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A SIMPLE AND SENSITIVE RP-HPLC METHOD FOR SIMULTANEOUS DETERMINATION OF HYDROXYCHLOROQUINE SULPHATE AND NITAZOXANIDE IN BINARY COMBINATION

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Keywords:

Hydroxychloroquine sulphate (HCQ), Nitazoxanide (NTZ), High-Performance Liquid Chromatography, Development and Validation, Simultaneous determination, ICH guidelines

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ABSTRACT: The purpose of the present research was to develop a suitable, simple, precise, accurate, robust and reproducible RP-HPLC method for reliable simultaneous quantification of Hydroxychloroquine sulphate (HCQ) and Nitazoxanide (NTZ) in a synthetic mixture. The samples were assayed by the cyber lab HPLC instrument using a C18 (250mm, × 4.6mm, D., 5µm) –cyber-sil under isocratic conditions. The mobile phase consisted of Phosphate buffer (pH-3 adjusted with 1% OPA): Methanol: Acetonitrile (30:35:35 v/v/v) with a flow rate of 1.5 ml/min. The wavelength for detection was 266nm (Isoabsorptive point). The Retention time of HCQ and NTZ was 2.8 min and 9. 3min, respectively. The method was validated by determining system suitability, selectivity, sensitivity, linearity, inter-day and intra-day precision, accuracy, robustness and stability in accordance with the ICH guidelines. The calibration curve was linear over the concentration range, 2-10µg/ml for HCQ and 5-25µg/ml for NTZ respectively (n=6). The developed chromatographic method proved to be simple, precise, accurate, robust, and reproducible. Moreover, the samples showed stability at room temperature over a period of 48 h. Thus, this method would be employed for routine simultaneous quantification of Hydroxychloroquine sulphate and Nitazoxanide.

INTRODUCTION: Hydroxychloroquine sulphate is chemically 2-[4-[(7-chloroquinoline-4-yl) amino] pentylethylamino] ethanol; sulfuric acid **Fig. 1A** with Chemical formula $C_{18}H_{28}C_lN_3O_5S$ and Molecular weight 434g/mol. It is used as antimalarial drug [1]. Hydroxychloroquine sulfate (HCQ) is used in combination with Nitazoxanide for SARS-coronavirus 2 (SARS-CoV-2) [2].

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The ACE2 undergoes glycosylation for it to convert to an active form. When the SARS-CoV-2 S protein bind to it, the ACE2 receptor undergoes glycosylation and gets activated. HCQ prevents the glycosylation of ACE2 receptors. So, HCQ prevents entry of SARS-CoV-2 into the host organisms [3].

Nitazoxanide is chemically [2-[(5-nitro-1,3-thiazol 2yl) carbamoyl] acetate **Fig. 1B** with Chemical formula $C_{12}H_9N_3O_5S$ and Molecular weight 307.28g/mol. It is used as antiprotozoal [4]. The reason behind selecting NTZ for SARS-CoV-2 could be derived from its impact on the immune system in potentiating the production of type 1 interferon and bronchodilation of the airways

through inhibition of TMEM16A ion channels. NTZ inhibits the production of pro-inflammatory cytokines TNFα, IL-2, IL-4, IL-5, IL-6, IL-8 and IL-10 in peripheral blood mononuclear cells [5]. Several HPLC methods have been described for quantifying Hydroxychloroquine sulphate, alone or in combination with other drugs [6–19].

Moreover, several methods have been described for quantifying Nitazoxanide, alone or in combination with other drugs in various pharmaceutical preparations [20–28].

However, the simultaneous determination of Hydroxychloroquine sulphate and Nitazoxanide in combination has not been reported previously.

Thus, the present investigation aimed to develop a suitable, simple, precise, accurate, robust and reproducible-HPLC method for simultaneously estimating Hydroxychloroquine sulphate and Nitazoxanide in a synthetic mixture. The method was validated according to ICH guidelines [29, 30, 31].

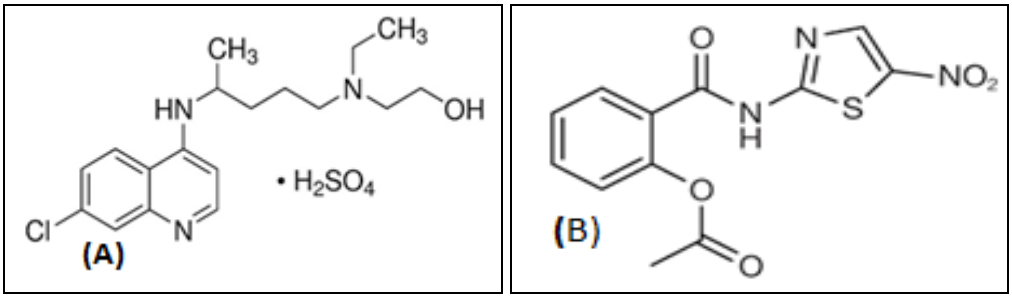

FIG. 1: CHEMICAL STRUCTURE OF (A) HYDROXYCHLOROQUINE SULPHATE AND (B) NITAZOXANIDE

MATERIALS AND METHODS:

Reagents and Material: Hydroxychloroquine sulphate and Nitazoxanide were kindly provided by Globela Pharma Pvt. Ltd. HPLC grade Methanol (Advent Chembio–Pvt. Ltd), HPLC grade Acetonitrile (Rankem), double distilled water, Potassium Hydroxide (ACS Chemicals) and Orthophosphoric acid (Chem think Lab) were used for development of method. All other reagents and solvents used in this study were of analytical grade.

Instrument and Chromatographic Conditions: Chromatographic analysis was performed using LC 100 HPLC system (CYBER LAB, USA), equipped with Column: C18 (250mm, × 4.6mm D., 5µm) – cyber-sil, Detector: D2 lamps-(UV 100 UV detector), DS-100 data system software.

The mobile phase consisted of phosphate buffer (1.0 M) pH-3 adjusted with 1% OPA: Methanol: Acetonitrile (30:35:35 v/v/v). HPLC with chromatographic conditions as flow rate 1.5ml/min, UV detection at wavelength 266nm, Injection volume 20µl, isocratic mode, and run time 15min were used to get the best separation.

Preparation of Standard Solutions: Accurately weighed HCQ and NTZ (25 mg each) were transferred to two separate 25 ml volumetric flasks. They were dissolved in methanol to get a stock solution having a strength of 1000µg/ml.

Further, Aliquot of 2.5 ml from the above standard stock solutions was pipette out into two separate 25ml of volumetric flasks. It diluted up to the mark with methanol to get a stock solution having a strength of 100µg/ml. Five standards with 2, 4, 6, 8, and 10 µg/ml concentrations for HCQ and 5, 10, 15, 20 and 25 µg/ml concentrations for NTZ were derived from the stock solution of HCQ and NTZ.

Preparation of Test Solution: The combination of HCQ and NTZ is under clinical trials, so we have prepared a synthetic mixture. The synthetic mixture of HCQ and NTZ was prepared in a ratio of 2:5. Common excipients like 400 mg of Microcrystalline cellulose, 300 mg of Hydroxypropyl methylcellulose, 900 mg of Lactose monohydrate, 200 mg of talc and magnesium stearate along with 10,000 mg of NTZ and 4,000 mg of HQC were transferred into motor pestle which is equivalent for 20 tablets.

A synthetic mixture equivalent to 20mg HCQ and 50mg NTZ was taken in a 100ml volumetric flask, dissolved in methanol, and sonicated for 10min. The solution was filtered with Whatman filter paper 42. From the filtrate, 0.5 ml solution was

taken and diluted to 10 ml with methanol to get a solution strength 10 µg/ml HCQ and 25 µg/ml NTZ. Then, 20 µl of the resulting solution was analyzed using the HPLC method described above.

Validation of RP-HPLC Method:

System Suitability: The system suitability was assured by determining the peak retention time, peak area, theoretical plates, and tailing or asymmetry factor for HCQ and NTZ. The acceptance criteria of % CV<2% for retention time and peak area, theoretical plates >2000 [32] and tailing factor <2.0.

The standard concentration of 6µg/ml HCQ and 15µg/ml NTZ was used to prepare the samples. The sample preparation was accomplished in accordance with the method described above in Section 2.4. Six replicate samples were assayed to determine the system's suitability.

Specificity: The specificity of the method was determined by analyzing standard and sample solutions of HCQ and NTZ in accordance with the method as described above. The excipients present in the synthetic mixture do not interfere in the measurement of HCQ and NTZ.

Sensitivity: The sensitivity of simultaneous determination of HCQ and NTZ was evaluated from the Limit of detection (LOD) and Limit of Quantification (LOQ) of the developed method. LOD and LOQ were calculated from the six-calibration curves.

The LOD and LOQ were calculated by using the formula.

$$LOD=3.3 \times \sigma / S$$

$$LOQ=10 \times \sigma / S$$

Where, σ = standard deviation of intercept of six calibration curves

S = mean slope of six calibration curves

Linearity: Five standard concentrations of 2, 4, 6, 8 and 10 µg/ml for HCQ and 5, 10, 15, 20 and 25 µg/ml for NTZ were prepared and analyzed according to the method as described above. The slope, intercept, and correlation coefficient (r2) were calculated for HCQ and NTZ regression analysis.

Accuracy: The accuracy was determined by calculating HCQ and NTZ percentage recovery. The spiking was done at levels 50%, 100%, and 150%. The samples were prepared according to the method described in above Section.

Thus, the final concentrations to be assayed by the described method were 6 µg/ml, 8 µg/ml, and 10 µg/ml for HCQ and 15 µg/ml, 20 µg/ml and 25 µg/ml for NTZ. The actual concentration was determined for each sample, and the mean percent recovery was calculated (n = 3).

Precision: For evaluating precision, repeatability, intra-day and inter-day variances were determined over a short interval of time, 1 day and 3 days, respectively. Standard concentrations of 6µg/ml for HCQ and 15µg/ml for NTZ were analyzed seven times for the repeatability study.

While, three concentrations 2, 6 and 10 µg/ml for HCQ and 5, 15 and 25 µg/ml for NTZ were analyzed for intra-day and inter-day precision study. All the samples were prepared and analyzed per the method described above. The % CV was calculated for each sample analyzed.

Robustness: The effect of slight deliberate variation in chromatographic parameters such as mobile phase flow rate and change in detection wavelength on peak area ratio was observed one by one.

The standard concentration 6µg/ml HCQ and 15µg/ml NTZ strength used in Robustness study. The mean peak area was determined for HCQ and NTZ and the % CV was calculated (n=3).

Stability: The standard solution of 4 µg/ml HCQ and 10 µg/ml NTZ was used to test the stability. The samples were prepared and analyzed according to the method described above.

The stability of HCQ and NTZ in the prepared sample was determined by analyzing concentration at 1, 6, 12, 24 and 48 h. The concentration was determined for HCQ and NTZ at each time point (n=3).

RESULT AND DISCUSSION: Hydroxy-chloroquine Sulphate and Nitazoxanide are soluble in methanol. In the present HPLC method, mobile

phases were used as mobile phases for the phosphate buffer (1.0 M) pH-3 adjusted with 1% OPA: Methanol: Acetonitrile (30:35:35 v/v/v). Separation was done using C18 Column (250mml, × 4.6mml, D., 5µm) –cyber-sil, D2 lamps-(UV 100 UV detector), DS-100 data system software. The described chromatographic conditions resulted in HCQ and NTZ retention at about 2.8 ± 0.29 and 9.3 ± 0.12 minutes, respectively **Fig. 2.**

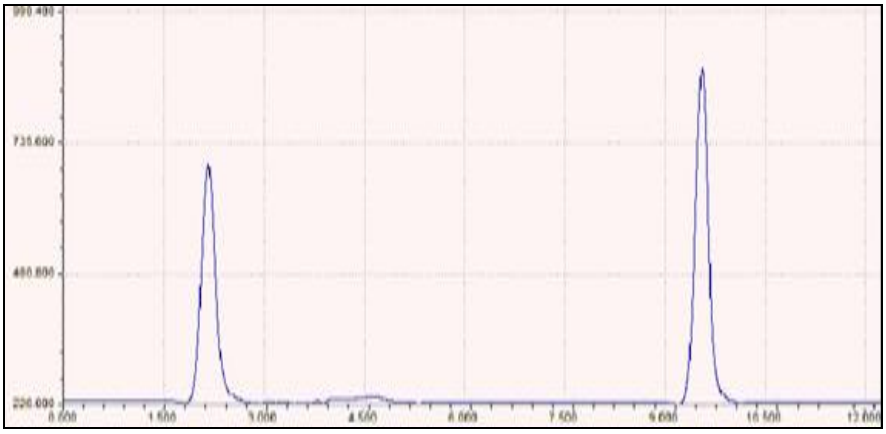

FIG. 2: CHROMATOGRAM SHOWING RETENTION TIME OF HCQ (2.7 MIN) AND NTZ (9.3 MIN)

According to the USP, the HPLC method is considered suitable when the CV of peak area < 2%, the tailing factor < 2, and the theoretical plates > 2000 [32]. The results of system suitability are shown in **Table 1**.

All the measured parameters are within the recommended limits. Thus, our results suggested that the described method was suitable for the simultaneous determination of HCQ and NTZ.

TABLE 1: SYSTEM SUITABILITY ANALYSIS

Drugs	Parameters	Mean ± S.D. (n=6)	%CV
HCQ	Retention Time(min)	2.866 ± 0.01027	0.3582
	Theoretical Plate	4436.388 ± 25.427	0.5731
	Tailing Factor	1.247 ± 0.9714	0.9714
NTZ	Retention Time(min)	9.3895 ± 0.0449	0.4786
	Theoretical Plate	7188.305± 51.577	0.7175
	Tailing Factor	1.647± 0.01211	0.7354
Resolution		7.328 ± 0.0402	0.5486

The excipients present in the synthetic mixture during testing specificity do not interfere with the measurement of HCQ and NTZ. No additional peak interfering with the analyte-peaks was seen in the chromatogram **Fig. 3A, B, C**. Accordingly, our results suggested that the described analytical method demonstrated specificity.

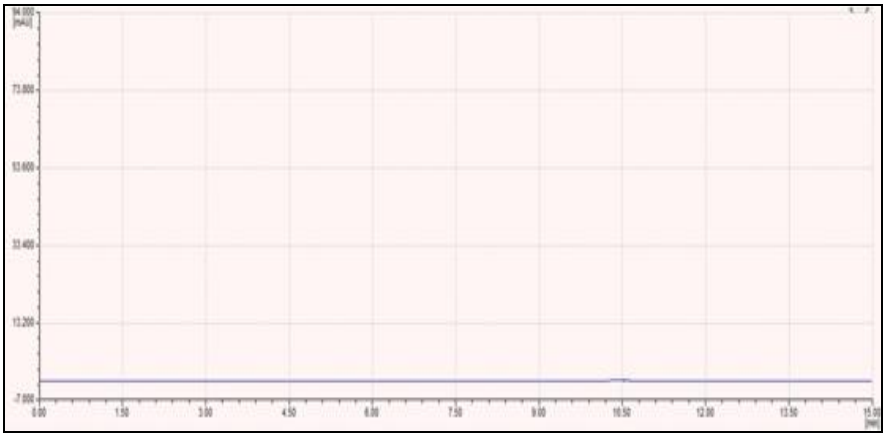

FIG. 3A: CHROMATOGRAM OF MOBILE PHASE

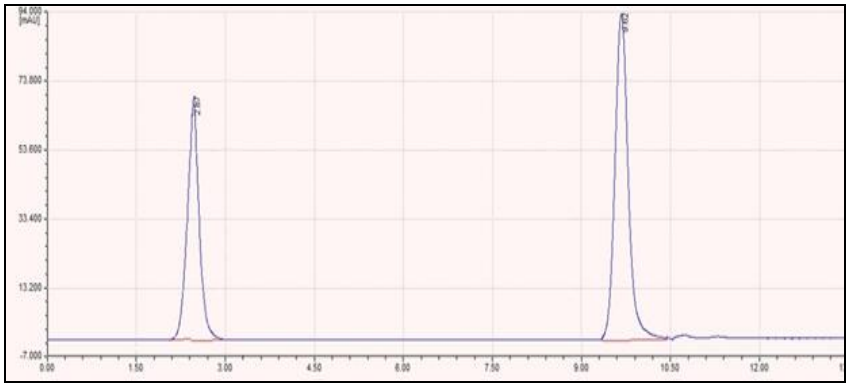

FIG. 3B: CHROMATOGRAM OF SAMPLE HCQ (10µG/ML) AND NTZ (25µG/ML)

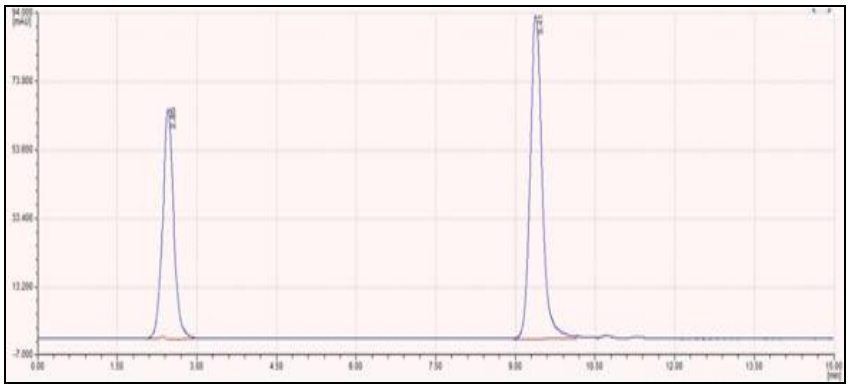

FIG. 3C: CHROMATOGRAM OF STANDARD HCQ (10µG/ML) AND NTZ (25µG/ML)

The Limit of detection (LOD) and Limit of Quantification (LOQ) of the developed method was calculated from the six-calibration curve. LOD and LOQ of HCQ were found to be 0.07 µg/ml and 0.21 µg/ml, respectively. While, LOD and LOQ of NTZ was found to be 0.25 µg/ml and 0.75 µg/ml respectively. Which shows sensitivity of the developed method. The range for constructing the calibration curves of HCQ and NTZwas 2-6 µg/ml and 5-25 µg/ml, respectively. **Fig. 4A, B**. The response was linear throughout the range for both analytes (r^2 = 0.9997 and r^2 = 0.9998, respectively). The Overlay Chromatogram of different concentrations of HCQ (2-10 µg/ml) and NTZ (5-25 µg/ml) is shown in **Fig. 5** and the regression analysis is shown in **Table 2**. According to the ICH guidelines, the calibrators should not deviate by more than 15% of the nominal concentrations [31]. All the concentrations determined were within 85–115% of the corresponding nominal concentrations; therefore, the method exhibited linearity.

TABLE 2: REGRESSION ANALYSIS DATA OF HCQ AND NTZ

	HCQ	NTZ
Regression line equation	y = 8380.3x + 1507.8	y = 2964.7x + 847.5
Regression coefficient (R^2)	0.9997	0.9998

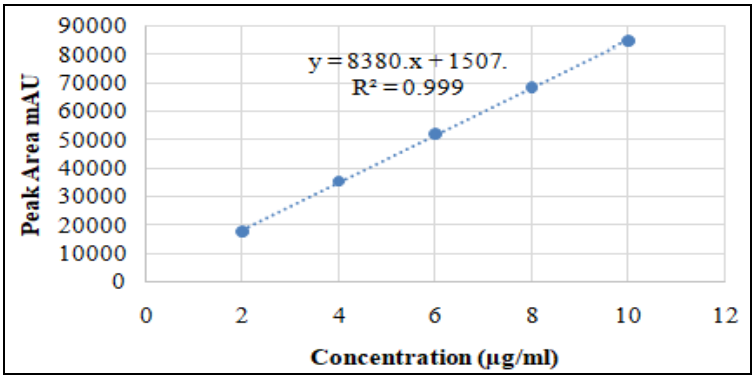

FIG. 4A: CALIBRATION CURVE OF HCQ

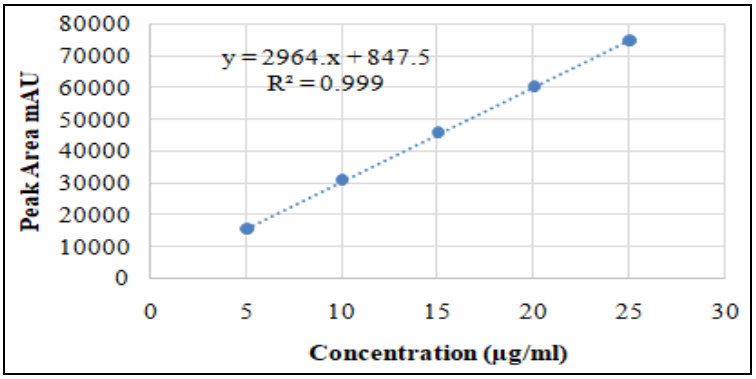

FIG. 4B: CALIBRATION CURVE OF NTZ

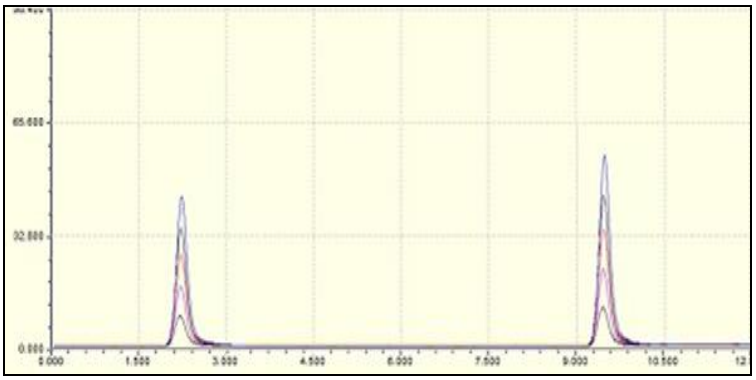

FIG. 5: OVERLAY CHROMATOGRAM OF DIFFERENT CONCENTRATIONS OF HCQ (2-10 µG/ML) AND NTZ (5-25 µG/ML)

The accuracy was assessed by the percent recovery method. The accuracy data are shown in **Table 3**. According to the ICH guidelines, the mean value should be within 15% of the nominal value, [31].

All the accuracy values were within 98–102% of the recovery range. Thus, our results suggested that the described method was accurate.

TABLE 3: ACCURACY STUDY DATA OF HCQ AND NTZ

Level	HCQ				NTZ			
	Conc. From Synthetic mixture (µg/ml)	Amount of Std. added (µg/ml)	amount recovered (µg/ml)* Mean ± SD	% Recovery	Conc. from Synthetic mixture (µg/ml)	Amount of Std. added (µg/ml)	amount recovered (µg/ml)* Mean ± SD	% Recovery
50%	4	2	2.03± 0.012	101.67%	10	5	5.01±0.023	100.20%
100%		4	4.03 ± 0.084	100.91%		10	9.98±0.179	99.80%
150%		6	5.97±0.012	99.54%		15	15.23±0.144	101.59%

The precision was determined by % CV. The inter-day and intra-day precision data are shown in **Table 4**. According to the ICH guidelines, the precision determined at each concentration should not exceed 2% of the CV [31]. All the tested samples showed the % CV within the accepted limits, suggesting that the described method was precise.

TABLE 4: PRECISION DATA OF HCQ AND NTZ

HCQ			NTZ		
Conc. (µg/ml)	Mean peak area ±SD	% CV	Conc. (µg/ml)	Mean peak area ±SD	% CV
Repeatability					
6	52773.88±445.52	0.8431	15	461375.20±214.05	0.4610
Intraday Precision					
2	17654.38±219.68	1.2444	5	15539.1±217.04	1.3968
6	53288.68±572.18	1.0738	15	45603.78±337.27	0.7396
10	84054.55±899.31	1.0699	25	73938.64±878.64	1.1875
Interday Precision					

2	17354.68±245.64	1.4155	5	15731.29±271.04	1.5812
6	54092.64±727.88	1.3456	15	45894.73±437.13	1.0832
10	85498.62±955.51	1.1175	25	74454.17±1120.23	1.5046

The effect of minor intentional changes in the described chromatographic conditions is shown in **Table 5.** "System suitability" and "robustness" were performed to ensure the reliability of our method. The slight variations in the mobile phase flow rate and change in detection wavelength did not result in considerable differences in the analytes' retention time and peak area ratio. Thus, the described method exhibited robustness.

TABLE 5: ROBUSTNESS DATA OF HCQ AND NTZ

Parameter	Change in condition	Mean Peak Area ± SD (n=3)		% CV	
		HCQ	NTZ	HCQ	NTZ
Change in detection wavelength	265nm	53954±362.72	45495 ±162.96	0.6723	0.3582
	267nm	53693 ±383.65	45595 ±335.86	0.7149	0.7366
Change in flow rate	1.4 ml/min	53621 ±284.77	45528±137.13	0.5311	0.4331
	1.6ml/min	53759 ±288.88	45763±183.46	0.5002	0.4140

The stability test of HCQ and NTZin the samples was performed at 1, 6, 24 and 48 h **Table 6**. The test was carried out in triplicate at each time point. The concentration results at various time points were not significantly different from one another.

TABLE 6: STABILITY OF SOLUTION

Time (hr)	Conc. (µg/ml) ± SD (n=3)	
	HCQ	NTZ
1	5.99±0.032	15.12±0.034
6	5.96±0.014	15.19±0.101
24	5.94±0.023	15.01±0.079
48	5.90±0.056	14.98±0.094

Application of the proposed method for analysis of HCQ and NTZ in synthetic mixture. The concentrations of HCQ and NTZ in synthetic mixture were determined using the developed HPLC method. The % assay values are given in **Table 7.**

TABLE 7: ASSAY OF HCQ AND NTZ IN SYNTHETIC MIXTURE

Sr. no.	Drug	%Assay* ± SD (n=3)	%CV
1	HCQ	100.65±0.012	0.3033
2	NTZ	100.98±0.057	0.5689

CONCLUSION: All the parameters for HCQ and NTZ met the criteria of the ICH guidelines for the method validation and found to be suitable for routine quantitative analysis. The result of linearity, accuracy, precision proved to be within limits with lower limits of detection and quantification. So, developed method is accurate, sensitive, specific, robust, and precise.

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CONFLICTS OF INTEREST: The authors declare that they have no competing interests.

REFERENCES:

1. Hydroxychloroquine sulfate. Pubchem. https://pubchem.ncbi.nlm.nih.gov/compound/Hydroxychloroquine-sulfate (accessed 01.12.2021)
2. Satarker SA, Banerjee TN, Agarwal S and Madhavan TN: Hydroxychloroquine in COVID-19: potential mechanism of action against SARS-CoV-2. Current Pharmacology Reports 2020; 6: 1-9.
3. Hydroxychloroquine and Nitazoxanide Combination Therapy for COVID-19. National Library of Medicine (US). https://www.coronavirus.gov. (accessed 2.12.2021)
4. Nitazoxanide. Pubchem. https://pubchem.ncbi.nlm.nih.gov/compound/Nitazoxanid. (accessed1.12.2020)
5. Khatri M and Mago P: Nitazoxanide Comstat Combination for COVID19: An unexplored potential therapy. Chemical biology letters 2020; 7: 27-98.
6. Xiong X, Wang K and Tang T: Development of a chiral HPLC method for the separation and quantification of hydroxychloroquine enantiomers. Sci Rep 2021; 11: 8017.
7. Singh A, Roopkishora, Singh CL, Gupta R, Kumar S and Kumar M: Development and Validation of Reversed-Phase

High Performance Liquid Chromatographic Method for Hydroxychloroquine Sulphate. Indian J Pharm Sci 2015; 77(5): 586-591.

8. Tett SE, Cutler DJ and Brown KF: High-performance liquid chromatographic assay for hydroxychloroquine and metabolites in blood and plasma, using a stationary phase of poly (styrene divinylbenzene) and a mobile phase at pH 11, with fluorimetric detection. J Chromatogr 1985; 344: 241-248.
9. Emami J, Kazemi M and Salehi A: *In-vitro* and *In-vivo* Evaluation of Two Hydroxychloroquine Tablet Formulations: HPLC Assay Development. J Chromatogr Sci 2021; 59(1): 71-78.
10. Parvinizadeh, Farnaz & Daneshfar: "Fabrication of magnetic metal-organic framework molecularly imprinted polymer for extraction of anti-malaria agent hydroxychloroquine." New J. Chemistry 2019; 43-52.
11. Dongala T, Katari NK, Palakurthi AK: Stability Indicating LC Method Development for Hydroxychloroquine Sulfate Impurities as Available for Treatment of COVID-19 and Evaluation of Risk Assessment Prior to Method Validation by Quality by Design Approach. Chromatographia 2020; 83: 1269–1281
12. Volin P: Simple and specific reversed-phase liquid chromatographic method with diode-array detection for simultaneous determination of serum hydroxychloroquine, chloroquine and some corticosteroids. J Chromatogr B Biomed Appl 1995; 666(2): 347-353.
13. Andrew J. McLachlan; Susan E. Tett and David J: Cutler "High-performance liquid chromatographic separation of the enantiomers of hydroxychloroquine and its major metabolites in biological fluids using an α 1 -acid glycoprotein stationary phase. The Journal of Chromatography 1991; 119-27.
14. Cardoso CD and Bonato PS: Enantioselective analysis of the metabolites of hydroxychloroquine and application to an *in-vitro* metabolic study. J Pharm Biomed Anal 2005; 37(4): 703-708.
15. Dongala T, Ettaboina SK and Katari NK: A Novel RP-HPLC-DAD Method Development for Anti-Malarial and COVID-19 Hydroxy Chloroquine Sulfate Tablets and Profiling of In-Vitro Dissolution in Multimedia. Res Sq [Preprint]. 2020 May 5: rs.3.pex-880.
16. Rudraraju AV, Hossain MF, Shrestha A, Amoyaw PNA and Tekwani BL: *In-vitro* Metabolic Stability Study of New Cyclen Based Antimalarial Drug Leads Using RP-HPLC and LC-MS/MS. Mod Chem Appl 2014; 129: 2329-6798.
17. Qu Y, Noe G, Breaud AR, Vidal M, Clarke WA, Zahr N, Dervieux T, Costedoat-Chalumeau N and Blanchet B: Development and validation of a clinical HPLC method for the quantification of hydroxychloroquine and its metabolites in whole blood. Future Sci OA 2015; 1(3): 345-349.
18. Shrivastava A, analytical methods for the determination of Hydroxychloroquine in various matrices. International Journal of Applied Pharmaceutics 2020; 12(4): 55-61.
19. Yimin Wei, Gloria A. Nygard & Shoukry KW Khalil: A HPLC Method for the Separation and Quantification of the Enantiomers of Hydroxychloroquine and Its Three Major Metabolites. J Liq Chromatogr 1994; 17: 16, 3479-3490.
20. Jadhav VY, Gandhi SV and Dhaval ND: "RP-HPLC determination of nitazoxanide in bulk and different tablet formulations."Eurasian J AnalChem 2008; 3: 212-220.
21. Rane V and Shinde D: "Simultaneous High-Performance Liquid Chromatographic Determination of Nitazoxanide and Ofloxacin in Tablet Formulation." Eurasian J Anal Chem 2009; 4: 304-310.
22. Sharma, Shivam, Anil Bhandari & Choudhary: "RP-HPLC Method for Simultaneous Estimation of Nitazoxanide and Ofloxacin in Tablets." Indian J Pharm Sci 2011; 73.
23. Kalta RR, Sharma R and Chaturvedi SC: Simultaneous RPHPLC Determination of Nitazoxanide and Ofloxacin in Combined Tablet Dosage Form. Indian J Pharm Sci 2008; 70(4): 491-494.
24. Ruiz-Olmedo MI, Gallegos-Perez JL, Calderon-Gonzalez KG, Franco-Perez J and Jung-Cook H: Sensitive high performance liquid chromatographic assay for nitazoxanide metabolite in plasma. Pharmazie 2009; 64(7): 419-422.
25. Sharma S: Development and Validation of a Dissolution method with Isocratic High-Performance Liquid Chromatographic Determination of Nitazoxanide and Ofloxacin in Pharmaceutical Dosage form." Am.-Eurasian J Sci Res 2011; 6: 182-187
26. Hegazy M, Kessiba A, El Gindy AE and Abdelkawy M: Validated stability indicating RP-HPLC for quantitation of nitazoxanide in presence of its alkaline degradation products and their characterization by HPLC-tandem mass spectrometry. J Chromatogr Sci 2014; 52(9): 1071-1081.
27. Shalan S, Nasr JJ and Belal F: Determination of tizoxanide, the active metabolite of nitazoxanide, by micellar liquid chromatography using a monolithic column. Application to pharmacokinetic studies. Anal. Methods 2014; 6(21): 8682–8689.
28. Jadhav AS, Pathare DB and Shingare MS: A Validated Stability Indicating RP-LC Method for Nitazoxanide, a New Antiparasitic Compound. Chroma 2007; 66: 595-600
29. ICH, "Q2 (R1) validation of analytical procedure, test and methodology," in Proceedings of the International Conference on Harmonization, Geneva, Switzerland, 2005.
30. ICH, Q2A. Validation of Analytical Procedures, Consensus Guidelines, ICH Harmonized Tripartite Guidelines, 1994.
31. ICH, Q2B. Validation of Analytical Procedures: Methodology, Consensus Guidelines, ICH Harmonized Tripartite Guidelines 1996
32. Shabir GA: Validation of high-performance liquid chromatography methods for pharmaceutical analysis: understanding the differences and similarities between validation requirements of the US Food and Drug Administration, the US Pharmacopeia and the International Conference on Harmonization. J Chromatogr A 2003; 987(1): 57–66.

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