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Validated Stability Indicating Eco-friendly RP-HPLC Method for the Concurrent Quantification of Gabapentin and Diclofenac K in Wastewater and Pharmaceutical Formulations

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Abstract
The concurrent detection of gabapentin (GAP) and diclofenac K (DIC) in pharmaceutical formulations and wastewater has been made more approachable by developing and validating a precise Eco-friendly HPLC method. We evaluated the environmental impact of the RP-HPLC method using AGREEprep. This ensured that the technique was effective and sustainable. Separation by HPLC was accomplished on a C18, 5 µm Hypersil column (150 mm × 4.6 mm) with a mobile phase composed of monobasic phosphate buffer pH 6.2: Methanol in a ratio of 50:50 and pumped at 1.5 mL/min. UV was detected at a wavelength of 210 nm and 275 nm for GAP and DIC, respectively. Obtaining retention times (Rt) of 1.30 min and 9.58 min, respectively. Limits of quantitation and detection, as well as specificity, linearity, precision, accuracy, robustness, and stability, were all validated for this technique per ICH requirements. The method was specific, precise, accurate, and reproducible. The linearity study was established for GAP and DIC in the 3-50 µg/mL range. It was discovered that the limits of both detection and quantification were 0.93 µg/mL and 2.82 µg/mL for GAP, whereas the results were 1.25 µg/mL and 3.78 µg/mL for GAP and DIC, respectively. Good accuracy, recovery, and precision of drugs from their commercial pharmaceutical formulations (99.01, 100.35%) and wastewater samples (100.84, 100.52%) for GAP and DIC, respectively. This approach has been effectively used for the quantitative measurement of GAP and DIC in commercial tablets and wastewater, and it is robust for minor or deliberate adjustments to the chromatographic variables.

Keywords: Gabapentin; Diclofenac K; Eco-friendly HPLC; Wastewater; ICH guidelines; Validation.

1. Introduction

Developing combination therapies remains an elusive goal for researchers and clinicians alike. Combination therapy has revealed increased efficacy and improved results relative to their respective monotherapies, like the combination of amoxicillin and clavulanic acid as an antibiotic and carbidopa, levodopa for Parkinson's dopamine replacement. In cases of neuropathic pain, anticonvulsant drugs like gabapentin are valuable, whereas opioids and NSAIDs are typically inefficient and have low efficacy [1-3]. Since NSAIDs adversely affect the stomach, liver, and kidneys, their mixture with other pain modulators, such as antidepressants and antiepileptic agents, is significantly recommended in recent guidelines to reduce these adverse effects [4]. A growing interest has been in incorporating environmentally sustainable practices in quantitative analysis by implementing green analytical methods. Enhancing the safety and health conditions of analysts, a pivotal aspect of the field, and mitigating the environmental ramifications of analytical procedures constitute the primary impetuses for this initiative. Adopting environmentally sustainable analytical methods represents a promising advancement for the pharmaceutical sector. The analytical community has adopted a proactive stance

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toward sustainability and environmental responsibility. Integrating ecologically sustainable practices in novel methodologies is of utmost importance, and the potential influence of solvents and waste generation on the overall ecological soundness of the technique was meticulously evaluated. The advancement in the promotion of sustainability within the field of analytical chemistry is noteworthy and justifies the need for continued endeavors to attain more substantial progress [5,6]. The chemical name for the GAP seen in (**Fig. 1a**) is 1-(aminomethyl)cyclohexane acetic acid. $C_9H_{17}NO_2$ is its chemical formula, and its molecular weight is 171.24. DIC, a cyclo-oxygenase inhibitor, analgesic, and anti-inflammatory drug, as depicted in (**Fig. 1b**), has the chemical name potassium [2-[(2,6-dichlorophenyl) amino] phenyl] acetate. Its officially recognized chemical formula is $C_{14}H_{10}Cl_2KNO_2$, and its molecular weight is 334.2.

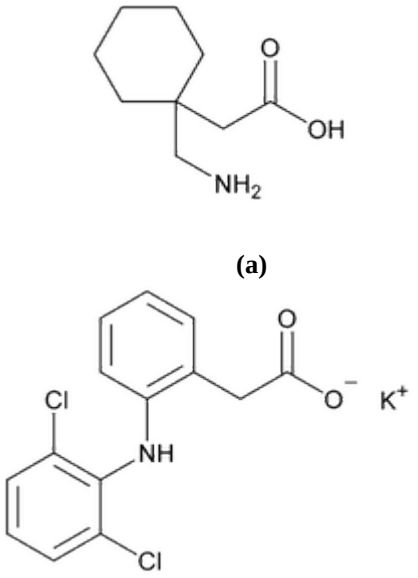

Fig.1 Chemical structures of (a) GAB and (b) DIC.

The HPLC method for GAP and the titrimetric approach for DIC were formally documented in the British Pharmacopoeia (BP) and the United States Pharmacopoeia (USP) [7,8]. Hurley et al., 2002 [9] reported the synergistic interaction between GAP and naproxen. Also, Ortega-Varela et al., 2004 [10] reported the synergistic interaction between GAP and metamizole concerning antinociception. Jiménez-Andrade et al., 2003 [11] have reported the synergistic interaction between DIC and codeine. Several studies have stated the safety and productivity of the mixture of GAP and DIC in treating neuropathic pain in rats [12, 13]. The association of GAP and NSAIDs is considered a dynamic therapy for postoperative pain and functional recovery enhancement after surgery [14]. Chromatographic techniques enhance sensitivity and precision while decreasing solvent consumption and processing time [15]. Several analytical methods for quantifying GAP and DIC, individually or in a mixture with other drugs, are available in the literature, such as individually quantitative LC-MS/MS analysis of paracetamol and DIC in human plasma using GAP as an internal standard [16], LC/MS technique for identifying and quantifying active pharmaceutical compounds including GAP and DIC, in the Ceyhan River [17], UHPLC/MS for the detection of drugs in environmental and wastewater samples [18], estimation of GAB and concurrent drugs in plasma HPLC/FD [19], Liquid-liquid extraction for the detection of gabapentin in human serum [20, 21], quantification of gabapentin and its main byproduct in pharmaceuticals [22], and UV methods [23-27]. Following a meticulous analysis, we have established a comprehensive comparison table to evaluate the recovery and sensitivity of GAB and DIC in wastewater, see **Table 1**. Our objective was to compare our work with previous studies [28-32]. We discovered that references [16,17] mentioned the simultaneous estimation of both drugs using LC-MS/MS, but with a low recovery of 70-87%. Based on our analysis of the cited references and previous methods, we can confidently conclude that no HPLC method has been utilized to simultaneously estimate GAB and DIC, confirming our current work's novelty. The uniqueness of our study is that no previous studies dealt with the simultaneous determination and quantitation of the two drugs. Only one LC/MS study has reported determining paracetamol and Diclofenac in human plasma using gabapentin as an internal standard. Nevertheless, it has limitations as no direct analysis of the two drugs has been conducted. The resolution between gabapentin and Diclofenac seemed to be low

as the retention time of Diclofenac was 2 minutes, whereas that of gabapentin was 1.1 minutes, whereas the resolution between the two drugs in our method 22.17 with higher theoretical plates. We also established AGREEprep to evaluate the proposed method, showing that it is more sustainable and environmentally friendly. We expect to release a new drug design form including this combination as several studies revealed this combination's synergetic effect in treating epilepsy and neuropathic pain in rats. However, no HPLC method that could be used in wastewater for the quantitative analysis of the two drugs simultaneously has been reported before. So, this article aims to develop a sensitive, fast, simple, economical, and Eco-friendly HPLC method for quantifying GAP and DIC in pharmaceutical formulations and wastewater samples.

Table 1. Comparison of the proposed method with the previous studies regarding recovery and sensitivity.

GAB			
Method	LOQ (µg/mL)	Recovery	References
LC-MS/MS	2.059	86.98%	[14]
LC-MS/MS	0.00033	70-110%	[15]
UHPLC-MS/MS	0.01	80 - 100%	[16]
In our study	2.82	100.84	
DIC			
Method	LOQ (µg/mL)	Recovery	References
LC-MS/MS	0.00002	70-110%	[15]
ESI-MS/MS	0.01	95.52%	[26]
UPLC/TQD-MS	0.001	94.4 ± 5.2%	[27]
HPLC-MS/MS	0.053	80-120%	[28]
solid phase extraction (SPE) coupled with high-performance liquid chromatography and diode array detection (HPLC-PDA)	0.01	85 ± 2.5	[29]
HPLC-MS/MS	0.2809	66.7-83.3%	[30]
In our study	3.78	100.52%	

2. Materials and Methods

2.1. Materials

2.1.1. Chemicals

GAP and DIC were purchased from Hikal Ltd. and Amoli, India. Methanol HPLC grade from Lichrosolv, bi-distilled water, sodium hydroxide, phosphoric acid, and monobasic potassium phosphate were procured from (Scharlau, Spain), and lactose monohydrate was purchased from (Shandong Deshang Chemical, China). GAP and DIC K were obtained from local pharmacies as commercially available tablets. All commercial tablets examined were current with shelf life and had been packed in original packaging.

2.1.2. Instruments

The used HPLC was model Agilent series 1200, Digital pH meter (Thermo Orion star A 211), and HPLC Column with the following specification C18 (150 mm × 4.6 mm, 5µm).

2.1.3. Mobile phase Preparation

The mobile phase was prepared using monobasic potassium dihydrogen phosphate buffer (50%):

Methanol (50%). The buffer was established by dissolving 3.811 gm of potassium dihydrogen phosphate anhydrous in 800 mL bi-distilled water, adjusting the pH of the solution at 6.2 using 0.1 N NaOH or 0.1 N phosphoric Acid.

Methods and General Procedure

2.2.1. Preparation of Stock and Working Standard Solutions

The standard (individual and mixed) stock solutions of 500 ppm of each Active Pharmaceutical Ingredient (API) were prepared in bi-distilled water with various concentrations and placed in amber-colored vials for immediate injection. The standard working solutions of GAB and DIC (3-50 ppm) were prepared by adequately diluting the stock solution with the bi-distilled water.

2.2.2. Analysis of marketed formulations

The development of an accurate, precise analytical method permits the quantitative analysis of different APIs, even at trace levels, without interference. Two different formulations of GAB (800 mg/tab) and (100 mg/cap) and DIC (50 mg/tab) and (50 mg/sachet) were used to prepare sample solutions to be analyzed using the proposed validated HPLC method. Upon comparison of the experimental results obtained from the analysis of the concerned APIs in all the commercial formulations with the standard solutions of these APIs of the same concentrations, the recoveries were found to be in the range of 99.01% for GAB and 100.84% for DIC. Moreover, our proposed HPLC method requires no sophisticated software for the quantitative analysis of the studied APIs.

2.2.3. Wastewater Sample Preparation

The previous study [33] clarifies that if no antibiotics are detected in the wastewater collected from the sewage system of the water station, it is possible to spike the drugs to the collected samples. Due to the negative impact of the residues of our constituents GAB and DIC on the ecosystem and human health, our validated method was used to determine GAB and DIC in wastewater quantitatively. The recovery tests shown in Tables 5 and 6 were performed on wastewater samples with GAB, and DIC spiked at a concentration of 2.5, 5, and 8 µg/mL, resulting in good recovery (100.35%, 100.52 %) for GAB and DIC, respectively. Both GAB and DIC weren't detected in the wastewater collected from the sewage system of the industrial wastewater from Beni-Suef governorate, Egypt. The wastewater sample was collected in 1 L sterile glass from the sewage system of the industrial wastewater from the Beni-Suef governorate. The wastewater sample was centrifuged at 11,000 rpm for 10 min. The supernatant was filtered through a 0.45 µm nylon syringe filter to eliminate contamination and protect the column from undesirable particles [35].

2.2.4. Chromatographic Conditions

The procedure was carried out using isocratic elution at a flow rate of 1.5 mL/min and an injection volume of 100 µL on a Hypersil C18 (150 mm × 4.6 mm 5 µm) column maintained at 25 °C. After three minutes of measuring at 210 nm, the wavelength was shifted to 275 nm.

2.2.5. Calibration Curves

Each drug under study has a 1 mg/mL stock standard solution developed using the solvent. From these stocks, a suitable sequence of dilutions was established. Therefore, the calibration curves for GAB and DIC were constructed using solutions to generate concentrations of the normal range (3-50 ppm).

3. Results and discussion

3.1. Method development and optimization

Several variables, such as flow rate, HPLC column length and pore size, mobile phase composition, and wavelength, were studied comprehensively while developing the suitable HPLC technique. The optimum flow rate was determined to be 1.5 mL/min after testing rates between 0.5 and 2 mL/min. We also tried C8, C18, phenyl, and cyano columns ranging from 100 mm to 250 mm before settling on the C18, 5µm Hypersil column (150 mm × 4.6 mm), providing higher accuracy and faster resolution than others. A chromatogram showing distinct, sharp peaks at 210 nm for GAB and 275 nm for DIC was obtained by scanning in the wavelength range (200-400 nm). Methanol/water (50:50, v/v), acetonitrile/water (50:50, v/v), monobasic potassium dihydrogen phosphate buffer (pH 2.5): methanol (50:50, v/v), and monobasic potassium dihydrogen phosphate buffer (pH 5.0): methanol (50:50, v/v)

were all tried, but none of them produced definite, sharp peaks. With distinct and good peak shape at high resolution, the mobile phase of monobasic phosphate buffer pH 6.2: Methanol by ratio 50:50 was shown to be the optimal development method for separating GAB and DIC, respectively as depicted in **Fig.2**. The column temperature was kept at 25°C.

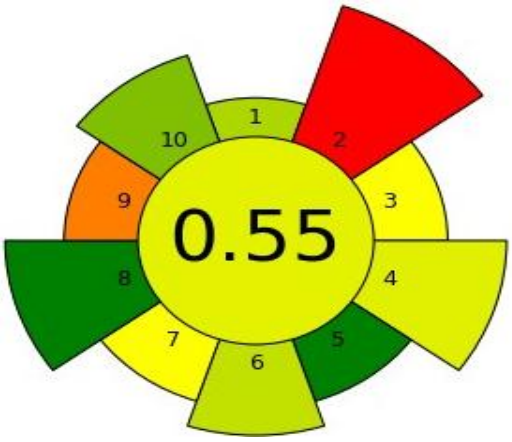

Fig. 2 HPLC chromatogram of Gabapentin and Diclofenac K recorded at the concerned wavelengths 210 nm and 275 nm.

3.2. Application of AGREEprep metrics

The current work used AGREE prep which was used to assess the ecological impacts of various sample preparation techniques. The AGREEprep approach streamlines the procedure by incorporating evaluation with the ten guiding principles of ecologically responsible sample preparation. With scores ranging from 0 to 1 and a score of 1 denoting the ideal level of performance, this system consists of ten distinct stages that evaluate each individual's ability [36]. Each of the ten sectors is represented graphically differently, as seen in **Fig.3**. With a value of 0.55, the results in **Fig.4** demonstrate the ecological effectiveness of our methodology. These figures proved to be very helpful in determining the method's effectiveness and allowed us to make fair and accurate assessments of its success. Overall, the results demonstrated that the proposed method was a safe and efficient way to prepare samples for analysis.

3.2. Method validation

In preliminary trials, different wavelengths were used to simultaneously determine both active pharmaceutical ingredients (APIs), revealing the impossible analysis of the two concerned APIs using a single wavelength.

1.	**Sample preparation placement** Sample preparation placement: On-line/In situ	0.66	1
2.	**Hazardous materials** Mass [g] or volume [mL] of problematic materials: 75	0.0	5
3.	**Sustainability, renewability, and reusability of materials** 50-75% of reagents and materials are sustainable or renewable, but can only be used ONCE	0.5	2
4.	**Waste** Mass [g] or volume [mL] of waste: 1.5	0.56	4
5.	**Size economy of the sample** Mass [g] or volume [mL] of the sample: 0.02	1.0	2
6.	**Sample throughput** Hourly sample throughput: 14	0.62	3
7.	**Integration and automation** No. of sample prep. steps: 2 steps or fewer; degree if automation: Semi-automated systems	0.5	2
8.	**Energy consumption** Approximate energy consumption per analysis [W]: 1.5	1.0	4
9.	**Post-sample preparation configuration for analysis** Liquid chromatography, gas chromatography with quadrupole detection, etc.	0.25	2
10.	**Operator's safety** No. of distinct hazards: 1 hazard	0.75	3

Fig. 3 The criterion for Analytical Greenness Metric for Sample Preparation (AGREEprep).

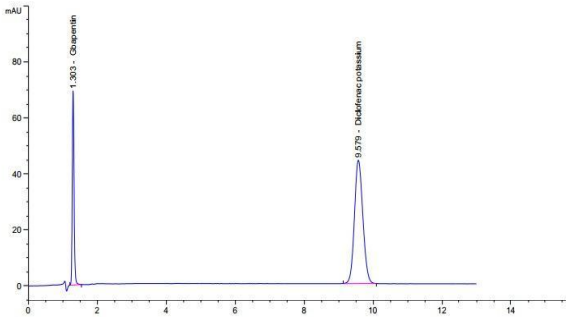

Fig. 4 The green metric AGREEprep for the proposed method.

The analytical method was optimized so that gabapentin (Rt =1.30 min) is monitored at 210 nm and diclofenac K (Rt = 9.58 min) is observed at 275 nm. According to current ICH guidelines and papers, the recommended analytical method for simultaneous estimating GAB and DIC in pharmaceutical formulations has been validated concerning system suitability, precision, LOD, LOQ, working ranges, linearity, accuracy, and recovery [37-40].

3.2.1. Linearity and range

A study has assessed the linearity of the calibration curves within the specified range of 3-50 µg/mL. Establishing linearity involved preparing of a series of six distinct concentrations of working standards GAB and DIC injected in duplicate for each concentration and then plotting the peak areas of each active ingredient against its concentration. Prepare a stock standard solution for each of API of concentration 500 ppm, then make serial dilutions of 3, 5, 10, 20, 30, and 50 ppm, as clarified in **Fig 5**. A regression line was calculated by the least-squares method with a coefficient of correlation (r), R2 ≥ 0.999, as shown in **Table 2**.

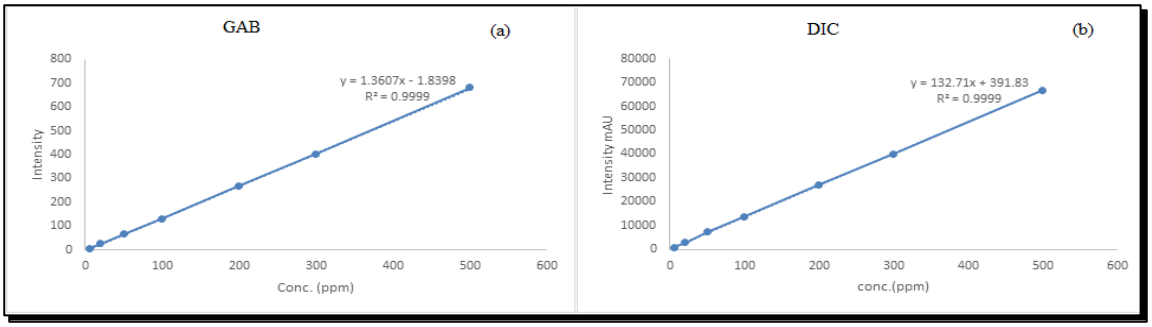

Fig. 5 Calibration curves for the determination of (a) GAB, and (b) DIC respectively.

Table 2. Regression and statistical parameters from the calibration curves of GAB and DIC.

Regression analysis	**GAB**	**DIC**
Parameters	Values	Values
Correlation coefficient (R^2)	0.9999	0.9999
Slope	1.3607	132.71
y-intercept	- 1.8398	391.83
Regression equation	y = 1.3607x - 1.8398	y = 132.71x + 391.83

3.2.2. LOD and LOQ

LOD is the minimum concentration of an analytical substance that can be measured with high precision and accuracy. LOQ refers to the minimum concentration of an analytical substance that can be accurately and precisely estimated. The established method had a low noise level, which made it difficult to determine the limit of quantification (LOQ) and limit of detection (LOD) using the signal-to-noise ratio. As displayed in **Table 3**, both LOD and LOQ were calculated from the ANOVA statistical by the equations using standard deviation and slope obtained from a calibration curve plotted using low concentrations of each API as displayed in **Fig 6**.

LOQ = 10 × standard error/slope (1)

LOD = 3.3 × standard error/slope (2)

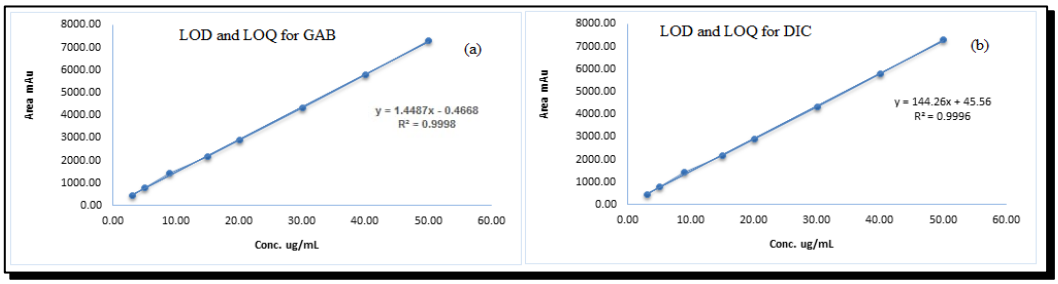

Fig.6 Calibration curve at low concentrations for determination of LOQ and LOD of GAB, and DIC respectively.

3.2.3. Precision

The precision of the measurements was evaluated through two methods: intra-day and inter-day precision. Intra-day precision was determined by preparing six spiked samples using lactose monohydrate and wastewater on the same day.

Table 3 Regression and statistical parameters from the calibration curves for determination of LOQ and LOD of GAB and DIC.

Parameter	**GAB**	**DIC**
Concentration range	3 -50 µg/mL	3 -50 µg/mL
Slope	1.4487	144.26
Intercept	-0.4668	45.56
Standard error	0.408	54.59
Determination Coefficient (R^2)	0.9998	0.9996
LOD	0.93 µg/mL	1.25 µg/mL
LOQ	2.82 µg/mL	3.78 µg/mL

Inter-day precision was determined by preparing six spiked samples on two different days. The following formula was used to compute the relative standard deviation: (RSD = (SD*100) / mean), it must be less than 2%, confirming that the method was precise, see **Table 4,5**.

Table 4 Results of repeatability and intermediate precision for DIC and GAB in commercial tablets.

Test	**DIC**		**GAB**	
	1st analyst (Interday)	**2nd analyst (Intraday)**	**1st analyst (Interday)**	**2nd analyst (Intraday)**
Test 1	99.25	100.53	97.86	98.75
Test 2	99.14	100.62	100.35	98.14
Test 3	99.19	100.94	98.34	98.46
Test 4	98.6	100.70	101.64	98.67
Test 5	99.82	100.64	100.63	97.97
Test 6	101.21	100.78	99.65	98.20
Average	99.54	100.70	99.75	98.37
SD	0.90	0.14205	1.43389	0.31152
RSD	0.91	0.14106	1.43758	0.31670
Pooled RSD (12 samples)	0.87		1.24	

Table 5 Results of repeatability and intermediate precision for DIC and GAB in wastewater samples

Test	**DIC**		**GAB**	
	1st analyst (Interday)	**2nd analyst (Intraday)**	**1st analyst (Interday)**	**2nd analyst (Intraday)**
Test 1	98.38	98.30	100.79	101.30
Test 2	98.53	98.18	103.35	101.19
Test 3	98.25	98.09	101.28	101.51
Test 4	99.47	98.02	104.69	101.22
Test 5	98.8	98.34	103.64	101.09
Test 6	100	98.34	102.63	101.15
Average	98.90603	98.21102	102.73	101.24
SD	0.68814	0.13884	1.47682	0.14696
RSD	0.69575	0.14137	1.43758	0.14515
Pooled RSD (12 samples)	0.60		1.24	

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3.2.4. Accuracy

The term "accuracy" refers to the degree of proximity between the results of a test obtained through a specific technique and the actual value. The user evaluated the method's precision by computing the mean recoveries percentage. The sample concentration levels were analyzed using the standard addition approach at three distinct levels, namely 50%, 100%, and 120%. The sample was prepared by adding a predetermined quantity of the analyte to a fixed amount containing lactose monohydrate. The resulting samples were analyzed in triplicate against a standard analyte concentration, as shown in **Table 6,7**.

Table 6. Results of accuracy for DIC in commercial tablets and wastewater samples

Test %	St add.n (ml) to 50 mL Flask		Calculated Amount mcg/mL		Amount found mcg/mL		Recovery %	
	Tablets	**Wastewater**	**Tablets**	**Wastewater**	**Tablets**	**Wastewater**	**Tablets**	**Wastewater**
50%	5	2.5	50.02	25.01	50.545	25.297	101.049	101.147
100%	10	5	100.04	50.02	101.110	50.147	101.070	100.254
150%	15	8	150.06	75.03	150.684	75.142	100.416	100.149
Minimum							100.42	100.15
Maximum							101.07	101.15
Average							100.84	100.52
SD							0.37	0.55
RSD%							0.36868	0.54540

Table 7. Results of accuracy for GAB in commercial tablets and wastewater samples.

Test %	**St add.n (mL) to 50 mL Flask**		**Calculated Amount mcg/mL**		**Amount found mcg/mL**		**Recovery %**	
	Tablets	**Wastewater**	**Tablets**	**Wastewater**	**Tablets**	**Wastewater**	**Tablets**	**Wastewater**
50%	**5**	**2.5**	50.02	24.76	49.422	24.707	98.804	99.776
100%	**10**	**5**	100.04	49.53	99.393	51.150	99.354	103.281
150%	**15**	**8**	150.06	74.29	148.364	72.805	98.870	98.004
Minimum							98.80	98.00
Maximum							99.35	103.28
Average							99.01	100.35
SD							0.30	2.69
RSD%							0.30308	2.67611

3.2.5. Specificity

The statement describes the analytical capability of accurately identifying the analyte even when it is present with alongside impurities or excipients. The experiment involved injecting blank samples (matrix) into the recommended HPLC system to assess whether the matrix would impact the primary peaks of the relevant analytes.

3.2.6. Robustness

The concept of robustness refers to the ability of a method to maintain its performance despite minor variations in its parameters. This method involves the determination of robustness based on specific criteria or factors such as a change in wavelengths (210 nm ± 2 nm) for GAB or (275 nm ± 2 nm) for DIC, change in flow rate (1.5 min/mL ± 0.1), and change in mobile phase composition ratio (Buffer: Methanol, 50:50 ± 1%), see **Table 8**.

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Table 8. Method robustness for the developed method.

Analyte	Chromatographic parameters	Column Temp. (°C)		Wavelength (nm)		MeOH ratio		Flow Rate	
GAB		22.5 °C	27.5 °C	*274	*276	49.00%	51.00%	1.45 min/mL	1.55 min/mL
	Assay %	98.62	97.75	98.52%	98.43%	98.13	98.81	97.96	98.83
	Retention time (Rt)	1.322	1.285	1.32	1.31	1.31	1.29	1.320	1.294
	Tailing factor	0.863	0.852	0.855	0.862	0.86	0.84	0.85	0.83
	Resolution	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
DIC	Assay %	99.96	99.12	99.45	98.92	99.24	100.05	99.69	100.32
	Retention time (Rt)	9.62	9.51	9.64	9.57	9.63	9.49	9.68	9.47
	Tailing factor	1.30	0.96	0.88	0.87	0.90	0.84	0.92	0.83
	Resolution	29.35	25.36	27.56	26.00	27.34	24.86	28.55	25.73

3.2.7. System suitability

According to European Pharmacopeia, some parameters should be fulfilled to confirm the suitability of the used system, like asymmetry, resolution, and the number of theoretical plates. The resolution between the peak of interest and any potential interference should be greater than 2.0 for accurate results. Additionally, the theoretical plates should be greater than 2000. There is a positive correlation between the number of theoretical plates and the efficacy of the column used. The observation of asymmetry factors closes to 1 suggests a minor degree of tailing in the quantification measurements. These results are consistent with the ICH guideline, which recommends a maximum asymmetry factor of 2.0 for accurate quantification, as shown in **Table 9**.

Table 9. Results of system suitability parameters for GAB and DIC.

Parameter	GAB		DIC	
	1st day precision	2nd day Precision	1st day precision	2nd day Precision
RSD % (Retention time)	0.218	0.218	0.404	0.474
RSD% (Peak area)	0.52	0.176	1.0152	0.18
Resolution	N/A	N/A	22.36	22.17
Tailing factor	0.54	0.54	0.24	0.23
Theoretical Plates	3825.67	3802.12	3700.15	3746.83

4. Conclusion

For the estimation of GAB and DIC in pharmaceutical formulations and wastewaters, the validated method was found to be uncomplicated, specific, precise, accurate, and reproducible, with high recoveries and accurate and precise quantitative results demonstrated with various analytical systems. The RP-HPLC method's greenness evaluation was conducted using the AGREEprep tool for sample preparation. This thoroughly examined the method's environmental impact, guaranteeing its efficacy and sustainability. The procedure was shown to be valid per European Pharmacopeia and ICH standards. We were able to make sensitive measurements of the analyte using a simple HPLC system, with limits of detection and quantification of 0.93 µg/mL and 2.82 µg/ mL, respectively, for GAB and 1.25 µg/ mL and 3.78 µg/ mL, respectively, for DIC. Standard solutions of GAB and DIC were made at known concentrations to ensure that samples of both ingredients would have the same retention time on the chromatogram.

Declaration of Competing Interest

The authors declare no conflict of interest.

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5. References

[1] Baillie, J. K., Power, I., The mechanism of action of gabapentin in neuropathic pain. Curr. Opin. Investig. Drugs (London, England: 2000), **7**(1), 33-39 (2006).

[2] Chincholkar, M., Analgesic mechanisms of gabapentinoids and effects in experimental pain models: a narrative review. Br. J. Anaesth, **120**(6),1315-1334 (2018).

[3] Harden, R.N., Pharmacotherapy of complex regional pain syndrome. Am. J. Phys. Med. Rehabil, **84**(3), S17-S28 (2005).

[4] Ghlichloo, I., Gerriets, V., Nonsteroidal Anti-inflammatory Drugs (NSAIDs). In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing, (2022).

[5] Imam, M.S., Batubara, A.S., Gamal, M., Abdelazim, A.H., Almrasy, A.A.y, Ramzy, S., Adjusted green HPLC determination of nirmatrelvir and ritonavir in the new FDA approved co-packaged pharmaceutical dosage using supported computational calculations, Sci Rep. **13**, 137(2023).

[6] Mohamed, D., Fouad, M.M., Application of NEMI, Analytical Eco-Scale and GAPI tools for greenness assessment of three developed chromatographic methods for quantification of sulfadiazine and trimethoprim in bovine meat and chicken muscles: Comparison to greenness profile of reported HPLC methods, Microchem. J. **157**, 104873 (2020).

[7] British Pharmacopoeia Stationary Office. Medicines and Healthcare Products Regulatory Agency, London, **2**, (2023).

[8] United States Pharmacopoeia Revision, NF 39. The United States Pharmacopoeia Convention Inc, **43** (4), (2023).

[9] Hurley, R. W., Chatterjea, D., Rose Feng, M., Taylor, C. P., Hammond, D. L., Gabapentin and pregabalin can interact synergistically with naproxen to produce antihyperalgesia. J. Am. Soc. Anesthesiol, **97**(5), 1263-1273 (2002).

[10] Ortega-Varela, L.F., Herrera, J.E., Medina-Santillán, R., Reyes-García, G., Rocha-González, H.I., Granados-Soto, V., Synergistic interaction between gabapentin and metamizol in the rat formalin test. In Proc West Pharmacol Soc, **47**, 80-83 (2004).

[11] Jiménez-Andrade, J.M., Ortiz, M.I., Pérez-Urizar, J., Aguirre-Bañuelos, P., Granados-Soto, V., Castañeda-Hernández, G., Synergistic effects between codeine and diclofenac after local, spinal and systemic administration. Pharmacol. Biochem. Behav, **76**(3-4), 463-471 (2003).

[12] Ibrahim, M.A., Abdelzaher, W.Y., Rofaeil, R.R., Abdelwahab, S., Efficacy and safety of combined low doses of either diclofenac or celecoxib with gabapentin versus their single high dose in treatment of neuropathic pain in rats. Biomed. Pharmacother, **100**, 267-274 (2018).

[13] Picazo, A., Castañeda-Hernández, G., Ortiz, M.I., Examination of the interaction between peripheral diclofenac and gabapentin on the 5% formalin test in rats. Life Sci, **79**(24),2283-2287 (2006).

[14] Narai, Y., Imamachi, N., Saito, Y., Gabapentin augments the antihyperalgesic effects of diclofenac sodium through spinal action in a rat postoperative pain model. Anesth. Analg, **115**(1), 189-193 (2012).

[15] Mohamed, M.A., Stability-Indicating New RP-UPLC Method for Simultaneous Determination of a Quaternary Mixture of Paracetamol, Pseudoephedrine, Chlorpheniramine, and Sodium Benzoate in (Cold–Flu) Syrup Dosage Form. J. AOAC Int, **105**,703-716 (2022).

[16] Bhatt, P., Saquib Hasnain, M., Nayak, A.K., Hassan, B., Beg, S., Development and validation of QbD-driven bioanalytical LC-MS/MS method for the quantification of paracetamol and diclofenac in human plasma. Anal. Chem. Lett., **8**(5), 677-691 (2018).

[17] Guzel, E.Y., Cevik, F., Daglioglu, N., Determination of pharmaceutical active compounds in Ceyhan River, Turkey: Seasonal, spatial variations and environmental risk assessment. Hum. Ecol. Risk Assess.: Int. J., **25**(8),1980-1995 (2019).

[18] Gracia-Lor, E., Martínez, M., Sancho, J.V., Peñuela, G. and Hernández, F., Multi-class determination of personal care products and pharmaceuticals in environmental and wastewater samples by ultra-high performance liquid-

chromatography-tandem mass spectrometry. Talanta, **99**, 1011-1023 (2012).

[19] Gurke, R., Rossmann, J., Schubert, S., Sandmann, T., Rößler, M., Oertel, R., Fauler, J., Development of a SPE-HPLC–MS/MS method for the determination of most prescribed pharmaceuticals and related metabolites in urban sewage samples. J. Chromatogr. B, 990,23-30 (2015).

[20] Martinc, B., Roškar, R., Grabnar, I., Vovk, T., Simultaneous determination of gabapentin, pregabalin, vigabatrin, and topiramate in plasma by HPLC with fluorescence detection. J. Chromatogr. B, **962**, 82-88 (2014).

[21] Bahrami, G., Kiani, A., Sensitive high-performance liquid chromatographic quantitation of gabapentin in human serum using liquid–liquid extraction and pre-column derivatization with 9-fluorenylmethyl chloroformate. J. Chromatogr. B, **835**(1-2), 123-126 (2006).

[22] Ciavarella, A.B., Gupta, A., Sayeed, V.A., Khan, M.A., Faustino, P.J., Development and application of a validated HPLC method for the determination of gabapentin and its major degradation impurity in drug products. J. Pharm. Biomed. Anal., **43**(5), 1647-1653 (2007).

[23] Goswami, A., Jiang, J.Q., Simultaneous quantification of gabapentin, sulfamethoxazole, terbutryn, terbuthylazine and diuron by uv-vis spectrophotometer. Biointerface Res. Appl. Chem., **8**(1), 3111-3117 (2018).

[24] De la Cruz, N., Giménez, J., Esplugas, S., Grandjean, D., De Alencastro, L.F., Pulgarin, C., Degradation of 32 emergent contaminants by UV and neutral photo-fenton in domestic wastewater effluent previously treated by activated sludge. Water research, **46**(6), 1947-1957 (2012).

[25] Abdulrahman, S.A., Basavaiah, K., Highly sensitive spectrophotometric method for the determination of gabapentin in capsules using sodium hypochloride. Turk J. Pharm. Sci, **9**(2),113-126 (2012).

[26] Adegbolagun, O.M., Thomas, O.E., Aiyenale, E.O., Adegoke, O.A., A new spectrophotometric method for the determination of gabapentin using chromotropic acid. Acta Pharm. Sci., **56**(3),93-110 (2018).

[27] Adegoke, O.A., Adegbolagun, O.M., Aiyenale, E.O., Thomas, O.E., New spectrophotometric method for the determination of gabapentin in bulk and dosage forms using p-dimethylaminobenzaldehyde. J. Taibah Univ. Sci., **12**(6), 754-764 (2018).

[28] Gopal, C.M., Bhat, K., Ramaswamy, B.R., Kumar, V., Singhal, R.K., Basu, H., Udayashankar, H.N., Vasantharaju, S.G., Praveenkumarreddy, Y., Lino, Y., Balakrishna, K., Seasonal occurrence and risk assessment of pharmaceutical and personal care products in Bengaluru rivers and lakes, India. J. Environ. Chem. Eng,**9**, 105610 (2021).

[29] Singh, V., Suthar, S., Occurrence, seasonal variations, and ecological risk of pharmaceuticals and personal care products in River Ganges at two holy cities of India. Chemosphere, **268**, 129331(2021).

[30] Lei, H., Yao, K., Yang, B., Xie, L., Ying, G., Occurrence, spatial and seasonal variation, and environmental risk of pharmaceutically active compounds in the Pearl River basin, South China. Frontiers of Environmental Science & Engineering, 17, 46 (2023).

[31] Hlengwa, N.B., Mahlambi, P.N., SPE-LC-PDA method development and application for the analysis of selected pharmaceuticals in river and wastewater samples from South Africa. Water SA, **46**, 514-522 (2020).

[32] Al-Odaini, N.A., Zakaria, M.P., Yaziz, M.I., Surif, S., Abdulghani, M., The occurrence of human pharmaceuticals in wastewater effluents and surface water of Langat River and its tributaries, Malaysia. Int. J. Environ. Anal. Chem, **93**, 245-264 (2013).

[33] Becze, A., Resz, M.A., Ilea, A. and Cadar, O., A Validated HPLC Multichannel DAD Method for the Simultaneous Determination of Amoxicillin and Doxycycline in Pharmaceutical Formulations and Wastewater Samples. Applied Sciences, **12**, 9789 (2022).

[34] Teixeira, S., Delerue- Matos, C., Alves, A., Santos, L., Fast screening procedure for antibiotics in wastewaters by direct HPLC- DAD analysis. J. Sep. Sci., **31**(16- 17), 2924-2931(2008).

[35] Wojnowski, W., Tobiszewski, M., Pena-Pereira, F., Psillakis, E., AGREEprep–Analytical greenness metric for sample preparation. TrAC Trends in Analytical Chemistry, 116553 (2022).

[36] Gujral, R.S., Haque, S.M., Development and validation of a new HPLC method for the determination of gabapentin. Int. J. Biomed. Sci, **5**(1), 63 (2009).

[37] Patel, K., Verriboina, S.K., Vasantharaju, S.G., Stability Indicating Assay Method Development and Validation of Simultaneous Estimation of Chlorzoxazone, Diclofenac Sodium and Paracetamol in Bulk Drug and Tablet by RP-HPLC. Res. J. Pharm. Technol., **14**(9), 5024-5028 (2021).

[38] ICH. Stability Testing of New Drug Substances and Products. Current step, Q1A (R2), **4**, 1-2(2003).

[39] Hassouna, M.E.M., Mohamed, M.A., Optimization and Modelling of Novel RP-UPLC Method for Simultaneous Determination of Cefradine, Cefalexin, Sodium Benzoate and Methylparaben in Some Biological Fluids. Application to Experimental Design. Egypt. J. Chem., **65**(9), 673-686 (2022).

[40] Mohamed, M.A., Nassar, H.F., Stability-indicating RP-UPLC method for determination of antihypertensive drugs and their degradation products in tablets: application to content uniformity and dissolution studies. J IRAN CHEM SOC, **20**,1-11(2023).