Research Article

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Analysis of some pharmaceuticals in the presence of their synthetic impurities by applying hybrid micelle liquid chromatography

Abstract: A stability-indicating hybrid micelle liquid chromatography accompanied by UV detection was developed for the simultaneous analysis of either paracetamol (PCA) or pseudoephedrine hydrochloride (PSU) with their synthetic impurities. Mixture I contains PCA with p-amino phenol and p-nitro phenol, while mixture II involves the estimation of PSU with benzaldehyde and benzoic acid. Both mixtures were separated using a C18 column that was thermostatically maintained at 40°C and operating under a flow rate of 1.5 mL/min, applying UV detection at 240 nm for mixture I and 220 nm for mixture II. In both cases, the mobile phase consisted of 0.1 M sodium dodecyl sulfate, acetonitrile, and triethylamine (90:10:0.3, v/v/v) and adjusted to pH 4 (mixture I) or pH 3.7 (mixture II) using 2.0 M O-phosphoric acid. The proposed method was validated and successfully applied to assay different pharmaceuticals containing PCA or PSU. Moreover, the stability-indicating nature of the proposed method was proved through applying photolytic degradation procedures for PCA.

Keywords: paracetamol, pseudoephedrine, impurities, degradation, micellar

1 Introduction

Potency and safety of pharmaceutical compounds are two major concerns during remedial treatment. Safety of any drug is judged by both its pharmacological-toxicological profile and the undesirable side effects which are in the vast majority, resulting from the impurities encountered in pharmaceutical formulations [1]. The International Conference of Harmonization (ICH) organization (ICH Q3A&ICHQ3B) specified the acceptable concentration limits of various impurities in the pharmaceutical active ingredient (PAI) or in the final dosage forms [2,3]. Determination of the permitted quantity of impurities has also been undertaken by the United States Pharmacopoeia (USP) [4] and the British Pharmacopoeia (BP) [5]. Consequently, both qualitative and quantitative profiling of impurities are getting attention from drug regulatory authorities. The ICH [2] documented that organic impurities are frequently encountered in PAI. Such impurities originate from different sources, namely, unreacted starting or intermediate materials through the synthetic process, presence of by-products, and appearance of degradation products either by degradation of end products or through aging and improper storage of PAI.

Paracetamol (PCA), acetamide, N-(4-hydroxy phenyl) [4] (Table 1), is a potent analgesic and antipyretic drug that is available over-the-counter and could be safely administered to children and elderly patients [6]. Some organic impurities are formed during the synthesis of PCA, including p-amino phenol (PAP) and p-nitro phenol (PNP) (Table 1). Since PCA is synthesized through an acetylation reaction between PAP and acetic anhydride [7], PAP is, thus, considered as a major organic impurity of PCA, where it being the starting material may be found in excess as unreacted form, or it may be present as a degradation product of PCA due to improper storage [7]. PNP may also be present but in a lower extent than PAP [8]. Both USP and BP specify the amount of PAP in PCA pure form to be 0.005% w/w [4,5], while its limit in the final products depends on the type of the dosage form [7]. The significance of PAP quantitation is based on the reported clinical studies of its teratogenic and nephrototoxic effects...
on humans [9]. On the other hand, PNP is believed to cause methemoglobinemia, a disorder accompanied by lower capability of carrying oxygen to the tissues [10].

Pseudoephedrine hydrochloride (PSU) – [(+)-(1S,2S)-2-methyl amino-1-phenyl propan-1-ol]hydrochloride [4] (Table 1) – a stereoisomer of ephedrine and an α adrenergic agonist [11] – is a sympathomimetic amine that is commonly combined with other ingredients for the symptomatic relief of cough and cold [11]. Its uses to control urinary incontinence and for the management of priapism have been documented [11].

Mass production of PSU involves reductive amination of L-phenylacetylcarbinol (L-PAC), and the intermediate product is produced via a biotransformation process using yeast, where benzaldehyde (BZH) acts as a substrate. BZH then condenses with acetaldehyde – which results from decarboxylation of pyruvic acid (a product of glucose glycolysis) by pyruvate decarboxylase, where the product of condensation is proved to be L-PAC [12]. Khan and Daugulis [13] reported benzoic acid (BZA) as a by-product of this synthesis. It is, thus, expected that both BZH and BZA are potential impurities that could be encountered in PSU pharmaceutical formulations.

It is reported that 55–75% of PSU is excreted in urine without any change [11]. Pseudoephedrine elimination half-life is documented to be pH dependent [14]. The presence of BZH or BZA – as impurities in PSU – is expected to modify the pH of the urine, causing a prolongation in the duration of action of PSU. This behavior may in turn intensify the common side effects experienced from sympathomimetic “tachycardia, insomnia, anxiety,” etc.

Simultaneous determination of PCA with both PAP and PNP was performed by applying various separation techniques such as conventional high-performance liquid chromatography (HPLC) [15,16] or by using microemulsion liquid chromatography and micellar electrokinetic capillary chromatography [8]. The assay of PCA with PAP and/or other impurities was carried out using HPLC [7,17–20], micellar electro kinetic capillary chromatography [21], and thin-layer chromatography densitometry [20,22]. Moreover, some electrochemical methods [23–26] were also applied to quantify the formerly mentioned analytes.

On the other hand, few reports were about the quantitation of impurities in PSU [27–29], where focusing on the determination of PSU itself as an impurity in methamphetamine was prevalent, since PSU is considered as one of the main precursors of methamphetamine synthesis [30]. As a consequence, many countries start to limit the prescription of PSU as an over-the-counter drug, providing a comprehensive measure against methamphetamine trafficking. The proposed method was fully validated, which allows its application for the analysis of different dosage forms containing PCA or PSU. Moreover, a photodegradation study was carried out to prove that the proposed method indicates the stability.

This study depends on using hybrid micelle liquid chromatography (HMLC) instead of applying traditional reversed phase HPLC, as HMLC is superior in many aspects, because it has a unique ability to simultaneously separate the pharmaceuticals carrying various charges and the neutral compounds whose polarities are diverse with distinguished reproducibility and remarkable selectivity, in addition to supporting the universal trend of applying green chemistry in chemical analysis [31]. The HMLC is considered safe for analysts; besides, it is well-known to be ecofriendly. These features arise from the fact that sodium dodecyl sulfate (SDS) is the main component in HMLC, and its safety as a surfactant has been proved [31]. Direct application of SDS to the skin shows negative carcinogenic effects. Besides, it was found to be negative in the bacterial mutation test (Ames test) [32]. Moreover, SDS prevents the evaporation of the small ratios of organic modifiers added to HMLC, which is another advantage to this mobile phase as it reduces the hazards usually encountered in organic–aqueous

### Table 1: Chemical structure of the studied compounds

| Studied species | Chemical structure | pKₐ   |
|-----------------|--------------------|-------|
| PCA             | ![Chemical structure of PCA](image) | 9.38  |
| PAP             | ![Chemical structure of PAP](image) | 5.48, 10.46 |
| PNP             | ![Chemical structure of PNP](image) | 7.15  |
| PSU             | ![Chemical structure of PSU](image) | 9.52, 13.89 |
| BZH             | ![Chemical structure of BZH](image) | 7.1   |
| BZA             | ![Chemical structure of BZA](image) | 4.2   |
mobile phases that consume a large amount of organic solvents [31]. Carrying the chemical nature of a fatty alcohol, SDS is liable to be degraded aerobically; consequently, HMLC waste is considered clean. When the analysis does not require a large number of sample injections, the hybrid micelle liquid mobile phase is susceptible to recycling [33]. Owing to the solubilizing power of micelles, the application of HMLC to dosage form extraction and quantification makes it an alternative candidate to the conventional mobile phases [34]. Another advantage which encouraged the authors to utilize HMLC is its compatibility with reversed phase columns and several detection modes [35,36].

In this study, a HMLC method based on UV detection was developed for the simultaneous analysis of either PCA or PSU with their synthetic impurities. The proposed method was validated and applied successfully to assay different pharmaceuticals containing PCA or PSU.

2 Experimental

2.1 Instrumentation

Chromatographic separation was carried out using a Shimadzu SPD-20A apparatus, a product of Kyoto, Japan, provided with a 20 µL loop and a UV/VIS detector. The instrument is supplied with a CTO-20A column oven and a DGU-207 degasser unit. Besides, a pH meter (Jenway, England, UK) was used.

2.2 Materials and reagents

PSU, of purity 99.65%, was provided by Sigma Pharmaceuticals, Cairo, Egypt. PCA, of purity 100.12%, was provided by SEDICO Pharmaceutical Company, Cairo, Egypt. PAP and PNP, having purity percentages of 98% and 99%, respectively, were purchased from Alfa Aesar Fine Chemicals, Fisher Scientific, USA, whereas BZH and BZA were imported from Oxford Lab. Fine Chemical, India.

Methanol and acetonitrile of HPLC grade were obtained from TEDIA Company, Inc., Fairfield, USA. SDS was imported from Sigma Aldrich, USA. Meanwhile, El Nasr Pharmaceutical Chemical Company, Egypt, supplied both O-phosphoric acid and triethylamine (TEA; both with 85% minimum assay).

2.3 Pharmaceutical dosage forms

Cetal® oral drops containing 100 mg of PCA in 1 mL and Cetal® suspension containing 250 mg of micronized PCA in 5 mL were supplied by Egyptian International Pharmaceutical Industries Co., Tenth of Ramadan City, Egypt. Injectamol® solution for IV infusion containing 10 mg PCA/mL was supplied by PHARCO B International for Pharma-Tech Co., Cairo, Egypt. Rhinostop® oral drops containing 25 mg of PSU and 2 mg of carbinoxamine maleate in 1 mL were provided by Medical Union Pharmaceuticals MUP, Egypt. Decongess-L® capsules containing 120 mg of PSU and 5 mg of loratadine were supplied by Pharania Pharmaceuticals, Egypt. All pharmaceutics were purchased from local pharmacies in Mansoura, Egypt.

2.4 Chromatographic separation conditions

Both mixtures were separated using a C18 Prontosil Kromasilus column (250 × 4.6 mm; León berg, Germany), which was maintained at 40°C throughout the analysis, applying a flow rate of 1.5 mL/min and adopting UV detection at 240 nm for mixture I (PCA with PAP and PNP) and at 220 nm for mixture II (PSU with BZH and BZA).

For both mixtures, the mobile phase consisted of 0.1 M SDS, acetonitrile, and TEA (90:10:0.3, v/v/v) and adjusted to pH 4 (mixture I) or pH 3.7 (mixture II) using 2 M orthophosphoric acid.

Routine steps aiming to maintain the stability of the chromatographic system were carefully followed, where ultrfiltration of the mobile phase was carried out using Chrom-Tech UK Nylon membrane filters having a pore size of 0.45 µm, followed by sonication for 30 min. After analysis, the system was first washed with deionized double-distilled water for 30 min and then with a mixture of distilled water and methanol in a ratio of 1:1 for another 30 min.

2.5 Calibration curve

All stock solutions were individually prepared as 1.0 mg/mL except for PSU which was prepared as 10.0 mg/mL. Each working standard solution was prepared by serial dilution of its corresponding stock solution in a 10.0 mL volumetric flask using methanol, to cover the linearity ranges of 3.0–100.0, 4.0–200.0, 10.0–400.0, 2.0–100.0, and 3.0–150.0 µg/mL for PCA, PAP, PNP, BZH, and BZA, respectively, and
0.2–3.0 mg/mL for PSU. Each calibration curve was constructed by plotting the final concentration of the analyte – in µg/mL or mg/mL – versus the average response (average peak area obtained by triplicate injection of each concentration). Regression equation for each analyte was deduced from the corresponding calibration curve.

2.6 Analysis of laboratory prepared mixtures

Different volumes of the stock solutions within the linearity range of either PCA with PAP and PNP (mixture I) or PSU with BZH and BZA (mixture II) were transferred to 10.0 mL volumetric flasks and completed to the mark with methanol. The procedures mentioned under “Calibration curve” were then followed to deduce the concentration of each analyte in both mixtures.

2.7 Analysis of different dosage forms

2.7.1 Oral drops and solution for IV infusion

Either 1.0 or 10.0 mL of Cetal® oral drops or Injectamol® solution for IV infusion was transferred to a 100.0 mL volumetric flask and diluted with methanol to the mark. Ten milliliters of Rhinostop® oral drops were transferred to a 25.0 mL volumetric flask and diluted to the volume with methanol. These aliquot volumes were selected to reach the final concentration of the stock solutions of 1.0 mg/mL for PCA and 10.0 mg/mL for PSU.

2.7.2 Capsules

Ten capsules were weighed and evacuated from their content. Capsular content equivalent to 250.0 mg PSU was transferred to a small conical flask and extracted with methanol (2 × 10 mL) with the aid of sonication. The extracts were filtered into a 25.0 mL volumetric flask. Few milliliters of methanol were used to wash the conical flask and complete the volume in the volumetric flask to reach a final stock solution concentration of 10.0 mg/mL.

2.7.3 Suspension

Cetal® suspension was shaken before extraction to avoid the presence of any settled particles, after which 2.0 mL of the suspension was quantitatively transferred to a conical flask, then the extraction steps mentioned for capsules were followed using a 100.0 mL volumetric flask for dilution, so as to prepare a stock solution with the final concentration of 1.0 mg/mL.

The stock solutions of all the extracted dosage forms were subjected to a serial dilution with methanol to prepare different concentrations within the linearity ranges of either PCA or PSU. The concentration of each drug in its pharmaceutical preparation was calculated by referring to its regression equation.

2.8 Photodegradation stability study

Two dosage forms of PCA were selected to carry out this study: Cetal® oral drops and Injectamol® solution for IV infusion (final concentration: 100.0 µg/mL PCA) extracts. Photodegradation procedures were performed with analogy to a previous report [16].

During this study, the methanolic solution of each dosage form was irradiated with a UV lamp (254 nm) for 1 h. The irradiated solutions were assayed using two steps, first immediately after exposure to UV radiation and the second time was 7 days later during this time the solutions were maintained at ambient temperature in the laboratory. In both cases, the solutions were injected in triplicates under the chromatographic conditions described for PCA. Quantitative determination of the analyte was performed by applying the specified regression equation.

Ethical approval: The conducted research is not related to either human or animal use.

3 Results and discussion

Optimization of chromatographic conditions aims to get adequate separation and to quantify the studied drugs together with their impurities. The studied factors for mobile phase optimization include concentration of surfactant, type and ratio of organic modifier, pH of the mobile phase, and the detection wavelength. During optimization of the chromatographic separation conditions for both mixtures, two factors were found to be in common: the column temperature and the flow rate.

To achieve high repeatability, the column thermostat was maintained at 40°C through the analysis, which
also assisted in lowering the applied pressure resulting from the organic modifier and the high flow rate [37]. In addition, a flow rate of 1.5 mL/min was found suitable regarding peak shape, so it was utilized during the assay.

Regarding mixture I: PCA with PAP and PNP, it was found that the retention time \( t_r \) of PCA was not affected by changing the surfactant concentration (from 0.05 to 0.15 M); however, distorted peak shape and poor separation from the solvent front were observed at lower concentrations (0.05–0.075 M), while \( t_r \) of both PAP and PNP gradually decreases by increasing the SDS molar concentration. The tailing of PAP peak was remarkable when the SDS strength was 0.15 M; therefore, 0.1 M SDS was chosen because it provides reasonable analysis time accompanied by peak symmetry and acceptable baseline separation (Figure 1As–Ds).

The pH of the mobile phase was also studied over the range of 3–6, and it was found that through the entire range, the retention times and peak responses of all three species were slightly affected (Figure 2As–Es), as the studied compounds possess the basic \( pK_a \) values (Table 1) and are completely ionized throughout the entire investigated pH range. Better resolution was achieved between the peaks of PCA and PAP at pH value \( \geq 4 \), however, at pH values ranging from 4.5 to 6, the peaks of all the three analytes were distorted. Based on these findings, this mixture was analyzed at pH 4.

The type and ratio of the organic modifier were also a matter of concern. Both methanol and 1-propanol succeeded in separating PCA from its impurities, but both resulted in distorted PCA peaks and poor baseline separation (Figure 3As and Bs). Acetonitrile, on the other hand, was able to overcome this drawback, providing optimum separation and well-shaped symmetric peaks. The ratio of acetonitrile (7–15%) was also investigated, as expected, by increasing the percentage of acetonitrile, the retention time of both PAP and PNP decreased gradually, while \( t_r \) of PCA was slightly affected. Although the use of 15% of acetonitrile enhanced the peak response of PAP, it resulted in a forked peak of PCA; thus, 10% acetonitrile was used to maintain the peak symmetry.

To achieve maximum sensitivity, different wavelength settings were tried, ranging from 220 to 270 nm. Despite both 220 and 230 nm resulted in higher sensitivity for both PAP and PNP, they were not used, as they yielded a very pronounced solvent front with poorly separated baseline. On the other hand, higher wavelength values (250–270 nm) enhanced the sensitivity of PCA but depressed that of PAP and PNP. Consequently, 240 nm was chosen to achieve the appropriate sensitivity parameter for the three compounds.

Concerning mixture II: PSU with BZH and BZA, it was found that separation of the three species is pH dependent. When the pH was lower than 3.5, the PSU eluted with the solvent front and poor resolution was obtained between BZH and BZA (Figure 4As). By increasing the pH to values higher than 4 (pH 4.5–5.5), the peaks of BZH and BZA showed poor resolution but the PSU showed acceptable separation from both the analytes (Figure 4Bs). Further elevation in pH (pH \( \geq 6 \)) resulted in total overlap of BZH and BZA to appear as a single peak; whereas the PSU elution order was altered, so that it appeared first (Figure 4Cs). Therefore, pH 3.7 was utilized and considered the most suitable for obtaining well-separated peaks. An interpretation of this behavior could be understood by knowing the \( pK_a \) values of the three compounds (Table 1). The basic \( pK_a \) values of PSU are 9.52 and 13.89 and its degree of ionization increases by decreasing the pH values. The pH ranges were selected in such a way that the PSU was completely ionized throughout the process. It was observed that at pH values lower than 3.5, PSU was eluted with the mobile phase owing to its increased polarity. Gradual increase in pH leads to depression of PSU ionization, and it was successfully separated under the described chromatographic conditions. On the other hand, the degree of ionization of BZA increases by increasing the pH over its \( pK_a \) (4.2), and its retention time starts to decrease dramatically to overlap with BZH.

The surfactant concentration was also studied over the range of 0.05–0.18 M. SDS (0.05 M) did not have enough elution strength to separate PSU that overlapped with the solvent front (at pH 3.7; Figure 5As), while higher concentrations succeeded to elute PSU. As a result, 0.1 M SDS was chosen as it achieved well-separated peaks accompanied by a short chromatographic run. An advantage of 0.1 M SDS over 0.075 M SDS was that it also successfully separated the three pharmaceuticals (Figure 5Bs). In spite of the fact that both 0.15 M and 0.18 M SDS succeeded in achieving separation and yielded shorter analysis time, they were not used, as 0.15 M resulted in an overlap of the peaks of BZH and PSU accompanied by distorted peak shapes (Figure 5Cs), while 0.18 M yielded tailed peaks of all three analytes (Figure 5Ds). It is to be noticed that the elution order was modified by using higher concentrations of SDS, i.e., at 0.15 M SDS, PSU eluted after BZH and BZA appeared later. Upon increasing the molar strength to reach 0.18 M, PSU was eluted first, followed by BZH and eventually by BZA.
The type and ratio of the organic modifier were also investigated in this study. The use of methanol did not provide the required separation of the three analytes, where the peaks of BZH and BZA were distorted and PSU had a long retention time (25 min), in addition to the poor baseline separation (Figure 6A). On the other hand, upon using 1-propanol, it was not possible to separate BZH from BZA as they appeared as a single peak; furthermore, the elution order was modified, where PSU appeared first followed by an overlapped peak of the two impurities (Figure 6B). Acetonitrile was the solvent of choice, as it could overcome the drawbacks of the two previous organic modifiers. Its ratio (7–15%) was also investigated to yield optimum separation parameters. It was found that $t_r$ of the three compounds decreased upon increasing the ratio of acetonitrile through this range, so 10% was chosen to yield a shorter chromatographic run and well-shaped separated peaks. To achieve the best sensitivity measures, 220 nm was adopted as a detection wavelength.

TEA is added to HPLC for two reasons, first to adjust the pH and second to increase the separation efficiency [31] through binding to the silanol groups of the stationary phase, causing a synergistic effect with the organic modifier in enhancing the efficiency. The effect of TEA percentage on separation efficiency of the proposed method was studied, and 0.3% was found to be the optimum ratio, as higher percentages (0.4–1%) cause elution of PCA with solvent front (mixture I) and poor resolution between PSU and its impurities (mixture II). Meanwhile, lower ratios of TEA resulted in asymmetric peaks for all the chemical compounds in both mixtures.

A representative chromatogram of PCA with its two impurities, PAP and PNP, is illustrated in Figure 1A, where separation was conducted using 0.1 M SDS, acetonitrile, and TEA (90:10:0.3, v/v/v) at pH 4 by adopting UV detection at 240 nm, using a flow rate of 1.5 mL/min, and maintaining the column at 40°C.

Figure 1B represents a chromatogram of PSU with its two impurities, BZH and BZA, where separation was carried out using a mobile phase composed of 0.1 M SDS, acetonitrile, and TEA (90:10:0.3, v/v/v) at pH 3.7, adopting 220 nm as a detection wavelength, and using a flow rate of 1.5 mL/min, where the column temperature was maintained at 40°C.

Figure 1: (A) A chromatogram representing PCA with its impurities PAP and PNP under the described chromatographic conditions, where (a) 25.0 µg/mL PCA, (b) 10.0 µg/mL PAP, and (c) 20.0 µg/mL PNP. (B) A chromatogram representing PSU with its impurities BZH and BZA under the described chromatographic conditions, where (a) 10.0 µg/mL BZH, (b) 10.0 µg/mL BZA, and (c) 1.0 mg/mL PSU.
3.1 Method validation

According to the USP [4], validation is the establishment of any analytical method through laboratory studies to ensure that the performance of the suggested method is in accordance with the preconceived application. The typical characteristics in method validation include linearity and range, limit of detection (LOD), limit of quantitation (LOQ), accuracy, precision, specificity, and robustness.

For mixture I, linearity was experienced over the range of 3.0–100.0, 4.0–200.0, and 10.0–400.0 µg/mL, for PCA, PAP, and PNP, respectively, with their corresponding LOD values of 1.5, 2.0, and 5.0 µg/mL and LOQ values of 2.5, 3.0, and 9.0 µg/mL. Regarding mixture II, the obtained linearity ranges were 2.0–100.0, 3.0–150.0 µg/mL, and 0.2–3.0 mg/mL for BZH, BZA, and PSU, respectively, with their corresponding LOD values of 1.0, 1.5, and 100.0 µg/mL and LOQ values of 1.5, 2.5, and 150.0 µg/mL. Both LOD and LOQ were experimentally determined through evaluating the concentrations which yield a signal–noise ratio of 3:1 and 10:1, respectively [4]. The low values obtained demonstrate the sensitivity of the proposed method.

Evaluation of the accuracy is performed through comparison of the concentrations of the studied compounds in both mixtures—whether alone or in laboratory prepared mixtures—applying both the proposed and the reference methods [15,27]. The high accuracy of the proposed method is demonstrated from the small values of $t$ test and variance ratio $F$ test [38]. Tables 2 and 3 list the obtained data where the concentrations

| Parameter | Concentration taken (µg/mL) | Concentration found (µg/mL) | % found | Reference methods [15,27], % found |
|-----------|----------------------------|----------------------------|---------|-----------------------------------|
| **Mixture I** |                             |                            |         |                                   |
| PCA       | 3.0                        | 2.94                       | 98.15   | 99.47                             |
|           | 10.0                       | 9.86                       | 98.55   | 99.22                             |
|           | 30.0                       | 29.84                      | 99.46   | 101.57                            |
|           | 50.0                       | 49.99                      | 99.97   |                                   |
|           | 70.0                       | 70.31                      | 100.44  |                                   |
|           | 100.0                      | 100.13                     | 100.13  |                                   |
| Average ± SD |                             |                            | 99.45 ± 0.92 | 100.09 ± 1.29                       |
| $t$ test  |                            |                            | 0.88*   |                                   |
| $F$ test  |                            |                            | 1.97*   |                                   |
| PAP       | 4.0                        | 4.01                       | 100.25  | 99.64                             |
|           | 20.0                       | 20.37                      | 101.84  | 100.84                            |
|           | 70.0                       | 71.15                      | 101.64  | 101.28                            |
|           | 120.0                      | 118.98                     | 99.15   |                                   |
|           | 150.0                      | 149.51                     | 99.67   |                                   |
|           | 200.0                      | 199.78                     | 99.89   |                                   |
| Average ± SD |                             |                            | 100.41 ± 1.09 | 100.59 ± 0.85                       |
| $t$ test  |                            |                            | 0.92*   |                                   |
| $F$ test  |                            |                            | 1.64*   |                                   |
| PNP       | 10.0                       | 9.85                       | 98.47   | 98.35                             |
|           | 100.0                      | 98.05                      | 98.05   | 99.48                             |
|           | 150.0                      | 149.51                     | 99.67   | 101.47                            |
|           | 200.0                      | 198.22                     | 99.11   |                                   |
|           | 300.0                      | 302.76                     | 100.92  |                                   |
|           | 400.0                      | 402.96                     | 100.74  |                                   |
| Average ± SD |                             |                            | 99.49 ± 1.17 | 99.77 ± 1.58                       |
| $t$ test  |                            |                            | 0.83*   |                                   |
| $F$ test  |                            |                            | 1.82*   |                                   |
| **Mixture II** |                            |                            |         |                                   |
| PSU       | 200.0                      | 196.52                     | 98.25   | 98.16                             |
|           | 500.0                      | 493.85                     | 98.77   | 99.73                             |
|           | 1000.0                     | 993.41                     | 99.34   | 99.33                             |
|           | 1500.0                     | 1485.92                    | 99.06   |                                   |
|           | 2000.0                     | 1963.22                    | 98.16   |                                   |
|           | 3000.0                     | 3013.51                    | 100.45  |                                   |
| Average ± SD |                             |                            | 99.01 ± 0.84 | 99.07 ± 0.82                       |
| $t$ test  |                            |                            | 0.24*   |                                   |
Table 2: continued

| Parameter | Concentration taken (µg/mL) | Concentration found (µg/mL) | % found | Reference methods [15,27], % found |
|-----------|-----------------------------|-----------------------------|---------|-----------------------------------|
| F test    |                             |                             | 1.05*   |                                   |
| BZH       | 2.0                         | 1.99                        | 99.33   | 99.34                             |
|           | 20.0                        | 19.74                       | 98.72   | 99.87                             |
|           | 30.0                        | 29.42                       | 98.06   | 101.28                            |
|           | 50.0                        | 49.06                       | 98.11   |                                   |
|           | 70.0                        | 71.15                       | 101.64  |                                   |
|           | 100.0                       | 101.28                      | 101.28  |                                   |
| Average ± SD |                   |                             | 99.52 ± 1.57 | 100.16 ± 1.01 |
| t test    |                             |                             | 0.79*   |                                   |
| F test    |                             |                             | 2.42*   |                                   |
| BZA       | 3.0                         | 3.05                        | 101.75  | 100.48                            |
|           | 20.0                        | 20.27                       | 101.35  | 101.84                            |
|           | 50.0                        | 50.04                       | 100.08  | 99.58                             |
|           | 70.0                        | 69.77                       | 99.67   |                                   |
|           | 100.0                       | 99.15                       | 99.15   |                                   |
|           | 150.0                       | 148.98                      | 99.32   |                                   |
| Average ± SD |                   |                             | 100.22 ± 1.09 | 100.63 ± 1.14 |
| t test    |                             |                             | 0.24*   |                                   |
| F test    |                             |                             | 1.09*   |                                   |

*The tabulated t and F values are 2.571 and 19.30, respectively, at \( p = 0.05 \) [38].

Table 3: Determination of the studied drugs in laboratory prepared mixtures using the proposed method

| Mixture I | Parameter | Taken (µg/mL) | % found | Reference method [15], % found |
|-----------|-----------|---------------|---------|--------------------------------|
|           | PCA       | PAP           | PNP     | PCA                           |
|           |           |               |         | PAP                           |
|           |           |               |         | PNP                           |
|           |           |               |         | PSU                           |
|           |           |               |         | BZH                           |
|           |           |               |         | BZA                           |
| 100.0     | 10.0      | 20.0          | 99.52   | 99.78                         |
| 90.0      | 9.0       | 18.0          | 98.64   | 98.64                         |
| 80.0      | 8.0       | 15.0          | 100.67  | 98.25                         |
| 70.0      | 7.0       | 15.0          | 101.08  | 99.67                         |
| 60.0      | 6.0       | 10.0          | 100.76  | 100.14                        |
| Average   |           |               | 100.13  | 99.29                         |
| SD        |           |               | 1.02    | 0.81                          |
| t test*   |           |               | 0.64    | 0.57                          |
| F test*   |           |               | 1.47    | 1.64                          |
| F test    |           |               | 3.74    | 3.18                          |

| Mixture II | Parameter | Taken (µg/mL) | % found | Reference method [27], % found |
|------------|-----------|---------------|---------|--------------------------------|
|            | PSU       | BZH           | BZA     | PSU                           |
|            |           |               |         | BZH                           |
|            |           |               |         | BZA                           |
| 3000.0     | 30.0      | 30.0          | 101.25  | 100.95                        |
| 2000.0     | 20.0      | 20.0          | 101.88  | 101.75                        |
| 1000.0     | 10.0      | 10.0          | 100.75  | 99.45                         |
| 800.0      | 8.0       | 8.0           | 100.06  | 98.45                         |
| 500.0      | 5.0       | 5.0           | 99.68   | 98.11                         |
| Average    |           |               | 100.72  | 99.74                         |
| SD         |           |               | 0.89    | 1.57                          |
| t test     |           |               | 0.78    | 0.68                          |
| F test     |           |               | 3.74    | 3.18                          |

*The tabulated t and F values are 2.776 and 19.25, respectively, at \( p = 0.05 \) [38].
were calculated from the regression equation of each drug.

According to USP [4], precision is the agreement among the obtained results when the analytical procedure is repeatedly applied on multiple homogenous samples. Assessment of precision was carried out by applying the proposed method on three different concentrations of each drug in both mixtures on the same day or on three different days. The data abridged in Table 4 illustrate acceptable precision as revealed from the small values of standard deviation.

Specificity is the ability to assay the analytes in the presence of expected components such as impurities, degradation products, and matrix components [4], and it was clearly manifested in this study.

The specificity of the proposed method could be demonstrated from the adequate separation of PCA and PSU from their impurities (Figure 1A and B). Moreover, the three selected chromatograms from the studied dosage forms (Figure 2A–C) illustrate the satisfactory separation of both drugs from inactive ingredients and separation of PSU from its coformulated pharmaceuticals. Furthermore, acceptable separation of PCA from its photolytic degradation product obtained after UV decomposition of pharmaceutical dosage forms containing PCA (Figure 3A–D) proves the specificity of the proposed method.

The robustness, which measures the capacity of the analytical method to be unaffected by the minor changes in the experimental conditions indicating its suitability through normal analysis conditions [4], could be illustrated in this study. This could be exemplified by referring to the optimization of mixture I, where the pH did not affect the retention times or the sensitivity of the three compounds, and the ability to use a variety of organic modifiers to separate them efficiently in spite of selecting acetonitrile; while in the case of mixture II, wide concentration range of SDS could be applied (0.1–0.18 M) to separate PSU from its impurities.

### 3.2 Applications

#### 3.2.1 Analysis of PCA and PSU in their dosage forms

The proposed method succeeded to quantitate PCA and PSU in their different pharmaceutical dosage forms (Figure 2A–C and Table 5). As the presented

| Parameter | Intraday precision | Mixture I | % found | Mixture II | % found |
|-----------|--------------------|----------|---------|------------|---------|
| Taken (µg/mL) | | PCA | PAP | PNP | PCA | PAP | PNP | PCA | PAP | PNP | PSU | BZH | BZA | PSU | BZH | BZA |
| 3.0 | 4.0 | 10.0 | 99.85 | 99.45 | 100.48 | 200.0 | 2.0 | 3.0 | 98.78 | 101.58 | 99.78 |
| 50.0 | 100.0 | 200.0 | 98.78 | 98.77 | 101.06 | 1500.0 | 50.0 | 75.0 | 98.36 | 101.44 | 98.91 |
| 100.0 | 200.0 | 400.0 | 99.36 | 100.06 | 99.32 | 3000.0 | 100.0 | 150.0 | 99.78 | 99.67 | 101.58 |
| Mean* | | 99.33 | 99.43 | 100.29 | | 98.97 | 100.89 | 100.09 | | 0.73 | 1.06 | 1.36 |
| SD | | 0.54 | 0.65 | 0.89 | | | | | | | |

| Parameter | Interday precision | Mixture I | % found | Mixture II | % found |
|-----------|--------------------|----------|---------|------------|---------|
| Taken (µg/mL) | | PCA | PAP | PNP | PCA | PAP | PNP | PCA | PAP | PNP | PSU | BZH | BZA | PSU | BZH | BZA |
| 3.0 | 4.0 | 10.0 | 99.65 | 100.08 | 98.78 | 200.0 | 2.0 | 3.0 | 101.75 | 99.75 | 100.46 |
| 50.0 | 100.0 | 200.0 | 98.77 | 101.58 | 99.63 | 1500.0 | 50.0 | 75.0 | 100.35 | 98.67 | 101.35 |
| 100.0 | 200.0 | 400.0 | 101.48 | 99.46 | 99.45 | 3000.0 | 100.0 | 150.0 | 99.65 | 101.25 | 99.13 |
| Mean | | 99.97 | 100.37 | 99.29 | | 100.58 | 99.89 | 100.31 | | 1.07 | 1.29 | 1.12 |
| SD | | 1.38 | 1.09 | 0.45 | | | | | | | |

*Each value represents the average of three determinations.
chromatograms demonstrated, the coformulated pharmaceuticals with PSU, carbinoxamine maleate and loratadine (Figure 2B and C), did not interfere with the separation or quantification of PSU as revealed from their retention times (8.2 and 4.6 min, respectively). On the other hand, the sample matrix was not an obstacle in PCA assay (Figure 2A). These facts emphasize on the specificity of the proposed method; moreover, the high recovery percentages and the low values of $t$ and $F$ tests confirm the accuracy of the method.

No impurities were detected in any of the tested pharmaceutical formulations of PSU (Figure 2B and C), while a minor impurity was observed at 2.7 min in PCA pharmaceuticals (Figure 2A).

3.2.2 Stability-indicating property of the proposed method

By analogy to a previous report [16], a forced degradation study was performed to assess the stability-indicating nature of the proposed method. Upon exposing methanolic solutions of Cetal® oral drops or Injectamol® solution for IV infusion (final concentration: 100.0 µg/mL PCA) to a UV

Figure 2: (A) A chromatogram representing Cetal® oral drop extracts, where (a) 50.0 µg/mL PCA and (b) minor impurity. (B) A chromatogram representing Rhinostop® oral drop extracts, where (a) 80.0 µg/mL carbinoxamine maleate and (b) 1.0 mg/mL PSU. (C) A chromatogram representing Decongess-L® capsule extracts, where (a) 41.67 µg/mL loratadine and (b) 1.0 mg/mL PSU.
Figure 3: (A) A chromatogram representing PCA (100.0 µg/mL) in Cetal® oral drop extracts after exposure to UV light for 1 h, where (a) PCA and (b) photolytic degradation product. (B) A chromatogram representing PCA (100.0 µg/mL) in the extract of Injectamol® solution for IV infusion after exposure to UV light for 1 h, where (a) PCA and (b) photolytic degradation product. (C) A chromatogram representing PCA (100.0 µg/mL) in Cetal® oral drop extracts after exposure to UV light for 1 h and leaving the solution for 7 days in the laboratory, where (a) PCA and (b) photolytic degradation product. (D) A chromatogram representing PCA (100.0 µg/mL) in the extract of Injectamol® solution for IV infusion after exposure to UV light for 1 h and leaving the solution for 7 days in the laboratory, where (a) PCA and (b) photolytic degradation product.
lamp at 254 nm for 1 h (Figure 3A and B), the degradation product which appeared in PCA dosage forms at 2.7 min was still persistent. When the same irradiated solutions were tested later after keeping them at controlled temperature in the laboratory for a period of 7 days, a remarkable increase in the concentration of the produced degradation product was detected as revealed from the enhancement in its peak area (Figure 3C and D). According to Martignac et al. [39], photolysis of PCA in the presence of oxygen suggests the

![Figure 4: Proposed pathway of the photolytic degradation of PCA.](image)

Table 5: Application of the proposed method to the analysis of the studied drugs in their pharmaceuticals

| Parameter                  | Taken (µg/mL) | Found (µg/mL) | % found | Reference methods [15,27], % found |
|----------------------------|---------------|---------------|---------|-----------------------------------|
| Cetal® oral drops          | 10.0          | 9.85          | 98.54   | 98.77                             |
|                            | 30.0          | 29.45         | 98.16   | 99.58                             |
|                            | 50.0          | 49.92         | 99.84   | 99.36                             |
|                            | 70.0          | 69.52         | 99.32   |                                   |
|                            | 100.0         | 100.48        | 100.48  |                                   |
| Mean ± SD                 |               |               | 99.27 ± 0.94 | 99.24 ± 0.42                    |
| t*                        | 0.44          |               |         |                                   |
| F*                        | 5.01          |               |         |                                   |
| Cetal® suspension          | 10.0          | 9.97          | 99.66   | 101.44                            |
|                            | 30.0          | 29.74         | 99.13   | 100.85                            |
|                            | 50.0          | 50.87         | 101.74  | 100.32                            |
|                            | 70.0          | 71.27         | 101.81  |                                   |
|                            | 100.0         | 100.29        | 100.29  |                                   |
| Mean ± SD                 |               |               | 100.53 ± 1.21 | 100.87 ± 0.56                   |
| T                         | 0.21          |               |         |                                   |
| F                         | 4.7           |               |         |                                   |
| Injectamol® solution for IV infusion | 10.0         | 9.88          | 98.77   | 100.78                            |
|                            | 30.0          | 29.69         | 98.98   | 99.37                             |
|                            | 50.0          | 50.74         | 101.47  | 99.81                             |
|                            | 70.0          | 70.08         | 100.11  |                                   |
|                            | 100.0         | 100.37        | 100.37  |                                   |
| Mean ± SD                 |               |               | 99.94 ± 1.1 | 99.99 ± 0.72                    |
| t                         | 0.46          |               |         |                                   |
| F                         | 2.33          |               |         |                                   |
| Rhinostop® oral drops     | 1000.0        | 999           | 99.85   | 100.97                            |
|                            | 1500.0        | 1,522         | 101.47  | 101.48                            |
|                            | 2000.0        | 2,027         | 101.35  | 99.61                             |
|                            | 2500.0        | 2,512         | 100.46  |                                   |
|                            | 3000.0        | 2,993         | 99.78   |                                   |
| Mean ± SD                 |               |               | 100.58 ± 0.81 | 100.69 ± 0.97                   |
| t                         | 0.18          |               |         |                                   |
| F                         | 1.43          |               |         |                                   |
| Decongess-L® capsules     | 1000.0        | 996           | 99.61   | 99.75                             |
|                            | 1500.0        | 1,486         | 99.05   | 99.61                             |
|                            | 2000.0        | 2,009         | 100.47  | 100.47                            |
|                            | 2500.0        | 2,534         | 101.37  |                                   |
|                            | 3000.0        | 3,032         | 101.05  |                                   |
| Mean ± SD                 |               |               | 100.31 ± 0.97 | 99.94 ± 0.46                    |
| t                         | 0.29          |               |         |                                   |
| F                         | 4.45          |               |         |                                   |

*The tabulated t and F values are 2.776 and 19.25, respectively, at p = 0.05 [38].
formation of 4-aminophenol, which undergoes further oxidation to p-benzoquinone (Figure 4).

The significance of carrying out this stability study arises from the fact of the ecotoxicity of the photodegradation product of PCA, which has been studied by Sugiara [40] via the luminescent bacteria test after exposing PCA to UV light. Hence, the proposed method serves as both a qualitative and a quantitative tool of this toxic product, aiming to protect the ecosystem and improve public health.

Selection of UVC radiation (254 nm) for photolysis of PCA was made in accordance with a previous report which proved inefficient photodegradation of PCA by UVA (315–400 nm) or by UVB (280–315 nm) [41], as UVC has a high potential energy and consequently shows more photolysis efficiency.

On the other hand, PSU was not a subject of concern in this stability study, owing to its high stability and resistance to photolytic degradation as reported in its monograph on analytical profile for drug substances [42].

4 Conclusion

A HMLC method was developed for the simultaneous determination of PCA and PSU with some of their synthetic impurities. The method was subjected to full validation, which enables its application to analysis of the concerned drugs in their pharmaceuticals; moreover, photodegradation of PCA was carried out to prove the stability-indicating property of the proposed method. The proposed method carries many advantages over the reported articles, as for example the short chromatographic run especially for mixture I, where the separation of the three studied drugs was accomplished in 9 min. This is superior to other reports using conventional aqueous–organic HPLC techniques where PNP causes a delay in the chromatographic run time, 33.37 min [15] and 12 min [16]. Besides, better resolution was achieved between PCA and PAP in the proposed method unlike other research from the literature [18]. These facts illustrate the contribution of this work to the field of drug analysis and emphasize on its significance.

Conflict of interest: The authors declare no conflict of interest.

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