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QBD based Method Development and Validation for Anti-Inflammatory Drugs in Bulk and Fast Dissolving Tablets

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Abstract: In this study, we use quality by design (QBD) to develop an HPLC technique for measuring metformin hydrochloride (M-HCl) that is simple, fast, precise, accurate, and economical. Several considerations informed the use of design of experiments (DoE) to determine the optimal parameters for the HPLC technique testing. The CMPs were determined by a risk assessment that used an Ishikawa model. The factor screening tests used a two-factor three-level method. Two independent variables—the buffer's pH and the mobile phase's composition—formed the basis of the statistical models. To go further into the response surface approach and the implications of these individual components, we used central composite design (CCD). Because of this, we were able to evaluate the method's stability using the key analytical attributes (CAAs), which include retention duration, peak area, and symmetry factor. Both CAAs were optimised simultaneously using the desirability function. For the most precise and optimal findings from the contour plot, the mobile phase should consist of a 70/30 (v/v) mixture of methanol and a 0.02 M acetate buffer with a pH of 3. The flow rate should be 1 mL/min. This was carried out in an oven set at 35 °C with 235 nm UV detection on a Thermoscientific ODS HypersylTM chromatographic column (250 × 4.6 mm, 5 µm). The optimal test parameters were validated in accordance with ICH standards. The findings made it quite evident that the QBD technique might be used to enhance the HPLC method of testing M-HCl. Researchers used this technique to measure the M-HCl content of tablets and observe the dissolving properties of both standard and extended-release M-HCl in a petri dish.

Keywords: metformin hydrochloride; quality by design (QBD); central composite design (CCD); critical analytical attributes (CAA); HPLC; validation

1. Introduction

Metformin, N,N-dimethylimidodicarbonimidic diamide, or 1,1-dimethylbiguanide is another name for this compound. It has a molecular weight of 129.16 and a freezing point ranging from 223-226 °C. The hydrochloride salt of the biguanide metformin is known as M-HCl (Figure 1). High blood sugar, a symptom of type 2 diabetes mellitus, is treated with it.

Metformin has shown promise in reducing cancer risk in animal studies [1,2]. In addition to preventing glioma, endometrial, colon, breast, and stomach cancers, it decreases the overall cancer mortality rate. (3–7).

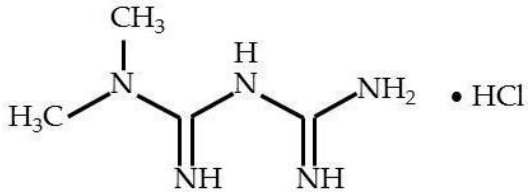

Figure 1. Chemical structure of metformin hydrochloride (M-HCl).

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Metformin is often the first medication offered to patients diagnosed with Type 2 diabetes. Metformin hydrochloride has a trifecta of action. To begin with, it inhibits gluconeogenesis and glycogenolysis, which in turn reduces glucose synthesis in the liver. Secondly, it improves the absorption and utilisation of insulin by muscles, which increases their insulin sensitivity. Last but not least, it mitigates the intestinal glucose absorption [8].
Separate administration of metformin reduces glycosylated haemoglobin levels by about 1.5 percent [9]. One may get it as 500 mg, 850 mg, or 1000 mg IR tablets, 500 mg, 750 mg, or 1000 mg ER tablets, 500 mg in a 5 mL liquid solution, or 500 mg in a powder sachet [10]. Researchers have shown that individuals using the extended-release prescription version of metformin had more success in controlling their blood sugar and cholesterol levels. Metformin XR has greater gastrointestinal tolerability when taken less often throughout the day because the active ingredient is released more gradually and under control. Not only does this reduce the frequency and intensity of adverse effects, but it also increases adherence to treatment by decreasing the number of daily dosages.

According to the quality by design (QBD) approach, the analytical process's quality should be considered throughout its design phase. Instead of focussing on reviewing the final analysis findings, it suggests incorporating quality into the process design from the start. Quality by Design (QBD) is an approach to product and process development that is goal-oriented, method-oriented, and scientifically sound [35,36]. This strategy is identical to the one proposed in the preliminary FDA process validation guide [37]: Validation of methods typically consists of three stages: Step One: Developing the Method Outline the requirements and circumstances of the approach, and mention its primary controls. Verify that the method is capable of executing its intended function in Stage 2, Method Qualification. Step 3 of Verifying the approach in Stage 3: Make sure the approach remains under control even when utilised often.

Following these procedures improves the efficacy and reliability of scientific approaches.

Although regulatory agencies do not specify a procedure for analytical QBD, a comparable approach based on product QBD may be used. Results from analytical quality by design (AQBD) are just like results from process QBD: well-known, useful, and dependable all the way through the product's lifespan. It is possible to reframe the ATP, CMA, and DOE as QTTP, CQA, and DOE, respectively, in terms of quality target product profile (QTTP), critical method attributes (CMA), retention time, peak area, symmetry factor, tailing factor, resolution between adjacent peaks, plate count, etc. [38,39].

Finding the ATP is the first step in the QBD analysis method's process of examining technology. The next stage is to establish the CMAs. In order to improve analytic techniques and get a better understanding of the statistics that impact their efficacy, a MODR is required throughout the development process. Discovering critical method parameters (CMPs) is the objective in order to identify high-risk elements that significantly impact analytical performance. These may include technique parameters, material qualities, instrument-related aspects, and operational parameters of the instrument. These should be identified via studies that evaluate risks and screen for potential factors. After that, the appropriate experimental projects should be used to optimise them for better technique performance. The CMAs then determine the optimal chromatographic conditions based on what was learnt during the research stage and the screening trials [35,38,40-42].

Every kind of analysis has its own unique CMP. Most often occurring mistakes in high-performance liquid chromatography (HPLC) procedures include material selection, instrument configuration, mobile phase parameters (such as buffer type, concentration, pH, organic modifier, elution technique), column design and preparation, and researcher error. Any kind of risk may be located and evaluated using the Ishikawa fishbone model (Figure 2).

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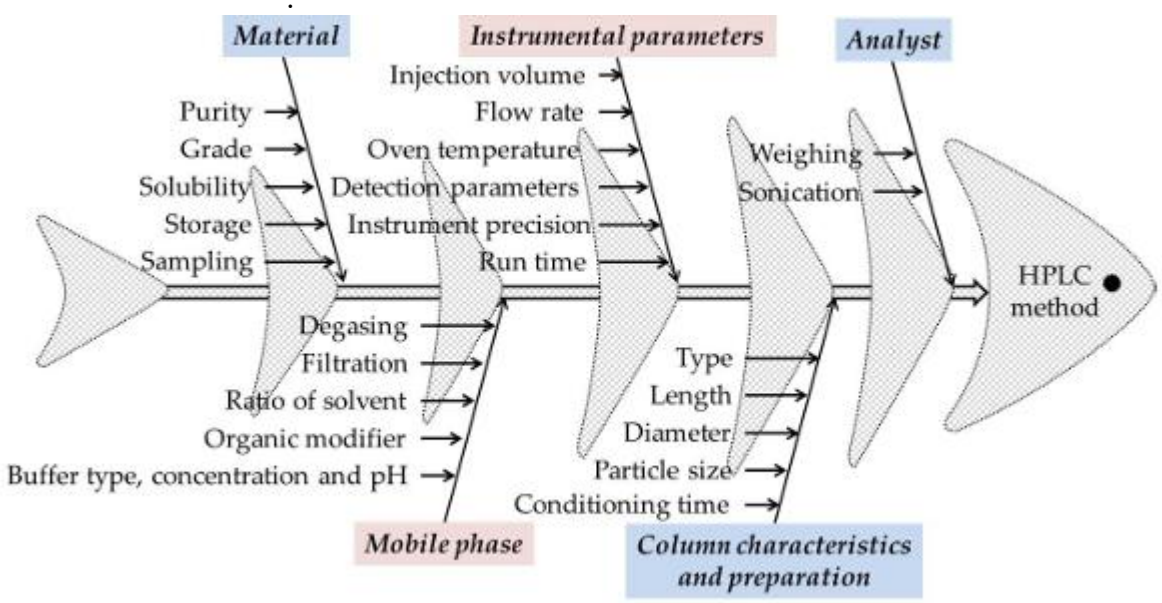

Figure 2. The Ishikawa (fishbone) diagram to identify potential variables in HPLC method development.

To summarize, in order to establish a scientific process that incorporates QBD ideas, the following must be done: At the beginning of the project, the steps include conducting a literature search and a rudimentary risk assessment. We need to locate ATP, CMAs, and risk assessments. Making the procedure more efficient, and creating procedures using DOE MODR; moreover, a control plan and risk evaluation Proof that the A QBD technique works Constantly keeping an eye on the procedure.

In light of the above, the objective of this study was to implement the required measures, one of which was to develop and refine an HPLC technique for quantifying M-HCl in accordance with QBD standards. Verifying the method's efficacy in accordance with the ICH Q2 (R1) Guidelines was the subsequent stage [47]. and to investigate the dissolving process of tablets containing M-HCl using the enhanced and established HPLC technique.

2. Materials and Methods

A. Chemicals and Reagents

Metformin hydrochloride (97%) was purchased from Sigma Aldrich Chemie Gmbh (Steinheim, Germany), sodium acetate (CH3COONa, 99.0%) was obtained from Silal Trading SRL (Bucharest, Romania), sodium phosphate dibasic (Na2HPO4, 99.0%) was obtained from Sigma-Aldrich Chemie GmbH (Steinheim, Germany), potassium dihydrogen phosphate (KH2PO4, 99.5%) was obtained from Utchim SRL (Râmnicu Vâlcea, Romania), potassium chloride (KCl, 99.0%) was obtained from Chemical Company S.A. (Iasi, Romania), orthophosphoric acid (H3PO4, 85%) was obtained from Chemical Company S.A. (Iasi, Romania), glacial acetic acid (CH3COOH, 99.84%) was obtained from Chimreactiv SRL (Bucharest, Romania), hydrochloric acid (HCl, 37.0%) was obtained from Chemical Company S.A. (Iasi, Romania), sodium hydroxide (NaOH, 98.5%) was pu The 18.2 MΩ cm ultrapure water was supplied by a local pharmaceutical company. We purchased 500 mg to 1000 mg tablets of industrial M-HCl from a local pharmacy.

B. Equipment

Along with a quaternary pump (type G1311A), degasser (type G1322A), oven column compartment (type

G1316A), and diode array detector (DAD type G1315B), the Agilent Technologies 1200 liquid chromatograph was equipped with Agilent ChemStation 32 software (Rev. B.03.02). The Santa Clara, California, USA headquarters of Agilent Technologies is where you may find all of them. For the chromatography, a column made by Thermoscientific called ODS Hypersyl TM (250 4.6 mm, 5 µm) was used. Other instruments used were pH-meter, inoLAB pH 7110 (Xylem Analytics Germany GmbH, Weilheim, Ger- many), water bath Biobase (model SY-1L4H, Biobase Biodustry, Shandong, Co., Ltd., Jinan, China), ultrasonic bath Biobase (model UC-40A, Biobase Biodustry, Shandong, Co., Ltd., Jinan, China), analytical balance PIONEER® Analytical OHAUS PX124M (Ohaus Corpora- tion, Parsippany, NJ, USA), dissolution apparatus SR 8 Plus Dissolution Test Station (model 73-100-104, Hanson Research, Chatsworth, CA, USA), microliter™ Syringes, 20 µL Hamil- tonne Bonaduz AG (CH-7402 Bonaduz, Switzerland), Transferpette® Dig. 100–1000 µL (article 704180, Brand GMBH + CO KG, Wertheim, Germany) and Rotilabo®-Mikroliterpipette 0.5–5.0 mL (article TA 26.1, Carl Roth GmbH, Karlsruhe, Germany).

C. Preparation of Standard Solutions

Until the solution reached 50 mL, more ultrapure water was added to 103.1 mg of M-HCl (the reference material, 97%) that had been soaked in 25 mL of water with a resistivity of 18.2 MΩ cm. The result was a 2 mg/mL (2000 µg/mL) concentration in the answer. Any further dilution of standard solutions was likewise accomplished using ultrapure water.

Choosing the mobility phase and preparing for it the mobile phases were tested using varying concentrations of acetonitrile, water, methanol, and buffers with varying pH values. Using a mobile phase and a flow rate of 1 mL/min produced peaks with excellent CMAs (retention duration, peak area, and symmetry factor) 30/70 methanol and 20 mM acetate buffer (pH 3). One litre of floating phase is prepared by mixing 300 millilitres of methanol with 700 millilitres of 0.02 M acetate buffer (pH = 3). After the mixture had reached room temperature, the mobile phase was subjected to sonication for fifteen minutes. Subsequently, it was filtered under pressure using 0.45 µm membrane filters.

D. Method Development and Optimization Using QBD Approach

Identification of ATP, CMAs, and Risk Assessment

The process of identifying ATP began with a literature search and an initial risk assessment. The research that followed presented several ways for measuring M-HCl by HPLC, and the development of these methods relied on the application of QBD principles. A variety of mobile phase components (such as buffer type, buffer pH, organic modifier type, and buffer to organic modifier ratio) were associated with the chosen CMPs. The Ishikawa fishbone diagram was used for the purpose of risk identification and evaluation (Figure 2).

Method Development and Optimization

To achieve a satisfactory separation of M-HCl while maintaining acceptable values for the system suitability parameters, such as retention time, peak area, and symmetry factor, chromatograms were recorded for different compositions and mobile phase ratios using KH2PO4 and CH3COONa acetate buffers with different pH values, as well as methanol and acetonitrile as organic modifiers.

First, we used a mobile phase consisting of a mixture of methanol and water, phosphate buffer, and acetate (all with a concentration of 0.02 M and pH = 3). Then, we substituted acetonitrile for methanol to study how the aqueous phase and organic modifier affected the separation performance.

We use a central composite design (CCD) with star points at the centre of each face of the factorial space [46,48-50] to examine how the buffer pH and the ratio of the organic solvent to the buffer solution affect the mobile phase composition. Three levels of each component (-1, 0, +1) are required for this type. The mobile phase's composition and the buffer's pH were chosen as independent variables (factors selected based on the preliminary study). The design matrix with the chosen factors at low (-1), medium (0), and high (+1) values for eleven experimental runs with triplicate testing at the centre point (0, 0) is shown in Table 1. The suggested independent

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factors were symmetry factor, retention duration, and peak area, whereas the dependent variables were these same metrics. Retention duration was limited to a minimum while peak area and symmetry factor were limited to maximums as dependent variables.

The following procedures were carried out in the experimental design: utilising MATLAB and Statistics Toolbox Release 2020a (The MathWorks, Inc., Natick, MA, USA) software, the optimal response for second-order polynomial exploring quadratic response surfaces [40,46], and calculating the desirability function. The mobile phase composition and pH of the buffer solution were also considered at three different levels.

Y is the measured response for each combination of factor levels, β0 is the intercept, and β1 to β5 are regression coefficients obtained from experimental runs of the observed experimental values of Y. The independent variables A and B are coded for levels, and the equation (1) states that $Y = \beta0 + \beta1A + \beta2B + \beta3AB + \beta4A2 + \beta5B2$. The symbols AB, A2, and B2 stand for interaction and quadratic terms, equally. We also used the MATLAB software to create a desirability plot and a surface response plot for the dependent variables.

Run	Run Order	Coded Factor Level	
		Factor A	Factor B
1	7	−1	−1
2	9	−1	0
3	4	−1	1
4	8	0	−1
5	5	0	0
6	1	0	0
7	2	0	0
8	6	0	1
9	3	1	−1
10	11	1	0
11	10	1	1
		Level of Factor	
Parameter	Low (−1)	Intermediate (0)	High (+1)
A: Buffer pH	3	4	5
B: Buffer content (%)	70	80	90

Table 1. Design matrix as per central composite design (CCD) for optimization of the HPLC methodof metformin hydrochloride (M-HCl).

Risk Assessment and Control Strategy

The CMAs ensure the optimised method remains efficient and operational by checking it throughout its life. In order to study ruggedness and robustness, the method's parameters and performance were tested under various intentionally created conditions, such as different reagents, analysts, and days. To test the method's resilience, we made small adjustments to the CMPs (methanol supply, mobile phase flow, and buffer solution pH), and to test its ruggedness, we switched analysts and ran the analyses on different days. All three of these metrics—retention time, peak area, and symmetry factor—must have RSD% acceptability limits below 2%.

After the technique was developed, a control strategy was put in place to guarantee that the results and the method's performance stayed within the predetermined ATP. This approach included a planned set of controls of certain parameters.

Method Validation

In accordance with the ICH Q2 (R1) Guidelines, the HPLC technique for M-HCl was assessed for linearity, limit of detection (LOD), limit of quantification (LOQ), precision, and accuracy [47].

A calibration curve was created for 19 standard solutions with concentrations ranging from 10 to 2000 µg/mL in order to ascertain the linearity. We ran duplicate analyses on each solution, and then utilised integration to find the peak regions; these we then used to build a calibration curve with the relevant M-HCl concentration. With

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the help of Excel's regression function and some mathematical estimations of the degree of linearity (r2 for the coefficient of determination, the slope, and the intercept with the ordinate of the regression line), we were able to find the equation of the regression line.

Using the formulae Signal/Noise > 3 for LOD and Signal/Noise > 10 for LOQ, we were able to determine the quantification limit (LOQ) and the detection limit (LOD), where signal is the peak area for the signal and noise is the peak area for noise, respectively. In order to determine these bounds, the linearity study yields the equation of the regression curve:

$$LOD = \frac{3 \times Area_{Noise} - Intercept}{Slope} \text{ and } LOQ = \frac{10 \times Area_{Noise} - Intercept}{Slope} \quad (2)$$

The new HPLC technique was assessed for its accuracy using two metrics: intra-day precision for repeatability and inter-day precision for intermediate precision. Three solutions of M-HCl at concentrations of 700, 1000, and 1300 µg/mL were tested in triplicate on the same day in order to determine repeatability. Similarly, the answers mentioned above were examined for three days in a row to find the intermediate precision. The RSD% was used to indicate the method's repeatability and intermediate precision. The analytical approach was applied to recovery tests using the standard addition method to assess the method's accuracy. This included spiking sample solutions with three different concentrations of M-HCl, which had previously been tested. To make it short, 2 mL was achieved by adding 2000 µg/mL of M-HCl (0.4, 0.7, and 1.0 mL) and water (1.0, 0.7, and 0.4 mL) to 0.6 mL of 1000 µg/mL M-HCl solution. The results showed that solutions containing 700, 1000, and 1300 µg/mL of M-HCl were accomplished. The precision was measured by the percentage of final concentrations that were recovered. At lower concentrations, the method's accuracy was also examined; here, it was computed as a percentage of relative error (RE%) using the following equation:

$$\text{RE\%} = \frac{|C_M - C_R|}{C_R} \times 100 \quad (3)$$

where C_M and C_R are the measured and real concentration, respectively.

E. Stability Studies for M-HCl Solutions

We created solutions with concentrations of 1000 and 2000 µg/mL to conduct the stability research of M-HCl solutions throughout time. At first, these solutions were examined. After 24 and 48 hours, they were kept in the fridge, at room temperature, and in a water bath set at 37 ◦C. The recovery of M-HCl was determined in every instance.

Tablet M-HCl Content Determination

First, twenty pills were crushed into powder form after their average weight (in milligrammes) was established for each tablet in accordance with the requirements of the European Pharmacopoeia, 10th edition [51]. A concentration of 1000 µg/mL of designed M-HCl (tablets) was achieved by dissolving 500 mg of powdered tablets in 500 mL of ultrapure water. This was done to produce samples of the tablets. After passing the solution through 13 mm PTFE filters with a pore size of 0.45 µm, it was subjected to analysis according to the procedure conditions. The following equation was used to compute the percent concentration (C%) of M-HCl:

$$C\% = \frac{M_m}{A \cdot a} \cdot \frac{P_A - Int}{S} \cdot 50 \quad (4)$$

where Mm is average weight of a tablet calculated for 20 tablets (in g), A is the declared content (in mg), a is the quantity of tablet powder (in g), PA is the peak area, and Int and S are the intercept and the slope of the regression line, respectively.

F. Dissolution Studies

In vitro dissolution tests were performed according to the specifications of the “2.9.3. Chapter 5.17, "Recommendations on methods for dosage forms testing," and Section 5.16 of the 10th Edition of the European Pharmacopoeia [51] provide the dissolution test for solid pharmaceutical forms.

Using apparatus 2, often known as the paddle apparatus, the dissolving experiments were conducted at a temperature

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of 37 ◦0.5 ◦C. A stomach fluid simulation with a pH of 1.2 (3.7 g KCl, 7.5 mL concentrated HCl, and distilled water up to 1000 g) and an intestinal fluid simulation with a pH of 6.8 (6.8 g KH2PO4, 22.4 mL 1M NaOH solution, and distilled water up to 1000 g) dissolving media were each created for the dissolution investigations. The dissolving media's pH was measured using a pH-meter, and a solution was made up of concentrated HCl or 1 M NaOH as needed to bring it down to the correct range. The cylindrical vessel was prepared by placing the test sample (tablet) on its base and then removing any air bubbles from the test surface.

specimen; the machinery was turned on, and the speed of rotation was fine-tuned. The dissolving test was conducted for 2 hours under the following circumstances on conventional release tablets (CP-2, CP-3, and CP-4 samples, which included both tablets and film-coated tablets): 500 millilitres of a solution of dissolving media for stomach fluid simulation at a pH of 1.2, 37 degrees Celsius, 0.5 revolutions per minute, and 2 hours of running time. The disintegration test was conducted for 24 hours under particular working circumstances for the following prolonged-release pills, CP-1 and CP-5: simulated gastric fluid pH = 1.2; temperature 37 0.5 ◦C; and 60 rpm for the first two hours. Simulated intestinal fluid with a pH of 6.8, a temperature of 37 0.5 ◦C, and a speed of 60 rpm was used in lieu of the dissolving media. After each sample, 2 mL of media was removed and replaced with the same amount of new dissolving medium in the cylindrical vessel to maintain a consistent volume at 37 ◦C. We collected samples for conventional release tablets at 5, 10, 15, 30, 45, 60, 90, and 120 minute intervals, and for extended release tablets, we collected samples at 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, and 24 hour intervals. Nylon filters with a pore size of 0.45 µm and a diameter of 25 mm were used to filter the samples after they were collected at the specified time at a distance of at least 10 mm from the vessel wall and the surface of the dissolving media. The specified procedure was used to analyse the samples. A dilution factor (DF) was included into the equation used for calculations when the area of the M-HCl peak was found to be bigger than that of standard M-HCl (1300 µg/mL). This process was repeated twice to dilute the solution. Six pills were used for the dissolving test.
The following equations were used to determine the amount of M-HCl released:

$$C_I\% = DF \cdot \frac{P_{A(tx)} - Int}{S} \cdot \frac{500}{1000} \cdot \frac{100}{A} \quad (5)$$

$$C_{II}\% = DF \cdot \frac{P_{A(tx-1)} - Int}{S} \cdot \frac{500}{1000} \cdot \frac{2}{500} \cdot \frac{100}{A} \quad (6)$$

$$C\% = C_I\% + C_{II}\% \quad (7)$$

Where

$C\%$ = percentage release in the dissolution medium;
$C_I\%$ = percentage concentration calculated for the first sampling;
$C_{II}\%$ = percentage concentration calculated in the 2 mL taken previously;
DF = dilution factor (1 or 2);
P_A = peak area (mAU min);
Int and S = intercept and slope of the regression line respectively;
A = declared content (mg);
tx = current sampling time;
$tx - 1$ = previous sampling time.

The first sample was obtained at 5 minutes (for conventional release pills) and 1 hour (for prolonged-release tablets) at simulated stomach fluid pH = 1.2 using Equation (5); subsequent samples were taken using Equation (7). Since simulated intestinal fluid with a pH of 6.8 was employed in lieu of the dissolving media for prolonged-release tablets, Equation (5) was applied to the three-hour sample and Equation (7) to the following samples. At a pH of 1.2 (the pH of the simulated stomach fluid), the data were combined with the final concentration.

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3. Results and Discussions

A. Method Development and Optimization Using QBD Approach

A retention time of less than 10 minutes, together with an area and symmetry of the peak as big as feasible, were the target ATP values for the assay and dissolution study of M-HCl from tablets using the HPLC method with UV detection. Isocratic elution with a buffer or organic modification as the mobile phase is necessary for CMAs to conduct an use a C18 chromatographic column with UV detection, aqueous sample with a concentration of about 1000 µg/mL, minimising retention time, maximising peak area, and ensuring symmetry of the corresponding peak. The ability to use the HPLC separation process for mass spectrometry (MS) detection is the final and most crucial feature.

When it comes to high-performance liquid chromatography (HPLC) analysis, the majority of studies have documented the utilisation of a non-polar stationary phase (C18) in various chromatographic columns (with lengths ranging from 50 to 250 mm, inner diameters of 3.9 or 4.6 mm, and particle sizes of 4 or 5 µm). The mobile phase is typically a combination of two or three solvents, such as acetonitrile, methanol, water, phosphate buffer, ammonium buffer, or tetrahydrofuran. The pH of the aqueous phase is typically between 2.8 and 7.0.

The CMPs chosen for the next study have a high risk assessment of variability due to factors like the instrument's precision and the mobile phase, which includes factors like the buffer's type, pH, organic modifier type, and the ratio of the buffer to the organic modifier, according to the Ishikawa diagram and the results of the preliminary experiments.

Review of Experiment Findings and Final Method Conditions Selection

We set out to investigate how the mobile phase's composition changed depending on the buffer solution's pH and the buffer solution to organic solvent ratio. Selected a facial CCD. These technique conditions were evaluated using the CCD methodology. The initial stage included checking the retention duration, peak area, and symmetry factor conditions. Different chromatographic circumstances resulted for M-HCl as a result of this. Whereas the quality of HPLC separation is unaffected by purposeful modifications in the method's parameters, the acceptable value falls within those ranges. In order to optimise the selection of CMPs that really affected the technique performance, a two-factor CCD was used at three equidistant levels: low (1), moderate (0), and high (+1), based on the factor screening investigations. The CCD-recommended design matrix is summarised in Table 2. The experimental runs that were analysed for method CMAs (retention duration, peak area, height, tailing, and symmetry) all employed the same standard concentration.

Table 2. Design matrix as per the CCD for optimization of parameters for analysis of M-HCl.

Run	Run Order	Factor		Response-1	Response-2	Response-3
		pH	Buffer %	Retention Time	Peak Area	Symmetry Factor
1	7	3	70	5.27	2352.14	0.74
2	9	3	80	5.64	2183.85	0.58
3	4	3	90	6.32	1981.89	0.56
4	8	4	70	5.65	2079.55	0.70
5	5	4	80	6.03	1907.56	0.55
6	1	4	80	5.99	1911.25	0.56
7	2	4	80	6.05	1915.15	0.56
8	6	4	90	6.71	1827.62	0.53
9	3	5	70	6.04	1980.39	0.54
10	11	5	80	6.42	1813.09	0.49
11	10	5	90	7.10	1679.52	0.45

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For instance, chromatograms may be shown in Figure 3 for a mobile phase mixture of (a) 0.02 M acetate buffer (pH = 3)/methanol in 70/30, 80/20, and 90/10, v/v, and (b) 0.02 M acetate buffer (pH = 3, 4, and 5)/methanol in 70:30, v/v.

Space for Design

A CCD with eleven runs, which is the response surface research type, was used. The outcomes of the experiment were summarized in Table 2 and Figure 4, respectively, and they included using the suggested CCD design to assess the mobile phase composition and buffer pH in relation to the three responses: retention duration, peak area, and symmetry factor.

Given that Rt = 10.209 + 0.38833 A 0.19623 B + 0.0015567 B2, we may deduce that A is a positive coefficient (+0.38833). This coefficient may be understood as follows: with B held constant, a one-unit change in A will result in a 0.38833-unit change in retention time. However, due to the addition of the word B2 to the calculation, we can no longer interpret the negative coefficient of B (0.19623) in the same way as previously. The only thing we can tell is that retention time increases as B increases, given a constant value of A and a range of 70 to 90 for B. Therefore, a shorter retention period will be the result of a lower B.

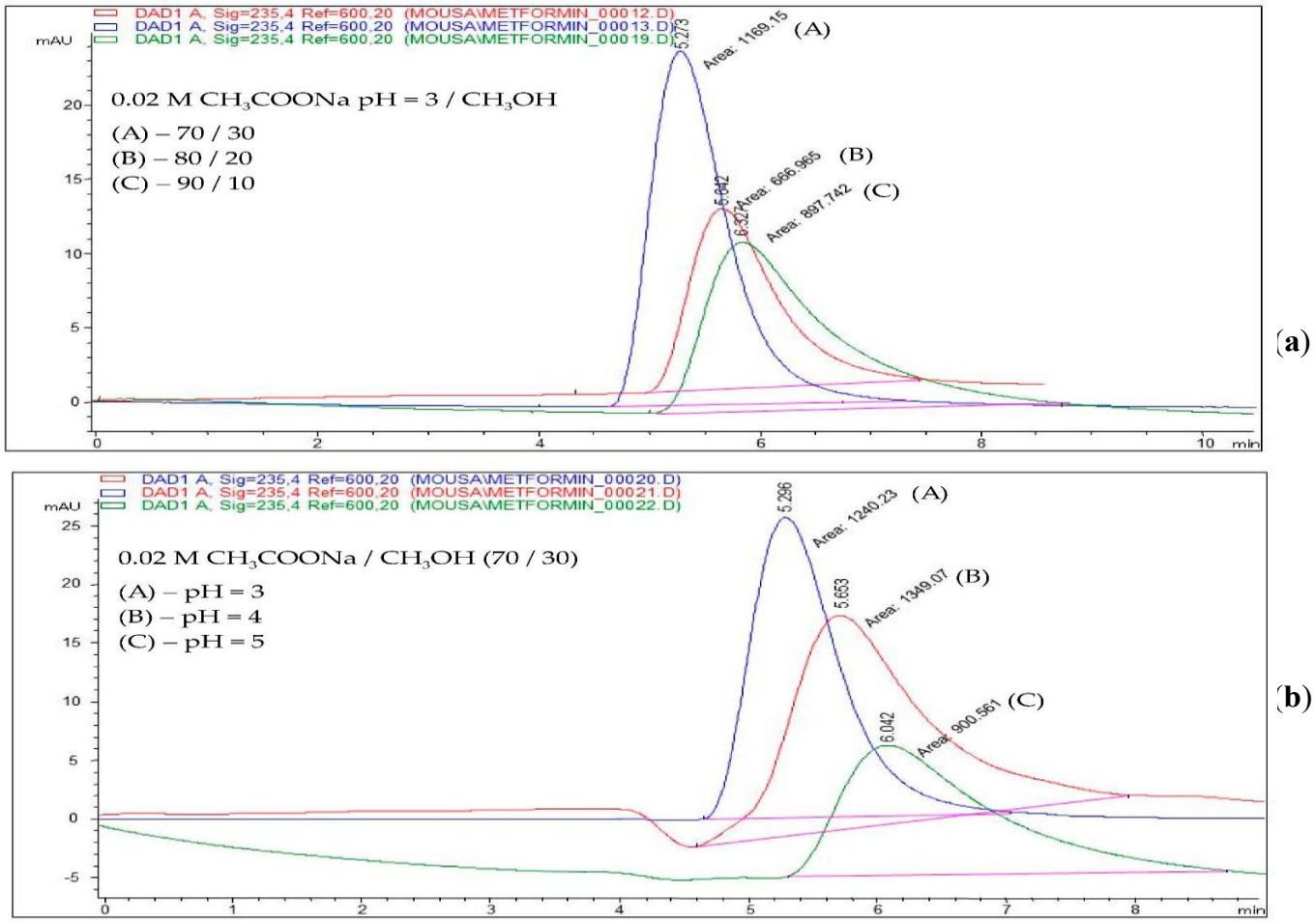

Figure 3. Overlayed chromatograms for a mobile phase consisting of a mixture of (**a**) 0.02 M acetatebuffer (pH = 3)/methanol in a ratio of 70/30, 80/20, and 90/10, respectively, *v*/*v*; (**b**) 0.02 M acetatebuffer (pH = 3, 4, and 5, respectively)/methanol in a ratio of 70/30, *v*/*v*.

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The peak area (Pa) equation, Pa = 4979.6 736.18 A 15.384 B + 70.254 A2, reveals that B is a negative coefficient, with a value of 15.384. With A held constant, we can deduce the following from this coefficient: a one-unit change in B will result in a 15.384-unit change in peak area. Not only is A's coefficient negative (736.18), but it can't be understood in the same way as B's since A2 is also a part of the equation. The only thing we can tell is that peak area decreases as A increases, with a constant value of B and an A range of 3 to 5. This means that a decrease in A will determine an increase in peak area. In other words, if we intersectthe peak area response surface by the plane of equation B = c (here, 70 c 90), then thecurve of junction point represents a decreasing function of A. Therefore, a decrease in A willdetermine an increase in peak area.

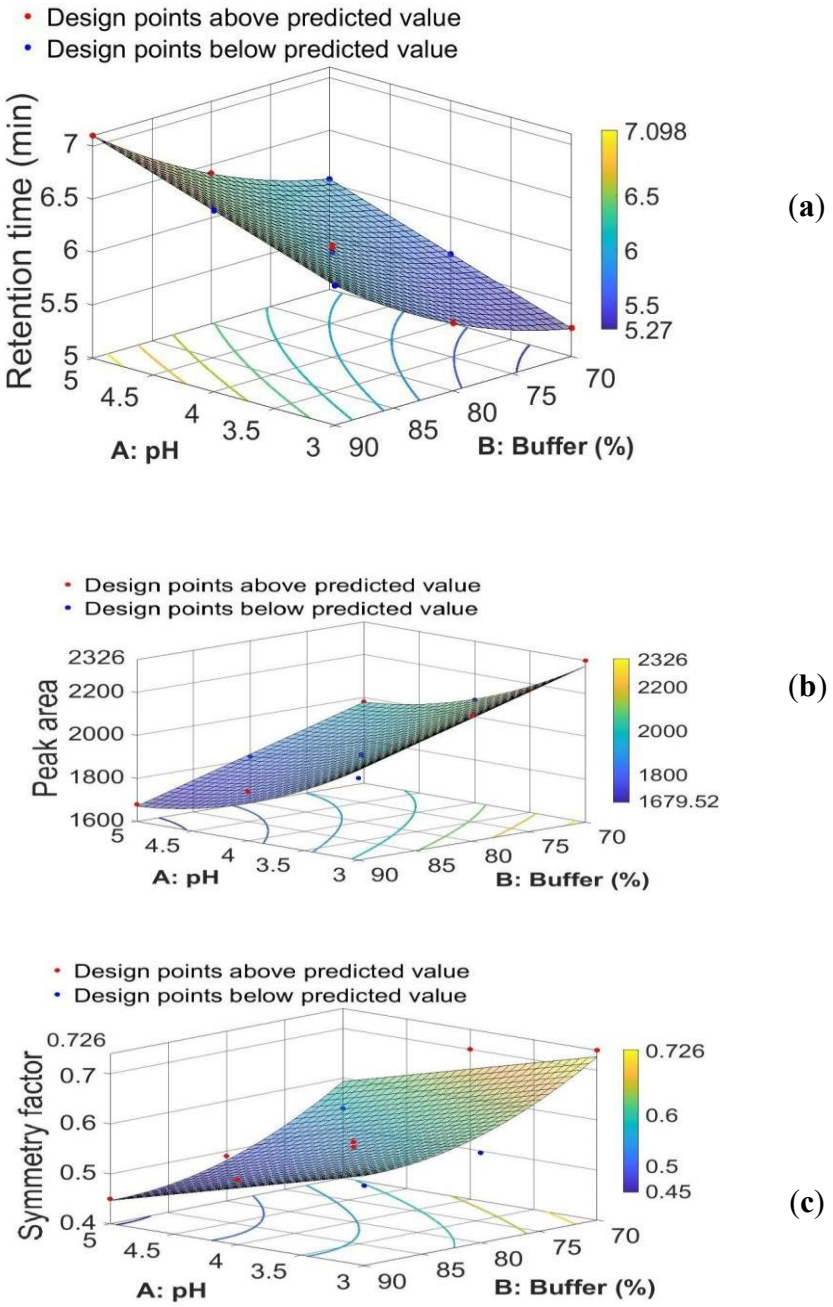

Figure 4. Three-dimensional (3D) response surface plot for (**a**) retention time, (**b**) peak area, and (**c**) symmetry factor, showing effect of % of buffer and pH in the mobile phase.

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Table 3. Statistical calculation of a second-order polynomial exploring quadratic response surfaceswith equation $Y = \beta_0 + \beta_1 A + \beta_2 B + \beta_3 AB + \beta_4 A^2 + \beta_5 B^2$.

	Retention Time			Peak Area			Symmetry Factor		
	Coefficient	t-Stat	*p*-Value	Coefficient	t-Stat	*p*-Value	Coefficient	t-Stat	*p*-Value
β_0	10.209	15.766	1.0004×10^{-6}	4979.6	16.805	6.4667×10^{-7}	3.876	3.2031	0.015
β_1	0.38833	56.702	1.3921×10^{-10}	−736.18	−5.1359	0.0013449	−0.0667	−5.2088	0.0012409
β_2	−0.19623	−12.063	6.1394×10^{-6}	−15.384	−12.78	4.1615×10^{-6}	−0.0692	−3.2763	0.03695
β_4	—	—	—	70.254	3.9349	0.0056404	—	—	—
β_5	0.0015567	15.324	1.2146×10^{-6}	—	—	—	0.00038667	3.0368	0.041091

All coefficients (β_0–β_5) are statistically significant at 95% confidence level.

Also, the symmetry factor (Sf) equation (3.876 x 0.0692 B + 0.00038667 B2) shows that A has a negative coefficient (0.0667). This coefficient may be understood in the following way: assuming B is constant, a one-unit change in A will result in a 0.0667-unit change in the symmetry factor. We can't apply the same logic to understand B's negative coefficient (0.0692) as we did for A now since B2 is in the equation as well. For any given A and some B between 70 and 90, the only thing we can say is that the symmetry factor is a decreasing function of B, such that as B decreases, the symmetry factor increases.

Based on Figure 4a-c and the equations for the retention time, peak area, and symmetry factor, it can be inferred that as the pH value (code factor A) and the percentage of sodium acetate buffer (code factor B) in the mobile phase composition decrease, the retention time value increases with the peak area and symmetry factor for the corresponding M-HCl peak decreases.

Enhanced Chromatographic Settings

To find the best solution, numerical optimisation was used to "trade off" different CAAs in order to maximise peak area and symmetry factor while minimising retention time. The goal was to get the desirability function near to 1. According to the optimised solution, all of the CAAs were within the required ranges, and the mobile phase composition, which consisted of a combination of 70/30 (v/v) methanol and 0.02 M acetate buffer (pH = 3), produced a desirability near to 1.0. Table 4 displays the optimised mobile phase pH and buffer content values together with the projected results. Figure 5 displays the three-dimensional desirability plot.

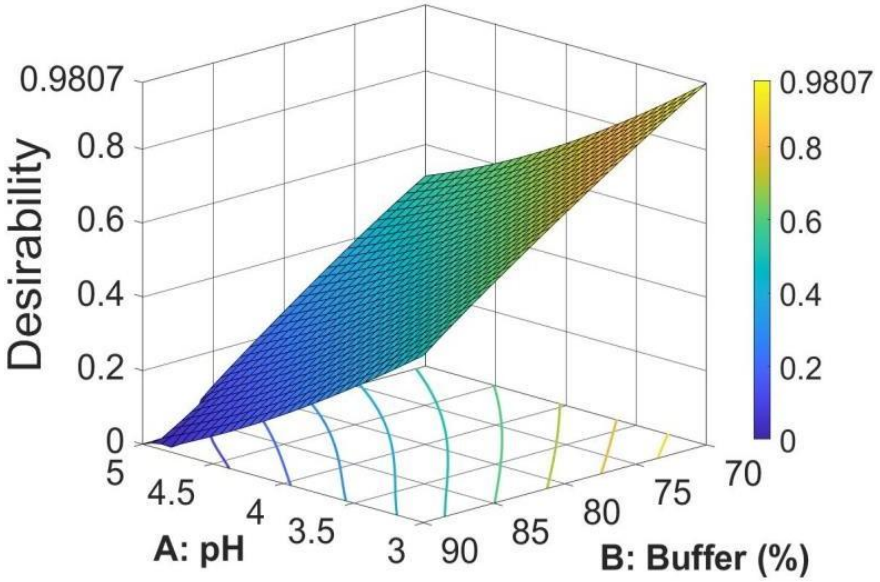

Figure 5. 3D surface plot for desirability for optimal formulation.

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Table 4. Obtained solution for optimized formulation.

pH	Buffer/Methanol	Retention Time (min)	Peak Area	Symmetry Factor	Desirability
3	70/30	5.27	2326.47	0.73	0.9807

Thus, the optimal mobile phase consists of a mixture of 0.02 M acetate buffer(pH = 3)/methanol in a ratio of 70/30, v/v, conditions in which the method is fasterand has a higher sensitivity. The final chromatographic conditions for M-HCl are shown in Table 5.

Table 5. Final chromatographic conditions.

Parameters	Values
Stationary phase (column)	Thermoscientific ODS Hypersyl^{TM} chromatographic column; (250 × 4.6 mm, 5 µm)Mobile phase 0.02 M acetate buffer (pH = 3)/methanol (70/30, *v*/*v*)
Flow rate (mL/min)	1
Column temperature	35 °C
Injection volume (µL)	20
Detection wavelength (nm)	235

The technique becomes somewhat more sensitive (peak area rises) and quicker (retention time lowers) when a larger proportion of organic modifier is employed. For instance, a mobile phase consisting of 0.02 M CH3COONa (pH = 3)/CH3OH in a ratio of 60/40 would work. But there are three main reasons why we went with a 70/30 split: (i) lowering expenses (by reducing the amount of methanol used) and (iii) reducing pollution (by disposing of as little organic solvent as waste)—when the buffer solution makes up less than 70% of the mobile phase composition, a negative peak appears before the corresponding peak of M-HCl, making integration difficult and decreasing the precision of the peak integration.

Evaluating Risk and Implementing Control Measures

A 1000 µg/mL solution of M-HCl was used for the robustness and ruggedness experiments. In order to conduct the robustness research, the values of the CMPs were purposefully varied, including the use of different sources of methanol, the mobile phase flow rate, and the pH of the buffer solution. Separate analysts made the toughness study's conclusions on separate days. Retention duration, peak area, and symmetry factor values were all below 2% RSD in both instances.

The quality of the data and the method's performance should both fall within the predetermined ATP, thus we devised a set of controls for several factors as a control approach. These parameters include sample preparation and storage conditions, measurements taken, and the doubling of control procedures.

B. Method Validation

Linearity

The suggested approach was tested for linearity in accordance with the ICH recommendations [47]. The examination of linearity throughout the concentration range of 10-2000 µg/mL required the preparation of three sets of working solutions. The chromatograms that resulted from subjecting each of these samples to the aforementioned conditions allowed us to calculate the area of the peaks associated with M-HCl. The concentration range of 10-2000 µg/mL was linear for M-HCl (r2 = 0.9999). In the regression equation, the variables PA (peak area) and C (concentration of M-HCl in µg/mL) were substituted, yielding a result of 56.361.

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C. Limit of Detection (LOD) and Limit of Quantification (LOQ)

Using the peak area for noise (1.51212) and Equation (2), the detection and quantifica-tion limits were calculated:

$$LOD = (3 \times 1.51212 + 56.361)/2.4266 = 25.09\ \mu g/mL \quad (8)$$

$$LOQ = (10 \times 1.51212 + 56.361)/2.4266 = 29.46\ \mu g/mL \quad (9)$$

Precision and Accuracy

In order to get the same number of chromatograms, the identical solution containing 1000 µg/mL of M-HCl was injected six times to test the system's precision. We calculated the mean, standard deviation, and relative standard deviation percentage from the chromatograms by measuring the peak regions. According to Table 6, the RSD% number that was achieved is below 2%.

Table 6. System precision.

No.	Peak Area	Statistics
1	2369.7	
2	[illegible]	Mean = 2376.7
3	[illegible]	SD = 7.5048
4	[illegible]	RSD = 0.3158%
5	2389.7	
6	2375.3	

The three different doses of M-HCl (700, 1000, and 1300 µg/mL) were injected to assess the repeatability and intermediate precision. Sets of three duplicates of each concentration were examined on the same day for repeatability (intra-day) determination. On three separate days, three duplicates were examined to achieve intermediate accuracy, also known as inter-day. Both instances show that the approach was accurate, with RSD% values below 2%.

To ascertain the precision, the recovery trials were conducted with three distinct solutions at three distinct concentration levels (700, 1000, and 1300 µg/mL), and the ultimate recovery was computed. Return rates vary from 98.12 to 100.93%, with an average of 99.75%. In Table 7 we can see the method's accuracy and precision.

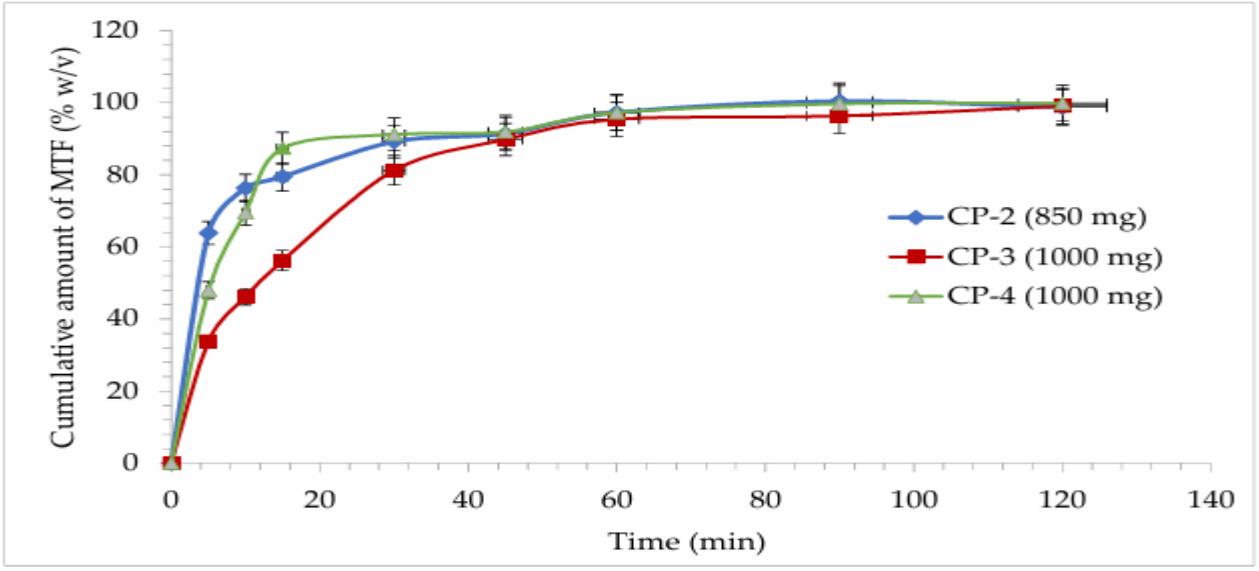

Figure 6. Release profile of M-HCl from conventional tablets.

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It is found that at the initial sampling of prolonged-release tablets containing 500 mg and 1000 mg of M-HCl, simulated gastric fluid with pH = 1.2 has a release of M-HCl from the CP-1 sample of 41.74% and from the CP-5 sample of 23.57%. This is in accordance with the European Pharmacopoeia, 10th edition [51] acceptance criteria, as it prevents "dose dumping." After two hours, formulation CP-1 has a maximum release of 49.71% in the simulated stomach juice with pH = 1.2, while CP-5 has a maximum release of 35.96% (Figure 7). The release of around 50% of M-HCl from prolonged-release tablets will serve as the second dissolution test specification point. In contrast to formulation CP-5, which released 53.17% of M-HCl after 4 hours in simulated intestinal fluid with pH = 6.8, the first formulation under study exhibits a much quicker release profile, reaching the second point after 2 hours in simulated gastric fluid with pH = 1.2 (CP-1: 49.71%). The guarantee of full release, which is defined as at least 80% attained in a simulated intestinal fluid pH = 6.8, is the last specification point outlined in the European Pharmacopoeia. This threshold is achieved after 5 hours for the CP-1 test (81%), with a maximum release of 100.04% at the conclusion of the test; 81.21% is released after 8 hours for the CP-5, with a maximum of 99.87% after 24 hours (Figure 7). The European Pharmacopoeia, 10th edition's "2.9.3. Dissolution test for solid dosage forms" and "5.17. Recommendations on methods for dosage forms testing" both state that the two samples under analysis are compliant and fit the release profile for extended-release dosage forms [51].

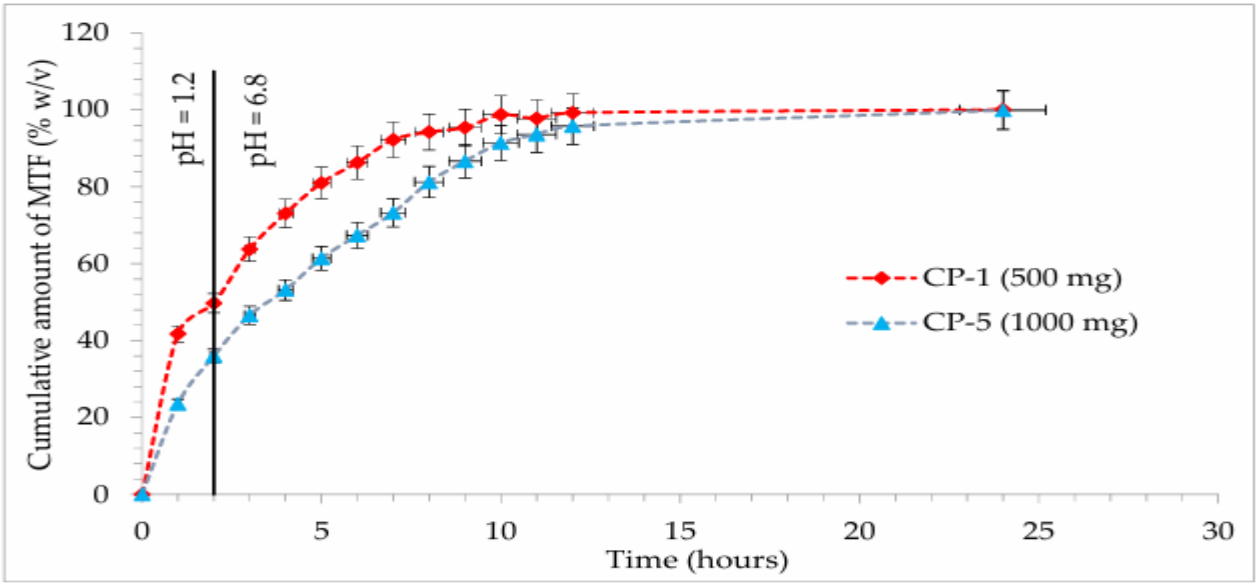

Figure 7. Release profile of M-HCl from prolonged-release tablets.

Conclusions

By examining the interactions of two variables pertaining to the mobile phase (the aqueous phase's pH and the ratio of methanol to acetate buffer) at three distinct levels, the paper outlines the development of an HPLC method for the QBD approach's determination of M-HCl using a central composite design. Following second-order polynomial calculations that explored quadratic response surfaces for retention duration, peak area, and symmetry factor as functions of buffer pH and buffer-to-methanol ratio, the optimised CMPs were discovered. The mobile phase composition of the optimised solution was a combination of 0.02 M acetate buffer (pH = 3) and methanol in a 70/30 (v/v) ratio; in this instance, the desirability function was quite near to 1 (desirability function = 0.9807).

The ICH Q2 (R1) Guidelines were followed in the complete validation of the optimised procedure. With good detection and quantification limits (LOD = 25.09 µg/mL and LOQ = 29.46 µg/mL, respectively), good precision (RSD < 2%) and accuracy (mean recovery = 99.75%) in the range of 700–1300 µg/mL, and a mean percent error of 1.07% in the range of 40–2000 µg/mL, the method demonstrated good linearity (r2 = 0.9999) in the studied range (10–2000 µg/mL). The M-HCl solutions are sufficiently stable at room temperature 24 hours after production, according to the data obtained. It is advised that samples be stored in a refrigerator if they were

examined more than twenty-four hours later.

The M-HCL test from tablets and dissolving experiments were conducted using the optimised and validated technique. All of the pharmaceutical items examined in laboratory investigations had an M-HCl percentage concentration between 97.78 and 99.20%, with a 5% allowable variance. In the case of dissolving experiments, a release of more than 98% was recorded for all formulations under study after 2 hours for conventional-release tablets. According to European Pharmacopoeia, 10th edition, "2.9.3. Dissolution test for solid dosage forms" and "5.17. Recommendations on methods for dosage forms testing," the two samples examined in the case of prolonged-release tablets are compliant and fit the release profile for extended-release dosage forms.

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