DEVELOPMENT AND VALIDATION OF A SINGLE HPLC METHOD FOR THE DETERMINATION OF THIRTEEN PHARMACEUTICALS IN BULK AND TABLET DOSAGE FORM

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ABSTRACT. The aim of this study was to develop and validate a high performance liquid chromatography (HPLC) method for the determination of thirteen selected pharmaceutical compounds (metformin, amoxicillin, chloroquine, theophylline, trimethoprim, caffeine, norfloxacin, ciprofloxacin, acetylsalicylic acid, doxycycline hyclate, metronidazole, albendazole and cloxacillin) in bulk and tablet dosage form. Chromatographic separation using a Kromasil C18 column, gradient elution with aqueous formic acid (0.1%), methanol and acetonitrile, a UV absorption wavelength of 250 nm and a mobile phase flow rate of 1 mL/min over a 22 min run time was optimized for complete separation of the selected target compounds. The method was validated and results for: linearity, precision, sensitivity, accuracy, specificity, suitability and method robustness were obtained and met the ICH guidelines. Calibration curve correlation coefficients ranged from 0.9985 – 0.9998 and the percentage relative standard deviations for repeated analysis was below 5%, indicating acceptable method precision. The limits of detection (LODs) and quantification (LOQs) ranged from 0.020 – 0.27 µg/L and 0.080 – 0.91 µg/L, respectively. The accuracy study yielded recoveries in the ranges 86.0 – 102% for pure compounds and 90.9 – 106% for compounds in tablet dosage form. The method is robust for small or deliberate changes to the chromatographic parameters and found to be appropriate for analysis of tablets for the determination of the thirteen pharmaceuticals.

KEY WORDS: Pharmaceuticals, Bulk determination, Tablet dosage, High performance liquid chromatography, Method development, ICH guidelines

INTRODUCTION

Pharmaceuticals are synthetic or natural chemicals that are used extensively in human and veterinary medicine to prevent illness. They are also used as growth promoters that contain active ingredients designed to have pharmacological effects and confer significant benefits [1, 2]. After administration, a part of the consumed pharmaceuticals is excreted as metabolites and unchanged parent compounds largely through the urine and feces [3]. Classification of pharmaceuticals into various classes may be based on their pharmacological properties, such as their therapeutic effects. Those selected for this study are frequently prescribed drugs that are also detected in the environment.

Antibiotics are natural, semi-synthetic or synthetic drugs used as antibacterial, antifungal or anti-parasitic agents and are important medicines [4]. Widely used antibiotics include amoxicillin (AMOX), which is used to combat bacterial infections [5], and ciprofloxacin (CIP) a synthetic fluoroquinolone derivative with broad-spectrum activity against many pathogenic gram-positive bacteria [6]. Cloxacillin (CLA) belongs to the semi-synthetic β-lactam antibiotics

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used against staphylococci that produce beta-lactamas [7], while doxycyclinhyclate (DOXH), is a broad spectrum tetracycline antibiotic with a large range of antibacterial activities [8]. A member of the fluoroquinolones third-generation antibiotics, norfloxacin (NOR), is used for the treatment of diseases caused by Campylobacter, Escherichia coli, Shigella and Vibrio cholera and used to treat gonorrhea and urinary tract infection [9]. The antibiotic trimethoprim (TMP) is used for protozoal infections [10], while metronidazole (MTZ) is prescribed for treatment and prophylaxis of anaerobic bacterial infections and specific bacterial infections [11].

Antihelminthics are drugs that are used to eject parasitic worms from the gastro-intestinal tract by either stunning or killing them [12]. Albendazole (ABZ) is one of several broad-spectrum benzimidazole parasiticides used for treatment of parenchymal neurocysticercosis [13].

Anti-malarial drugs such as chloroquine (CHQ) are used for treating fever caused by malaria. Chloroquine is also prescribed to decrease the symptoms of rheumatoid arthritis and to treat systemic and discoid lupus erythematosus in adults [14].

Nonsteroidal anti-inflammatory drugs (NSAIDs) are used for treatment of fever, pain and inflammation. Acetyl salicylic acid (ASA), also known as aspirin, with the chemical name 2-acetoxybenzoic acid, is one of the most widely used drugs in the world and exerts analgesic, antipyretic and anti-inflammatory effects [15, 16].

Central nervous system stimulants are a group of drugs that produce an increase in mental and motor activity. 1,3,7-Trimethylxanthine, commonly referred to as caffeine (CAF) acts as a stimulant psychotrope and as a mild diuretic [17], while theophylline (THP) displays anti-inflammatory activities by inhibiting the activity of CD4 lymphocytes in vitro and through mediator release from mast cells [18].

Anti-diabetic metformin (MET) is used in the treatment of diabetes mellitus II, since it suppresses the production of hepatic glucose, and thus attenuates hyperglycemia [19].

Many authors have described the determination of pharmaceuticals in pure, tablet dosage form and real sample using a range of analytical techniques. Analytical techniques including liquid chromatography (LC) and gas chromatography (GC) are commonly employed for the separation and determination of compounds in mixtures, but liquid chromatography is mostly used to determine pharmaceuticals, since they are mostly non-volatile [20, 21]. Liquid chromatographic methods for amoxicillin [22], ciprobendazole [23], chloroquine [24], acetylsaliclyc acid [16], caffeine [25], metronidazole [26], theophylline [27] and ciprofloxacin [28] have been reported. Spectrophotometric methods for amoxicillin [29], cefoxacilin [30], ciprofloxacin [31], trimethoprim [32], ciprobendazole [23], chloroquine [33], metronidazole [26] and for metformin [34] has been reported. Electrochemical methods for metronidazole [35], caffeine and theophylline [36], ciprobendazole [37] and acetylsaliclyc acid [38]; a kinetic method for amoxicillin [5] and a method using chemiluminescence for norfloxacin [39] are available in literature.

To the best of our knowledge there is no single HPLC method reported for the simultaneous determination of these selected pharmaceutical compounds of different therapeutic classes. This study attempts to develop a simple, accurate, precise and stable analytical chromatographic method, which can separate and determine thirteen selected pharmaceutical drugs simultaneously in a single optimized method in bulk and commercial tablet dosage form. The proposed method has been developed and validated as per the ICH guidelines for the analysis. Developing such an analytical method is more cost-effective than changing parameters for each analyte for the analysis of real samples. The developed method is suitable for laboratories that are not equipped with highly specialized state-of-the-art instrumentation. The method will still give a sufficiently accurate determination of the selected compounds.
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EXPERIMENTAL

Chemicals, reagents and equipment

All the chemicals used for method development were analytical grade. The HPLC grade methanol (>99%) and HPLC grade acetonitrile (>99.9%) were from Fisher Scientific (UK), the formic acid (>96%) from Sigma Aldrich (Germany) and acetic acid (99%) from Fisher Chemicals (UK). Ultra-pure water used throughout the study was generated using a Millipore system (Direct-Q 3 UV with pump, South Africa). Pharmaceutical standard compounds (assigned purity ≥99%), listed in Table 1, were a kind donation from Addis Pharmaceuticals (Adigrat, Ethiopia) and tablets containing the target active compound were purchased from the local market in Addis Ababa, Ethiopia. Chromatographic separation was performed on an Agilent HPLC 1260 Series equipped with quaternary pump, auto sampler and diode array detector from Agilent Technologies (Singapore, Germany). Data acquisition and processing were accomplished with LC Chemstation software (Agilent Technologies).

Table 1. Selected pharmaceuticals of different therapeutic classes used for method development.

| Therapeutic class | Molecular formula | Molecular weight (g/mol) | Structure of the compound | Maximum absorption wavelength, $\lambda_{\text{max}}$ (nm) |
|-------------------|-------------------|--------------------------|---------------------------|------------------------------------------------------|
| Antibiotics       |                   |                          |                           |                                                      |
| Amoxicillin       | $\text{C}_{16}\text{H}_{25}\text{N}_{3}\text{O}_{7}\text{S}$ | 365.5                    | ![Amoxicillin Structure](image) | 335                                                  |
| Ciprofloxacin     | $\text{C}_{17}\text{H}_{18}\text{FN}_{3}\text{O}_{3}$ | 331.3                    | ![Ciprofloxacin Structure](image) | 280                                                  |
| Cloxacillin       | $\text{C}_{10}\text{H}_{19}\text{ClN}_{3}\text{NaO}_{6}\text{S}$ | 435.8                    | ![Cloxacillin Structure](image) | 254                                                  |
| Doxycyclinehyclate| $\text{C}_{12}\text{H}_{14}\text{N}_{3}\text{O}_{6}$ | 444.4                    | ![Doxycyclinehyclate Structure](image) | 273                                                  |
| Norfloxacin       | $\text{C}_{9}\text{H}_{13}\text{FN}_{3}\text{O}_{3}$ | 319.3                    | ![Norfloxacin Structure](image) | 273                                                  |
Chromatographic separation

Chromatographic separation of the thirteen selected pharmaceutical compounds was achieved using a Kromasil C18, 5 µm (4.6 mm x 150 mm x 5 µm) column (Sigma Aldrich, Stockholm, Sweden) held at ambient temperature. A combination of 0.1% aqueous formic acid, acetonitrile and methanol was used as mobile phase at a flow rate of 1 mL/min. The detector was set to

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detect at 250 nm UV absorbance. An aliquot of 10 μL of a mixture of the selected compounds were injected into the system. The total run time was 22 min.

**Preparation of standard solutions**

Mixed stock solutions of twelve pharmaceutical standards (200 μg/mL) were prepared by dissolving 10 mg with ultra-pure water/methanol (50:50, v/v) in 50 mL volumetric flask. A stock solution of ciprofloxacin, which is insoluble in methanol, was dissolved in ultra-pure water (200 μg/mL) and stored in the refrigerator. Series of working standard solutions for the method development were prepared daily with a mixture of ultra-pure water/methanol (90:10, v/v).

**Preparation of tablet sample solutions**

For the preparation of the sample solutions used for method validation, an accurately weighed amount of powder equivalent to 10 mg of each tablet was transferred completely to a 50 mL volumetric flask and dissolved with ultra-pure water/methanol (50:50, v/v). The ciprofloxacin powdered tablet was dissolved in ultra-pure water only. All the tablet powders were sonicated in the solvent for about 30 min and then filtered through Whatman filter paper number 1 (110 mm). The volume was adjusted to 50 mL followed by filtration through a 0.22 μm membrane filter. Mixtures of series of sample solutions were prepared by dissolving with ultra-pure water and methanol (90:10, v/v).

**Parameter optimization**

Separation in liquid chromatography is highly affected by different factors including mobile phase (both solvent type and composition), the addition of additives, absorption wavelength and mobile phase flow rate. Different solvents (water, acetonitrile and methanol) and additives (formic acid and acetic acid) were evaluated for the separation of thirteen pharmaceutical compounds. The mixed standard solution was scanned in the wavelength region of 190–400 nm for wavelength selection for proper separation. The effect of mobile phase flow rate was also investigated using a different flow rate of 0.6, 1 and 1.3 mL/min. The chromatographic parameters were evaluated by taking both the resolution and symmetry of the peaks into account.

**Validation of the analytical method**

The developed method for the determination of selected pharmaceuticals was evaluated as per the ICH Q2 [40] guidelines protocol for, linearity, sensitivity, precision, accuracy, specificity, robustness, and system suitability.

**RESULTS AND DISCUSSION**

**Mobile phase and additive selection**

The mobile phase was selected according to the physico-chemical properties of pharmaceutical drugs to provide good separation. Separation in liquid chromatography is highly affected by mobile phase, both solvent type and composition. Different solvents (water, acetonitrile and methanol) and additives (formic acid and acetic acid) were evaluated. From the examined mobile phase composition and additives, a combination of formic acid in water, acetonitrile and methanol resulted in the best resolution and a short run time. The effect of the concentration of
formic acid in the water on separation was also examined and 0.1% formic acid yielded the best resolution between analytes. The composition of the solvent was evaluated by taking both the resolution and symmetry of the peak into account. The optimum mobile phase comprised three solvents. Separation of the analytes was accomplished over 22 min using gradient elution with A (water with 0.1% formic acid), B (acetonitrile) and C (methanol). The gradient was applied as follows: program, 90-50% A, 1-20% B and 9-30% C (8 min), held at 50% A, 20% B and 30% C (7 min) before changing to 50-30% A, 20-50% B and 30% C (2 min), to 30-5% A, 50-90% B, 30-5% C (3 min) and finally, to 5-90% A, 90-1% B and 5-9% C (2 min).

**Wavelength selection**

The optimum wavelength should be the one which shows good absorbance for all the selected compounds. The mixed standard solution was scanned in the wavelength region of 190–400 nm. It is evident from Figure 1 that 250 nm yielded the largest overall relative peak height for all the analytes compared to those obtained at 240 nm, 270 or 340 nm. It should be noted that Table 1 shows that three compounds (amoxicillin, metronidazole and chloroquine) have a maximum UV absorption at 340 nm or close to 340 nm. However, Figure 1 shows that only chloroquine had a good absorbance at 340 nm while amoxicillin and metronidazole do not have good absorbance at 340 nm. This is because the absorbance of a compound depends on the type of solvent, concentration and molar absorptivity. This indicates that amoxicillin and metronidazole have smaller molar absorptivity in the solvent used. No absorption of amoxicillin and metronidazole at 340 nm may also be due effect of the other compounds present in the mixture. Therefore, 250 nm was selected as an optimum wavelength at which all the thirteen compounds showed good absorbance. It should also be noted that using a wavelength of 250 nm (the selected wavelength) would certainly diminish the sensitivity of the detector towards analytes with UV max above 300 nm. Although this would not really matter for pharmaceutical products containing high concentrations of the active ingredient, since the analytes are easily detected, small differences in concentrations would not be picked up for these compounds.

![Figure 1. Detector response of 13 selected pharmaceutical drugs analysed using four different UV wavelengths to enable selection of the optimal wavelength.](image)

**Mobile phase flow rate optimization**

The interaction of the compound with the stationary and the mobile phase affects the resolution of the peaks, in addition to the flow rate of the mobile phase. It was observed that the increase
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in flow rate (1.3 mL/min) decreased the retention time of all analyte compounds, it adversely affected the resolution of some compounds. A faster mobile phase flow limits the interaction of analyte with the stationary phase. While decreasing the flow rate (0.6 mL/min) increased the retention times and total run time, it caused and leads to broadening of the peaks and yielded poorly resolved peaks, compared to 1.0 mL/min which was selected as optimum flow rate (Figure 2). In this study the first peak, which was due to the elution of metformin, was controlled by avoiding contamination with frequent injection in the system by running the blank after each run.

![Chromatograms obtained using different mobile phase flow rates](image)

Figure 2. Chromatograms obtained using different mobile phase flow rates (a) 0.6 mL/min, (b) 1 mL/min and (c) 1.3 mL/min.

Validation of the analytical method

Linear range

The ability of the assay to give data that is directly proportional to the concentration of analyte that the sample contains is referred to as linearity. Similarly, the range refers to the highest and lowest concentration of the analyte that can be detected by the detector of the method with an appropriate accuracy, precision, and linearity [41]. The linearity of the method measured as peak areas against the corresponding concentrations of the analyte compounds were evaluated using the correlation coefficients (R²) as reported in (Table 2).
Table 2. Linear range, regression equation and correlation coefficient for each of the 13 selected pharmaceuticals when using the developed method.

| Pharmaceutical compound       | Linear range (µg/mL) | Regression equation          | R²  |
|-------------------------------|----------------------|-----------------------------|-----|
| Acetyl salicylic acid         | 1.0 - 100            | y = 3.6181x + 1.3002         | 0.9998 |
| Albendazole                   | 0.50 - 100           | y = 8.8529x + 13.169         | 0.9988 |
| Amoxicillin                   | 2.5 - 100            | y = 2.4260x + 0.2300         | 0.9998 |
| Caffeine                      | 0.50 - 100           | y = 10.167x + 6.0244         | 0.9996 |
| Chloroquine                   | 0.50 - 100           | y = 11.150x + 7.5555         | 0.9997 |
| Ciprofloxacin                 | 1.0 - 100            | y = 9.8126x + 5.7892         | 0.9987 |
| Cloxacillin                   | 0.70 - 100           | y = 3.422x + 2.7597          | 0.9991 |
| Doxycycline hyclates          | 1.0 - 100            | y = 0.9325x + 5.5146         | 0.9987 |
| Metformin                     | 0.50 - 100           | y = 9.0233x + 7.2918         | 0.9993 |
| Metronidazole                 | 1.0 - 100            | y = 4.4886x + 9.6939         | 0.9985 |
| Norfloxacin                   | 0.70 - 100           | y = 7.5441x + 1.4492         | 0.9998 |
| Theophylline                  | 0.50 - 100           | y = 11.644x + 8.8687         | 0.9994 |
| Trimethoprim                  | 0.25 - 100           | y = 16.536x + 9.2685         | 0.9997 |

**Sensitivity**

The limits of detection (LOD) and limits of quantification (LOQ) for each of the analytes were calculated from the standard deviation of the response and slope of the calibration curve of pharmaceutical compounds using the formula as per ICH guideline, 3.3σ/s and 10σ/s, respectively, where σ is the standard deviation of the response and s the slope of the calibration curve. The results are reported in Table 3, which shows that the LODs ranged from 0.02–0.27 µg/L and the LOQs from 0.08–0.91 µg/L.

Table 3. LODs, LOQs and precision obtained for the method developed for the simultaneous determination of selected pharmaceutical compounds.

| Pharmaceutical compound       | LOD (µg/L) | LOQ (µg/L) | Precision %RSD | Repeatability (%RSD, n = 3) | Reproducibility (%RSD, n = 3) |
|-------------------------------|------------|------------|----------------|-------------------------------|-------------------------------|
| Acetyl salicylic acid         | 0.070      | 0.23       | 2.8            | 5.1                           |                               |
| Albendazole                   | 0.040      | 0.15       | 1.7            | 4.3                           |                               |
| Amoxicillin                   | 0.27       | 0.91       | 1.2            | 4.9                           |                               |
| Caffeine                      | 0.020      | 0.08       | 1.1            | 2.0                           |                               |
| Chloroquine                   | 0.030      | 0.12       | 1.4            | 3.3                           |                               |
| Ciprofloxacin                 | 0.030      | 0.09       | 1.2            | 5.1                           |                               |
| Cloxacillin                   | 0.16       | 0.53       | 1.3            | 2.6                           |                               |
| Doxycycline hyclates          | 0.16       | 0.54       | 3.1            | 3.9                           |                               |
| Metformin                     | 0.15       | 0.33       | 3.0            | 5.2                           |                               |
| Metronidazole                 | 0.10       | 0.58       | 1.3            | 3.9                           |                               |
| Norfloxacin                   | 0.080      | 0.27       | 1.7            | 4.1                           |                               |
| Theophylline                  | 0.050      | 0.16       | 1.4            | 3.1                           |                               |
| Trimethoprim                  | 0.020      | 0.10       | 1.3            | 5.3                           |                               |

**Precision**

The precision of the developed method was evaluated by performing intra-day and inter-day precision studies using a concentration of 1 µg/mL of each analyte in solution. The measured peak areas were used to calculate the percent relative standard deviations (%RSDs). Intra-day
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Precision was carried out by analysing three replicates of a mixture of the standard pharmaceuticals on the same day. The measured peak areas were used to calculate the percentage relative standard deviations (%RSDs). The inter-day precision study was performed on three consecutive days by analyzing the mixture of all thirteen pharmaceutical drugs in triplicate and the % RSDs were calculated (Table 3). The result obtained for the precision study were regarded as acceptable for analysis, due to the small % RSDs that ranged from 1.1-5.3%, which were lower than the stipulated values of 15% [42].

Table 4. Results of the recovery studies of the developed HPLC method.

| Pharmaceutical compound | Spiked amount | Recovery (%) | Mean recovery± SD | Recovery (%) | Mean recovery ± SD |
|-------------------------|---------------|--------------|-------------------|--------------|-------------------|
| Acetylsalicylic acid    | 50 %          | 86.8         | 86.3±1.0          | 89.4         | 90.9±10           |
|                         | 100 %         | 85.2         | 81.4              |              |                   |
|                         | 150 %         | 87.0         | 102               |              |                   |
| Albenzolide             | 100 %         | 89.7         | 89.2              |              |                   |
|                         | 150%          | 103          | 93.8±7.7          | 97.8         | 95.1±5.2          |
|                         | 150 %         | 89.0         | 98.5              |              |                   |
| Amoxicillin             | 50 %          | 88.9         | 99.6              |              |                   |
|                         | 100 %         | 86.0         | 93.0              |              | 97.0±3.5          |
|                         | 150 %         | 85.5         | 98.5              |              |                   |
| Caffeine                | 50 %          | 92.5         | 83.1              |              | 91.9±8.2          |
|                         | 100 %         | 92.6         | 99.4              |              |                   |
|                         | 150%          | 95.6         | 93.1              |              |                   |
| Chloroquine             | 50 %          | 88.7         | 84.7±2.4          | 83.7         | 94.9±11           |
|                         | 100%          | 85.2         | 107               |              |                   |
|                         | 150%          | 89.9         | 83.9              |              |                   |
| Ciproflaxacin           | 50 %          | 89.9         | 102±10            | 89.4         |                   |
|                         | 100%          | 85%          | 97.4±7.0          |              |                   |
|                         | 150%          | 111          | 100               |              |                   |
| Cloxacillin             | 50 %          | 96.0         | 110               |              | 106±4.0           |
|                         | 100%          | 90.0         | 104               |              |                   |
|                         | 150%          | 91.6         | 103               |              |                   |
| Metformin               | 50 %          | 89.4         | 92.2±3.5          | 92.5         | 94.9±11           |
|                         | 100%          | 87.2         | 105               |              |                   |
|                         | 150%          | 85.9         | 107               |              |                   |
| Doxycycline hyclates    | 50 %          | 108          | 107               | 117          | 101±9.6           |
|                         | 100%          | 93.7         | 95.2              |              |                   |
|                         | 150%          | 97.9         | 94.7              |              |                   |
| Metronidazole           | 50 %          | 94.8         | 101±10            | 115          |                   |
|                         | 100%          | 113          | 104±12            | 106          |                   |
|                         | 150%          | 95.5         | 106               |              |                   |
| Norflaxacin             | 50 %          | 85.6         | 95.3±9.2          | 98           | 99.6±9.2          |
|                         | 100%          | 96.3         | 109               |              |                   |
|                         | 150%          | 104          | 91.3              |              |                   |
| Theophylline            | 50 %          | 85.2         | 87.9±2.8          | 105          | 101±6.1           |
|                         | 100%          | 87.6         | 105               |              |                   |
|                         | 150%          | 90.9         | 93.8              |              |                   |
| Trimethoprim            | 50 %          | 86.5         | 86.3±0.8          | 102          | 98.5±11           |
|                         | 100%          | 85.4         | 85.8              |              |                   |
|                         | 150%          | 86.9         |                   |              |                   |
Accuracy

To study the accuracy of the proposed method, recovery studies were carried out by applying standard addition at three different levels. A known amount of the mixture of pharmaceutical compounds (50%, 100% and 150%) of 10 mg/L was added to a pre-analyzed standard and sample solution. The percentage recoveries were calculated as reported in Table 4. Recoveries ranged from 86.0–102% for pure compounds and from 90.9–106% for tablet dosage forms, which are within the acceptable range [42]. The preparation of the solution and the ingredients other than the active compounds in the marketed tablet might be the reason for the difference in the recovery.

Specificity

The specificity of the developed HPLC method was established by injecting the blank (solvent) and placebo solution without active pharmaceutical ingredient, into the HPLC system using the optimized conditions. The representative chromatogram of blank and placebo is shown in Figure 3 (a) and (b), respectively. No peaks were detected at the retention times corresponding to any of the target analytes considered in this study.

Figure 3. Chromatogram of the blank solvent (a) and placebo solution (b) using the developed method.

Robustness

The robustness of an analytical procedure refers to its capability to remain unaffected by small, but deliberate variations in method parameters [43] and provides an indication of its reliability during application. Robustness of the developed method was investigated after minor modifications of conditions including changes to the flow rate of the mobile phase, percent of additive (formic acid) in the mobile phase, and detector wavelength for absorption. The results
Development of a single HPLC method for the determination of thirteen pharmaceuticals (Table 5) revealed that the developed method is robust, and the peaks are well separated and elute with acceptable symmetry and resolution.

Table 5. Results of the method robustness tests.

| Compounds            | Parameters changed for robustness study | Flow rate (mL/min) | Wavelength (nm) | % Formic acid |
|----------------------|----------------------------------------|--------------------|-----------------|---------------|
|                      |                                        | 0.8    | 1.0       | 1.2   | 248 | 250   | 252 | 0.05 | 0.1   | 0.15 |
| Albendazole          | Peak symmetry                          | 1.116  | 0.913    | 0.879 | 0.379 | 0.913 | 0.927 | 1.077 | 0.913 | 0.919 |
|                      | % RSD                                 | 0.362  | 1.731    | 3.267 | 1.971 | 1.731 | 2.962 | 0.166 | 1.731 | 2.146 |
|                      | Rt (min)                              | 16.03  | 16.17    | 15.55 | 16.07 | 16.17 | 16.08 | 16.18 | 16.17 | 16.62 |
| Amoxicillin          | Peak symmetry                          | 0.883  | 0.218    | 0.911 | 0.585 | 0.218 | 0.47  | 0.905 | 0.218 | 0.837 |
|                      | % RSD                                 | 0.557  | 1.205    | 3.566 | 0.838 | 1.205 | 3.077 | 2.722 | 1.205 | 2.939 |
|                      | Rt (min)                              | 5.344  | 4.910    | 4.541 | 4.823 | 4.910 | 4.812 | 4.584 | 4.910 | 3.758 |
| Acetyl salicylic acid| Peak symmetry                          | 1.038  | 0.995    | 0.995 | 0.782 | 0.995 | 1.100 |
|                      | % RSD                                 | 4.641  | 2.778    | 2.158 | 1.794 | 2.778 | 2.963 | 3.608 | 2.778 | 0.307 |
|                      | Rt (min)                              | 11.53  | 11.51    | 11.11 | 11.42 | 11.51 | 11.50 | 11.16 | 11.51 | 11.12 |
| Caffeine             | Peak symmetry                          | 0.960  | 0.995    | 0.988 | 0.988 | 0.995 | 0.995 | 0.611 | 0.995 | 1.005 |
|                      | % RSD                                 | 3.057  | 1.142    | 4.339 | 2.050 | 1.142 | 0.544 | 3.629 | 1.142 | 2.204 |
|                      | Rt (min)                              | 7.731  | 7.417    | 7.064 | 7.032 | 7.417 | 7.293 | 7.162 | 7.417 | 7.167 |
| Chloroquine          | Peak symmetry                          | 1.735  | 0.676    | 0.630 | 1.46  | 0.676 | 1.592 | 0.650 | 0.676 | 0.393 |
|                      | % RSD                                 | 3.125  | 1.430    | 3.110 | 2.478 | 1.430 | 0.878 | 0.660 | 1.430 | 1.257 |
|                      | Rt (min)                              | 6.241  | 5.900    | 5.623 | 5.634 | 5.900 | 5.476 | 5.626 | 5.900 | 4.940 |
| Ciprofloxacin        | Peak symmetry                          | 0.770  | 0.717    | 0.866 | 0.913 | 0.717 | 0.908 | 0.897 | 0.717 | 0.630 |
|                      | % RSD                                 | 2.564  | 1.198    | 2.894 | 3.398 | 1.198 | 0.676 | 3.994 | 1.198 | 1.684 |
|                      | Rt (min)                              | 8.308  | 8.166    | 7.918 | 8.008 | 8.166 | 8.014 | 7.700 | 8.166 | 7.518 |
| Clavulanic acid      | Peak symmetry                          | 0.911  | 0.925    | 0.912 | 0.270 | 0.925 | 0.968 | 0.948 | 0.925 | 0.856 |
|                      | % RSD                                 | 3.832  | 1.323    | 3.909 | 2.008 | 1.323 | 0.785 | 3.578 | 1.323 | 2.096 |
|                      | Rt (min)                              | 19.278 | 19.39    | 18.85 | 19.03 | 19.39 | 18.99 | 19.23 | 19.393| 19.16 |
| Metformin            | Peak symmetry                          | 0.672  | 0.503    | 0.515 | 1.002 | 0.503 | 0.999 | 0.406 | 0.503 | 0.721 |
|                      | % RSD                                 | 2.818  | 3.020    | 1.961 | 1.961 | 3.020 | 3.030 | 1.171 | 3.020 | 0.969 |
|                      | Rt (min)                              | 1.635  | 1.334    | 1.185 | 1.333 | 1.334 | 1.332 | 1.348 | 1.334 | 1.333 |
| Doxycycline          | Peak symmetry                          | 0.844  | 0.859    | 0.851 | 0.659 | 0.859 | 0.740 | 0.247 | 0.859 | 0.846 |
| Bicyclates           | % RSD                                 | 4.545  | 3.104    | 3.040 | 4.140 | 3.104 | 2.433 | 3.101 | 3.104 | 2.674 |
|                      | Rt (min)                              | 11.76  | 11.82    | 11.56 | 11.52 | 11.82 | 11.45 | 11.78 | 11.82 | 11.35 |
| Metronidazole        | Peak symmetry                          | 1.019  | 0.953    | 1.009 | 0.771 | 0.953 | 1.245 | 0.948 | 0.953 | 1.154 |
|                      | % RSD                                 | 1.742  | 1.294    | 0.754 | 2.286 | 1.294 | 3.116 | 3.838 | 1.294 | 0.881 |
|                      | Rt (min)                              | 13.11  | 13.19    | 12.67 | 13.04 | 13.19 | 13.01 | 12.24 | 13.19 | 12.33 |
| Norfloxacin          | Peak symmetry                          | 0.780  | 0.714    | 0.712 | 1.037 | 0.714 | 1.026 | 0.671 | 0.714 | 0.547 |
|                      | % RSD                                 | 1.890  | 1.713    | 4.006 | 1.389 | 1.713 | 1.949 | 2.163 | 1.713 | 0.881 |
|                      | Rt (min)                              | 8.114  | 7.960    | 7.699 | 7.654 | 7.960 | 7.556 | 7.494 | 7.960 | 7.269 |
| Theophylline         | Peak symmetry                          | 0.963  | 0.942    | 1.003 | 0.792 | 0.942 | 0.783 | 0.637 | 0.942 | 0.952 |

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System suitability

The system suitability test of a chromatographic method is used to verify that the chromatographic system is adequate for application to samples. The parameters considered for this test include retention time, resolution (to the adjacent peak); peak symmetry and number of theoretical plates. These parameters were investigated using the optimized chromatographic conditions. The results (Table 6) reflect good performance and acceptable levels for all the selected analytes.

Table 6. System suitability results determined for the developed chromatographic method.

| Pharmaceutical compound | Retention time (min) | Resolution | Peak symmetry | Theoretical plates (N) |
|-------------------------|----------------------|------------|---------------|-----------------------|
| Acetyl salicylic acid   | 11.5                 | 3.7        | 0.995         | 341762                |
| Albendazole             | 16.2                 | 13         | 0.953         | 28382                 |
| Amoxicillin             | 4.8                  | 9.0        | 0.218         | 18916                 |
| Caffeine                | 7.4                  | 8.5        | 0.955         | 212283                |
| Chloroquine             | 5.9                  | 5.0        | 0.676         | 139880                |
| Ciprofloxacin           | 8.2                  | 47         | 0.717         | 262543                |
| Cloxacillin             | 19.4                 |            | 0.925         | 2071273               |
| Metformin               | 1.3                  | 30         | 0.503         | 13950                 |
| Doxycycline hyclates    | 11.8                 | 14.5       | 0.859         | 311071                |
| Metronidazole           | 13.2                 | 21         | 0.953         | 251295                |
| Norfloxacin             | 7.6                  | 3.2        | 0.714         | 250709                |
| Theophylline            | 6.2                  | 12.5       | 0.942         | 170141                |
| Trimethoprim            | 7.0                  | 5.8        | 0.691         | 174425                |

CONCLUSION

A simple, accurate, precise and robust HPLC method has been developed for the determination of thirteen selected pharmaceutical drugs in bulk and marketed tablet dosage form using a single optimized condition. The developed method was validated using ICH guidelines, which proves the reliability of the proposed method. The accuracy of the method was validated by percentage recovery and found to be in the acceptable range. The system suitability parameters were within limit, hence it was concluded that all the systems were suitable to perform further analysis. The development of such an analytical method has many advantages, since different products can be analysed in sequence without changing the instrumental conditions. This is a cost-effective approach for laboratories that lack highly specialized state-of-the-art instrumentation, since it streamlines the laboratory workflow. As new samples come in for quality control, they can be analyzed without any changes to the instrumental conditions.
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