

# Tomorrow's quantitation with the TSQ Fortis mass spectrometer: quantitation of phenylephrine hydrochloride for QA/QC laboratories

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## Goal

Development of a robust, reliable, and reproducible quality control workflow for the analysis and quantitation of phenylephrine hydrochloride for QA/QC using a triple quadrupole mass spectrometer.

## Application benefits

- Develop robust and easy quantitation workflows with reproducibility, reliability, and the required sensitivity for phenylephrine HCl in Milli-Q® water
- Implement easy-to-use and easy-to-implement quantitation workflows that allow quantitation of any molecule type in matrix regardless of user expertise and experience

## Introduction

Phenylephrine HCl is a nasal decongestant that provides relief from discomfort caused by colds, allergies, and hay fever.<sup>1</sup> Quantitation of phenylephrine HCl in biological matrices (e.g., plasma) bears unique significance due to determining its optimal dosage as a nasal decongestant and also quantifying it as a drug of abuse if consumed. In addition, developing robust and accurate quantitation assays for quantifying phenylephrine HCl in water is critical for quality control (QC) and quality

assurance (QA) processes. These QA/QC methods not only provide the concentration of phenylephrine HCl but also analyze and quantify any impurities that may have been added or formed during the manufacturing process. There have been several reports of quantitative assays of phenylephrine hydrochloride (phenylephrine HCl) that used a host of technologies.<sup>2</sup> These ranged from chromatographic to electrochemical and spectrophotometric techniques. Although liquid chromatography (LC) with ultraviolet or fluorescence detection has been well established, LC methods often face serious limitations due to poor sensitivity and longer analysis times.

Liquid chromatography coupled to triple quadrupole mass spectrometry (QqQ, also represented as MS/MS) offers some significant advantages, especially in offering higher sensitivity, selectivity, and productivity with remarkable reproducibility for quantitation assays. However, despite the outstanding quality of data and productivity gains offered by LC-MS/MS, for the typical QA/QC environment, minimizing the cost per sample continues to be a big challenge. The use of high-end QqQs often adds complexity to the challenge of

successfully addressing the cost per sample issue, which in turn, can affect organizational profitability. Striking an optimal balance between choosing the right LC and MS platforms that can offer quality data while addressing other organizational challenges is extremely important for every QA/QC laboratory. In this report, we offer a robust, reliable, reproducible quantitation assay of phenylephrine HCl in Milli-Q water with a Thermo Scientific™ Vanquish™ Flex UHPLC system, Thermo Scientific™ TSQ Fortis™ triple quadrupole mass spectrometer, and Thermo Scientific™ TraceFinder™ 4.1 software.

## Experimental

### Sample preparation

A 100 mg/L phenylephrine HCl stock solution was prepared by dissolving 10 mg of phenylephrine HCl in a 100 mL volumetric flask using Milli-Q water. The stock solution was further diluted as shown in Table 1.

The QC analysis of phenylephrine HCl was carried out by preparing three different QC samples from the parent stock solution. The final concentrations of phenylephrine HCl in the QC A, QC B, and QC C solutions were 0.01, 0.002, and 0.001 ppm, respectively (Table 2).

**Table 1. Dilution of phenylephrine HCl stock dilution using Milli-Q water**

Calibration Level	Sample ID	Prep From	Stock (mL)	Mobile Phase A (mL)	Final Volume (mL)	Final Conc. (mg/L)
Cal 06	A	Stock	1.0	99.0	100.0	1.0
Cal 05	B	A	1.5	8.5	10.0	0.150
Cal 04	C	A	2.0	18.0	20.0	0.100
Cal 03	D	C	5.0	5.0	10.0	0.050
Cal 02	E	C	1.0	9.0	10.0	0.010
Cal 01	F	E	5.0	7.5	12.5	0.004

**Table 2. Preparation of QC solutions from phenylephrine HCl stock solution**

Sample ID	Prep From	Stock (mL)	Mobile Phase A (mL)	Final Volume (mL)	Final Conc. (mg/L)
QC A	A	1.0	99.0	100.0	0.01
QC B	QC A	2.0	8.0	10.0	0.002
QC C	QC A	1.0	9.0	10.0	0.001

## Liquid chromatography

Liquid chromatographic analysis was done using the Vanquish Flex UHPLC system equipped with a Thermo Scientific™ Hypersil GOLD™ column (50 × 2.1 mm, 1.9 μm) that was conditioned and operated at 30 °C. A 2 μL injection volume was used for all analyses following the gradient indicated in Table 3 and Figure 1.

## Mass spectrometry

The TSQ Fortis mass spectrometer was used for this analysis with positive heated electrospray ionization (HESI) mode. The experimental conditions were

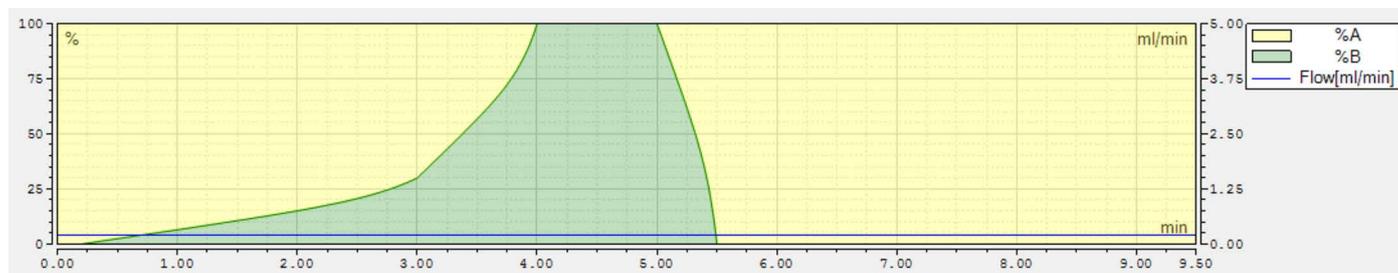
optimized with a static spray voltage of 3500 V, a cycle time of 0.3 s, and both Q1 and Q3 resolutions maintained at 0.7 Da FWHM. The ion transfer tube and vaporizer temperatures were maintained at 300 °C and 225 °C, respectively. The HESI probe position was optimized following the instrument control software guide and was locked. The SRM table and other critical MS features for all the target analytes are listed in Table 4.

## Software

Data acquisition and processing were conducted using TraceFinder software version 4.1.

**Table 3. LC gradient information**

Time (min)	Flow (mL/min)	%B	Curve
<b>Equilibration</b>			
0.000	0.200	0.0	5
<b>Run</b>			
0.000	0.200	0.0	5
0.200	0.200	0.0	6
3.000	0.200	30.0	6
4.000	0.200	100.0	6
5.000	0.200	100.0	6
5.500	0.200	0.0	6
9.500	0.200	0.0	6



**Figure 1. LC gradient profile for the analysis of phenylephrine HCl in water**

**Table 4. Optimized mass spectrometer transitions for phenylephrine HCl analyzed in this experiment**

Compound	Precursor (m/z)	Product (m/z)	Collision Energy (V)	Min Dwell Time (ms)	Tube Lens (V)
Phenylephrine HCl	168.1	91.0	23	149	55
Phenylephrine HCl	168.1	150.0	10	149	55

## Results and discussion

The superior performance and outstanding resolution offered by the Vanquish Flex UHPLC, combined with the robustness, selectivity, and sensitivity offered by the TSQ Fortis MS, enabled efficient quantitation workflows for phenylephrine HCl in water. The superior performance of the LC-MS/MS platform solution not only enabled identification but also routine quantitation of analytes without contamination of the ESI-MS/MS instrument source.

The calibration curve (Figure 2) highlights the linearity and range of sensitivity that were addressed by this quantitation workflow. A five-point linear calibration curve showed a minimum  $R^2$  value of 0.9993 (Figure 2), when a linear curve fitting with weighting  $1/x$  was applied to the curve. The lower limit of quantitation (LLOQ) calibration standard shows an acceptable CV value of 4.72%. Representative chromatograms of phenylephrine HCl at 4  $\mu\text{g/L}$  and that of the blank sample are shown in Figure 3.

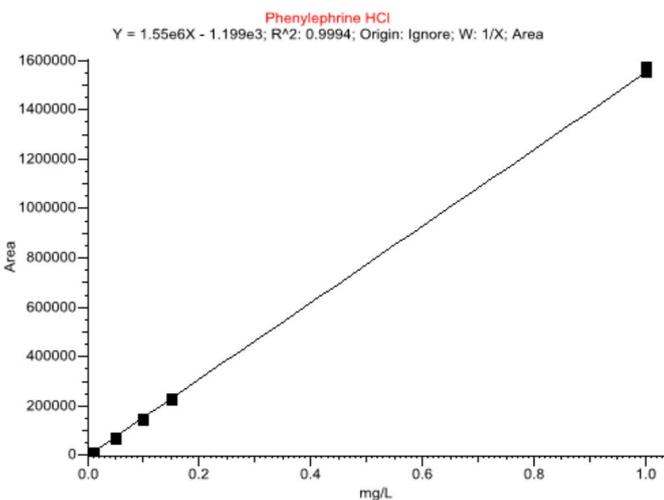
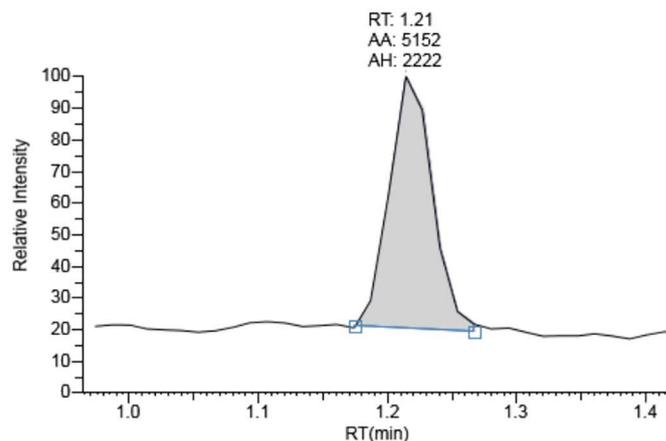


Figure 2. Calibration curve of phenylephrine HCl in Milli-Q water

Cal01-6 Phenylephrine HCl m/z: 150.04



Blank-04 Phenylephrine HCl m/z: 150.04

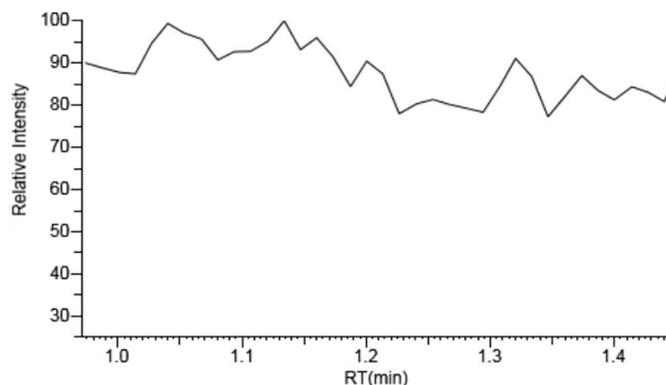
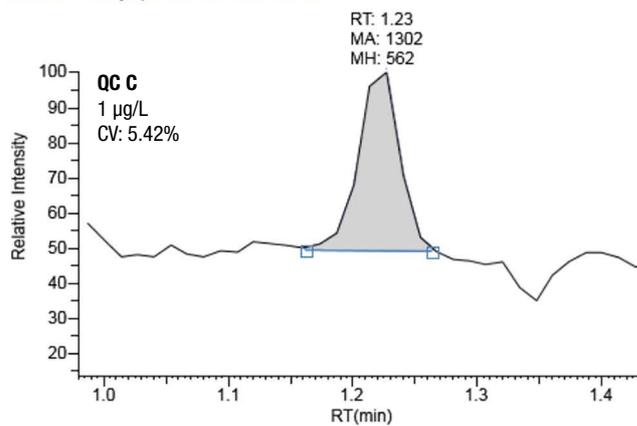


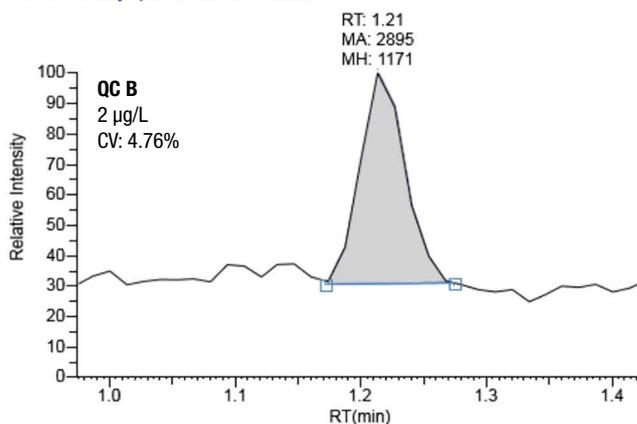
Figure 3. Chromatogram of phenylephrine HCl in LLOQ solution with a concentration of 4  $\mu\text{g/L}$  (top) and that of the blank sample (bottom)

For the three analytical batch runs (Figure 4), the precision (% CV) ranged from 2.19% (QC C) to 4.76% (QC B) to 5.42% (QC A). The concentration ranges for the three shown QC samples are 10 µg/L (QC A) to 2 µg/L (QC B) to 1 µg/L ppm (QC C). The correlation coefficients ( $R^2$ ) for these analytical batches of phenylephrine HCl were > 0.99. The results obtained for the analysis of the QC batches are well within the acceptable quantitation, precision, and accuracy limits.

QC3-59 Phenylephrine HCl m/z: 150.04



QC2-50 Phenylephrine HCl m/z: 150.04



QC1-41 Phenylephrine HCl m/z: 150.04

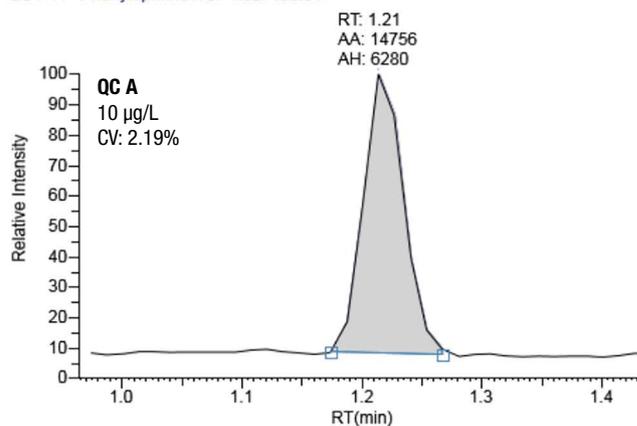


Figure 4. QC chromatograms of phenylephrine HCl at 10 µg/L (bottom), 2 µg/L (middle), and 1 µg/L (top)

## Conclusions

Robust, reproducible, sensitive, and affordable quantitation workflows for phenylephrine HCl are extremely important for determining the dosage amount, concentration in samples, and ensuring purity. An LC-MS/MS method for the quantitation of phenylephrine HCl in water was successfully developed using the Vanquish Flex UHPLC system and the TSQ Fortis mass spectrometer. The same quantitation workflow can also be transferred to quantify phenylephrine HCl in biological matrices. Five separate batches were analyzed for this study, with a linear fit  $R^2$  value of 0.9993. The precision and accuracy results for the QC samples in all batches addressed the expectations.

## References

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