HPLC Method for Simultaneous Determination of Ascorbic acid, Phenylephrine, Paracetamol, Caffeine in Their Pure and Dosage Forms

Yara Elkady, Sobhy M. El-Adl, Mohamed Baraka, Mahmoud M. Sebaiy*

Department of Medicinal Chemistry, Faculty of Pharmacy, Zagazig University, Zagazig, Egypt

*Corresponding Author: Mahmoud M. Sebaiy, Department of Medicinal Chemistry, Faculty of Pharmacy, Zagazig University, Zagazig, Egypt

Abstract: An isocratic HPLC method has been developed for determination of ascorbic acid, phenylephrine, paracetamol, and caffeine in their pure and tablet forms. Separation was carried out at room temperature on a Kinetex 2.6 C18 100Å (4.6 mm × 100 mm) column using a mobile phase of 0.05 M potassium dihydrogen phosphate buffer (pH 3.50 by ortho-phosphoric acid): acetonitrile: methanol (70:20:10). The flow rate was 1 mL/min, maximum absorption was measured at 220 nm and linearity was in the range of 1-50 µg/mL for all drugs. The retention times of ascorbic acid, phenylephrine, paracetamol and caffeine were reported to be 1.83, 2.94, 3.74 and 5.13 minutes, respectively, indicating a very short analysis time compared with other reported methods. Also, limits of detection were reported to be 0.76, 0.82, 0.47 and 0.24 µg/mL for ascorbic acid, phenylephrine, paracetamol, and caffeine respectively, showing a high degree of the method sensitivity. The proposed method was validated in terms of linearity, accuracy, precision and robustness according to ICH guidelines and results were compared statistically with reference methods in respect of precision and accuracy.

Keywords: HPLC; ascorbic acid; phenylephrine; paracetamol; caffeine; ICH guidelines

1. INTRODUCTION

Ascorbic acid (ASC), is chemically (2R)-2-[(1S)-1,2-dihydroxyethyl]-3,4-dihydroxy-2H-furan-5-one (Figure 1). It is a water-soluble vitamin (Vitamin C) and synthesized by eukaryotes only. It was isolated from the adrenal cortex by Albert Szent-Györgyi in 1928 [1]. It is found naturally in certain foods, and added to others, and available as a dietary supplement. Unlike most animals, humans cannot endogenously synthesize ASC, so it is an important dietary compound [2]. ASC is an essential component of plant and animal antioxidant systems which can be characterized as complex redox networks with mutual interactions and synergistic effects, including metabolites and enzymes [3]. ASC is necessary for the biosynthesis of collagen, L-carnitine, cholesterol as well as some neurotransmitters [4]. It also has the potential involvement in cancer and cardiovascular diseases [5-7]. The effects of vitamin C in the treatment of ocular disease was investigated, illustrating that ascorbate influences cataract development [8]. High-performance liquid chromatography (HPLC) [9-13], thin layer chromatography (TLC) [14-17] and spectrophotometric methods [18-20] were developed for the quantification of ASC in pharmaceutical and biological samples.

Phenylephrine (PHE), is chemically (1R,2S)-respectively (1S,2R)-2-methylamino-1-phenylpropan-1-ol (Figure 1). PHE is an α1-adrenergic receptor agonist; it is a potent, synthetic, sympathomimetic element that has been reported to have significant vasoconstrictor confidants when intravenously dosed. PHE appears to have no effect on the heart's beta receptor cells. When given intravenously, it blunts the heart rate but increases the stroke output thereby causing diastolic and systolic pressures to rise [21]. PHE is a drug used for nasal congestion, hypertension, sinusitis and rhinitis [22]. PHE is now the preferred vasopressor during the elective caesarean section but it can trigger bradycardia reflex [23]. PHE was determined by several methods such as HPLC [24-26], TLC [27-30], spectrophotometric [31-33] and spectrophotofluorimetric methods [34].

Paracetamol (PAR), is chemically N-(4-hydroxyphenyl)acetamide, (Figure 1). As an analgesic and anti-inflammatory agent, PAR or acetaminophen is commonly used [35]. It is a safe and effective analgesic agent used to treat viral and bacterial infections as well as to alleviate pain associated with migraine, backache, cephalagia and post-operative pain fevers [36]. HPLC methods were developed for the quantification...
ion of PAR [37-40]. Also, TLC methods were used in several experiments for the evaluation of PAR [41,42]. In addition, highly sensitive UV spectrophotometric methods were used for the characterization of PAR in different samples [38,43,44].

Caffeine (CAF), is chemically 1,3,7-trimethyl-1H-purine-2,6 (3H,7H)-dione (Figure 1). It is a natural alkaloid commonly used in the food industry as the most beneficial psychostimulant for motor stimulation, mood improvement, information processing and cognitive / motor output in beverages or foods. CAF can have both positive and negative effects on the health [45]. It is on the one hand, powerful stimulant on the central nervous system and also induces the cardiac muscle. On the other hand, its high levels have visible gastrointestinal tract irritation and may induce anxiety, insomnia, irritability and headaches resulting from the "caffeinism" syndrome [45]. Several analytical methods such as HPLC [46-49], capillary electrophoresis (CE) with UV-detection [51], gas chromatograph with flame ionization detection (GC-FID) [52], TLC [53-55], voltammetry [56], synchronous fluorescence [57] and spectrophotometric method [58, 59] had been developed for the determination of CAF in several matrices.

![Figure 1](image.png)

**Figure 1.** Chemical structures of ascorbic acid (ASC), phenylephrine (PHE), paracetamol (PAR), caffeine (CAF)

To the best of our knowledge, there is a reported method [13] for the simultaneous chromatographic separation of ASC, PHE, PAR, and CAF but it some limitations were reported in respect of high linearity range and LODs. As such, the present work introduces a simple, rapid, reproducible and very sensitive chromatographic method with lower LOD values that has been established and validated for the determination of ASC, PHE, PAR, and CAF in their pure forms and in their tablet dosage form according to ICH guidelines [60].

2. **MATERIALS AND METHODS**

2.1. **Instrumentation**

High performance liquid chromatography (HPLC) apparatus is equipped with Surveyor quaternary pump with Intel vacuum degasser (Agelint 1100), a Surveyor autosampler plus (Thermo Scientific Co., USA), Kinetex 2.6 μ C18 100A (4.6 mm × 100 mm) column (Thermo Scientific Co. USA), Autosampler vials 1.8 mL screw cap (Thermo Scientific, USA), and Surveyor photodiode array detector (PDA) (Thermo Scientific Co. USA). A computer with a software chromo quest 5 (Surveyor Thermo Scientific Co. USA), has been used for data collection and analysis. Consort P400® digital pH-meter was used for pH adjustment.

2.2. **Chemicals and Reagents**

All solvents and reagents were of HPLC analytical grade. Acetonitrile and methanol HPLC grade were supplied by Fischer scientific (Loughborough, UK), while ortho-phosphoric acid was purchased from Merck (Darmstadt, Germany) and water used in all the experiments was obtained from Milli-RO and Milli-Q systems (Millipore, Bedford, MA). Standard powders of ASC, PHE, PAR and CAF were kindly supplied by EIPIICO (Egypt) .
2.3. Chromatographic Conditions
HPLC was connected with Kinetex 2.6 μ C18 100A (4.6 mm × 100 mm) column as a stationary phase. A mixture of 0.05 M potassium dihydrogen phosphate buffer (pH 3.50 by ortho-phosphoric acid), acetonitrile, methanol a ratio of 70:20:10 (v/v/v) was freshly prepared and used as an isocratic mobile phase. The mobile phase was pumped at a flow rate of 1 mL/min. The injection volume was 10 µL and the column was maintained at ambient temperature while the eluent was monitored at 220 nm. All chromatographic conditions are illustrated in Table 1.

Table 1. Chromatographic Conditions for the proposed method

| Parameters          | Conditions                                                                 |
|---------------------|----------------------------------------------------------------------------|
| Column              | Kinetex 2.6 μ C18 100A (4.6 mm × 100 mm)                                   |
| Mobile phase        | 0.05 M potassium dihydrogen phosphate buffer (pH 3.50 using ortho-phosphoric acid): acetonitrile: methanol (70:20:10, v/v/v) |
| UV detection, nm    | 220                                                                        |
| Flow rate, mL/min   | 1                                                                          |
| Injected volume, µL | 10                                                                         |
| Temperature         | Ambient                                                                   |

2.4. Preparation of Standard Stock Solution and Construction of Calibration Curves
Standard stock solution of ASC, PHE, PAR and CAF (100 µg/mL) was prepared by dissolving 10 mg of each pure drug in 100 mL water. Then, Standard solution was diluted by methanol to get final concentrations of 1, 5, 10, 15, 20 and 50 µg/mL for all drugs for construction of calibration plots. The mixture was injected in triplicate and chromatographed under the previously mentioned conditions. A linear relationship was obtained when average drug standard peak areas were plotted against the corresponding concentrations for each drug and regression equations were computed.

2.5. Pharmaceutical Preparations
10 tablets (6 mg ASC, 0.5mg PHE, 40 mg PAR and 3.5 CAF) were weighed and finely powdered. An accurately weighed portion from the powdered tablets equivalent to the average concentration of one tablet was transferred into a 100 mL volumetric flask. 80 mL of water were added and sonicated for 20 minutes then the volume was completed with diluent to 100 mL and filtered. Further dilution was performed to obtain the required concentration range of the drug mixture.

3. RESULTS AND DISCUSSION
3.1. Optimization of Chromatographic Conditions
Several trials were done to obtain the optimized chromatographic condition for determination of ASC, PHE, PAR, and CAF. First, chromatographic detection was performed at 220, 215, and 210 nm using a PDA detector and the optimal wavelength was set at 220 nm. Second trials were carried out by changing mobile phase composition to reach the optimum separation with good resolution where the mobile phase 0.05 M potassium dihydrogen phosphate buffer (pH 3.50 by ortho-phosphoric acid), acetonitrile, methanol at a ratio of 70:20:10, v/v/v was chosen as the optimum one based on faster separation and good peak resolution. Final trials were carried out to show the effect of different flow rates and optimum separation was achieved at a flow rate of 1 mL/min. Under these conditions, ASC, PHE, PAR, and CAF in pure form were separated and eluted at 1.83, 2.94, 3.74 and 5.13 minutes, respectively as illustrated in Figure 2(A) and in dosage form as illustrated in Figure 2(B). However, the optimum mobile phase showed symmetrical peaks (0.86 < T < 1.22), capacity factor (1 < k < 10), resolution > 2 and theoretical plates > 2000 which are in agreement with the CDER values recommendation [61]. Table 2 show all system suitability parameters of the proposed HPLC method for simultaneous determination of these four drugs in pure form.
HPLC Method for Simultaneous Determination of Ascorbic acid, Phenylephrine, Paracetamol, Caffeine in Their Pure and Dosage Forms

Figure 2. Typical HPLC chromatograms obtained from ASC, PHE, PAR and CAF using Kinetex 2.6 µC18 100A (4.6 mm × 100 mm) column in (A) pure form and (B) pharmaceutical formulation using mobile phase of 0.05 M potassium dihydrogen phosphate buffer (pH 3.50 by ortho-phosphoric acid): acetonitrile: methanol (70:20:10, v/v/v). Other chromatographic conditions are stated in Table 1

Table 2. System suitability parameters for ascorbic acid (ASC), phenylephrine (PHE), paracetamol (PAR) and caffeine (CAF) in their pure form

| Parameters                        | ASC  | PHE  | PAR  | CAF  | Reference values [61]     |
|-----------------------------------|------|------|------|------|---------------------------|
| Retention time, \( t_r \)         | 1.86 | 2.92 | 3.37 | 5.16 |                           |
| Capacity factor, \( k' \)         | 2.21 | 2.41 | 2.11 | 2.31 | Accepted \( k' \) value \((1-10)\) |
| Peak asymmetry (Tailing factor, \( T \)) | 1.08 | 1.22 | 0.91 | 0.86 | Accepted \( T \) value \( \leq 2 \) |
| Theoretical plates, \( N \)       | 5886 | 6769 | 6233 | 7561 | Accepted \( N \) value \( > 2000 \) |
| Resolution, \( R_s \)             | 8.32 | 7.68 | 6.63 | 9.52 | Accepted value \( > 2 \)     |
| Selectivity (Separation factor, \( \alpha \)) | 8.25 | 8.02 | 8.22 | 8.16 |                           |

3.2. Method Validation

The proposed method was validated according to ICH guidelines [61] in terms of specificity, linearity, precision, accuracy, robustness, limit of detection and limit of quantification.

3.2.1. Specificity

Specificity, is the ability of an analytical method to distinguish the analyte from other chemicals in the sample. The specificity of the method was assessed by deliberately adding impurities into a sample containing the analyte and testing how well the method can identify the analyte. It was found that there was no interference due to excipients found in tablet formulation as seen in Figure 2(B).

3.2.2. Linearity

Six different concentrations of the drug mixture were specified for linearity studies in the range of 1-50 μg/mL for all drugs as seen in Table 3. A linear relationship was established by plotting concentrations against corresponding peak areas. The correlation coefficient was around 0.999 indicating good linearity as shown in Figure 3. Also, the regression equations were found to be \( y=5.086x + 1.093 \), \( y=4.911x + 1.301 \), \( y=7.124x + 1.074 \), and \( y=8.15x -1.015 \), for ASC, PHE, PAR, and CAF respectively.
HPLC Method for Simultaneous Determination of Ascorbic acid, Phenylephrine, Paracetamol, Caffeine in Their Pure and Dosage Forms

Figure 3. Calibration curves of ASC, PHE, PAR, and CAF using the proposed HPLC method

Table 3. Results of analysis for the four drugs in pure form using the proposed method

|        | ASC | PHE | PAR | CAF |
|--------|-----|-----|-----|-----|
| Take n µg/mL | Foun d µg/mL | Recove ry % | Take n µg/mL | Foun d µg/mL | Recove ry % | Take n µg/mL | Foun d µg/mL | Recove ry % | Take n µg/mL | Foun d µg/mL | Recove ry % |
| 1      | 0.97 | 97.34 | 1    | 1.01 | 101.12 | 1    | 0.98 | 97.93 | 1    | 0.98 | 98.01 |
| 5      | 4.98 | 99.61 | 5    | 4.89 | 97.86 | 5    | 4.98 | 99.61 | 5    | 4.91 | 98.13 |
| 10     | 10.05 | 100.52 | 10   | 9.87 | 98.72 | 10   | 9.92 | 99.16 | 10   | 9.80 | 98.02 |
| 15     | 15.06 | 100.37 | 15   | 15.13 | 100.87 | 15   | 15.11 | 100.77 | 15   | 14.71 | 98.07 |
| 20     | 19.94 | 99.69 | 20   | 20.18 | 100.88 | 20   | 20.19 | 100.98 | 20   | 19.62 | 98.12 |
| 50     | 49.02 | 98.04 | 50   | 49.93 | 99.85 | 50   | 49.91 | 99.81 | 50   | 49.83 | 99.67 |

|        | Mean | ±SD  | ±RSD | ±SE  | Variance | LOD (µg/mL) | LOQ (µg/mL) |
|--------|------|------|------|------|----------|--------------|--------------|
| ASC    | 99.26 | 1.28 | 1.29 | 0.57 | 1.66     | 0.76         | 2.53         |
| PHE    | 99.88 | 1.33 | 1.34 | 0.60 | 1.79     | 0.82         | 2.73         |
| PAR    | 99.71 | 1.11 | 1.12 | 0.50 | 1.25     | 0.47         | 1.57         |
| CAF    | 98.33 | 0.65 | 0.66 | 0.29 | 0.42     | 0.24         | 0.80         |

3.2.3. Limits of Detection and Quantification

Limit of detection (LOD) of an analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value. Limit of quantification (LOQ) is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy. LOD = 3.3 S/K and LOQ = 10 S/K, were used for the values calculation where S is the standard deviation of three replicate determination values under the same conditions and K is the slope of calibration graph. LODs were reported to be 0.76, 0.82, 0.47 and 0.24 µg/mL, while LOQs were calculated to be 2.53, 2.73, 1.57 and 0.80 µg/mL for ASC, PHE, PAR, and CAF respectively (Table 3). The results show that the proposed method is highly sensitive in comparison with reported methods [13] and applicable not only for pharmaceutical analysis but also for pharmacokinetic studies.
3.2.4. Precision

The precision of the method was calculated in terms of repeatability and intermediate precision (intra-day and inter-day precision). Standard deviation (SD) of five replicate determinations using the same solution containing pure drug during the same day and five consecutive days were calculated as shown in Table 4. The SD values (1.30 to 1.78) for intra-day and those (0.10 to 3.35) of inter-day precision were in the acceptable range and showed that the proposed method has an adequate precision in respect of the simultaneous determination of the 4 cited drugs in their pharmaceutical formulation.

| Drugs | Conc. µg/mL | Intra-day | Inter-day |
|-------|-------------|-----------|-----------|
|       | Mean ± SD   | RSD%      | Mean ± SD | RSD%      |
| ASC   | 5           | 96.54 ± 1.57 | 1.63      | 96.10 ± 1.26 | 1.13 |
|       | 10          | 97.43 ± 1.58 | 1.62      | 95.90 ± 2.10 | 2.12 |
|       | 15          | 97.29 ± 1.57 | 1.62      | 96.00 ± 1.88 | 1.96 |
| PHE   | 5           | 96.19 ± 1.78 | 1.86      | 96.30 ± 0.10 | 0.94 |
|       | 10          | 97.09 ± 1.73 | 1.79      | 99.69 ± 3.35 | 3.37 |
|       | 15          | 99.23 ± 1.75 | 1.26      | 101.50 ± 2.83 | 2.79 |
| PAR   | 5           | 98.90 ± 1.33 | 1.34      | 96.90 ± 2.10 | 2.18 |
|       | 10          | 98.46 ± 1.30 | 1.32      | 97.10 ± 0.61 | 0.63 |
|       | 15          | 100.06 ± 1.32 | 1.32     | 99.34 ± 0.94 | 0.95 |
| CAF   | 5           | 99.58 ± 1.33 | 1.34      | 98.37 ± 0.50 | 0.54 |
|       | 10          | 97.15 ± 1.67 | 1.67      | 100.07 ± 0.91 | 0.91 |
|       | 15          | 100.63 ± 1.32 | 1.31     | 99.13 ± 0.20 | 0.21 |

3.2.5. Accuracy and recovery

Accuracy was assessed using 9 determinations over 3 concentration levels of 5, 10, and 15 µg/mL covering the specified ranges. The results showed excellent recoveries with lower SD values as seen in table 5.

| Drugs | ASC | PHE | PAR | CAF |
|-------|-----|-----|-----|-----|
|       | Mean ± SD | RSD% | Mean ± SD | RSD% | Mean ± SD | RSD% | Mean ± SD | RSD% |
| Conc. µg/mL | 5    | 96.69 ± 2.57 | 2.66 | 97.50 ± 0.86 | 0.88 | 99.47 ± 0.63 | 0.64 | 98.20 ± 2.05 | 2.09 |
|        | 10   | 99.79 ± 1.25 | 1.25 | 97.93 ± 1.69 | 1.73 | 98.83 ± 0.94 | 0.95 | 99.80 ± 2.07 | 2.08 |
|        | 15   | 96.79 ± 3.13 | 3.22 | 101.90 ± 1.61 | 1.61 | 100.79 ± 0.39 | 0.39 | 99.30 ± 2.04 | 2.05 |

3.2.6. Robustness

Robustness of an analytical procedure is a measure of its capacity to remain unaffected by small variations in method parameters. In the proposed method, a small variation in the flow rate and mobile phase composition showed a negligible effect on the on the results as revealed by small SD values (SD ≤ 3.97) for all applied changes (Table 6).

| Drugs | ASC | PHE | PAR | CAF |
|-------|-----|-----|-----|-----|
|       | Mean ± SD | Mean ± SD | Mean ± SD | Mean ± SD |
| pH 3  | 93.12 ± 0.49 | 96.17 ± 2.63 | 99.60 ± 0.81 | 99.00 ± 0.78 |
| pH 4  | 93.19 ± 0.58 | 97.49 ± 3.92 | 100.11 ± 2.29 | 99.20 ± 0.33 |
| Mobile phase (68:22:10) | 94.90 ± 0.54 | 99.75 ± 2.62 | 96.60 ± 0.81 | 100.58 ± 0.78 |
| Mobile phase (72:18:10) | 95.10 ± 0.59 | 101.2 ± 3.97 | 97.14 ± 2.25 | 100.70 ± 0.33 |

3.2.7. Application on Pharmaceutical Preparation

The proposed method was successfully applied on pharmaceutical preparation containing ASC, PHE, PAR, and CAF. Results obtained were established in Table 7, showing a high degree of accuracy and precision where excipients and impurities did not show interference on the selected values. Also, results...
HPLC Method for Simultaneous Determination of Ascorbic acid, Phenylephrine, Paracetamol, Caffeine in Their Pure and Dosage Forms

obtained were compared to those obtained by reference methods [11,26,37,47] where Student’s t-test and F-test were performed for comparison. Results shown in Table 8 indicated that calculated t and F values were less than tabulated ones for the 4 drugs which in turn indicate that there is no significant difference between proposed method and reference ones relative to precision and accuracy.

Table 7. Results of analysis of ASC, PHE, PAR and CAF in pharmaceutical formulations

| DRUG | Take n µg/mL | Found µg/mL | Recovery % | Take n µg/mL | Found µg/mL | Recovery % | Take n µg/mL | Found µg/mL | Recovery % | Take n µg/mL | Found µg/mL | Recovery % |
|------|--------------|-------------|------------|--------------|-------------|------------|--------------|-------------|------------|--------------|-------------|------------|
| ASC  | 3            | 2.97        | 99.02      | 0.25         | 0.24        | 99.28      | 20           | 19.29       | 96.49      | 1.75         | 1.73        | 99.27      |
|      | 6            | 5.99        | 99.99      | 0.50         | 0.48        | 96.70      | 40           | 39.40       | 98.51      | 3.5          | 3.47        | 99.32      |
|      | 9            | 9.02        | 100.29     | 0.75         | 0.74        | 98.90      | 60           | 59.09       | 98.49      | 5.25         | 5.19        | 99.01      |
|      | 12           | 12.09       | 100.81     | 1            | 0.98        | 98.09      | 80           | 78.31       | 97.88      | 7            | 7.02        | 100.3      |
|      | 18           | 17.96       | 99.81      | 1.5          | 1.47        | 98.52      | 120          | 119.6       | 99.67      | 10.5         | 10.46       | 99.70      |

Mean | 99.99 | 98.24 | 98.21 | 99.53 |
±SD  | 0.66  | 1.14  | 1.15  | 0.52  |
±RSD | 0.65  | 1.16  | 1.18  | 0.52  |
±SE  | 0.29  | 0.51  | 0.51  | 0.23  |
Variance | 0.43 | 1.31 | 1.34 | 0.27 |

Table 8. Statistical analysis of results obtained by the proposed HPLC method applied on pharmaceutical formulations compared with reference methods

| DRUG | Recovery ± SD | Reference Method | Student t-values | F-values |
|------|---------------|------------------|------------------|----------|
|      | Proposed Method | N | Reference Method | N | [11] | [26] | [37] | [47] | 0.65 (1.94)² | 4.71 (6.39)² | 0.64 (2.13)² | 7.93 (9.28)² | 1.24 (2.13)² | 7.77 (9.28)² | 2.07 (2.13)² | 6.48 (9.28)² |
| ASC  | 100.00 ± 0.37 | 4 | 100.30 ± 0.17 | 4 |      |      |      |      | 0.65 (1.94)² | 4.71 (6.39)² | 0.64 (2.13)² | 7.93 (9.28)² | 1.24 (2.13)² | 7.77 (9.28)² | 2.07 (2.13)² | 6.48 (9.28)² |
| PHE  | 100.60 ± 0.29 | 3 | 100.40 ± 0.10 | 3 |      |      |      |      | 0.64 (2.13)² | 7.93 (9.28)² | 1.24 (2.13)² | 7.77 (9.28)² | 2.07 (2.13)² | 6.48 (9.28)² |      |      |
| PAR  | 100.50 ± 0.36 | 3 | 100.00 ± 0.13 | 3 |      |      |      |      | 1.24 (2.13)² | 7.77 (9.28)² | 2.07 (2.13)² | 6.48 (9.28)² |      |      |
| CAF  | 99.43 ± 0.13 | 3 | 99.73 ± 0.05 | 3 |      |      |      |      | 2.07 (2.13)² | 6.48 (9.28)² |      |      |

4. CONCLUSION

A simple, precise, accurate, valid, robust, highly sensitive and reliable HPLC method was established for determination of ascorbic acid, phenylephrine, paracetamol and caffeine in bulk and pharmaceutical preparation. In the proposed method, the chromatographic resolution was achieved within 6 minutes for the four drugs. Linearity was observed over a concentration range of 1-50 µg/mL for all drugs. The method has been successfully applied for the analysis of tablet formulation in respect of quality control in addition to performing statistical comparison with reference methods showing no significant differences.

REFERENCES

[1] Szent-Gyorgyi, A Observations on the function of peroxidase systems and the chemistry of the adrenal cortex: Description of a new carbohydrate derivative. Biochem,1928, 1387–1409.
[2] Padayatty, S.J. and Levine, M. Vitamin C: The known and the unknown and Goldilocks. Oral Dis. 2016., 463–493.
[3] Paciolla, C., Paradiso, A. and de Pinto, M.C. Cellular redox homeostasis as central modulator in plant stress. In Redox State as a Central Regulator of Plant-Cell Stress Responses; Gupta, D.K., Palma, J.M., Corpas, F.J., Eds.; Springer: Cham, Switzerland.; pp.2016, 1–23.
HPLC Method for Simultaneous Determination of Ascorbic acid, Phenylephrine, Paracetamol, Caffeine in Their Pure and Dosage Forms

[4] Li, Y. and Schellhorn, H.E., New developments and novel therapeutic perspectives for vitamin C. J Nutr 2007,71-84.
[5] Pena E., Roa F. J., Inostroza E., Sotomayor K., Gonzalez M., Gutierrez-Castro F. A., et al. Increased expression of mitochondrial sodium-coupled ascorbic acid transporter-2 (mitSVCT2) as a central feature in breast cancer. Free Radic. Biol. Med.2019, 283–292.
[6] Nyysönen K., Parviainen M.T., Salonen R., Tuomainen T. J. and Salonen J.T., Vitamin C Deficiency and Risk of Myocardial Infarction: Prospective Population Study of Men from Eastern Finland. Br. Med. J.2016:634–638.
[7] Salvayre, R., Negre-Salvayre, A. and Camar, C., Oxidative theory of atherosclerosis and antioxidants. Biochimie. 2015:281–296.
[8] Lopes de Jesus, C.C., Atallah, A.N., Valente, O. and Moca Trevisani, V.F., Vitamin C and superoxide dismutase (SOD) for diabetic retinopathy. Cochrane Database Syst Rev, 2008, CD006695.
[9] Kim, Y. and Ha, N. and Kim, M-G. Simultaneous determination of L-ascorbic acid and dehydroascorbic acid in human plasma. Analytical methods. 2015,7-10.
[10] Clark, Z. D. and Frank, E. L., Development and implementation of an HPLC-ECD method for analysis of vitamin C in plasma using single column and automatic alternating dual column regeneration. Practical Laboratory Medicine, 2016, 25-37.
[11] Garnero, C. and Longhi, M. Development of HPLC and UV spectrophotometric methods for the determination of ascorbic acid using hydroxypropyl-β-cyclodextrin and triethanolamine as photostabilizing agents. Analytica chimica acta., 2010, 159-166.
[12] Kandár, R., Drábková, P. and Hampl, R., The determination of ascorbic acid and uric acid in human seminal plasma using an HPLC with UV detection. Journal of Chromatography B,2011, 2834-2839.
[13] Koblová, P., Sklenářová, H., Brabcová, I. and Solich, P., Development and validation of a rapid HPLC method for the determination of ascorbic acid, phenylephrine, paracetamol and caffeine using a monolithic column. Analytical Methods, 2012, 1588-1591.
[14] Pyka-Pająk, A., Dolowy, M., Parys, W., Bober, K. and Janikowska, G. A Simple and Cost-Effective TLC-Densitometric Method for the Quantitative Determination of Acetylsalicylic Acid and Ascorbic Acid in Combined Effervescent Tablets. Molecules,2018, 3115.
[15] Trineeva, O. V., Safonova, E. F. and Slivkin, A. I. Method Development for Quantitative Determination of Ascorbic Acid by High-Performance Thin-Layer Chromatography. Pharmaceutical Chemistry Journal, 2018, 938-944.
[16] Peter, E.L., Kaligirwa, A. and lukwago, T. Stability indicating high-performance thin-layer chromatography method for estimation of ascorbic acid in Hibiscus sabdariffa L. aqueous extract. Journal of complementary medicine research, 2019, 50–57.
[17] Alam, P., Kamal, Y.T., Alqasoumi, S.I., et al. HPTLC method for simultaneous determination of ascorbic acid and gallic acid biomarker from freeze dry pomegranate juice and herbal formulation. Saudi Pharmaceutical Journal. 2019,:975-980.
[18] Shishehbore, M. R. and Aghamiri, Z. A highly sensitive kinetic spectrophotometric method for the determination of ascorbic Acid in pharmaceutical samples. Iranian journal of pharmaceutical research : IJPR, 2014, 373–382.
[19] Fadhel, D. H., Spectrophotometric determination of ascorbic acid in aqueous solutions. Al-Nahrain Journal of Science, 2012, 88-94.
[20] Salkić, M. and Selimović, A., Spectrophotometric determination of L-ascorbic acid in pharmaceuticals based on its oxidation by potassium peroxymonosulfate and hydrogen peroxide. Croatica Chemica Acta, 2015, 73-79.
[21] Ok, S. H., Bae, S. I., Kwon, S. C., Park, J. C., Kim, W. C., Park, K. E., Shin, I. W., Lee, H. K., Chung, Y. K., Choi, M. J. and Sohn, J. T. Bupivacaine-induced Vasodilation Is Mediated by Decreased Calcium Sensitization in Isolated Endothelium-denuded Rat Aortas Precontracted with Phenylephrine. The Korean journal of pain, 2014,. 229–238.
[22] Desjardins, P. J. and Berlin, R. G., Efficacy of phenylephrine, Br. J. Clin. Pharmacol. 2007, 555-556.
[23] Kinsella, S.M., Carvalho, B., Dyer, R.A., et al., International consensus statement on the management of hypotension with vasopressors during caesarean section under spinal anaesthesia. Anaesthesia. 2018,71–92.
[24] Yadav, O. M., and H. K. Jain., RP-HPLC method development and validation for simultaneous estimation of phenylephrine hydrochloride and ebastine in tablet dosage form. International Journal of Pharmacy and Pharmaceutical Sciences, Sept. 2014. 466-70.
[25] Sanchaniya, P. M., Mehta, F. A. and Uchadadiya, N. B., Development and validation of an RP-HPLC method for estimation of chlorpheniramine maleate, ibuprofen, and phenylephrine hydrochloride in
combined pharmaceutical dosage form. Chromatography Research International, 2013.

[26] Pirol, O., Sukuroglu, M. and Ozden, T. . Simultaneous determination of Paracetamol, Phenylephrine hydrochloride, Oxolamine citrate and Chlorpheniramine maleate by HPLC in pharmaceutical dosage forms. E-Journal of Chemistry, 2011,8

[27] El Yazbi, F. A., Hassan, E. M., Khamis, E. F., Ragab, M. A. and Hamdy, M. M., Development and validation of a high-performance thin-layer chromatographic method for the simultaneous determination of two binary mixtures containing ketorolac tromethamine with phenylephrine hydrochloride and with febuxostat. Journal of chromatographic science, 2016, 819-828.

[28] Bhola, R. P., Jagadale, P. D., Chitlange, S. S. and Wankhede, S. B. A Simple and Sensitive HPTLC Method for Simultaneous Analysis of Phenylephrine hydrochloride and Ketorolac tromethamine in Combined Dose Formulation. Analytical Chemistry Letters, 2015, 206-215.

[29] El-Kimary, E. I., Khamis, E. F., Belal, S. F. and Abdel Moneim, M. M. . Robust Chromatographic Methods for the Analysis of Two Quaternary Mixtures Containing Paracetamol, Codeine, Guaifenesin and Pseudoephedrine or Phenylephrine in their Dosage Forms. Journal of Chromatographic Science, 2019, 828-837.

[30] Hegazy, M. A., Al-Ghobashy, M. A., Eltanany, B. M. nd Khattab, F. I., 'Purity Indicating TLC Method for Quantitative Determination of Phenylephrine and Dimethindine Maleate in Presence of Dimethindine Maleate Impurity: 2-ethyl pyridine in Nasal Gel. J Pharmaceut Res, 2016, 1-6.

[31] Al-Sabta, T. .Spectrophotometric Assay of Phenylephrine Hydrochloride Using 4- Aminoantipyrine and Copper (II). Pakistan Journal of Analytical and Environmental Chemistry, 2010, 01-07.

[32] Khoshayand, M. R., Abdollahi, H., Ghaffari, A., Shariatpanahi, M. and Farzanegan, H., Simultaneous spectrophotometric determination of paracetamol, phenylephrine and chlorpheniramine in pharmaceuticals using chemometric approaches. Daru : journal of Faculty of Pharmacy, Tehran University of Medical Sciences, 2010, 292–297.

[33] Kazemipour, M. and Ansari, M. . Derivative spectrophotometry for simultaneous analysis of chlorphiramine maleate, phenylephrine HCl, and phenylpropanolamine HCl in ternary mixtures and pharmaceutical dosage forms,.2015.

[34] Saad R.A., Salim, M. M. and Hammad, S. F. , Synchronous spectrofluorometric methods for simultaneous determination of diphenhydramine and ibuprofen or phenylphrine in combined pharmaceutical preparations. Luminescence, 2020, 550-560.

[35] Kachoosangi, R. T., Wildgoose, G. G. and Compton, R. G.. Sensitive adsorptive stripping voltammetric determination of paracetamol at multiwalled carbon nanotube modified basal plane pyrolytic graphite electrode, Anal. Chim. Acta. 2008: 54–60.

[36] Shahrokhi, S. and Asadian, E. . Simultaneous voltammetric determination of ascorbic acid, acetaminophen and isoniazid using thionine immobilized multi-walled carbon nanotube modified carbon paste electrode, Electrochim. Acta.2010: 666–672.

[37] Abdelaleem, E. A., Naguib, I. A., Hassan, E. S. and Ali, N. W., HPTLC and RP-HPLC methods for simultaneous determination of paracetamol and pamabrom in presence of their potential impurities. Journal of pharmaceutical and biomedical analysis,2015, 22-27.

[38] Palur, K., Archakam, S. C. and Koganti, B. . Chemometric assisted UV spectrophotometric and RP-HPLC methods for simultaneous determination of paracetamol, diphenhydramine, caffeine and phenylephrine in tablet dosage form. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 2020,118801.

[39] Abdelwahab, N. S., Abdelrahman, M. M., Boshra, J. M. and Taha, A. A. . Different stability-indicating chromatographic methods for specific determination of paracetamol, dantrolene sodium, their toxic impurities and degradation products. Biomedical Chromatography, 2019, 45-98.

[40] Abdelaleem, E. A. and Abdelwahab, N. S. . Validated stability indicating RP-HPLC method for determination of paracetamol, methocarbamol and their related substances. Analytical Methods, 2013, 541-545.

[41] Mostafa, N. M. . Stability indicating method for the determination of paracetamol in its pharmaceutical preparations by TLC densitometric method. Journal of Saudi Chemical Society, 2010, 341-344.

[42] Abdelaleem, E. A. and Abdelwahab, N. S. . Stability-indicating TLC–densitometric method for simultaneous determination of paracetamol and chloroxazone and their toxic impurities. Journal of chromatographic science, 2013, 187-191.

[43] Ashour, A., Hegazy, M. A., Abdel-Kawy, M. and. ElZeiny, M. B., “Simultaneous spectrophotometric determination of overlapping spectra of paracetamol and caffeine in laboratory prepared mixtures and pharmaceutical preparations using continuous wavelet and derivative transform,” Journal of Saudi Chemical Society, vol. 19, pp. 186–192, 2012.
HPLC Method for Simultaneous Determination of Ascorbic acid, Phenylephrine, Paracetamol, Caffeine in Their Pure and Dosage Forms

[44] Vichare, V., Mujgond, P., Tambe, V. and Dhole, S. N., “Simultaneous spectrophotometric determination of paracetamol and caffeine in tablet formulation,” International Journal of PharmTech Research, vol. 2, no. 4, pp. 2010, 2512–2516.

[45] Reissig, C.J., Strain, E.C. and Griffiths, R.R., Caffeinated energy drinks-A growing problem, Drug Alcohol Depend. 2009.: 1–10.

[46] Alvi, S. N. and Hammami, M. M. Validated HPLC method for determination of caffeine level in human plasma using synthetic plasma: application to bioavailability studies. Journal of chromatographic science, 2011, 292-296.

[47] Tsvetkova, B.G., Kostova, B.D., Rachev, D.R., Peikova, L.T. and Pencheva, I.P. HPLC assay and stability studies of tablets containing paracetamol and caffeine, International Journal of Pharmaceutical Sciences Review and Research. 2013, 138-142.

[48] Fernando, C. D. and Soysa, P. Simple isocratic method for simultaneous determination of caffeine and catechins in tea products by HPLC. SpringerPlus, 2016, 970.

[49] Dewani, A. P., Dabhade, S. M., Bakal, R. L., Gadewar, C. K., Chandewar, A. V. and Patra, S., Development and validation of a novel RP-HPLC method for simultaneous determination of paracetamol, phenylephrine hydrochloride, caffeine, cetirizine and nimesulide in tablet formulation. Arabian journal of chemistry, 2015, 591-598.

[50] Tzanavaras, P.D. and Themelis, D.G. Development and validation of a high-throughput high-performance liquid chromatographic assay for the determination of caffeine in food samples using a monolithic column, Analytica Chimica Acta. 2007: 89-94.

[51] Khasanov, V. V., Slizhov, Y.G. and Khasanov, V. V. Energy drink analysis by capillary electrophoresis, Journal of Analytical Chemistry. 2013, 357–359.

[52] Pasias, I., Kiriakou, I. Proestos, and C. Development of a Rapid Method for the Determination of Caffeine in Coffee Grains by GC-FID—A Fully Validated Approach, Antioxidants. 2017, 6.

[53] Sharma, P., Murthy, P. and Shivhare, P., Validated high-performance thin layer chromatographic method for caffeine quantification in beverages and edibles. International Journal of Toxicology, 2014, 31-36.

[54] Oellig, C., Schunck, J. and Schwack, W., Determination of caffeine, theobromine and theophylline in Mate beer and Mate soft drinks by high-performance thin-layer chromatography. Journal of Chromatography A, 2018., 208-212.

[55] Bhawani, S. A., Albishiri, H. M. and Rengarajan, R. Microemulsion thin-layer chromatographic separation of caffeine and paracetamol and their determination in formulated tablet and in spiked urine sample by HPLC. Analytical Chemistry Letters, 2014, 207-212.

[56] Tyszczuk-Rotko, K. and Bęczkowska, I., Nafion covered lead film electrode for the voltammetric determination of caffeine in beverage samples and pharmaceutical formulations, Food Chemistry. 2015, 24-29.

[57] Žiak, L., Májek, P., Hroboňová, K., Čacho, F. and Sádecká, J., Simultaneous determination of caffeine, caramel and riboflavin in cola-type and energy drinks by synchronous fluorescence technique coupled with partial least squares, Food Chemistry. 2014, 282-286.

[58] Somya, K. V., Ravishankar, K., Basha, D. P. and Kiranmayi, G. V. N., “Estimation of caffeine and sodium benzoate in caffeine and sodium benzoate injection by isosorption method (isobestic method),” International Journal of Pharmaceutical, Chemical and Biological Sciences, 2011., 26–31.

[59] Bharate, S. S., Kolhe, S. R. and Bharate, S. B., “Development of validated spectrophotometric method for simultaneous estimation of acetylsalicylic acid and caffeine in pure and tablet dosage form,” Journal of Advanced Research, 2011, 34–41.

[60] Guidance for industry: Q2B validation of analytical procedures: Methodology. International Conference of Harmonization (ICH). (https://www.fda.gov/downloads/drugs/guidances/ucm073384.pdf), 1996.

[61] CDER center for drug evaluation and research; Reviewer guidance; Validation of chromatographic methods.. (https://www.fda.gov/downloads/drugs/guidances/ucm134409.pdf), 1994.

Citation: Mahmoud M. Sebaiy, et.al, ‘HPLC Method for Simultaneous Determination of Ascorbic acid, Phenylephrine, Paracetamol, Caffeine in Their Pure and Dosage Forms’, International Journal of Advanced Research in Chemical Science, 7(6), pp. 7-16. DOI: https://doi.org/10.20431/2349-0403.0706002

Copyright: © 2020 Authors, This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.