RSC Advances

PAPER

View Article Online
View Journal | View Issue

Cite this: *RSC Adv.*, 2026, **16**, 15216

Green RP-HPLC method for the simultaneous determination of octenidine and phenoxyethanol in antiseptic formulations: integration of a lean six sigma approach

Safaa Hussein Salah El-Din,[ab] Amr M. Mahmoud,[c] Sherine S. Diab *[c] and Amany Morsi[b]

Received 29th November 2025
Accepted 10th March 2026

DOI: 10.1039/d5ra09244b

rsc.li/rsc-advances

A green and efficiency-oriented Reversed Phase High Performance Liquid Chromatography (RP-HPLC) method was developed for the simultaneous determination of octenidine dihydrochloride and phenoxyethanol in antiseptic formulations. Unlike previously published studies, the present work offers a fully detailed, optimized, and rigorously validated isocratic method providing simultaneous quantification with a short run time of approximately 8 minutes. Excellent resolution was achieved using an Agilent Zorbax cyanopropyl column (250 × 4.6 mm, 5.0 μm) with a mobile phase of acetonitrile and water (each containing 0.1% trifluoroacetic acid) at 60 : 40 (v/v), a flow rate of 1 mL min^{-1}, and detection at 270 nm. The method exhibited linear ranges of 0.5–3.0 μg mL^{-1} for octenidine dihydrochloride and 3.0–50.0 μg mL^{-1} for phenoxyethanol, and was validated in accordance with the International Council for Harmonization (ICH) guidelines. Greenness assessment confirmed a low environmental impact, while Lean Six Sigma analysis demonstrated excellent efficiency and robustness. The method was successfully applied to pure standards and commercial antiseptic formulations, supporting its suitability for routine quality control.

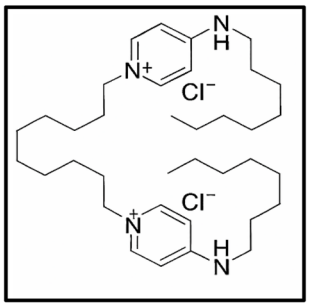
1 Introduction

Antiseptics are chemical agents used on the skin to reduce microbial levels and minimize the risk of surgical site infections. Broad-spectrum antiseptics, which target a wide range of pathogens, are widely favored in dermatologic surgery.[1] Octenidine dihydrochloride [OCT] exhibits a broad antimicrobial spectrum against Gram-positive and Gram-negative bacteria, including methicillin-resistant *Staphylococcus aureus* (MRSA), due to its non-specific yet potent adsorption and interaction with bacterial cell wall and membrane structures. It is also effective against plaque-forming bacteria such as *Actinomyces* and *Streptococcus* spp., *Chlamydia*, *Mycoplasma*, and fungi.[2,3] Octenidine dihydrochloride; [*N*,*N*′-(1,10-decanediyldi-1[4*H*]-pyridinyl-4-ylidene)-bis-(1-octanamine)dihydrochloride], Fig. 1a, is a cationic, surface-active compound. Unlike quaternary ammonium compounds such as benzalkonium chloride and guanidines such as chlorhexidine, OCT does not contain amide or ester functionalities in its molecular structure; therefore, it does not produce the toxic byproduct 4-chloroaniline.[2] Phenoxyethanol [PE]; 2-phenoxyethyl alcohol, ethylene glycol, 2-monophenyl ether, Fig. 1b[4] is a commonly used preservative in both cosmetic and non-cosmetic products. In cosmetics, it serves as a preservative offering antiseptic properties effective against Gram-negative and Gram-positive bacteria, mold, and yeast. Additionally, it is utilized in lubricants, greases, metalworking fluids, coatings, inks, and toners.[5] OCT and PE are used in combination as an antiseptic product for wound treatment and topical skin (0.1% OCT and 2% PE).[6,7] Octenidine-containing disinfectants can be used as an alternative to alcohol-based disinfectant specially during coronavirus disease (COVID-19) pandemic. While the World Health Organization (WHO) recommends frequent hand washing and alcohol-based disinfectants to inactivate severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) by disrupting its lipid membrane, OCT combined with PE can be used as a viable option as it has similar efficacy against enveloped viruses. Unlike alcohol-based disinfectants, this combination is non-irritating, non-allergic and has a prolonged biocidal effect (up to 48 hours) compared to the short-lived action of alcohol. Additionally, it allows easier large-scale preparation, as it can be dissolved in water, reducing the need for transporting flammable alcohol-based solutions.[8] A review of the literature indicates that octenidine dihydrochloride and phenoxyethanol have been analyzed in mixtures using High-Performance Liquid Chromatography (HPLC), though not simultaneously.[6,7]

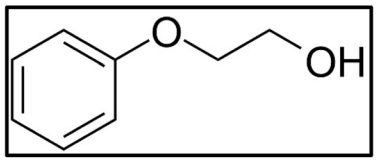
[a]*Postgraduate Program in Pharmaceutical Analytical Chemistry, Faculty of Pharmacy, Cairo University, Cairo 11562, Egypt*

[b]*Egyptian Drug Authority, Cairo, Egypt*

[c]*Pharmaceutical Analytical Chemistry Department, Faculty of Pharmacy, Cairo University, Cairo 11562, Egypt. E-mail: sherine.diab@pharma.cu.edu.eg*



a Octenidine Dihydrochloride Chemical Structure



b Phenoxyethanol Chemical Structure

Fig. 1 (a) Octenidine dihydrochloride chemical structure. (b) Phenoxyethanol chemical structure.

Additionally, these compounds have been individually analyzed alongside their metabolites or in combination with other drugs using various analytical techniques including; ultraviolet (UV) spectrophotometry.[9–11] Fourier Transform Infrared Spectroscopy (FTIR),[12] HPLC,[10,13–17] Ultra-Performance Liquid Chromatography (UPLC),[18,19] Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS),[20–22] and voltammetry.[23]

Previous analytical studies quantified octenidine dihydrochloride and phenoxyethanol separately, and no validated method has been reported for their simultaneous determination. Although one publication mentioned both analytes together, it did not provide chromatographic details—such as retention times, chromatograms, or validation data—to confirm actual simultaneous separation.[6,7] The present work therefore provides the first fully described and truly simultaneous HPLC method for both compounds in a short isocratic run with clear and reproducible resolution. Moreover, this is the first study to comprehensively assess the environmental sustainability of a simultaneous method for this analyte pair using different tools, in addition to incorporating Lean Six Sigma evaluation to confirm operational efficiency and robustness. This combination establishes the proposed method as a novel, practical, and environmentally responsible approach suitable for routine use in analytical laboratories.

The objective of this study is to establish a rapid, isocratic HPLC method capable of accurately quantifying octenidine dihydrochloride and phenoxyethanol simultaneously in their pure forms and in commercial antiseptic formulations. The method was designed to support routine quality control by providing a short run time and reduced solvent consumption, contributing to improved analytical efficiency and lower operational cost. Environmental sustainability was considered throughout method development, the proposed method was validated in accordance with International Council for Harmonization (ICH) guidelines,[24] demonstrating acceptable accuracy and precision.

2 Experimental

2.1 Instrumentation

The Agilent 1260 HPLC system (Agilent, United States) was equipped with a quaternary pump, a diode array detector, and an automatic injector with a 50.0 μL loop. The stationary phase utilized was an Agilent Zorbax Cyanopropyl column (250 × 4.6 mm, 5.0 μm) (Agilent, United States).

2.2. Materials and reagents

2.2.1 Chemical and reagents. Bi-distilled water; from a Milli-Q system (Millipore, Bedford, MA, USA), trifluoracetic acid, acetonitrile HPLC grade and methanol HPLC grade (Fisher Scientific, Loughborough, Leicestershire, UK).

2.2.2 Authentic samples. Phenoxyethanol (99.99%) and octenidine dihydrochloride (99.50%) were purchased from Sigma-Aldrich (St. Louis, MO, USA).

2.2.3 Antiseptic samples. The Octe-Macro Wound Antiseptic Spray (batch no. 01) contains 0.1% OCT and 2% PE, while the Octe-Macro Wound Antiseptic Gel (batch no. 01) contains 0.1% OCT and 1% PE. Both products are manufactured by Macro Pharmaceuticals and were purchased from a local pharmacy in Cairo, Egypt.

Placebo spray and gel matrices, containing all excipients of the respective formulations without OCT and PE, were kindly supplied by the manufacturer.

2.3. Standard solutions

A standard stock solution (1.0 mg mL^{-1}) of OCT and PE was individually prepared using methanol. Subsequently, standard solutions of OCT (10.0 μg mL^{-1}) and PE (100.0 μg mL^{-1}) were separately prepared using a mixture of acetonitrile and water (60 : 40, v/v).

2.4. Laboratory prepared mixtures

Aliquot portions of the standard stock solutions of OCT (10.0 μg mL^{-1}) and PE (100.0 μg mL^{-1}) were transferred into a series of 10 mL volumetric flasks to prepare mixtures in various ratios, including the market ratio. The volume was then adjusted to 10.0 mL using a mixture of acetonitrile and water (60 : 40, v/v).

2.5. Chromatographic conditions

(RP-HPLC) analysis was conducted using an Agilent Zorbax cyanopropyl bonded column (250 × 4.6 mm, 5.0 μm) as the stationary phase. Isocratic elution was performed at room temperature with a mobile phase composed of solution A and solution B in a (60 : 40, v/v) ratio, where solution A consisted of

acetonitrile containing 0.1% trifluoroacetic acid, and solution B comprised water containing 0.1% trifluoroacetic acid. The flow rate was maintained at 1.0 mL min^{-1}, with UV detection at 270.0 nm. Samples were injected in triplicate, with an injection volume of 10.0 μL.

2.6. Procedure

2.6.1 Construction of calibration curves. Aliquots of OCT and PE were separately transferred from their respective standard solutions (10.0 μg mL^{-1} and 100.0 μg mL^{-1}) and transferred into two separate series of 10 mL volumetric flasks. The volumes were adjusted to the mark using an acetonitrile-to-water mixture (60 : 40, v/v) to achieve final concentration of 0.5, 0.6, 0.8, 2.0, 2.5 and 3.0 μg mL^{-1} for OCT and 3.0, 5.0, 10.0, 30.0, 40.0 and 50.0 μg mL^{-1} for PE. A 10.0 μL aliquot of each prepared solution was injected in triplicate under the previously described operating conditions. Calibration curves were constructed by plotting peak area against the corresponding concentrations (μg mL^{-1}), and the regression equations were subsequently determined.

2.6.2 Assay of antiseptic spray and gel. An accurately weighed sample of approximately 0.1 g of Octe-Macro Wound Antiseptic Spray, claimed to contain 0.1% OCT and 2.0% PE, and 0.1 g of Octe-Macro Wound Antiseptic Gel, claimed to contain 0.1% OCT and 1.0% PE, were each transferred into separate 10 mL volumetric flasks. The volume was brought to the mark with methanol, followed by sonication for 5 minutes and filtration through a 0.22 μm NYLON syringe filter. A 1.0 mL aliquot of each filtrate was then transferred into a 10 mL volumetric flask and diluted to the mark with an acetonitrile-to-water mixture (60 : 40, v/v), yielding final concentrations of 1.0 μg mL^{-1} OCT and 20.0 μg mL^{-1} PE for the spray, and 1.0 μg mL^{-1} OCT and 10.0 μg mL^{-1} PE for the gel. Final solutions were analyzed in triplicate using the previously described method. The concentrations were determined based on the corresponding regression equations, and the mean recovery percentage was subsequently calculated.

2.7. Application of six sigma methodology

The process capability index (C_{pk}) is a key statistical tool used in analytical methods to assess the performance and consistency of measurement processes. By evaluating how closely an analytical method operates within its specified limits, C_{pk} enables laboratories and quality control teams minimize variability, improve accuracy, and ensure reliable results. A high C_{pk} value indicates that the method is well-controlled and produces consistent data, even with inherent variations, whereas a low C_{pk} signals the need for method optimization. In analytical applications, a C_{pk} of at least 1.33 (corresponding to 4-sigma level) is typically targeted to meet stringent quality standards and ensure confidence in test results. Regular monitoring of C_{pk} allows analysts to maintain method robustness, reduce errors, and comply with regulatory requirements.[25–27]

2.8. Greenness assessment of the developed method (green metrics)

The adoption of green chemistry principles has become essential in modern chemical laboratories, creating a need for reliable tools to assess the environmental impact of analytical procedures. In this study, three distinct green metrics were employed to evaluate the eco-friendliness of the developed RP-HPLC method: National Environmental Method Index (NEMI),[28,29] analytical eco-scale,[30] and Green Analytical Procedure Index (GAPI).[31–35] Together, these tools provide a comprehensive profile of the method's sustainability by examining factors such as hazardous chemical use, energy efficiency, and waste generation.

2.8.1 National environmental method index (NEMI label). The assessment utilizes a circular diagram divided into four quadrants to evaluate the analytical procedure's greenness. Each quadrant turns green only when its specific condition is met: (1) no chemicals are persistent, bioaccumulative, and toxic (PBT)-listed, (2) none are classified as D-, F-, P-, and U-listed hazardous wastes, (3) the sample pH remains non-corrosive (2–12), and (4) total waste generated is <50 g. Full green shading indicates full compliance with these environmental criteria.[28,29]

2.8.2 Analytical eco-scale. The analytical eco-scale is a semi-quantitative tool that evaluates the greenness of analytical methods using a scoring system ranging backward from 100. A score of 100 represents an ideal green method with no significant environmental impact. Penalty points are deducted based on various non-green aspects of the analytical procedure, including the type and quantity of reagents used, potential hazards, energy consumption, and waste generation. According to this scale, methods scoring below 50 are considered environmentally inadequate, those scoring above 50 are deemed acceptable, and methods achieving scores greater than 75 are classified as excellent green analytical procedures.[30]

2.8.3 Green analytical procedure index (GAPI). It is a pictogram that classify the greenness of each step of an analytical procedure using a color scale: green, yellow and red, representing low, medium and high environmental impact, respectively. In GAPI symbol five pentagrams are used to evaluate and quantify the analytical procedure. Each pictogram is divided into segments, with each segment corresponding to a specific step of the analytical procedure. A segment is colored green when it meets certain environmental criteria.[31–35]

3 Results and discussion

3.1. Method development

OCT and PE are commonly present in antiseptic formulations; therefore, a method for their simultaneous determination is essential for quality control laboratories. An extensive literature review revealed the absence of any reported method for the simultaneous quantification of both drugs. Consequently, we aimed to develop a simple and efficient HPLC method utilizing isocratic elution with a short runtime of 8 minutes. Preliminary chromatographic trials were conducted using conventional

reversed-phase C18 and C8 columns. The C18 column did not achieve satisfactory simultaneous separation of OCT and PE within a single run. In contrast, the C8 column enabled separation of the two analytes; however, very broad peaks and excessively prolonged retention times were observed, resulting in poor chromatographic efficiency and impractically long analysis time. Different organic modifiers were also investigated during method development. Methanol-based mobile phases were evaluated but resulted in prolonged retention times, broad peak shapes, and unsatisfactory separation. Therefore, acetonitrile was selected as the organic component due to its stronger elution power, improved peak symmetry, and enhanced chromatographic efficiency. Given that OCT is a polar compound (a bispyridine-type cationic antiseptic),[36,37] the use of a cyanopropyl bonded stationary phase was subsequently explored. This stationary phase was selected for its multimodal retention properties, which combine moderated hydrophobicity with polar interactions. The cyanopropyl bonded column's reduced hydrophobicity, compared to a conventional C18 phase, prevented excessive retention of the large, hydrophobic octenidine cation, while its cyano group can engage in dipole–dipole interactions and weak hydrogen bonding. These polar interactions are crucial for retaining and resolving the polar functional groups present in both analytes. The pyridine rings of OCT and the ether and hydroxyl groups of PE can interact specifically with the stationary phase, fine-tuning their separation. Water was acidified with trifluoroacetic acid (TFA) to enhance chromatographic performance. TFA served as a key ion-pairing reagent, forming a neutral complex with the protonated octenidine to control its retention and improve peak shape by suppressing undesirable interactions with residual silanols on the stationary phase.[38] This synergistic combination resulted in a selective, efficient, and robust method for the analysis of these two chemically distinct compounds. Various ratios of solution A and solution B were investigated, with optimal separation observed at a (60 : 40, v/v) ratio. The optimum resolution was achieved with a flow rate of 1.0 mL min^{-1}. A detection wavelength of 270.0 nm was selected, as it provided good sensitivity for the simultaneous detection of both OCT and PE. The average retention times were 3.8 ± 0.04 minutes for PE and 7.0 ± 0.02 minutes for OCT, Fig. 2.

3.2. Method validation

The analytical method was rigorously validated following ICH guidelines,[24] with comprehensive evaluation of the following parameters: linearity, specificity, accuracy, precision (repeatability and intermediate precision), robustness, detection limit (LOD), quantification limit (LOQ), and system suitability (as per USP requirements).[4]

3.2.1 Linearity. The proposed method demonstrated excellent linearity over the concentration ranges of 0.5–3.0 μg mL^{-1} for OCT and 3.0–50.0 μg mL^{-1} for PE. Calibration curves were

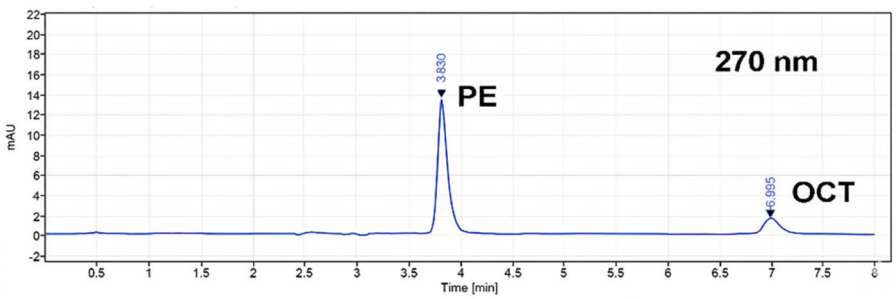


Fig. 2 A typical HPLC chromatogram of a mixture of OCT and PE detection at 270.0 nm.

Table 1 Assay validation and regression data for determining OCT and PE *via* the proposed chromatographic technique

Parameter		OCT	PE
Linearity (μg mL^{-1})		0.5–3.0	3.0–50.0
Regression equation		$y = 28.636x + 0.1947$	$y = 5.5496x + 0.5421$
Correlation coefficient (r)		0.9999	0.9999
Accuracy (mean ± RSD%)		100.06% ± 1.25	99.41% ± 1.28
Laboratory prepared mixtures ($n = 5$)[a]			
LOD (μg mL^{-1})		0.15	1.0
LOQ (μg mL^{-1})		0.5	3.0
Repeatability[b]	Spray matrix	99.52% ± 0.32	99.70% ± 0.63
Mean ± RSD%	Gel matrix	100.34% ± 1.11	99.84% ± 0.49
Intermediate precision[c]	Spray matrix	99.91% ± 0.93	99.71% ± 0.48
Mean ± RSD%	Gel matrix	100.65% ± 1.12	99.90% ± 0.41

[a] Mean of five independently prepared mixtures, each measured in triplicate. [b] The intra-day ($n = 9$), average of nine determinations of spiked placebo repeated three times within day. [c] The inter-day ($n = 9$), average of nine determinations of spiked placebo in three successive days.

constructed using six concentration levels for each analyte, providing adequate data points to reliably establish linearity. The regression analysis revealed high correlation coefficients ($r \geq 0.999$), indicating a strong linear relationship between peak area and concentration within the studied ranges. These results confirm the suitability of the method for accurate quantitative analysis of both analytes in antiseptic formulations, Table 1.

3.2.2 Precision. The precision of the proposed method was assessed through evaluations of repeatability and intermediate precision. Repeatability was determined by conducting the analysis on the same day, using the same analyst and instrument, while intermediate precision was evaluated over three consecutive days. A total of nine determinations were performed using placebo samples spiked with the corresponding standard solutions, with three replicates at each concentration level. For the spray matrix, the concentrations were 0.75, 1.0, and 1.25 μg mL^{-1} for OCT and 15.0, 20.0, and 25.0 μg mL^{-1} for PE. For the gel matrix, the concentrations were 0.75, 1.0, and 1.25 μg mL^{-1} for OCT and 7.5, 10.0, and 12.5 μg mL^{-1} for PE. The concentrations were calculated using the corresponding regression equations, and the percentage recoveries along with the relative standard deviation (RSD%) were determined. The low RSD% values confirm the satisfactory precision of the developed method Table 1.

3.2.3 Accuracy. The accuracy of the developed method was evaluated using two approaches: laboratory prepared mixtures (Section 2.4) and recovery studies. In the laboratory prepared mixtures, known ratios of OCT and PE were analyzed within the studied concentration range. In the recovery study, placebo matrices (spray and gel) were spiked with known amounts of the corresponding standard solutions, and the percentage recoveries were assessed across the concentration ranges of 0.75–2.75 μg mL^{-1} for OCT and 7.5–45.0 μg mL^{-1} for PE. The concentrations were calculated using the corresponding regression equations, and the mean percentage recoveries were determined. The results presented in Tables 1 and 2 confirm the high accuracy of the proposed method.

3.2.4 Limit of detection (LOD) and limit of quantification (LOQ). LOD and LOQ were determined using the signal-to-noise (S/N) ratio approach, corresponding to S/N ratios of approximately 3 : 1 and 10 : 1, respectively. The obtained values in Table 1 indicate the adequate sensitivity of the proposed method.

3.2.5 Specificity. Specificity refers to the ability of the method to accurately quantify OCT and PE in the presence of matrix components typically found in the formulation. Solvent blank, placebo matrices, and commercial formulations were analyzed under the optimized chromatographic conditions. No interfering peaks were observed at the retention times of OCT and PE and adequate baseline separation was achieved with a resolution > 2 between the two analytes. In addition, peak purity analysis using the diode array detector confirmed that the chromatographic peaks of both analytes were spectrally homogeneous with purity values exceeding 995, indicating the absence of co-eluting components. The absence of matrix interference was further supported by recovery study, which showed recoveries ranging from 99.64% to 100.14% for both analytes in both gel and spray matrices. The results presented in Tables 2 and 4 validate the specificity of the proposed method.

3.2.6 Application to commercial antiseptic spray and gel. The proposed methods were successfully applied for the determination of OCT and PE in Octe-Macro Wound Antiseptic Spray and Octe-Macro Wound Antiseptic Gel. The obtained results demonstrated high recovery, aligning with the labeled amounts, as presented in Table 2.

3.2.7 Recovery study. Known concentrations of OCT (0.75–2.75 μg mL^{-1}) and PE (7.5–45.0 μg mL^{-1}) were added to placebo formulations containing all excipients without the active ingredients and analyzed in triplicate using the previously described method. The obtained percentage recoveries in Table 2 were close to 100% with acceptable precision, confirming the accuracy of the proposed method and the absence of matrix interference.

3.2.8 Robustness. Robustness was evaluated by introducing small, deliberate variations in chromatographic conditions, including changes in mobile phase composition (±2% acetonitrile) and flow rate (1.0 ± 0.1 mL min^{-1}). Spiked placebo samples (at 100% of the nominal assay concentration) and standard solutions of the same concentration were analyzed under the modified conditions. The results obtained were compared in terms of precision with those obtained under normal operating conditions. The RSD% values remained low under all tested variations and did not exceed 1.17% for both analytes in spray and gel matrices, confirming that these minor changes had no significant effect on method performance and demonstrating its robustness.

3.2.9 Statistical comparison with reported methods. Statistical analysis was performed to compare the results obtained by the proposed method with those reported[10,13] as

Table 2 Determination of OCT and PE in antiseptic formulations and application of the recovery study using the proposed method

	Drug	
Parameter	OCT	PE
Assay of commercial spray formulation[b] (mean ± RSD%) $n = 6$[a]	100.46% ± 1.33	100.05% ± 0.87
Assay of commercial gel formulation[c] (mean ± RSD%) $n = 6$[a]	100.11% ± 1.28	100.96% ± 1.40
Recovery study (spray matrix) (mean ± RSD%) $n = 6$[a]	100.14% ± 0.80	99.64% ± 0.81
Recovery study (gel matrix) (mean ± RSD%) $n = 6$[a]	100.02% ± 0.81	99.67% ± 1.01

[a] Average of triplicates measurements. [b] Octe-Macro Wound Antiseptic Spray. Batch no. 01. [c] Octe-Macro Wound Antiseptic Gel. Batch no. 01.

Table 3 Statistical analysis of the proposed method and the reported methods of Oct and PE in the antiseptic formulations

Drug	Method	Matrix	Mean ± RSD%	St. dev.	Variance	*N*	*t*-test[a]	*F*-ratio[a]
OCT	Proposed	Spray	100.46% ± 1.33	1.33	1.78	6	0.07	1.17
	Reported[b10]		100.40% ± 1.44	1.44	2.08	6		
	Proposed	Gel	100.11% ± 1.28	1.28	1.64	6	0.01	1.12
	Reported[b10]		100.10% ± 1.36	1.36	1.84	6		
PE	Proposed	Spray	100.05% ± 0.87	0.87	0.76	6	0.92	1.09
	Reported[c13]		100.52% ± 0.91	0.91	0.83	6		
	Proposed	Gel	100.96% ± 1.40	1.41	1.98	6	1.65	2.13
	Reported[c13]		99.81% ± 0.97	0.96	0.93	6		

[a] Theoretical values at $P = 0.05$ ($t = 2.228$, $F = 6.26$). [b] Reported HPLC method for separation of OCT using C18 column, mobile phase consisting of acetonitrile : phosphate buffer (40 : 60, % v/v), pH 6.4, the flow rate 1.0 mL min^{-1} and detection at 285.0 nm. [c] Reported HPLC method for separation of PE using C18 column, mobile phase consisting of acetonitrile : water (50 : 50, % v/v), column oven is 30 °C, the flow rate 1.0 mL min^{-1}.and detection at 270.0 nm.

Table 4 System suitability test results of the proposed RP-HPLC method for determination of OCT and PE simultaneously

Parameter	PE	OCT
(RSD%) < 2	0.12	0.37
Resolution R_s > 2	—	12.47
Tailing factor $T \leq 2$	1.35	1.31
Capacity factor K > 2	2.83	5.99
Plates number N > 2000	5993	8218

presented in Table 3, revealed that there are not any statistically significant differences between the two methods. This conclusion is supported by the fact that the calculated *t*- and *F*-values were lower than the corresponding theoretical values, indicating the reliability and equivalence of the developed method.

3.2.10 System suitability determination. The system suitability parameters were evaluated in accordance with USP guidelines[4] to ensure the proper performance of the HPLC system during analysis. The assessed parameters included precision (RSD%), resolution (*R*), tailing factor (*T*), capacity factor (*K*), and theoretical plates (*N*). The obtained values in Table 4 complied with the recommended acceptance criteria, confirming the adequacy of the chromatographic system.

3.3. Lean six sigma technique

In our study, Minitab Statistical Software (version 14) was used to generate a comprehensive process capability six-pack analysis for the assay data of the finished product. Statistical evaluation revealed excellent process control, as evidenced by *X*-bar and *R* charts consistently maintaining compliance with regulatory specifications. Analysis of 20 samples with 5 subgroups demonstrated homogeneous and random data distribution, confirming process stability. Capability analysis through multiple statistical tools (including process capability charts, probability plots, and histograms) confirmed that our optimized analytical method consistently exceeded the minimum C_{pk} threshold of 1.33. As illustrated in Fig. 3(a and b), the method achieved exceptional C_{pk} values of 1.62 for OCT and 3.74 for PE, providing additional validation of the method's robustness. The results demonstrate that the proposed analytical approach offers substantial improvements in both precision and accuracy, suggesting strong potential for implementation in quality control applications.

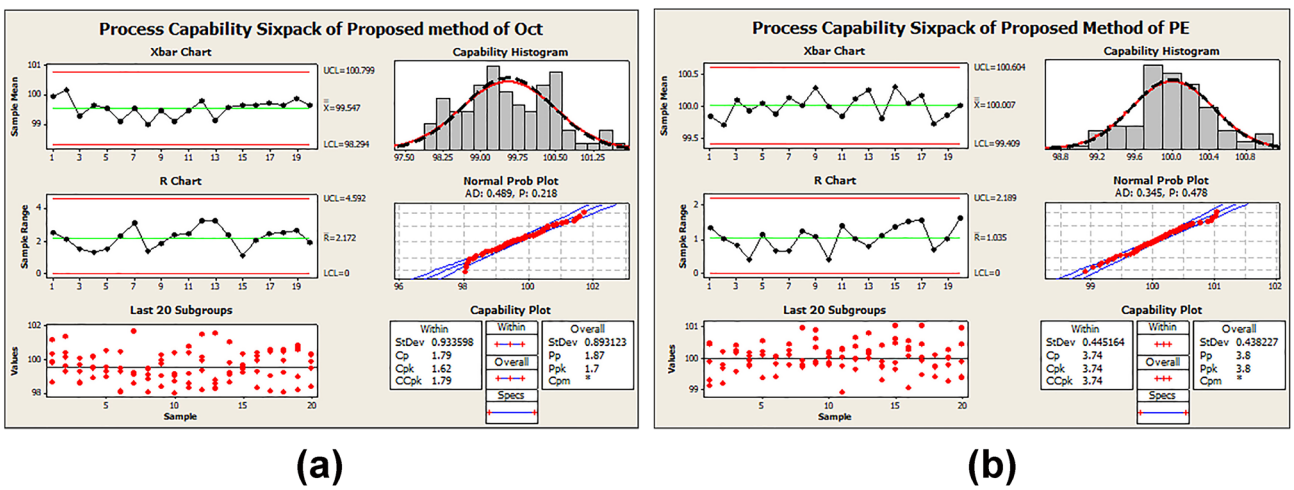


Fig. 3 Process capability six-packs for the proposed method of (a) OCT and (b) PE.

3.4. Greenness assessment of the developed method (green metrics)

3.4.1 National environmental method index (NEMI label). The proposed method demonstrates excellent green chemistry profiles, as evidenced by full compliance with all four evaluation criteria represented in the circular diagram (Fig. 4). None of the chemicals used listed as PBT substances,[39] and all reagents are absent from the D, F, P, and U hazardous waste classifications.[40] Additionally, the sample pH remains neutral (pH 7) and the total waste generation is maintained below 50 grams. This comprehensive adherence to green chemistry principles confirms the environmental sustainability of the method.

3.4.2 Analytical eco-scale. The analytical eco-scale score of 92 indicates that the method is environmentally friendly, exerting minimal impact on health, safety, and the environment, as detailed in Table 5.

3.4.3 Green analytical procedure index (GAPI). The method demonstrates strong alignment with green chemistry principles in most aspects, as reflected by the dominant green areas in the GAPI pictogram, Fig. 5. However, one specific area (marked red) requires attention, and moderate-impact areas (yellow) could be further optimized to enhance sustainability. The results are listed in Table 6.

3.4.4 Comprehensive discussion on greenness. The combined evaluation using NEMI, analytical eco-scale, and GAPI provides a robust and holistic understanding of the environmental profile of the proposed method. Each metric assesses greenness from a different perspective: NEMI offers a quick qualitative screening based on reagent toxicity and waste generation; the analytical eco-scale provides a semi-quantitative score reflecting penalties for hazardous reagents and energy consumption; while GAPI delivers a detailed, pictographic evaluation of the entire analytical workflow. The three tools are highly complementary. NEMI confirms foundational compliance with green principles, the high eco-scale score (92) numerically verifies its excellent environmental performance and operational safety, and GAPI identifies specific aspects that could be further optimized to enhance sustainability. The apparent minor discrepancies, particularly GAPI's identification of yellow/red zones despite the other tools' positive results, do not indicate inconsistency but rather highlight the value of a multi-tool approach; GAPI's granularity reveals opportunities for future refinement that the broader-scope NEMI and eco-scale do not capture. Collectively, the convergent results from these distinct tools provide a defensible and multi-faceted validation, demonstrating that the developed method is not only environmentally sustainable but also represents a safer, greener alternative for routine quality control.

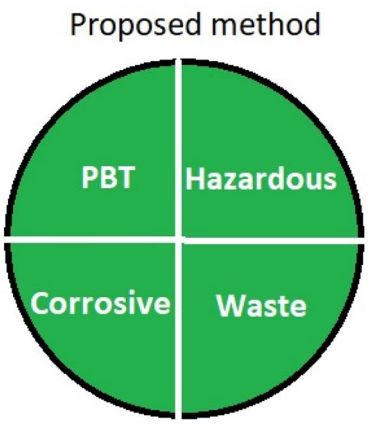


Fig. 4 NEMI pictogram for the proposed HPLC method.

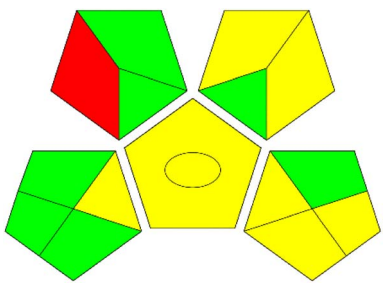


Fig. 5 GAPI pictogram for the proposed method.

Table 5 Analytical eco-scale score for proposed RP-HPLC method for OCT and PE

Method item	Value			Penalty points
Acetonitrile	Amount	4.8 mL	<10 mL	Amount PP × hazard PP 1 × 2 = 2
	Hazard	More severe hazard		
Trifluoroacetic acid	Amount	0.008	<10 mL	Amount PP × hazard PP 1 × 2 = 2
	Hazard	More severe hazard		
pH	2.5			0
Washing and conditioning time	Normal			0
Waste	1–10 mL			3
HPLC	≤1.5 kWh per sample			1
Occupation hazards	Emission of vapor to air			0
Total penalty points				8
Analytical eco scale score	≥75 excellent green method ≥50 acceptable green method <50 inadequate green method			92

Table 6 Green analytical procedure index parameters description for proposed method for determination of OCT and PE

	Proposed method	
Category	Type	Color
Sample preparation		
Collection (1)	At-line	Yellow
Preservation (2)	None	Green
Transport (3)	None	Green
Storage (4)	None	Green
Type of method: direct or indirect (5)	Simple procedure (filtration)	Yellow
Scale of extraction (6)	None	Green
Solvent/reagent used (7)	Non green solvent	Red
Additional treatment (8)	None	Green
Reagent and solvents		
Amount (9)	<10 mL	Green
Health hazard (10)	Moderate toxicity	Yellow
Safety hazard (11)	Flammable	Yellow
Instrument		
Energy (12)	≤1.5 kWh per sample	Yellow
Occupational hazard (13)	None	Green
Waste (14)	<10 mL	Yellow
Waste treatment (15)	Passivation	Yellow
Additional mark: quantification		
Circle in the middle of GAPI	Yes Qualitative and quantitative method	

4 Conclusion

The proposed HPLC method offers significant advantages for the simultaneous determination of OCT and PE, achieving excellent resolution with exceptionally high sensitivity and without interference from each other or from the matrix. Unlike previous methods, it employs isocratic elution with a remarkably short run time of approximately 8 minutes. Statistical comparison with established methods revealed no significant differences in the results, confirming the method's accuracy and reliability. Furthermore, the method was rigorously evaluated using Lean Six Sigma, NEMI, analytical eco-scale, and GAPI tools, demonstrating its environmental friendliness and strong adherence to green chemistry principles. Based on these advantages, the proposed method is highly suitable for routine application in quality control laboratories for both pure substances and antiseptic formulations.

Author contributions

Safaa Hussein: conceptualization, methodology, formal analysis, validation, writing – original draft. Amr M. Mahmoud: writing – review & editing, supervision. Sherine S. Diab: writing – review & editing, supervision. Amany Morsi: writing – review & editing, supervision.

Conflicts of interest

There is no conflict to declare.

Glossary

Accuracy: The closeness of the test results obtained by the method to the true value

Calibration curve: A graphical representation of the relationship between the detector response and known concentrations of analyte

C_{pk}(process capability index): A statistical tool used to measure how well a process is performing relative to its specification limits

Greenness assessment: Evaluation of the environmental impact of an analytical method using green chemistry metrics such as NEMI, eco-scale, and GAPI

Isocratic elution: A chromatographic technique where the mobile phase composition remains constant throughout the run

LOD (limit of detection): The lowest amount of analyte that can be detected, but not necessarily quantified

LOQ (limit of quantification): The lowest amount of analyte that can be quantitatively determined with acceptable precision and accuracy

Octenidine dihydrochloride (OCT): A cationic antiseptic compound with broad-spectrum antimicrobial activity

Phenoxyethanol (PE): An aromatic ether alcohol used as a preservative and antiseptic in pharmaceutical and cosmetic products

Precision: The degree of agreement among repeated measurements under unchanged conditions

RP-HPLC (reversed-phase high performance liquid chromatography): A chromatographic technique using a non-polar stationary phase and a polar mobile phase to separate analytes

Robustness: The ability of an analytical procedure to remain unaffected by small variations in method parameters

Six sigma: A set of techniques and tools for process improvement aimed at minimizing variability and defects

Trifluoroacetic acid (TFA): A strong organic acid used as an ion-pairing agent in chromatography to improve peak shapes

UV detection: A method of detecting compounds in chromatography based on their absorption of ultraviolet light

Data availability

The data supporting this article have been included as part of the supplementary information (SI). Supplementary information is available. See DOI: https://doi.org/10.1039/d5ra09244b.

Acknowledgements

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors. The author declares that there are no acknowledgments to include.

References

1 R. S. Bednarek, A. Nassereddin and M. L. Ramsey, in *StatPearls*, StatPearls Publishing, Treasure Island (FL), 2025.
2 N.-O. Hübner, J. Siebert and A. Kramer, *Skin Pharmacol. Physiol.*, 2010, **23**, 244–258.
3 M. Greener, *Wounds*, 2011, **7**, 74–78.
4 United States Pharmacopeia, *The United States Pharmacopeial Convention*, Rockville, USP 44-NF 39, 2021.
5 M. Kwon, J. B. Park, M. Kwon, J. Song, C. S. Yeo and S. H. Bae, *Arch. Toxicol.*, 2021, **95**, 2019–2036.
6 J. Stahl, M. Braun, J. Siebert and M. Kietzmann, *BMC Vet. Res.*, 2011, **7**, 44.
7 J. Stahl, M. Braun, J. Siebert and M. Kietzmann, *Int. Wound J.*, 2010, **7**, 62–69.
8 B. Clanner-Engelshofen, L. E. French and M. Reinholz, *Eur. J. Dermatol.*, 2020, **30**, 613–614.
9 F. Roustaei, M. Baghdadi, A. Marjani and M. Alimoradi, *Heliyon*, 2024, **10**, e27143.
10 V. Chougule, M. Rajmane, N. Chougule, A. Desai, V. Rohile and N. Inamdar, *Am. J. PharmTech Res.*, 2022, **12**, 179–188.
11 R. Lohar, V. Patil, R. Gaikwad and S. Patil, *World J. Pharm. Pharmaceut. Sci.*, 2016, **5**, 1206–1213.
12 R. Lohar, V. Patil, V. Yeligar and S. Patil, *World J. Pharmaceut. Res.*, 2017, **6**, 1292–1304.
13 G. E. Kızılçay, S. Toker and D. Matur, *Istanbul J. Pharm.*, 2024, **54**, 96–101.
14 Z. M. F. Hui, *J. Environ. Hyg.*, 2024, **14**, 448–452.
15 G. A. Shabir, *Indian J. Pharm. Sci.*, 2010, **72**, 421–425.
16 G. A. Shabir, T. K. Bradshaw, G. Q. Shar and S. A. Arain, *Indian J. Pharm. Sci.*, 2010, **72**, 312–317.
17 M. Borremans, J. Van Loco, P. Roos and L. Goeyens, *Chromatographia*, 2004, **59**, 47–53.
18 T. Jäger, M. Bader, L. Kuhlmann, T. Göen, A. Hartwig, M. a. K. Commission and M. Arand, *The MAK Collection for Occupational Health and Safety*, 2023, vol. 8, p. Doc093.
19 V. Esposito, F. Luciani, A. Battistone, C. von Hunolstein and F. Marino, *Biologicals*, 2021, **69**, 15–21.
20 S. Langen, P. Lötscher, J. Prost, C. Bovet, E. Vögelin and C. Surke, *Int. Wound J.*, 2024, **21**, e14425.
21 T. Jäger, E. Eckert, E. Leibold and M. Bader, *Anal. Methods*, 2022, **14**, 4408–4417.
22 T. H. Kim, M. G. Kim, M. G. Kim, B. S. Shin, K.-B. Kim, J. B. Lee, S. H. Paik and S. D. Yoo, *Talanta*, 2015, **144**, 29–38.
23 M. Jakubczyk and S. Michalkiewicz, *J. Electrochem. Soc.*, 2019, **166**, H291–H296.
24 International Council for Harmonisation, *ICH Harmonised Guideline: Validation of Analytical Procedures Q2(R2)*, 2023.
25 International Organization for Standardization, *ISO 22514-4:2016 Statistical Methods in Process Management—Capability and Performance—Part 4: Process Capability Estimates and Performance Measures*, ISO, Geneva, 2016.
26 T. Y. A. Alanazi, S. M. Mahgoub, H. A. Ahmed, M. A. Almalki, B. R. Alsehli, M. A. Abdel-Reheim, O. A. Mohammed and M. A. Mohamed, *Rev. Anal. Chem.*, 2025, **44**, 20230083.
27 H. M. Nassef, H. A. Ahmed, M. A. El-Atawy, T. Y. A. Alanazi and M. A. Mohamed, *Green Chem. Lett. Rev.*, 2024, **17**, 2301315.
28 M. Tobiszewski, M. Marć, A. Gałuszka and J. Namieśnik, *Molecules*, 2015, **20**, 10928–10946.
29 M. Tobiszewski, *Anal. Methods*, 2016, **8**, 2993–2999.
30 A. Gałuszka, Z. M. Migaszewski, P. Konieczka and J. Namieśnik, *TrAC, Trends Anal. Chem.*, 2012, **37**, 61–72.
31 J. Płotka-Wasylka, *Talanta*, 2018, **181**, 204–209.
32 A. S. Al-Wasidi, H. A. Ahmed, M. F. A. Alshammari, S. S. Nafee and M. A. Mohamed, *Arch. Pharm.*, 2024, **357**, 2400256.
33 H. A. Ahmed, M. A. El-Atawy, H. M. Nassef, M. S. Amin, M. Jaremko, A.-H. Emwas, O. A. Mahmoud and M. A. Mohamed, *Sustain. Chem. Pharm.*, 2024, **38**, 101469.
34 T. Y. A. Alanazi, M. A. Almalki, M. A. Mohamed and H. F. Nassar, *Microchem. J.*, 2023, **194**, 109359.
35 Y. Syrgabek, M. Alimzhanova, P. A. García-Encina, J. J. Jiménez and R. López-Serna, *Trends Environ. Anal. Chem.*, 2023, **39**, e00206.
36 R. Köck, L. Denkel, A. T. Feßler, R. Eicker, A. Mellmann, S. Schwarz, C. Geffers, N.-O. Hübner and R. Leistner, *Pathogens*, 2023, **12**, 612.
37 *Equine Surgery*, ed. J. A. Auer and J. A. Stick, Elsevier, Saunders, St. Louis, Mo, 4th edn, 2012.
38 M. Dong and B. E. Boyes, *LC-GC Eur.*, 2018, **31**, 572–583.
39 *PBT-List Details-SRS|US EPA*, https://cdxapps.epa.gov/oms-substance-registry-services/substance-list-details/156, accessed 27 November 2025.
40 O. US EPA, *Defining Hazardous Waste*, https://www.epa.gov/hw/defining-hazardous-waste-listed-characteristic-and-mixed-radiological-wastes, accessed 27 November 2025.