Effect of Metoclopramide on the Excretion Rate of Paracetamol Using HPLC–DAD

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A simple, precise, accurate and robust high-performance liquid chromatography assay was developed and validated for the simultaneous analysis of metoclopramide and paracetamol in human urine. The drugs were isolated from urine samples by solid-phase extraction using C8 cartridges, then analyzed on a C18 reversed-phase column using a mixture of aqueous phase (water containing 0.2% TEA adjusted to pH 3 using ortho-phosphoric acid) and methanol in a ratio of 80:20 (v/v). The method was found to be linear for both drugs in a concentration range of 0.5 to 160 μg/mL using a concentration of 10 μL/mL of internal standard (theophylline) in urine samples (r > 0.999). The accuracy of the method was higher than 91.73% (percentage of the grand mean of recoveries) and the precision was lower than 3.4% (overall percentage of relative standard deviation) for both metoclopramide and paracetamol.

The method was applied to the determination of the drugs in urine samples obtained from male volunteers, following the administration of two formulations, one containing paracetamol alone (Paracetamol®) and the other containing a mixture of paracetamol and metoclopramide (Migracicid®). Determination of the drugs was conducted and the effect of increasing the rate of absorption, consequently increasing the mean urinary excretion of paracetamol due to the presence of metoclopramide in the pharmaceutical formulation, was recorded.

Introduction
Metoclopramide HCl (MCP) (Figure 1A) is a drug with prokinetic and anti-emetic properties. It stimulates the motility of the upper gastrointestinal tract. As a result, both gastric emptying and duodenal peristalsis are increased (1). MCP is used in disorders of decreased gastrointestinal motility, such as nausea and vomiting, with migraine and with cancer therapy (1). Paracetamol (PCM) (Figure 1B) is an analgesic, antipyretic and weak anti-inflammatory drug (1). Mixtures of MCP and PCM are claimed to reduce the frequency and severity of headaches and to assist in allaying the nausea and vomiting that often accompany a migraine attack (2). MCP, which acts as an antiemetic, is reported to increase the absorption of PCM by enhancing the action of acetylcholine (1, 2). Increasing the action of acetylcholine causes the muscles at the entry to the stomach to tighten and the muscles at the exit of the stomach to relax (1, 2). It also increases the contraction of the muscles in the stomach itself, which speeds the passage of the stomach contents through the stomach into the intestine (1, 2, 3). This physically helps to prevent vomiting, but is also useful in migraine attacks because it speeds the passage of the PCM into the intestine, allowing it to be absorbed and to relieve the headache more quickly (2). This mixture is mostly used in a conventional tablet form containing 5 and 500 mg of MCP and PCM, respectively. Up to approximately 80% of the dose of MCP is excreted in the urine in 24 h with approximately 10–20% as the unchanged drug (4).

Approximately 90% of the orally introduced PCM is excreted in the urine in 24 h; of the excreted material, 1–4% is unchanged (4). The biological half-time of MCP and PCM are 4–6 and 1–3 h, respectively (4).

The British Pharmacopoeia (BP) (5) recommends titrimetric methods for the evaluation of the raw material of each drug. For the assay of their dosage forms, the BP describes different spectrophotometric and liquid chromatographic methods. The United States Pharmacopoeia (USP) (6) recommends spectrophotometric and titrimetric methods for their raw materials. For the assay of their dosage forms, liquid chromatographic methods are used.

MCP has been determined in biological fluids using spectrophotometry (7), spectrfluorimetry (8), voltammetry (9), high-performance liquid chromatography (HPLC) (10, 11), liquid chromatography–mass spectrometry (LC–MS) (12–14) and gas chromatography–mass spectrophotometry (GC–MS) (15). PCM also has been determined using spectrophotometry (16), spectrfluorimetry (17), voltammetry (18), HPLC–MS (19–23), GC–MS (24), HPLC (25, 26) and high-performance capillary electrophoresis (27). HPLC methods used for the assay of MCP or PCM in biological fluids sometimes require specific solvent and evaporation of organic solvents, or a combination with a liquid–liquid extraction (LLE) step that is time-consuming and hinders the degree of automation (13, 28). Recently, a new solid-phase extraction (SPE)–HPLC method has been developed that is based on a new technology using molecularly imprinted polymers (MIPs) as sorbent material in solid-phase extraction (MISPE) for the sample clean-up technique for the determination of MCP in biological fluids (11). However, this new method requires time-consuming preparation, and optimization of a new polymer to be selective to a single analyte; additionally, the extraction recoveries obtained from this new technique are comparable to conventional SPE (11). Finally, this technology is not well developed in many clinical laboratories. The proposed study focused on a simple SPE procedure followed by an HPLC–diode-array detection (DAD) method because of its wide availability in ordinary laboratories and its sufficient sensitivity and selectivity, especially in assaying mixtures in human urine. SPE is a more popular clean-up technique than LLE, due to factors such as convenience, cost, time saving and simplicity. SPE can reduce the time required for
The purpose of this study is to develop a simple SPE method for the simultaneous extraction of MCP and PCM from human urine using theophylline (THP) (Figure 1C) as internal standard (IS). The DAD detector offers advantages over the conventional ultraviolet (UV) detector in confirming the peak purity and identity, which makes it suitable for the determination of the investigated drugs in urine. The method was validated by evaluating the recovery, selectivity, linearity, accuracy, precision, stability and robustness. Finally, the method was applied to investigate the hypothesis that a faster absorption rate of PCM is achieved from the formulation mixture with MCP than the formulation with no MCP (2). The method was used to determine the drugs in urine samples from volunteers administered two formulations, one containing the PCM alone (Paracetamol® containing 500 mg PCM) and the other containing mixture of PCM and MCP (Migracid® containing 500 mg PCM and 5 mg MCP). Determination of the drugs was conducted and the effect of increasing the rate of absorption, consequently increasing the mean urinary excretion (MUE) of PCM, calculated as percentage of administered dose due the presence of MCP, was recorded. A one sided t-test was used to test whether this increase in the MUE of PCM in volunteers receiving Migracid® was significantly higher than the MUE of PCM in volunteers receiving Paracetamol®.

**Experimental**

**Chemicals and reagents**

MCP was supplied by Alexandria Company for Pharmaceuticals (Alexandria, Egypt). PCM and THP (IS) were donated by Pharco Pharmaceutical Industries (Alexandria, Egypt). HPLC-grade methanol (Sigma–Aldrich Chemie GmbH, Buchs, Switzerland), TEA (BDH, Poole, UK), analytical grade ortho-phosphoric acid and high purity distilled water were used. The pharmaceutical preparations assayed in the study were Migracid® tablets, labeled to contain 5 mg MCP and 500 mg PCM per tablet, and Paracetamol® tablets, labeled to contain 500 mg PCM per tablet. All final solutions were filtered using a 0.45 μm hydrophilic PVDF (Millipore Millex-HV, Germany) before injection.

**Instrumentation and chromatographic conditions**

Analysis of MCP and PCM was conducted using an HPLC–DAD system consisting of an Agilent 1200 series (Agilent Technologies, Santa Clara, CA) quaternary pump, vacuum degasser, diode array and multiple wavelength detector (G1315 C/D and G1365 C/D) connected to a computer loaded with Agilent ChemStation Software. A Rheodyne manual injector with 20 μL loop was used. The column was Zorbax SB-C18 (4.6 × 150 mm, 5 μm particle size) (Agilent). A mobile phase consisting of an aqueous phase (water containing 0.2% TEA adjusted to pH 3 using ortho-phosphoric acid) and methanol in a ratio of 80:20 (v/v) was found to give best results. Isocratic elution was performed with a flow rate of 1 mL/min at ambient temperature. Elution was monitored in the full UV region and the best detection was achieved at 273 nm. Separation of the analyzed drugs was performed with the SPE vacuum manifold (Agilent Technologies); the solid-phase adsorbents used were C8 cartridges (Agilent Technologies, AccuBond SPE OCTYLE Cartridges, 500 mg, 3 mL).

**Preparation of stocks and standard solutions**

Stock solutions of MCP and PCM were prepared at a concentration of 0.1 mg/mL by dissolving appropriate amounts of these drugs in methanol. In the same way, THP (as IS) was prepared at a concentration of 0.01 mg/mL. The working standard solutions were prepared by diluting appropriate portions of MCP and PCM solutions in 10 mL volumetric flasks, and then these solutions were completed to the mark with water, adjusted to pH 3 with ortho-phosphoric acid. Calibration curves were constructed with six blank urine samples spiked with 1 mL of these standard solutions of each drug, plus 0.1 mL of the IS. Linear regression analysis was performed.

**Urine collection**

To six healthy male volunteers, who followed a two-week washing period, two tablets of Migracid® containing 10 mg MCP and 1,000 mg PCM were administered, along with 250 mL of water. Urine samples were collected before dosing and after 6, 12 and 24 h following drug administration. At the end of each collection period, the volume of urine was measured and a 50 mL urine sample was kept frozen at −20°C until analysis. The urine samples were thawed gently at ambient temperature and a 1 mL urine volume was then sampled. The same procedures were conducted upon administration of two tablets of Paracetamol® after a washing period of two weeks.

**SPE procedures**

Before extraction, the cartridge was conditioned with 3 mL of methanol followed by 3 mL of water. A blank urine sample of 1 mL was spiked with 1 mL of the working standard solutions of MCP and PCM and 0.1 mL of THP as IS. This spiked urine sample was introduced to the cartridge with (0.2%, v/v) ortho-
phosphoric acid. Elution was performed with 5 mL of methanol. The eluate was evaporated to dryness under nitrogen and the residue was dissolved in a 100 μL diluting solvent consisting of water, adjusted to pH 3, and methanol in a ratio of 80:20. The urine samples collected post-dose were spiked with only 0.1 mL of THP as IS and the same procedures were conducted. A 20 μL aliquot of the final solutions was introduced to the HPLC system after filtering using 0.45 μm hydrophilic PVDF.

Results and Discussion

Method development

Sample extraction and clean-up

For the successful separation of analytes in a biological matrix, sample pretreatment before HPLC analysis is generally required (11). It is well known that urine samples have many endogenous substances that interfere with the chromatographic separation of analytes, causing difficulties with their quantitation. Chromatograms of a standard synthetic mixture of the drugs and the extract of the spiked blank urine with these drugs are shown in Figure 2. The figure shows that, after SPE procedures, no interfering peaks were co-eluted with the drugs. The SPE technique was investigated for the selective elution of MCP, PCM and IS (THP). The SPE conditions described in this method were selected after investigating different parameters. A better clean-up method was found by using C8 OCTYLE cartridges, (0.2%, v/v) ortho-phosphoric acid and 5 mL methanol as eluting organic solvent, which effectively eliminated the interfering peaks and resulted in high recoveries of both drugs and IS; this will be discussed in the next section.

Recovery from the urine

The absolute recovery of MCP, PCM and IS (THP) was assessed by direct comparison of their peak areas from extracts of blank urine samples that had been spiked with known concentrations of both drugs, versus those found by direct injection of standards of the same concentration prepared in the diluting solvent. The recovery of the IS was simultaneously determined in the same solutions. The mean recoveries ± relative standard deviation (RSD, %) for MCP and for PCM at concentration levels of 0.5, 40 and 160 μg/mL and for the IS at 10 μg/mL are shown in Table I. The results in Table I provide evidence that there was no major loss during sample processing. Chromatograms of a standard synthetic mixture of the drugs

![Figure 2](https://academic.oup.com/chemsci/article-abstract/51/4/383/416419)

**Figure 2.** Chromatograms of a synthetic mixture of 40 μg/mL of PCM and MCP in a ratio of 1:1, using 10 μg/mL THP as IS in: standard solution (A); spiked urine after SPE (B).
and the extract of the spiked blank urine with the same concentrations of these drugs are shown in Figure 2.

Optimization of chromatographic conditions

The chromatographic conditions described in this assay were selected after exploring different types of columns and different mobile phases consisting of aqueous mixtures of methanol and acetonitrile in different ratios. Better chromatographic separation of the analytes was achieved using a C18 reversed-phase column with a mobile phase consisting of water containing 0.2% TEA and adjusted to pH 3 with ortho-phosphoric acid–methanol (80:20, v/v).

Because both drugs are soluble in methanol, and because PCM has more solubility in methanol than MCP (4), if the percentage of methanol is increased, the drugs, especially PCM, will rapidly co-elute with urine interferences and the PCM peak will overlap with MCP. If the percentage of methanol is less than 20% of the mobile phase, the retention time ($t_r$) of MCP will be much higher than PCM (Figure 3A).

Different pH values were examined from the range of 2.5 to 7, and the optimum pH, providing the most symmetric, well-defined peak within a reasonable retention time, was found to be 3 (Figure 3B). PCM and MCP possess pKa values of 9.5 and 9.3, respectively (4), so as the pH of the mobile phase decreases, these basic nitrogenous drugs will be ionized and rapidly eluted with acceptable peak shapes. pH values higher than 3 (more basic pH) caused a low-response, tailed MCP peak. The peak shape of MCP is more affected by the pH change, because it contains more basic nitrogenous centers than PCM (Figures 1A and 1B), so MCP requires a more acidic pH to elute in a suitable retention time (not too late) and with a good peak shape (small peak width).

Various concentrations of TEA were tested at the selected pH. A concentration of 0.2% in the aqueous phase was found to be optimum. The addition of TEA in the aqueous phase did not greatly change the $t_r$ of the peaks, but it was important in increasing the sharpness and decreasing the tailing in the peaks of the drugs. This may be because TEA in an acid medium can be used to block residual silanol groups on the silica gel backbone of bonded-phase columns. This is useful for the analysis of ionized nitrogenous compounds that might interact with these silanols.

Several wavelengths were examined. The optimum wavelength was found to be 273, which provided reasonable absorbance of both drugs and provided minimum interference of the urine endogenous substances.

Under the selected conditions, several ISs were tried to choose the most suitable one for the analysis. THP was selected because it was eluted with acceptable recovery after SPE procedures, had a moderate response, eluted between the drugs, had a good peak shape and no interfering substances of urine samples were found at its retention time. A concentration of 10 μg/mL was found to be optimum.

The DAD detector of the HPLC instrument enhances the power of HPLC; it is an elegant option for assessing method specificity and peak purity by comparison of recorded spectra during peak elution. This was very useful in confirming the peak purity and identity of each drug, because no endogenous substances or metabolites were co-eluted or interfered with the drugs (Figure 4). As a result, no interfering peaks due to urine components or metabolites eluted at the retention times of MCP, PCM or IS (THP) (Figure 2).

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Table I
Extraction Recovery of MCP, PCM and the IS, THP, After the SPE Procedures.

<table>
<thead>
<tr>
<th>Nominal concentration (μg/mL)</th>
<th>MCP</th>
<th>THP</th>
<th>PCM</th>
</tr>
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<tbody>
<tr>
<td>0.5</td>
<td>88.13</td>
<td>88.76</td>
<td>97.45</td>
</tr>
<tr>
<td>40</td>
<td>89.89</td>
<td>87.72</td>
<td>95.45</td>
</tr>
<tr>
<td>160</td>
<td>88.20</td>
<td>86.58</td>
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Recovery (%)

<table>
<thead>
<tr>
<th>Mean (%)*</th>
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<tr>
<td>88.07</td>
</tr>
<tr>
<td>87.69</td>
</tr>
<tr>
<td>95.98</td>
</tr>
</tbody>
</table>

RSD (%)†

| 2.13     |
| 1.24     |
| 1.35     |

Grand mean (%)‡

| 90.57    |
| 89.11    |
| 87.29    |

Overall RSD (%)§

| 4.68     |
| 2.03     |
| 5.20     |

*The mean of all recoveries of the same concentration for each drug ($n = 3$) and for the IS ($n = 9$).
†The percentage RSD of the recoveries of the same concentration for each drug ($n = 3$) and for the IS ($n = 9$).
‡The mean of all recoveries of different concentrations for each drug ($n = 9$).
§The percentage RSD of the recoveries of different concentrations for each drug ($n = 9$).

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Figure 3. Variation of the retention factors of a synthetic mixture of PCM and MCP as a function of: percentage of methanol in the mobile phase (A); pH of the aqueous phase (B).
Under these conditions, the chromatographic characteristics of the mixture are summarized in Table II, which shows that the proposed HPLC method permitted adequate resolution of the mixture’s components and IS (good resolution and selectivity values) within a reasonable run-time. PCM was eluted at 4.5 min, THP at 6.2 min and MCP at 11 min (suitable retention factors). In addition, high column efficiency was indicated from the large number of theoretical plates. The degree of peak asymmetry was also evaluated using the tailing factor, which did not exceed the critical value (1.2), indicating an acceptable degree of peak asymmetry.

**Method validation**

**Linearity**
Linearly was tested over three days at six concentration levels, ranging from 0.5 to 160 μg/mL for MCP and PCM using a concentration of 10 μg/mL of the IS in urine samples. Peak area ratios were plotted versus the respective concentrations of MCP and PCM. Good linearity was achieved \((r = 0.9995, 0.9996)\) for MCP and PCM, respectively. The calibration curves had the regression equations \(y = 0.026x - 0.015\) and \(y = 0.037x + 0.018\) for MCP and PCM, respectively; where \(y\) is the peak area ratio of the drug to the IS and \(x\) is the concentration of the drug.

**Accuracy and precision**
Accuracy and precision (intra-day and inter-day) were determined by measuring blank plasma samples spiked with known concentrations of each drug in three synthetic mixtures of PCM: MCP (1:320, 1:1 and 320:1), as cited in Table III. The concentration of IS in spiked plasma was 10 μg/mL. Extraction, analysis and determination of the recovery and RSD (%) was conducted for each drug. The obtained results of recovery and RSD% indicated good accuracy and precision, respectively. Data for these experiments are shown in Table III.

**Limits of detection and quantitation**
The limit of detection (LOD) and limit of quantitation (LOQ) were determined at signal-to-noise ratios (S/N) of 3 and 10, respectively (33). LOD and LOQ were obtained for each drug as follows: 0.2 and 0.5 μg/mL for MCP and 0.15 and 0.5 μg/mL for PCM, respectively.

**Stability of urine samples**
The stability of MCP and PCM in urine samples was investigated with spiked samples at the same concentration levels used for precision and accuracy. Spiked samples were analyzed after different storage conditions: immediately, after staying for 2 and 24 h at room temperature (RT), and after one and two freeze/thaw cycles stored at \(-20^\circ C\). The obtained results are shown in Table IV. The results show that mean recoveries and RSD are between 90.2, 95.1% and 1.22, 3.14%, respectively, which confirms the stability of both drugs in urine samples under different storage conditions.

**Robustness of the method**
The robustness of the proposed method was evaluated by analyzing three different concentration levels of MCP and PCM, as conducted for accuracy and precision, but at intentional slight variations of the selected parameters. The RSD% of peak areas and the SD of retention factor (K') values of MCP and PCM were calculated after introducing small, deliberate changes in the method parameters. The intentional slight variations that were applied to these parameters included: percentage of methanol, pH values of the aqueous phase, percentage of TEA in the aqueous phase, flow rate of the mobile phase and detection wavelength. Results presented in Table V show RSDs of the peak areas of the drugs less than 2% and nearly unchanged retention factors, indicating the robustness of the developed HPLC method.

<table>
<thead>
<tr>
<th>Table II</th>
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<tr>
<td>Chromatographic Characteristics of MCP, PCM and THP by the Proposed HPLC Method</td>
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<tr>
<td>(t_r) (min)</td>
</tr>
<tr>
<td>PCM</td>
</tr>
<tr>
<td>THP</td>
</tr>
<tr>
<td>MCP</td>
</tr>
</tbody>
</table>

*Number of theoretical plates retention factor.

1Selectivity.

2Resolution.

3Tailing factor.
Application of the method for the analysis of drugs in urine samples

The present method was applied to perform the determination of the urine concentration of MCP and PCM after oral administration of two tablets of Migracid, which contain 10 mg MCP and 1,000 mg PCM. The chromatogram in Figure 5 shows an example of an 0–6 h post-dose showing the two drugs and the IS. The identity and the purity of the drug peaks in the urine samples were confirmed using the DAD detector of the HPLC and by spiking the samples with the standard drug solutions. Figure 6 shows the MUE for six healthy volunteers calculated as a percentage of the administered doses of MCP and PCM after administering two tablets of Migracid, and of PCM alone upon administrating two tablets of Paracetamol. The effect of MCP in increasing the rate of absorption, consequently increasing the MUE of PCM, calculated as a percentage of the administered dose, is shown in Figure 6. This difference was analyzed for significance by applying a one-sided t-test (33). The MUE of PCM in volunteers that received the PCM with MCP in the same dosage form (Migracid) was significantly higher than the MUE of PCM in volunteers that received the PCM alone in its dosage form (Paracetamol) at P < 0.05 (Table VI).
Conclusion

A simple SPE method with reversed-phase HPLC–DAD was developed and validated for the simultaneous determination of MCP and PCM in human urine. The method is selective, precise, accurate and robust. The method was applied for the determination of the drugs in urine samples from volunteers that received two pharmaceutical formulations. One of these formulations contained both MCP and PCM, and the other contained PCM alone. The differences in the MUE of PCM were tested for significance by using a one-sided t-test.

Table VI

<table>
<thead>
<tr>
<th>Time interval</th>
<th>Migracid*</th>
<th>Paracetamol*</th>
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<tbody>
<tr>
<td>0–6 h</td>
<td>2.25 ± 0.0067</td>
<td>1.02 ± 0.0058</td>
</tr>
<tr>
<td>6–12 h</td>
<td>1.50 ± 0.013</td>
<td>1.07 ± 0.041</td>
</tr>
<tr>
<td>Total 24 h</td>
<td>3.76 ± 0.005</td>
<td>2.09 ± 0.059</td>
</tr>
</tbody>
</table>

*Mean urinary excretion of PCM as percentage administered dose ± standard deviation for six volunteers.

References