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Comparative Quantitative Analysis of Cetirizine Dihydrochloride by HPLC (High Performance Liquid Chromatography) and q_NMR (Quantitative Nuclear Magnetic Resonance) Techniques

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Factor	Lower level	Upper level
% Organic phase	40	70
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Buffer Concentration (mM)	20	40
Flow rate (mL/min)	1	2
Injection volume (µL)	5	20

Table 2. *Matrix of Plackett-Burman Screening*

Buffer pH	Buffer Concentration (mM)	Organic %	Flow Rate	Injection Volume (uL)
2.1	20	40	2	20
2.1	20	40	1	5
2.1	20	70	2	20
2.1	40	40	1	5
2.1	40	70	2	5
2.1	40	70	1	20
2.5	20	40	1	20
2.5	20	70	2	5
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2.5	40	40	2	5
2.5	40	40	2	20
2.5	40	70	1	20

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Injection volume (µL)	5	10

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Buffer Concentration	Organic %	Injection Volume
30	50	7.5
30	65	10
30	65	5
30	80	7.5
40	50	10
40	50	5
40	65	7.5
40	65	7.5
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50	50	7.5
50	65	10
50	65	5
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60 µg/mL	120%
80 µg/mL	160%
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Concentration (µg/mL)	Mean Area	%CV	N
98.000	426.1232	0.2632647	3
78.400	346.6939	0.0999582	3
58.800	257.0439	0.08409429	3
39.200	166.6318	0.1448752	3
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	Average	%CV	
0	225.65	0.2166	100.0
8 days	227.00	0.3084	100.6

Table 9. *Average retention time from 3 trials at nominal concentration ran 8 days apart from one another.*

Time	**Retention Time (min)**		**Percent of initial**
	Average	%CV	
0	1.861	0.03103	100.0
8 days	1.849	0.06246	99.4

Table 10. *Results of HPLC Precision analysis of 6 samples at the same nominal concentration.*

Sample	**Peak area**
1	224.1618
2	224.7716
3	224.6507
4	224.4123
5	224.7069
6	224.8817
Mean	224.598
SD	0.24153
%CD (±2%)	0.10754

Table 11. *Shows the %CV for n = 3 trails of each concentration for each set of protons in NMR analysis.*

Concentration (mM)	**%CV**		
	Aromatic Protons	5.14 ppm Protons	4.31 ppm Protons
98.240	0.4833	0.1373	0.5400
78.590	0.3931	0.3991	0.8029
58.940	0.1113	0.2994	0.3495
39.300	0.3965	0.7217	0.1972
19.650	0.3612	3.1987	2.4465

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Sample	Percent of nominal (µg/mL)			Amount of Cetirizine (µg/mL)			
	%	Concentration	Concentration Corrected	Total	Spike	Recovered	% Recovery (n=3)
1	50	25.98	25.47	35.31	9.882	9.656	99.53%
2	100	52.27	51.25	65.50	9.882	10.25	103.8%
3	150	78.84	77.29	87.31	9.882	10.014	101.34%
Mean						10.035	101.54%
Standard Deviation						0.2096	0.02121
%CV						2%	2%

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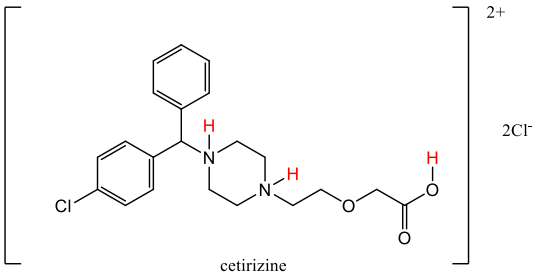
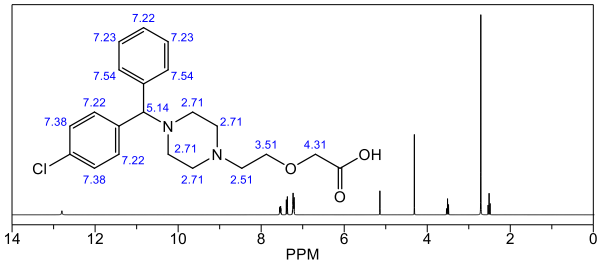


Figure 1. *cetirizine dihydrochloride (acidic protons are red)*



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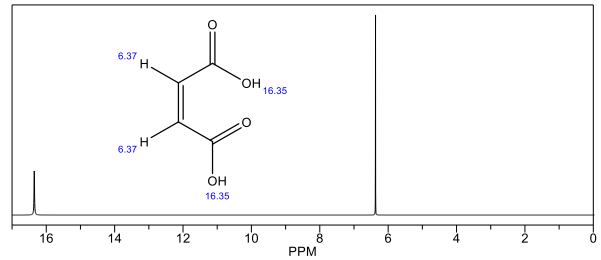

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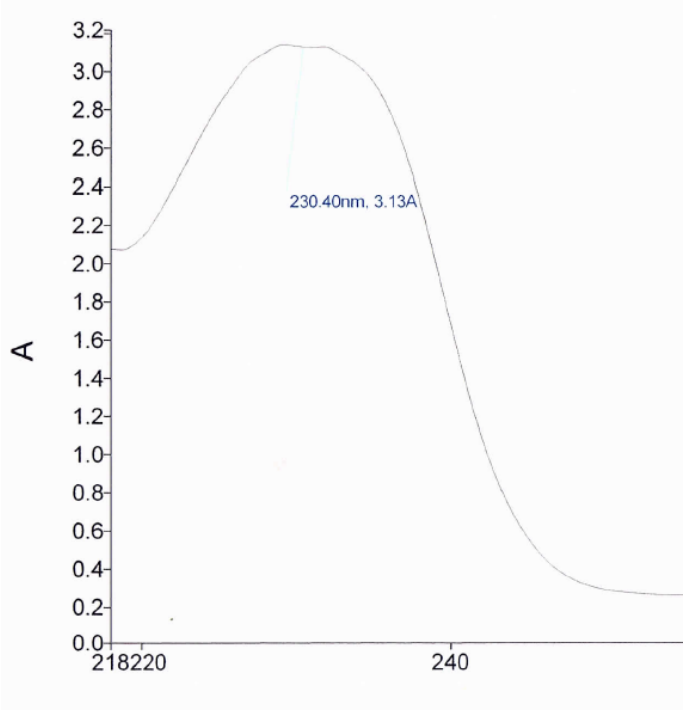

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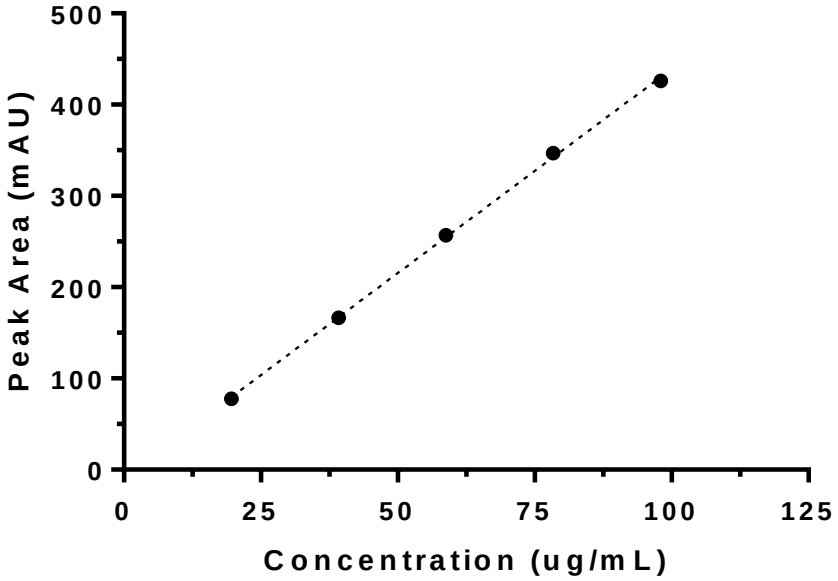

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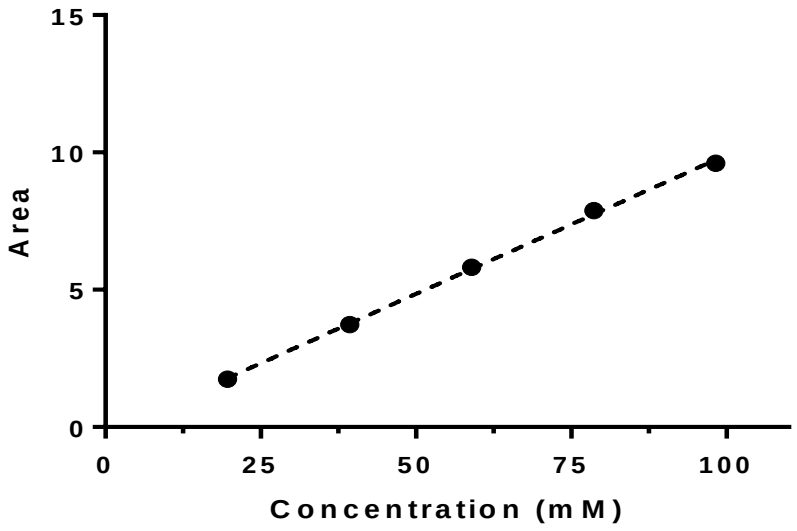

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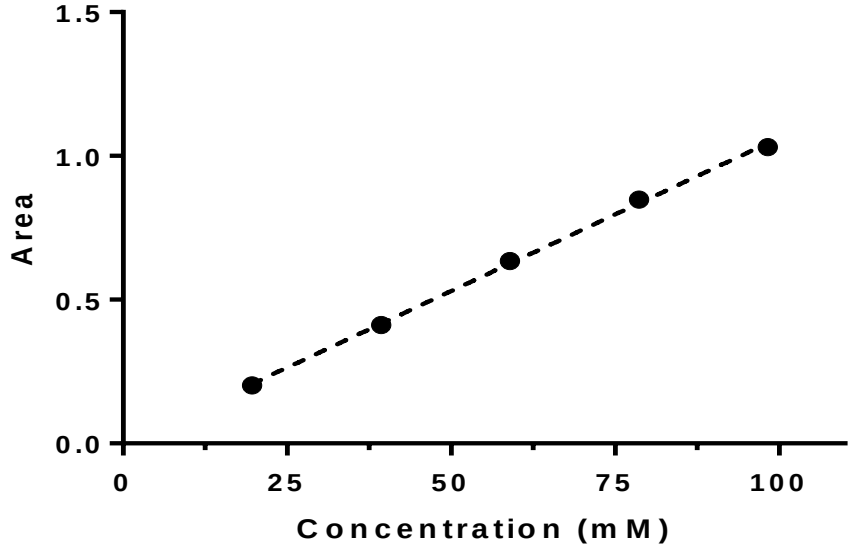

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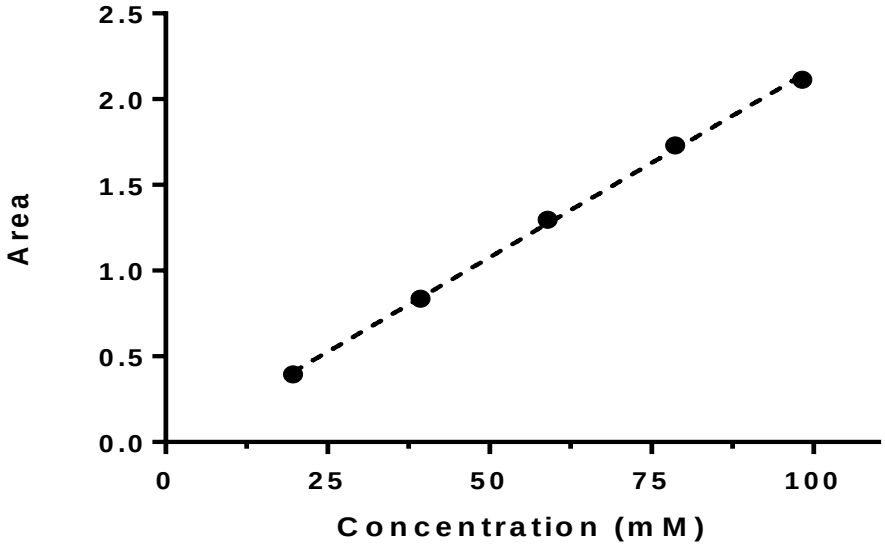

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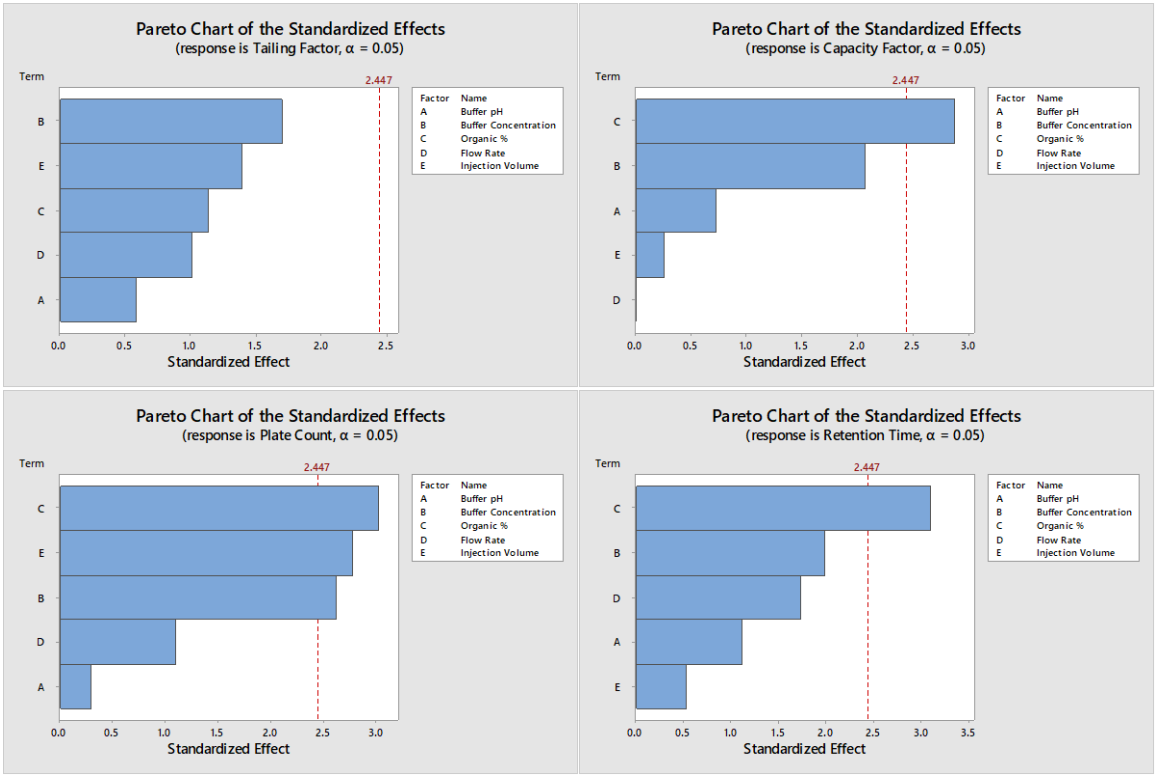

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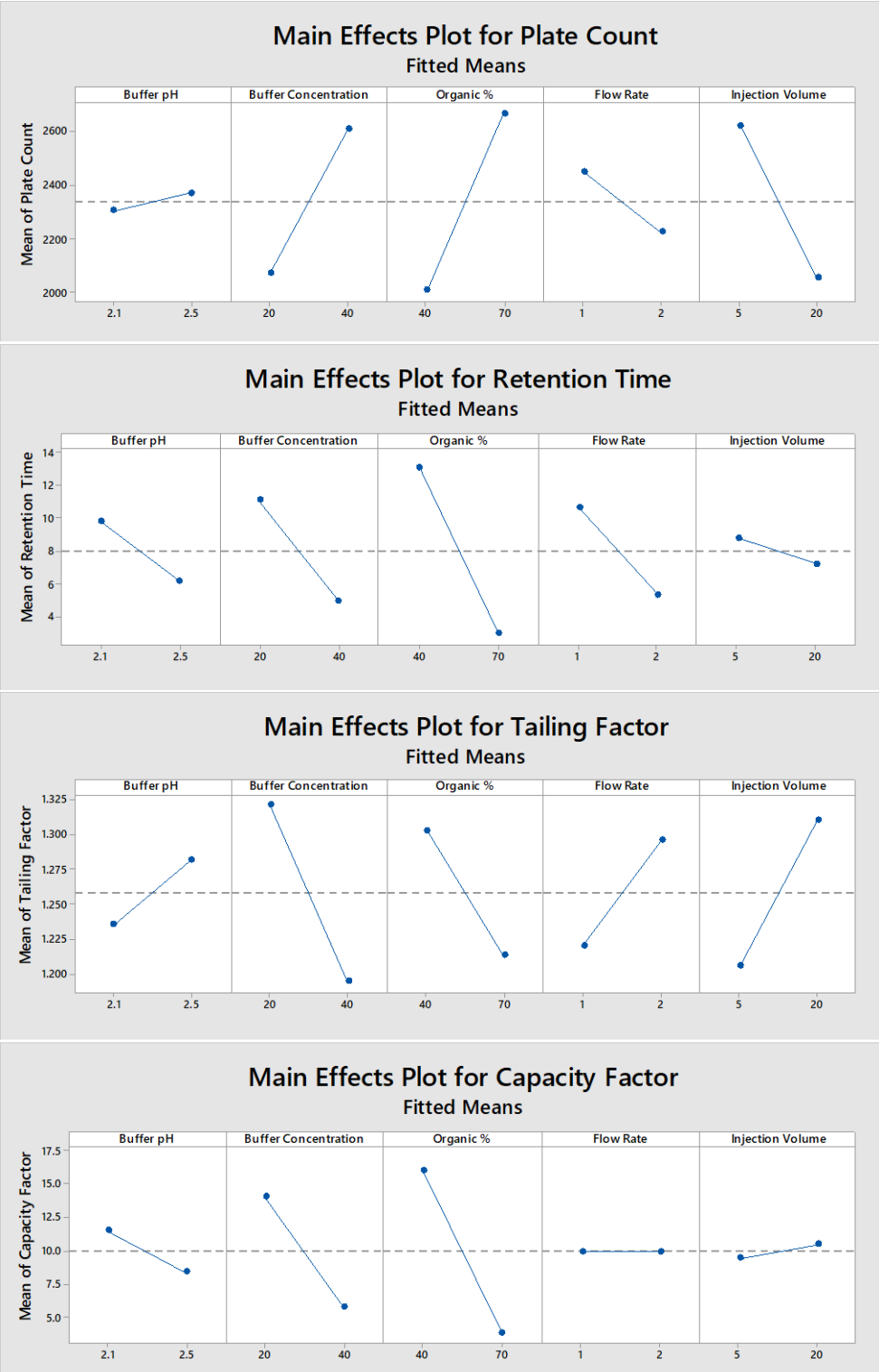

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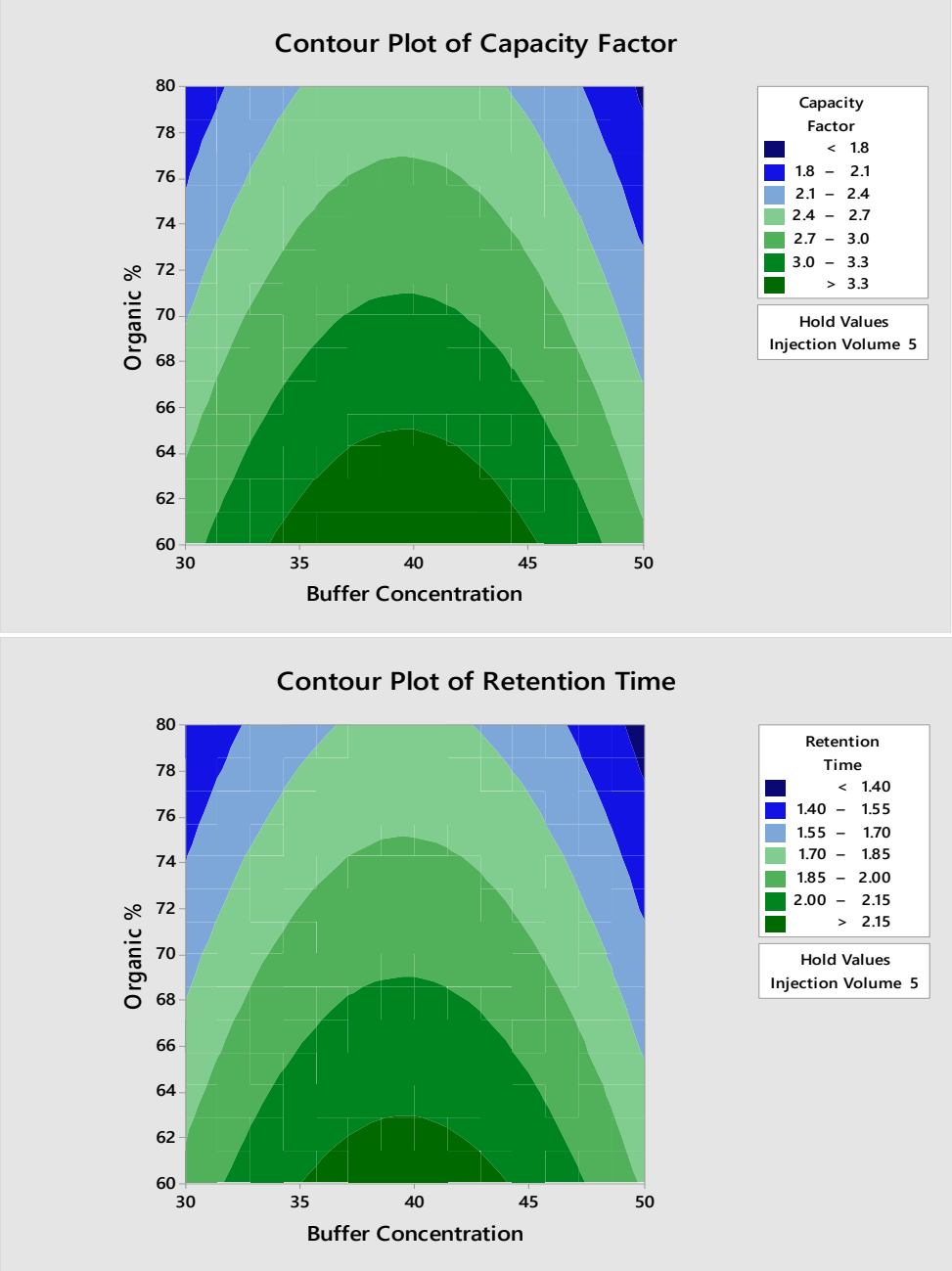

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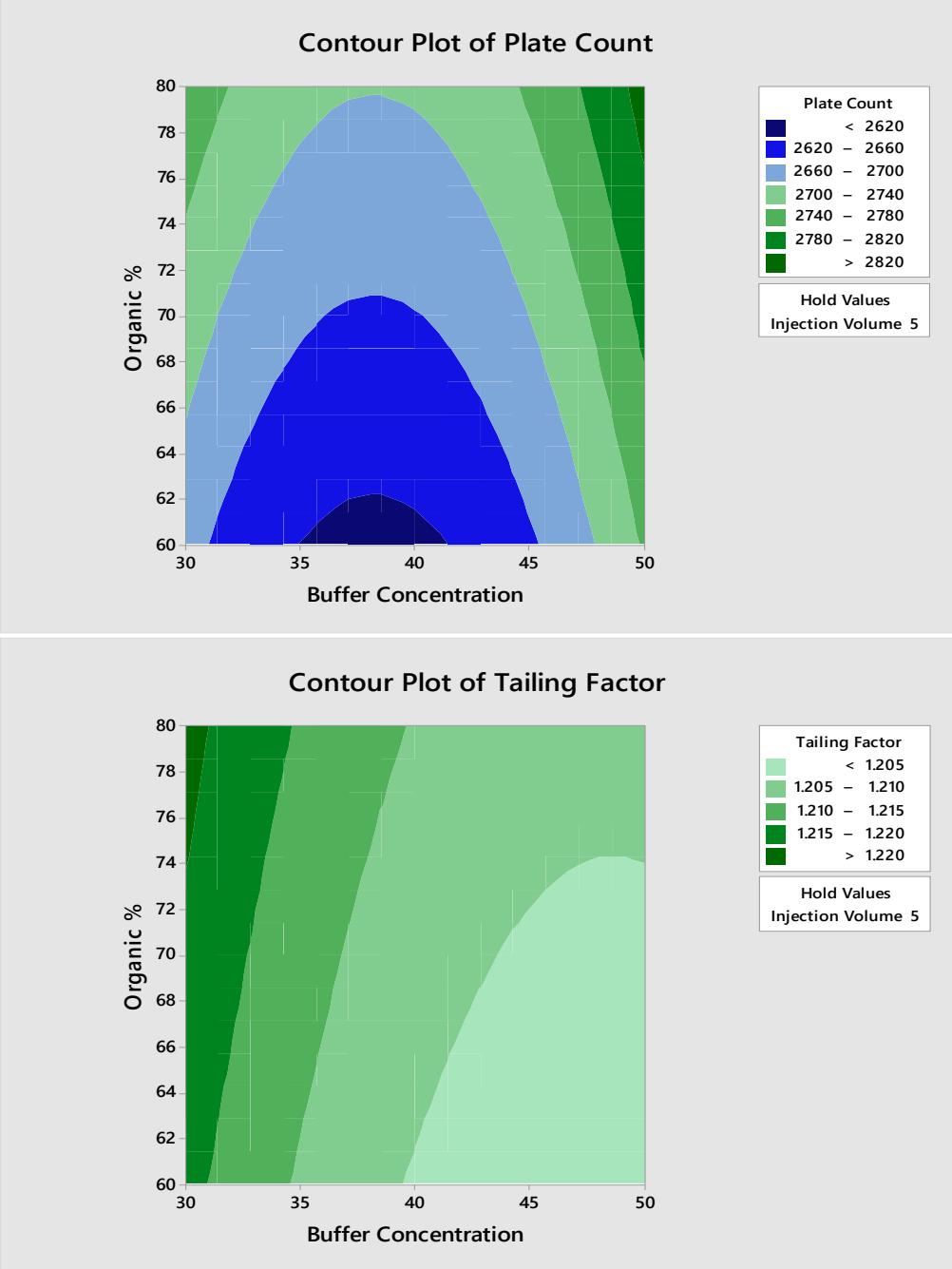

Figure 12. *Contour plots of plate count and tailing factor*

ABSTRACT

In the pharmaceutical industry, the primary method of analysis comes from High-Performance Liquid Chromatography (HPLC) and Gas chromatography (GC). Although these methods are excellent in their ability to quantitate data there exists another option that isn't readily used in quantitative analysis. Quantitative Nuclear Magnetic Resonance (qNMR) exists as another viable alternative, unlike HPLC and GC techniques qNMR does not require any specific chromatographic conditions or the constant generation of mobile phase waste. This work looks to develop, optimize and compare quantitative methods of analysis for Cetirizine Dihydrochloride by HPLC and qNMR.

HPLC methods of analysis adhere to a Design of Experiment[1,2] approach which employs a screening stage followed by a later optimization stage. A Plackett-Burman design assessed the potential critical analytical attributes (CAA) (Buffer Concentration, Buffer pH, Organic % and Injection volume) by evaluating the responses (Capacity Factor, Plate Count, Tailing Factor and Retention Time). A Box Behnken design further optimized results by evaluating main interactions and their quadratic effects.

Optimization results show optimal chromatographic conditions occurring with a 2.1 pH 40 mM phosphate buffer with Acetonitrile 22:78 v/v as a mobile phase at a flow rate of 2 mL/min and an injection volume of 5 uL. Optimal conditions showed a 4% deviation in retention time from the predicted DOE value. Validation of the HPLC method based on the guidelines outlined by ICH Q2R1[3] showed a high degree of linearity (r square value of 0.9994), accuracy, precision, and stability.

In a D_2O based solvent system using maleic acid as an internal standard, the q-NMR analysis looks at the peak area of three sets of protons in cetirizine with respect to the olefinic protons peak area of maleic acid. When plotted over a concentration range of 20 -100mM linearity from all three cetirizine peaks show r square values greater than 0.998. Triplicate runs of each calibration sample show the highest %CV being 0.483, which indicates excellent precision. NMR waste generation is relatively small in comparison to the HPLC method, compared to the 1.5 L acetonitrile used in HPLC qNMR analysis only required 25 mL of deuterated water. Method development was also drastically shorter at roughly 2 hours when compared to the 15 hours allocated to the HPLC method.

The results of this study show that not only is qNMR up to the task of quantitative analysis but is capable of doing so while significantly decreasing the generation of waste and time of analysis.

1. INTRODUCTION

Cetirizine dihydrochloride[4–9] is an over the counter 2nd generation histamine h1 antagonist. It is typically available in pill form and comes in 10 mg dosages. Cetirizine dihydrochloride is frequently used in the treatment of hay fever, hives, itching and allergy symptoms. Name brands include Zyrtec and Alleroff.

Cetirizine's structure contains three acidic protons shown in red in figure 1. Each proton refers to a separate pKa value, care must be taken so that deprotonation does not occur during HPLC analysis as this could affect results. To prevent deproteinization from occurring a phosphate buffer is utilized.

Typical methods of analysis are often only as reliable as the design of the experiment they are analyzing. By applying a DoE (design of experiment)[1,2] based approach, a reliable method is able to be quickly developed. The DoE works as a two-step process, first by screening the available factors to determine the critical analytical attributes (CAA)[10–12]. CAA's are factors that when manipulated show a statistically relevant change in the experiment's response. The responses[13] Capacity Factor, Plate Count, Tailing Factor and Retention Time are all looked at in this study.

After CAA's are determined an optimization is carried out in order determine the specific level for each CAA in order to generate an optimal response. In this work retention time is looked to be maximized while minimizing plate count. Tailing factor is to be held as close to one as possible while also maintaining a capacity factor of greater than two in order to ensure good separation.

Unlike in HPLC, qNMR has no need for the design of any complex method, instead it looks to directly analyze a sample which is prepared in a deuterated solvent. Quantitation is achieved through comparison of the relative peak area of two separate species. The strength of qNMR lies in its response being directly proportional to the number of protons of the species under analysis, therefore this response remains the same for all compounds.

NMR of Cetirizine gives rise to several sets of peaks, however, only three groups are of interest for the sake of this study. The aromatic protons occurring between 7-8 ppm as well as protons found at 5.14 and 4.31 ppm (figure 2) are used to evaluate Cetirizine's concentration. A solvent system prepared from maleic acid in D2O is used as an internal standard to generate a peak to reference off of. Maleic acid has a single peak occurring at 6.37 ppm (figure 3) for its olefinic protons, this peak does not interfere with any of the peaks from Cetirizine and there for is a suitable internal standard.

2. EXPERIMENTAL

2.1. Instrumentation

A Lambda 35 UV/Vis spectrometer is used to determine the maximum UV absorption. pH meter used is an OAKTON ION 700. The HPLC (Agilent 1260 Infinity) instrument is equipped with a model G4225A degasser, model G1312B binary pump, model G1329B auto loader, model G1316A column compartment and a model G1315D diode array detector. A Bruker 300 Ultrashield is used for qNMR analysis

2.2. Materials

The Cetirizine (Ak Scientific Inc.) used for calibration standards is 98% purity. The Cetirizine used for analysis is the brand GoodSense with a product name of “All Day Allergy”. Each tablet is stated to contain 10 mg of Cetirizine. All water used is filtered through a Millipore Synergy UV-R system. Anhydrous sodium phosphate monobasic (Fisher) 99% purity, 85% phosphoric acid (Fisher) and Acetonitrile (Honeywell) HPLC grade are used throughout the experiment. For NMR analysis Deuterium oxide is used as well as Maleic acid. All DoE (Design of Experiment) analysis is computed using MiniTab18.

2.3. DoE (Design of Experiment) Screening

For HPLC analysis a max absorbance of cetirizine is determined by UV-VIS, shown in figure 4.

A Plackett-Burman design[1,14] is used to evaluate five factors for statistical influence. These factors are percent organic in mobile phase, buffer pH, buffer concentration, flow rate, and injection volume. These factors are evaluated on plate count, retention time, tailing factor, and capacity factor. Table 1 shows the levels for the factors and the execution of the Plackett-Burman design is outlined in table 2, all runs are conducted in triplicate.

2.4. DoE (Design of Experiment) Optimization

A Box Behnken design[10,12] is used to evaluate the factors outlined in table 3. Buffer pH and flow rate are kept constant at 2.1 and 2 mL/min respectively.

These factors are evaluated on plate count, retention time, tailing factor, and capacity factor. Table 3 shows the levels for the factors and the execution of the Box Behnken design is outlined in table 4, all runs are conducted in triplicate.

2.5. Sample preparation

Cetirizine tablets are prepared by crushing ten tablets to a fine powder with a mortar and pestle. The theoretical Cetirizine concentration can then be determined by dividing the mass of the tablets by ten to determine the average mass of an individual tablet. Since each tablet has a theoretical cetirizine concentration of 10 mg this can be used to determine to amount of tablet powder required to contain a specific amount of cetirizine (table 5).

2.6. Validation

Validation[15] is carried out according to ICH Q2R1[3] guidelines. All calibration standards and tablet samples are passed through a 0.2 µm membrane filter.

2.6.1 Accuracy

Spiked samples are prepared in triplicate at three levels across a range of 50-150% of the target concentration. The percent recovery is then calculated. Each concentration is run in triplicate to insure precision.

2.6.2 Precision (Repeatability)

Six samples at nominal concentration are prepared from tablet and are analyzed for %CV.

2.6.3 Range

ICH guidelines[3] specify a minimum of five concentration levels, along with certain minimum specified ranges. For assay, the minimum specified range is 80–120% of the theoretical content of active.

2.6.4 Linearity

A calibration curve comprising of five concentrations is established between 20-100 µg/mL is evaluated for linearity. Each concentration is run in triplicate to insure precision.

2.6.5 Stability in analytical solutions

Peak area of nominal sample is analyzed fresh and after a one-week time period to measure the stability of the solutions after an extended period of time.

3. RESULTS AND DISCUSSION

3.1. DoE (Design of Experiment) Screening

UV/Vis analysis of Cetirizine in 0.05 M 2.11 pH Phosphoric-Acid (Sodium) buffer solution is carried out in order to determine its lambda max under these conditions. Referring to figure 4, a lambda max of 230 nm is observed and used for HPLC analysis.

Analysis of the pareto charts from the Plackett-Burman screening design show that flow rate and buffer pH are statistically insignificant as shown in figure 9. Based on these results the CAA's are determined to be buffer concentration, Organic % and Injection volume.

The main effects plot shown in figure 10 show higher buffer concentrations resulted in improved tailing factor, plate count and reduced retention time. Higher organic concentration resulted in improved tailing factor, plate count and reduced retention time. Lower injection volume showed improved tailing factor, plate count and reduced retention time. Buffer pH and flow rate show no apparent impact, although flow rate has a high influence on retention time maximizing it would only give favorable results. Buffer pH will be kept at a minimum, flow rate is held at 2 mL/min with a buffer pH of 2.1 for all further optimization runs.

3.2. DoE (Design of Experiment) Optimization

Tables 3 and 4 respectively show the factors with their upper and lower levels as well as the actual run parameter's. Figures 11 and 12 show the contour plots generated from the optimization run parameters. Ideal conditions based on contour plots, occur at an organic % of 78 and buffer concentration of 40mM. Injection volume was favored at the lower volume of 5 µL across all runs. Based on the contour plots, these regions allow for slight variance while maintaining a constant response.

3.2.1. Chromatographic conditions

The mobile phase consists of a 40 mM Phosphoric-Acid (Sodium) Buffer solution with Acetonitrile mixed by binary pump at a ratio of 22:78 respectively. pH of the phosphoric-Acid (Sodium) Buffer Solution has an apparent pH of 2.1 at its final concentration of 40 mM. All analysis is performed at ambient temperature using a C_{18}, 53 x 7 mm i.d., reverse phase column (Grace, 3 µM). The diode array detector is set to monitor the 230-nm wavelength which represents where Cetirizine absorbs at its max. The injection volume is set to 5 µL with a flow rate of 1 mL-min^{-1}.

3.3. HPLC Validation

3.3.1 Accuracy

Method accuracy was determined by the method of standard addition utilizing a 9.882 µg/mL spike (Table 12). This spike was performed on three separate concentrations of cetirizine prepared in 20 mM phosphate buffer at 2.1 pH and run in triplicate. The recovered spike amount shows a %CV of 2 with an average 1.54% deviation from the actual spike amount across all levels.

3.3.2 Range

The method works at a range of 20 µg/mL to 100 µg/mL or 40% to 200% of the nominal concentration of 50 µg/mL.

3.3.3 Linearity

A linear calibration graph (Figure 5) of peak area is plotted against concentration generating an R square of 0.9994 over the working range outlined in table 6 and 7. A %CV for n = 3 of below 1% (Table 7) for all standard solutions is obtained which displays the precision of this chromatographic method.

3.3.4 Stability in Analytical Solution

Stability of Cetirizine in solution as well as the buffers stability showed negligible change in peak area and retention time as shown in table 8 and 9.

3.3.5 Precision (Repeatability)

Six samples prepared from tablet at nominal concentration showed a %CV of under 1 percent in peak area measurements (Table 10). This shows the excellent precision of the method.

3.4 Time and Solvent Consumption

Method development, validation as well as analysis took approximately 15 hours to complete. HPLC runs were automated as allowed and completed over the course of a two-week period. All runs used freshly prepared samples as well as buffer solution prepared on the day of analysis. This generated a total of 1.5 L of acetonitrile waste and approximately 2 L of phosphate buffer waste.

3.4 NMR Analysis

Analysis of Cetirizine by NMR showed excellent linearity for all three proton sets analyzed with an R square 0.9988 or better as shown in figures 6, 7 and 8.

Precision for each trial were also excellent showing an %CV of less than 1 across the concentration range for the aromatic protons and a maximum %CV of 3.1987 low concentration for the 5.14 ppm proton (Table 11).

3.4.1 Time and Solvent Consumption

Unlike the HPLC experiment the NMR experiment did not require any method development time. Due to the nature of NMR the samples are able to be ran directly prepared in deuterated water. This made analysis and preparation time only require approximately 2 hours to complete. The solvent consumption also is kept at a minimal using only 25 mL of deuterated water.

4. CONCLUSION

The HPLC Method presented showed excellent precision and accurately determines the Cetirizine dihydrochloride content in tablet form. Like the HPLC method the q-NMR method also shows excellent precision. Both methods show excellent linearity with R Square values greater than 0.99 for all calibration curves. Waste and time of analysis for both methods varied greatly however, with q-NMR taking significantly less time while generating far less waste.

This shows q-NMR can be a viable alternative in the analysis of pharmaceuticals. By using q-NMR over HPLC you can expect to see a drastic reduction in the generation of waste as well as method development and analysis time.

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