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ECO-FRIENDLY NON-DERIVATIZED HPLC APPROACH FOR SIMULTANEOUS DETERMINATION OF PREGABALIN, MILNACIPRAN, AND DULOXETINE EMPLOYED IN FIBROMYALGIA TREATMENT

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A novel, eco-friendly high performance liquid chromatography (HPLC) method was developed for the simultaneous determination of pregabalin (PRE), milnacipran hydrochloride (MIL), and duloxetine hydrochloride (DLU), FDA-approved fibromyalgia drugs. This method addresses challenges posed by their structural diversity and PRE's poor UV absorbance. By eliminating derivatization, it avoids hazardous reagents and reduces waste. Separation was achieved on a C18 column using a mobile phase of 45% 0.03M sodium dihydrogen phosphate buffer (pH 3.5), 30% acetonitrile, and 25% methanol at flow rate 0.9 ml/min, with UV detection at 210 nm. The column temperature was 30°C, and the injection volume was 20 µl. Linearity ranges were extended to 100–1600 µg/ml for PRE, 2–40 µg/ml for MIL and DLU, allowing direct dosage form analysis for combined formulations without PRE derivatization, with quantification limits of 91.200, 1.326, and 1.872 µg/ml, respectively. Validated per ICH guidelines and applied to pure drugs and formulations. Greenness assessments using analytical greenness metrics (AGREE) and complementary green analytical procedure index (Complex GAPI) confirmed its sustainability.

Keywords: *High performance liquid chromatography, pregabalin, milnacipran, duloxetine, environmental sustainability*

INTRODUCTION

Fibromyalgia (FM) is a chronic disorder defined by neurological abnormalities. The primary symptoms of this syndrome include muscle and joint stiffness, insomnia, anxiety, depression, and an inability to perform everyday tasks[1,2]. Studies conducted in Europe and South America report a prevalence range of 3.3% to 8.3%, and the likelihood of FM tends to increase with age[3,4] the exact cause and underlying mechanisms of FM remain unidentified[5]. In terms of the disease and treatment approaches, it is advisable to combine nonpharmacologic measures with medications for most FM patients[6]. Medications include tricyclic -pain relievers, selective serotonin reuptake inhibitors (SSRIs), and norepinephrine reuptake inhibitors (SNRIs). Additionally, anticonvulsants such as gabapentin and pregabalin have shown beneficial effects[7]. Three medications have received FDA approval for FM treatment: pregabalin (PRE), duloxetine HCL (DLU), and

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milnacipran HCL (MIL)[8]. Combination therapy has also been authorized, demonstrating increased effectiveness when combining PRE with DLU [9] or PRE with MIL[10].

PRE, chemically described as (S)-3-(aminoethyl)-5-methyl hexanoic acid, has a structure similar to that of γ-aminobutyric acid (GABA). It is used to manage neuropathic pain [11] (**Fig. 1a**).

MIL is an antidepressant drug that is chemically detailed as (2-(amino methyl)-N, N-diethyl-1-phenylcyclopropanecarboxamide) hydrochloride and acts as a serotonin-norepinephrine reuptake inhibitor (SNRI) [12] (**Fig. 1b**).

DLU is chemically detailed as (+) -(S)-N-methyl-3-(naphthalen-1-yloxy)-3-(thiophen-2-yl) propan-1-amine hydrochloride. DLU is an SNRI employed in the treatment of major depressive disorders [13] (**Fig. 1c**).

Previous research has left a gap in the literature regarding the analysis of ternary mixtures involving PRE, MIL, and DLU. Additionally, the published techniques for the simultaneous determination of PRE and DLU are not applicable to pharmaceutical formulations due to their limited linearity within the relevant pharmaceutical concentration range[14,15] . The diverse chemical structures of these drugs, combined with the absence of chromophores in some cases, complicate their determination in complex mixtures. Moreover, existing methods for PRE determination often require derivatization of PRE due to low UV absorbance [16–18]. This step not only adds complexity to the analytical procedure but also raises concerns regarding environmental sustainability, as it may involve the utilization of hazardous reagents and additional waste disposal.

Green analytical chemistry (GAC) is an approach in analytical chemistry that highlights the development and application of techniques that are environmentally sustainable, economically viable, and socially responsible. It incorporates green chemistry principles to lower the environmental impact of analytical processes.[19–27].

In response to these challenges, we aim to develop a method with enhanced linearity that can analyze this ternary mixture without the need for derivatization of PRE, whether in combined or individual dosage forms. The method will focus on minimizing run time and waste production, offering an efficient and environmentally sustainable solution. Our goal is to create a versatile analytical technique that maintains high accuracy and sensitivity while prioritizing eco-friendliness. This approach will not only expand the applications of our method in pharmaceutical analysis but also align with contemporary environmental sustainability goals, promoting safer practices in the laboratory.

An optimized HPLC-UV detection approach was selected for its proven sensitivity, accuracy, precision, and robustness. The approach concentrates on enhanced linearity and reducing run time and waste output. Using the same HPLC column and solvent ratio helps reduce the time needed to achieve equilibrium between the stationary and mobile phases. As a result, this work has established and validated a straightforward HPLC method under consistent conditions for various applications in combined or individual dosage forms.

The analytical greenness metrics (AGREE) and the complementary Green Analytical Procedure Index (complex GAPI) were used to evaluate the method's greenness and ensure that it was environmentally friendly [28–30] .The investigations yielded results that suggest that the process complies with GAC and exhibits environmental sustainability, as per the rules set forth by ICH, 2005[31]. The approach was validated and applied to pure medication powders and pharmaceutical formulations.

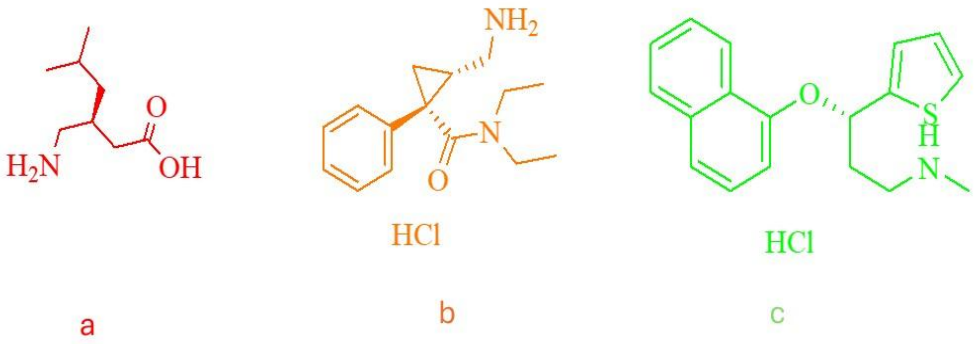

Fig. 1: Chemical structure of PRE (a), MIL (b), and DLU (c).

MATERIALS AND METHODS

Chemicals and reagents

A Millipore water filtration system (Bedford, USA) was used to create double-distilled water. Methanol and acetonitrile of HPLC quality were used. El-Nasr Co. in Egypt provided orthophosphoric acid and sodium dihydrogen orthophosphate monohydrate. PRE, MIL, and DLU were procured from Sigma Aldrich, with certified purities of 100.5%, 99.5%, and 99.2%, respectively.

Pregabid D 75/20® capsules contain 75 mg of PRE and 20 mg of DLU per capsule. The Myodonia 50® tablets included 50 mg of MIL per tablet, the Kimirca 150® capsules contained 150 mg of PRE per capsule, and the Cymbalta 60® capsules contained 60 mg of DLU per capsule and were obtained from a community pharmacy.

Instrumentation

A Shimadzu HPLC system (LC-20AT series, Shimadzu, Japan) with a multiple wavelength detector (SPD-20AD Model), electrical balance, sonicator, pH meter for buffer pH adjustment, and filtration pump was used. The instrument is controlled using LC solution version 1.2 (Shimadzu, Kyoto, Japan) for data preparation and acquisition.

Preparation of Stock and Working Solutions:

PRE

100 mg of PRE was dissolved in 50 mL of the mobile phase and sonicated for 10 minutes, resulting in a 2000 µg/ml stock solution. Working solutions ranging from 100 to 1600 µg/ml were created in 10 mL measuring flasks by withdrawing specific volumes from the stock solution and adjusting the volume to the mark with the mobile phase.

MIL and DLU

A total of 25 mg of each MIL and DLU mixture was dissolved in 100 mL of the mobile phase in two separate measuring flasks and sonicated for 10 minutes, resulting in a stock solution A with a concentration of 250 µg/ml. Stock solution A was further diluted by adding 10 mL and completing the volume with the mobile phase to the mark in a 50 mL measuring flask, resulting in a stock solution B of each drug with a concentration of 50 µg/ml. Working solutions ranging from 2 to 40 µg/ml were created in 10 mL measuring flasks by withdrawing specific volumes from stock solution B and completing the volume to the mark with the mobile phase.

Chromatographic Conditions

Chromatography was performed with a Prontosil ODS C18 column measuring 15 cm by 4.5 mm I.D. with a particle size of 5 µm. The instrument was operated at a flow rate of 0.9 ml/min, with UV detection set at 210 nm. The column was kept at a temperature of 30°C. The mobile phase was composed of 45% (0.03 M) sodium dihydrogen phosphate buffer, customized to a pH of 3.5 using orthophosphoric acid, combined with 30% acetonitrile and 25% methanol. The injection volume was 20 µL.

Pharmaceutical dosage forms

Pregabid D 75/20®

Ten capsules were emptied accurately and weighed; in a 100 mL measuring flask, the powder in an exact amount equal to 75 mg of PRE and 20 mg of DLU was added. The mobile phase was put into the flask to the mark, and it was sonicated for 10 minutes. Following filtering, 1.6 mL of the filtrate was transferred to a 10 mL measuring flask and filled with the mobile phase to the mark.

Kimirca 150 ®

Ten capsules were accurately emptied and weighed, and in a 50 mL measuring flask, the powder in an exact amount equal to 150 mg of PRE was added. The mobile phase was put into the flask to the mark, and it was sonicated for 10 minutes. Following filtering, 5 mL of the filtrate was put into a 10 mL measuring flask, and the mobile phase was filled to the mark.

Myodonia 50®

Ten tablets were accurately weighed and crushed, and in a 100 mL measuring flask, an exact amount of powder equal to 50 mg of MIL was added. The mobile phase was put into the flask to the mark, and it was sonicated for 10 minutes. Following filtering, 2 mL of the filtrate was put into a 100 mL measuring flask, which was filled with the mobile phase to the mark.

Cymbalta 60®

Ten capsules were emptied accurately and weighed. In a 100 mL measuring flask, an exact amount of powder equal to 60 mg of DLU was added. The mobile phase was put into the flask to the mark, and it was sonicated for 10 minutes. Following filtering, 2 mL of the filtrate was put into a 100 mL measuring flask, which was filled with the mobile phase to the mark.

RESULTS AND DISCUSSION

Optimization of the proposed HPLC approach

The optimization of an HPLC method represents a critical stage in guaranteeing dependable and reproducible results within the domain of GAC for the analysis of pharmaceutical compounds. The principal objective was to reduce or eliminate the reliance on hazardous reagents while meeting the system suitability parameters.

Column selection

C18 columns offer high resolution and efficiency for separating complex mixtures, especially when dealing with organic molecules, owing to their long carbon chain and hydrophobic interactions. As the most popular and widely used column, C18 is available in various particle sizes, lengths, and pore sizes, allowing users to select columns that best fit their needs. To minimize solvent usage and create an eco-friendlier approach, we assessed columns with reduced lengths, lower internal diameters, and finer particle sizes such that a Prontosil ODS C18 column measuring 15 cm by 4.5 mm I.D. with a particle size of 5 µm was selected and showed symmetrical peaks and a short analysis time of 4 min.

Wavelength selection

Based on the spectral analysis depicted in (**Fig. 2**) and the improved baseline, a wavelength of 210 nm was selected as the point where all drugs exhibited reliable absorbance. According to the UV spectrum of pregabalin, the compound exhibits very low absorbance, even at high concentrations. However, in the lower UV range (220 nm and below), the absorbance becomes more sensitive to pregabalin. Based on this observation, we selected 210 nm as the optimal detection wavelength. While wavelengths lower than 210 nm could further enhance sensitivity, they would significantly affect the baseline quality. This is supported by the UV cut-off values, with methanol and acetonitrile having cut-offs at 205 nm and 190 nm, respectively. Below these wavelengths, solvent interference becomes a concern. The official pharmacopeial method for pregabalin determination, as outlined in the USP 2020, recommends detection at 205 nm. Blank chromatogram reveals no interference from the mobile phase at the selected wavelength (**Fig. 3**)

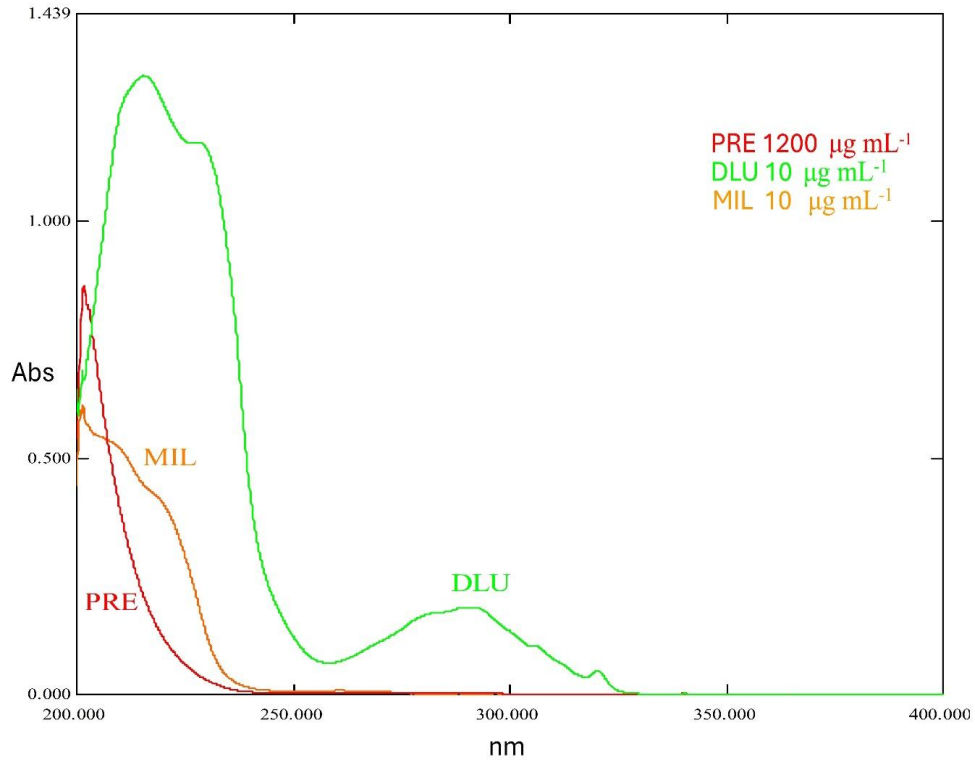

Fig. 2: UV spectrum of, PRE 1200 µg/ml, MIL 10 µg/ml, and DLU 10 µg/ml.

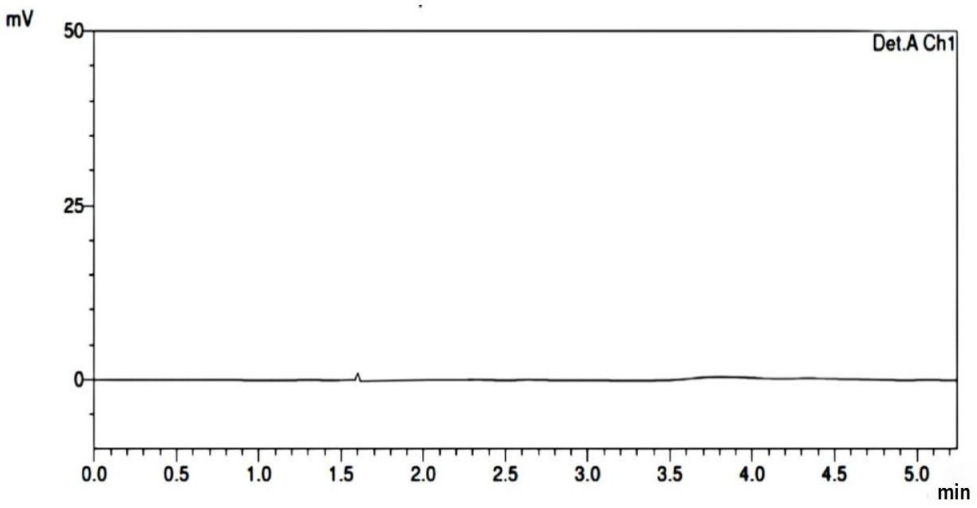

Fig. 3: Blank chromatogram using the specified chromatographic conditions

Mobil phase optimization

The optimization of the mobile phase involved several trials aimed at improving the HPLC method. The different trials used 0.03 M phosphate buffer, and the pH was adjusted to 3.5. In the first attempt, a mixture of 70% phosphate buffer and 30% acetonitrile at a flow rate of 1 ml/min resulted in an extended run time, with DLU showing a delayed retention time of 13.025 minutes and tailing. In the second attempt, a combination of 60% phosphate buffer, 15% acetonitrile, and 25% methanol, also at 1 ml/min, resulted in an even longer run time, with the DLU retention time delayed to 19.206 minutes and still showing tailing. The third attempt, using 65% phosphate buffer, 15% acetonitrile, and 20% methanol with the same flow rate, led to a loss of the duloxetine peak until 20 minutes. The fourth trial, which involved the use of 55% phosphate buffer, 25% acetonitrile, and 20% methanol, reduced the duloxetine retention time to 7.206

minutes. In the fifth attempt, with 55% phosphate buffer, 25% acetonitrile, and 20% methanol, the flow rate was reduced to 0.9 ml/min, and the run time was decreased to approximately 6 minutes. Finally, in the sixth and most successful trial, a mobile phase comprising 45% phosphate buffer, 30% acetonitrile, and 25% methanol at a flow rate of 0.9 ml/min yielded favorable results. This setup achieved a concise run time of less than 5 minutes and satisfactory system suitability parameters in terms of resolution

tailing factors and theoretical plate counts, making it the chosen option for the optimized mobile phase in alignment with GAC principles. The combination of acetonitrile 30% and methanol 25% offered a balanced elution strength and polarity. Acetonitrile, being less viscous and more eluting, resulted in sharper peaks and shorter retention times. Methanol fine-tuned the selectivity, especially between closely eluting analytes, contributing to enhanced resolution and reduced peak tailing. Phosphate Buffer 45% maintained a stable pH and played a critical role in maintaining consistent ionization and solubility of the analytes. This was particularly important for the basic drugs duloxetine and milnacipran, as well as for the highly polar pregabalin. The buffer also supported stable interaction with the stationary phase and contributed to improved peak shapes. pH was particularly suited to the characteristics of each compound:

Buffer concentration and pH selection

The impact of pH was examined throughout the pH range of 3.0-4.5 by employing 0.03 M sodium dihydrogen phosphate buffer in the mobile phase. Duloxetine & Milnacipran (pKa 9–10): Both are basic compounds and remain fully protonated at pH 3.5. This minimizes variability in ionization and suppresses interactions with residual silanol groups, thereby reducing peak tailing and improving symmetry Pregabalin zwitterionic (pKa 4.2 and 10.6). At pH 3.5, the carboxyl group is mostly protonated (neutral), and the amino group remains protonated (+ve), resulting in a net positive charge. Higher pH would lead to increased ionization, excessive polarity. This moderate polarity facilitates adequate retention on the C18 column and improves peak shape Overall, pH 3.5 provided an optimal balance for all three analytes—ensuring sufficient retention for pregabalin while enhancing peak shape and resolution for duloxetine and milnacipran.

Furthermore, the effects of different buffer concentrations were assessed. Among the investigated concentrations, peak forms that were well resolved were produced by 0.0٣ M buffer.

Flow rate and column temperature selection

In HPLC, there is a general trend that increasing the flow rate and reducing the column length tends to decrease the retention time. However, it is essential to assess the potential impact on system suitability parameters carefully. During optimization, the flow rate changed from 0.8 to 1 ml/min, 0.9 ml/min was the ideal flow rate since it resulted in shorter retention time and improved peak outlines. Temperatures below 25°C can lead to longer run times and reduced chromatographic efficiency, while temperatures above 40°C may increase the risk of analyte degradation. Therefore, a column temperature of **30°C** was selected as an optimal compromise to ensure both efficiency and analyte stability.

System suitability parameters

System suitability testing is a fundamental component of chromatographic techniques.[32–34]. Parameters such as the resolution (Rs), number of theoretical plates (N), and tailing factor (T) were measured to validate the proper operation of the HPLC method. The results of the system suitability parameters were computed, as shown in (**Table 1**), whereas the found values conformed to reference values, indicating good results[35].The chromatogram obtained after applying the optimized HPLC method is depicted in (**Fig. 4**).

Validation and applications

The optimized HPLC approach was validated in accordance with the ICH,2005 guidelines[31], as shown in (**Table 2**).

Table 1: Parameters required for the system suitability test of the HPLC method.

`Parameters	PRE	MIL	DLU	Reference value [35]
Retention time (Rt)	1.989	2.533	3.932	-
Resolution (Rs)	-	3.196	6.946	>1.5
Number of theoretical plates (N)	2054.101	3788.870	4357.588	>2000
Tailing factor (T)	1	1	1.634	<2

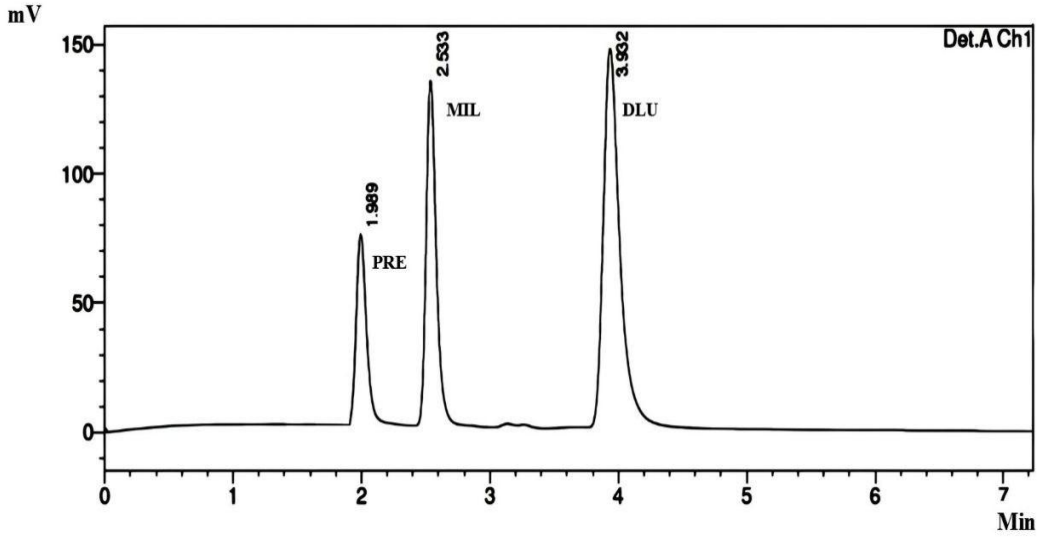

Fig. 4: HPLC chromatogram of PRE 800 µg/ml, MIL 10 µg/ml, and DLU 10 µg/ml.

Table 2: Regression parameters and validation of PRE, MIL and DLU by the proposed method.

Parameters		**PRE**	**MIL**	**DLU**
Linearity (µg/ml)		100-1600	2-40	2-40
Regression equation		486.970x + 2939.400	69596x + 6331.5	154686x + 12881
Correlation coefficient r		0.9999	0.9999	0.9999
Accuracy (Mean ± SD) [a]		100.295 ± 1.850	99.911 ± 1.130	99.533 ± 1.987
LOD (µg/ml)		30.300	0.438	0.618
LOQ (µg/ml)		91.200	1.326	1.872
Intraday Precision RSD% [b]		1.123	0.147	0.300
Interday Precision RSD% [C]		1.370	0.230	0.353
	Flow rate	1.852	0.852	1.521
	pH	1.130	0.750	1.240
	Buffer concentration	1.850	0.845	1.720

(a) Average of five determinations.
(b) Repeatability (n=9), average of three concentrations of PRE (200,800,1200 µg/ml), MIL (4,7.5,15 µg/ml), and DLU (4,7.5,15 µg/ml) repeated three times within the day (intra-daily).
(c) The inter-daily Precision (n=9), average of three concentrations of PRE (200,800,1200 µg/ml), MIL (4,7.5,15 µg/ml), and DLU (4,7.5,15 µg/ml) repeated three times on three successive days.
(d) Robustness (slight alteration to the method) (n=9).

Linearity

Accurate aliquots of PRE, MIL, and DLU were extracted from their respective working standard solutions and subsequently inserted into three distinct sets of 10 mL measuring flasks. The volume of each flask was then adjusted exactly to the mark value via the mobile phase to produce solutions with concentrations ranging from 100–1600, 2–40 and 2–40 µg/ml respectively. The solutions were injected into the HPLC system using the established chromatographic settings. To establish a relationship between peak area ratios and concentrations µg/ml, calibration curves were constructed, and regression equations were calculated (**Fig. 5**).

Accuracy

The accuracy of the HPLC approach was estimated by determining the concentrations of various pure samples of PRE, MIL, and DLU via the corresponding regression equations. The calculated recovery percentages revealed excellent accuracy.

Precision

Repeatability (intraday variation) was assessed by repeating the assay at three different concentrations in triplicate on the same day (200, 400, and 800 µg/ml) for PRE and (4, 7.5, and 15 µg/ml) for MIL and DLU. The percentage of relative standard deviation (RSD%) was calculated and revealed outstanding intraday precision.

Intermediate precision (interday variation) was assessed by determining solutions of the same concentrations as those used for repeatability on three successive days. The RSD% was computed and revealed outstanding interday precision.

Specificity

By applying the described approach to analyze multiple laboratory-created mixtures comprising the components under study in varying ratios within the linearity range, specificity was guaranteed. By computing the % recoveries and evaluating the tiny standard deviation (SD) values, as indicated in (**Table 3**). There is no evidence of excipients' interference in the applications. The commonly used excipients in these dosage forms, such as starch, microcrystalline cellulose, magnesium stearate, croscarmellose, sodium starch glycolate, and titanium dioxide, are insoluble in the mobile phase and therefore do not interfere with measurements or even soluble ones such as mannitol and sorbitol showing negligible absorbance at the selected wavelength. The specificity of the procedure was verified.

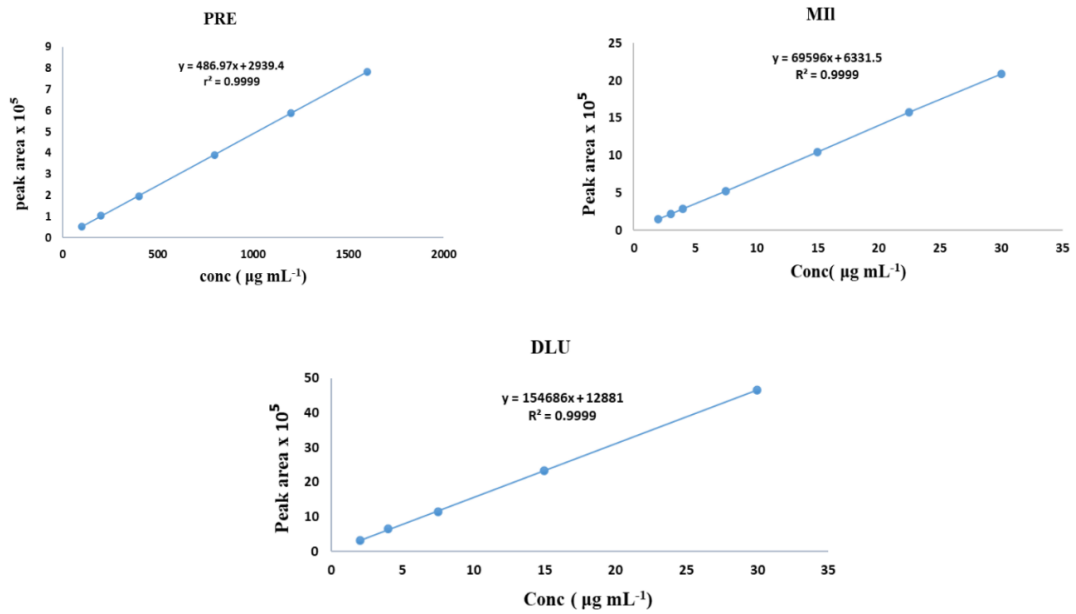

Fig. 5: calibration curves for PRE (100-1600) µg/ml, MIL (2-40) µg/ml, and DLU (2-40) µg/ml.

Table 3: Results of laboratory-created mixtures of PRE, MIL, and DLU by the proposed Method.

	PRE (µg/ml)	R	MIL (µg/ml)	R	DLU (µg/ml)	R
	200	100.968	6	100.356	10	99.320
	400	98.315	30	99.256	15	98.652
	600	101.558	10	101.256	25	100.236
	1000	99.217	20	100.654	4	101.810
SD		**1.507**		**0.838**		**0.795**

Detection and quantitation limits

The following formulas were employed to determine the limits of detections (LOD) and quantification (LOQ): LOQ = 10 (SD of regression residuals/slope) and LOD = 3.3 (SD of regression residuals/slope). The strong sensitivity of the suggested HPLC method has been proven by its low LOD and LOQ values.

Robustness

Robustness was assessed by intentionally altering the experimental conditions; small changes in pH (3.5 ± 0.1) ,buffer concentration (0.03 M ± 0.002 M), and flow rate (0.9± 0.05) ml/min were considered, while other factors were held constant. The areas of the studied drugs were recorded under these altered conditions, and the results were expressed as RSD% and revealed reliable results.

Applications to pharmaceutical formulations and utilization of the standard addition approach

The designed chromatographic techniques were successfully used to determine Pregabid D 75/20®, Kimirca 150®, Myodonia 50®, and Cymbalta 60®. This demonstrated the suitability of the recommended techniques for determining PRE, MIL, and DLU without the interference of any excipients or contaminants that might be present in pharmaceutical formulations, and the application of the standard addition approach to pharmaceutical formulations was employed; the results are presented in (**Table 4**).

Table 4: Results of multiple pharmaceutical formulations and applications of standard addition technique.

Pharmaceutical[a]	Mean ± SD			Application of standard addition techniques					
	PRE (µg/ml)	MIL (µg/ml)	DLU (µg/ml)	PRE (µg/ml)		MIL (µg/ml)		DLU (µg/ml)	
				added	R	added	R	added	R
`Pregabid D 75/20®	98.235 ± 1.152		99.569 ± 0.953	200	98.65			4	99.454
				300	99.56			6	100.23
				400	100.3			8	100.74
				Mean ± SD	99.486 ± 0.800			**Mean ± SD**	100.141 ± 0.648
Kimirca 150®	99 ± 0.953			200	99.65				
				300	98.57				
				400	100.3				
				Mean ± SD	99.512 ± 0.886				
Myodonia 50®		98.1 ±1.235				4	99.562		
						6	100.26		
						8	98.565		
						Mean ± SD	99.462 ± 0.851		
Cymbalta 60®			101.2 ±.652					4	101.36
								6	99.2
								8	100.21
								Mean ± SD	100.257 ± 1.086

(a) Average of three determinations.

Evaluation of Greenness

Agree

It stands out as the current leading metric for evaluating eco-friendliness via analytical methods. It boasts comprehensiveness by encompassing all 12 principles of GAC. What makes AGREE particularly attractive is its flexibility, allowing for the application of weights to the principles, its user-friendly nature (resulting in a color-coded pictogram), and its ease of use facilitated by freely available software.

The 12 crucial fundamentals of GAC are embedded in the input parameters of AGREE, and users have the flexibility to assign various weights to these parameters, providing an added layer of customization. These input parameters are then transformed into a final score ranging from 0 to 1. The graphical representation of the result looks like a clock, with a central score and color indicative of the final assessment. The color can range from dark green (score of 1) to dark red (score of 0)[28]. The resulting graph score (0.66) in (**Fig.** 6) shows the remarkable eco-friendliness of the proposed method, achieving a high score, indicated by a color closer to dark green, which signifies the method's superior adherence to green analytical concepts.

Complex GAPI

Recently, complex GAPI has emerged as a noteworthy semiquantitative tool, gaining considerable consideration, confidence, and approval in the chemical society. This tool enhances the traditional GAPI metric by introducing an additional hexagonal area into the original GAPI graph. Its foundation lies in CHEM21 parameters, encompassing the different steps and procedures occurring before the general analytical methodology and the final analysis. This means that each step of an analytical method can be thoroughly assessed, including sample gathering, transportation, preservation, storage, preparation, and preliminary procedures prior to the actual analysis.

A notable advantage of Complex GAPI is its user-friendly interface, facilitated by shareware software designed for generating Complex GAPI pictograms. These pictograms transition through colors, from green to yellow to red, offering a visual representation for the assessment and quantification of every step before the general analytical methodology and the final analysis[29].

Remarkably, the method proposed in this context is deemed environmentally friendly, as evident from the green pictograms and the E-factor. The E factor, which serves as a measure of the efficiency of a chemical process, is lower in the present method as displayed in (**Fig. 7**). This signifies reduced waste generation, improved environmental impact, and enhanced sustainability. Taking together, these attributes highlight the superior eco-friendliness of the suggested method, which aligns with the fundamentals of GAC.

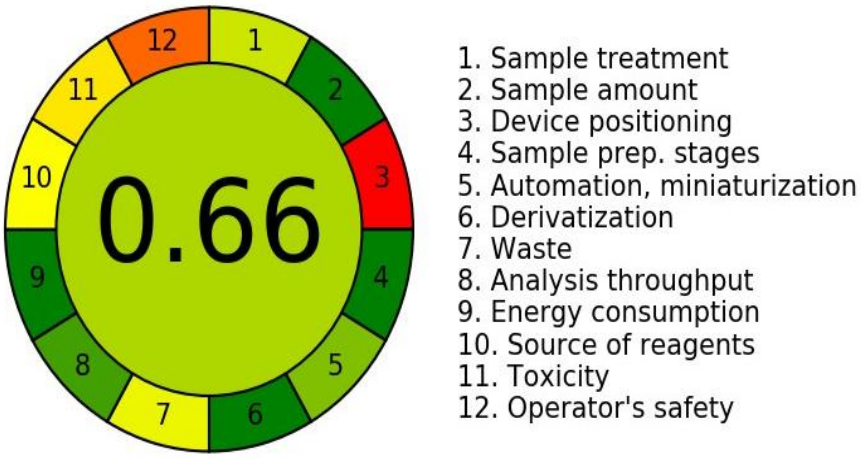

Fig. 6: Assessment of the greenness of the method using AGREE tool.

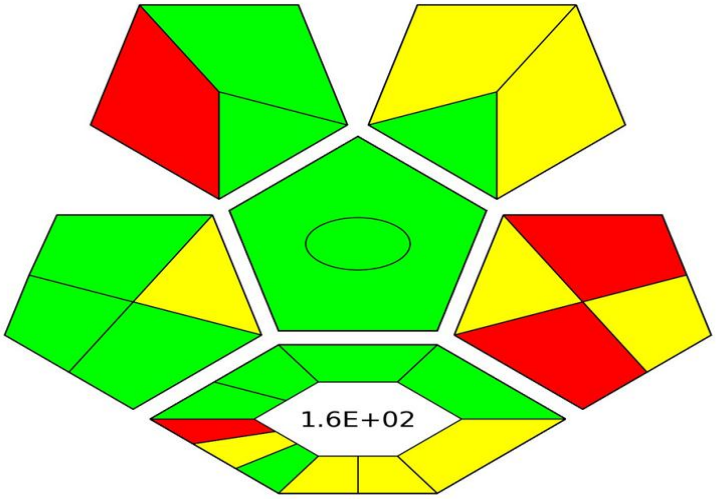

Fig. 7: Assessment of the greenness of the method using complex GAPI tool.

Comparative Study And Statistical Analysis

The Proposed Method offers better performance in terms of linearity**,** sensitivity (LOD and LOQ)**,** retention time, and waste production compared to the Reported Methods[11-13] as detailed in **(Table 5).** A statistical comparison was made between the findings from the published techniques of analysis[11-13] and the optimized HPLC approach for pharmaceutical formulations. As indicated in (**Table 6**), T and F tests verified that there was no discernible difference between the new approach and the published ones.

Table 5: Comparison of Analytical Parameters for Proposed and Reported Methods.

Parameter	Proposed Method	Reported Method [11] (PRE)	Reported Method[12] (MIL)	Reported Method [13] (DLU)
Linearity (µg/ml)	PRE: 100-1600 MIL: 2-40 DLU: 2-40	200-800	10-60	12-60
Mobile Phase	45% 0.03M NaH_2 PO_4 (pH 3.5), 30% Acetonitrile, 25% Methanol	Acetonitrile, Buffer (30:70)	Water, Methanol (55:45)	Phosphate Buffer, Acetonitrile Methanol (50:30: 20)
RT (min)	PRE: 1.989 MIL: 2.533 DLU: 3.932	3.1	~2.8	11.03
λ (nm)	210 (all)	210	254	231
LOD (µg/ml)	PRE: 30.300 MIL: 0.438 DLU: 0.618	-	3.08	0.432
LOQ (µg/ml)	PRE: 91.200 MIL: 1.326 DLU: 1.872	-	9.35	1.112

Table 6: The statistical analysis results between the proposed and reported methods on pharmaceutical formulations.

	Proposed method			Reported method [11]	Reported method [12]	Reported method [13]
	PRE	MIL	DLU	PRE	MIL	DLU
Mean	99.537	99.842	99.165	99.628	99.692	98.93
SD	0.734	0.979	1.201	0.868	1.148	1.032
Variance	0.539	0.96	1.443	0.753	1.318	1.06
N	5	5	5	5	5	5
Student t-test	0.441	0.415	0.151			
F	1.400	1.748	1.355			

The theoretical t-and F-values at P=0.05 were 2.31 and 6.39; respectively.

Conclusion

A novel environmentally friendly HPLC method has been created for the simultaneous estimation of a ternary mixture of FDA-approved medications—PRE, MIL, and DLU—used to treat fibromyalgia. This method eliminates the need for derivatization of PRE, focusing on reducing run time and minimizing waste generation, which aligns with modern sustainability goals. The method underwent validation according to ICH principles and demonstrated characteristics such as simplicity, sensitivity, accuracy, precision, and robustness, making it suitable for various applications. Additionally, an assessment of the method's environmental influence was conducted via AGREE and complex GAPI assessments. The results were found to be acceptable, confirming the method's environmental sustainability.

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طريقة كروماتوغرافيا سائلة عالية الأداء صديقة للبيئة بدون اشتقاق للتحديد المتزامن للبريجابالين، ميلناسيبران، ودولوكستين المُستخدمة في علاج الألم العضلي الليفي

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تم تطوير طريقة جديدة وصديقة للبيئة باستخدام تقنية الكروماتوغرافيا السائلة عالية الكفاءة لتحديد كميات ثلاثة أدوية معتمدة لعلاج الألم العضلي الليفي في نفس الوقت. هذه الأدوية هي البريجابالين، ميلناسيبران هيدروكلوريد، ودولوكستين هيدروكلوريد. تعالج هذه الطريقة الصعوبات الناتجة عن الاختلاف في التركيبات الكيميائية لهذه الأدوية وضعف قدرة البريجابالين على امتصاص الأشعة فوق البنفسجية. من خلال تجنب خطوات التعديل الكيميائي، تم تجنب استخدام المواد الكيميائية الضارة وتقليل النفايات.

تم عملية الفصل باستخدام عمود من نوع C18 مع خليط من ثلاثة مكونات: محلول فوسفات الصوديوم بتركيز ٠،٠٣ مولار ودرجة حموضة ٣،٥ بنسبة ٤٥٪، وأسيتونيتريل بنسبة ٣٠٪، وميثانول بنسبة ٢٥٪، بمعدل تدفق ٠،٩ مل لكل دقيقة. تم الكشف عن المواد عند طول موجي ٢١٠ نانومتر. كانت درجة حرارة العمود ٣٠ درجة مئوية، وحجم العينة المحقونة ٢٠ ميكرولتر.

تم توسيع نطاقات القياس لتتراوح بين ١٠٠ إلى ١٦٠٠ ميكروغرام لكل مللتر للبريجابالين، وبين ٢ إلى ٤٠ ميكروغرام لكل مللتر لكل من الميلناسيبران والدولوكستين. هذا يسمح بتحليل الأشكال الدوائية مباشرة دون الحاجة إلى تعديل البريجابالين كيميائيًا، مع حدود كمية تبلغ ٩١،٢٠٠، ١،٣٢٦، و١،٨٧٢ ميكروغرام لكل مللتر على التوالي. تم التحقق من دقة الطريقة وفقًا للمعايير الدولية المتبعة، وتم تطبيقها على الأدوية النقية والمستحضرات الصيدلانية. كما أكدت تقييمات الاستدامة باستخدام أداتيAGREE و Complex GAPI على أن هذه الطريقة صديقة للبيئة.