DEVELOPMENT AND VALIDATION OF A HPLC METHOD FOR THE SIMULTANEOUS DETERMINATION OF BROMHEXINE, CHLORPHENIRAMINE, PARACETAMOL, AND PSEUDOEPHEDRINE IN THEIR COMBINED COLD MEDICINE FORMULATIONS

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DEVELOPMENT AND VALIDATION OF A HPLC METHOD FOR THE SIMULTANEOUS DETERMINATION OF BROMHEXINE, CHLORPHENIRAMINE, PARACETAMOL, AND PSEUDOEPHEDRINE IN THEIR COMBINED COLD MEDICINE FORMULATIONS

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☐ *A simple and efficient liquid chromatographic method has been developed and validated for the simultaneous determination of bromhexine, chlorpheniramine, paracetamol, and pseudoephedrine in common cold medications (tablets and syrups). The separation of the analytes was achieved within 10 min, employing a mixture of 10 mM triethylamine-phosphoric acid buffer (pH 4.0) and MeOH (35:65, v/v) as isocratic mobile phase, pumped at 1.0 mL min^{-1} through a cyano column (5 μm particle size). The analytes were detected at 215 nm. Statistical experimental designs and graphic representations (response surface methodologies, Pareto charts) were used for selecting the proper detection wavelength, optimizing the mobile phase composition, and assessing method robustness.*

The linearity of the calibration (r > 0.99, n = 21) in the relevant ranges (up to 130% of the expected concentrations of the analytes in the formulations), method accuracy (bias < 2.0%), repeatability (RSD < 2.0%) and intermediate precision, were verified. In addition, specificity (peak purities with photodiode array detector > 0.9997) and method robustness were evaluated, and system suitability parameters were determined.

The validated method was successfully employed for the routine analysis of various commercial tablet and syrup pharmaceutical preparations against the common cold, showing satisfactory analyte recoveries and RSD values.

Keywords bromhexine, chlorpheniramine, experimental design, paracetamol, pseudoephedrine, validated HPLC determination

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INTRODUCTION

Medications against the common cold come in different forms (tablets, syrups, etc.) and usually contain a complex mixture of nitrogenous compounds as active ingredients. These are usually present in varying and very different proportions, have diverse properties inherent to their formulation and desired action, and often possess some similar physical and chemical properties, which turn difficult their separation. Moreover, in the case of HPLC analyses, these basic drugs strongly interact with the stationary phases, causing peak asymmetry and lowering separation efficiency.[1] Due to these characteristics, quality control of preparations against the common cold always offers an interesting analytical challenge.[2]

Bromhexine (BRO), chlorpheniramine (CPA), paracetamol (acetaminophen, PAR), and pseudoephedrine (PSE) are nitrogenous compounds (Figure 1) widely used as active ingredients in combined cold medicine formulations due to their mucolytic (BRO), antihistaminic (CPA), antithermic (PAR), and decongestant (PSE) activities.[3] Their commercial associations exhibit important mass differences among the analytes (PAR:CPA up to 125:1, *w/w*) and many of the active principles (PSE, CPA) have rather poor chromophores, posing an additional challenge to their simultaneous quantification.

Spectrophotometric[4] electrophoretic (CE,[5] MEEKC[6]), gas chromatographic,[7,8] and liquid chromatographic techniques with various detections (HPLC-MS,[9] HPLC-conductimetry[10]) and employing elution gradients[11,12] have been proposed for the analyses of mixtures of active ingredients in cold medicine formulations.

A literature survey revealed the interest in the determination of PAR, PSE, CPA, and BRO in pharmaceutical formulations, in combinations of
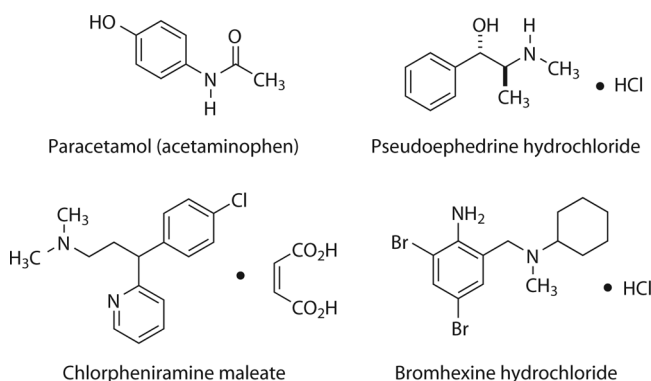

FIGURE 1 Chemical structures of paracetamol, pseudoephedrine hydrochloride, chlorpheniramine maleate, and bromhexine hydrochloride.

two[1,13,14] or three[15,16] of these drugs, or with other active principles.[17–20] However, it also evidenced the lack of a method suitable for the simultaneous determination of all four compounds in their polydrug associations.

The official USP 32 assay for pharmaceutical formulations containing at least three active principles among PAR, CPA, dextromethorphan, and PSE employs a multi-ingredient mobile phase and requires separate sample preparation and HPLC runs for the different drug substances, accepting analyte tailing factors (t_f) to be as high as 2.5.[21] This is a highly time- and solvent-consuming approach.

The development of new rapid and efficient analytical techniques for the determination of the active principles in multi-ingredient pharmaceuticals is part of the current need to ensure their quality. However, whereas isocratic HPLC-UV remains the preferred and most efficient approach toward this analytical problem,[22] only a few methods for the determination of cold medicine ingredients in their complex mixtures have been statistically optimized taking into account experimental design strategies.[23,24]

Therefore, the purpose of this study was to develop and validate a HPLC methodology for the simultaneous determination of BRO, CPA, PAR, and PSE in their combined tablet and syrup formulations, which has no literature precedents. Experimental design techniques were employed for development and validation of the proposed method, as a rational, cost-effective and convenient tool to speed up the process.[25]

EXPERIMENTAL

Apparatus and Software

The separations were performed on a Varian Prostar 210 liquid chromatograph controlled by Star software (Varian, Inc., Palo Alto, CA), which included two isocratic pumps, a manual injector fitted with a 20-μL loop and a variable dual-wavelength UV-Vis detector. Specificity studies were carried out in a HP 1100 liquid chromatograph, fit with a photodiode array detector. Chromatograms were monitored and processed using Chemstation software (Agilent, Wilmington, DE).

The experimental designs, data analysis and response surfaces were performed with Design Expert v. 7.1 (Stat-Ease Inc., Minneapolis, MN). Statistical analyses were carried out in SPSS v. 9 (SPSS, Inc., Chicago, IL).

Reagents and Materials

Analytical-grade reagents (Merck, Darmstadt, Germany), HPLC-grade solvents (J. T. Baker, Mexico) and double-distilled water were employed

for the preparation of the samples and for chromatographic analyses. The experiments were performed with pharmaceutically-certified bromhexine hydrochloride, chlorpheniramine maleate, paracetamol (Saporiti, Buenos Aires), and pseudoephedrine hydrochloride (Laboratorios Lazar, Buenos Aires). Tablet and syrup products (six brands) were acquired in a local pharmacy store. Tablets (except brand 1, which does not contain CPA) declared to contain BRO (8 mg), CPA (4 mg), PAR (500 mg), and PSE (60 mg) per tablet. Syrup labels declared to contain BRO (80 mg), CPA (40 mg), PAR (2500 mg), and PSE (600 mg) every 100 mL solution.

Preparation of Solutions

Stock standard solutions of BRO (1600 $mg\,L^{-1}$), CPA (800 $mg\,L^{-1}$), PAR (2000 $mg\,L^{-1}$), and PSE (6000 $mg\,L^{-1}$) were prepared in MeOH and stored in light-resistant containers, where they demonstrated to be stable at least 90 days. Working standard solutions of BRO (32.0 $mg\,L^{-1}$), CPA (16.0 $mg\,L^{-1}$), PAR (1000 $mg\,L^{-1}$) and PSE (240 $mg\,L^{-1}$) were freshly prepared by dilution of the stock standard solutions with mobile phase. Solutions containing mixtures of the analytes were freshly prepared, by mixing appropriate volumes of the corresponding working standard solutions and completing to the mark with mobile phase. All dilutions were performed in volumetric flasks.

Commercial Tablets and Syrups

Tablets. Twenty tablets were accurately weighed and crushed in a mortar. An appropriate weight of the resulting fine powder was transferred to a 50-mL volumetric flask, dissolved with 30 mL MeOH and submitted to ultrasound irradiation for 10 min to ensure dissolution of the powder. Then, Et_3N-H_3PO_4 buffer (10 mM, pH 4.0) was added to the mark and mixed. An aliquot of the fine suspension (8 mL) was centrifuged (10 min at 1900 $\times$ *g*) and 1.0 mL of the supernatant was transferred to a 10-mL volumetric flask, making up to volume with mobile phase.

Syrups. 3 mL of the syrup was transferred to a 50-mL volumetric flask, diluted with 30 mL MeOH and completed to the mark with Et_3N-H_3PO_4 buffer (10 mM, pH 4.0). An aliquot (1.0 mL) of the solution was transferred to a 10-mL volumetric flask and diluted to the mark with mobile phase.

The solutions were filtered through a 0.45-μm Millipore filter before injection. All preparations were performed in triplicate for each brand.

Chromatographic Conditions

Under the optimized conditions, the separation was achieved at 30°C on a Microsorb-MV 1005 CN column (250 mm $\times$ 4.6 mm, 5 μm particle size)

using a mobile phase containing $Et_3N-H_3PO_4$ buffer (10 mM, pH 4.0) and MeOH (35:65, v/v) pumped at a flow rate of 1.0 mL min^{-1}. Prior to any analysis, the mobile phase was degassed and filtered using 0.45-μm nylon filters. Analytes in the eluate were detected at 215 nm.

RESULTS AND DISCUSSION

Method Development and Optimization

Detection Wavelength

The UV spectra of BRO, CPA, PAR, and PSE, dissolved in the mobile phase are exhibited in Figure 2. BRO, CPA and PSE keep the relationships found in pharmaceutical formulations; however, because of its high proportion in the formulations and its absorption characteristics, the spectrum of PAR was taken with a 100 times more diluted sample. It can be observed that PSE exhibits reasonable absorbances at wavelengths below 220 nm. On the other hand, the more absorbing analyte (PAR) exhibits a minimum at 219 nm. Taking into account the disparate concentrations of the analytes and their dissimilar spectral characteristics, a one factor response methodology strategy was coupled to Derringer's desirability function, with the goals of minimizing the absorption of PAR, while maximizing those of the remaining analytes. This pointed out to 215 nm as the most desirable detection wavelength. Derringer's desirability function can be expressed as in Eq. 1:[26]
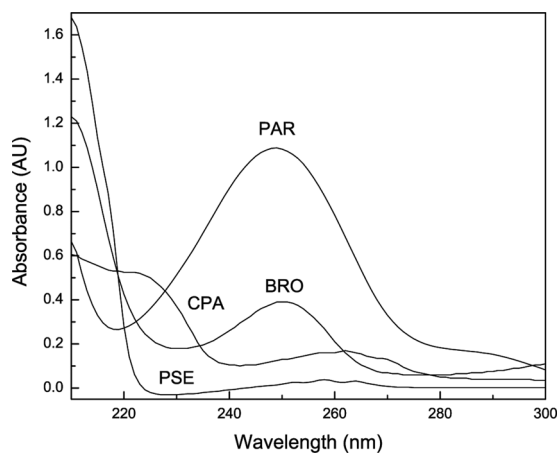

FIGURE 2 Absorption spectra of the analytes in the 210-300 nm region. BRO (16 mg L^{-1}), CPA (8.0 mg L^{-1}), PAR (10 mg L^{-1}), and PSE (48 mg L^{-1}) in the optimized mobile phase. BRO, CPA, and PSE are in the same proportion as in the studied compounded mixtures.

$$D = [d_1 w_1 \times d_2 w_2 \times \ldots \times d_n w_n]^{1/n} \qquad (1)$$

where d_i is the individual desirability function of the ith response, w_i is its corresponding weight ($w_i = 1$ in this case), n is the number of responses (in this study, $n = 4$), and D is the global desirability. The value of D ($0 \leq D \leq 1$), indicates the degree with which the combination of the different criteria complies with the desired conditions.

Screening Experiments

Previously published methodologies for the separation of three of the analytes of interest proved not to be useful.[15,16,19,20] Aiming to achieve the separation of all four analytes, the effects of some chromatographic parameters such as type of column, mobile phase composition, and pH of the buffer solution were investigated.

An initial column screening step was carried out, testing C_{18}, C_8 and CN columns with different mobile phases containing MeOH ($20 - 60\%$) and 4.5 mM potassium phosphate buffer (pH $2.8 - 4.8$). Under these conditions, in the isocratic mode, the C_{18} and C_8 columns were unable to properly resolve between CPA, PAR and PSE, while resulting in late elution of BRO ($t_r > 30$ min). Better separation was observed with the cyano column,[27] albeit with the analytes exhibiting poor peak shapes.

The addition of amines is a common solution to the problem of peak tailing in reversed-phase HPLC.[28] Various alkylamines[29–31] have been used as organic modifiers for the separation of amines.[12] Considering the structures of the relevant analytes, the effect of employing phosphate buffers with different primary (cyclohexylamine and *n*-hexylamine) and tertiary (triethylamine and tripentylamine) amines (10 mM) on peak shape (tailing factor) and analyte resolution was studied with the aid of a Plackett-Burman experimental design. The results revealed that tertiary amines outperformed primary amines in furnishing more symmetric peaks; among the former, use of triethylamine was chosen for further mobile phase optimization.

Optimization of the Composition of the Mobile Phase

The optimum composition of the mobile phase was determined with a series of samples containing 10 mM Et_3N-H_3PO_4 buffer (pH $= 3.0 - 5.0$) and MeOH (50–70%), prepared according to a central composite experimental design.

Peak resolution and the length of the separation were analyzed, with the goals of maximizing the former and minimizing the latter. Use of response surface methodologies (Figure 3A) indicated that the optimum mobile phase was a 35:65 (v/v) mixture of Et_3N-H_3PO_4 buffer (pH 4.0)
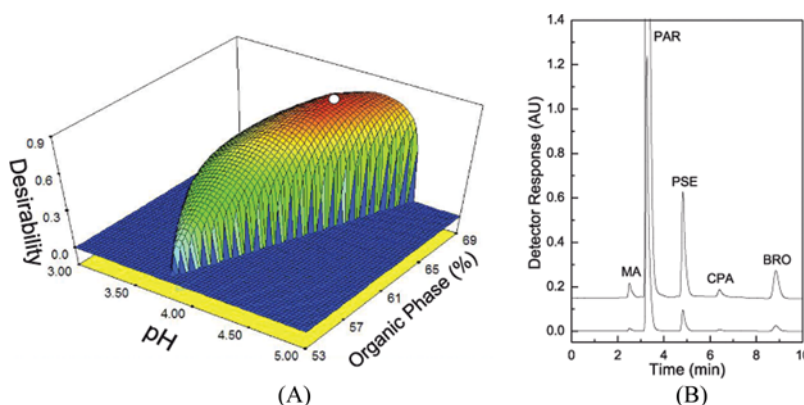
FIGURE 3 A) Optimization of the composition of the mobile phase. Desirability plot; the white dot indicates the optimum mobile phase composition. B) Typical chromatogram of standard solution for the separation of PAR, PSE, CPA, and BRO (MA = maleate). (Color figure available online.)

and MeOH. Figure 3B shows a typical chromatogram of a mixed standards solution under the optimized conditions, where baseline separation of the relevant analytes was achieved within 10 min. Maleate anion (from chlorpheniramine maleate) eluted at the dead time, as demonstrated by injection of a standard of sodium maleate.

Method Validation

The optimized method was validated in agreement with the ICH guideline.[32] Accordingly, method linearity in the relevant working ranges, precision, accuracy, specificity, and robustness were evaluated. System suitability was also determined.

Range and Linearity

Method range and linearity were evaluated with seven mixtures of standards at the following concentrations: 100.0–400.0 mg L^{-1} for PAR, 12.0–48.0 mg L^{-1} for PSE, 0.80–3.20 mg L^{-1} for CPA, and 1.60–6.40 mg L^{-1} for BRO, covering up to 130% of the expected concentration of the analytes in their different formulations.

Samples were injected in triplicate and calibration curves were plotted from the standard drug concentrations versus peak areas of the individual drugs. The calibration curves were defined by the equations shown in Table 1, and the residuals were spread uniformly and at random around the regression lines, passing the normality distribution test ($p < 0.05$). In addition, correlation coefficients were higher than 0.99 and the confidence intervals of the intercepts contained the zero, confirming method linearity.

TABLE 1 Results of Method Validation

Parameters	PAR	PSE	CPA	BRO
Linear range (mg L^{-1})	100.0–400.0	12.0–48.0	0.80–3.20	1.60–6.40
Linearity – Regression equation				
$b \pm SD_b$ ($\times 10^8$)	3.36 ± 0.04	2.56 ± 0.03	3.28 ± 0.06	8.30 ± 0.11
$a \pm SD_a$ ($\times 10^6$)	2.00 ± 1.15	0.96 ± 0.87	-0.28 ± 0.13	-0.17 ± 0.46
r ($n = 21$)	0.9990	0.9993	0.9929	0.9967
Normality test for residuals ($p < 0.05$)	Passed	Passed	Passed	Passed
Precision				
Repeatability (Rec., % ± SD)[a]				
Low level (80%)	101.8 ± 1.1	101.3 ± 1.5	101.3 ± 1.5	100.7 ± 1.5
Medium level (100%)	99.3 ± 1.5	99.6 ± 1.1	99.6 ± 1.6	101.1 ± 0.7
High level (120%)	99.8 ± 0.6	99.8 ± 0.4	100.9 ± 1.1	99.8 ± 0.9
Intermediate precision (Rec., % ±SD)[b]				
Low level (80%)	101.1 ± 1.6	101.4 ± 1.4	100.8 ± 1.2	100.2 ± 1.7
Medium level (100%)	101.2 ± 1.3	101.3 ± 0.9	100.5 ± 1.6	101.3 ± 1.3
High level (120%)	100.9 ± 0.7	101.7 ± 0.9	101.5 ± 1.4	101.1 ± 1.4
Overall analyte recovery (% ±RSD)	101.1 ± 1.2	101.5 ± 1.1	100.9 ± 1.4	100.9 ± 1.5
ANOVA – $F_{\text{Between days}}$ ($F_{(0.95, 1, 17)} = 4.451$)	0.483	0.367	1.939	1.062
ANOVA – $F_{\text{Between analysts}}$ ($F_{(0.95, 2, 17)} = 3.592$)	1.521	2.487	1.599	2.332
Specificity (Peak purity factor)	0.9997	0.9998	0.9998	0.9998
Limit of detection (mg L^{-1})			0.21	0.24
Limit of quantification (mg L^{-1})			0.69	0.94
Accuracy (Bias, %)				
Low level (80%)	– 0.4	– 0.3	– 1.2	+1.8
Medium level (100%)	+0.3	+1.6	+1.7	+1.7
High level (120%)	+1.2	+1.4	+1.5	+1.1

[a]Nine replicates of a combination of standards at three levels.
[b]Triplicate injections of 2 independent sets of samples at three concentration levels each.

Precision

Method precision was verified in its repeatability and intermediate precision aspects. For assessing repeatability, the ICH guideline Q2(R1)[32] offers two alternatives; one of them requires triplicate determinations with independently prepared samples at low, medium and high analyte levels, while the other demands six replicate determinations of the 100% level. Accordingly, nine independent mixed standards samples containing the four analytes at 80%, 100%, and 120% of their corresponding expected concentrations in the pharmaceutical product were injected and the RSD (%) of their recoveries were determined. The observed RSD levels (Table 1), which were below 2%, were considered satisfactory.

For verification of the intermediate precision, the samples were injected at random during two different days by three independent analysts. Drug recoveries were determined and analyzed by means of ANOVA tests. This evidenced that the outcome of the determination was statistically similar regardless the day of the assay and the analyst carrying out the determinations. In addition, in all cases almost quantitative and consistent

(RSD < 2%) drug recoveries were recorded. These results confirmed that the method is precise.

Accuracy

Method accuracy was demonstrated by evaluating analyte recoveries from a pre-assayed pharmaceutical formulation sample, containing 60% of the declared amounts of the drugs, which was fortified with known amounts of the four analytes, to reach concentration levels of 80%, 100%, and 120% of the expected drug concentrations in the pharmaceutical dosage form. The bias data obtained [analyte recovered (%) – analyte in the sample (%)] did not exceed ±2% (Table 1), meaning that essentially quantitative recoveries were achieved. This confirmed that the method enables the accurate determination of the analytes.

Specificity

To prove that the proposed simultaneous determination is free from interferences, mixed standard solutions and samples of commercial tablets and syrups were determined with the aid of a diode array detector. It was observed (Figure 4) that the excipients did not interfere with the determination of the active principles and with their retention times. These were observed as perfectly separated peaks, with retention times of 3.25 ± 0.01 min, 4.99 ± 0.07 min, 6.69 ± 0.12, and 8.89 ± 0.24 min for PAR, PSE, CPA, and BRO, respectively. Interestingly, excipients in the syrups (Figure 4B) eluted at t_0 (2.4 min).

In addition, the peak purity function furnished values of 0.9997, 0.9998, 0.9998, and 0.9998 for these analytes, respectively, demonstrating the absence of underlying peaks. Therefore, the method can be considered as specific for the intended purpose.

Limits of Detection and Quantification

The ICH guidelines do not specifically require calculation of the limits of detection (LOD) and quantification (LOQ) for principal analytes in their formulations. However, in order to assess that the validated concentration ranges of the analytes were above their LOQ values, the LOD and LOQ were determined for CPA and BRO, the active principles with the lowest concentrations, employing the ICH method based on the calibration curve.[32] The LOD values were $0.21 \, \mathrm{mg \, L^{-1}}$ for CPA and $0.24 \, \mathrm{mg \, L^{-1}}$ for BRO; the corresponding LOQ values, determined by the use of the same method, were 0.69 and $0.94 \, \mathrm{mg \, L^{-1}}$, respectively. These values fall below the lowest expected analyte concentrations in the samples.
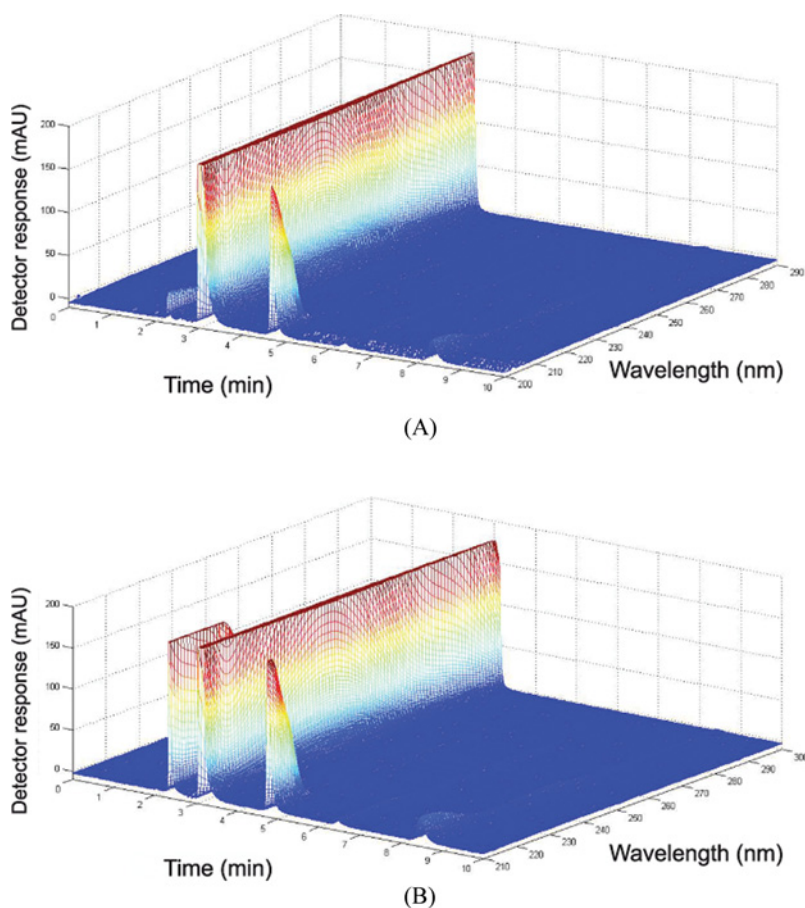
FIGURE 4 Method specificity. Typical chromatograms of A) a tablet sample and B) a syrup sample, acquired with a photodiode-array detector. Chromatographies were carried out on a Microsorb-MV 1005 CN column (250 mm × 4.6 mm, 5 μm particle size) thermostatized at 30°C, employing $Et_3N-H_3PO_4$ buffer (10 mM, pH 4.0) and MeOH (35:65, v/v) as the mobile phase, pumped at 1.0 mL min^{-1}. (Color figure available online.)

Robustness

The robustness of the method was assessed by purposely causing small changes to the optimized procedure, according to a 12 injection Plackett-Burman experimental design and examining their effect on the recovery of the analytes, as proposed by Massart et al.[33] Thus, the pH of the aqueous phase was modified in ±0.2 units (3.8–4.2), the proportion of the organic mobile phase content was changed in ±2% (63–67%), the concentration of the buffer was varied in ±3.0 mM (7 − 13 mM), and the temperature was adjusted in ±3.0°C (27–33°C) in accordance with the experimental design. The standardized effects were plotted on Pareto charts and analyzed. In all cases, the *t*-values for the studied chromatographic

parameters were found to be below the critical value ($t_{crit} = 2.30$), being the pH of the aqueous mobile phase the most influential factor and PAR and PSE the most affected determinations. Overall drug recoveries (mean $\pm$RSD) were $100.4 \pm 1.3\%$ for PAR, $100.4 \pm 1.1\%$ for PSE, $99.8 \pm 1.7\%$ for CPA, and $101.3 \pm 1.7\%$ for BRO, respectively. The peaks were always observed completely and satisfactorily resolved. These data confirmed method robustness within the experimental domain.

System Suitability

System suitability parameters must be checked to ensure that the system is working correctly during the analysis. The test was carried out under the conditions of USP 32[21] and BP 2009,[34] by injecting five replicates of a solution containing $300.0 \, \text{mg L}^{-1}$ PAR, $36.0 \, \text{mg L}^{-1}$ PSE, $2.40 \, \text{mg L}^{-1}$ CPA, and $4.80 \, \text{mg L}^{-1}$ BRO. The observed RSD values for repeated injections were 0.75%, 0.28%, 0.95%, and 0.60%, respectively (Table 2), in full compliance with the commonly accepted values ($\leq 2\%$). Method performance data including column efficiencies (N) capacity factors (k), selectivities (α), resolutions between adjacent peeks (R_s), and tailing factors (t_f) are also listed. All parameters were within acceptable limits.

Stability of Solutions

For the establishment of the stability of standard stock solutions, four standard stock solutions were prepared and stored at 4°C. For the measurements, freshly diluted solutions were periodically injected during 90 days and their peak area differences from the beginning were calculated. No additional peaks were detected and peak area differences did not exceed 2%, confirming stock solution stability for at least 90 days.

Application: Analysis of Commercial Samples

The validated HPLC method was applied to the simultaneous determination of the analytes in samples corresponding to four commercial brands

TABLE 2 Results of the System Suitability Test

Parameter Analyte	PAR	PSE	CPA	BRO
Retention time (t_r, min)	3.25 ± 0.01	4.99 ± 0.07	6.69 ± 0.12	8.89 ± 0.24
Resolution (R_s)	7.8 ± 0.4	6.2 ± 0.2	7.2 ± 0.2	
Tailing factor (t_f)	1.5	1.5	1.4	1.3
Theoretical plates (N)	5600	7500	8100	8500
RSD (%, $n = 5$)	0.75	0.28	0.95	0.60

TABLE 3 Application. Assay of Commercial Tablets and Syrups

Analyte Brand No (Dosage form)	PAR			PSE			CPA			BRO		
	Declared (mg)	Found (mg)[a]	Recovery (%)[a]	Declared (mg)	Found (mg)[a]	Recovery (%)[a]	Declared (mg)	Found (mg)[a]	Recovery (%)[a]	Declared (mg)	Found (mg)[a]	Recovery (%)[a]
1 (Tablets)	500	515 ± 0.6	103.4 ± 0.6	60	59.6 ± 0.7	99.4 ± 0.7	—[b]	—[b]	—[b]	8	8.12 ± 0.6	101.5 ± 0.6
2 (Tablets)	500	512 ± 1.3	102.4 ± 1.3	60	59.2 ± 0.8	98.7 ± 0.8	4	3.98 ± 1.3	99.5 ± 1.3	8	7.98 ± 0.4	99.8 ± 0.4
3 (Tablets)	500	500.5 ± 0.2	100.1 ± 0.2	60	59.7 ± 0.8	99.5 ± 0.8	4	3.96 ± 1.4	98.9 ± 1.4	8	7.98 ± 1.3	99.8 ± 1.3
4 (Tablets)	500	499.5 ± 1.2	99.9 ± 1.2	60	59.8 ± 0.9	99.7 ± 0.9	4	3.98 ± 1.3	99.5 ± 1.3	8	8.0 ± 0.5	100.3 ± 0.5
5 (Syrup)	2500	2532.5 ± 1.3	101.3 ± 1.3	600	593.4 ± 0.7	98.9 ± 0.7	40	40.3 ± 0.9	100.8 ± 0.9	80	81.4 ± 0.8	101.8 ± 0.8
6 (Syrup)	2500	2497.5 ± 0.6	99.9 ± 0.6	600	595.2 ± 0.6	99.2 ± 0.6	40	39.5 ± 0.9	98.7 ± 0.9	80	79.1 ± 0.6	98.9 ± 0.6

[a]Mean ±RSD (%); $n = 3$.
[b]This formulation does not contain CPA.

of tablets and two of syrups of the pharmaceutical association among PAR, PSE, CPA, and BRO. Analyses were carried out in triplicate and the results (mean and RSD) are shown in Table 3.

The data showed recoveries between 98.7% and 103.4% of label claim of the different analytes, with satisfactory precision (RSD $\leq 1.4\%$, $n = 3$). These results confirmed that the amount of each active principle in the samples was close to the declared content and that all the associations met the general requirements (90–110% of the declared content) for all of their active principles.[34]

In all cases, no additional peaks which could interfere with the determination of the analytes were observed and the determination of drugs in lower concentrations was accurately achieved in the presence of high content of PAR, with good recovery and precision. Therefore, the proposed method can be confidently employed for the quality control of tablets and syrups containing the pharmaceutical combination of BRO, CPA, PAR, and PSE.

CONCLUSIONS

A reliable and rapid liquid chromatography method for the simultaneous determination of BRO, CPA, PAR, and PSE in various pharmaceutical preparations has been developed and validated. Experimental designs were employed for rational method optimization and demonstration of its suitability for the intended purpose. The method is capable of separating the active principles within 10 min, despite their widely different properties. It is also able to achieve their quantification in spite of the important abundance differences among the analytes (PAR:CPA up to 125:1, w/w). In addition, the results indicate that the method is sensitive, linear, precise, accurate, specific, and robust with regards to the mixture under investigation. Therefore, the method can be safely applied to the quality control of combined cold medicines containing BRO, CPA, PAR, and PSE.

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