Simultaneous quantification of teneligliptin hydrobromide and metformin hydrochloride: an improved HPTLC method with implementation of Plackett-Burman design

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Abstract: Simple, rapid, sensitive, robust, and validated high-performance thin-layer chromatography was developed for the simultaneous estimation of anti-diabetic drugs Teneligliptin hydrobromide (TH) and Metformin hydrochloride (MH) in bulk and pharmaceutical formulation. HPTLC system equipped Camag Linomat V, TLC Scanner IV, Twin Trough chamber, Dual-wavelength UV Cabinet, and win CATS software V1.4.7 were used during the study. Separation of analytes was achieved using a pre-coated silica gel G60 F254 aluminum sheets 10x10 cm<sup>2</sup>, with the thickness of 0.2 mm as stationary phase and a mobile phase comprising of Methanol: Ammonium sulfate (0.5 %w/v): Triethylamine (9: 2.7: 0.5 v/v/v). The detection wavelength was set at 237 nm. The R<sub>f</sub> value of the TH and MH was 0.63 and 0.19, respectively. The method was validated using a dynamic linear range of 4-28 ng/band and 100-700 ng/band for TH and MH, respectively. The developed method was validated as per the ICHQ2 (R1) guideline. The statistical analysis proved that the process is accurate with 98.80–99.38 % w/v and 99.66–99.96 % w/v for TH and MH, respectively, and precise with less than 2 % RSD. The Plackett-Burman design was applied to check the robustness of the developed method. Finally, the method is termed as linear, precise, accurate, and robust. It can be implemented successfully for the routine analysis of pharmaceutical dosage forms comprising TH and MH and is appropriate for regulatory submission under regulatory flexibility.

Keywords: Teneligliptin hydrobromide; metformin hydrochloride; high-performance thin-layer chromatography; ICH guidelines; simultaneous estimation; quality by design. © 2021 ACG Publications. All rights reserved.

1. Introduction

Teneligliptin, a novel dipeptidyl peptidase 4 inhibitor, recently approved for the treatment of diabetes mellitus (Type 2 DM). Chemically it is ((2S,4S)-4-[4-(5-methyl-2-phenyl pyrazole-3-yl)piperazin-1-yl][pyrrolidin-2-yl]-(1,3-thiazolidine-3-yl)methanone;hydrate;hydrochloride [1]. Metformin is a derivative of a biguanide, and for many years, it is prescribed as an oral anti-diabetic drug. It is a drug of choice for the management of type 2 diabetes mellitus. It acts by reducing glucose production by the liver. Chemically it is 3-(diaminomethylidene)-1,1-dimethylguanidine (Figure 1). The combination of Teneligliptin and Metformin with adjunct to diet and exercise, plays an important role in controlling the glycemic level.

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For the quality control aspects, it is essential to develop analytical methods for such combination products [2]. Various sophisticated analytical techniques are described in the literature to analyze Metformin and Tenelegliptine either individually or in combination with other drugs by UV Spectrometry method [3-4], HPLC [5-11], Ultrafast LC method [12-13], LC-MS/MS methods [14-16], Stability indicating HPLC method [17] and HPTLC method [18-20]. However, the HPTLC analytical method for simultaneous estimation of Metformin and Tenelegliptine is not available. So Precise, accurate, and sensitive method for simultaneous determination of TH and MH using HPTLC was planned and validated as per Q2 (R1) guideline.

Robustness testing is an essential part of analytical method validation. It generally determines an analytical method or procedure's capacity, which remains unbiased while changing the experimental condition. Robustness is essential because sometimes small variations in the experimental parameters or process make considerable differences in results. The analyst needs to develop such a method to not affect the method’s accuracy by these minor experimental conditions. For the robustness evaluation, the two-level factorial design is considered the most suitable. However, fractional factorial design, like Plackett-Burman designs, has been employed in robustness evaluations [21-23]. By looking at the above consideration, it was planned to assess robustness parameter using widely accepted Plackett-Burman designs.

2. Experimental

2.1. Materials and Methods

Quantitative HPTLC was performed on liquid chromatography, Camag Linomat V, Hamilton Syringe (100µL), Camag TLC Scanner IV (scanning speed up to 100mm/s, spectral range 190-800nm), Camang Twin Trough chamber, Dual-wavelength UV Cabinet, and Camag winCATS software V1.4.7. Teneligliptin (TH) is 99.95% w/w, and metformin (MH) 99.45%w/w was obtained from Pure Chem Pvt Ltd, Ankleshwar, India. The solvents used methanol, Triethylamine, and Ammonium Sulfate of AR grade were purchased from Loba Chemicals. The tablet of Afoglip M 500 containing 20 mg of TH and 500 mg MH were obtained from a local pharmacy.

2.2. Selection of Mobile Phase for HPTLC

In the initial trial, mobile phase comprised of methanol and ammonium sulfate (5% w/v) (7.5:2.5 v/v/v) was tried but this mobile phase was not suitable for separation of TH and MH. Later on, triethylamine was added into the previous mobile phase, and proportion was set as methanol: ammonium sulfate (0.5%w/v): triethylamine (9:2.5:0.5 v/v/v). In this method, both drugs were detected, but peak tailing was found in TH and MH peaks. The method was then carried out with the mobile phase ammonium sulfate (1%w/v): methanol: triethylamine (8:4.2:0.4 v/v/v). All peaks were detected; however, tailing on the peaks were observed. The method was then carried out with the mobile phase consisted of methanol: ammonium sulfate (0.5%w/v): triethylamine (9:2.7:0.5v/v/v). This method gives both peaks better resolution and reproducibility; hence this mobile phase was selected for HPTLC.
2.2. Preparation of Solutions

Preparation of the mobile phase and diluents: Optimized mobile phase consisted of a mixture of methanol: ammonium sulfate (0.5%w/v); triethylamine in ratio 9: 2.7: 0.5 v/v/v respectively.

Wavelength optimization and selection by TLC spots were scanned over 200-400 nm for optimized wavelength detection. The spectra of TH and MH have been taken for the same. During this exercise, it was considered the 237 nm for detection of both the drugs.

Stock standard solutions prepared by approximately 10 mg of TH has been precisely weighted and transferred to a 10 mL volumetric flask and dissolved in 10 mL of methanol to give a 1000 µg/mL (Solution A) stock prepare a stock solution of 5000 µg/mL (solution B).

Working standard solutions prepared by taking about 2 mL solution was taken from solution A, and 1 mL from solution B in 10 mL volumetric flask and make up the volume by mark with methanol. This final solution was a concentration of 200 µg/mL of TH and 500 µg/mL of MH (Solution C). Another solution was made by taking a 1 mL solution from solution C in a 10 mL volumetric flask and make up the volume by mark with methanol. This solution was of a concentration of 20 µg/mL of TH and 50 µg/mL of MH.

Sample solution for assay was prepared by weighed and crushed twenty tablets. Tablet powder equivalent to 20 mg of TH and 500 mg of MH has been transferred to a 100 mL flask. Twenty milliliters of methanol were added to the volumetric flask and sonicated for 45 min, filtered through Whatman filter paper No. 41, and make up the volume by mark with methanol to prepare solution concentration 200 µg/mL of TH, and 5000 µg/mL MH. From that, 1 mL solution was transferred to 10 mL volumetric flask and diluted with methanol to make 20 µg/mL of TH and 500 µg/mL MH. 1 mL solution was further transferred to a 10 mL volumetric flask and make up the volume by mark with methanol to prepare a solution containing 2 µg/mL of TH and 50 µg/mL MH.

Working standard solution (14 µL) or sample solution for analysis of formulation (10 µL) was spotted on the pre-washed TLC plate and developed with mobile phase Methanol: Ammonium sulphate (0.5%w/v): Triethylamine in ratio 9: 2.7: 0.5 v/v/v respectively. With Camag TLC Scanner-V using winCATS V 1.4.7 software, photometric measurements were taken at 237 nm in the absorbance/reflectiveness mode.

2.3. Methods

The sample solution for analysis of formulation (10 µL) and standard working solution (14 µL) carried out on a pre-washed TLC plate using a semi-automatic spotter under a nitrogen stream.

The IR lamp has been used for drying purposes, and the plate was developed in a saturated chamber for at least 15 min using mobile phase. The oven is used for drying the plate after development. Photometric measurements were taken at 237 nm in the absorbance/reflectiveness mode incorporating the track optimization option.

A linearity study checks that the sample solutions are within a concentration range where the analytes’ response directly relates to concentration. The accuracy was computed using a standard method of addition. The fixed amount of pre-analyzed standard solution was added with a known amount of standard drug [14,24]. The precision was conducted by intra-day and inter-day precision by using three standard solutions of TH and MF. Specificity is the method to compare R<sub>d</sub> of the sample solution and standard drug solution [15,25]; however, no interference observed in the separation and resolution of MH and TH indicates the method is specific. Standard solutions of TH and MH calculated the method's detection limit on the TLC plate. Using the S/N method, the peak-to-peak noise around the analyte retention time is calculated, and subsequently, the analyte concentration that would yield a signal equal to a specific value of noise to signal ratio is measured. An S/N ratio of 3 is generally accepted for estimating LOD, and an S/N ratio of 10 is used for estimating LOQ. This method is commonly applied to analytical methods that exhibit baseline noise [16, 25-27].

2.4. Application of Placket–Burman (PB) design

The developed HPTLC method validated for robustness using Placket–Burman (PB) design with twelve experiments. Different kind of seven HPTLC trials was planned and analyzed, i.e., modification in
the proportion of benzene and methanol in mobile phase composition by keeping one of it constant, modification in mobile phase volume, alteration in saturation time, detection at a different wavelength, change selection of bandwidth, modification in a run distance of solvent. Each factor was analyzed at two factorial levels. The study's limitation has been chosen based on the range of errors most commonly observed in an analytical laboratory. The experimental designs for the HPTLC method were create using the Design Expert 9 software. In each experimental condition, the area for TH and MH were observed.

3. Results and discussion

3.1. Chromatographic Separation

In the development of the HPTLC method, which is accurate, specific, sensitive, and reliable for the aspect of determination of TH and MH in combined dosage form, The Silica gel G60 F254 aluminum sheet (10x10 and 10x20 cm) was used as the stationary phase. The mobile phase comprises of methanol: ammonium sulfate (0.5%w/v): triethylamine in ratio 9: 2.7: 0.5v/v/v. Both the drug were detected and scanned at 237 nm. The representative densitogram indicating TH and MH is displayed in Figure 2.

Figure 2. Densitogram of MH (700ng/band) \(R_f = 0.19\pm0.3\) and TH (28ng/band) \(R_f = 0.63\pm0.2\)

In developing methods, the effects of different parameters are carried out by changing parameters one by one and keeping other parameters constant. In the development of this method, a systemic study of the effects of significant parameters was conducted by changing one parameter at a time and controlling all others.

3.2. Optimized Chromatographic Conditions of TH and MH Parameters

Based on all the trials, HPTLC separation was optimized on aluminum plates pre-coated with silica gel 60 F254 (10x10 cm², layer thickness 0.2 mm) as the stationary phase using methanol: ammonium sulphate: triethylamine (9: 2.7: 0.5 v/v/v) as the mobile phase. Methanol was used as diluent. Entire experiments were carried out at room temperature, and chamber saturation time was kept for 15 minutes. Quantification was achieved based on densitometric analysis of teneligliptin hydrobromide and metformin hydrochloride over the concentration range of 4–28 ng/band and 100–700 ng/band, respectively, at 237 nm. The method yielded compact and well-resolved bands at \(R_f\) of 0.63 and 0.19 for TH and MH, respectively, in a run distance of 8 cm. The linear regression analysis for the calibration plots produced \(r^2 = 0.993\) and \(r^2 = 0.991\) for TH and MH, respectively.
Several experiments were performed to get a sharp chromatographic peak that doesn’t interfere with baseline separation peaks. During this trial, the flow rate of the mobile and solvents ratio of the mobile phase changed many times. For ideal separation of the drugs, AR grade methanol: ammonium sulphate (0.5% w/v): triethylamine (9: 2.7: 0.5 v/v/v) proved to be the best combination because the chromatographic peak obtained was better solved and free of tailing and fronting shown in Figure S1.

3.4. Method Validation

3.4.1. Linearity

The linearity of response for the present method was determined by analyzing TH and MH's standard solution in the range of 4-28 ng/band and 200-700 ng/band, respectively, as shown in Figure S1. Correlation coefficients were found to be r² = 0.992 and 0.991, respectively. The linear regression equation was y = 2.6412x + 1803.60 for TH and y = 6.3369x + 261.03 for MH, where y is the peak area and x is the concentration in ng/band (see Table S1, and Figure S2, in supporting information).

3.4.2. Accuracy

Recovery experiments were conducted to determine the accuracy of the method for the quantification of TH and MH. Recovery of the method was checked at around working concentrations for target compounds in triplicate. Average recoveries were determined as 99.75 % for TH and 98.9 % for MH (Table 1 and Table 2).

### Table 1. Accuracy data of TH (n=3)

| Level (%) | Sample Concentration (ng/band) | Standard added (ng/band) | Total concentration (ng/band) | Amount found (ng/band) | Recovery (%) | Mean area ± SD | % RSD |
|-----------|-------------------------------|--------------------------|-----------------------------|-----------------------|--------------|----------------|-------|
| 50        | 12                            | 8                        | 20                          | 7.98                  | 99.75        | 99.72±0.20     | 0.203 |
|           |                               |                          |                             | 7.99                  | 99.89        |                |       |
|           |                               |                          |                             | 7.96                  | 99.50        |                |       |
|           |                               |                          |                             | 11.96                 | 99.66        |                |       |
| 100       | 12                            | 12                       | 24                          | 11.97                 | 99.75        | 99.66±0.09     | 0.090 |
|           |                               |                          |                             | 11.95                 | 99.58        |                |       |
|           |                               |                          |                             | 16.01                 | 100.06       |                |       |
| 150       | 12                            | 16                       | 28                          | 15.98                 | 99.87        | 99.96±0.095    | 0.095 |
|           |                               |                          |                             | 15.96                 | 99.75        |                |       |

### Table 2. Accuracy data of MH (n=3)

| Level (%) | Sample Concentration (ng/band) | Standard added (ng/band) | Total concentration (ng/band) | Amount Recovered (ng/band) | Recovery (%) | Mean ± SD | RSD (%) |
|-----------|-------------------------------|--------------------------|-----------------------------|---------------------------|--------------|-----------|---------|
| 50        | 300                           | 200                      | 500                         | 196.21                    | 98.10        | 98.98±0.69 | 0.70    |
|           |                               |                          |                             | 197.55                    | 98.77        |            |         |
|           |                               |                          |                             | 198.99                    | 99.49        |            |         |
|           |                               |                          |                             | 296.11                    | 98.70        |            |         |
| 100       | 300                           | 300                      | 600                         | 298.06                    | 99.35        | 98.80±0.50 | 0.59    |
|           |                               |                          |                             | 295.08                    | 98.36        |            |         |
|           |                               |                          |                             | 396.80                    | 99.20        |            |         |
| 150       | 300                           | 400                      | 700                         | 398.28                    | 99.57        | 99.38±0.18 | 0.18    |
|           |                               |                          |                             | 397.55                    | 99.38        |            |         |
3.4.3. Precision

Intra-day precision - the RSD % was calculated, and it was found to be 0.82 - 1.01 and 0.93 - 0.115 for TH and MH, respectively, which are well within the acceptable criteria of not more than 2.0. The result was shown in Table 3.

| Conc. (ng/band) | Peak area (Mean ± SD) | %RSD |
|----------------|-----------------------|------|
|                | TH                    | MH   | TH    | MH    |
| 8              | 200                   | 1800.36± 18.36 | 1537.66± 16.31 | 1.01 | 1.06 |
| 12             | 300                   | 1841.66± 15.27 | 2228.60± 25.76 | 0.82 | 1.15 |
| 16             | 400                   | 1850.00± 18.02 | 2946.73± 27.53 | 0.97 | 0.93 |

Inter-day precision - the RSD % was calculated, and it was 1.06 - 1.13 and 1.19 - 1.27 for TH and MH, respectively, which are well within the acceptable criteria of not more than 2.0. The result was shown in Table 4.

| Conc. (ng/band) | Peak area (Mean ± SD) | %RSD |
|----------------|-----------------------|------|
|                | TH                    | MH   | TH    | MH    |
| 8              | 200                   | 1808.00 ± 19.52 | 1539.00 ± 18.52 | 1.13 | 1.2 |
| 12             | 300                   | 1857.40 ± 19.76 | 2252.00 ± 28.74 | 1.06 | 1.27 |
| 16             | 400                   | 1868.00 ± 21.28 | 2949.60 ± 35.30 | 1.07 | 1.19 |

3.4.4. Specificity

The purity of the peaks was confirmed by a high degree of correlation, i.e., 0.99, by comparison of peaks of MH and TH, which was scanned at peak end, peak apex, and peak start. The correlation spectra of sample drugs with standards were shown in Figure 3, while TH and MH's peak purity's demonstrated in Table 5. The correlation obtained was 0.9995, 0.9998 for MH and TH, respectively. Identity of the spots in overlay spectra of sample and standard confirmed the correlation and peak purity shown in Figure 4.

**Figure 3.** (a) Correlation spectra of MH from standard and sample solution (b) Correlation spectra of TH from standard and sample solution

| Sr. No. | Drug component | Type      | r (S, M) | r (M, E) |
|---------|----------------|-----------|----------|----------|
| 1       | MH             | Standard  | 0.9997   | 0.9996   |
| 2       | MH             | Formulation | 0.9991  | 0.9994  |
| 3       | TH             | Standard  | 0.9998   | 0.9997   |
| 4       | TH             | Formulation | 0.9997  | 0.9996  |
3.4.5. Limit of Detection (LOD) and Limit of Quantification (LOQ)

LOD for TH was found 0.3 ng/band while MH was detected at a 27 ng/band concentration. LOQ for TH was 3 ng/band, and MH was 85 ng/band.

3.4.6. Robustness Using QbD Approach

Robustness was performed using Placket–Burman (PB) design to validate the HPTLC method considering a total of twelve experiments [17, 21]. In this design, seven HPTLC conditions were designed and analyzed (Table 6, 7). A combined standard solution of TH (12 ng/band) and MH (300 ng/band) was analyzed at each design of experiment. All the experiments were taken three times and performed in random order.

Table 6. Twelve - experiment Plackett–Burman (PD) design for seven factors

| List of Experiment | A | B | C | D | E | F | G |
|--------------------|---|---|---|---|---|---|---|
| 1                  | -1| 1 | 1 | -1| 1 | 1 | 1 |
| 2                  | 1 | -1| -1|-1 | 1 | -1| 1 |
| 3                  | 1 | -1| 1 | 1 |-1 | 1 | 1 |
| 4                  | 1 | 1 | -1| 1 | 1 | 1 |-1 |
| 5                  | 1 | 1 | -1|-1 | 1 | -1| 1 |
| 6                  | -1| 1 | -1|-1 | 1 | 1 |-1 |
| 7                  | -1| 1 | 1 | 1 | -1| 1 | 1 |
| 8                  | -1| 1 | 1 | 1 | -1| -1|-1 |
| 9                  | 1 | -1| 1 | 1 | -1| -1| -1 |
| 10                 | 1 | 1 | 1 | -1|-1 | -1| 1 |
| 11                 | -1| -1| -1|-1 | -1|-1 |-1 |
| 12                 | -1| -1| 1 | 1 | -1| 1 | 1 |

Plackett-Burman (PB) design shown that method was robust. The obtained graphs presented in Figure 5. The Pareto graph shown in Figures 5 indicates the relationship of bar length to the estimated effect of that factors divided by the pseudo standard error defined by Lenth (Lenth's PSE). Based on the Pareto Chart, the effects of each factor were identified. Both main effects and terms of interaction are statistically negligible, as absolute values of main effects are below the critical p-value, (Table 8).
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Table 7. Plackett-Burman experimental design with seven factors for HPTLC

| Factor Label code | Significant factor | Modification Levels |
|-------------------|-------------------|---------------------|
|                   |                   | (-)     | (0)     | (+)     |
| A                 | Modification in proportion of Methanol in mobile phase composition (mL) | 8.8  | 9  | 9.2  |
| B                 | Altering the amount of Ammonium sulphate in mobile phase composition (mL) | 2.6  | 2.7  | 2.8  |
| C                 | Deviation in the amount of Mobile phase volume | 12.1  | 12.2  | 12.3  |
| D                 | Altering in saturation time for TLC development (min) | 13  | 15  | 17  |
| E                 | Detection at different wavelength (nm) | 235  | 237  | 239  |
| F                 | Modifying in band width (mm) | 4  | 6  | 8  |
| G                 | Alteration in solvent run distance (cm) | 7.5  | 8  | 8.5  |

Figure 5. (a) Pareto chart (t –value vs effect) for MH and (b) Pareto chart (t –value vs effect) for TH

Table 8. P-Value for robustness study

| Factor | P-Value |
|--------|---------|
|        | MH      | TH      |
| A      | 0.2997  | 0.2848  |
| B      | 0.2180  | 0.6445  |
| C      | 0.4668  | 0.2006  |
| D      | 0.2721  | 0.2076  |
| E      | 0.7468  | 0.2983  |
| F      | 0.7386  | 0.5709  |
| G      | 0.9604  | 0.2752  |

P-Value: > 0.05, which means that the factor is significantly not affected by the response. So here, no value is lower than 0.05 means the method is robust.

3.5. Assay of Marketed Formulation by HPTLC Method

The % assay of TH and MH were calculated by taking five replicates of the test sample (Table 9). The % mean assay of TH and MH were 99.99 % and 100.08 %, respectively, which lies within the acceptable limit of 95-105 %, which shows that the method is applicable for marketed formulation analysis.
Table 9. Assay of formulation (Tablet) by HPTLC method (Where, n=5)

| Labeled claim (%W/W) | Peak area | % Assay |
|----------------------|-----------|---------|
| TH | MH | TH | MH | TH | MH |
| 20 | 500 | 1836 | 2201 | 100.01 | 100.01 |
| 20 | 500 | 1833 | 2205 | 99.83 | 100.18 |
| 20 | 500 | 1834 | 2206 | 99.89 | 100.22 |
| 20 | 500 | 1833 | 2199 | 99.83 | 99.90 |
| 20 | 500 | 1835 | 2203 | 99.94 | 100.09 |
| MEAN | | | | 99.99 | 100.08 |
| SD | | | | 0.07 | 0.12 |
| % RSD | | | | 0.07 | 0.12 |

4. Conclusions

In a development of the HPTLC method for estimating TH and MH in tablet formulation, by trials and applying QbD, it has shown the mobile phase composition of methanol: ammonium sulphate (0.5% w/v): triethylamine (9: 2.7: 0.5 v/v/v) was more appropriate for simultaneous estimation of both the drugs and Plackett-Burman (PB) design indicate the method was robust and suitable for simultaneous estimation of TH and MH. With these optimal conditions, baseline separation of both drugs with a reasonable resolution and optimized retention factor was achieved. The HPTLC method has been validated according to Q2 (R1) of the ICH Guidelines. The percentage of assay results for combination drugs is found to be within approval requirements (98-102 %); the result summary is shown in Table 10.

Table 10. Summary of results of HPTLC method

| Parameters | TH | MH |
|------------|----|----|
| Linearity (ng/band) | 4-28 | 100-700 |
| Linearity equation | $y = 2.6412x + 1803.60$ | $y = 6.3369x + 261.03$ |
| Correlation co-efficient | 0.993 | 0.991 |
| Recovery study (Mean %RSD) | 0.12 | 0.49 |
| Intraday Precision (%RSD) | 0.82-1.01 | 0.93-1.15 |
| Interday Precision (%RSD) | 1.06-1.07 | 1.19-1.27 |
| Repeatability Precision (%RSD) | 0.74 | 0.79 |
| LOD (ng/spot) | 0.3 | 27 |
| LOQ (ng/spot) | 3 | 85 |
| Assay % | 99.99 | 100.08 |

This research involves systematically developing a simple, fast, accurate, and cost-effective HPTLC method for simultaneous TH and MH estimation. A better percentage of recoveries and proper validation showed the appropriateness and efficiency of the proposed analytical procedures. The optimized HPTLC method was validated as per ICH guidelines. All system suitability parameters are observed in optimal condition, and it was found with the range for TH and MH estimation. The validation analysis helped identify the best conditions by verifying that the approach was selective, specific, accurate, linear, precise, and robust. A statistical method was robust, and it can be design and proven by using Plackett-Burman (PB) approach. The percentage assay values for formulations research were found to be between 98-102%. In conclusion, a simple, selective, sensitive, and accurate HPTLC method was developed and validated under ICH guidelines Q2 (R1) for the routine quality of the dosage form analyzes containing TH and MH. Consequently, this approach can be implemented successfully for the routine consists of dosage form analysis comprising TH and MH and is appropriate for regulatory submission under regulatory flexibility.
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Supporting Information

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