Simultaneous Densitometric and UPLC Methods for the Determination of Paracetamol and Metoclopramide HCL

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Authors’ contributions
This work was carried out in collaboration among all authors. Author AANM designed the study, performed the statistical analysis and wrote the first draft of the manuscript. Authors NSR and MMF wrote the protocol and managed the analyses of the study. Author AANM managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT
Two simple, accurate and precise methods were presented for the simultaneous quantitative estimation of paracetamol and metoclopramide hydrochloride. The first is based on TLC separation of the two drugs, followed by densitometric measurement at 274 nm using a developing system composed of dichloromethane - methanol - conc. ammonia (8:2:0.05 by volume). The second method is based on UPLC separation of the cited drugs at 230 nm using C18 column and a mobile phase of 0.1% ortho-phosphoric acid (PH 3.5) – acetonitrile (70: 30, v/v). Regression analysis of Beer’s plots showed good correlations (r = 0.9996 - 0.9998) over concentration ranges of 1.5 – 15 and 0.1-0.6 μg/spot or 5-25 and 0.25 – 1.5 μg/mL for paracetamol and metoclopramide HCL using the two suggested methods, respectively. The proposed methods were also successfully applied to analyze both drugs in their pharmaceutical formulation; Migracid® tablets.

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with mean recoveries ranging from 99.03 to 101.1%. The results obtained were validated as per ICH guidelines and statistically analyzed and found to be in accordance with those given by a reported method.

Keywords: Paracetamol; metoclopramide HCL; TLC; UPLC.

1. INTRODUCTION

Paracetamol is a non-steroidal anti-inflammatory drug having mild analgesic and antipyretic properties. It is chemically known as N-(4-hydroxyphenyl) acetanilide [1]. It acts by inhibiting prostaglandin (PG) synthesis in CNS. It has less effect on cyclooxygenases in peripheral tissues, which accounts for its weak anti-inflammatory activity [2]. Metoclopramide hydrochloride is 4-amino-5-chloro-N-(2-diethylaminoethyl)-2-methoxy benzamide [1]. It acts centrally by blocking dopamine D₂ receptors in the chemoreceptor trigger zone in the central nervous system and peripherally by enhancing the action of acetylcholine at muscarinic nerve endings in the gut [3]. The combination of the two active ingredients, paracetamol and metoclopramide is used for treatment of migraine, gastritis and heartburn.

Literature survey revealed that liquid chromatography [4-8], electrochemical [9-11] and spectrophotometric [12-15] methods are available for determination of paracetamol. Moreover, metoclopramide hydrochloride was determined individually using electrochemical [16-19], liquid chromatography [20-23] and spectrofluorimetric [24] methods. Meanwhile, few methods were reported for the simultaneous determination of both drugs in pharmaceutical formulations and biological fluids including spectrophotometric methods [25-28], liquid chromatography [29-34], electrophoresis [35] and densitometric methods [36,37]. Fortunately, searching the literature found no UPLC method reported for the analysis of both drugs. Thus the objective of this work was to develop simple and sensitive methods for simultaneous determination of both drugs in their combined dosage form.

2. MATERIALS AND METHODS

2.1 Instrumentation
- Agilent 1100 Ultra HPLC with binary pump and UV detector (USA).
- Phenomenex Kinetex C₁₈ 100A column (4.6 mm, 100 mm)
- Camag TLC densitometric scanner, with WINCATS computer software (Switzerland).
- CamagLinomat, autosampler (Switzerland) with a constant application rate of 10 μL·s⁻¹.
- Chromatographic tank (25 × 25 × 9 cm).
- UV lamp with short wavelength, 254 nm (Desega-Germany).
- Precoated TLC plates, silica gel 60 GF₂₅₄ (20 × 20 cm), (Flukachemie, Switzerland).
- Jenco digital pH/temp meter with Jenway double function glass electrode (UK).

2.2 Pure and Market Samples
Metoclopramide HCL and paracetamol, kindly supplied by Egyptian Pharmaceutical Company (EPICO), 10th of Ramadan, Egypt; purity of which are 99.91 and 99.93%, respectively as stated by the supplier. Migracid® tablet; batch no. 03160509, labeled to contain 500mg paracetamol and 10 mg metoclopramide HCL; the product of Chemical Industries Development (CID), Cairo, Egypt.

2.3 Chemicals and Reagents
- Dichloromethane, methanol, acetonitrile; HPLC grade(Sigma - Aldrich, USA).
- Conc. ammonia (El-Nasr Pharmaceutical Chemicals Co., Cairo, Egypt)
- Ortho-phosphoric acid (Fisher, UK).

2.4 Standard Solution
Stock solutions of the two cited drugs; 10 mg mL⁻¹ paracetamol and 0.1 mg mL⁻¹ metoclopramide HCL were prepared by dissolving 500 mg paracetamol or 5 mg metoclopramide in 50 mL methanol. Working solutions; 0.1 and 0.01 mg mL⁻¹ of the two studied drugs, respectively were prepared by further dilution with the same solvent. The solutions were found to be stable for three weeks when kept in the refrigerator.

2.5 Procedures
2.5.1 TLC method
2.5.1.1 Chromatographic conditions
Analysis was performed on pre-coated 20 × 20 cm TLC aluminum silica gel 60 GF₂₅₄ plates. Plates were spotted 1.5 cm apart from each
other and 1.5 cm apart from the bottom edge. The chromatographic tank was pre-saturated with the mobile phase for 10 min, then developed by ascending chromatography using dichloromethane - methanol - conc. ammonia (8: 2:0.05, by volume) as a mobile phase. The plates were air dried, detected under UV lamp (254 nm) and scanned under the following conditions:

- Slit dimensions: 6.0 × 0.3 μm,
- Wavelength: 274 nm,
- Scanning speed: 20 mm/s,
- Data resolution: 100 nm/step,
- Measurement mode: absorption,
- Result output: chromatogram and integrated peak area.

### 2.5.1.2 Construction of calibration curve

In a series of 10-ml volumetric flasks, aliquots of standard paracetamol solution (10 mg mL⁻¹) equivalent to (1.5, 3, 6, 9, 12, 15 mg) or standard solution of metoclopramide (0.1 mg mL⁻¹) equivalent to (0.1, 0.2, 0.3, 0.4, 0.5, 0.6 mg) were transferred separately and diluted to volume with methanol. Ten μL of each solution were applied to a TLC plate following the previously described chromatographic conditions and scanned at 274 nm. Calibration curves were plotted representing the recorded area under the peak against corresponding drug concentration in μg/spot.

### 2.5.2 UPLC method

#### 2.5.2.1 Chromatographic conditions

At ambient temperature, isocratic separation was carried out on Phenomenex kinekt C₁₈ 100A column (100 mm, 4.6 mm i.d, 2.6 μm) using a mobile phase of 0.1% ortho-phosphoric acid (pH 3.5) – acetonitrile (70: 30, v/v). The mobile phase was filtered and degassed before being pumped at flow rate of 1 mL min⁻¹ and UV detection was carried out at 230 nm. UPLC chromatograms revealed that paracetamol was clearly separated from metoclopramide HCL at retention times of 2.074 and 2.845 minutes for both drugs, respectively.

#### 2.5.2.2 Construction of calibration curve

Aliquots of working solution of paracetamol (0.1 mg mL⁻¹) containing (0.05, 0.1, 0.15, 0.2, 0.25 mg) or metoclopramide working solution (0.01 mg mL⁻¹) equivalent to (0.0025, 0.005, 0.0075, 0.01, 0.0125, 0.015 mg) were transferred into a series of 10- mL volumetric flasks and adjusted to volume with the mobile phase. Ten μL of each solution were injected into the column and chromatographed under the conditions described. Calibration graphs were constructed by plotting the peak area against the corresponding drug concentration in μg mL⁻¹ and the regression equations were computed.

### 2.5.3 Laboratory prepared mixtures

#### 2.5.3.1 TLC method

Into a set of 10-mL volumetric flasks, aliquots of standard paracetamol solution (10 mg mL⁻¹) equivalent to (5, 7.5, 10 mg) were transferred and mixed with solutions equivalent to (0.1, 0.15, 0.2mg) metoclopramide from (0.1 mg mL⁻¹) metoclopramide stock solution. Volumes were completed to the mark with methanol, and then 10 μL of each solution were spotted on TLC plate following the above mentioned specific chromatographic conditions and scanned at 274 nm. Peak areas of the obtained chromatograms were measured and the concentration of each drug was calculated from their corresponding regression equation.

![Paracetamol](image1.png)  ![Metoclopramide HCL](image2.png)

**Fig. 1. Chemical structure of paracetamol and metoclopramide HCL**
2.5.3.2 UPLC method

Accurate aliquots of paracetamol solution (0.1 mg mL\(^{-1}\)) containing (0.125, 0.15,0.2 and0.25 mg) together with volumes of metoclopramide working solution (0.01 mg mL\(^{-1}\)) equivalent to (0.0025, 0.003, .004 and .005 mg) were transferred into a set of 10-mL volumetric flasks and mixed together to prepare four mixtures and then diluted to the volume with the mobile phase. Ten μL of each solution were injected into the UPLC column and analyzed under the previous conditions. The corresponding chromatograms were monitored at 230 nm and drugs concentrations were calculated from the corresponding regression equation.

3. Application to Pharmaceutical Formulations

Ten Migracid® tablets were accurately weighted, finely powdered and mixed well. A weight equivalent to 1000 mg paracetamol and 20 mg metoclopramide HCL was transferred into into100 - mL volumetric flask, extracted three times with 25 mL methanol, adjusted to volume with the same solvent then filtered. The obtained solution labeled to contain 10 mg mL\(^{-1}\) paracetamol and 0.2 mg mL\(^{-1}\) metoclopramide HCL was analyzed by the densitometric and UPLC method.

3. RESULTS AND DISCUSSION

Two sensitive and selective chromatographic methods were developed and validated for the simultaneous determination of paracetamol and metoclopramide HCL. Best chromatographic conditions were adequately selected on the basis of peak shape, recoveries of the selected drugs and time required for analysis.

3.1 Method Development

3.1.1 TLC method

A quantitative TLC densitometric method was developed for the determination of paracetamol and metoclopramide HCL, depending mainly on the difference in RF values of the two drugs. Initial studies were carried out to achieve good separation in which different developing systems were tried such as dichloromethane-methanol, dichloromethane - methanol- acetonitrile and acetonitrile - methanol - conc. ammonia in different ratios. Best separation with almost well-defined spots was achieved using a mobile phase of dichloromethane - methanol - conc. ammonia (8: 2:0.05, by volume).

Different wavelengths were tried and it was found that well defined symmetric bands were observed at R\(_f\) 0.75 for paracetamol and 0.28 for metoclopramide when measured densitometrically at 274 nm; Fig. 2.

3.1.2 UPLC method

Different mobile phases were tried to separate the two studied drugs using variable solvents with different ratios as 0.1% ortho- phosphoric acid- acetonitrile, acetonitrile - methanol and acetonitrile - phosphate buffer in different ratios. Best separation was obtained using a mixture of 0.1% ortho- Phosphoric acid - acetonitrile (70: 30, v/v). Different wavelengths (200 - 400 nm) and flow rates (0.3 - 1.5 mL min\(^{-1}\)) were also tested; much better detector response was obtained at 230 nm with flow rate of 1.0 mL min\(^{-1}\). Fig. 3 illustrated that the peaks of paracetamol and metoclopramide HCL were clearly separated at reasonable retention times of 2.074 and 2.85 min, respectively.

3.2 Method Validation

The proposed methods were validated according to ICH guidelines [38].

3.2.1 Linearity

For the densitometric method, linear relationship was found to exist between peak areas of the separated spots and the corresponding drug concentration over the range of 1.5 - 15 μg/spot for paracetamol and 0.1-0.6 μg/spot for metoclopramide HCL; Figs. 4 and 5. While, linearity between the peak area and the drug concentration covers the range of 5-25 μg mL\(^{-1}\) and0.25-1.5 μg mL\(^{-1}\) for paracetamol and metoclopramide HCL, respectively using UPLC method Figs. 6 and 7. The obtained high correlation coefficient (0.9996-0.9998) indicates good obedience to Beer's law. Regression parameters were calculated and presented in Table 1.

3.2.2 Accuracy

Accuracy of the proposed methods was evaluated by application of the standard addition technique, in which known amounts of standard pure drugs were added to previously analysed concentration of the pharmaceutical formulation.
For densitometric method, recovery of standard added showed mean recoveries of added ± SD of 100.33% ± 1.49 and 99.82% ± 1.88, for the two studied drugs, respectively, while for UPLC method recovery of standard added showed mean recoveries of added ± SD of 100.53% ± 1.67 and 100.33% ± 1.32 for the two drugs, respectively; Table 1.

**Fig. 2.** Densitometric chromatogram of mixture of metoclopramide HCL (0.2 μg/spot) and paracetamol (10 μg/spot) at 274 nm

**Fig. 3.** UPLC chromatogram of paracetamol (5 μg mL⁻¹) and metoclopramide HCL (1.5 μg mL⁻¹) mixture

**Fig. 4.** Calibration curve of peak area to the corresponding concentration of paracetamol at 274 nm by TLC method
Fig. 5. Calibration curve of peak area to the corresponding concentration of metoclopramide HCL at 274 nm by TLC method

Fig. 6. Calibration curves of peak area to drug concentration of paracetamol at 230 nm by UPLC method

Fig. 7. Calibration curves of peak area to drug concentration of metoclopramide HCL at 230 nm by UPLC method
Table 1. Regression and validation parameters for the determination of paracetamol and metoclopramide HCL by the proposed methods

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Densitometric method</th>
<th>UPLC method</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Paracetamol</td>
<td>Metoclopramide</td>
</tr>
<tr>
<td>Amax (nm)</td>
<td>274</td>
<td>230</td>
</tr>
<tr>
<td>Linearity range</td>
<td>1.5-15(μg/spot)</td>
<td>0.1-0.6(μg/spot)</td>
</tr>
<tr>
<td>Slope±SE</td>
<td>1138.22±11.49</td>
<td>14650.57±151.9</td>
</tr>
<tr>
<td>Intercept±SE</td>
<td>5638.75±104.6</td>
<td>1161.13±59.16</td>
</tr>
<tr>
<td>SE of residual</td>
<td>134.47</td>
<td>63.54</td>
</tr>
<tr>
<td>Correlation coefficient (r²)</td>
<td>0.9996</td>
<td>0.9996</td>
</tr>
<tr>
<td>Accuracy (R %)</td>
<td>100.33%</td>
<td>99.82 %</td>
</tr>
<tr>
<td>Precision (RSD%)</td>
<td>Intraday</td>
<td>0.36-1.73</td>
</tr>
<tr>
<td></td>
<td>Interday</td>
<td>0.25-0.81</td>
</tr>
<tr>
<td>LOD</td>
<td>0.35 μg/spot</td>
<td>0.013 μg/spot</td>
</tr>
<tr>
<td>LOQ</td>
<td>1.06 μg/spot</td>
<td>0.039 μg/spot</td>
</tr>
</tbody>
</table>

3.2.3 Precision

For densitometric method triplicate analysis at three concentration levels (1.5, 6, 12 μg/spot) for paracetamol and (0.1, 0.3, 0.6 μg/spot) for metoclopramide HCL were performed in the same day to study the intraday precision. The interday precision were analyzed by repeating the above procedure for three successive days using the same drug concentrations. This method show intraday precision (RSD %) ranged from 0.36% to 1.73% and 0.4% to 0.7% while interday precision was from 0.25% to 0.81% and 0.58% to 1.34% for paracetamol and metoclopramide HCL, respectively.

In UPLC method, nine determinations were carried out over three concentration levels (5, 10, 20 μg mL⁻¹) and (0.5, 1, 1.5 μg mL⁻¹) for paracetamol and metoclopramide HCL respectively; each in triplicate on the same day and on three successive days using the above mentioned procedure. Paracetamol show intraday precision (RSD %) from 0.22% to 1.23% and interday precision was from 0.48% to 0.64% while for metoclopramide HCL intraday precision (RSD%) range was 0.13% to 1.11% and interday precision was from 0.56% to 1.54%; Table 1.

3.2.4 Specificity

Specificity was revealed by analyzing laboratory prepared mixtures of paracetamol together with metoclopramide HCL in different ratios. Densitometric method was valid for simultaneous determination of both drugs with mean recoveries% ± SD of 100.62%±1.18 and 100.62% ± 1.25 for the two studied drugs, respectively; while UPLC method showed mean recoveries% ± SD of 100.28±0.53 and 100.79±1.62 for the two studied drugs, respectively; Table 2.

3.2.5 Robustness

The robustness of the proposed densitometric method was assessed by small variations in the mobile phase ratios. It was observed that no significant difference in Rf value upon introduction of variation in ammonia volume (from 0.45 - 0.55 mL); RSD% didn’t exceed 1.05% and 1.12% for paracetamol and metoclopramide HCL; respectively. Whereas, altering the ratio of 0.1% ortho-phosphoric acid–acetonitrile (± 2%) didn’t affect the system suitability parameters of UPLC method confirming robustness of the proposed methods as shown in Table 3.

3.2.6 System suitability test

In UPLC method, system suitability test was performed to ensure system performance before or during the drug analysis where the capacity factor (K’), selectivity factor (α), resolution factor (R) and number of theoretical plates (N) were calculated, from which the system was found to be suitable; Table 4.

Statistical analysis of the results obtained by the two proposed methods compared with that of a reported method [27] revealed no significant differences between them within a probability of 95%; Table 5. However, the proposed methods were more sensitive and selective.
Table 2. Determination of paracetamol in mixtures with metoclopramide HCL by the proposed methods

<table>
<thead>
<tr>
<th>Densitometric Method</th>
<th>Paracetamol</th>
<th>Metoclopramide</th>
<th>Ratio Paracetamol: Metoclopramide</th>
<th>Recovery % of paracetamol</th>
<th>Recovery % of metoclopramide</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5</td>
<td>0.1</td>
<td>50:1</td>
<td>101.4</td>
<td>99.2</td>
</tr>
<tr>
<td></td>
<td>7.5</td>
<td>0.15</td>
<td>50:1</td>
<td>99.3</td>
<td>101.4</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>0.2</td>
<td>50:1</td>
<td>101.2</td>
<td>101.3</td>
</tr>
<tr>
<td>Mean±RSD</td>
<td></td>
<td></td>
<td></td>
<td>100.62±1.18</td>
<td>100.62±1.25</td>
</tr>
<tr>
<td>UPLC Method</td>
<td>12.5</td>
<td>0.25</td>
<td>50:1</td>
<td>99.93</td>
<td>101.72</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>0.3</td>
<td>50:1</td>
<td>101.08</td>
<td>101.62</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>0.4</td>
<td>50:1</td>
<td>100.11</td>
<td>101.49</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>0.5</td>
<td>50:1</td>
<td>100.02</td>
<td>98.35</td>
</tr>
<tr>
<td>Mean±RSD</td>
<td></td>
<td></td>
<td></td>
<td>100.28±0.53</td>
<td>100.79±1.62</td>
</tr>
</tbody>
</table>

*Ratio of dosage form

Table 3. Robustness results for the determination of paracetamol and metoclopramide HCL by the proposed methods

<table>
<thead>
<tr>
<th>Method</th>
<th>Parameters</th>
<th>Changed condition</th>
<th>%RSD</th>
<th>Paracetamol</th>
<th>Metoclopramide</th>
</tr>
</thead>
<tbody>
<tr>
<td>TLC</td>
<td>Volume of ammonia (0.5 ml)</td>
<td>±0.05 ml</td>
<td>1.05</td>
<td>1.12</td>
<td></td>
</tr>
<tr>
<td>UPLC</td>
<td>Mobile phase</td>
<td>±2%</td>
<td>0.79</td>
<td>1.09</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ratio 0.1% ortho-phosphoric acid (pH 3.5): acetonitrile (70: 30, v/v).</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 4. Parameters of system suitability test of the proposed UPLC method

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Obtained value</th>
<th>Reference value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Column capacity (K')</td>
<td>4.22</td>
<td>1–10 acceptable</td>
</tr>
<tr>
<td>Selectivity factor (α)</td>
<td>7.05</td>
<td>≥1</td>
</tr>
<tr>
<td>Resolution factor (R)</td>
<td>15.84</td>
<td>R &gt;2</td>
</tr>
<tr>
<td>Number of theoretical plates (N)</td>
<td>8554</td>
<td>The higher the value, the more efficient the column is</td>
</tr>
<tr>
<td>Paracetamol R&lt;sub&gt;T&lt;/sub&gt; = 2.074</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Metoclopramide HCL R&lt;sub&gt;T&lt;/sub&gt; = 2.845</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 5. Statistical analysis of the results obtained by the proposed methods and reported method [27] for the determination of paracetamol and metoclopramide HCL in their pharmaceutical formulations

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Densitometric method</th>
<th>UPLC method</th>
<th>Reported method [27]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Paracetamol</td>
<td>Metoclopramide</td>
<td>Paracetamol</td>
</tr>
<tr>
<td>Linearity range</td>
<td>1.5-15 (µg/spot)</td>
<td>0.1-0.6 (µg/spot)</td>
<td>5-25 (µg mL⁻¹)</td>
</tr>
<tr>
<td>N</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Mean %</td>
<td>101.1</td>
<td>99.03</td>
<td>100.61</td>
</tr>
<tr>
<td>SD</td>
<td>1.54</td>
<td>0.78</td>
<td>0.75</td>
</tr>
<tr>
<td>Variance</td>
<td>2.38</td>
<td>0.61</td>
<td>0.56</td>
</tr>
<tr>
<td>t-</td>
<td>1.03(2.31)</td>
<td>1.28(2.31)</td>
<td>1.8(2.31)</td>
</tr>
<tr>
<td>F-</td>
<td>1.36(6.39)</td>
<td>4.41(6.39)</td>
<td>5.79(6.39)</td>
</tr>
</tbody>
</table>

Reported method [27] involves spectrophotometric estimation of metoclopramide hydrochloride and Paracetamol in tablet dosage form through simultaneous equation method at 248.6 nm and 275.6 nm using methanol as solvent.
4. CONCLUSION

In the present work, TLC–densitometric and UPLC methods were developed for the determination of paracetamol and metoclopramide HCL. All the obtained results were satisfactory, confirming the applicability, accuracy, and precision of these methods. Both methods can be useful successfully in the routine qualitative and quantitative analyses of the studied drugs in their pharmaceutical preparations.

TLC method has the advantages of high sensitivity, short run time and large sample capacity; although two other methods were reported for the drugs determination, yet the proposed method showed much more sensitivity.

Regarding the developed UPLC method, it can be recommended as being fast and sensitive. Being compared with the other reported HPLC methods, the developed method revealed shorter analysis time (less time consuming; a higher number of analyses per unit of time can be performed) with respect to some of them and more sensitivity than the others; thus being more applicable in routine analysis of the studied drugs.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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