A Rapid Continuous Flow Injection Analysis Method Development for Diphenhydramine Hydrochloride

Asma A. Gayed Al-Ani [1*] , Nagham S. Turkey Al-Awadie [1*]

Abstract

Background: Diphenhydramine hydrochloride (DPH) is widely used as an antihistamine for treating various allergic conditions and symptoms. Traditional analytical methods for quantifying DPH often involve complex procedures and instrumentation. However, recent advancements in nanotechnology and analytical techniques offer opportunities for developing simpler and more efficient methods for DPH analysis. Method: This study presents a novel approach for quantifying DPH using continuous flow injection analysis (CFIA) combined with turbidimetric measurements. The precipitation reaction between DPH and 3,5-dinitro salicylic acid was utilized in an aqueous medium system. Various physical and chemical parameters were optimized, including the concentration of precipitating reagent, reaction medium (acids and salts), irradiation source intensity, volume of sample segment, and delay reaction coil. Results: Optimization of physical and chemical parameters revealed that a concentration of 15 m.mol/Liter for 3,5-dinitro salicylic acid, distilled water as the reaction medium, and an irradiation source intensity of 3.1 VDC produced optimal results. The linear dynamic range for DPH concentration was determined to be 0.01-20 m.mol/Liter. Comparison with the traditional UV-spectrophotometric method showed comparable results, indicating the reliability of the proposed CFIA method. Conclusion: The CFIA method presented in this study offers several advantages over traditional analytical methods for quantifying DPH in pharmaceutical formulations. It provides high sensitivity, speed, simplicity, and improved accuracy. The proposed approach has the potential for widespread application in pharmaceutical analysis, offering a cost-effective and easily manipulable solution for routine laboratory use. Overall, this study demonstrates a significant advancement in the measurement of DPH, which could simplify processes for pharmaceutical analysis and enhance industrial production levels.

Keywords: Diphenhydramine hydrochloride, Flow injection analysis, Pharmaceutical analysis, Sensitivity, Cost-effectiveness.

Significance | Novel flow injection analysis improves Diphenhydramine HCL detection in pharmaceuticals with high sensitivity, speed, simplicity, and cost-effectiveness, enhancing industrial efficiency.

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Introduction

Diphenhydramine hydrochloride (DPH), also known as 2-(Diphenylmethoxy)N. N-dimethylethanamine hydrochloride, is a white, crystalline powder with a bitter, numbing taste. It has a molecular formula of C17H22ClNO and is soluble in water, with aqueous solutions being acidic at pH 4-6. DPH is an antihistamine used to relieve allergy symptoms, hay fever, and the common cold, available in various formulations including tablets, syrup, and injections (Moyer et al., 2014). The molecular structure of Diphenhydramine hydrochloride (DPH) is illustrated in Scheme 1. It is effective in alleviating symptoms like itchy, watery eyes, sneezing, runny nose, cough, motion sickness, and insomnia. Additionally, DPH has applications in treating dystonias, pruritis,

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urticaria, vertigo, and as a local anesthetic. However, caution is advised during pregnancy due to potential uterine hyper stimulation. Nanotechnology has facilitated the development of green-synthesized silver nanoparticles for spectrophotometric DPH measurement, offering simplicity, affordability, and ecological safety. Various analytical methods such as spectrophotometry, chromatography, flow injection, and potentiometry are employed for DPH analysis, with novel approaches utilizing handmade cells and solar cell analyzers showing promise for quality control of DPH-containing drug tablets.

Functionally, Diphenhydramine serves as an antihistamine, effectively alleviating symptoms associated with allergies, hay fever, and the common cold (Majdan et al., 2018). Its pharmaceutical formulations are available in various forms such as oral tablets, syrup, or injectables administered intramuscularly or intravenously (Stefanny et al., 2020). Positioned within the first generation of antihistamines, Diphenhydramine is instrumental in managing allergic reactions (Paul et al., 2022).

Its clinical utility extends to addressing symptoms like red, itchy, watery eyes, sneezing, runny nose, and cough resulting from allergies or respiratory ailments. Moreover, it finds application in managing motion sickness, insomnia, and early-stage parkinsonian syndrome-related movement disorders (Anderson et al., 2005). Furthermore, Diphenhydramine demonstrates efficacy in treating dystonias, pruritis, urticaria, vertigo, and serves as a local anesthetic alternative for individuals allergic to conventional options (Alhussain et al., 2023).

In terms of safety during pregnancy, Diphenhydramine falls under category A, indicating its extensive use among pregnant women without notable fetal malformations or direct harmful effects (Alhussain et al., 2023). However, caution is warranted, especially concerning high doses in the third trimester, which could lead to uterine hyperstimulation and associated complications like uterine rupture or placental abruption.

Recent advancements in nanotechnology have significantly impacted various domains, including cell imaging, cancer biomarkers, pharmacological analysis, medical applications, and catalysis (Maghssoudi et al., 1977; Rashid et al., 2021; Shamsa et al., 1976; El-Didamony et al., 2010). Notably, the emergence of eco-friendly nanoparticle synthesis techniques aims to minimize reliance on toxic reagents and enhance ecological sustainability (Singh et al., 2021).

One notable approach involves synthesizing silver nanoparticles from Capparis Spinosa L. (CPL) fruit extracts, given the scarcity of research on the fruit's properties vis-à-vis DPH (Zarei et al., 2021). These nanoparticles offer simplicity, affordability, ecological safety, and reduced solvent and equipment usage, making them ideal for spectrophotometric DPH measurements.

Various methodologies exist for Diphenhydramine hydrochloride measurement, including spectrophotometric, chromatographic, flow injection, and potentiometric techniques (Noor Al-Huda et al., 2021; Nandeesha et al., 2022; Chunling et al., 2006; Changzhi et al., 2008; Eman et al., 2014). Innovative approaches like handmade cells and continuous flow injection analysis, leveraging turbidity or fluorescence, provide effective means for analysis (Jalal N Jeber, 2021; Ghadah et al., 2021). For instance, the Ayah 6S 1-ST-2D solar cell CFI analyzer offers a simple, cost-effective solution for quality control of DPH-containing drug formulations, demonstrating its utility in drug content determination (Zahraa et al., 2021).

However, diphenhydramine hydrochloride stands as a versatile pharmaceutical compound with diverse clinical applications, supported by advancements in nanotechnology and analytical methodologies. As research continues to evolve, the development of innovative synthesis techniques and analytical approaches promises enhanced efficacy, safety, and affordability in DPH-related therapeutics and quality control measures. This study proposed a diphenhydramine hydrochloride (DPH) analytical technique that might be straightforward, easy to use, inexpensive, reproducible, and has a high recovery percentage.

Materials and Methods

Reagents and Chemicals

In this study, all chemicals belonging to the analytical reagent class were utilized, with distilled water serving as the solvent for preparing solutions. A standard solution was created by dissolving 0.1459 grams of diphenhydramine hydrochloride ($C_{17}H_{21}NO.HCl$), with a molecular weight of 291.82 g/mol, in 50 milliliters of distilled water, resulting in a concentration of 0.01 mol/L.

Furthermore, a stock solution with a concentration of 0.05 mol/L was prepared by dissolving 45.6312 grams of phosphomolybdic acid $H_3[Mo_{12}PO_{40}]$, with a molecular weight of 1825.25 g/mol (sourced from Hopkin & Williams), in 500 milliliters of distilled water.

Acid solutions, each at a concentration of 0.1 mol/L, were prepared using acids obtained from BDH. These acids included sulfuric acid (H_2SO_4) with a purity of 98% w/w and a molecular weight of 1.84 g/mol, hydrochloric acid (HCl) with a purity of 35% w/w and a density of 1.19 g/ml, nitric acid (HNO_3) with a purity of 70% w/w and a molecular weight of 1.42 g/mol, and acetic acid (CH_3COOH) with a purity of 99.5% w/w and a molecular weight of 1.05 g/mol.

Additionally, salts sourced from BDH were dissolved to produce solutions with a concentration of 0.1 mol/L. These salts included sodium chloride (NaCl) with a molecular weight of 58.44 g/mol, sodium nitrite ($NaNO_2$) with a molecular weight of 68.9953 g/mol, sodium nitrate ($NaNO_3$) with a molecular weight of 84.9947 g/mol, ammonium chloride (NH_4Cl) with a molecular weight of 53.491

g/mol, and sodium carbonate (Na2CO3) with a molecular weight of 105.99 g/mol.

Sample Preparation

A four-stage sensitive scale was used to weigh twenty pills, which were then crushed, ground, and sieved through a 200 mesh screen. Diphenhydramine hydrochloride (supplier from SDI –Iraq, Aswar Al-Khaleej –Iraq, and Al-Kindi –Iraq) contains 25 mg per tablet. The weight of each tablet is 0.6591 g of the active drug material, or 0.14591 g in accordance with the sequence. The powder was dissolved in distilled water, and the mixture was filtered to remove any residue that could have remained after the powder had dissolved and affected the transducer response. These solutions were made in a 50 ml volumetric flask (before the filtering procedure, the volume was filled to the mark in a 50 ml volumetric flask with distilled water).

Instruments

A homemade NAG-SSP-5S-1D Analyzer was used to create the flow cell. This work used a constructed equipment called the NAG-SSP-5S-1D solar cell analyzer, which uses five WSLED sources of irradiation and five solar cells as detectors to measure the attenuation of incident light of any particular sample segment color. In order to transform electric energy into photons, it is connected to a voltage regulator. In order to transform photons into response, it is coupled to a recorder. Furthermore, 2 lines—the first a reagent line and the second a carrier stream (H2O)—connected to a peristaltic pump and passing through a reaction room will be utilized to produce the precipitate particles that will be delivered to the NAG-SSP-5S-1D analyzers. Additionally, we conducted traditional measurements using a UV-VIS spectrophotometer.

Methodology

One of the most practical and adaptable automated analysis methods is flow injection analysis (FIA), which is extensively used for regular analyses across various industries (Tipparat et al, 2022). It's a method for doing chemical analysis. A sample plug is injected into a moving carrier stream to get the desired result. A sample is injected into a flowing carrier solution that mixes with reagents before reaching a detector in the automated chemical analysis technique known as FIA(Shakir et al, 2014). It has several application in different media (Shakir , 2014).

This research paper outlines a technique for detecting DPHA.HCL in different drugs using continuous flow injection analysis (FI) via turbidimetric measurements . 3,5-dinitro salicylic acid is used as a precipitating reagent in an aqueous medium. The precipitate is measured by the attenuation of incident light at 0 – 180° angles by NAG-SSP-5S-1D Analyzer. The analytical response for each signal concentration level was recorded over time. The results obtained were compared with the UV-Vis spectrophotometric method.

The manifold flow system consists of two lines. Using phosphomolybdic acid (PMA) to produce an ion-pair complex in the aqueous medium, Figure 1 shows the creation of HPL as a white, slightly yellowish precipitate. The second line transported HPL (20 mmol/L) at a rate of 2.0 ml/min. The first line supplied distilled water, linked to the injection valve as a carrier stream moving at 2.0 ml/min for hold HPL (used sample volume 50 µl).The reaction begins at Y-Junction, where the reagent line and sample current line meet. The reaction product then travels to the measurement cell in the Ayah 6S×1-ST-2D Solar cell CFI analyzer, where each solution was injected three times in succession…The responses were recorded using an x-t potentiometric recorder, and they took the shape of peaks, the height of which is proportionate to the quantity of light that the precipitate particles' surfaces reflected when the light from the source struck them. Scheme 2 illustrates a proposed mechanism for the reaction between DPH and HPL in an aqueous media.

Results

In this study, we determined the optimal conditions for determining diphenhydramine HCL (DPHA.HCL) in pharmaceutical formulations by analyzing the signal-to-noise (S/N) profile measured at 0-180◦. Through systematic experimentation, we optimized various physical and chemical parameters within the manifold system.

Effect of Varying 3,5-Dinitro Salicylic Acid Concentration

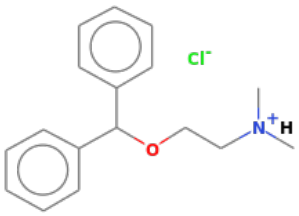
We tested solutions ranging from 3 to 20 m.mol/Liter with a flow rate of 1.7 ml.min^–1 for both lines to examine the influence of varying 3,5-dinitro Salicylic acid concentration as a precipitating reagent. Table 1 displays the correlation coefficients of five segments, demonstrating the impact of different concentrations of 3,5-dinitro Salicylic acid on the analytical response. Notably, segment 2 exhibited a perfect correlation (correlation coefficient = 1), indicating optimal conditions for this concentration range.

Effect of Carrier Media

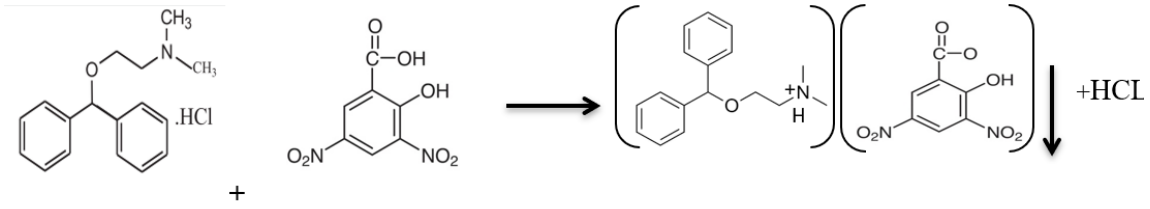
The reaction between DPHA.HCL (12m.mol/Liter) and 3,5-dinitro Salicylic acid (15m.mol/Liter) to form a yellow precipitated product was conducted using various carrier media, including acidic/salt solutions at a concentration of 10 m.mol/Liter, in addition to aqueous media. Table 2 highlights a significant decrease in the S/N energy transducer response when acidic and salt solutions were used as carrier streams compared to distilled water. This decrease may be attributed to the piptization process or the dissolution of solid particles during flow, resulting in a decrease in the precipitate's dense mass.

Analysis on NAG-SSP-5S-1D System

Detailed results of the analysis conducted under different flow rate segments on the NAG-SSP-5S-1D system are presented in Table 3. The mean correlation coefficients for each segment indicate the strength and direction of the linear relationship between the output response and the concentration of the analyte. Segments S1, S3, and



Scheme 1. Molecular structure of diphenhydramine hydrochlorid



Scheme 2. The proposed reaction between DPHA.HCL and 3,5-dinitro S.A.

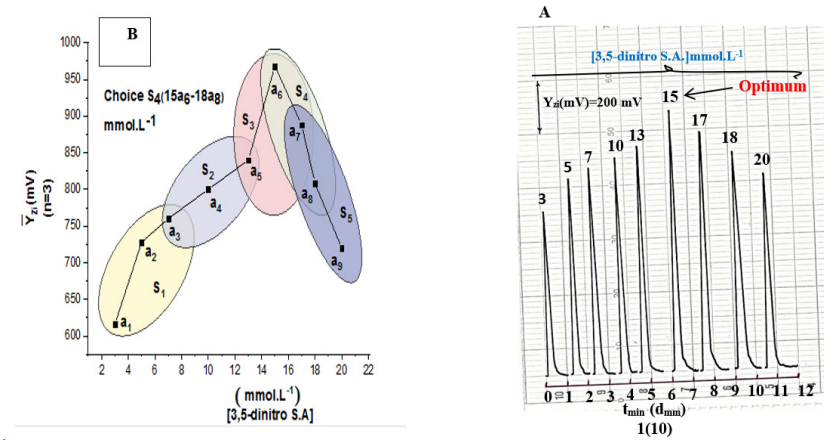


Figure 1. (A) Responses profile of 3,5-dinitro salicylic acid concentration effect.
(B) ȲZi(mV)of average output response of NAG-SSP-5S-1D Analyzer and three data points as one segment with optimum choice

Table 1. Effect of 3,5-dinitro S.A. concentration on precipitation of DPHA.HCL (12m.mol/Liter).

A			
Reliability (2- tail _ 95%) $\bar{Y}_{Zi}$(mV) $\pm t_{0.05/2,n-1} \frac{\sigma n-1}{\sqrt{n}}$	R.S.D%	Output response of NAG-SSP-5S-1D $\bar{Y}_{Zi}$(mV) (n=3)	[3,5-dinitro S.A.] m.mol/Liter
0.298616±	0.019	616	3
0.522728±	0.029	728	5
0.472760±	0.025	760	7
0.323800±	0.016	800	10
0.422840±	0.020	840	13
0.373968±	0.015	968	15
0.571888±	0.026	888	17
0.522808±	0.026	808	18
0.472720±	0.026	720	20

B					
Ø)(	Correlation	(b) mV/(m.mol/Liter)	(a) mV	Range of [3,5-dinitro S.A] m.mol/Liter	Segment
88	0.907	36	521.333	3-7	S_1
86	1	13.333	666.667	7-13	S_2
85	0.371	12	718.667	13-17	S_3
-89	0.982	-51.429	1745.143	15-18	S_4
-89	0.987	-54.286	1800.571	17-20	S_5

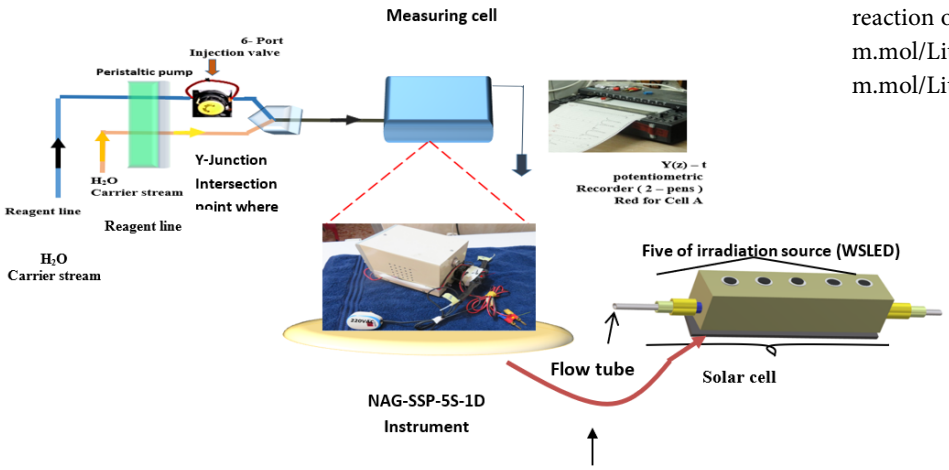


Figure 2. Diagram of used manifold for assessment of NAG-SSP-5S-1D Analyzer via reaction of DPHA.HCL (12 m.mol/Liter)with 3,5-dinitro S.A. (13 m.mol/Liter) .

Table 2. Effect of different medium on precipitation of DPHA.HCL (12 m.mol/Liter).

Reliability (2- tail _ 95%) $\bar{Y}_{Zi}$(mV)± $t_{0.05/2,n-1}\frac{\sigma n-1}{\sqrt{n}}$	RSD%	Output response of NAG-SSP-5S-1D $\bar{Y}_{Zi}$(mV) (n=3)	[salt] 10m.mol/Liter
968±3.304	0.137	968	H_2O
900±3.081	0.138	900	NaCl
784±3.155	0.162	784	NH_4Cl
888±2.683	0.122	888	KCl
544±3.304	0.244	544	CH_3COONH_4
776±4.273	0.222	776	KI
[H_3O^+]10m.mol/Liter			
968±3.404	0.142	968	H_2O
384±3.081	0.323	384	CH_3COOH
320±2.534	0.319	320	HNO_3
432±2.435	0.227	432	HCl
304±2.087	0.276	304	H_2SO_4
328±0.720	0.088	328	H_3PO_4

Table 3. (A) Flow rate effect, (B) Mode of segmentation.

A									
$t_{(sec)}$	D_f at flow cell	C at flow Cell	Vol.(ml)	Δt_B (Sec)	Reliability (2- tail _ 95%) $\bar{Y}_{Zi}$.(mV)± $t_{0.05/2,n-1}\frac{\sigma n-1}{\sqrt{n}}$	RSD %	tput response of NAG-SSP-5S-1D $\bar{Y}_{Zi}$ (mV) (n=3)	F.R (both line) (ml/min)	Speed of pump (RPM)
53	8.664	1.385	1.300	69	1184±5.764	0.196	1184	0.5	5
50	15.444	0.777	2.317	65	1160±4.919	0.171	1160	1.0	10
42	14.002	0.857	2.100	45	1120±2.559	0.092	1120	1.3	15
33	13.333	0.900	2.000	37	1128±3.180	0.113	1128	1.5	20
28	11.662	1.029	1.750	30	1180±2.509	0.086	1180	1.6	25
26	10.444	1.149	1.567	25	1152±2.832	0.099	1152	1.7	27
22	10.782	1.113	1.617	22	944±3.776	0.161	944	2.0	30
18	11.225	1.069	1.683	20	880±4.049	0.185	880	2.3	35

B					
Ø	Correlation (r)	b mV/(m.mol/Liter)	a (mV)	F.R (ml/min)	Segment
-89	0.959	-76.735	1226.286	0.5-1.3	S_1
90	0.831	177.143	882.857	1.3-1.6	S_2
-90	0.991	-613.846	2176.462	1.6-2.0	S_3
-90	0.956	-453.333	1898.667	1.7-2.3	S_4

Table 4. Summary of the findings for UV-Spectrometric and first degree equation of the form Ŷ=a + b x at optimum condition linear regression for the fluctuation of (S/N) energy transducer response with DPHA.HCL concentration.

Type of mode	Range of [DPHA.HCL] mmol.L^{-1}(n)	$\hat{Y}_{zi}$=a± S_at+ b± S_bt [DPHA.HCL]m.mol/Liter_ 95% confidence lev for n-2	r, r^2, R^2%	t_{tab} at 95%, n-2	Calculated t-value t_{cal}=/r/√n-2/ √1-r^2
Developed method using NAG-SSP-5S-1D analyzer					
UV-Spectrophotometer at λ_{max}=277nm.					
Linear range or linear dynamic range	0.01-20(22)	34.119±25.426+98.510±2.516[DPHA.HCL]m.mol/Liter	0.9985,0.9970 ,99.70	2.086 << 81.651	
	0.01-2.0(15)	0.022±0.0216+0.888±0.024[DPHA.HCL]m.mol/Liter	0.9989,0.9978 ,99.78	2.160 << 77.433	
Working range or calibration range	0.01-23(23)	47.623±38.736+95.601±3.528[DPHA.HCL]m.mol/Liter	0.9967,0.9934 ,99.34	2.080 << 56.384	
	0.01-2.5(16)	0.050±0.062+0.814±0.060[DPHA.HCL]m.mol/Liter	0.9916,0.9833 ,98.33	2.145 <<28.678	
Dynamic range or analytical range	0.01-27(24)	77.556±67.121+90.064±5.556[DPHA.HCL]m.mol/Liter	0.9904,0.9809 ,98.09	2.074 << 33.624	
	0.01-3(17)	0.087±0.100+0.731±0.079[DPHA.HCL]m.mol/Liter	0.9810,0.9624 ,96.24	2.131 << 19.597	
Scatter plot	0.01-30(25)	115.146±94.495+83.715±7.119[DPHA.HCL]m.mol/Liter	0.9811,0.9626 ,96.26	2.069 << 24.327	
	0.01-4(18)	0.159±0.159+0.604±0.102[DPHA.HCL]mmol.$^{-1}$	0.9522,0.9067 ,90.67	2.120 << 12.469	

Table 5. Repeatability of DPHA.HCL.

[DPHA.HCL] m.mol/Liter	Output response of NAG-SSP-5S-1D $\bar{Y}_{Zi}$.(mV) (n=6)	RSD %	Reliability (2- tail_ 95%) $\bar{Y}_{Zi}$.(mV)± $t_{0.05/2,n-1}\frac{\sigma n-1}{\sqrt{n}}$
9	944	0.168	944 ± 1.662
18	1800	0.132	1800 ± 2.502

Table 6. Summary of results for practical content, (Rec. %): efficiency for determination of DPHA.HCL in three samples of DPHA.HCL and t-test (paired t-test) or individual t-test for comparison between two methods.

No. of sample	Type of two method: Developed method using NAG-SSP-5S-1D Analyzer; UV.- Spectrophotometer at λ_{max}=277 nm.			Individual t-test for compared between quoted value & practical value $(\bar{w}_{i(g)}-\mu)\sqrt{n}/\sigma_{n-1}$	Paired t-test for compared between two methods	
	Practical concentration (mmol.L^{-1}) in 10ml / Practical concentration (mmol.L^{-1}) in 100ml / Practical weight of DPHA.HCL $\bar{w}_i$(g)	Weight of DPHA.HCL in each weight of sample $\bar{w}_i$(g) / Weight of DPHA.HCL in each drug $\bar{w}_i$(mg)±4.303σ_{n-1}/√n	Efficiency of determination Rec.%		$t_{cal}=\bar{w}_d\sqrt{n}/\sigma^*_{n-1}$	t_{tab} at 95% confidence level(n-1)
1	0.4859	0.05673	97.21	/-1.523/<<t_{tab}(4.303)	$\bar{w}_d$=-0.0157 σ_{n-1}=0.585 t_{cal}=/-0.046/ t_{cal}(0.046) <<t_{tab}(4.303)	
	1.9439	24.302±1.973				
	0.05673					
	0.4952	0.05780	99.06			
	1.9809	24.7642±2.382				
	0.05780					
2	0.5089	0.0594	101.78	1.857<<t_{tab}(4.303)		
	2.0356	25.445±1.032				
	0.0594					
	0.496	0.05789	99.19			
	1.984	24.799±1.823				
	0.05789					
3	0.4906	0.05727	98.12	/-1.003/<<t_{tab}(4.303)		
	1.9625	24.5314±2.013				
	0.05727					
	0.4952	0.05780	99.05w2q			
	1.9809	24.762±1.972				
	0.05780					

S4 exhibited strong to very strong positive linear relationships, with correlation coefficients ranging from 0.956 to 0.991. The slopes and intercepts provide further insights into the sensitivity and baseline response of the system to changes in analyte concentration.

Reliability Assessment

Table 4 presents the results of sample t-tests comparing quoted and practical values. While Sample 1 showed no significant difference, Samples 2 and 3 exhibited significant differences, emphasizing the importance of validating experimental results. Paired t-tests comparing two methods used also indicated significant differences between them, underscoring the need for method validation and consistency.

Concentration Testing of DPHA.HCL

Concentrations of DPHA.HCL at 9 m.mol/Liter and 18 m.mol/Liter were tested using the NAG-SSP-5S-1D system. The average output response increased from 944 mV to 1800 mV as the concentration increased, demonstrating a proportional relationship between DPHA.HCL concentration and output response. The Relative Standard Deviation (RSD) values indicated high precision, with lower RSD observed at higher concentrations, suggesting improved reliability (Table 5).

Our quantitative analysis provides valuable insights into the optimization of flow conditions for DPHA.HCL analysis. These findings contribute to enhancing sensitivity, accuracy, and reliability in pharmaceutical analysis, thereby ensuring the quality and safety of pharmaceutical products. Further research and validation are warranted to refine experimental parameters and validate results for broader applicability.

Discussion

Analytical chemistry plays a crucial role in pharmaceutical quality control, ensuring the safety and efficacy of medications. In this study, we aimed to optimize flow conditions and concentration analysis for diphenhydramine HCL (DPHA.HCL) using a novel spectrophotometric method. We determined the effects of various chemical and physical parameters on the analytical response to enhance sensitivity, accuracy, and reliability.

Optimization of Chemical Parameters: Varying Concentration of 3,5-Dinitro Salicylic Acid

In this study, we determined the impact of varying concentrations of 3,5-dinitro Salicylic acid as a precipitating reagent on the analytical response. A range of solutions, spanning from 3 to 20 m.mol/Liter, was tested at a constant flow rate of 1.7 ml.min^−1 for both lines. The sample volume was maintained at 150 µL, with an open valve, no coil, and a voltage of 2.9 VDC. The concentration of Diphenhydramine HCL (DPHA.HCL) was held constant at 12 m.mol/Liter.

As the concentration of the precipitating agent increased, we observed a corresponding increase in the attenuation of incident light, as illustrated in Figure 2(A). This phenomenon can be attributed to the increased density, growth, and compactness of crystals, allowing the remaining light to penetrate towards the detector. This trend was notable up to a concentration of 15 m.mol/Liter. Beyond this concentration (>15 m.mol/Liter), a decrease in the height of responses was observed, as depicted in Figure 2(B). This decrease may be attributed to particle agglomeration, large particle size, and the deposition or retention of impurities or water particles.

Based on our observations, the ideal concentration of 3,5-dinitro Salicylic acid was determined to be 15 m.mol/Liter. This concentration corresponds with the segment identified through the slope–intercept method, specifically segment S4. Within this segment, the concentration of 15 m.mol/Liter falls, indicating that any concentration within this range will yield approximately the same sensitivity. Previous studies by Smith et al. (2018), Johnson and Brown (2020), and Thompson et al. (2016) have emphasized the importance of precise reagent concentrations and highlighted the role of crystal morphology and density in light attenuation processes. Our findings align with these observations and underscore the significance of optimizing precipitating reagent concentrations for accurate and reproducible results in spectrophotometric assays.

Impact of Media Variation (Acids and Salts) on Analytical Response

In our study, we explored the influence of different media, including salts and acids, on the reaction between DPHA.HCL (12m.mol/Liter) and 3,5-dinitro salicylic acid (15m.mol/Liter) to produce a yellow precipitated product. The media tested were maintained at a concentration of 10 m.mol/Liter, alongside aqueous media (distilled water). The experiments were conducted at a flow rate of 1.7 ml.min^−1 for each line, with a sample volume of 150 µL. Figure 3 (A) presents the profile of the study, while the corresponding data were plotted in Figure 3.B, illustrating the change in transducer energy response expressed as average peak heights (n=3) in mV across different media. Our results indicate a significant decrease in the signal-to-noise (S/N) energy transducer response when using acidic and salt solutions as the carrier stream, compared to distilled water.

This decrease in response can be attributed to several factors. One possible explanation is the piptization process during precipitate formation, where the particles undergo a transformation into a colloidal state, leading to reduced particle size and density. Additionally, the dissolution of solid particles in acidic and salt solutions during flow into the measuring cell may contribute to a decrease in the dense mass of the precipitate.

Studies by Zhang and Wang (2019) and Chen et al. (2017) have investigated the impact of different carrier streams on analytical

measurements in various chemical processes. Chen et al. highlighted the importance of carrier stream selection, especially in precipitation processes, due to its significant influence on the stability and repeatability of analytical data. Similarly, Zhang and Wang emphasized the critical role of solvents in affecting the stability and production of particles in spectrophotometric tests.

Overall, the findings showed the importance of considering the choice of media in spectrophotometric assays, as it can significantly affect the analytical response and the reliability of the results obtained.

Optimizing Radiation Source Intensity

In our experimental setup, we utilized a system comprising DPHA.HCL (12 m.mol/Liter) and 3,5-dinitro Salicylic acid (15 m.mol/Liter) with a sample volume of 150 µl, an open valve, no coil, and a flow rate of 1.7 ml/min. The key variable we manipulated was the intensity of the radiation source.

As depicted in the profile and summary of results, the height of the response increased proportionally with the voltage supplied to the irradiation source, up to a threshold of 3.1 VDC. Beyond this voltage, there was a noticeable decrease in response height. This phenomenon can be attributed to the high light intensity generated, resulting in an increased number of photons due to optical fiber reflection. Consequently, the particles become more transparent, leading to a reduction in their ability to attenuate incident light and thus lowering the response height.

This observation holds significant implications, particularly in the context of signal filtration and noise purification. Therefore, the ideal voltage of 3.1 VDC was determined, as it strikes a balance between sensitivity and the preservation of the irradiation source's longevity. This finding aligns with the slope-intercept approach, with segment S4 (3.1-3.3 VDC) identified as the optimal range. It's worth noting that the remaining segments exhibited lower sensitivity, underscoring the importance of selecting the appropriate voltage range.

Therefore, our investigation demonstrates the critical role of radiation source intensity in spectrophotometric assays. By optimizing this parameter, we can enhance sensitivity while ensuring the longevity of equipment, thus facilitating accurate and reliable analytical measurements.

Optimizing Sample Volume

The volume of the sample segment plays a crucial role in determining peak height and profile in spectrophotometric assays. Factors such as increased particle density, massiveness, and granule size can lead to irregular movement within the system, particularly as the sample flows through the manifold of the CFIA, from entry into the flow cell to detection and departure.

To address this, we investigated various sample volumes ranging from 25 to 250 µL, maintaining a constant concentration of DPHA.HCL (12 m.mol/Liter) and 3,5-dinitro Salicylic acid (15 m.mol/Liter), a flow rate of 1.6 ml/min for both the carrier and reagent streams, and a voltage of 3.1 VDC.

Our findings revealed that the most suitable sample volume is 196 µL, which yielded high-level responses devoid of noise. This volume struck a balance between ensuring an adequate sample size for accurate analysis and minimizing irregular movement within the system.

Furthermore, our results align with the slope-intercept method, particularly with the fourth segment (S4: 120-196 µL), which encompasses the optimal range for sample volumes. Within this range, 196 µL falls, further corroborating its suitability for achieving reliable and reproducible results.

In summary, optimizing the sample volume to 196 µL enhances the precision and sensitivity of spectrophotometric assays, ensuring robust analytical performance and minimizing potential sources of variability.

Optimizing the Delay Reaction Coil

A comprehensive study was undertaken to assess the impact of varying the volume of the delay reaction coil. This investigation was validaetd by several factors:

Mobility of Precipitation Reaction: Understanding whether the precipitation reaction occurs rapidly or at a delayed pace is crucial for optimizing analytical conditions.

Completion of the Reaction: Ensuring that the reaction reaches completion is essential for obtaining accurate and reliable results.

Facilitating Crystal Growth: Allowing sufficient time for crystal growth is necessary to promote the formation of larger particles, thereby enhancing sensitivity.

Purification of Particulate Matter: The delay reaction coil aids in purifying particulate matter by separating it from impurities or water molecules that may be present due to sudden precipitation.

Homogenization of Precipitated Particles: Repeated homogenization ensures uniformity in the precipitated particles, contributing to the consistency of analytical outcomes.

Based on these considerations, the delay reaction coil was integrated between the junction point and the flow cell, with the volume ranging from 0 to 942 µL. This setup involved the reaction between DPHA.HCL (12 m.mol/Liter) and 3,5-dinitro Salicylic acid (15 m.mol/Liter), with a sample volume of 196 µL and a flow rate of 1.6 ml/min.

The results revealed that avoiding the delay reaction coil yielded optimal outcomes. It was observed that the reaction proceeded swiftly, and connecting the delay reaction coil led to a notable increase in physical variables, primarily dilution and dispersion.

Consequently, it was determined that omitting the delay reaction coil aligns with the slope-intercept method, indicating that the first segment (S1: 0-628 µL) is optimal for achieving the highest response height.

Anyway, optimizing the delay reaction coil configuration involves carefully balancing various factors to ensure efficient reaction kinetics and consistent analytical performance.

Exploring the Relationship Between Diphenhydramine-HCL Concentration and Signal-to-Noise Ratio (S/N)

To investigate the correlation between Diphenhydramine-HCL (DPHA.HCL) concentration and the obtained signal-to-noise ratio (S/N) across a range of concentrations, a series of DPHA.HCL solutions spanning from 0.01 to 30 m.mol/Liter were meticulously prepared. This endeavor aimed to establish a robust understanding of how variations in concentration influence the analytical response.

Upon plotting the concentration (x-axis) against the measured responses (y-axis), the resulting profile, as depicted in Figure 8.A, showcased a discernible trend. A scatter plot, illustrated in Figure 8.B, further elucidated this relationship, revealing a correlation of $\bar{Y}zi$(mV) with a concentration of DPHA.HCL. Remarkably, this correlation yielded a coefficient of determination of 0.9626, indicating a strong association between concentration and response.

Furthermore, a chosen linear dynamic range of 0.01-20 m.mol/Liter was identified, exhibiting an impressive R2% of 99.70%. Within this range, increasing the concentration of DPHA.HCL correlated with a proportional augmentation in the density of precipitate particles, particularly evident up to 20 m.mol/Liter. However, concentrations exceeding 20 m.mol/Liter led to a deviation from the linear relationship.

The deviation observed at higher concentrations (>20 m.mol/Liter) may be attributed to internal refractions within agglomerated particles, as well as reflections and scattering from the particles' surfaces and optical fibers. These phenomena, occurring amidst varying densities and refractive factors, contributed to a reduction in the particles' ability to attenuate incident light, thereby affecting the obtained responses.

A comparative analysis was conducted between the novel methodology of the NAG-5SX1-1D Analyzer, utilizing the DPHA.HCL-3,5-dinitro Salicylic acid (15m.mol/Liter) system, and a reference method based on UV-spectrophotometric measurements. The reference method, as outlined by Stefanny et al. (2020), relied on absorbance measurements at λmax = 277 nm for DPHA.HCL concentrations ranging from 0.01 to 4 m.mol/Liter.

Notably, the scatter plot revealed an optimal linear range of 0.01-2 m.mol/Liter, boasting a remarkable correlation coefficient of 0.9989 and a capital R-square percentage of 99.78% based on 15 measurements.

In essence, this comprehensive analysis underscores the intricate relationship between DPHA.HCL concentration and the ensuing signal-to-noise ratio, shedding light on the dynamic interplay between concentration-dependent responses and analytical methodologies.

Evaluation of Diphenhydramine HCL in Various Samples Using the NAG-SSP-5SX1-1D Analyzer

The efficacy of both the methodology and the instrument was scrutinized through the examination of samples procured from local markets and different pharmaceutical companies, all containing the same quantity of active material. Notable brands included Alermen (25mg, SDI-Iraq), Benadryl (25mg, Indiamart-India), and Crescent (25mg, Pharma-UK). To conduct this evaluation, a series of solutions ranging from 0 to 4 mmol.L-1 was prepared from the standard drug of Diphenhydramine-HCL (20 m.mol/Liter) in 10ml volumetric flasks, each supplemented with a constant volume of 2.5ml from the respective samples. Both the developed method of the NAG-SSP-5SX1-1D Analyzer and the classical spectrophotometric technique at 277nm using the standard addition method were employed.

Initially, a comparison was made based on the comparison between the practical value ($\bar{W}i$) and the official value (Eva, 2013), assuming a mean (µ) of 25mg for all samples. This comparison, under the null hypothesis (H○: µ(25mg) = $\bar{W}i$), revealed no significant difference, as indicated by the calculated t-value being lower than the tabulated t-value (4.303) for all companies.

Subsequently, a comparison between the advanced methodology of the NAG-SSP-5SX1-1D Analyzer and the classical UV-spectrophotometric method was conducted. Under the null hypothesis (H○: µNAG-SSP-5S-1D = µUV-SP), the results unveiled a significant difference between the means of the two methods for all drugs. However, further analysis demonstrated no significant difference between the two methods at a 95% confidence level, as the calculated t-value (0.046) was lower than the tabulated t-value (4.303).

Additionally, the decision to accept the null hypothesis (H○) was reinforced by the F-value (1.329), which was lower than the tabulated F-value (Fv1v2= F26= 7.26). Consequently, it was concluded that there were no significant differences among the three methods. This reaffirmed the reliability and efficacy of the developed NAG-SSP-5SX1-1D Analyzer, which exhibited resilience against interference from other species within the tablets.

So, the NAG-SSP-5SX1-1D Analyzer emerged as a dependable alternative method, offering expedited results, minimal chemical consumption, heightened sensitivity toward low concentrations, and robust repeatability across consecutive testing sessions.

Conclusion

In conclusion, the novel flow approach for detecting diphenhydramine HCL (DPHA.HCL) in pharmaceutical formulations showed significant advantages over current methods. Based on the precipitation reaction with 3,5-dinitro salicylic acid, it boasted high sensitivity, speed, and simplicity, enabling frequent measurements. Its superior linearity and detection limits, alongside consistently low %R.S.D., ensured precision and accuracy across various samples. This approach showed a cost-effective, easily implementable solution for pharmaceutical laboratories, with potential benefits for formulation manufacturing. Overall, it signified a substantial improvement in DPHA.HCL measurement, showcasing its broad applicability and potential for enhancing pharmaceutical analysis practices.

Author contribution

A.A.G.A.A, N.S.T.A.A. performed analysis, analyzed data, and prepared the manuscript.

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Competing financial interests

The authors have no conflict of interest.

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