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Evaluation of six eco-analytical tools for the sustainable HPLC analysis of a quinary anti-cold drug mixture

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We present a novel, eco-friendly RP-HPLC method for determining five key components commonly found in anti-cold pharmaceutical formulations simultaneously: Paracetamol (PAR), an analgesic and antipyretic; pseudoephedrine (PSE), an antihistamine; chlorpheniramine (CHL) and preservatives methylparaben (MET) and propylparaben (PRO). In cold medications, these compounds are often incorporated to treat fever, nasal congestion, and allergic rhinitis symptoms. Chromatographic separation was achieved using an Inert Sustain C18 column (250 × 4.6 mm, 5 µm) under gradient elution mode, with a green mobile phase of ethanol and 0.1% trifluoroacetic acid (pH 2.2), a flow rate of 1.0 mL/min, and ambient temperature (25 °C). Detection was performed using PDA with time-programmed wavelength scanning: 214 nm for PSE, 244 nm for PAR, and 264 nm for CHL, MET, and PRO, ensuring optimal sensitivity and selectivity for each analyte. An analysis of the method went beyond its analytical performance by incorporating six advanced sustainability metrics: MoGAPI, Complex MoGAPI, MoGSA, BAGI, RAPI, VIGI, CACI, and GEARS, which are all designed to provide a multidimensional evaluation of factors such as environmental impact, operational efficiency, and innovation. The STABLE toolkit was applied to all compounds to provide quantitative stability scores and identify vulnerabilities under stress conditions. A low toxicity and biodegradable organic solvent, ethanol, was selected for the organic solvent, while trifluoroacetic acid provided optimal peak resolution under acidic conditions. Analytical validation confirmed excellent linearity ($r^2 > 0.999$), precision (%RSD < 2%), and sensitivity, in compliance with ICH guidelines. Using sustainable metrics for routine pharmaceutical analysis is an important step toward eco-friendly chromatographic practices, which is pioneered in this study. Furthermore, it fosters responsible innovation in pharmaceutical quality control by ensuring regulatory compliance and analytical reliability.

Keywords Anti-Cold drug mixture, RP-HPLC, Six sustainability metrics, Green solvent, Environmental impact

The most prevalent infectious illness that affects people is the common cold. Sneezing, sore throat, coughing, hoarse voice, and nasal stuffiness and discharge are the main symptoms associated with colds, however most observers consider them to comprise rhinitis symptoms with varying degrees of pharyngitis[1]. Antihistamines in combination with oral decongestants and/or analgesics may offer some relief from cold symptoms, although they have a limited impact on cough. So, decongestants, cough suppressants, and paracetamol are examples of active component combinations that can be used as over-the-counter (OTC) drugs[2]. The common cold and cough symptoms are treated with a mixture of PAR, PSE, and CHL, along with the preservatives MET and PRO in combined dosage form 123 syrup. PAR have analgesic and antipyretic effect which is chemically

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known as *N*-(4-hydroxyphenyl) acetamide) Fig. 1a[3] The potent first-generation alkyl amine antihistamine CHL Fig. 1b, inhibits the H1-receptors, is known as 3-(p-chlorophenyl)−3-(2-pyridyl)-N, Ndimethyl propylamine. Its mild sedative effects make it popular for treating the symptoms of the common cold and allergic rhinitis. PSE is (1 S,2 S)−2-(methylamino)−1 phenylpropan-1-ol hydrochloride Fig. 1c It is a stereoisomer of ephedrine and functions similarly. PSE is taken orally to alleviate the symptoms of nasal congestion. Additionally, it frequently appears in pharmaceutical dosage forms along with other substances that help relieve the symptoms of colds and coughs[4].The nature of medicinal liquid solutions' constituents makes them especially susceptible to microbial development. Preservatives are added to such preparations to protect them against product formulation alterations and deterioration. Therefore, preservatives, such as parabens, are an essential component of liquid dosage formulation due to their antibacterial and antifungal characteristics. Among the alkyl esters of p-hydroxybenzoic acid are parabens, such as MET Fig. 1d and PRO Fig. 1e, which have a long history of application and a low toxicity character. In view of their antimicrobial and anti-fungal attributes, MET and PRO are recognized as good preservatives[5,6].

As the need for efficient and effective over-the-counter medications grows daily, pharmaceutical companies are adding additional ingredients to their dosage forms. Therefore, there is a need for quick, precise, and cost-effective techniques of assessing such mixtures. So different techniques were found in the literature review for determining each drug either alone or in combination with other drugs in pharmaceutical or biological fluids. Fluorescence spectrophotometric[7], CE[8–10], UV-Spectrophotometry[11–15], LC/MS[16,17], and HPLC[18–31] and TLC[32–34]. Based on a

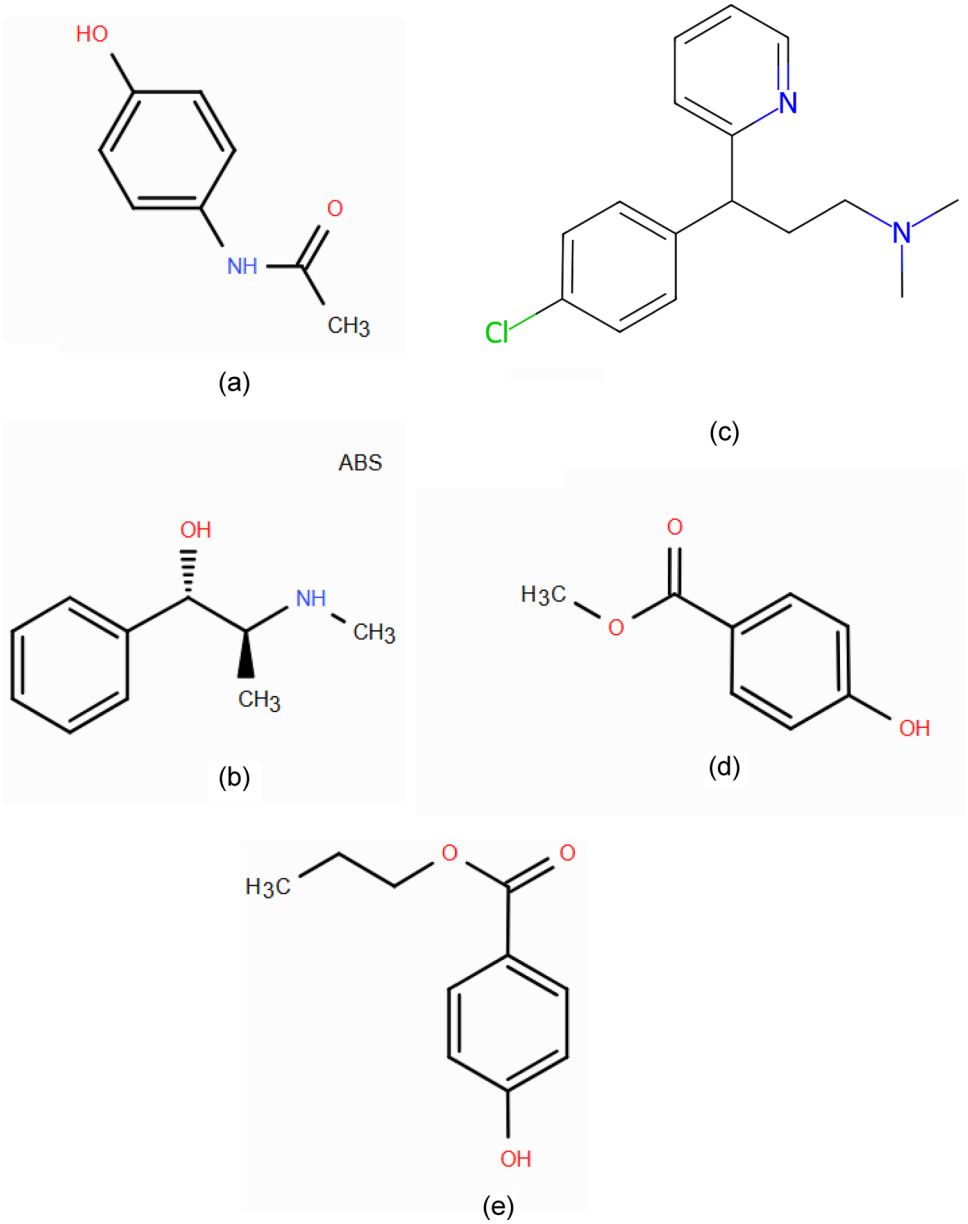


Fig. 1. Chemical structure of (**a**) PAR, (**b**) PSE, (**c**) CHL, (**d**) MET, and (**e**) PRO.

survey of the literature, no study has been found that simultaneously determines paracetamol, pseudoephedrine, chlorpheniramine maleate, and the preservatives methylparaben and propylparaben in a single run using an HPLC technique. Therefore, the goal of this study was to create a straightforward and accurate HPLC-PDA system for the simultaneous measurement of these medications in pharmaceutical dosage forms. Our approach opens up opportunities for analytical solutions that are ecologically conscious, accurately usable, economically viable, and adhere to sustainable development principles while ensuring high levels of quality control.

Results and discussion

Finding sustainable analytical methods that minimize waste and the usage of hazardous materials while preserving high analytical efficiency ranks as one of the primary issues facing pharmaceutical research today. This study sought to develop a practical and safe technique for measuring PAR, CHL, PSE, MET, and PRO concurrently. To the best of the information we have, no alternative methods exist for determining these five medications simultaneously using HPLC-PDA in single dosage form.

Methods development and optimization

To obtain the best resolution and clearly defined symmetry of all eluted peaks within the suitable total time span, a variety of trials were conducted before the stated chemicals were adequately and optimally separated from one another chromatographically.

A wide range of mobile phase combinations were tested; ethanol, methanol, and acetonitrile were the most commonly used organic solvents. Unfortunately, both acetonitrile and methanol showed broad, overlapping peaks with poor separation. But when ethanol was used, peak shape, resolution factor, and retention duration were improved, leading to better separation of the eluted materials. In light of this, ethanol was used as the organic phase. Noise or drifting was eliminated and baseline stability was increased when trifluoro acetic acid was utilized as an aqueous component of the mobile phase rather than other buffer types like phosphate buffer.

Buffer pH (2–5.0): Sharper peaks and less tailing were produced at a pH of 2.2. The peak form weakened with increasing tailing and decreasing theoretical plate count at higher pH values. Therefore, it was discovered that acidic conditions were ideal for producing symmetric peaks and reliable retention.

Additionally, better results were obtained at ambient temperature (about 25 °C) due to the low viscosity of the mobile phase, which eliminated the need to raise the column temperature. A fixed flow rate of 1.0 mL min^{-1} was used. It is remarkable that the chromatographic performance is significantly enhanced when 80% ethanol is used in water as a diluent.

By using an Inert Sustain C18 HPLC Column, 5 µm, 250 × 4.6 mm and Avantor Hichrom C18 (4.6 mm × 150 mm, 5 µm) columns were evaluated. The resolution of the first one was considerably improved. After scanning at multiple wavelengths (210–280 nm) with regard to sensitivity, the ideal detection wavelength for the mentioned compounds was 214 nm for PSE, 244 nm for PAR, and 264 nm for CHL, MET, and PRO. All of the suggested analytes achieved a satisfactory response and increased sensitivity with the least amount of noise. The five substances' ideal chromatographic separation was accomplished under typical chromatographic circumstances. A representative chromatogram of the five medications was displayed in Fig. 2.

Validation of the recommended procedure

The suggested approach was validated in accordance with the principles set by the International Council for Harmonization (ICH)[35].

System suitability

As advised by USP and ICH Q2(R1), standard chromatographic formulae were used to calculate system appropriateness parameters, in which standard solutions were injected six times in duplicate to assess resolution (Rs) values, theoretical plate numbers (N), capacity factor (K′) and tailing factors, the HPLC technique showed outstanding performance. Table 1 details the remarkable reproducibility of peak retention periods with RSD < 2%, demonstrating the chromatographic system's durability.

Ranges of the linearity

Using the chromatographic parameters listed above, the linearity of the suggested HPLC technique was evaluated by analyzing the five analytes at various concentration levels. The concentration ranges of 32–320 µg mL^{-1}, 30–270 µg mL^{-1}, 2–20 µg mL^{-1}, 6–120 µg mL^{-1} and 2–15 µg mL^{-1} yielded acceptable linearity for PAR, PSE, CHL, MET, and PRO, with correlation coefficients (r) of 0.9999, provided lowest standard error estimates. The acquired data is expressed in Table 2.

Sensitivity

Following ICH recommendations, the slope and standard deviation of the responces were used to calculate the LOD and LOQ in order to evaluate the method's sensitivity. The suggested approach has sufficient sensitivity to accurately determine and measure PAR, PSE, CHL, MET, and PRO at minimal concentrations, as shown in Table 2 low LOQ and LOD values guaranteed the suggested HPLC technique's sensitivity and validated its suitability for additional evaluation of the drug's levels in pharmaceutical products.

Accuracy and precision

For each analyte, samples have been investigated at three concentration levels within the specified linearity zone in order to assess accuracy. A range of three levels concentrations were examined for the examined analytes. According to ICH Q2(R1) recommendations, the recovery (%R) of the known concentrations was determined to demonstrate the method's accuracy. Intra-day variability (repeatability) and inter-day variability (intermediate

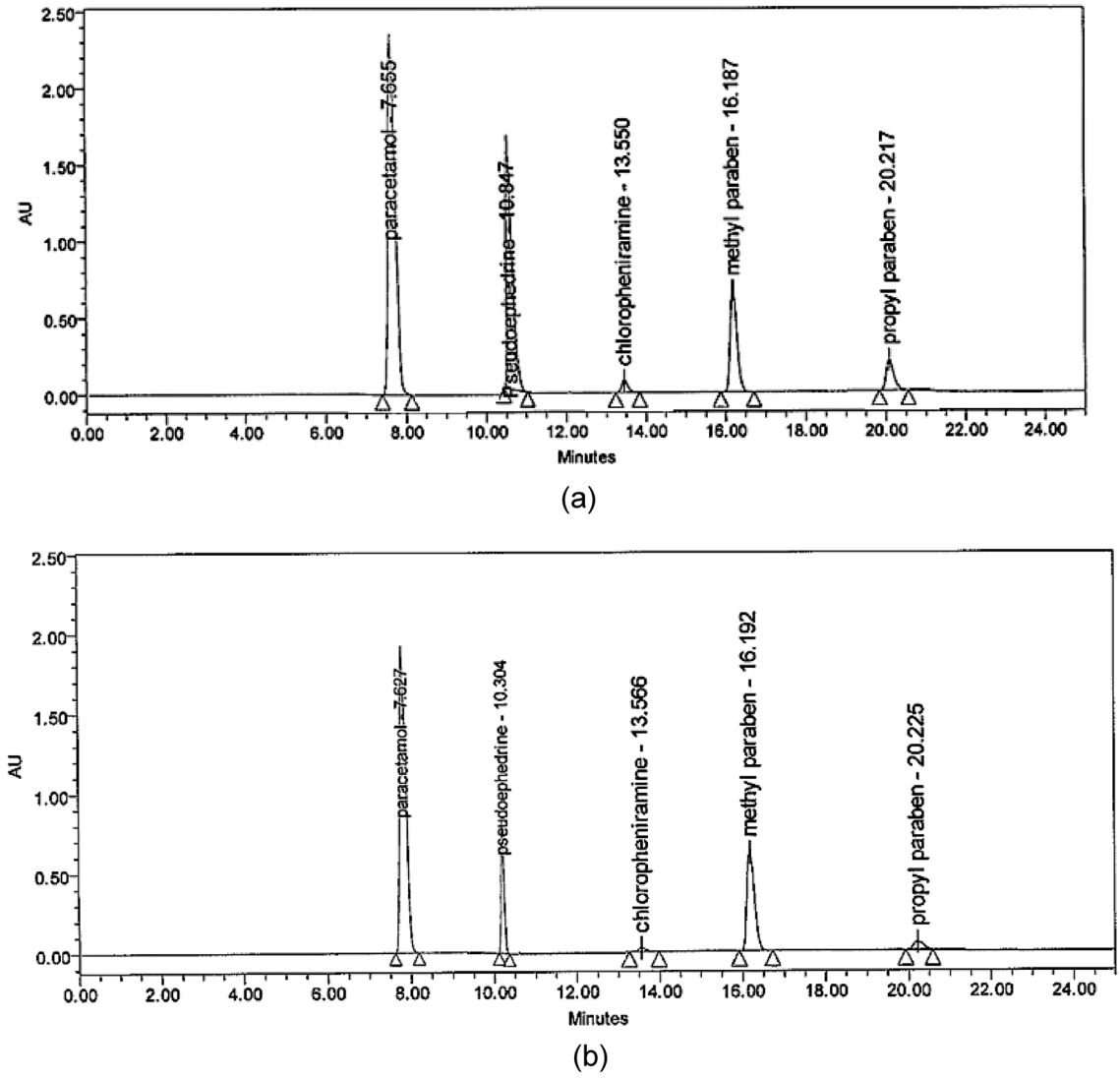


Fig. 2. An analysis of (**a**) chemical compounds and (**b**) dosage form in quinary mixtures using an Inert Sustain C18 column (250 × 4.6 mm, 5 µm) under gradient elution mode, with a green mobile phase of ethanol and 0.1% trifluoroacetic acid (pH 2.2), a flow rate of 1.0 mL/min, and ambient temperature (25 °C). Detection was performed using PDA with time-programmed wavelength scanning: 214 nm for PSE, 244 nm for PAR, and 264 nm for CHL, MET, and PRO, ensuring optimal sensitivity and selectivity for each analyte.

Suitability parameter	PAR	PSE	CHL	MET	PRO
Retention Time (min)	7.655	10.847	13.550	16.187	20.217
% RSD tR (± 10%)	0.4	0.3	0.1	0.1	0.2
Resolution (NLT 2.0)	N/A	4.8	3.7	5.2	6.1
Theoretical plates (NLT 2000)	4282	3890	5632	4768	5106
Tailing factor (NMT 2.0)	1.1	1.3	1.2	0.9	1.0

Table 1. An analysis of factors that affect the suitability of a HPLC system.

precision) were evaluated in order to determine precision. Triplicate analyses at each concentration level were performed within a single day to measure intra-day precision, and three days in successive days were used to evaluate inter-day precision. The results were expressed using the relative standard deviation (%RSD), which was found to be less than 2%, demonstrating the reproducibility and repeatability of the approach under examination (Table 3).

Parameter	Drugs				
	PAR	PSE	CHL	MET	PRO
Wavelength	244 nm	214 nm	264 nm	264 nm	264 nm
Range (µg/mL)	32–320	30–270	2–20	6–120	2–15
Coefficients of determination (R^2)	0.9999	0.9999	0.9999	0.9999	0.9999
Slope (b)	21319.15	32499.97	26091.51	126107.65	115549.60
RSD of the slope (Sb%)	1.8	1.3	0.8	0.5	0.4
Intercept (a)	−1993.16	85715.52	−68.08	18098.75	−38884.94
S_a	5677.97	8872.39	356.07	2087.65	1105.68
S_b	66.95	111.84	67.90	72.35	210.03
$S_{y/x}$	16888.18	24359.33	1093.79	7594.12	2333.38
LOD [a]	0.87 µg/mL	0.90 µg/mL	0.05 µg/mL	0.06 µg/mL	0.03 µg/mL
LOQ [a]	2.66 µg/mL	2.73 µg/mL	0.14 µg/mL	0.17 µg/mL	0.09 µg/mL

Table 2. Investigating the HPLC-based regression for quinary anti-cold drug mixtures.

Parameters	HPLC				
Relative concentrations %	PAR	PSE	CHL	MET	PRO
	*Recovery (%)	*Recovery (%)	*Recovery (%)	*Recovery (%)	*Recovery (%)
50	100.3	101.9	101.9	101.9	99.1
	99.8	102.4	99.2	102.2	98.3
	99.0	101.7	101.3	102.0	98.4
Mean ± RSD	99.7%±0.66	102.0%±0.35	100.8%±1.4	102.0%±0.17	98.6%±0.45
100	98.5	99.2	102.1	100.5	98.5
	98.6	99.5	101.0	100.6	98.5
	99.1	99.5	99.2	100.8	98.2
Mean ± RSD	98.7%±0.33	99.4%±0.17	100.7%±1.5	100.6%±0.15	98.4%±0.18
150	99.3	99.0	101.4	101.1	98.1
	99.1	99.2	102.1	100.9	98.8
	98.9	98.9	100.8	100.9	98.9
Mean ± RSD	99.1%±0.20	99.1%±0.15	101.4%±0.64	101.0%±0.11	98.6%±0.45

Table 3. Evaluation of the presented HPLC method for the trueness and recovery of quinary anti-cold mixtures. * The results represent the average of two injections.

Selectivity

All analytes had outstanding resolution in selectivity tests using mixtures made in the lab. There was no evidence of common excipient interference in the analysis of placebo samples. The examined recovery values of the tested samples, which demonstrated the selectivity of the proposed method. The method's specificity across various matrix compositions was further confirmed by commercial formulation analysis., there is no interference in the chromatograms. The collected data was deemed appropriate and validated the suggested method's selectivity and specificity were illustrated in (Table 4).

Specificity

In a forced degradation study, five active ingredients were used in a quinary anti-cold pharmaceutical formulation: PAR, PSE, CHL, MET, and PRO. A variety of stress conditions were evaluated, including heat, acid (1 N HCl), base (1 N NaOH), and oxidation (3% H_2O_2). Chromatographic analysis was used to quantify the extent of degradation, and purity angles and purity threshold values were used to confirm the method's specificity (Figures S2- S6). According to the results, PSE was the most stable compound under all conditions, with minimal degradation. However, MET is extremely sensitive to alkaline conditions and degrades by over 99% under these conditions. Similarly, PAR and PRO were significantly degraded by acidic and basic conditions, whereas CHL was moderately degraded by oxidation (Figure S1). All purity angles fell below the purity threshold, indicating that the analytical method was effective at separating active ingredients from degradation products. Thus, the method would be suitable as a stability-indicating assay (Table 4).

Robustness

By analyzing the samples under investigation with only little conscious modifications to the authorized chromatographic parameters, the method's robustness was achieved. Factors were investigated: a slight variation in the pH of the mobile phase (± 0.05), and a flow rate (± 0.10 mL min^{-1}). There were no discernible changes

Name	Purity angle	Purity threshold	Amount %	Degradation %	Degradation Peak tR (min)	Drugs
Standard (No degradation)	0.062	0.262	N/A	N/A	7.633	
Heat degradation	0.051	0.261	98.79%	1.21%	7.638	
Acid degradation 1 N	0.086	0.280	73.71%	26.29%	7.631	PAR
Base degradation 1.0 N	0.153	0.355	53.00%	47.00%	6.808	
Oxidation degradation 3.0%	0.055	0.262	98.42%	1.58%	7.631	
Name	Purity angle	Purity threshold	Amount %	Degradation %	Degradation Peak tR (min)	Drugs
Standard (No degradation)	0.911	1.225	N/A	N/A	10.294	
Heat degradation	0.944	1.188	98.78%	1.22%	10.270	
Acid degradation 1 N	0.978	1.218	99.35%	0.65%	10.293	PSE
Base degradation 1.0 N	0.980	1.235	99.00%	1.00%	10.306	
Oxidation degradation 3.0%	0.944	1.187	98.66%	1.34%	10.269	
Name	Purity angle	Purity threshold	Amount %	Degradation %	Degradation Peak tR (min)	Drugs
Standard (No degradation)	0.739	0.985	N/A	N/A	12.498	
Heat degradation	0.786	0.966	98.98%	1.02%	12.483	
Acid degradation 1 N	0.809	0.940	99.00%	1.00%	12.500	CHL
Base degradation 1.0 N	0.666	0.935	96.10%	3.90%	12.459	
Oxidation degradation 3.0%	0.655	0.878	91.80%	8.20%	12.489	
Name	Purity angle	Purity threshold	Amount %	Degradation %	Degradation Peak tR (min)	Drugs
Standard (No degradation)	0.073	0.348	N/A	N/A	16.441	
Heat degradation	0.169	0.346	99.71%	0.29%	16.443	
Acid degradation 1 N	0.141	0.328	89.18%	10.82%	16.454	MET
Base degradation 1.0 N	15.084	17.445	0.81%	99.19%	16.483	
Oxidation degradation 3.0%	0.074	0.346	99.53%	0.47%	16.449	
Name	Purity angle	Purity threshold	Amount %	Degradation %	Degradation Peak tR (min)	Drugs
Standard (No degradation)	0.174	0.385	N/A	N/A	20.745	
Heat degradation	0.402	0.637	97.88%	2.12%	20.755	
Acid degradation 1 N	0.493	0.723	87.90%	12.10%	20.760	PRO
Base degradation 1.0 N	22.603	26.221	58.38%	41.62%	20.748	
Oxidation degradation 3.0%	0.422	0.649	99.57%	0.43%	20.762	

Table 4. Specificity profile of quinary anti-cold mixtures in active pharmaceutical dosage form.

Parameters	HPLC				
Dosage form	**PAR**	**PSE**	**CHL**	**MET**	**PRO**
One Two Three Syrup	Assay (%)	Assay (%)	Assay (%)	Assay (%)	Assay (%)
	100.1	102.2	102.9	104.8	103.4
	100.1	102.4	102.0	104.3	103.6
	98.3	101.7	102.3	104.0	103.7
	100.5	101.7	101.7	104.3	103.6
	97.5	102.1	102.2	104.2	103.5
	101.6	101.6	101.5	104.2	103.4
Mean ± RSD	99.7%±1.5	101.9%±0.3	102.1%±0.5	104.3%±0.3	103.5%±0.1

Table 5. Testing assay of quinary anti-cold mixtures in pharmaceutical dosage form.

to the five compounds' peak areas. According to the findings in Table S1, the averaged RSD was less than 2%. Therefore, the suggested approach can be regarded as dependable method.

Employment in oral Preparation

The concentrations of the APIs in their combined oral dosage form were determined using the established HPLC–PDA method, demonstrating the method's dependability for the analysis of the chosen drugs. With no discernible interference from excipients or formulation additives, the technique showed outstanding selectivity. The suggested method's accuracy and precision were further confirmed by applying a typical standard addition procedure. The results in Table 5 prove the accuracy of the procedure in measuring the active ingredients in oral formulation.

Sustainability assessment

Sustainability is a multifaceted, sophisticated idea that includes cost, performance, safety, waste reduction, and greenness. Evaluating analytical methodologies' eco-friendliness is crucial since they offer essential information about possible effects on the economy and environment. However, sustainability is a wide the opinion that encompasses many different ideas, and no one technique can fully evaluate environmental sustainability across all pertinent factors. As a result, the current study uses an integrated different new tools to allow for a more comprehensive examination from various favorable aspects, such as ecological responsibility, safety concerns, waste minimization techniques, and economic effectiveness[36–42].

MoGAPI metric

The conventional GAPI metric has been replaced by more informative and insightful metrics, such as the recently released Modified Green Analytical Procedure Index (MoGAPI) tool in September 2024[43], comparable to the conventional GAPI, this newly introduced metric looks at the entire analytical approach by examining fifteen different specific elements one at each stage. This includes sampling, followed by method category (qualitative or quantitative), collecting the sample, quantity of the reagents and solvents of choice, the hazards they pose, and the use of energy, prior to getting at the actual amount of waste produced and the possibility of its treatment. Furthermore, it is faster and easier to calculate using free software than the traditional GAPI. The developed method obtained an outstanding environmentally friendly approach rating of 90, as shown in Table 6, after this statistic was applied to it.

Complex MoGAPI metric

A sophisticated hazard assessment tool that improves on conventional ComplexGAPI approaches is the ComplexMoGAPI. By taking into account elements like harmful effects, bioaccumulation, and environmental dedication, it provides an extensive evaluation of chemical dangers and produces a holistic hazard profile. When exact risk assessments are required for regulatory evaluations, this methodology is essential[44]. By creating a total ranking and taking into account a variety of options within each area, it determines the overall environmental

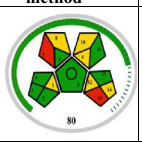
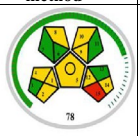
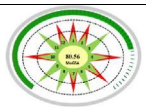
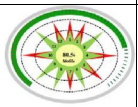
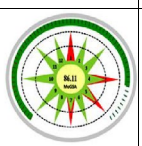
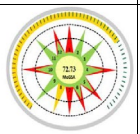
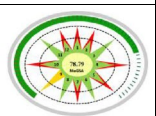
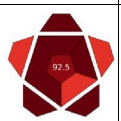
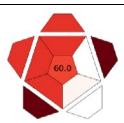
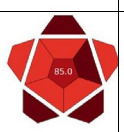
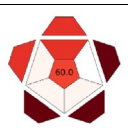
	Proposed method	Reported method [13]	Reported method [14]	Reported method [20]	Reported method [21]	Reported method [26]	Reported method [30]	Reported method [33]
MoGAPI [43]								
Complex MoGAPI metric [45]								
MoGSA metric [46]								
BAGI metric [47]								
RAPI metric [48]								
VIGI metric [50]								

Table 6. Evaluation of potential HPLC methods and published methods from a sustainable perspective.

sustainability of an analytical procedure. Because of the yellow and green measures, the created technique received a score of 95 from ComplexMoGAPI, which indicates significant environmental sustainability (Table 6). The application is available as a free download on bit.ly/ComplexMoGAPI[45].

MoGSA metric

Several factors, including the type of solvents and reagents used, energy consumption, and waste generation, are taken into consideration when evaluating the environmental impacts of chemical operations using the Modified Green Star Area (MoGSA) scale. You may get this application currently at https://bit.ly/MOGSA[46]. MoGSA offers user convenience and accessibility by including open-source software, encouraging more widespread utilization of ecologically friendly chemical techniques. In keeping with the global effort to lower environmental and health hazards in the chemical sector, this creative approach promotes the continual advancement and application of sustainable and eco-friendly chemical processes. According to Table 6, this approach produced a MoGSA value of 96.3, reflecting an excellent degree of environmental compatibility.

BAGI metric

The BAGI determine evaluates 10 important parameters to provide an exhaustive assessment of the "blueness," or usefulness, of an analytical procedure. These consist of the kind of examination carried out, the quantity of analytes in the equipment used, the effectiveness of sample manufacturing, the requirements for sampling, the speed of analysis (the number of samples examined per hour), the relevant reagents and components, the need for preliminary concentration, the level of automated processes, and the volume of sample needed. A scale with 1 representing the minimum and 10 representing the highest is used to evaluate these characteristics[47]. Calculating the geometric mean of the user's scores yields the final BAGI grade.For fast evaluations, contact BAGI's official website at https://bagi-index.anvil.app[41]. With an impressive BAGI score of 75 in this study, our method demonstrated exceptional accessibility (Table 6).

RAPI metric

A new assessment tool called the Red Analytical Performance Index (RAPI) has similar to the BAGI basis. It makes use of an easy-to-understand, freely available Language application (https://mostwiedzy.pl/rapi). The widely accepted ideas, excellent laboratory procedures, and the ICH verification requirements served as a reference for choosing the assessment variables. Since there are many aspects that determine quantitative abilities, we chose the most fundamental and expected measures, i.e., those that are relevant to any variety of statistical techniques[48]. The logo in the form of a star is comparable to but different from the one employed by BAGI. The graphical evaluation of each criterion is shown by measuring the light brightness of various areas on a scale from white (0) to intense red (10).The striking colors used were motivated by the "reds" successive color pattern created for the Matplotib Sect[49].. The method's excellent ability, automated advantages, adequate linear span, and reasonable parameters for validation are all highlighted in its exceptionally high RABI score of 92.5 Table 6.

VIGI metric

The Greenness and Blueness evaluates offer important information on the sustainability of the environment and the usefulness of analytical techniques in real-world situations. The "violetness" VIGI tool, which was launched in 2025, was created especially to measure and illustrate creativity in statistical methods. Ten unique innovation-related factors are evaluated by VIGI and displayed in a decagonal icon producing the visual representation of the results. Sample collection and equipment, data analysis and deployment of software, white chemical analysis acceptance, regulatory compliance, resources and reagent originality, the computerization degree, multifaceted methodology, sensitivity improvement, and scientific novelty are some of the factors that the VIGI regulations uses to assess analytical advancement. The final VIGI number is determined by taking a geometric average of the scores assigned to each requirement, which ranges from 1 to 10[50]. With a score of 85, our evaluation of the suggested methodology using VIGI included remarkable distinctive characteristics as shown in Table 6.

Green assessment of solvent selection using GEARS

To support the use of ethanol as the organic modifier, the Green Environmental Assessment and Rating of Solvents (GEARS) measure was used to compare ethanol with methanol and acetone in ten sustainability measures, which are toxicity, biodegradability, renewables, volatility, thermal stability, flammability, environmental impact, efficiency, recyclability, and affordability. The GEARS score of ethanol was 80% (recommended), whereas that of methanol and acetone was 77% and 73%, respectively (Fig. 3). Ethanol proved to be good in such critical green criteria as low toxicity (LD50 > 2000 mg/kg), superior biodegradability (> 60%), and renewable (> 75%), as well as outstanding thermal stability and recyclability. Even though ethanol is not free of limitations compared to other solvents, including its significant volatility and medium flammability capacity, its overall environmental profile and sustainability benefits make it the most suitable option[51]. Comparatively, methanol had moderate toxicity and substantial flammability hazards. In contrast, acetonitrile had the lowest score because of its excessive toxicity and safety hazard, high efficiency and recycling. These results affirm the use of ethanol as a green substitute for RP-HPLC (Figure S7).

Click analytical chemistry index (CACI)

CACI is a complex indicator intended to assess the feasibility of approaches to analysis outside of environmental factors. CACI is a collection of dimensions as opposed to green indices, otherwise known as BAGI, which concentrate mainly on the concept of sustainability, as it incorporates several dimensions like sample size, complexity of preparation, feasibility, cost per analysis, ability to run multiple analyses, applicability to various

Fig. 3. An analysis of sustainability parameters using GEARS confirms (**a**) ethanol as the most environmentally friendly solvent over (**b**) methanol and (**c**) acetonitrile.

matrices, portability, degree of automation and sensitivity. CACI offers minimal subjectivity to operational efficiency and practicality through a numerical score[52]. The method proposed has a CACI score of 83 (Fig. 4), meaning that it is highly practical in terms of practical application. This score shows that the method is efficient and secure in its application. Major factors that have contributed to this successful performance are that the sample preparation required is minimal. It uses commercially available reagents and normal laboratory tools and equipment and has a minimal per-sample cost (≤ $10). Moreover, the method can be employed to identify a

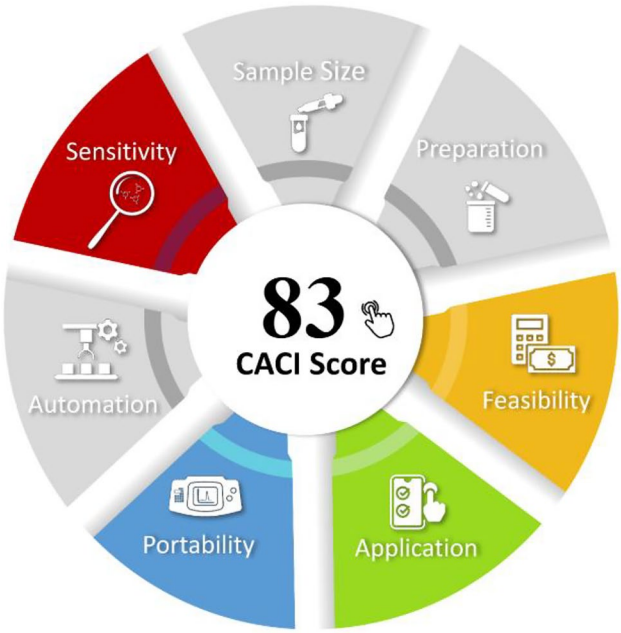


Fig. 4. Evaluation of the proposed method using CACI showed high efficiency, multi-analyte capability, and broad applicability.

variety of analytes (≥ 3) in a variety of matrices (3). This improves its breadth and cost-efficiency significantly over single-analyte analysis methods. Portability, semi-automation, detection below 1% of the target concentration, and other features further confirm its suitability for quality control and regulatory applications (Figure S8). In comparison to BAGI which only focuses on environmental sustainability, CACI offers a broad evaluation of practicality, including feasibility, the range of applications and operation. Having a rating of more than 80 indicates that the technique is not only green, but also very practical and applied to a wide range of situations.

A comparative study of green HPLC against conventional methods

The relative analysis of the suggested green HPLC techniques and methods reported [13,14, 20, 21, 26, 30, 33] earlier shows that there are major differences in the aspects of sustainability, safety and analytical performance. The given method exhibits better adherence to green chemistry principles, with high scores reflected in the various measures of greenness, such as MoGAPI, ComplexMoGAPI, MoGSA, BAGI, RAPI, and VIGI (Table 6). The fact that green and yellow areas dominate in the pictograms of the proposed method shows that environmental impact is minimal. This improves the operator's safety. Alternatively, reported UV-based approaches[13,14] use hydrochloric acid as the solvent which is corrosive and environmentally dangerous leading to reduced sustainability scores. In the same way, HPLC techniques based on extraction procedures (method[26]) and other HPLC techniques [20, 21, 30, 33] that rely on acetonitrile and methanol have numerous red zones, indicating the use of toxic and non-renewable reagents.

Regarding the robustness and greenness views, the proposed approach scored highest in terms of RAPI (92.5) and VIGI scores (85) compared to the reported approaches with scores of between 60 and 87.5 and 60–75, respectively. This highlights the fact that the method is analytically reliable and at the same time environmentally friendly.

By comparing LOD and LOQ values of the target analytes (Table 7), analytical performance was evaluated. The suggested method had a good sensitivity with LOD of 0.03 to 0.90 µg/mL and LOQ of 0.09 to 2.66 µg/mL. Some of the reported techniques have marginally lower LODs (method 13 to PAR at 0.05 µg/mL), however, these techniques tend to not be linked to LOQ. Some of them use hazardous solvents or require complicated extraction procedures and are not practical or sustainable Method[26] obtained very low LLOQs because of sophisticated extraction methods but such methods are expensive, operationally and harmful to the environment.

Generally, the proposed approach to green HPLC provides a reasonable trade-off between performance and environmental friendliness in analysis. Removing the need for corrosive acids, toxic organic solvents and the tedious extraction process, the method operates safely for the operator. It has a less environmental impact, and can still be applied to routine pharmaceutical analyses. These discoveries support the increased interest in sustainability in the development of methodological tools without compromising reliability and accuracy.

Stability toolkit for the appraisal of bio/pharmaceuticals' level of endurance (STABLE)

STABLE is a sophisticated assessment system that is aimed at measuring the stability of pharmaceutical substances under different stress conditions. It is a combination of forced-degradation data, including acidic, basic, oxidative, thermal, and photolytic environments, into one composite score for drug endurance. In addition to numerical points, STABLE determines structural deficiencies and emphasizes key precautions

Method	PAR (µg/mL)	PSE (µg/mL)	CHL (µg/mL)	MET (µg/mL)	PRO (µg/mL)
Proposed Green Method	LOD: 0.87 LOQ: 2.66	LOD: 0.90 LOQ: 2.73	LOD: 0.05 LOQ: 0.14	LOD: 0.06 LOQ: 0.17	LOD: 0.03 LOQ: 0.09
Reported method[13]	LOD: 0.05 LOQ: N/A	LOD: 0.08 LOQ: N/A	LOD: 0.08 LOQ: N/A	LOD: 0.05 LOQ: N/A	LOD: 0.05 LOQ: N/A
Reported method[14]	LOD: 0.09 LOQ: 0.27	LOD: 0.08 LOQ: 0.24	LOD: 0.08 LOQ: 0.24	LOD: 0.07 LOQ: 0.21	LOD: 0.07 LOQ: 0.21
Reported method[20]	LOD: 0.07 LOQ: 0.21	LOD: 0.02 LOQ: 0.05	LOD: 0.46 LOQ: 1.38	LOD: N/A LOQ: N/A	LOD: N/A LOQ: N/A
Reported method[21]	LOD: N/A LOQ: N/A	LOD: N/A LOQ: N/A	LOD: N/A LOQ: N/A	LOD: 0.20 LOQ: 0.65	LOD: 0.42 LOQ: 1.35
Reported method[26]	LOD: N/A LOQ: N/A	LOD: N/A LLOQ: 0.5 ng/mL	LOD: N/A LLOQ: 0.25 ng/mL	LOD: N/A LLOQ: 5.0 ng/mL	LOD: N/A LLOQ: 5.0 ng/mL
Reported method[30]	LOD: 0.92 LOQ: 2.60	LOD: 0.42 LOQ: 1.07	LOD: N/A LOQ: N/A	LOD: N/A LOQ: N/A	LOD: N/A LOQ: N/A
Reported method[33]	LOD: 1.45 LOQ: 4.40	LOD: 0.50 LOQ: 1.51	LOD: N/A LOQ: N/A	LOD: N/A LOQ: N/A	LOD: N/A LOQ: N/A

Table 7. Comparison of LOD and LOQ values for the proposed green HPLC method versus literature-reported methods. * Not applicable (N/A).

related to manufacturing, packaging and storage. This methodology is used to provide the overall picture of any vulnerability of a compound[53]. This facilitates the process of risk reduction and remains in terms of regulatory stability. This is the combined STABLE analysis and forced-degradation analysis (Fig. 5), which gives a detailed picture of the stability profile of the five drugs studied. With a STABLE score of 66 indicating its extreme susceptibility to alkaline (47.00% degradation) and acidic environments (26.29% degradation) and high stability to heat (1.21% degradation), oxidative stress (1.58% degradation), and photolytic stress (no degradation when exposed to sunlight), PAR is highly liable to degradation processes. PSE obtained the highest STABLE scores (71) and exhibited outstanding stability under all stress conditions. Degradation percentages were less than 1.34 even under photolytic stress. CHL with 71 was also not significantly degraded by heat and acid (≈ 1%), base (3.90%), or oxidative (8.20%) and no *light* degradation occurred. MET was the lowest in STABLE (63) because it is most vulnerable to alkaline hydrolysis (99.19), moderate acid degradation (10.82%), and does not degrade under heat, oxidation, or sunlight. PRO scored 67, with a greater degradation rate in the presence of base (41.62%), moderate degrees of acid (12.10%), but was stable in the presence of heat, oxidation and light. In general, alkaline hydrolysis became the most significant stress factor especially in MET, PAR and PRO (Figure S9). Oxidative and thermal conditions had minimal effects and photolytic conditions produced no degradation effect on any compound.

Conclusion

For the first time, a unified green analytical strategy is established in this work for the simultaneous chromatographic analysis of a Quinary medication combination of PA, CH, and PS with the preservatives MP and PP. Moreover, the proposed method integrates these sustainability metrics along with a green mobile phase, making it unique among HPLC methods for evaluating these drugs collectively. Thus, the research we performed constitutes a new addition to medicinal quality control as well as green analytical chemistry. Such methods are appropriate for regular quality control tests because they exhibit outstanding linearity, accuracy, and precision over a broad concentration range. An extensive examination of the sustainability characteristics of the developed method using a range of evaluating tools, such as MoGAPI, Complex MoGAPI, MoGSA, BAGI, RAPI, VIGI, CACI, and GEARS tools, offers an in-depth overview of their efficiency, environmental responsibility, ecological impact, and potential for creativity. According to this, this approach integrates analytical skills with real environmentally conscious procedures to provide a quick, selective, and eco-friendly solution in regular inspection procedures for pharmaceutical testing. In addition to its excellent specificity, the method has also demonstrated its suitability as a stability indicator, with all purity angles remaining below their respective purity thresholds. Furthermore, quantitative stability scores were determined for all compounds by applying the STABLE toolkit under stress conditions.

Experimental

Instruments and common chromatographic conditions

- -The Waters 2998 PDA Detector offers a 190–800 nm range with ± 1 nm accuracy, 1.2 nm bandwidth, and 512 photodiodes. It features a deuterium lamp, 10 mm TaperSlit flow cell (8.4 µL), 1000 psi pressure limit, and ≤ 10 µAU noise. Sampling rate reaches 80 points/sec.
- -The Quinary Mixture (PA, CH, PS, MP and PP) were separated using an Inert Sustain C18 HPLC Column, 5 µm, 250 × 4.6 mm. with a gradient elution of Mobile phase solution A: 0.1% TFA (Trifluoracetic acid), solution B: ethanol (pH 2.2) at a flow rate of 1.0 mL/min. The column oven temperature was regular at 25 °C. Gradient elution was performed, starting with 90% 0.1% TFA for 15 min, followed by a linear decrease to 90% over 0 to 25 min. The whole program for gradient elution is included in Supplementary Table S2.

Fig. 5. An analysis of the overall stability of the drugs (**a**) PAR, (**b**) PSE, (**c**) CHL, (**d**) MET, and (**e**) PRO evaluated under acidic, basic, oxidative, thermal, and photolytic stress conditions.

Chemicals, materials and reagents

- - HPLC-grade ethanol (Cat. No. 459836; ≥99.9% purity) was purchased from Sigma-Aldrich in St. Louis, MO, USA. Because of its superior quality and compatibility with green chemistry principles, it behaved as the main solvent. Using a Milli-Q Advantage A10 water purification system (MilliporeSigma, Bedford, MA, USA), high-purity deionized water was obtained and utilized for all applicable processes and dilute solutions.
- - PAR; purity:98.9%, CHL; purity: 98.5%), PSE; purity: 99.5%w/w, MET; purity: 99.7%, and PRO; purity:99.7% according to the manufacturer's certificate.

– 123 oral suspension (BNo.1927) was a brand-name drug that was studied. Each 5.0 mL contains 160 mg PAR, 1.0 mg CHL and 15.0 mg PSE, 5.0 mg MET and 1.0 mg PRO. The medicine was purchased from a licensed regional pharmacy.

Standard and working solutions
Independent stock solutions of PAR, CHL, PSE, MET, and POR were made by dissolving 100.0 mg of each in 50.0 mL of a mixture of ethanol and water in the following ratios: 20: 80. The same solvent mixture was used to complete each one after it had been sonicated for 15 min. Appropriate volumes of the stock solutions were transferred to distinct 25.0 mL volumetric flasks to create working solutions, which were then diluted to their designated levels using the same mixture of ethanol and water in ratios of 20: 80.

Calibration curves
To assess the linearity range of each drug using various serial dilutions from the stock solutions, five solutions of every compound were prepared, with concentrations ranging from 32 to 320 µg mL^{-1} for PAR, 2–20 µg mL^{-1} CHL, 30–270 µg mL^{-1} PSE, 6–120 µg mL^{-1} MET, and 2–15 µg mL^{-1} PRO. After that, injected into the HPLC system in triplicate in accordance with "Chromatographic conditions stated above" The regression equation was then computed after peak area data were plotted against the five suggested medication concentrations to create a calibration curve for each analyte. Linearity, accuracy, precision, limit of quantitation, limit of detection, specificity, and robustness were all evaluated for the analytical method in compliance with the ICH criteria.

Pharmaceutical Preparation assay
In a 100 mL volumetric flask, 5 mL of the pharmaceutical dosage form (123 oral suspension) was dissolved in 50 mL of diluent. The mixture was then sonicated for five minutes to dissolve it and finished to the appropriate level. A 50 mL volumetric flask was filled with 10 mL of the solution, which was then diluted to volume, combined with a diluent, and filtered using filter paper with 0.45 µm pore sizes.

Application to created laboratory combinations
Within their calibration limits, various synthetic mixtures with various ratios of PAR, CHL, PSE, MET and PRO, were created. Precise aliquots of each analyte stock solution were transferred into 10.00 mL volumetric flasks to obtain these mixtures. The total volume was then adjusted using the mobile phase to achieve different concentration proportions within the specified linearity ranges. The ideal HPLC conditions outlined above were used to properly homogenize and analyze the produced solutions. For every analyte, peak areas were measured and the chromatograms were recorded. Using the corresponding calibration formulae, the concentrations of PAR, CHL, PSE, MET and PRO in the produced mixtures were determined. The percentage recovery (%R) for every analyte was computed in order to assess the procedure's accuracy.

Data availability
All data generated or analyzed during this study are included in this published article.

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Asma S. Al-Wasidi contributed to the conceptualization, data analysis, and supervision of the study. Lateefa A. Al-Khateeb was responsible for methodology development and data curation. Noha S. Katamesh* led the experimental work, formal analysis, and manuscript writing. Afnan S. Batubara contributed to validation and visualization. Hoda A. Ahmed participated in the investigation and review & editing. Mahmoud A. Mohamed contributed to the analytical method development and sustainability assessment. All authors reviewed and approved the final manuscript.

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