ISSN 0975-2366
DOI:https://doi.org/10.31838/ijpr/2020.12.04.608

Research Article

Development of The Stable, Reliable, Fast and Simple RP-HPLC Analytical Method for Quantifying Diphenhydramine-Hcl (DPH) In Pharmaceuticals

H. N. K. AL-SALMAN[1*], ERFAN A. S. ALASSADI[1], RAJAA HUSSEIN FAYADH [2], HUSSEIN H. HUSSEIN [1], EKHLAS QANBER JASIM[1]
[1]Department of Pharmaceutical Chemistry, College of Pharmacy, University of Basrah, Iraq.
[2]Department of Anesthesia, Medical Technical Institute, Southern Technical University, Basrah, Iraq,
*Corresponding Author
E-mail:hsennaserh@yahoo.com,
Received: 09.10.20, Revised: 07.11.20, Accepted: 22.12.20

ABSTRACT
Context: In this manuscript, a high-performance liquid chromatography (HPLC) technique for the estimation of Diphenhydramine HCl (DPH)in pharmaceuticals was described and developed. Methods: The reversed-phase HPLC (RP-HPLC) process was urbanized and the results obtained to determine the form of Diphenhydramine. Chromatographic analysis was performed in HPLC-ultraviolet (HPLC-UV) system with Ion Pac column; Arcus EP-C18; 5 µm, 4.6 mm× 250 mm, with Methanol: acetonitrile: water: 10mM Heptan sulfonate and 13 mM Triethylamine, (10:26:64) at pH 3.3 as mobile phase, at a flow rate of 1.0 ml/min. UV detection in the HPLC system was performed at 254 nm.Results: The strategy was approved for exactness, accuracy, particularity, linearity, and affectability. The maintenance time for the Diphenhydramine was 9.9 min. Alignment plots were straight over the focus ranges 1–5 µg/L for the Diphenhydramine HCl (DPH). The limit of detection was 1.04473µg/ml and the limit of quantitation was 3.16585µg/ml. The exactness of the proposed strategy was controlled by recuperation studies and discovered to be from 96.0% to 100%. Conclusion:The modern HPLC-UV method was used in the analysis of samples of commercial medicine and under a registered trademark in the Iraqi Ministry of Health, and the reliability of the proposed method for analysis was verified. All research results indicate that accuracy, precision, and quality are within acceptable limits, and the results additionally demonstrated that there is no critical contrast between the qualities acquired from the proposed strategy.

Keywords: Diphenhydramine-HCl (DPH), Stress Degradation, DPH Crud

INTRODUCTION

Diphenhydramine is used asan antiprotozoal, chemical name AUPIC:[2-(benzhydryloxy)-N,N-dimethylethan-1-amine],[(2- (diphenyl methoxy) -N], or [N-dimethyl ethyl amine)] ($C_{17}H_{22}N_2O_4$, Mol. Wt. 318.4 g/mol), Diphenhydramine is a whitecrystalline powder or light white odorless, soluble in water, almost soluble in dichloromethane, chloroform, and soluble acetone in ethanol, . Its melting point is 166-170°C, The structural structure is illustrated in Figure. 1.

Diphenhydramine [(2- (diphenyl methoxy) -N] or [N-dimethyl ethyl amine)] is an ethanolamine derivative, which acts as an antihistamine H1 receptor antagonist with anticholinergic side effects. The ether bond is subject to acid hydrolysis. Therefore, the target compounds for urolysis after acid cleavage are the corresponding carbenols.

Diphenhydramine is marketed under the trade name Benadryl by McNeil Consumer Healthcare in the U.S., Canada, and South Africa. Trade names in other countries include Dimedrol, Daedalon, and Nytol. It is also available as a generic medication.

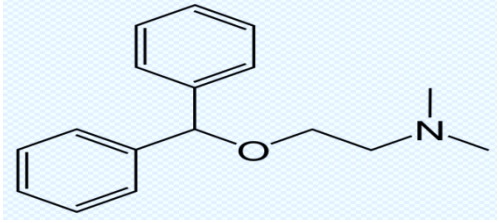


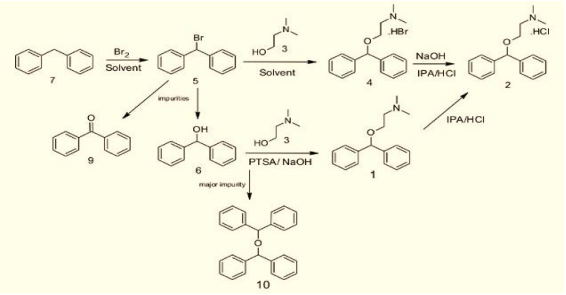
Fig.1: chemical stracture of Diphenhydramine

N, N-(diphenylmethane)-2, or Diphenhydramine is a first-generation antihistamine used to treat various conditions including hypersensitive indications and irritation, the regular cold, a sleeping disorder, movement infection, and extrapyramidal side effects. Diphenhydramine also has local anesthetic properties and has been used as such in people allergic to common local anesthetics such as lidocaine.

Diphenhydramine is used clinically as an antihistamine, antitussive, analgesic and hypnotic drug. The main metabolites are dimethyl, dimethyldiphenhydramine and diphenyl methoxyacetic acid (DPMA). In addition, one hydroxylated and twice one phenyl radical followed by methylated one of the hydroxy groups. The first phase metabolites can be conjugated with glucuronic acid or sulfuric acid. Diphenhydramine can lead to severe poisoning and has also been abused in drug offenses[1-6].

Synthesis

The different methods of circulating the compound diphenhydramine hydrochloride (Scheme 1) have several disadvantages, including the low efficiency of the central active atom by 39% and the fact that the reaction requires very high temperatures, as well as the use of bromine in the reaction, which may cause corrosion in the reaction tube and the formation of dangerous substances from inorganic wastes in addition to Therefore, using the molar equivalent of beta-colored sulfonic acid is considered a hazardous waste and is not economically feasible. One of the difficulties in working with this reaction is the need for a PFA tubular reactor with a temperature resistance greater than 175 degrees Celsius, and the use of hydrochloric acid will be a salt that may cause toxic impurities to 2-chloropropane. [7-9].



Scheme 1: Synthesis of Diphenhydramine HCl (DPH)

The objective of the study

The objective of the study was to develop and verify the RP-HPLC method with an ultraviolet (UV) detector for the quantitative determination of Diphenhydramine HCl (DPH) in pharmaceuticals.

EXPERIMENTAL

Instrumentation

LC-100 series S-HPLC features fully automatic digitalcomputer control. Its electronic circuit design, internalmechanical structure design, processing technology, functions of cinematography workstation, and the technicalcriteria make it leading instruments with excellent stabilityand reliability. The LC100-type HPLC-UV consists of adouble-beam optical spectrometer (Angstrom Advanced Inc.USA), type UV-100 PC with 1 cm light frequency quartz cell is utilized and it is joint with to IBM agreeable with the PC. The product was UPVC rendition Matlab, R2003b was utilized for the reason chemometric strategies, the halfway least squares (PLS) were amazing with PLS_Toolbox for use with Matlab R2003b, VP pumps,and variable frequency programmable UV indicator. Peakareas were coordinated utilizing an Angstrom Advanced Inc. LCsolution programming program. The chromatographic separationand measurement was performed on Ion Pac section; ArcusEP-C18 (250 mm × 4.6 mm; molecule size 5 µm) analyticalcolumn kept up at room temperature. In the portable stage, drug standard arrangements and tablet test arrangements werefiltered through a millipore layer channel before injectioninto the HPLC framework [10-12].

CHEMICALS AND REAGENTS

Pure Standard

Standard DPH with claimed purity of 99%, Based on the company's factory certificate and Awarded by the Industries Pharma SamarraAlfayhaa®Iraq under NO. DH02821114, for medical devices and pharmaceuticals.

Market Sample

DPH-Samarra Alfayhaa® No. 81902-Samarra contains 25 mg per tablet of Samarra® Pharma.

Configure the Samples for Measurement

- HPLC grade solution (Sigma-Aldrich®).
- Stock standard solutions of DPH were prepared inMethanol: acetonitrile: water: 10mM Heptan sulfonate and 13 mM Triethylamine, (10:26:64)

at pH 3.3 to prepareconcentration of 1 mg/ml from DPH.

• DPH(standard solution) was preparedin Methanol: acetonitrile: water: 10mM Heptan sulfonate and 13 mM Triethylamine, (10:26:64) at pH 3.3to prepare the concentration of 1.0, 2.0, 3.0, 4.0, and5.0 µg/ml.

The sample Modernization

To perform sample Modernization, the streamlined PLS alignment set was enlarged with various examples of DPH-Samarra® tablets containing known sums from standard DPH-25mg tablets were produced by Samarra®. One known focus to three obscure convergences of tests containing various groupings of each was added reason for done the underlying adjustment and the prescient capacity of the refreshed example was checked utilizing outer approval tests, at that point figure the perform test refreshing for every segment utilizing the created strategy RP-HPLC with three centralizations of the additional refreshing examples.

PROCEDURE

Standard Drug Solution

The mobile phase was used as a solvent for the planning of standard arrangements. A standard stock arrangement of DPH (500 µg/ml) was set up by dissolving a precisely gauged measure of DPH (50 mg) in 100 ml of versatile stage in a 250 ml volumetric flagon. The cup was then made sufficient with the portable stage. The stock arrangement was weakened suitably with the versatile stage to set up the working standard arrangements of DPH (1, 2, 3, 4, and 5 µg/ml).

Chromatographic Conditions

Table 1 shows the values of the basic parameters obtainedusing the reverse-phase chromatography system (RP-HPLC).

Table 1: Parameters of RP-HPLC method

Mobile phase	Methanol: acetonitrile: water: 10mM Heptan sulfonate and 13 mM Triethylamine, (10:26:64) at pH 3.3
Flow rate	1.0 ml/minute
Detection wavelength	254 nm
Column temperature	Room temperature
Injection volume	20 µL
Run time	10 min

The Calibration Curve

Alignment bends of the proposed strategy were preparedover focus scope of 1–5 µg/ml for DPH. Arrangement wasprepared in three-fold and 20 µl of every arrangement was injectedonto the section. The pinnacles were resolved at 254 nm.The adjustment bend of DPH was developed byplotting the pinnacle zone versus focus.

Theexertion degeneration Studies [13-20]

The effort degeneration Studies were done utilizing different ICH recommended pressure conditions, for example, acidic, basic, oxidative, warm, and photolytic effort.

Acid degeneration

Around 60 mg from tablet powder of DPH was taken in a 100 ml volumetric cup. 5 ml of 0.1 N HCl was added to the jar and kept at 70–80 °C reflux condition for 2–3 h. After fulfillment of the pressure, the arrangement was killed utilizing 0.1 N NaOH and finished sufficient with the versatile stage. Hydrolysis of DPH may be hydrochloric acid. One such reaction is hydrolysis, splitting with water."Hydrolysis of amine is stimulated by any acid or base [Figure 2].

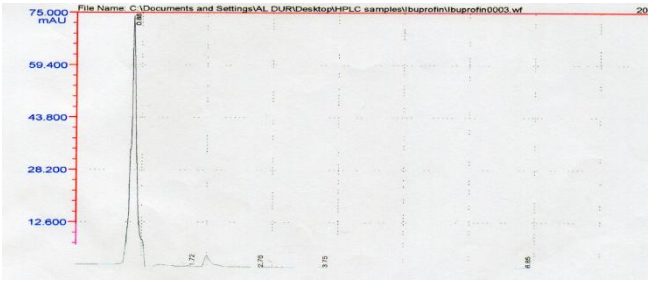


Fig.2: Chromatogram of acid degeneration

Base degeneration

When using bases such as NaOH or potassium hydroxide to suppress amine, results of amine salt. 60 mg from tablet powder of DPH was taken in a 100 ml volumetric carafe. 5 ml of 0.1 N NaOH was added to the jar and kept at 70–80°C reflux condition for 2–3 h. After consummation of the pressure, the arrangement was killed utilizing 0.1 N HCl and finished sufficient with versatile stage [Figure 3].

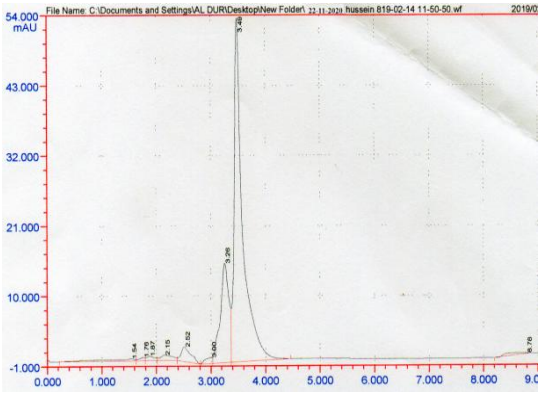


Fig.3: Chromatogram of Base degeneration

Oxidative degeneration

About 60 mg from tablet powder of DPH and 5 ml of 20% H_2O_2 were added in a 100 ml volumetric flask. The flask was kept at 70–80°C reflux condition for 2–3 h. After culmination of the pressure, the jar was finished sufficient with the portable stage [Figure 4].

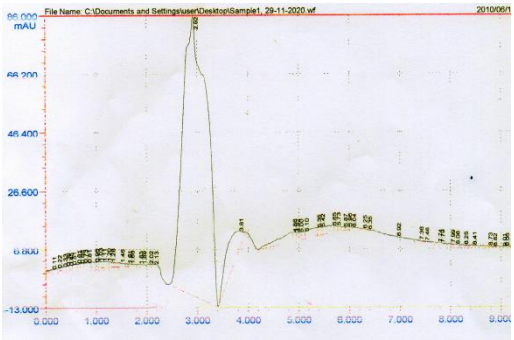


Fig.4: Chromatogram of oxidative degradation

Photolytic degeneration

For the photolytic degeneration study, 60 mg from tablet powder of DPH e was transferred into a glass Petri dish and put in direct daylight for 2–3 h. After consummation of the pressure, the tablet powder was moved to a 100 ml volumetric cup and made sufficient with the portable stage. The infrared spectrum of the solution is then analyzed. The process of decomposition in this way leads to the partial disintegration of the DPH compound and the uncontrolled interference with pharmaceutical additives and this is evident in Figure 5, where the peaks of HPLC-UV appear irregular and sometimes overlapping.

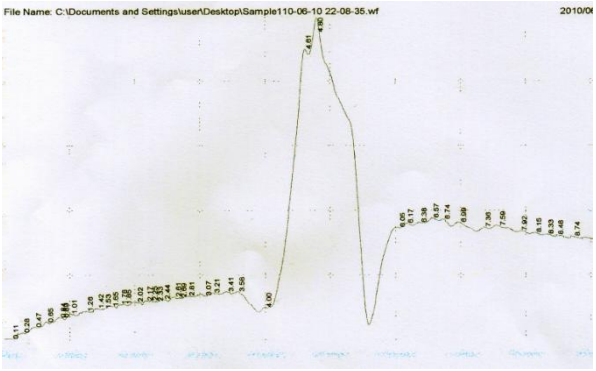


Fig.5: Chromatogram of photolytic degeneration

Thermal degeneration

For this, 60 mg from tablet powder of DPH was taken in a glass Petri dish and placed in a hot air oven at 105°C for 2–3 h. After a specified time, the tablet powder was transferred to a 100 ml volumetric flask and made up to the stain with the mobile segment. Increasing the temperature of the DPH solution >100 °C indicates that it is difficult to control the synthetic structure of the DPH and thus obtain the complete thermal dissolution of the compound; this is shown in Figure 6.

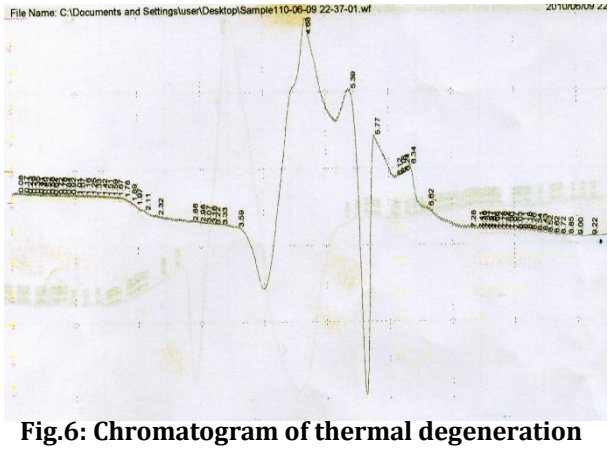


Fig.6: Chromatogram of thermal degeneration

Infrared Spectrophotometer OF DPH [21-25]

The infrared spectra were recorded for the compounds prepared in the form of a potassium bromide disk KBr using a device of the type FTIR-84005-SHIMADZU, country of origin Germany and in the region (4000−500 cm^{-1}) at room temperature, the spectra were recorded at Basra University / College of Education for Pure Sciences

For DPH-Standard

The infrared spectrum of DPH-Standard [Figure 7, Table 2], shows important peaks for stretching and bending vibrations which are in agreement with its structure. The FTIR spectrum of standard-DPH appears weak peaks at 3030 and 72891 cm^{-1} can be assigned to aromatic and aliphatic stretching of C-H. Medium peaks at 756-651 cm^{-1} rang can be attributed to aromatic bending of C-H bonds. The peaks of aromatic C=C appears at the rang 1598-1348 cm^{-1}.

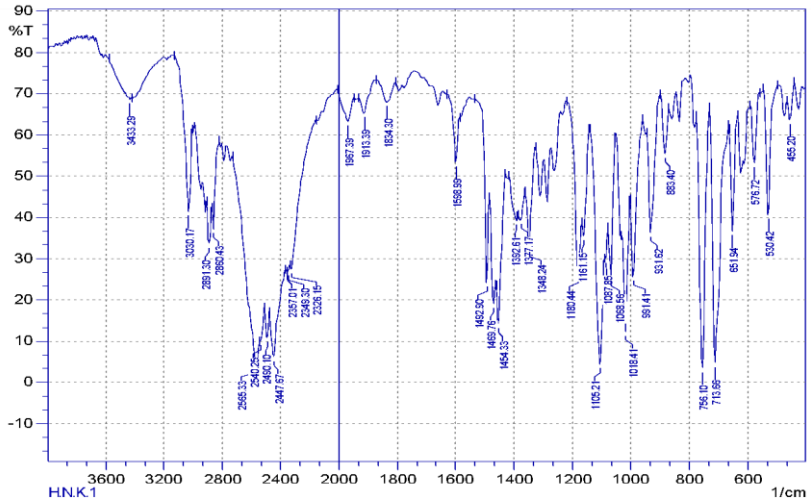


Fig.7: IR spectrum of DPH standard

Table 2: Characteristic absorption bands in the infrared spectra of DPH

Functional groups	DPH-sample(cm^{-1})	DPH-Standard (cm^{-1})
ν(C-H)stretchingaromatic	3030 weak peaks	3030
ν(C-H)stretching aliphatic	72891weak peaks	72891
ν(C-H) aromatic Medium peaks	760	756-651
ν(C=C), aromatic	1450	1598-1348
M-OH)Ambient humidity)ν	3400	3400

For DPH-Sample

The infrared spectrum of the DPH sample [Fig. 8, Table 2] shows peaks corresponding to the peaks of the standard model where the vibrations show extension and curvature corresponding to its structure. The FTIR spectrum of the standard DPH appears weak peaks at 3030 and 72891 cm^{-1} can be assigned to aromatic and aliphatic expansion of C-H. The medium peaks at 760 cm^{-1} can be attributed to the aromatic bending of the C-H bonds. C = C aromatic peaks appear in the range 1450 cm^{-1}.

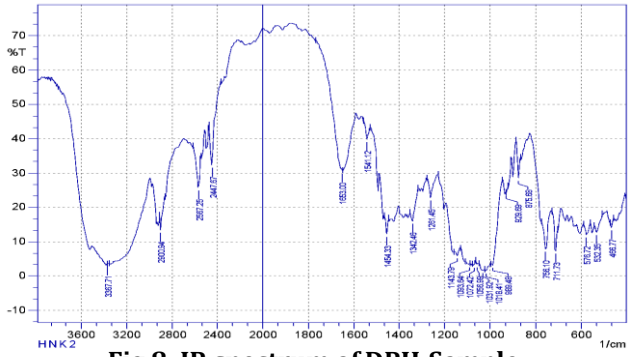


Fig.8: IR spectrum of DPH-Sample

DISCUSSION OF THE RESULTS

The Optimization of HPLC conditions

The chromatographic conditions were developed to separateall the degradation products from the peaks of DPH.During the process of HPLC method optimization, severaltrials were taken using Ion Pac Arcus EP-C18; 5 µm,4.5 mm × 250 mm, with the use of suitable organic phase,Methanol: acetonitrile: water: 10mM Heptan sulfonate and 13 mM Triethylamine, (10:26:64) at pH 3.3 and 1 ml/minflow rate. The wavelength was observed at 270 nm[26]. Theretention instant for DPH was 4.815 min. Good peakshape was observed of the new analytical method [Figure 9].

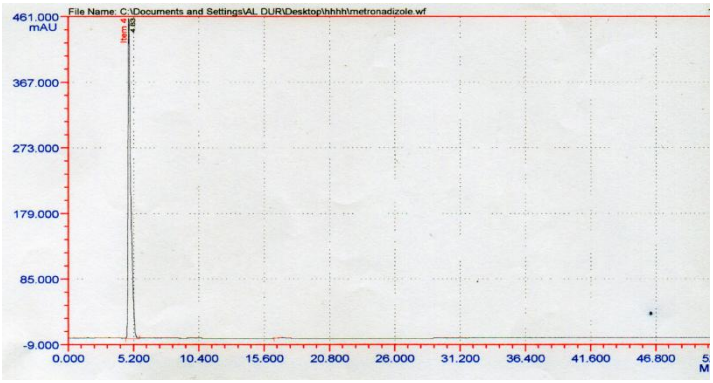


Fig.9: Chromatogram obtained after method optimization

The System Suitability

Studies were carried out for the purpose of adapting the HPLC-UV system. The standard DPH (3 µg/mL) was used through three replicas of the same concentration that was replicated using the optimal method. Table 3 illustrate the system suitability. These results meet the requirements of the separation method and DPH estimates in various pharmaceuticals[27].

Table 3: System suitability

Parameters	Value of DPH	Recommended limits
Retention time	2.815 (%RSD 0.289)	RSD≈ 0.101
Peak area	577,025.2 (%RSD 0.223)	RSD≤ 1
USP plate count	1980	≥2000−2500
USP tailing factor	2.14	≤1.9−2.5
Resolution	4.83 min	≥5

The Validation of Method and Assay

In accordance with ICH guidelines[28], the new chromatographic method HPLC-UV and parameters such as specificity, linearity range, and sensitivity, regression, precision, accuracy, and rigidity were used to validate the method used. To assess the method validity, the effect experimental conditions on the peak areas of the analytes were scrutinize. The validity of the technique was checked at a concentration of 3 µg/ml for DPH. Table 4 summarized all the results. The results revealed that the peak areas for the drugs were unaffected by littletransform in flow rate, the work of art of the mobile phase, temperature, and detection wavelength, indicating significant validity of the method.

Table 4: Results of method robustness

Parameter	DPH (3 µg/ml)		
	Found (µg/ml)	%Recovery	%RSD
Analyst	3.0	100.0	0.201
System	3.0	100.0	0.992

The Specificity

The specificity of the proposed scheme was studied using the study of forced deprivation. The analysis was performed to ensure that the proposed method was able to separate DPH from the potential degradation products generated during the study of forced degradation. Studies were performed using acid, base, oxidation, photolysis, and heat for the tablet sample at a concentration of 3 µg/ml of DPH. Table 5 shows the results of forced decomposition. Chromatograms shapes are shown in Figures 2-6. The highest percentage of deterioration occurred under the alkaline conditions of the drug[29]. The lowest percentage of the degradation of DPH occurred in the case of thermal and in the case of photosynthesis. One peak degradation was observed in decomposition products. Other degradation products due to stress do not interfere with the detection of DPH, so the method can be considered as an indicator of stability.

Table 5: Results of forced degradation studies

Type of degradation	DPH (60 µg/ml)	
	%Recovery	%Degradation
Undegraded	100	0.090
Acid	98.323	1.241
Base	96.021	4.082
Oxidative	92.944	3.832
Photolytic	90.102	1.666
Thermal	95.541	1.001

The Linearity Range and Sensitivity

Under the ideal test conditions, a straight relationship was set up by plotting the pinnacle regions for the medication against the medication fixation (µg/ml). The focus range was discovered to be (1–5) µg/ml for DPH. The straight relapse investigation of the information gave from the accompanying conditions:

$y = 7.057x + 0.523$ ($R^2 = 0.998$) for DPH

On the assumption that: y = peak area, x= convergence of the medication (µg/mL), and R^2 = Regression coefficient[30].The high estimations of relapse coefficients with a little catch demonstrate the great linearity of the adjustment bend shows in Figure 10.

The Regression

The understanding of the proposed process was assessed by calculating limit of quantitation (LLOQ) and edge of detection (LLOD). The LOD and LLOQ were calculated as follows[31]:

$LLOQ = 10 \times SD/S$; $LLOD = 3.3 \times SD/S$

Where, SD = standard deviation of the drug rejoinder and S = Slope of the calibration curve. LLOD values were found to be 1.04473µg/ml while LLOQ values were established to be 3.16585µg/ml. These values make obvious the satisfactory sensitivity of the planned technique for the analysis of the selected drug. Table 6 shows the results of regression statistics of the anticipated process [32].

Table 6: Regression statistics of the proposed scheme

R^2	0.998
Standard error	0.7660
Standard error estimate	0.7303
Intercept	0.523
Slope	7.057
LLOD (μg/ml)	1.04473
LLOQ (μg/ml)	3.16585

The Accuracy

For the pre-analysis tablet sample solutions, a known amount of standard solution was added at three dissimilarlevels, 10%, 20%, and 30%. The solutions were reanalyzed by the projectedtechnique. The percentage recovery was between 96% and 100% with a percentage of RSD<0.3%. The results indicate good accuracy of the process. The selectivity of the process was established by the non-interference of the excipients by means of the analysis of the analytes[33]. The results are summarized in Table 7.

Table 7: Summarized results of accuracy

Claimed conc. (μg ml^{-1})	Found conc. (μg ml^{-1})	Recovery±RSD
1	1.0	100±0.222
2	2	100±0.276
3	2.9	96.6±0.211
3.0 μg ml^{-1} for drugs (DPH-Samarra® tablets)	3.0	100±0.295

The Precision

The precision was established by analyzing DPH at a concentration of 3 μg/ml. The system precision was tested by be relevant the developed technique for the estimation of DPH in the pure standard DPH for three successive times (n = 3). The method exactness was tried by rehashed investigation of DPH in tablet samples for three successive times (n = 3). The results are summarized in Table 8. The percentage RSD values for system precision and method precision were ≤0.3%,showing that the proposed strategy has great accuracy in the investigation of DPH[34,35].

Table 8: Results of precision studies

Claimed conc. (μg ml^{-1})	Intraday		Interday	
	Found (μg ml^{-1})	Recovery±RSD%	Found (μg/ml)	Recovery±RSD%
1	1	100±0.222	1	100±0.200
2	2	100±0.276	2.1	110±0.233
3	2.9	96.6±0.211	2.87	95.6±0.201
4	3.88	97.6±0.281	4.1	102±0.204
5	5	100±0.295	4.9	97.4±0.244
3.0 μg/ml drug (DPH-Samarra® tablets)	3	100±0.295	2.9	96.6±0.283

The Applications of Method

The analytical method of DPH-25 mg was assessed by examining commercially available tablets (samarra pharm- tablets, samarra, Iraq, claiming to contain 25 mg of DPH). The percentage of Standard-DPH was found where the values were 100 ± 0.216%,while the ratio of DPH in DPH-25 (Limited samarra) was found where the values were 99± 0.212%. This result indicating the values of percentage recovery and RSD% that the proposed method was accurate and precise in DPH analysis in dosage forms. Table 9 summarized the results of the applications.

Table 9: Assay of DPH in tablets

Analyte	Labeled claim (mg)	Found (mg)	Mean (mg)	%Recovery	%RSD
Standard -DPH	25	25	25	100	±0.216
DPH-25	25	24.5	24.75	99	±0.212

CONCLUSION

This work described an HPLC system (LC100 Angstrom advanced) equipped with a UV detector for DPH determination in two commercial pharmaceutical drugs. This developed method considered as simple, inexpensive, and needs only a very small volume of the sample as well as used it is an ultraviolet detector makes this system very specific due to one peak in the chromatogram. In this application, there is no need for high sensitivity since the pharmaceutical drugs have a very low concentration. The method was validated as per the HPLC-UV guidelines and the created technique complies with Beer's law over the fixation scope of 1.0–5.0 µg/mL for drugs.

Based on the results, this study divulges the important analytical method used to decide the presence of DPH in the measurements structure. The created and approved security showing HPLC-UV technique for the measurement of DPH is straightforward, exact, exact, delicate, explicit, rough, and hearty. The proposed technique can, in this manner, be applied for routine examination of DPH in the tablet dose structure.

ACKNOWLEDGMENTS

The authors are grateful to the professors of the Pharmaceutical Chemistry Branch at the College of Pharmacy and the Southern Technical University / Department of Anesthesia Technologies for their assistance in preparing this manuscript.

AUTHORS CONTRIBUTIONS

This research was done individually in the laboratories of the College of the Pharmacy/ University of Basrah and the Southern Technical University / Department of Anesthesia Technologies. This research was completed over a period of 3 months with serious and continuous work, and therefore, excellent results were obtained in finding an easy and sensitive method to estimate the DPH.

REFERENCES

1. Ahmed A. A. Alsaad, Erfan A. S. Alassadi, H. N. K. Al-Salman, Hussein H. Hussein, The Simultaneous Determination of Ibuprofen and Paracetamol in Pharmaceutical Formulations by High-performance Liquid Chromatography with Ultraviolet Detection, Asian Journal of Pharmaceutics • Apr-Jun 2019 • 13 (2) | 141.
2. Eman Tariq Ali, Adheed Khalid Alsharrad, Falah Hassan Shari, H. N. K. Al-Salman, 17β -estradiol Hormone and Interleukin 1-beta Change Related to Menopause in the Women with Rheumatoid Arthritis, Asian Journal of Pharmaceutics • Apr-Jun 2019 • 13 (2) | 110.
3. Shah SR, Pradhan SD, Jain HK, Upadhyay UM. Method development and validation for simulataneous estimation of albendazole and praziquantel in bulk and in a synthetic mixture. J Taibah Univ Sci 2014;8:54-63.
4. Falah Hassan Shari, Abdulrazaq Almiahi, Ahmed Sami Abduljabbar,Ahmad Salahuddin, H. N. K. Al-Salman, Optimization of a micro-high-performance liquid chromatography method for determination of metronidazole benzoatein their standard powder and in dosagepharmaceuticals, International Journal of Green Pharmacy • Jan-Mar 2019 • 13 (1) | 48.
5. Erfan A. S. Alassadi, Ekhlas Qanber Jasim, Ahmed A. A. Alsaad, H. N. K. Al-Salman, Quantitative analysis of two penicillins in oral dosage form using modern high-performance liquid chromatography method, International Journal of Green Pharmacy • Jan-Mar 2019 • 13 (1) | 81.
6. Hussein N. AL-Salman, Eman T. Ali, Omar A. Almukhtar and Majid. S. Jabir, 2-benzhydrylsulfinyl-N-hydroxyacetamide Extracted from Fig: A good Therapeutic Agent against Staphylococcus Aureus, AIP Conference Proceedings 2213, 020223 (2020).
7. H. N. K. Al-Salman · Eman T. Ali · Majid Jabir3 · Ghassan M. Sulaiman3 · Shaker A. S. Al-Jadaan, *2-Benzhydrylsulfinyl-N-hydroxyacetamide-Na* extracted from fig as a novel cytotoxic and apoptosis inducer in SKOV-3 and AMJ-13 cell lines via P53 and caspase-8 pathway, European Food Research and Technology,1591–1608(2020).
8. Erfan A. S. Alassadi, Ekhlas Qanber Jasim , H. N. K. AL-Salman, Mazin N. Mosa, Comparative Study of an In Vitro Release Patterns of Ceftaroline Fosamil fom Chemically – Prepared Coated Hydroxyapatite Nanoparticles, Sys Rev Pharm 2020; 11(3): 797 805.
9. Khawla Salman Abd-Alrassol , H. N. K. AL-Salman, Ekhlas Qanber Jasim, Hussein H. Hussein, Determination and Evaluation of Doses of Metronidazole in Different Quantities and

Formulations with Multiple Spectroscopic Methods, Sys Rev Pharm 2020; 11(5): 130 139.
10. Rana D. S. ALkamil, Dawood CH. Al-Bahadily, Rasool Chaloob, Falah Hassan Shari, H. N. K. AL-Salman, Estimation of Truffles Extracts and Study the Biological Efficacy of Ethyl 6-methyl-2-oxo-4-(2-thienyl)-1,2,3,4- tetrahydropyrimidine-5-carboxylate (EMOTTC) as One of the Extracts against Ophthalmic Bacteria, Sys Rev Pharm 2020; 11(6): 878 887.
11. Eraga SO, Arhewoh MI, Chibuogwu RN, Iwuagwu MA. A comparative UV−HPLC analysis of ten brands of ibuprofen tablets. Asian Pac J Trop Biomed 2015;5:880-4.
12. Abdelaleem EA, Naguib IA, Hassan ES, Ali NW. HPTLC and RP-HPLC methods for simultaneous determination of paracetamol and pamabrom in presence of their potential impurities. J Pharm Biomed Anal 2015;114:22-7.
13. Al-Salman HN, Hussein HH, Maan AN. Quantitative analysis of cephradine using the modern high-performance liquid chromatographic method. Asian J Pharm 2018;12:228-34.
14. Loudiki A, Boumya W, Hammani H, Nasrellah H, El Bouabi Y, Zeroual M, *et al.* Ibuprofen analysis in blood samples by palladium particles-impregnated sodium montmorillonite electrodes: Validation using high performance liquid chromatography. Mater Sci Eng C Mater Biol Appl 2016;69:616-24.
15. Al-Salman HN, Qanber JE. Analytical methods for diagnosis a mixture of narcotic substances in seized materials. Int J Green Pharm 2018;12:216-26.
16. Elzanfaly ES, Zaazaa HE, Soudi AT, Salem MY. Development and validation of chromatographic methods for simultaneous determination of ibuprofen and famotidine in presence of related substances in pharmaceutical formulations. Acta Chim Slov 2015;62:45-51.
17. Al-Salman HN. Analysis methods and qualitative diagnosis chromatographic for mixture of narcotic substances in seized materials. Eur J Sci Res 2017;147:403-11.
18. Singh VD, Daharwal SJ. Development and validation of multivariate calibration methods for simultaneous estimation of paracetamol, enalapril maleate and hydrochlorothiazide in pharmaceutical dosage form. Spectrochim Acta A Mol Biomol Spectrosc 2017;171:369-75.
19. Ahmed DA, Abdel-Aziz O, Abdel-Ghany M, Weshahy SA. Stability indicating determination of albendazole in bulk drug and pharmaceutical dosage form by chromatographic and spectrophotometric methods. Future J Pharm Sci 2018;4:161-5.
20. Chavhan ML, Shirkhedkar AA, Surana SJ. Development and validation of a stability indicating RP-TLC/ densitometric method for determination of loratadine in bulk and in tablets. Arab J Chem 2017;10:825-30.
21. AL-Sowdani KH, Al-Salman HN. Semi-automatedhome-made HPLC-UV system for determination ofamoxicillin trihydrate (AMO) in antibiotic drugs.J Chem Biol Phys Sci 2016;6:31-8.
22. Danao KR, Hiradere SM, Moon RS, Kasture AV,Yeole PG. RP-HPLC simultaneous estimation ofmetronidazole and diloxanidefuroate in combination. IntJ Pharm Life Sci 2010;1:82-5.
23. Murali D, Rambabu C. Simultaneous determinationof metronidazole and furazolidone in combinedtablet dosage form: Development and validation of astability indicating HPLC method. Anal Chem Indian J2016;16:1-12.
24. Engle JP, Erstad BL, Anderson DC Jr. Bucklin MH,Chan A, Donaldson AR, *et al*. Minimum qualificationsfor clinical pharmacy practice faculty. Pharmacotherapy2014;34:e38-44.
25. Sebaiy MM, El-Shanawany AA, Adl SM, Abdel-Aziz LM, Hashem HA. Rapid RP-HPLC method forsimultaneous estimation of norfloxacin and tinidazole intablet dosage form. Asian J Pharm Anal 2011;1:79-84.
26. Smith C, Goldman RD. Alternating acetaminophen and ibuprofen for pain in children. Can Fam Physician 2012;58:645-7.
27. Hoang VD, Nhung NP, Aboul-Enein HY. Recent developments and applications of derivative spectrophotometry in pharmaceutical analysis. Curr Pharm Anal 2013;9:261-77.
28. ICH. Q2B, Harmonized Triplicate Guideline, Validation of Analytical Procedure Methodology. Geneva: IFPMA, Proceeding of the International Conference on Harmonization; 1996.
29. Ashour S, Khateeb M. Kinetic spectrophotometric determination of pravastatin in drug formulations via derivatization with 4-chloro-7-nitrobenzo-2-oxa-1, 3-diazole (NBD-Cl). Arab J Chem 2011;4:299-305.
30. Rajaa Hussein Fayadh , H. N. K. Al-Salman , Maan Al-Nuaim, Ekhlas Qanber Jasim, Analytical method for detecting and estimating of lanthanide ions paired with transitional elements, International Journal of Green Pharmacy • Apr-Jun 2019 • 13 (2) | 164.
31. Minal RG, Harpreet KP, Loni A, Shivsharan T.Devlopment and validation of RP-HPLC methodfor simultaneous estimation of metronidazole andnorfloxacin in bulk and tablet dosage form. Int J PharmPharm Sci 2012;4:241-5.
32. US DHHS, FDA, CDER. Guidance for Industry.Bioanalytical Method Validation. US Departmentof Health and Human Services, Food and DrugAdministration, Center for Drug Evaluation and Research(CDER), Center for Veterinary Medicine; 2011.
33. Sebaiy MM, El-Shanawany AA, Adl SM, Abdel-Aziz LM, Hashem HA. Rapid RP-HPLC method forsimultaneous estimation of norfloxacin and

tinidazole intablet dosage form. Asian J Pharm Anal 2011;1:79-84.
34. James WT, Leslie TW. Drugs used in the chemotherapyof protozoa infections. In: Goodman and Gilman'sPharmacological Basis of Therapeutics. 9th ed.New York: McGraw-Hill; 2007. p. 1105.
35. Ezzeldin E, El-Nahhas TM. New analytical method forthe determination of metronidazole in human plasma:Application to bioequivalence study. Trop J Pharm Res2012;11:799-805.