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Determination of Ciprofloxacin in Pharmaceutical Preparations Using (ZIC-HILIC) with UV Detection

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Abstract. Hydrophilic interaction chromatography (HILIC) method was developed to quantify ciprofloxacin in pharmaceutical samples using two ZIC_1-HILIC and ZIC_4-HILIC exchangers. These exchangers were produced by grafting polymerization a sequence of sulfobetaine precursors onto the surface of porous PS/DVB particles. The separation was carried out with the mobile phase acetonitrile and acetate buffer (85:15), flow rate of 0.5 mL/min and detected at 273 nm was used. The various spacer lengths were used to investigate the retention behavior of ciprofloxacin. It should be noted that an increase in the length of the chain leads to an increase in the ciprofloxacin retention time, as indicated in ZIC_4-HILIC exchanger. The retention behavior of ciprofloxacin with two exchangers was investigated, and the findings identified the hydrophilic interactions. The calibration curves for ciprofloxacin with two exchangers over a concentration range from 0.05 to 9 μg mL^{-1}, LOD (0.008 and 0.005 μg mL^{-1}), LOQ (0.024, 0.015 μg mL^{-1}), respectively. The proposed method has been shown to be appropriate for pharmaceutical samples. Additionally, the proposed method's results are compared to those obtained using the standard method, and their precision and accuracy are comparable.

INTRODUCTION

Reversed-phase high-performance liquid chromatography (RP-HPLC) is widely used in drug analysis. A common chromatographic position for this purpose involves using C18 columns, and that the mobile phase is usually composed of an aqueous solution and an organic solvent [1]. But it is noted that the analysis with this type of columns needs a long time. In addition to that, some analyzes, such as high-polar compounds and hydrophilic compounds, are difficult to maintain using C18 columns. To solve these problems, hydrophilic interaction liquid chromatography (HILIC) is used, which is an excellent alternative to C18 columns [2, 3]. The mobile phase used in HILIC is polar solvents, the most commonly used, acetonitrile and water [4].

In HILIC mode, the retention mechanism is significantly different from the C18 columns. The separation depends on the drug distribution between the acetonitrile-rich mobile phase and the water-rich layer absorbed on the stationary polar phase [5]. There are multiple groups of stationary phases for HILIC, which fall into three classes, including neutral, charged, and zwitterionic phases [6]. This diversity in stationary phases gives HILIC the ability to separate and analyze many compounds. In this study, the HILIC mode was used to investigate the chromatographic interaction of ciprofloxacin. Ciprofloxacin (Fig.1) is a type of quinolone derivative of carboxylic acid. It's used to cure acute infections or infections that haven't responded to other antibiotics.

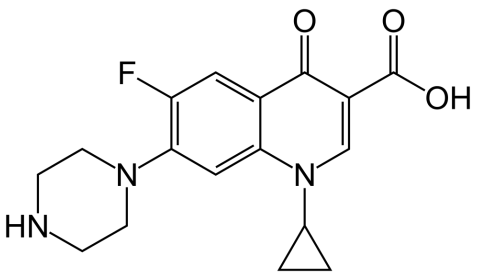

FIGURE 1. Ciprofloxacin chemical structure.

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Ciprofloxacin is used for bacterial infections treatments of the chest (including pneumonia) skin and bone infections are the most common types of infections [7-9]. Infections spread by sexual contact (STIs) conjunctivitis are a form of conjunctivitis. Infections in the eyes Otitis media (inflammation of the ear).

This drug is also used to treat urinary tract disorders [10], cardiovascular, gastrointestinal, diabetes and other digestive system disorders [11-13]. There are many analytical methods of measurement that can be achieved using several systematic techniques. Ciprofloxacin has been identified in pharmaceutical formulations using many techniques, including capillary electrophoresis [14, 15] and HPLC [16-18], which are often used to measure the amount of ciprofloxacin in medications, urine and plasma.

In light of this, the study aims to establish and validate a novel ZIC-HILIC-UV method for quantifying ciprofloxacin in pharmaceutical samples; this is the first goal of this study. The second goal of this study is to investigate retention behaviour for this medicine, using exchangers (ZIC_1-HILIC) and (ZIC_4-HILIC). Rasheed and co-workers had previously investigated the effect of chain length on charges between ZIC-HILIC exchangers [19-26]. It was discovered that the longer the chain between charges in ZIC-HILIC exchangers, the more significant interaction between the analyzes and the stationary phases. This study investigated the influence of chain length between the two charges using ZIC_1-HILIC and ZIC_4-HILIC. This goal was first investigated and has never been achieved in this class of drugs.

EXPERIMENTAL

Chemicals

Ciprofloxacin (purity 99%) was obtained from Sigma-Aldrich. HPLC grade acetonitrile (ACN), acetic acid (HAc), sodium acetate (NaOAc) were purchased from Merck. Both buffer materials and other chemicals used were of analytical reagent grade. A Millipore Milli-Q system (Millipore, USA) purification unit was used to provide high purity water. Millex® Syringe filter (0.45 μm) were used to filter the solution. Tablets of three different commercial companies, Neocipro 500 mg-Neophrma-UAE, Ciprofen 250 mg-Franklin Laboratories-India and Ciplox 100 mg- Cipla-India)

Instrumentation and chromatographic conditions

HPLC system (Merck Hitachi) with two exchangers (ZIC_1-HILIC and ZIC_4-HILIC) used for the ciprofloxacin separation. It is known that the zwitterionic molecule contains one methylene group between internal quaternary amines and outer sulfonic acids for (ZIC_1-HILIC), as well as for (ZIC_4-HILIC); it includes four methylene groups. Both ZIC_1-HILIC and ZIC_4-HILIC were prepared according to the previous work [22] onto the PS/DVB using PEEK exchangers (100 mm × 4 mm I.D.). HPLC device coupled with a UV detector (type L-4200) was used and a Merck Hitachi L-6200 Pump, in-line degasser with a 10 μL injection loop. The N2000 workstation's software was used to analyze the results. A pH 740 (WTW) and an ultrasonic bath were used. The mobile phase was filtered through Millex® Syringe filter (0.45 μm) and degassed. The UV-visible detector's wavelength was set to 273 nm. The flow rate was 0.5 mL /min. The temperature in the column oven is set to 40 °C.

Preparation of standard solution and pharmaceutical preparations

In the mobile phase, standard ciprofloxacin equal to 20 mg was dissolved and transferred into 100 ml of volumetric flasks. A filtration membrane filtered the resulting solution. The resulting solution was filtered using Millex® Syringe filter (0.45 μm). Individually weighed and triturated fifteen tablets of each of the two commercial pharmaceutical samples to produce a homogeneous mixture. A quantity of powder equal to 25 mg of commercial samples was dissolved in eluent and transferred to a volumetric flask with a volume of 100 mL. To ensure proper solubilization, the resulting solutions were sonicated for 15 minutes. Every commercial sample had a concentration of 250 μg mL^{-1}. Aliquots of each solution were diluted with eluent to a final concentration of 15 μg mL^{-1}.

RESULTS AND DISCUSSION

The chromatographic conditions shown in this study were derived from ZIC-HILIC, in which ciprofloxacin was selected as a retention mechanism for HILIC. This resulted in several experiments, including the effect of the ACN content, the impact of the buffer concentration and the eluent pH influence.

Investigate the effect of ACN content on ciprofloxacin retention

The choice of the mobile phase composition has an efficient and powerful effect on retaining compounds in the HILIC mode. It is frequently applied to the ZIC_1-HILIC and ZIC_4-HILIC exchangers to support compounds with poor water solubility when investigating specific influences. It was observed that when the level of acetonitrile was increased, the ciprofloxacin retention time increased (Fig. 2) at buffer acetate (4.75 pH-35 mM). Based on this effect, it became clear that ciprofloxacin behavior. Another indication that the hydrophobicity of ciprofloxacin is attributed to ciprofloxacin $logP_{OW}$ (-0.863).

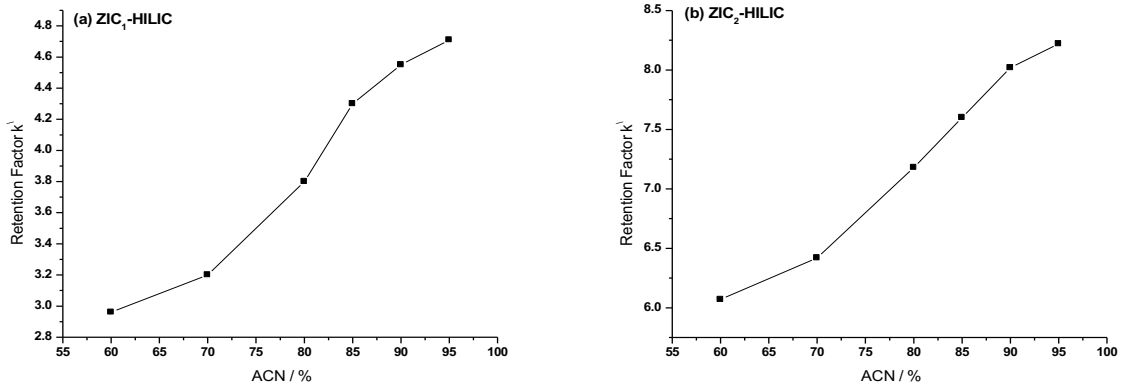

FIGURE 2. The effects of ACN fraction variance on ciprofloxacin behaviour (a) ZIC_1-HILIC exchanger (b) ZIC_4-HILIC exchanger.

The effect of salt concentration on ciprofloxacin retention

The aim of salt added to the mobile process represented by acetate buffer is to control the interaction between the charged analytes and the stationary phase. It is evident that as the concentration of salt increases, it will have an essential effect by reducing the electrostatic interactions of the charged analytes in the ZIC-HILIC exchangers. In this study, buffer acetate was used because of its strong solubility in the mobile phase. The effect of acetate buffer concentration on ciprofloxacin retention while maintaining a steady ACN content of 85% and pH 4.75 of buffer while varying the NaOAc/HAc buffer concentration (10-80 mM) in the mobile phase. Thus, when the concentration of buffer increases, the retention factor of ciprofloxacin increases (Fig. 3). This means that the exchangers (ZIC_1-HILIC and ZIC_4-HILIC) behave as HILIC material.

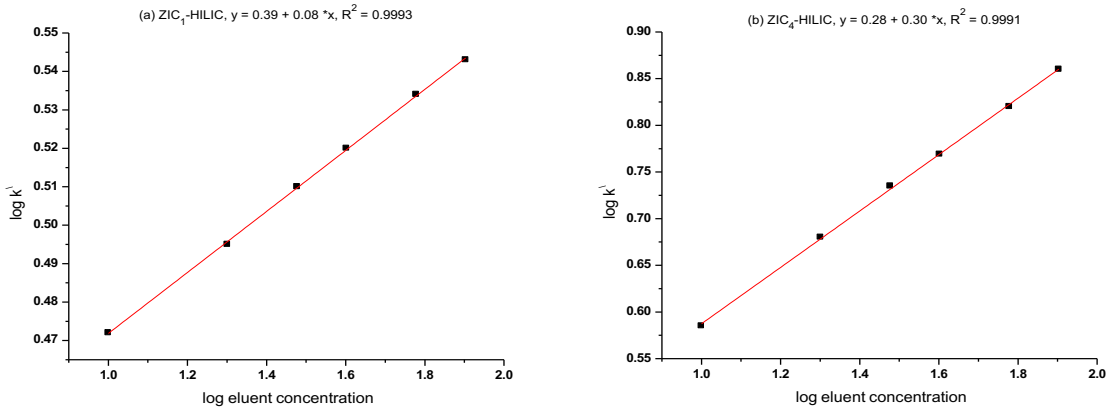

FIGURE 3. The effect of ciprofloxacin retention depending on variation buffer concentration (a) ZIC_1-HILIC exchanger (b) ZIC_4-HILIC exchanger.

The effect of eluent pH on ciprofloxacin retention

The eluent pH must be modified to complete the ciprofloxacin separation in HILIC mode. This significant effect is being investigated to resolve the strong electrostatic attraction between analyses and stationary HILIC content in charged states. To thoroughly study ciprofloxacin separation in HILIC mode, the eluent pH should be modified. At 35 mM buffer concentration and 85% ACN, the pH increased from 3 to 5.5. The retention factor of ciprofloxacin decreases, as seen in (Fig.5).

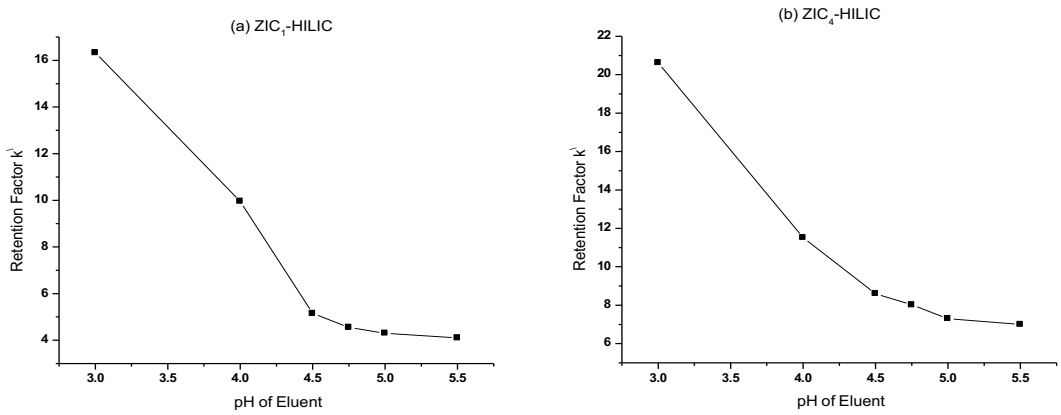

FIGURE 4. The effect of pH on ciprofloxacin retention (a) ZIC_1-HILIC exchanger (b) ZIC_4-HILIC exchanger.

Optimizing the separation of ciprofloxacin

After determining the optimal conditions for determining the ciprofloxacin separation mechanism using two exchangers (ZIC_1-HILIC and ZIC_4-HILIC), the chromatogram (Fig. 5) was obtained using acetate buffer 35 mM-pH 4.75 and 85% ACN. Ciprofloxacin retains the most in the ZIC_4-HILIC exchanger relative to the ZIC_1-HILIC column (Fig. 6). The inevitable reason for this is the presence of methylene groups between charged groups in exchangers. The ZIC_4-HILIC exchanger exhibits the highest ciprofloxacin retention when the exchangers are arranged geometrically. As a result, spacers between exchanger charges should affect ciprofloxacin retention.

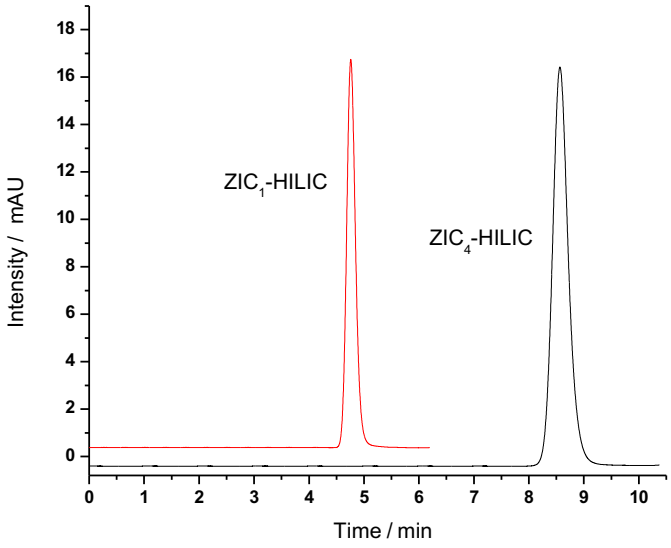

FIGURE 5. Chromatogram for the separations of ciprofloxacin using ZIC_1-HILIC and ZIC_4-HILIC exchangers.

Calibration graph

The calibration curves for the two columns demonstrated linearity over the ciprofloxacin concentration ranges of 0.05 to 9 µg mL^{-1} (fig.6). The coefficient of determination (R2) for the curve obtained by linear regression was 0.9996 for the ZIC_1-HILIC exchanger and 0.9994 for the ZIC_4-HILIC exchanger. Ciprofloxacin had a detection limit of 0.008, 0.005 and a quantification limit of 0.024, 0.015 µg mL^{-1} for two exchangers.

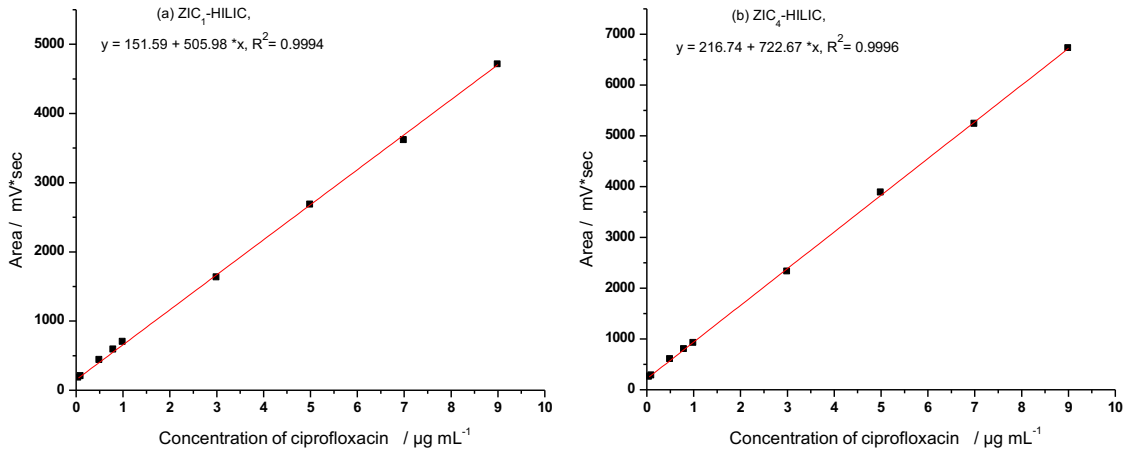

FIGURE 6: Calibration graph for ciprofloxacin using ZIC_1-HILIC and ZIC_4-HILIC exchangers.

Determination of ciprofloxacin in pharmaceutical preparations

The methods proposed were successfully applied to the determination of ciprofloxacin in three pharmaceutical formulations containing (tablets and injection). Table 1 summarizes the statistical data derived from the study of commercially available samples. The relative standard deviation (RSD) values were less than 0.8 percent, suggesting that the procedure was precise. The recovery values obtained ranged from 99.20 to 102.40 percent, confirming the proposed method's accuracy. Table 1 summarizes the percentages of RSD and recovery.

TABLE 1. Statistical data obtained in the analysis of ciprofloxacin in pharmaceutical preparations using ZIC_1-HILIC and ZIC_4-HILIC exchangers.

Name of drug	Theoretical Conc. (µg mL^{-1})	Found Conc. (µg mL^{-1})	Rec. %	RSD % n=7
		ZIC_1-HILIC		
Neocipro 500 mg	5.00	5.12	102.40	0.22
Ciprofen 250 mg	5.00	5.07	101.40	0.71
Ciplox 100 mg	5.00	4.98	99.60	0.43
		ZIC_4-HILIC		
	5.00	5.05	101.20	0.34
	5.00	5.10	102.00	0.26
	5.00	4.96	99.20	0.65

These findings are compared to those obtained using the British Pharmacopoeia protocol [27] in order to determine the HILIC method's expertise and effectiveness. The t-test and variance ratio F-test results (Table 2), both of which were 95% confident, were used as statistical analyses. The calculated values of t and F did not meet theoretical expectations, indicating that the accuracy of ciprofloxacin determination in pharmaceutical forms is comparable to both approaches.

TABLE 2. Statistical data obtained in the comparison of the proposed methods with official method for ciprofloxacin analysis using ZIC_1-HILIC and ZIC_4-HILIC exchangers.

Name of drug	ZIC_1-HILIC	ZIC_4-HILIC	Official method [27]	t-Test (theor.)	F-Test (theor.)
Neocipro 500 mg	102.40	101.20	101.55	0.7177* (2.7764)	2.8192* (19.000)
Ciprofen 250 mg	101.40	102.00	100.87	0.9715** (2.7764)	2.9126** (19.000)
Ciplox 100 mg	99.60	99.20	99.87		

*For ZIC_1-HILIC exchanger
** For ZIC_4-HILIC exchanger

CONCLUSIONS

Today, ZIC-HILIC-UV is a robust technique for precise and quantitative measurements of minimal levels of analytes in pharmaceutical samples. A new, quick and sensitive ZIC-HILIC-UV method was described to analyse ciprofloxacin pharmaceutical samples; besides this, the technique has better linearity, precision, accuracy, and sensitivity validation results. To obtain a complete and adequate separation of this drug used in this study, it is necessary to control and study some of the requirements that understand the separation process for ciprofloxacin. One of these essential requirements is to check the effect of acetonitrile ACN content on the separation of ciprofloxacin. It was found that when the acetonitrile components were increased, the behavior of these exchangers reached the highest level of performance in the separation of ciprofloxacin. The same applies to studying the effect of changing the concentration of the buffer solution used and regulating the pH, which provided a fuller understanding of the separation in these exchangers by controlling the separation time and the results achieved. These requirements give an understanding of the behavior of ciprofloxacin in ZIC-HILIC exchangers. This method was an applicable analytical procedure for identifying and quantifying ciprofloxacin in pharmaceutical samples.

REFERENCES

1. B. V. Rao, G. N. Sowjanya, A. Ajitha and V. U. M. Rao, World Journal Of Pharmacy And Pharmaceutical Sciences, 2015, 4, 405-423.
2. B. Buszewski and S. Noga, Analytical and bioanalytical chemistry, 2012, 402, 231-247.
3. W. Jian, R. W. Edom, Y. Xu and N. Weng, Journal of separation science, 2010, 33, 681-697.
4. P. Jandera, Analytica chimica acta, 2011, 692, 1-25.
5. F. Zhang, G. Shen, S. Ji and B. Yang, Journal of liquid chromatography & related technologies, 2015, 38, 349-352.
6. P. Jandera, Journal of separation science, 2008, 31, 1421-1437.
7. S. Kirmusaoglu, S. Yurdugül, A. Metin and S. Vehid, Urology journal, 2017, 14, 3028-3034.
8. J. N. Walker, A. L. Flores-Mireles, C. L. Pinkner, H. L. Schreiber, M. S. Joens, A. M. Park, A. M. Potretzke, T. M. Bauman, J. S. Pinkner and J. A. Fitzpatrick, Proceedings of the National Academy of Sciences, 2017, 114, E8721-E8730.
9. I. Baraboutis, E. Tsagalou, J. Lepinski, I. Papakonstantinou, V. Papastamopoulos, A. Skoutelis and S. Johnson, European journal of clinical microbiology & infectious diseases, 2010, 29, 1095-1101.
10. S. Karakonstantis and D. Kalemaki, Infection, 2018, 46, 293-301.
11. S. N. Buss, A. Leber, K. Chapin, P. D. Fey, M. J. Bankowski, M. K. Jones, M. Rogatcheva, K. J. Kanack and K. M. Bourzac, Journal of clinical microbiology, 2015, 53, 915-925.
12. K. J. Aldred, R. J. Kerns and N. Osheroff, Biochemistry, 2014, 53, 1565-1574.
13. A. D. Rowan-Nash, B. J. Korry, E. Mylonakis and P. Belenky, Microbiology and Molecular Biology Reviews, 2019, 83.
14. R. Bosma, J. Devasagayam, A. Singh and C. M. Collier, Scientific Reports, 2020, 10, 1-8.
15. K.-H. Bannefeld, H. Stass and G. Blaschke, Journal of Chromatography B: Biomedical Sciences and Applications, 1997, 692, 453-459.

16. Z. Liu, P. Moate, B. Cocks and S. Rochfort, Journal of agricultural and food chemistry, 2014, 62, 11568-11574.
17. J. Vella, F. Busuttil, N. S. Bartolo, C. Sammut, V. Ferrito, A. Serracino-Inglott, L. M. Azzopardi and G. LaFerla, Journal of Chromatography B, 2015, 989, 80-85.
18. S. N. Muchohi, N. Thuo, J. Karisa, A. Muturi, G. O. Kokwaro and K. Maitland, Journal of Chromatography B, 2011, 879, 146-152.
19. F. H. Abdulla and A. S. Rasheed, Current Pharmaceutical Analysis, 2020, 16, 935-941.
20. M. A. Abbas and A. S. Rasheed, Journal of the Chemical Society of Pakistan, 2018, 40, 89-94.
21. A. S. Rasheed, B. A. Al-Phalahy and A. Seubert, Journal of chromatographic science, 2017, 55, 52-59.
22. A. S Rasheed and A. Seubert, Current Chromatography, 2016, 3, 4-11.
23. A. Alknanei and A. S. Rasheed, International Journal of Drug Delivery Technology, 2020, 10, 344-348.
24. A. S. RASHEED, A. W. QASSIM and S. K. ABDULRAHMAN, International Journal of Pharmaceutical Research, 2020, 12.
25. Y. Abd Al-Hakeem and A. S. Rasheed, International Journal of Drug Delivery Technology, 2020, 10, 349-353.
26. Z. M. Hussein and A. S. Rasheed, Indian Journal of Forensic Medicine & Toxicology, 2021, 15, 2403.
27. B. Pharmacopoeia, 2020, I 589-591.