (used as reference spectra) |
| Pramoxine HCl | 10.104 | 119.67 | 1.36 | 533,715 | 998.13 | |

**Table 8.** System suitability and specificity—multisymptom relief cream sample.

| Analyte | RT (min) [RT Ratio] | USP Resolution (R) | USP Tailing (T) | USP Plates (N) | UV Purity* | UV Confirmation Match Factor* |
|---|---|---|---|---|---|---|
| Phenylephrine HCl | 2.221 [1.00] | NA** | 1.23 | 14,424 | 999.65 | 997.54 |
| Pramoxine HCl | 10.114 [1.00] | 26.23 | 1.31 | 584,023 | 998.80 | 999.42 |
| Unknown 2*** | 10.231 [NA] | 2.40 | NA | NA | NA | NA |

**Table 9.** System suitability and specificity—multisymptom relief cream sample treated with HCl.

| Analyte | RT (min) [RT Ratio] | USP Resolution (R) | USP Tailing (T) | USP Plates (N) | UV Purity* | UV Confirmation Match Factor* |
|---|---|---|---|---|---|---|
| Phenylephrine HCl | 2.223 [1.00] | NA** | 1.17 | 14,294 | 999.80 | 995.79 |
| Pramoxine HCl | 10.122 [1.00] | 26.63 | 1.23 | 597,857 | 999.26 | 998.39 |
| Unknown 2*** | 10.238 [NA] | 2.35 | NA | NA | NA | NA |

**Table 10.** System suitability and specificity—multisymptom relief cream sample treated with NaOH.

| Analyte | RT (min) [RT Ratio] | USP Resolution (R) | USP Tailing (T) | USP Plates (N) | UV Purity* | UV Confirmation Match Factor* |
|---|---|---|---|---|---|---|
| Phenylephrine HCl | 2.225 [1.00] | NA** | 1.15 | 14,478 | 999.54 | 994.87 |
| Pramoxine HCl | 10.124 [1.00] | 26.95 | 1.35 | 623,962 | 999.38 | 997.30 |
| Unknown 2*** | 10.239 [NA] | 2.38 | NA | NA | NA | NA |

**Table 11.** System suitability and specificity—multisymptom relief cream sample treated with $H_2O_2$.

| Analyte | RT (min) [RT Ratio] | USP Resolution (R) | USP Tailing (T) | USP Plates (N) | UV Purity* | UV Confirmation Match Factor* |
|---|---|---|---|---|---|---|
| Phenylephrine HCl | 2.226 [1.01] | NA** | 1.17 | 14,830 | 999.44 | 996.38 |
| Pramoxine HCl | 10.123 [1.00] | 26.66 | 1.29 | 598,724 | 999.21 | 998.40 |
| Unknown 2*** | 10.238 [NA] | 2.35 | NA | NA | NA | NA |

* Per the Agilent OpenLab CDS Data Analysis v2.7 Reference Guide, a match factor of 0 indicates no match, a factor > 990 indicates significant similarity, and a factor of 1,000 indicates identical spectra. The closer the UV confirmation match factor is to 1,000, the more similar the UV spectrum at the apex of the sample peak is to that of the standard. The closer the peak purity match factor is to 1,000, the more likely that the peak is pure. The lower the peak purity match factor, the more likely coeluted peaks with significantly different UV spectra are hidden within the peak.
** No preceding peaks were present to calculate the resolution.
*** Matrix peak adjacent to pramoxine HCl

## System suitability

Critical separations in the sample chromatogram were evaluated based on the resolution (how well the peak is separated from the preceding peak) of components that eluted closest together.[11] OpenLab software was used to calculate the resolution (R) of pramoxine HCl in the standard, sample, and samples treated with degradants (Tables 7 to 11). Note that no preceding peaks were present to calculate the R value of phenylephrine HCl. Since an unidentified low-level matrix peak (Unknown 2) was observed adjacent to pramoxine HCl in the sample chromatogram, it was considered a critical separation, so the RT and R of Unknown 2 were also calculated (Tables 8 to 11). A resolution of 2.0 or higher indicates that peaks are well separated (baseline resolved). The resolution of pramoxine HCl and Unknown 2 in the samples was ≥ 2.35.

Peak shape was also evaluated because a symmetrical, well-shaped (Gaussian) peak facilitates more accurate quantitation relative to poorly shaped (non-Gaussian), nonsymmetrical peaks. Peak shape is expressed as the tailing factor (T) in Tables 7 to 11. A perfect symmetrical peak has a tailing factor of 1. A tailing factor < 2 is generally considered appropriate for similar applications. The tailing factors of phenylephrine HCl and pramoxine HCl in the standard and sample chromatograms were ≤ 1.35.

The theoretical plate count was used to evaluate separation quality and efficiency, or the ability of the analytical column to distinguish between different analytes. The theoretical number of plates (N) pertaining to phenylephrine HCl and pramoxine HCl in the standard and sample chromatograms are shown in Tables 7 to 11. The InfinityLab Poroshell 120 EC-C8 column exhibited excellent separation quality and efficiency (N ≥ 13,490) throughout the standard and sample acquisitions.

## Linearity and range

The linearity of an LC method is a measure of its ability to obtain test results proportional to the levels of the analytes of interest in the prepared sample.[11,12] The range is the interval between the upper and lower concentrations where the method provides suitable linearity.[11,12] The responses of the samples must fall within the linear range (preferably close to the response of the mid-level calibrators) for accurate quantitation.

Linearity was evaluated by plotting the responses against the corresponding concentrations of five standard levels. The standard curves are shown in Figures 5 and 6.

Linearity is expressed as the correlation coefficient ($R^2$) for the linear regression of each standard curve.[11,12] The working concentration range, standard responses, residual analysis, slope, intercept, and $R^2$ value of each curve are shown in Tables 12 and 13. The $R^2$ values were ≥ 0.99975, indicating a linear relationship between the response and concentration of each standard within the respective working range.

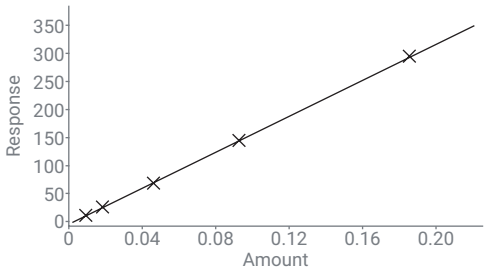



**Figure 5.** Phenylephrine HCl standard curve.

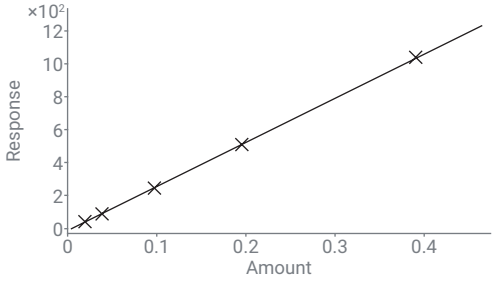



**Figure 6.** Pramoxine HCl standard curve.

**Table 12.** Linear analysis of phenylephrine HCl.

| Standard Level | Standard Amount (mg/mL) | Response (mAU) | Slope | Intercept | $R^2$ |
|---|---|---|---|---|---|
| 1 | 0.009* | 11.638 | 1,597.127 | −2.961 | 0.99975 |
| 2 | 0.019 | 25.627 | | | |
| 3 | 0.046 | 68.852 | | | |
| 4 | 0.093 | 144.637 | | | |
| 5 | 0.185** | 293.942 | | | |

*Working range minimum
**Working range maximum

**Table 13.** Linear analysis of pramoxine HCl.

| Standard Level | Amount (mg/mL) | Response (mAU) | Slope | Intercept | $R^2$ |
|---|---|---|---|---|---|
| 1 | 0.020* | 45.626 | 2,684.356 | −8.941 | 0.99978 |
| 2 | 0.039 | 90.628 | | | |
| 3 | 0.098 | 245.742 | | | |
| 4 | 0.196 | 514.156 | | | |
| 5 | 0.392** | 1,042.562 | | | |

*Working range minimum
**Working range maximum

## Precision

**Overview:** Precision is the degree of agreement between replicate measurements of the same material.[11,12] It is generally expressed as the degree of variability between measurements or percentage residual standard deviation (%RSD).[11,12] Three levels of precision were tested, including LC system precision (repeatability of injections), method precision (intra-assay repeatability), and intermediate precision (intraday/intra-analyst reproducibility). The expected level of precision is a function of concentration.[12] For phenylephrine HCl (1% w/w in cream) and pramoxine HCl (0.22% w/w in cream), the expected level of precision is 2 to 4% RSD.[12] The variance between the results of each of the three precision experiments (system repeatability, intra-assay repeatability, and method reproducibility) was ≤ 1.42% RSD, indicating that overall precision of this analytical procedure was acceptable.

**System precision:** LC system precision was evaluated by performing six replicate injections of the working standard, using the method. System precision is expressed as the %RSD of the average responses (peak areas) of phenylephrine HCl and pramoxine HCl (Table 14). For reference, Table 14 also shows the precision of the RT for each analyte. The %RSD of the response was ≤ 0.26%, and the %RSD of the RT was ≤ 0.08%.

**Method precision:** Intra-assay repeatability is the level of variance between multiple extractions of the same sample prepared and analyzed under the same conditions.[11,12] Six replicate multisymptom relief cream samples were prepared and injected using the method on the same day and by the same analyst. Method precision is expressed as the %RSD of the average levels of phenylephrine HCl and pramoxine HCl determined in the samples (Table 15). The %RSD of the replicate test results was ≤ 0.95%.

**Table 15.** Method precision (n = 6 sample preparations).

| | % w/w | |
|---|---|---|
| **Sample** | **Phenylephrine HCl** | **Pramoxine HCl** |
| 1 | 0.219 | 0.916 |
| 2 | 0.215 | 0.899 |
| 3 | 0.218 | 0.914 |
| 4 | 0.216 | 0.898 |
| 5 | 0.216 | 0.903 |
| 6 | 0.218 | 0.916 |
| **Average** | **0.217** | **0.908** |
| **%RSD** | **0.71** | **0.95** |

**Table 14.** System precision (n = 6 injections of 0.1 mg/mL phenylephrine HCl + 0.2 mg/mL pramoxine HCl).

| | Peak Area (mAU) | | Retention Time (min) | |
|---|---|---|---|---|
| **Injection** | **Phenylephrine HCl** | **Pramoxine HCl** | **Phenylephrine HCl** | **Pramoxine HCl** |
| 1 | 136.55 | 502.86 | 2.228 | 10.163 |
| 2 | 136.81 | 503.06 | 2.230 | 10.163 |
| 3 | 137.05 | 504.19 | 2.225 | 10.160 |
| 4 | 136.66 | 503.22 | 2.226 | 10.158 |
| 5 | 137.40 | 504.48 | 2.227 | 10.159 |
| 6 | 137.16 | 506.42 | 2.229 | 10.161 |
| **Average** | **136.94** | **504.04** | **2.23** | **10.16** |
| **SD** | **0.32** | **1.33** | **0.00** | **0.00** |
| **%RSD** | **0.24** | **0.26** | **0.08** | **0.02** |

**Intemediate precision:** Method reproducibility was demonstrated by calculating the %RSD of test results obtained from the multisymptom relief cream samples, prepared and analyzed by different analysts on separate days using independently prepared standards and calibration curves. Intermediate precision is expressed as the combined %RSD of the levels of phenylephrine HCl and pramoxine HCl determined in the two sets of samples (Table 16). The combined %RSD was ≤ 1.42%.

**Table 16.** Intermediate precision (n = 12 sample preparations).

| Sample | | % w/w | |
|---|---|---|---|
| | | Phenylephrine HCl | Pramoxine HCl |
| Analyst 1, Day 1 | 1 | 0.218 | 0.909 |
| | 2 | 0.219 | 0.917 |
| | 3 | 0.218 | 0.926 |
| | 4 | 0.220 | 0.935 |
| | 5 | 0.221 | 0.922 |
| | 6 | 0.222 | 0.925 |
| Analyst 2, Day 2 | 1 | 0.227 | 0.948 |
| | 2 | 0.223 | 0.935 |
| | 3 | 0.222 | 0.930 |
| | 4 | 0.226 | 0.944 |
| | 5 | 0.221 | 0.925 |
| | 6 | 0.227 | 0.947 |
| **Average** | | **0.222** | **0.930** |
| **Combined %RSD** | | **1.42** | **1.30** |

## Accuracy

Method accuracy or trueness is defined as the closeness of agreement between a reference value (true value) relative to a test result acquired as per the method.[11,12] Accuracy was determined by preparing and analyzing six placebo samples spiked with low, mid, and high levels of phenylephrine HCl + pramoxine HCl standard solution. The accuracy experiment was performed twice on different days with different preparations of standard solution for confirmation purposes. Recovery was calculated by dividing the determined analyte level by the respective spike level in each sample. Accuracy is expressed as the percentage recovery of the 12 samples shown in Tables 17 and 18. The expected level of recovery with respect to the analyte concentrations was 92 to 105%.[12] The recovery of phenylephrine HCl was 97 to 101%, and the recovery of pramoxine HCl was 95 to 101% (Tables 17 and 18). Therefore, method accuracy was considered acceptable.

**Table 17.** Phenylephrine HCl accuracy (n = 12 spiked placebos).

| Sample | Spike Level (mg/mL) | Level Determined (mg/mL) | Recovery (%) |
|---|---|---|---|
| 1 | 0.0290 | 0.0291 | 100.5 |
| 2 | 0.0290 | 0.0293 | 101.0 |
| 3 | 0.0309 | 0.0312 | 101.0 |
| 4 | 0.0309 | 0.0310 | 100.3 |
| 5 | 0.0580 | 0.0570 | 98.3 |
| 6 | 0.0580 | 0.0571 | 98.6 |
| 7 | 0.0617 | 0.0605 | 98.0 |
| 8 | 0.0617 | 0.0616 | 99.8 |
| 9 | 0.0869 | 0.0845 | 97.2 |
| 10 | 0.0869 | 0.0851 | 97.8 |
| 11 | 0.0926 | 0.0902 | 97.4 |
| 12 | 0.0926 | 0.0898 | 97.0 |

**Table 18.** Pramoxine HCl accuracy (n = 12 spiked placebos).

| Sample | Spike Level (mg/mL) | Level Determined (mg/mL) | Recovery (%) |
|---|---|---|---|
| 1 | 0.0631 | 0.0625 | 98.9 |
| 2 | 0.0631 | 0.0631 | 100.0 |
| 3 | 0.0653 | 0.0662 | 101.4 |
| 4 | 0.0653 | 0.0655 | 100.3 |
| 5 | 0.1262 | 0.1240 | 98.2 |
| 6 | 0.1262 | 0.1242 | 98.4 |
| 7 | 0.1306 | 0.1280 | 98.0 |
| 8 | 0.1306 | 0.1297 | 99.3 |
| 9 | 0.1893 | 0.1822 | 96.2 |
| 10 | 0.1893 | 0.1832 | 96.8 |
| 11 | 0.1959 | 0.1883 | 96.1 |
| 12 | 0.1959 | 0.1868 | 95.4 |

## Robustness

Robustness is a measure of the reliability and resistance of a method against slight variations in analytical procedures.[11] Robustness is generally expressed as the level of variance (%RSD) between test results acquired with small, deliberate variations in the test procedure.[11,14] A robustness test is not necessarily restricted by acceptance criteria.[14] Rather, the robustness test indicates that the method is sufficiently accurate and repeatable at the recommended set points defined across an established range.[12] Robustness against varying sample weights for extraction (100 to 300 mg), extraction times (sonication for 5, 10, or 15 minutes), and syringe filters used for sample cleanup (nylon versus polytetrafluoroethylene, PTFE) was investigated.

Per the method, samples containing approximately 100, 200, or 300 mg of the multisymptom relief cream were prepared in duplicate at each level and analyzed. Next, six samples were extracted by sonication for 5, 10, or 15 minutes in duplicate and analyzed. Lastly, six samples were extracted, with one portion of each homogenous extraction solution filtered with a 0.2 µm nylon syringe filter and another portion filtered with a 0.2 µm PTFE syringe filter for comparison. The results of each experiment are shown in Tables 19 to 21, wherein robustness against each variable is expressed as the %RSD of the averaged test results. The levels of robustness against varying sample weights, sonication times, or syringe filters were ≤ 3.88% RSD across a range of 100 to 300 mg of cream sample, ≤ 2.17% RSD across a range of 5 to 15 minutes of sonication, and ≤ 1.13% RSD for cleanups with nylon versus PTFE filtration, respectively.

**Table 19.** Robustness against varying sample weights (n = 6 sample preparations).

| Sample | Sample Weight (mg) | % w/w | |
|---|---|---|---|
| | | Phenylephrine HCl | Pramoxine HCl |
| 1 | 102.08 | 0.225 | 0.921 |
| 2 | 100.79 | 0.233 | 0.948 |
| 3 | 179.90 | 0.219 | 0.916 |
| 4 | 216.90 | 0.215 | 0.899 |
| 5 | 296.77 | 0.211 | 0.891 |
| 6 | 299.90 | 0.211 | 0.903 |
| **Average** | | **0.219** | **0.913** |
| **SD** | | **0.009** | **0.020** |
| **%RSD** | | **3.88** | **2.24** |

**Table 20.** Robustness against varying extraction (sonication) times (n = 6 sample preparations).

| Sample | Sonication Time (min) | % w/w | |
|---|---|---|---|
| | | Phenylephrine HCl | Pramoxine HCl |
| 1 | 5 | 0.212 | 0.895 |
| 2 | | 0.215 | 0.899 |
| 3 | 10 | 0.219 | 0.916 |
| 4 | | 0.215 | 0.899 |
| 5 | 15 | 0.210 | 0.925 |
| 6 | | 0.223 | 0.917 |
| **Average** | | **0.216** | **0.908** |
| **SD** | | **0.005** | **0.012** |
| **%RSD** | | **2.17** | **1.35** |

**Table 21.** Robustness against varying membrane filter types (n = 6 sample preparations filtered using two different techniques to make 12 total test results).

| Sample | Syringe Filter Type | % w/w | |
|---|---|---|---|
| | | Phenylephrine HCl | Pramoxine HCl |
| 1 | Nylon | 0.227 | 0.946 |
| 2 | | 0.224 | 0.940 |
| 3 | | 0.223 | 0.933 |
| 4 | | 0.228 | 0.951 |
| 5 | | 0.221 | 0.927 |
| 6 | | 0.226 | 0.945 |
| 1 | PTFE | 0.227 | 0.948 |
| 2 | | 0.223 | 0.935 |
| 3 | | 0.222 | 0.930 |
| 4 | | 0.226 | 0.944 |
| 5 | | 0.221 | 0.925 |
| 6 | | 0.227 | 0.947 |
| **Average** | | 0.225 | 0.939 |
| **SD** | | 0.003 | 0.009 |
| **%RSD** | | 1.13 | 0.95 |

## Matrix extension

Three products, including a moisturizing anti-itch lotion, a nighttime cough syrup, and a daytime cough syrup were prepared and injected per the method. The sample chromatograms are shown in Figures 7 to 9. The chromatograms were used to evaluate system suitability and specificity in each sample matrix. The system suitability parameters and a comparison of the label claim (API level listed on the product label) to the level of API determined in each sample assay are shown in Tables 22 to 24.

The RTs of the target peaks in each matrix were consistent with the corresponding standards (RT ratios = 1.00). The UV confirmation match factors were ≥ 997.80 and the peak purity match factors were ≥ 998.33, meeting the recommended criteria of > 990. Acceptable chromatographic performance was also demonstrated in each matrix (R = 25.8 or NA, T ≤ 1.25, N ≥ 13,504). Reasonable levels of pramoxine HCl or phenylephrine HCl were quantitated in the respective samples relative to the label claims (96 to 104% of label claims). These results suggest that the method may be applied to other matrices, which is potentially useful for QC test purposes, since phenylephrine HCl and pramoxine HCl are used as APIs in a wide range of OTC products. Further investigation beyond the scope of this study is necessary to evaluate the accuracy and precision of the analysis in each matrix.

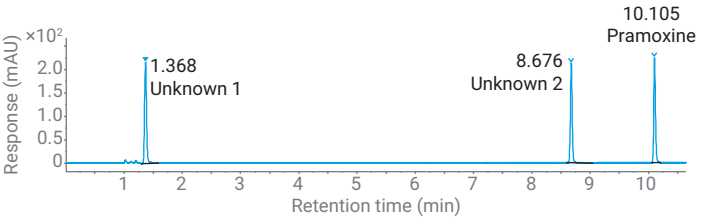



**Figure 7.** Chromatogram of the itch relief moisturizing lotion sample.

**Table 22.** System suitability and specificity for the itch relief moisturizing lotion.

| Analyte | RT (min) [RT Ratio] | USP R | USP T | USP N | UV Purity | UV Match | Label Claim (%) | Level Determined (%) |
|---|---|---|---|---|---|---|---|---|
| Pramoxine HCl | 10.105 [1.00] | 25.8 | 1.25 | 539,370 | 998.33 | 999.95 | 1 | 1.04 (104% of label claim) |

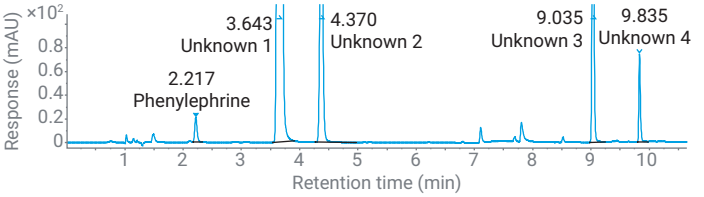



**Figure 8.** Chromatogram of the nighttime severe cold and flu syrup sample.

**Table 23.** System suitability and specificity for the nighttime severe cold and flu syrup.

| Analyte | RT (min) [RT Ratio] | USP R | USP T | USP N | UV Purity | UV Match | Label Claim (%) | Level Determined (%) |
|---|---|---|---|---|---|---|---|---|
| Pramoxine HCl | 2.217 [1.00] | NA | 1.21 | 13,526 | 999.68 | 997.80 | 5 mg per 15 mL (0.033%) | 0.032 (96.3% of label claim) |

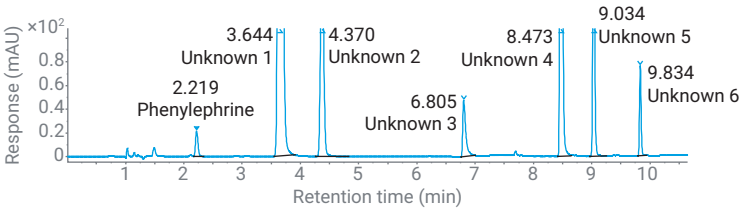



**Figure 9.** Chromatogram of the daytime severe cold and flu syrup sample.

**Table 24.** System suitability and specificity for the daytime severe cold and flu syrup.

| Analyte | RT (min) [RT Ratio] | USP R | USP T | USP N | UV Purity | UV Match | Label Claim (%) | Level Determined (%) |
|---|---|---|---|---|---|---|---|---|
| Pramoxine HCl | 2.219 [1.00] | NA | 1.22 | 13,504 | 999.58 | 997.87 | 10 mg per 30 mL (0.033%) | 0.032 (96.3% of label claim) |

The unidentified peaks in the cough syrup chromatograms are very likely the responses of other APIs listed on the product labels, such as acetaminophen 2.17%, dextromethorphan HBr 0.067%, and guaifenesin 1.33%. This suggests that the method may also be useful for combined testing of additional compounds in cough medicines with multiple APIs. Further investigation beyond the scope of this study is needed to confirm the identities of the unknown peaks with reference standards.

## Conclusion

An Agilent 1260 Infinity II LC system, Agilent 1290 Infinity DAD, and Agilent InfinityLab Poroshell 120 EC-C8 column (3.0 × 150 mm, 2.7 µm) were used to overcome the challenges of previous techniques for phenylephrine HCl and pramoxine HCl testing. Traditional approaches severely impact laboratory throughput because they require individual sample extraction and acquisition methods for each analyte and use outdated, inefficient HPLC column technology with buffer salts that cause cumbersome LC troubleshooting. This method resolves such challenges using LC-friendly mobile phases to combine and modernize test methodologies for OTC products, including topical treatments and cough and cold medicines. Suitable levels of specificity (RT ratio = 1, UV match ≥ 998, peak purity ≥ 999), system suitability (R ≥ 2.35 or NA, T ≤ 1.35, N ≥ 13,490), linearity ($R^2$ ≥ 0.99975), system precision (≤ 0.26% RSD), method precision (≤ 0.95% RSD), intermediate precision (≤ 1.42%), accuracy (95 to 101% recovery), and robustness (≤ 3.88% RSD) were demonstrated in a representative multisymptom relief cream. This method can be used to improve process efficiency, LC system fitness, column longevity, cost-effectiveness, as well as sustainability of OTC product testing, manufacturing regulatory compliance, and product development.

## References


1. USP Monograph Pramoxine Hydrochloride Cream. Current DocID: GUID-8571FEED-22A1-4645-82E7-B3C2083E50B2_1_en-US. DOI: uuw8f. Retrieved October 2024.
2. USP Monograph Phenylephrine HCl. Current DocID: GUID-3A30DAC5-A752-4351-A62B-4FCB7490364C_2_en-US. DOI: av5cy. Retrieved October 2024.
3. USP Monograph Phenylephrine HCl in Nasal Solution. Current DocID: GUID-2F4BD480-2965-43D8-B9A2-8F719D67248B_2_en-US. DOI: fah4y. Retrieved October 2024.
4. Upadhyay, Y.; Sharma, N.; Sarma, G. S.; Rawal, R. K. Application of RP-HPLC Method in Dissolution Testing and Statistical Evaluation by NASSAM for Simultaneous Estimation of Tertiary Combined Dosages Forms. *J. Pharm. Anal.* **2015**, *5(5)*, 307–315. DOI: 10.1016/j.jpha.2014.11.001. PMID: 29403944; PMCID: PMC5762241.
5. Sanchaniya, P.; Mehta, F.; Uchadadiya, N. Development and Validation of an RP-HPLC Method for Estimation of Chlorpheniramine Maleate, Ibuprofen, and Phenylephrine Hydrochloride in Combined Pharmaceutical Dosage Form. *Chromatogr. Res. Int.* **2013**. DOI: 10.1155/2013/424865.
6. Dewani, A. P.; Dabhade, S. M.; Bakal, R. L.; Gadewar, C. K.; Chandewar, A. V.; Patra, S. Development and Validation of a Novel RP-HPLC Method for Simultaneous Determination of Paracetamol, Phenylephrine Hydrochloride, Caffeine, Cetirizine and Nimesulide in Tablet Formulation. *Arab. J. Chem.* **2015**, *8(4)*, ISSN 1878–5352.
7. Ibrahim, F.; El-Deen, A. K.; Shimizu, K. Comparative Study of Two Different Chromatographic Approaches for Quantitation of Hydrocortisone Acetate and Pramoxine Hydrochloride in Presence of Their Impurities. *J. Food Drug Anal.* **2018**, *26(3)*, 1160–1170. DOI: 10.1016/j.jfda.2017.12.008. PMID: 29976408; PMCID: PMC9303017.

8. Tavlarakis, P.; Greylinga, J.; Snow, N. H. Simultaneous Determination of Pramoxine HCl and Benzalkonium Chloride in Wound Care Solutions by HPLC. *Anal. Methods* **2010**, *2*, 722–727.
9. USP Harmonized Standards Home Page. Supplement USP Stage 4 Harmonization, Official, December 1, 2022, latest revision in April 2023.
10. Fu, R.; Grover, M.; Freeman, R.; Long, W. Understanding the Latest Revisions to USP <621>; *Agilent Technologies white paper*, publication number 5994-6618EN, **2023**.
11. U.S. Department of Health and Human Services Food and Drug Administration Center for Drug Evaluation and Research (CDER) Center for Biologics Evaluation and Research (CBER). Q2(R1) Validation of Analytical Procedures: Text and Methodology Guidance for Industry. November 2005 ICH. Retrieved October 2024.
12. AOAC Official Methods of Analysis - Appendix F: AOAC Guidelines for Standard Method Performance Requirements, **2016**.
13. OpenLab CDS Data Analysis v2.7 Reference Guide; *Agilent Technologies reference guide*, publication number D0013815 Rev. A, **2022**.
14. Little, T. Establishing Acceptance Criteria for Analytical Methods. *BioPharm Int.* **2016**, *29(10)*.

**www.agilent.com**

DE-003924


Printed in the USA, March 13, 2025
5994-8113EN

Agilent
Trusted Answers