مجلة جامعة دمشق للعلوم الطبية المجلد: (40) العدد:3 (2024) الصفحات: (92-103)

Novel Green RP-HPLC Method for Simultaneous Determination of Sodium Diclofenac and Benzyl Alcohol as Bulk Mixtures and in Pharmaceutical Injectable Forms

Ola Mahmod Younes*

*Doctor in applied analytical chemistry-Department of food and analytical chemistry -Faculty of pharmacy-Damascus university.
Ola31.Younes@damascusuniversity.edu.sy

Abstract:

Background: Determination of active substances in dosage forms besides the inactive ingredients, including preservatives is required as a complete analytical procedure. Therefore, a new practical, accurate, and green RP-HPLC method was developed to determine Sodium Diclofenac and Benzyl Alcohol simultaneously in bulk and pharmaceutical injectable forms.

Material and Methods: The method is based on using of Ethanol: Sodium acetate 0.1%, pH =7(50: 50) v/v as mobile phase, and C8 5µ(4.6*150mm) Inert Sustain GL Sciences column at room temperature 〖25〗^° C, the flow rate was 1mL/min, the UV detector was set at 254 nm and the injection volume was 10 µL. Moreover, the greenness of the method was evaluated using the AGREE tool.

Results and Discussion: The validity of the developed method is achieved according to ICH guidelines, it revealed good linearity over the concentration range of 20-100 ppm for Sodium Diclofenac and 50-150 ppm for Benzyl Alcohol with the correlation coefficients were obtained 〖R〗^2=0.9998,0.9999 respectively. The LOD and LOQ were 1.65 and 5.00 ppm for Sodium Diclofenac and 1.21 and 3.66 ppm for Benzyl Alcohol. The Precision was studied as intra- and inter-day with relative standard deviations not more than 2%, and the accuracy by mean recoveries ranged between 100.06 – 101.10% for Sodium Diclofenac and 100.10 -101.83% for Benzyl Alcohol. The developed method was successfully applied to determine both compounds in marketed injectable forms. Finally, the AGREE tool revealed a high degree of greenness for the developed method.

Conclusion: The results showed that the developed method is green and suitable for the precise, accurate and rapid determination of Sodium Diclofenac and Benzyl Alcohol in bulk drugs and ampoules.

Keywords: RP- HPLC, Sodium Diclofenac, Benzyl Alcohol, Injectable Forms, Method Validation, Greenness Assessment.

Submitted: 7/11/2024 **Accepted:17/12/2024**

ISSN: 2789-7214 (online)
http://journal.damascusuniversity.edu.sy

طريقة جديدة خضراء للتفريق اللوني بالطور السائل العكوس عالي الأداء للتحديد المتزامن لديكلوفيناك الصوديوم والكحول البنزيلي كمزيج وسيط وفي الأشكال الصيدلانية الحقنية

علا محمود يونس*

*دكتور مدرس– اختصاص الكيمياء التحليلية التطبيقية– قسم الكيمياء التحليلية والغذائية– كلية الصيدلة – جامعة دمشق.

الملخّص:

خلفية البحث وهدفه: يعد تحليل المادة الفعالة في الأشكال الصيدلانية الى جانب المواد غير الفعالة من ضمنها المواد الحافظة أمراً مطلوباً كإجراء تحليلي متكامل، لذلك تم تطوير طريقة كروماتوغرافيا سائلة عالية الأداء بالطور العكوس وهي طريقة جديدة عملية ، دقيقة وخضراء للتحديد المتزامن لكل من ديكلوفيناك الصوديوم والكحول البنزيلي كمواد أولية وفي الأشكال الصيدلانية الحقنية.

مواد البحث والطرائق: تعتمد الطريقة على استعمال الإيتانول: خلات الصوديوم 0.1 (50: 50) (pH=7) % حجم/حجم كطور متحرك و عمود C8 بأبعاد InertSustain GL Sciences 5 (4.6*150mm) µ في درجة حرارة الغرفة 25°C، كان معدل التدفق 1 مل / دقيقة، عند طول موجة 254 نانومتر وحجم الحقنة 10ميكرولتر، علاوة على ذلك تم تقييم خضرة الطريقة المطورة باستخدام أداة.AGREE

النتائج والمناقشة: تم التحقق من مصدوقية الطريقة المقترحة وفقًا للمبادئ التوجيهية للمؤتمر الدولي للتنسيق والمواءمة حيث أظهرت علاقة خطية ممتازة ضمن مجال تركيز 20–100 ppm من أجل ديكلوفيناك الصوديوم ومعامل ارتباط قدره 0.9998 و50–150 ppm من أجل الكحول البنزيلي مع معامل ارتباط وقدره 0.9999، وكان حد الكشف والمقايسة الكمية 1.65 و 5.00 ppm من أجل ديكلوفيناك الصوديوم و 1.21 و 3.66 ppm من أجل الكحول البنزيلي. درست الدقة خلال اليوم الواحد وبين أيام مختلفة فكانت الانحرافات المعيارية النسبية لا تزيد عن 2%، وتراوحت المضبوطية بمتوسط استعادة من100.06% – 101.10 من أجل ديكلوفيناك الصوديوم و من 100.10–101.83% من أجل الكحول البنزيلي .تم تطبيق الطريقة المطورة بنجاح لتحديد كل من المركبين في الأشكال التجارية الحقنية. وأخيراً، أظهرت نتيجة أداة AGREE درجة خضرة عالية للطريقة المطورة.

الاستنتاج: أظهرت النتائج أن الطريقة المطورة خضراء ومناسبة للتحديد السريع والدقيق والمضبوط لكل من ديكلوفيناك الصوديوم والكحول البنزيلي في المواد الأولية وفي الأمبولات .

الكلمات المفتاحيّة: التفريق اللوني بالطور السائل العكوس عالي الأداء، ديكلوفيناك الصوديوم، الكحول البنزيلي، الأشكال الحقنية، مصدوقية الطريقة، تقييم خضرة الطريقة.

تاريخ الإيداع: 2024/3/12 تاريخ القبول: 2024/5/28

1- Introduction:

Sodium Diclofenac (DC) is a widely used drug administered globally. Chemically it is known as sodium 2-[(2,6- dichlorophenyl) amino] phenyl acetate, (Fig.1) . DC is a synthetic non-steroidal anti-inflammatory drug (NSAID) with analgesic, anti-inflammatory, and antipyretic properties. It is mainly used for the relief of pain and inflammation in various medical conditions [1].

DC is an official drug listed in both the British Pharmacopoeia (BP) [2] and the United States Pharmacopeia (USP)[3] where assay of the raw material is carried out by potentiometric nonaqueous titration. High-performance liquid chromatography (HPLC) is employed for the analysis of different dosage forms in both references. However, the determination of DC in injectable forms is officially included only in the Indian Pharmacopoeia (IP) [4]. Quantification methods of DC either alone or in combination with other active substances, in various pharmaceutical dosage forms or plasma samples, are extensively documented in literature. These methods include Spectrophotometric and spectrofluorometric techniques [5-7], as well as liquid chromatography coupled with different detectors [8-15].

Benzyl alcohol (BA) (Fig.2) [16] is commonly used as a preservative in multidose injectable pharmaceutical formulations. For this purpose, concentrations ranging from of 0.5 to 2.0% are employed, with such levels generally well tolerated in clinical use [17]. Recent guidelines permit BA content to reach up to 4% in final injectable formulations [18].

Moreover, it is widely used in cosmetic products and has shown toxicity in neonates and infants. However, it is generally recognized as safe by the FDA at concentrations up to 5% in adults. [17,19]. It is used as cosolvent in formulations to enhance the solubility of drugs such as DC [20]. According to the literature, the determination of BA could be depending on gas chromatography technique [3,21,22], beside HPLC methods to determine BA as raw material and in pharmaceutical dosage forms [24 -27] or in plasma [28].

Benzyl alcohol dose not have an official HPLC method in pharmacopeia, as the official method is potentiometric titrimetry. However, USP provide us with chromatographic methods to control its content in another materials [3].

BA is added to the Sodium Diclofenac injectable solutions as both preservative and cosolvent agent. Therefore, it is essential to ensure that its concentration does not exceed the acceptance criteria in final product to achieve the maximum benefit with minimum hazardous effects. Some spectrophotometric methods for simultaneous determination of DC and BA have been reported using mathematical derivation [29,30].

In the light of previous information, it is important to quantify and control both of them, DC as an active pharmaceutical ingredient, and BA as a preservative.

HPLC technique is obviously one of the crucial approaches to control and quantify the drugs [31,32], therefore, it was chosen for recent work.

To the best of our knowledge, there is no HPLC method for the simultaneous determination of Sodium Diclofenac and Benzyl Alcohol as bulk mixture or in injectable dosage forms.

Our method development adhered to the principles of green chemistry, by using green solvents and achieving short retention time. Therefore, the AGREE tool was employed to assess the greenness of the process.

The evaluation criteria are taken from the 12 principles of green analytical chemistry (sampling procedure, amount of sample, position of analytical device, steps of analytical procedure, degree of automation, derivation agent if it used, amount of waste, number of analytes in a single run, energy used in the method, type of reagents, toxic reagents if they involved, safety of the operator) [33]. The final result is transformed into a unified 0 –1 scale [34]. The tool is freely available software and can be downloaded from https://mostwiedzy.pl/en/wojciech-wojnowski,174235-1/AGREE.

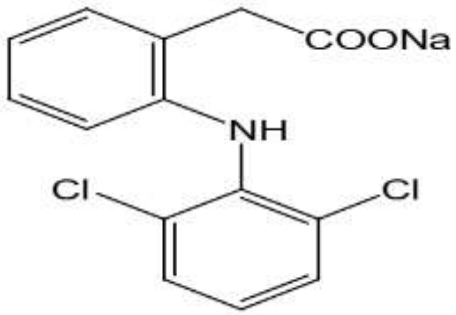


Fig. 1: Chemical structure of Sodium Diclofenac

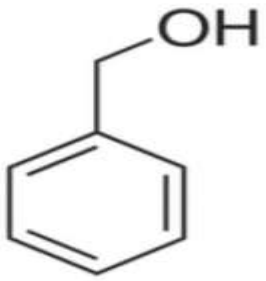


Fig. 2: Chemical structure of Benzyl Alcohol

This work introduces a new HPLC–PDA method for the simultaneous determination of DC and BA in bulk and injectable dosage forms. The method was validated according to ICH guidelines.

2-Materials and Methods:

2.1. Instrumentation

A Shimadzu HPLC system LC 2030C 3D plus (Shimadzu Corporation, Japan), equipped with a PDA detector, an auto sampler, column oven (Shimadzu Corporation, Japan) was used in the separation. The column used was a C8 5µ (4.6*150mm) InertSustain GL Sciences IncJapan. Ultrasonic bath -BANDELIN SONOREX, Balance A and D Company Limited, Japan. pH meter WTW-inolab pH7310p, paper filter-Albet made in EEC , PTFE syring filter 0.45µm.

2.2. Materials and Reagents

Sodium hydroxide (99% purity), Sodium acetate (99% purity), from SIGMA-ALDRICH. Ortho phosphoric acid 85% from HIMEDIA-INDIA. Isopropanol, Ethanol, gradient HPLC grades from Scharlou-SPAIN. Methanol, HPLC gradient from Merck-Germany. Sodium Diclofenac (99.9% purity) standard Benzyl Alcohol (99.5% purity) were kindly provided by Miamed pharmaceutical industries.

Ampouls (containing Sodium diclofenac 75mg/3 ml) obtained from Syrian market (different pharmaceutical companies A, B, C, D, E). Notably, Benzyl Alcohol content was not specified on the drug labels.

2.3. Chromatographic conditions

The chromatographic separation was performed using an InterSustain C8 Column 5 µm (4.6 x150mm) (GL Sciences, Japan), temperature $25^{\circ}C$, and mobile phase consisted of Ethanol: (0.1%) sodium acetate (50: 50) v/v. The mobile phase was degassed by sonication for 15 min and filtered through 0.45µm millipore membrane filter before use. (Fig. 3) shows the separation which was performed at a flow rate of 1 mL/min, and UV detection at 254 nm, injection volume 10 µl.

2.4. Standard Solution

Each stock solution of DC and BA was prepared at concentration of 1000 ppm , using mobile phase as a solvent and stored in refrigerator at $4^{\circ}C\ temperature$, then each stock solution was diluted to prepare five DC working solutions at concentration range of (20-100) ppm and five BA working solutions at concentration range of (50-150) ppm.

2.5. Method validation

The developed method was validated in accordance with the International Conference on Harmonization (ICH) guidelines. Thus, it was validated for linearity, limit of detection (LOD), limit of quantification (LOQ), selectivity, precision, accuracy and robustness [35].

2.5.1. Linearity:

It was evaluated by plotting calibration curve using five concentrations: 20-40-60-80-100 ppm of DC and 50-75-100-125-150 ppm for BA against their corresponding peak areas, each concentration was injected in triplicate. The slope, intercept and correlation coefficient R^2 of the calibration curve were calculated.

2.5.2. Limit of detection (LOD) and limit of quantification (LOQ):

They were calculated based on LOD (3.3 SD/S) and LOQ (10 SD / S), where SD the standard deviation

of the response and S the slope of the calibration curve.

2.5.3. Selectivity:

The selectivity of the developed method was evaluated by analyzing potential interference from common ampoule excipients. It was confirmed by the absence of overlapping peaks in the chromatograms.

2.5.4. Precision:

It was valuated as repeatability through intra-day (three replicates within the same day) and inter-day (three replicates across three different days) measurements at three concentration of each compound, then the results were statistically analyzed using relative standard deviation (RSD %).

2.5.5. Accuracy:

Accuracy of the developed method was evaluated using recovery and mean recovery at three different concentrations of each compound.

2.5.6. Robustness:

Robustness is a measure of method capacity to remain unaffected by small but deliberate variations in the chromatographic method parameters and provides an indication of its reliability; flow rate was chosen as a parameter, then relative standard deviation (RSD %) was calculated.

2.6. Determination of Sodium Diclofenac and Benzyl Alcohol in pharmaceutical injection forms

A quantity equivalent to 50 mg (2ml) of DC (each ampoule contains 75 mg of DC presented in a volume of 3 ml, so its concentration is 25 mg/ ml) was transferred into volumetric flask and diluted up to 50 ml with mobile phase. After filtration, 3 mL of this solution was further diluted to 50 ml using the same solvent. Finally, the practical content was calculated based on calibration curves of both compounds.

3- Results and discussion:

Choosing the optimal conditions for mixture separation by HPLC occupies the most important step, as analyst should balance between solvent and time consuming without affecting both the validity of the method and system suitability parameters. for this reason, many attempts were tried to give the best results.

3.1. Mobile phase composition

First of all, acetonitrile was avoided due to its hazardous effect on both analyst and environment. Greener solvents like methanol, isopropanol, ethanol were experienced.

Isopropanol as an organic part of mobile phase caused turbidity during mixing, so it was excluded.

While methanol in the percentage of 60%: sodium acetate 0.1% gave relatively long retention time and a tailing factor more than 2, when the flow rate increased up to 1.5 ml/min and more, to reduce time, the number of theoretical plates was below 2000. Finally, ethanol was tried in the percentage of 60%: sodium acetate 0.1%, and the resulted retention times were surprisingly shorter, but the resolution was below 1.5.

When editing the percentage of ethanol to 50%, the results were much better and all parameters were within acceptable criteria as shown in (Table 1).

3.1.1. pH adjustment of mobile phase

The pH of the mobile phase plays a critical role in the separation process. Several pH values were tested, and a pH of 7 was determined to be optimal based on the resolution (Rs).

3.1.2. flow rate and column temperature adjustment

The effect of column temperature (25± 5°C) on peak separation was found to be negligible. A flow rate of 1 ml/min showed acceptable results while, less flow rate caused longer retention time and wider peaks. When flow rate is more than 1 ml/min, lower Rs was resulted.

3.2. System suitability:

Table 2 summarizes the system suitability parameters for the developed method under the optimal conditions.

Table 2: System suitability

	DC	BA
Retention time (t_R)	4.522	3.002
Capacity factor (K)*	3.522	2.002
Resolution (Rs)	5.24	
Tailing factor(T)	1.228	1.24
Number of theoretical plates (NTP)	2602	2904

*Dead time 1 min

3.3 method validation

3.3.1. Linearity

The calibration curve obtained for each standard showed good linear relationship within the concentration ranges of 20–100 ppm for Sodium Diclofenac (DC) and 50–150 ppm for Benzyl Alcohol (BA)

The correlation coefficient for the calibration curve observed was 0.9998, 0.9999 for DC, BA respectively. (Table 3) (Fig 4).

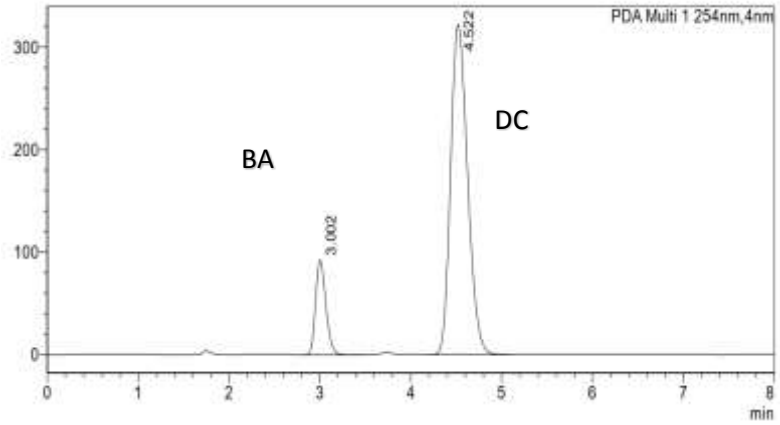
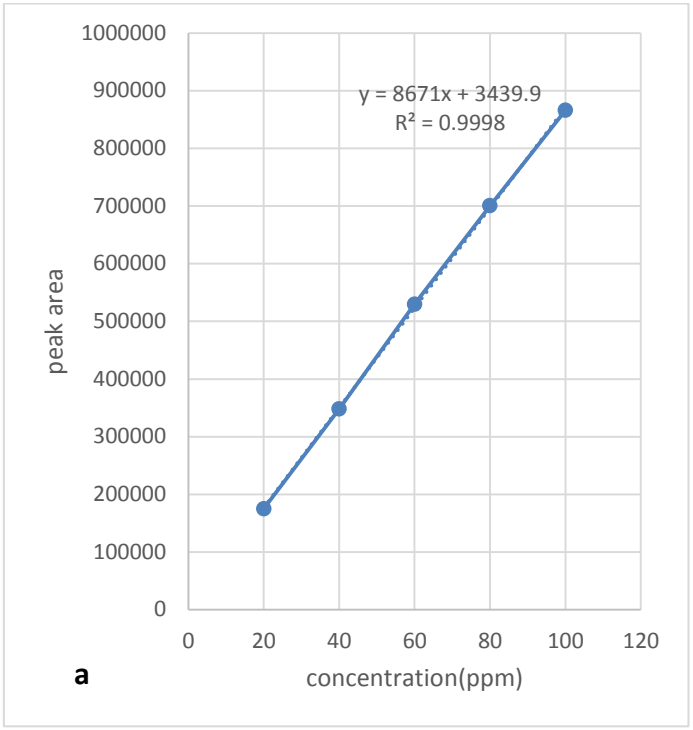


Fig. 3: Chromatogram of BA, DC under optimal conditions

Table 1 : Optimization of the chromatographic conditions for the determination of both DC and BA by the developed method (flow rate 1 ml/min, 25 c, detection wavelength at 254 nm)

Parameters			**T** **DC** **BA**	**t_R (min)** **DC** **BA**	**NTP** **DC** **BA**	**Rs**
Mobile phase composition	Organic phase 60%	Aqueous phase 40%				
	Isopropanol	Sodium acetate 0.1%	-	-	-	
	Methanol		2.10 2.20	4.10 8.30	1800 1920	1.80
	Ethanol		1.30	2.77 2.49	1937 2349	1.20
Optimal composition	Organic phase 50%	Aqueous phase 50%	1.22	4.52	2622	5.24
	Ethanol	Sodium acetate 0.1%	1.23	3.00	2903	



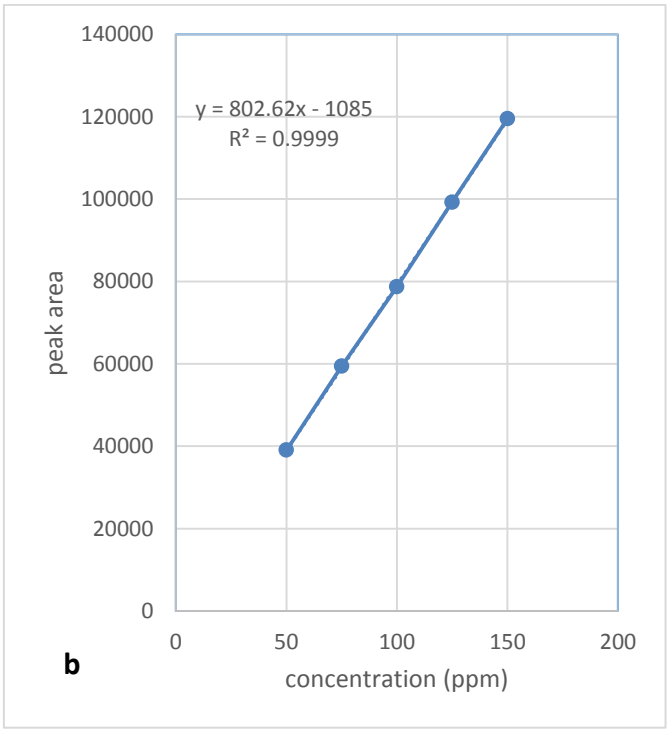


Fig. 4: Linearity of (a) Sodium Diclofenac and (b) Benzyl Alcohol by developed method

Table 3: linearity of the developed method

Concentration (ppm) for DC	Peak Area*	Statistical analysis
20	174798.00	Slope = 8761
40	348113.33	Correlation coefficient= 0.9998
60	529464.67	
80	700341.00	LOD = 1.65 ppm
100	865785.00	LOQ = 5.00 ppm
Concentration (ppm) for BA	**Peak Area***	**Statistical analysis**
50	39061.33	Slope=802.62
75	59411.33	Correlation coefficient= 0.9999
100	78703.33	
125	99227.00	LOD = 1.21 ppm
150	119480.67	LOQ = 3.66 ppm

* Average of three replicates

3.3.2. Accuracy and precision

Accuracy and precision of the developed method were evaluated across its range and the results showed the truth of the analytical procedure (table 4,5).

Table 4: Precision of the developed method

	Concentration (ppm)	withinday*		between day*	
		Found ±S.D* (ppm)	Precision (RSD %)	Found ± S.D* (ppm)	Precision (RSD %)
DC	20	20.05 ± 0.13	0.65	19.65 ± 0.11	0.56
	60	60.5 ± 0.15	0.25	60.31 ± 0.22	0.36
	100	100.6 ± 0.13	0.13	100.3 ± 0.42	0.42
BA	50	50.12 ±0.23	0.46	50.33 ± 0.45	0.89
	100	101.02±0.41	0.41	101.9 ±0.37	0.36
	150	150.4±0.66	0.43	151.3 ± 0.77	0.51

* Average of three separate determinations

Table 5: Accuracy of the developed method

Concentration (ppm)		Found (ppm)*		Recovery %	
DC	BA	DC	BA	DC	BA
20	50	20.21	50.05	101.05	100.10
60	100	60.04	101.83	100.07	101.83
100	150	101.10	152.57	101.10	101.71
				$Mean \mp SD = 100.73 \mp 0.58$	$Mean \mp SD = 101.22 \mp 0.96$

*** Average of three separate determinations**

3.3.3. selectivity

The selectivity was evaluated in the presence of common ampoule excipients. No interfering peaks were observed at the retention times of DC or BA, confirming the selectivity of the developed method. (Fig 5).

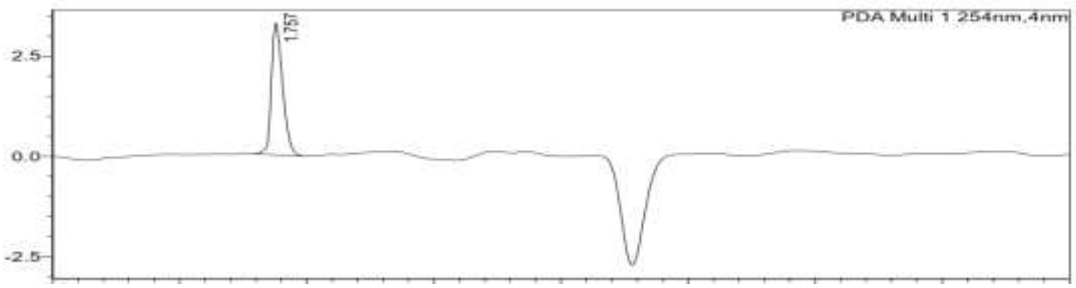


Fig. 5: selectivity, applying the developed method on common excipients

3.3.4. Robustness

The method's robustness was evaluated by testing slight variations in chromatographic conditions. Results as shown from table 6 demonstrated that, the small changes in flow rate had an insignificant impact on peak area.

Table 6: Robustness of the developed method

Flow rate (ml/min)	1.1	1	0.9	Average + SD	RSD%
Recovery % of DC	99.40	99.25	98.20	98.95±0.65	0.66
Recovery % of BA	99.12	99.27	99.33	99.24±0.11	0.11

3.4. Determination of Sodium Diclofenac and Benzyl Alcohol in pharmaceutical injectable forms

The results show that the developed method can be successfully applied to determine both compounds in ampoules (Fig. 6). Notably, BA concentration varies among marketed ampoules. Moreover, it exceeds the value of 4(w/v) % in some formulations (Table 7). It can be concluded that, controlling BA in the final products is crucial alongside the active pharmaceutical product.

3.5. Greenness assessment using the AGREE tool

AGREE metric as mentioned previously is a green analytical calculator. The calculation performed using this software depends on 12 parameters equal to the 12 principles of green analytical chemistry. Each principle or parameter contains a score range of 0–1 calculated based on the hazard to a particular principle's greenness. As the results show in (Fig.7), the final score is close to unity and the developed method is considered green [34] due to many factors: avoiding chemical derivation, using ethanol as a part of mobile phase beside the buffer, short retention time which reduces both the consumed solvents and the amount of resulted waste, moreover, it gives the advantages to analyze larger number of samples in a single run.

Table 7: Determination of DC, BA in ampoules by developed method*

Ampoule	Founded amount of DC (mg)**	Recovery % ± SD	Percentage of BA %
A	75.65	100.86 ± 0.24	3.80
B	75.51	100.68 ± 0.53	4.09
C	77.33	103.11 ± 0.44	5.12
D	77.85	103.80 ± 0.62	5.40
E	78.75	105.00 ± 0.67	4.40

*DC labeled amount is 75 mg /3ml, **Mean of five replicates, BA is not mentioned on the label

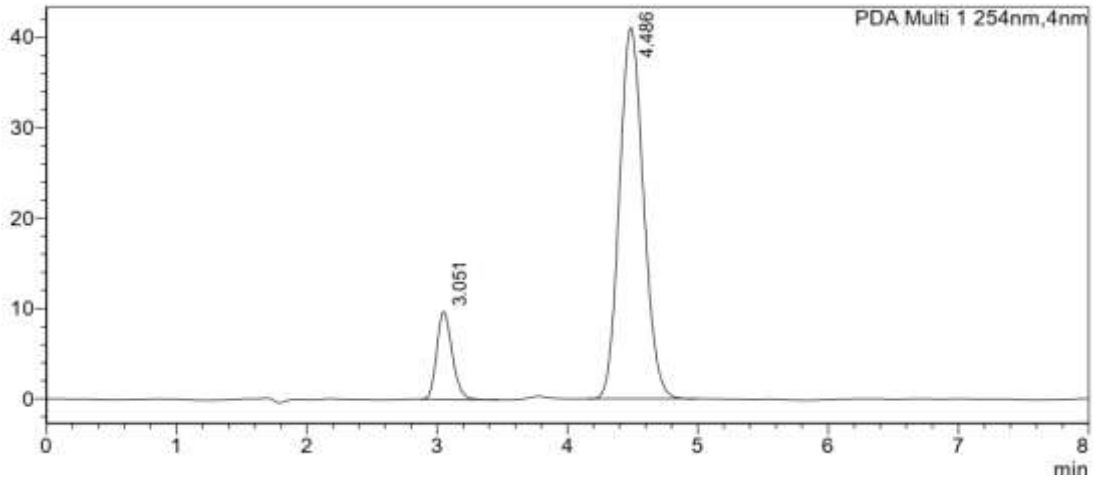


Fig. 6: Chromatogram of BA, DC in ampoule by the developed method

4.Conclusion

Control of not only the active pharmaceutical substances but also the preservatives and cosolvents, especially when they pose toxic effects, is important to ensure they are not excluding the acceptable range in injectable forms. In light of this concept, a simple, fast, precise, and green HPLC method was developed and validated to determine Sodium Diclofenac and Benzyl Alcohol simultaneously.

Throughout the development of the method, avoiding toxic solvents was a goal to enhance the greenness taking into account system suitability parameters. HPLC method was validated according to ICH guidelines and the greenness was evaluated using the AGREE tool. The results were excellent, as the run time was relatively short using ethanol in the mobile phase. Moreover, the method is suitable for routine analysis in quality control laboratories.

5. Acknowledgment

The authors are grateful to Miamed Pharmaceutical Industries for providing all the equipment and materials to achieve the research.

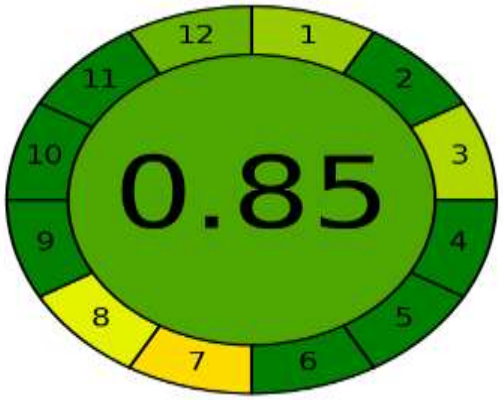


Fig. 7: AGREE tool score for the developed method

Funding information: this research is funded by Damascus university – funder No. (501100020595).

References:

1. Rashid O N, Bakir M H, Baban S O. Spectrophotometric determination of Diclofenac Sodium in pure form and in the pharmaceutical preparations. *Tikrit Journal of Pure Science*. 21 (3), 2016,76-80.
2. United State Pharmacopoeia, 46 .NF 41, 2023.
3. British Pharmacopoeia, 2022.
4. Indian Pharmacopoeia, vol 2, 2018.
5. Castillo M A, Bruzzone L. Indirect Fluorometric Determination of Diclofenac Sodium. *ANALYTICAL SCIENCES*, MARCH. 2006, VOL. 22.
6. Shivramchandra K**,** Agrawal Y.K. A Spectrophotometric determination of diclofenac sodium in tablets. *Journal of Pharmaceutical and Biomedical Analysis*. 9 (2), 1991, 97-100.
7. Alkassab R S, Hilal Y, Swed A. Development and Validation of a Simple Spectrophotometric Method for Quantitative Determination of Sodium Diclofenac in Modified Release Tablets. *Brazilian Journal of Analytical Chemistry*. 10(39), 2023, 70-79.
8. Belal T S, Bedair M M, Gazy A A., and GUIRGUIS K M. Validated Selective HPLC–DAD Method for the Simultaneous Determination of Diclofenac Sodium and Lidocaine Hydrochloride in Presence of Four of Their Related Substances and Potential Impurities. *Acta Chromatographica.* 27(3) 2015, 477–493.
9. Andraws G, Trefi S. Ionisable substances chromatography: A new approach for the determination of Ketoprofen, Etoricoxib, and Diclofenac sodium in pharmaceuticals using ion – pair HPLC. *Heliyon.* 6 ,2020, e04613.
10. Ahmed N R. High Performance Liquid Chromatographic Method for the determination of Diclofenac sodium in pharmaceutical preparations and in Environmental Samples. *Iraqi National Journal of Chemistry*.44, 2011,467-473.
11. Vasavi A, Visagaperumal D. Development and Validation of RP-HPLC method for simultaneous estimation of Eperisone Hydrochloride and Diclofenac Sodium in Pharmaceutical Dosage Form. *Journal of Advanced Pharmacy Education & Research* .3(4), 2013.
12. Bushra Tuwfeeq Alquadeib. Development and validation of a new HPLC analytical method for the determination of diclofenac in tablets. *Saudi Pharmaceutical Journal* .27, 2019, 66–70.
13. Atto R A. New method for determination of diclofenac sodium by High Performance Liquid Chromatography. *Tikrit Journal of Pharmaceutical Sciences.* 8(1), 602012.
14. Yilmaz B, Asci A, and Palabiyik S S. HPLC Method for Determination of Diclofenac in Human Plasma and Its Application to a Pharmacokinetic Study in Turkey. *Journal of Chromatographic Science*.49, 2011.
15. Rubim A M, Rubenick J B, Laporta L V, Rolim B. A simple method for the quantification of diclofenac potassium in oral suspension by high-performance liquid chromatography with UV-detection. *Brazilian Journal of Pharmaceutical Sciences*. 49(3), 2013.
16. El Sherbiny D, Wahba M. Development and validation
of liquid chromatographic methods for the estimation of the acceptance values of some hazardous preservatives in pharmaceutical formulations. A comparative study. *Journal of Taibah University for Science*.14(1), 2020:294-304.
17. Handbook of Pharmaceutical Manufacturing Formulations. Third edition, sterile products, volume six, 2020.
18. Meyler's. Side Effects of Drugs: The International Encyclopedia of Adverse Drug Reactions and Interactions. Fifteenth Edition, 2006.
19. Side A N. Miscellaneous Drugs, Materials, Medical Devices and Techniques. Chapter 47. Effects of Drugs Annual. 37, 2015:603-619.

20. Mehmood Y. Formulation development and evaluation of diclofenac sodium injection using benzyl alcohol (co-solvent), mixed solvency concept. *Edorium J Drug Res*.1,2015:1–8.
21. Lindeman C J, Rosolia A. Gas Chromatographic Assay for Benzyl Alcohol and Phenylethyl Alcohol in Pharmaceutical Formulations. *Journal of Pharmaceutical Sciences.* 58(1),1969:118-119.
22. Al-Zehouri J. Capillary Gas Chromatographic Method for the Determination of Benzyl Alcohol in Injectable Formulations. *International Journal of Pharmaceutical Sciences Review and Research* .8(2) May – June 2011; Article-002.
23. Sen I, Shandil. A and Shrivastava V S. Gas Chromatography-Mass spectrometric Determination of Benzyl alcohol in Injectable Suspensions. *Der Pharmacia Lettre***.** 3(2)2011: 218-225.
24. LIU Y, LAN W; SHI X. Determination of Benzyl Alcohol in Metamizole Sodium Injection by HPLC. *China Pharmacist*. (12),2018:1112-1114.
25. Hui Z, Hua-xin Y, Kang-sen X U. RP-HPLC Determination of Benzyl Alcohol . *Chinese Journal of Pharmaceutical Analysis*. 24, (4)1,2004:428-429.
26. V Khade, Mirgane S. High-performance liquid chromatography
method for the analysis of Benzyl Alcohol. *Journal of Scientific & Engineering Research*. 5, (11), November -2014.
27. Mohammed Khan K, Khan A R, Perveen S, Zia-Ullah, Ambreen N and Voelter W. High-Performance Liquid Chromatographic Quantitative Determination of Amcinonide and Benzyl Alcohol in Pharmaceutical Preparations. *The Open Analytical Chemistry Journal*. 2,2008:74-77.
28. Tan H S, Manning M A, Hahn M K, Tan H G, Kotagal U R. Determination of benzyl alcohol and its metabolite in plasma by reversed-phase high-performance liquid chromatography, *J Chromatogr*. Jul 17;568(1),1991:145-55.
29. Ghasemi J, Niazi A, and Ghobadi S. Simultaneous Spectrophotometric Determination of Benzyl Alcohol and Diclofenac in Pharmaceuticals Using Methods Based on the First Derivative of the Optical Density Ratio. *Pharmaceutical Chemistry Journal* .39(12),2005.
30. Ghasemi J, Niazia A and Ghobadib S. Simultaneous Spectrophotometric Determination of Benzyl Alcohol and Diclofenac in Pharmaceutical Formulations by Chemometrics Method. *Journal of the Chinese Chemical Society*.*52*, 2005:1049-1054.
31. Alzeer S. Extraction of caffeine and its metabolite paraxanthine from urine and measuring both of them by high-performance liquid chromatography. *Damascus University Journal for Medical Sciences*, 39(3),2023. (in Arabic)
32. Ghazal L, Almardini M A, Allous I. Development and validation of HPLC method for the assay of Etoricoxib in pharmaceutical dosage form. *Damascus University Journal for Medical Sciences*, 39(2),2023. (in Arabic)
33. Anastas P, Eghbali N. Green Chemistry: Principles and practice. *Chemical society Reviews.* 39,2010:301-312.
34. Kannaiah K P, Sugumaran A, Chanduluru H K, Rathinam S. Environmental impact of greenness assessment tools in liquid chromatography – *A review. Microchemical Journal*.170,2021: 106685.
35. International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use. ICH Harmonised Tripartite Guideline Validation of Analytical Procedures: Text and Methodology Q2(R1), 2005,1-13.

Statements and Declarations

Competing Interests:

- The corresponding author states that there is no conflict of interest.