Baghdad Science Journal

Volume 23 | Issue 3 Article 7

3-20-2026

# Determination of Trace Pyridoxine Hydrochloride (Vitamin B6) in Dosage Forms and Redbull Samples Using Dispersive Liquid-Liquid Microextraction Technique Coupled with Spectrophotometry

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## How to Cite this Article

Ahmed, Sally Ghanem and Khayoon, Wijdan Shakir (2026) "Determination of Trace Pyridoxine Hydrochloride (Vitamin B6) in Dosage Forms and Redbull Samples Using Dispersive Liquid-Liquid Microextraction Technique Coupled with Spectrophotometry," *Baghdad Science Journal*: Vol. 23: Iss. 3, Article 7.
DOI: https://doi.org/10.21123/2411-7986.5241

BAGHDAD SCIENCE JOURNAL 2026;23(3):834–847



RESEARCH ARTICLE

# Determination of Trace Pyridoxine Hydrochloride (Vitamin B6) in Dosage Forms and Redbull Samples Using Dispersive Liquid-Liquid Microextraction Technique Coupled with Spectrophotometry

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## ABSTRACT

This paper introduced a sensitive determination method measurement spectrophotometric coupled with dispersive liquid-liquid micro extraction based on charge transfer complex formation between pyridoxine hydrochloride and chloranil using alkaline buffer (pH = 10.5). After that, the extraction of charge transfer complex was conducted by injection a mixture of chloroform and acetone solvents followed by spectrophotometric measurement of tiny organic droplets at $\lambda = 535$ nm. The important parameters affecting the formation of the complex and efficiency of DLLME such as concentration of chloranil, volume and pH of buffer solution, reaction time and the optimum conditions of DLLME has been studied and optimized in detail. At the optimum conditions, linearity was obtained ranging from 0.025–0.85 $\mu$g mL$^{-1}$ and 0.05–0.75 $\mu$g mL$^{-1}$ for dosage form and Redbull, respectively at the optimal conditions. Limit of detection of 0.011, 0.019, 0.021, 0.018 and 0.036 $\mu$g mL$^{-1}$ for standard, Tablet (50 mg), Tablet (25 mg), injection and Redbull and LOQ 0.04, 0.03, 0.08, 0.06 and 0.18 were obtained, respectively. High enrichment factor of 73 was achieved, good recoveries of 96.4–102.0% were acquired. The medicinal determination of pyridoxine hydrochloride has been effectively expanded in dosage forms and Redbull.

**Keywords:** Chloranil, Dispersive liquid-liquid micro extraction, Pharmaceuticals preparations, Pyridoxine hydrochloride, Redbull, Spectrophotometry, Vitamin B6

## Introduction

Vitamin B6 or Pyridoxine hydrochloride (PN) (5-hydroxy-6-methylpyridine-3, 4-diyl) di methanol hydrochloride, Fig. 1 is the first vitamin in the B group to be separated, and the body's cells must remain intact and amino acid processing to occur in the diet. [1] It is a group of water-soluble forms of vitamin B and slightly soluble in alcohol and white crystalline powder. [2] It has a molecular structure of $C_8H_{11}NO_3$ HCl, with a molecular weight of 205.6 g.mol$^{-1}$.

Pyridoxine is a coenzyme involved in metabolizing nearly all amino acids and proteins in addition to the metabolism of sugar and fatty acids, body cell defense. [3] Vitamin B6 deficiency can lead to nerve and blood problems, as well as convulsions in children, and is required for the synthesis of the neurotransmitters serotonin and Gamma-aminobutyric acid (GABA), which regulate depression, pain perception, and anxiety. Therefore, it is used to treat premenstrual syndrome and compensate for a vitamin deficiency, particularly if it has resulted in neuritis or

Received 15 May 2024; revised 10 December 2024; accepted 12 December 2024.
Available online 20 March 2026

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https://doi.org/10.21123/2411-7986.5241

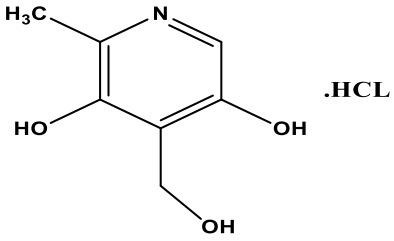



**Fig. 1.** Chemical structure of pyridoxine hydrochloride.

anemia. A higher dietary intake may be required during pregnancy, breast-feeding, or childhood growth. It has been recently shown that vitamin B6 is a potent antioxidant that may smother reactive oxygen species, including superoxide and singlet oxygen, as DNA synthesis and methylation are essential processes in 1-carbon metabolism, which is mediated by vitamin B6.[4] Although vitamin B6 is necessary for all living things, it can only be produced by microbes and plants. Vit.B6 is found as pyridoxine (PN), pyridoxal (PL), and pyroxamine (PM), which, during metabolic conversion, are phosphorylated at the 5-hydroxyl methyl position by a kinase that is necessary as a Co-factor in enzymatic processes.[5] The B vitamin complex is made up of eight water-soluble components that are frequently found together in the same foods and were once thought to be one.[6]

Recent research has focused on the charge transfer reaction. The spectrophotometric method, which uses a color charge-transfer complex formed with electron acceptors, has been used to investigate a variety of drugs.[7–10] Because they don't have a lone pair of electrons, some hydrochloride salts of amines don't react with $\delta$ and $\pi$ acceptors.[11] The non-availability of lone pair electrons prevents pyridoxine hydrochloride from reacting with chloranil.[12] Some researchers have reported the extraction methods for converting acidic drugs to basic forms. Several methods for the determination of Vit. B6.HCl such as capillary electrophoresis HPLC[13,14] planar HPLC[15,16] voltammetric,[17] spectrofluorimetry[18] and UV spectrophotometry[19,20] have been reported. However, all reported methods are cost-effective and require a long time. In addition, these methods are time-consuming and exhausting.

Several spectrophotometric methods for estimating pyridoxine hydrochloride in tablets have been reported, using various complicated techniques and expensive reagents.[21–23] As a result, spectrophotometric techniques are the most often utilized methods mentioned above due to their availability in analytical labs and ease of operation for the analyst.

DLLME was introduced by Rezaee et al.[24] The principle of this technique is based on using binary solvent mixtures composed of a small amount of an extracting solvent (i.e., chlorinating hydrocarbon compounds) and a larger amount of a disperser solvent (like methanol, ethanol, acetonitrile, and acetone). The disperser solvent is miscible in both aqueous and organic phases. A cloudy solution is produced once the mixture of extracting and disperser is rapidly injected into the sample solution. Droplet formation enhances the effective surface contact area between the organic layer and the aqueous phase, and the extraction equilibrium is reached faster. Separation is usually achieved by centrifugation[25] followed by analytical HPLC, GC, CE, and spectrophotometry instruments. Several advantages of DLLME such as simplicity, rapidity, small cost, low sample volume, high recovery, enrichment factor, and very short extraction time (a few seconds).[26] Numerous reports have used the DLLME approach with spectrophotometry to determine the preconcentration and level of carbamazepine,[27] folic acid,[28] procaine hydrochloride,[29] and mefenamic acid.[30] The proposed method is simple, sensitive, rapid, and reproducible, requiring low solvent volume and cost. It also reduces hazardous chlorinated solvent consumption, provides a high enrichment factor, and is determined at very low concentrations.

This study used a simple and quick method for converting acidic drugs to basic form. Pyridoxine hydrochloride was reacted with sodium bicarbonate solution, converting the solution to a basic medium. When chloranil solution was added to pyridoxine base solution (n donor), an instantaneous charge transfer complex of the n-z type was formed, with an absorption maximum of 535 nm.

## Materials and methods

### *Apparatus*

For all spectrophotometric measurements, absorbance was measured using a double beam 1800 PC UV visible spectrophotometer from Shimadzu, Japan, and matching 1-cm quartz cells.

### *Preparation of pyridoxine hydrochloride (100 $\mu$g mL$^{-1}$) (Fluka AG, Bushs SG)*

A stock solution of 1000 $\mu$g mL$^{-1}$ pyridoxine hydrochloride was made by dissolving 0.1g in the least quantity of deionized water, transferring it to a 100 mL volumetric flask, then adding more deionized water to get the volume up to 100 mL.

### *Preparation of chloranil (1%)*

Chloranil solution was prepared by dissolving 1g of chloranil in 100 ml acetone.

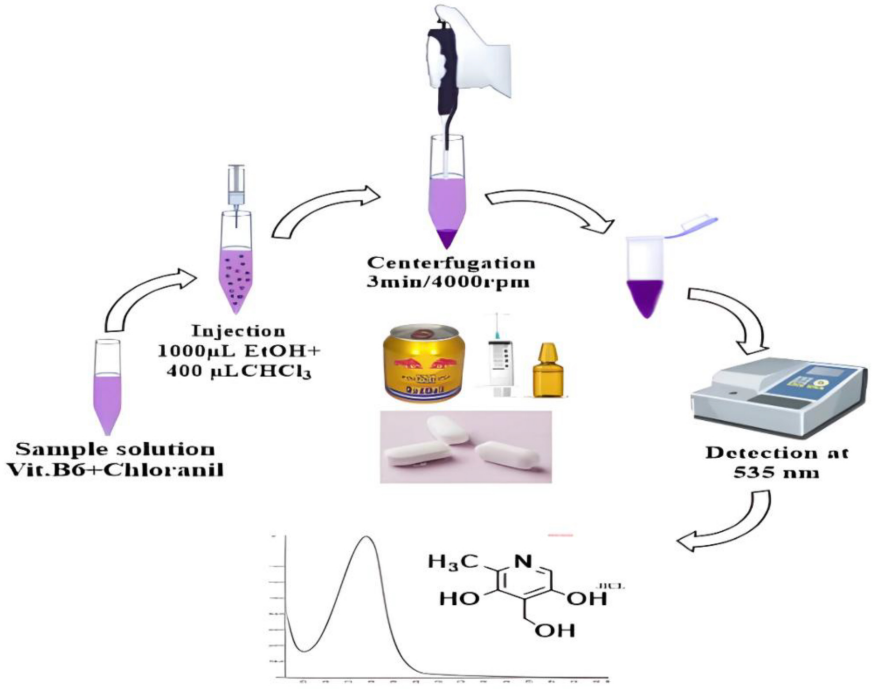



**Fig. 2.** Suggested DLLME steps for the detection of PN.

### *Preparation of buffer solution (pH = 10.5)*

Solution (A) (0.2 M) of anhydrous sodium carbonate was prepared by dissolving 2.21 g in 100 ml deionized water, and solution (B) sodium bicarbonate (0.2 M) was produced by dissolving 16.8g in 100 ml deionized water. Then, 40.5 ml of solution A was mixed with 9.5 ml of solution B in 200 ml, and the volume was completely marked with deionized water.

### *Preparation of NaCl solution*

A 10%–30% (w/v) was prepared by dissolving 10-30 mg of NaCl in 100 mL of deionized water.

### *Preparation of pyridoxine hydrochloride tablet*

The tablets (n = 5) of VitB6 (25 mg and 50 mg) were weighed, finely ground, and powdered and mixed. A 100 $\mu$g mL$^{-1}$ was prepared by weighing 0.01g of the drug powder, dissolving it in deionized water, filtering the mixture with Whatman No.1 filter paper, transferring the mixture to a 100 mL volumetric flask, and finally completing it with deionized water to the appropriate level.

### *Preparation of injection*

An injection sample was prepared by taking 1 mL from (100 mg/2 mL) in a 100 mL volumetric flask diluted to the mark with deionized water.

### *Preparation of Redbull sample*

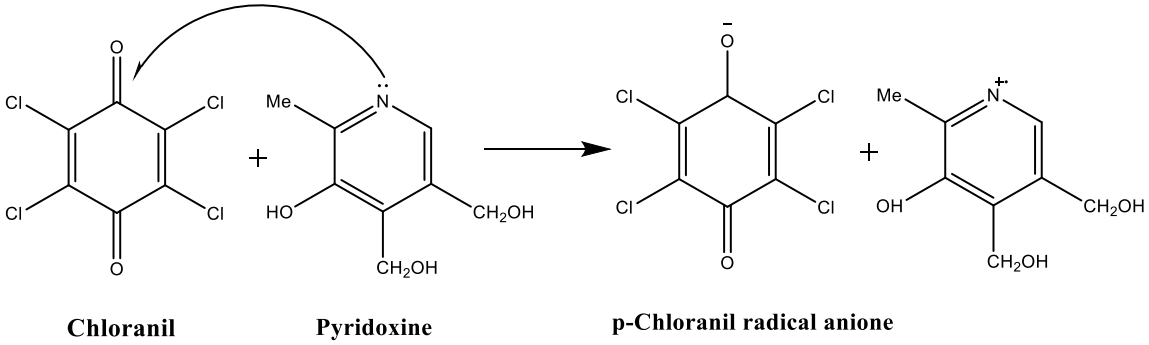
The Redbull was prepared by taking 1mL from (0.8 mg /100 mL) and then diluted to the mark with acetate buffer pH = 5.[31]

### *DLLME procedure*

A 1.0 mL of (100 $\mu$g mL$^{-1}$) pyridoxine hydrochloride standard or samples were put into a 10 ml measuring flask, mixed with 1.0 mL of buffer solution added and shaken (1 min.) followed by the addition of 1.0 ml of chloranil solution, instantaneously the color was produced. Next, a fast injection of 400 $\mu$L of chloroform extraction solvent and 1 mL of ethanol dispersive solvent was made into the sample solution. The aqueous phase was removed after the resultant tiny organic droplets were removed using a micro syringe and centrifuged for 3 minutes at 4000 rpm. Subsequently, the droplets were placed within a quartz cell to be measured spectrophotometrically against a black solution at ($\lambda$ = 535 nm). A blank solution was made using the same parameters without adding pyridoxine hydrochloride. The prescribed DLLME procedure's phases are outlined in Fig. 2.

### *Calibration of the curve of pyridoxine hydrochloride standard, tablet, injection, Redbull*

A series of solutions were prepared with different concentrations from 0.025–0.85 $\mu$g mL$^{-1}$ of 100




**Scheme 1.** Suggested chemical reaction of PN and chloranil complex.

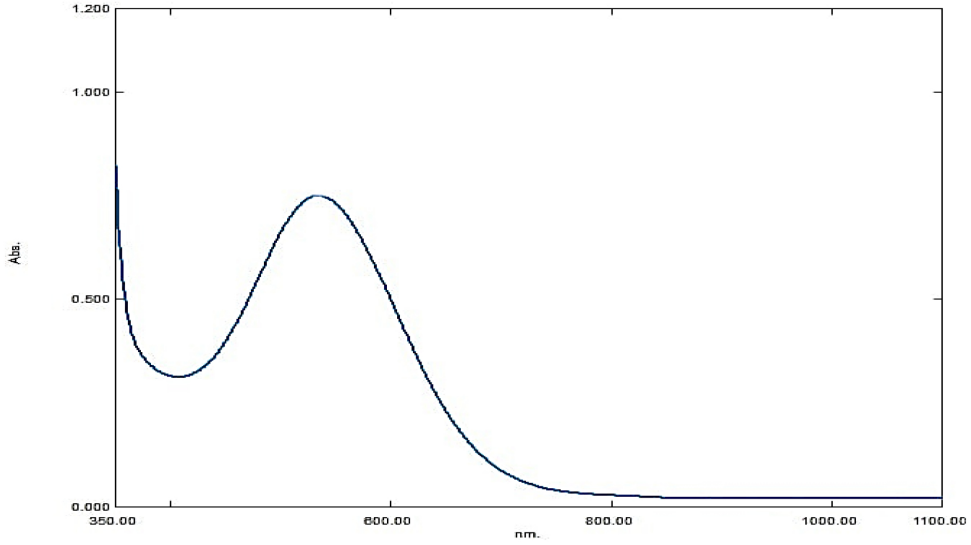



**Fig. 3.** UV Spectra for the chemical reaction of Pyridoxine hydrochloride and chloranil.

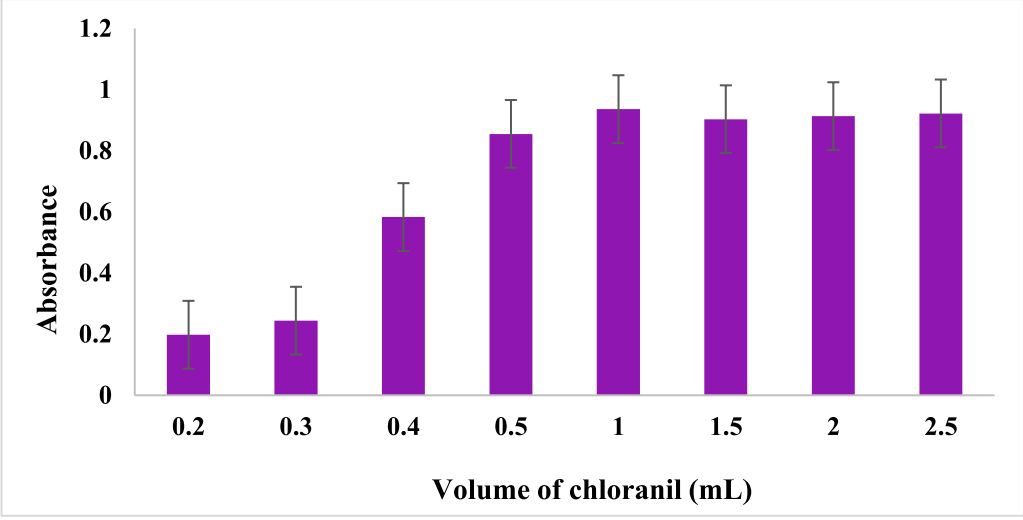



**Fig. 4.** Effect volume of chloranil (PN (0.5 ppm), buffer (10.2), ethanol (1 mL), chloroform (500 $\mu$L), (Centrifugation speed (5000 rpm) and time (5 min)).

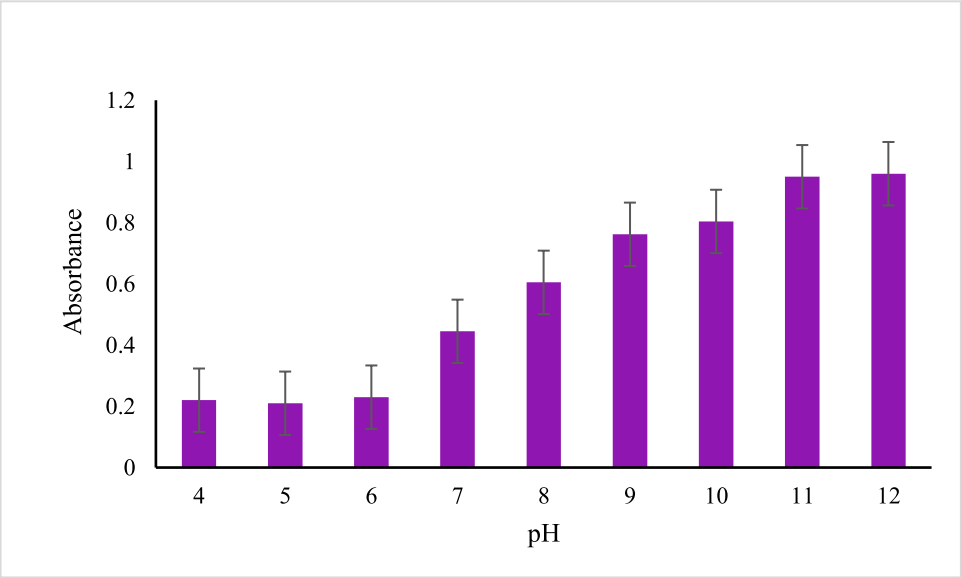



**Fig. 5.** Effect of pH media (PN (0.5 ppm), Chloranil (1 mL), ethanol (1 mL), chloroform (500 $\mu$L), (Centrifugation speed (5000 rpm) and time (5 min)).

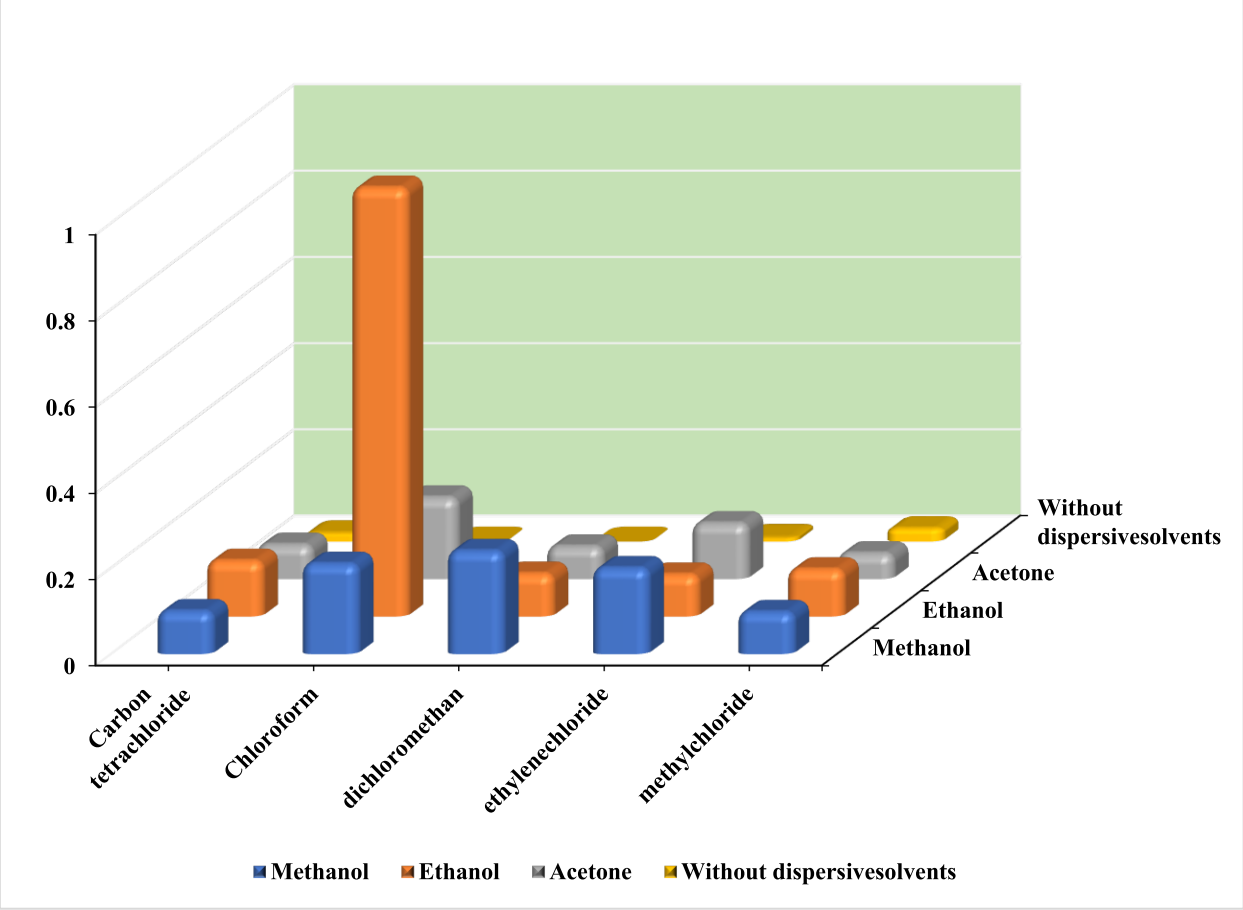



**Fig. 6.** Effect type of dispersive and extraction solvents.

$\mu$g mL$^{-1}$ ppm pyridoxine hydrochloride (standard, tablet, injection, and Redbull) were added and mixed with 1.0 mL of chloranil and 1.0 mL buffer. The volume was diluted to 10 mL with deionized water). Then, the solution was introduced to the DLLME procedure (section 2).

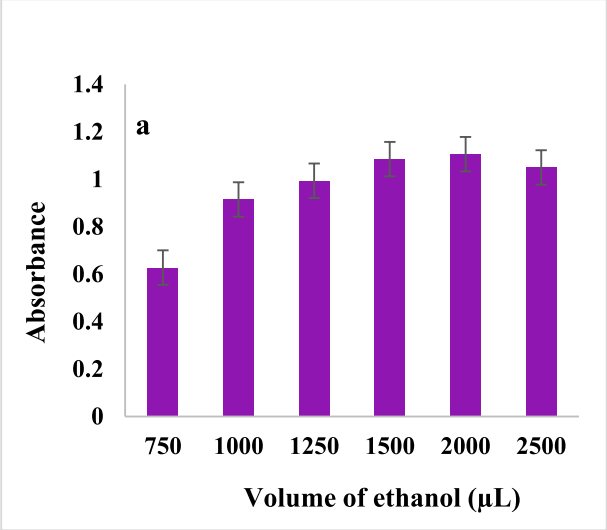
## Results and discussion

### Optimizing the reaction conditions

It was possible to study the impact on the colored product's absorbance by adjusting one at a time and observing the others as they remained constant. The effects of chloranil volume, buffer solution, salt addition, surfactant quantity, extraction, dispersive solvent type and volume, centrifugation time, and speed were studied.

### Absorption spectra

Charge-transfer complexation reactions often occur between good electron donors (aliphatic or aromatic amines) and electron acceptors. Therefore, it is necessary to convert the pyridoxine hydrochloride solution into a pyridoxine base solution to prepare the assay procedure for pyridoxine hydrochloride.[23] As a result, significant contact was achieved in the current work between chloranil (an electron acceptor) and Pyridoxine hydrochloride, which has an amino group on its structure (an electron donor). In Scheme 1 the proposed mechanism for the charge-transfer complex is shown in Scheme 1. The colored product's absorption spectra between pyridoxine hydrochloride and chloranil Fig. 3. The highest absorbance was measured at 535 nm, and the Charge-Transfer Complex was puller-shifted to exclude any possible interference.[32]



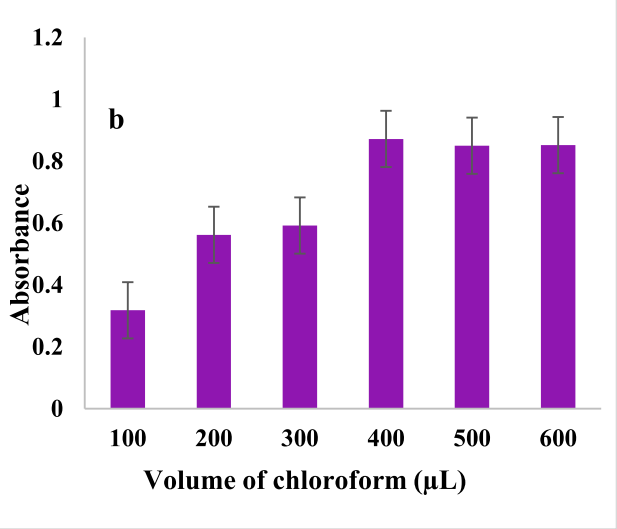




**Fig. 7.** Effect of volume a) dispersive solvent and b) extraction solvent (PN (0.5PP m), Chloranil (1 mL), (Centrifugation speed (5000 rpm) and time (5 min)).

### Effect of the reaction conditions

#### Effect of chloranil volume

This study examined the impact of varying volumes of (0.1M) chloranil reagent using 0.2–2.5 mL. As shown in Fig. 4, the Abs. Reached maximum after adding 1.0 mL of chloranil reagent and then remained constant. This volume is considered an optimum value since the suggested method aimed to consume the minimum amount of chemical reagent.

#### Effect of buffer solution type

The effect of different pH media was studied using an acidic and basic buffer Fig. 5. The results showed that pH 11.0 recorded the highest absorbance compared to the studied acidic buffers since the charge transfer reaction depends on the presence of non-protein amino groups. Therefore, pH 11.0 is ideal for complex formation.[21,33]

#### Effect of type of dispersive and extraction solvents

It's important to select the appropriate solvent for the DLLME process. Chlorinated solvents have been the extraction solvent of choice in all DLLME papers due to their density and capacity to extract the desired chemicals.[30] Carbon tetra chloride, chloroform, dichloromethane, ethylene chloride, and methyl chloride are examples of solvents.[34] In the DLLME approach, the choice of dispersive solvent is also crucial. It should be soluble in the extraction solvent and miscible in water so that the solvent can disperse into the aqueous phase as tiny particles, creating a hazy mixture (water/disperser solvent/extraction

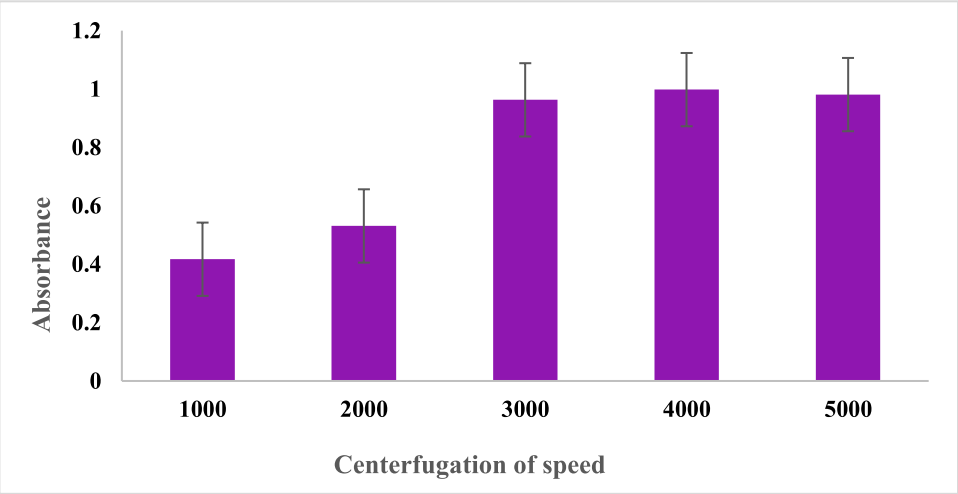



**Fig. 8.** Effect of centrifugation speed PN (0.5 PPm), Chloranil (1 mL), (Centrifugation speed (5000 rpm) and time (5 min)).

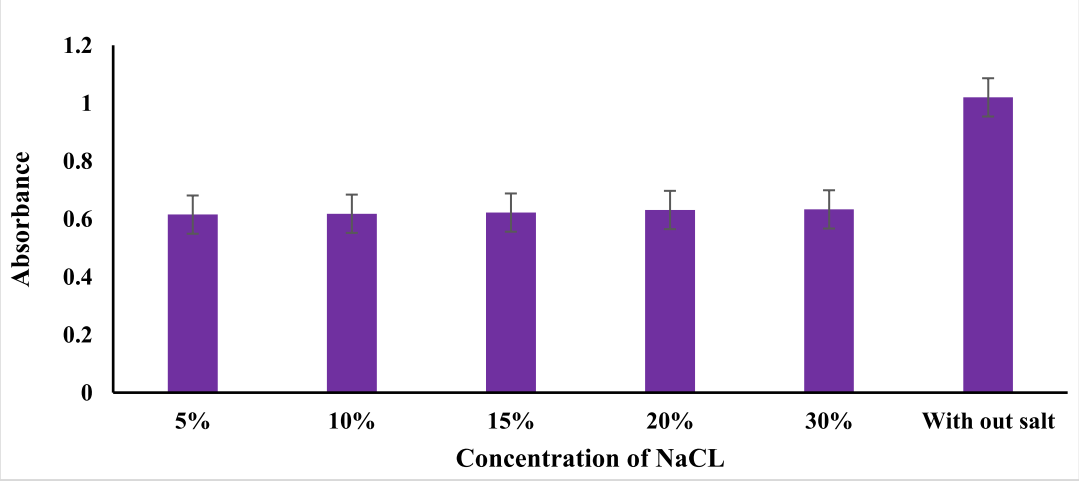



**Fig. 9.** Effect of ionic strength PN (0.5 PPm), Chloranil (1 mL), (Centrifugation speed (5000 rpm) and time (5 min)).

solvent).[35] Therefore, various solvents were studied, including methanol, ethanol, and acetone, combined with the chosen extraction solvents (Carbon tetra chloride, chloroform, dichloromethane, ethylene chloride, and methyl chloride). Fig. 6 demonstrates that a combination of ethanol and chloroform was the best to get the optimum extraction of the target analyte.

### *Effect of volume of extraction and dispersive solvents*

Studying the influence of extraction volume and dispersive solvents is important in developing the DLLME technique. Since both volumes can affect how dispersion forms, they must be adjusted, this led to an investigation into the effects of different chloroform $CHCl_3$ quantities (100–600 $\mu$L) combined with a fixed amount of dispersive solvent (1000 $\mu$L ethanol). Several dispersive solvent volumes were examined, ranging from 750 to 2000 $\mu$L. The results presented in Fig. 7a show that increasing the dispersive solvent volume from 750 $\mu$L to 2500 $\mu$L increases the analyte responses. Although using lower volumes of extractant leads to higher enrichment factors, operating with volumes less than 750 $\mu$L is difficult. However, when the volume of extraction solvent was increased, the volume of sediment phase and absorbance increased; therefore, the enrichment factor decrease.[36] Thus, 1000 $\mu$L was chosen for a good enrichment factor with less dispersive solvent.

The extracted organic phase's volume rose when the extraction solvent's volume was raised from 100 to 600 $\mu$L, which was not good for the DLLME procedure, leading to increased organic phase volume. Consequently, 400 $\mu$L was selected, Fig. 7b. Thus, 400 $\mu$L of chloroform and 1000 $\mu$L of ethanol were employed in the subsequent trials.

### *Effect of centrifugation speed and time*

From 1000 to 5000 rpm for 1–10 minutes, the impact of centrifugation time and speed on product absorbance was investigated. The maximum absorption was seen at 4000 rpm for five minutes Fig. 8. The surface area between the extraction solvent and the aqueous layer is infinitely large.[27] Therefore, this method is considered rapid, which is the most important advantage of the DLLME technique.

### *Effect of ionic strength*

In general, the aqueous solubility of an analyte decreases, and their extraction into the organic matter phase is enhanced by adding a salt.[37] The effect of ionic strength on DLLME extraction efficiency was investigated using 10%–30% (w/v) NaCl concentrations. There was no discernible difference in absorbance when the concentration of NaCl was increased from 10% to 30% Fig. 9. These conclusions led to the conduct of all future studies without salt.

### *Studying interference*

Studies were conducted to determine how interfering compounds affected the measurement of pyridoxine hydrochloride. The developed procedure was used to make sample solutions that contained 0.015 $\mu$g mL$^{-1}$ of pyridoxine hydrochloride and various concentrations of other potentially existing compounds. Results in Table 1 showed that most of the selected compounds did not interfere even if found in a 10-, 100-, or 1000-fold excess over the analyte concentration.

**Table 1.** Effect of interference molecules using 0.04 $\mu$g mL$^{-1}$ of pyridoxin hydrochloride.

| 10 times greater than standard | | | | |
|---|---|---|---|---|
| Excipient | RSD% | Found | Rec% | E% |
| Glucose | 3.37 | 0.040 | 100.0 | 0.08 |
| Sucrose | 4.61 | 0.038 | 99.89 | –0.10 |
| Lactose | 3.25 | 0.037 | 99.70 | –0.29 |
| Starch | 3.60 | 0.039 | 99.32 | –0.67 |
| Alanine | 4.21 | 0.039 | 99.13 | –0.86 |
| Creatinine | 2.21 | 0.039 | 99.32 | –0.67 |
| Urea | 2.41 | 0.038 | 98.37 | –1.62 |
| Uric acid | 2.79 | 0.039 | 98.75 | –1.24 |
| K$^+$ | 4.95 | 0.039 | 99.89 | –0.10 |
| Cystine | 1.74 | 0.039 | 98.56 | –1.43 |
| Glycine | 3.08 | 0.039 | 99.70 | –0.29 |
| 100 times greater than standard | | | | |
| Excipient | RSD % | Found | Rec% | E % |
| Glucose | 3.79 | 0.039 | 99.13 | –0.86 |
| Sucrose | 4.99 | 0.039 | 98.56 | –1.43 |
| Lactose | 3.56 | 0.039 | 98.56 | –1.05 |
| Starch | 3.68 | 0.039 | 98.94 | –2.19 |
| Alanine | 4.30 | 0.039 | 97.80 | –0.86 |
| Creatinine | 2.45 | 0.039 | 99.13 | –2.76 |
| Urea | 1.84 | 0.038 | 97.23 | –3.72 |
| Uric acid | 2.04 | 0.038 | 96.27 | –1.43 |
| K+ | 1.65 | 0.039 | 98.56 | –2.00 |
| Cystine | 0.88 | 0.038 | 97.42 | –1.43 |
| Glycine | 2.14 | 0.039 | 98.56 | –0.86 |
| 1000 times greater than standard | | | | |
| Glucose | 0.63 | 0.039 | 97.56 | –2.43 |
| Sucrose | 4.07 | 0.038 | 96.61 | –3.38 |
| Lactose | 2.16 | 0.039 | 97.80 | –2.19 |
| Starch | 2.91 | 0.039 | 97.56 | –2.43 |
| Alanine | 3.21 | 0.038 | 97.32 | –2.67 |
| Creatinine | 1.82 | 0.039 | 97.08 | –2.91 |
| Urea | 2.08 | 0.038 | 96.13 | –3.86 |
| Uric acid | 1.52 | 0.038 | 95.89 | –4.10 |
| Glycine | 1.99 | 0.038 | 96.13 | –3.86 |
| Cystine | 1.57 | 0.038 | 96.89 | –1.65 |
| K+ | 0.40 | 0.052 | 97.08 | –2.91 |

### *Calibration curve of pyridoxine hydrochloride*

The standard, drug, injection, and Redbull calibration graphs were constructed under optimum conditions by graphing absorbance vs. concentration of the standard, drug, injection, and Redbull Fig. 8. Table 2 summarizes the suggested method's analytical properties, including regression equation, linearity, limit of detection (LOD), limit of quantification (LOQ), linearity percentage ($r^2$%), and correlation coefficient (r). Based on 3Sb/m and 10Sb/m, the LOD and LOQ were determined (when Sb and m are the standard deviation of the blank and slope of the calibration graph, respectively.

### *Evaluation of the DLLME method*

Under optimal circumstances, the linearity of pyridoxine hydrochloride was examined at various B6 concentrations between 0.05 0.5 $\mu$g mL$^{-1}$ using a recently created DLLME technique. The extracted B6 was detected at 535 nm. Strong linearity was attained, as shown by the correlation coefficient of r = 0.9997, 2.17×10$^4$ L mol$^{-1}$ cm$^{-1}$, and 7.33 × 10$^{-3}$ $\mu$g mL$^{-1}$, respectively, were Sandal's sensitivity and molar absorptivity ($\varepsilon$) Table 2. Both intra-day and inter-day reproducibility were tested using concentrations of 0.015, 0.06, 0.12, and 0.45 $\mu$g mL$^{-1}$. Each concentration was repeated five times over five days. 3*Sb /m and 10*Sb /m were used to calculate the detection and quantification limits. S and m stand for the standard deviation of the blank and slope of a calibration graph, respectively Table 3.

### *Comparison with previously reported methods*

Table 4 compares the suggested approach to the published spectrophotometric approaches. DLLME

**Table 2.** Analytical parameters of Pyridoxine hydrochloride before and after DLLME.

| Parameters | Standard | | Real sample | | | |
|---|---|---|---|---|---|---|
| | Before DLLME | After DLLME | Tablet 50 mg | Tablet 25 mg | Injection 100 mg/2 mL | Redbull sample |
| Regression equation | 0.046x + 0.063 | 1.949x + 0.112 | 1.7x + 0.074 | 1.659x + 0.053 | 1.5807x + 0.0598 | 2.1083x + 0.129 |
| Correlation coefficient (r) | 0.9983 | 0.9997 | 0.9994 | 0.9995 | 0.9995 | 0.9992 |
| Linearity percentage $r^2$ % | 99.66 | 99.94 | 99.88 | 99.90 | 99.90 | 99.84 |
| Linear range ($\mu g\ mL^{-1}$) | 0.5–20 | 0.03–0.85 | 0.03–0.85 | 0.03–0.85 | 0.03–0.85 | 0.05–0.75 |
| Molar Abs. ($L\ mol^{-1}cm^{-1}$) | $3.3E^5$ | $1.3\ E^5$ | $9.4\ E^5$ | $7.6\ E^5$ | $8.1\ E^5$ | $9.0\ E^5$ |
| LOD[a] ($\mu g\ mL^{-1}$) | 0.46 | 0.011 | 0.013 | 0.011 | 0.022 | 0.013 |
| LOQ[b] ($\mu g\ mL^{-1}$) | 2.55 | 0.048 | 0.032 | 0.044 | 0.076 | 0.06 |
| Sandells sensitivity ($\mu g\ cm^{-2}$) | – | 0.094 | 0.072 | 0.053 | 0.067 | 0.038 |
| Enrichment factor[c] | – | 42 | 36 | 36 | 34 | 45 |

[a] Limit of detection; [b] limit of quantification; [c] Enrichment Factor was calculated from the slope of the calibration curve after and before DLLME.

**Table 3.** Accuracy and precision of the proposed method of pyridoxine hydrochloride Std.

| | Intra-day repeatability, (RSD, %,n=5) | | | | | Inter-day reproducibility, (RSD,%, n=25) | | | | | Rec.[b] % (n=5) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Tablet | | | | | Tablet | | | | | Tablet | | | |
| Conc. ($\mu g\ mL^{-1}$) | Std. | 50 mg[a] | 25 mg[b] | Injection[c] | Redbull[d] | Std. | 50 mg[a] | 25 mg[b] | Injection[c] | Redbull[d] | Std. | 50 mg[a] | 25 mg[b] | Injection[c] | Redbull[d] |
| 0.025 | 1.02 | 1.87 | 1.56 | 1.55 | 2.57 | 1.88 | 2.04 | 1.72 | 1.81 | 2.18 | 96.06 | 95.39 | 97.18 | 97.93 | 96.13 |
| 0.1 | 1.88 | 1.78 | 1.66 | 1.42 | 1.22 | 1.72 | 1.94 | 1.43 | 1.37 | 1.75 | 97.10 | 96.61 | 97.15 | 101.0 | 97.18 |
| 0.5 | 1.23 | 1.15 | 0.84 | 1.38 | 2.86 | 0.79 | 1.56 | 0.86 | 1.02 | 0.96 | 99.94 | 97.99 | 98.30 | 98.90 | 101.8 |
| 0.85 | 0.70 | 0.89 | 0.68 | 1.17 | 1.23 | 1.47 | 0.88 | 1.02 | 0.86 | 1.84 | 97.95 | 98.54 | 98.39 | 99.87 | 98.99 |

[a] Tablet 50 mg (SAMAVIT / SDI IRAQ); Tablet[b] 25 mg (AGP Limited/ Pakistan);[c] Injection 50 mg (Bangalore /India) and [d] Redball sample

**Table 4.** A comparison between the stated spectrophotometric techniques and the proposed DLLME method.

| Method | Sample | Linear range ($\mu g/mL$) | LOD ($\mu g/mL$) | LOQ ($\mu g/mL$) | Ref. |
|---|---|---|---|---|---|
| HPLC | Pharmaceutical preparations | 10–1250 | 2.2 | 7.34 | 15 |
| Cyclic voltammetric | Pharmaceutical formulations | 1–24 | 0.08 | 0.12 | 38 |
| Electrochemical Sensor | Pharmaceutical formulations | 0.25–77 | 0.4 | – | 39 |
| Flow injection | Tablets/injections | 1.0–10.0<br>10–100 | 0.5 | – | 40 |
| Chemiluminescent (using flow injection) | Tablet formulations and some dietary sources | 10–250 | 6.0 | – | 41 |
| Spectrophotometric | Pharmaceutical formulations | 0.4–24 | – | – | 21 |
| Spectrophotometric | Pharmaceutical Formulations and Wastewater Samples. | 2–28 | 0.36 | 1.18 | 42 |
| Spectrophotometric | Bulk drug and pharmaceutical preparations | 2–20 | – | – | 43 |
| Spectrophotometric | Pharmactical preparations as tables and injections | 0.125–2.5 | – | – | 44 |
| Spectrophotometric | Pharmaceutical preparation Redbull | 0.025–0.85<br>0.05–0.75 | 0.012<br>0.17 | 0.06<br>0.08 | Current study |

**Table 5.** Application using the suggested approach on Redbull and pharmaceutical formulation.

| Pharmaceutical formulation sample | Concentration of Vitamin B6 | | Recovery % (n = 5) | RSD% (n = 5) |
|---|---|---|---|---|
| | Present | Found | | |
| Tablet 50mg | 0.05 | 0.054 | 96.36 | 3.76 |
| (SAMAVIT) | 0.2 | 0.197 | 98.61 | 1.86 |
| (Iraq/ SDI) | 0.85 | 0.837 | 98.54 | 0.94 |
| Tablet 25mg | 0.05 | 0.049 | 97.48 | 1.13 |
| (Navidoxine) | 0.2 | 0.194 | 97.15 | 1.38 |
| (Iran/AGP) | 0.85 | 0.836 | 98.40 | 0.87 |
| Injection 100 mg/2ml | 0.05 | 0.056 | 102.0 | 2.96 |
| (Bangalore/India) | 0.2 | 0.200 | 97.24 | 1.69 |
| | 0.85 | 0.848 | 99.78 | 0.88 |
| Redbull sample | 0.05 | 0.050 | 101.13 | 3.75 |
| (PUNCH MONSTER) | 0.25 | 0.242 | 97.13 | 1.73 |
| 0.80 mg/100 ml | 0.75 | 0.731 | 97.99 | 1.98 |

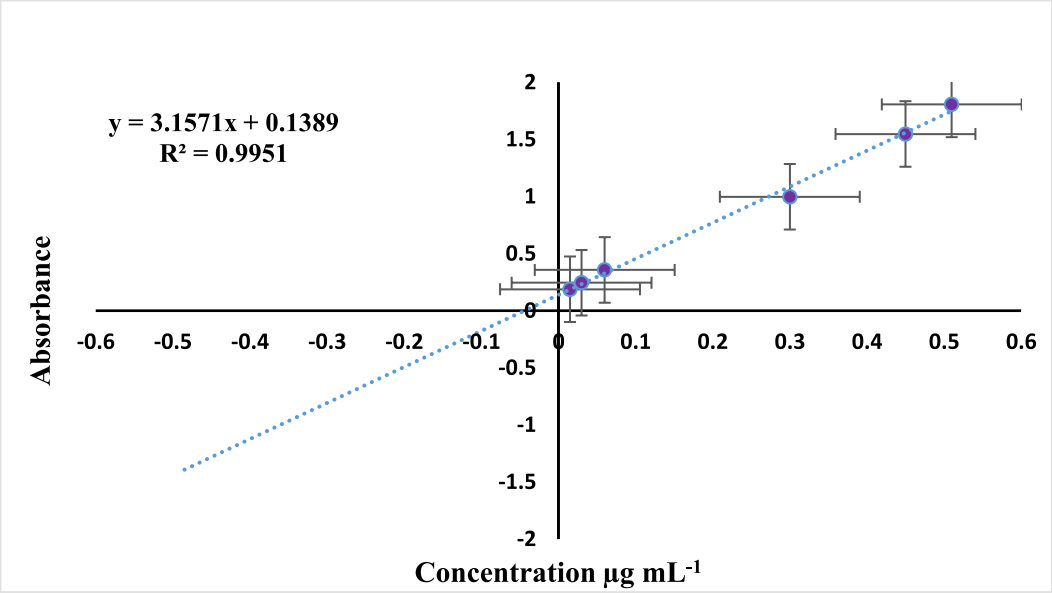



**Fig. 10.** Standard addition of PN tablet ((0.8 $\mu$g mL$^{-1}$) of PN tablet, Chloranil (1 mL), buffer (10.5), ethanol (1 mL), chloroform (400 $\mu$L), (Centrifugation speed (5000 rpm) and time (5 min)).

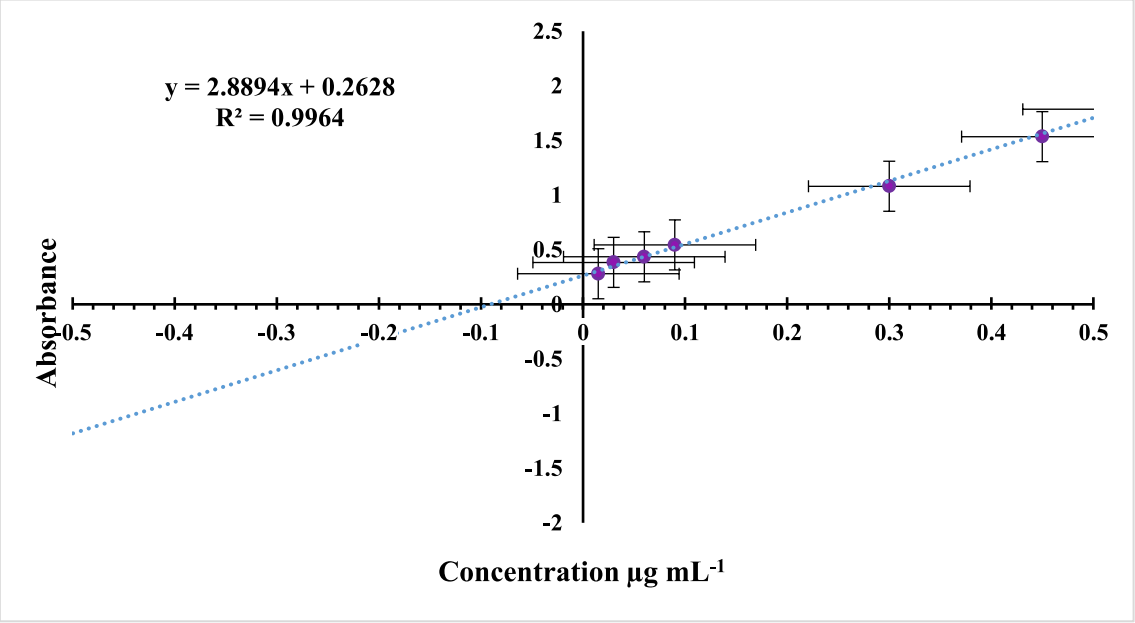



**Fig. 11.** Standard addition of PN injection ((0.8 $\mu$g mL$^{-1}$) of PN injection, Chloranil (1 mL), buffer (10.5), ethanol (1 mL), chloroform (400 $\mu$L), (Centrifugation speed (5000 rpm) and time (5 min)).

extracted a low concentration of this drug and gave a high recovery factor and lower detection and quantification limit than the other methods. This indicates that our method is more accurate.

## *Applications*

Using the proposed DLLME procedure, different concentrations (0.05, 0.2, and 0.85 $\mu$g mL$^{-1}$) of Vitamin B6 samples (Tablet 50 mg and 25 mg), Injection 50mg, and Redbull) were individually analyzed. The good recovery rate was 96.0–102.0% Table 5.

## *Standard addition method*

The standard additions approach was also used, as shown in Figs. 10 to 12, which illustrate the addition of varied concentrations (0.08–1.0$\mu$g mL$^{-1}$)

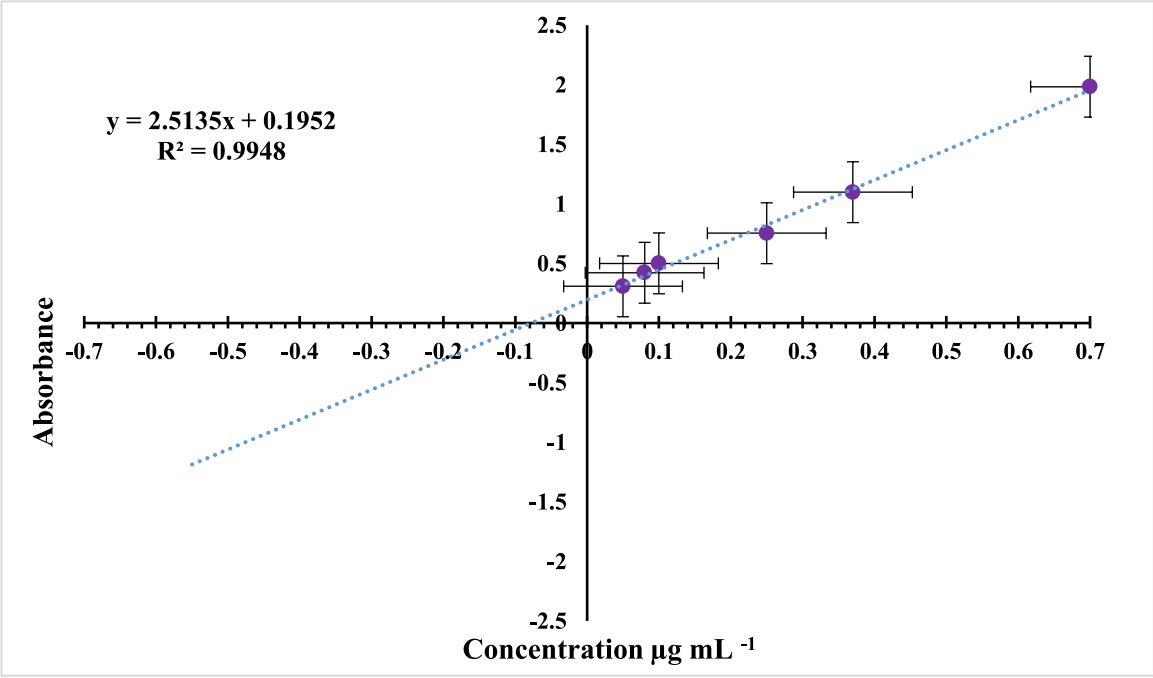



**Fig. 12.** Standard addition of Redbull ((0.8 $\mu$g mL$^{-1}$) of PN Redbull, Chloranil (1 mL), buffer (10.5), ethanol(1 mL), chloroform (400 $\mu$L), (Centrifugation speed (5000 rpm) and time (5 min)).

of a standard solution of 100 $\mu$g mL$^{-1}$ of PN to a fixed concentration (0.08 $\mu$g mL$^{-1}$) of pharmaceutical formulations and Redbull under the condition. Table 3. shows that they provided high precision and accuracy.

## Conclusion

The trace measurement of vitamin B6 in Redbull and pharmaceutical formulations was developed and assessed using a one-step DLLME approach and a spectrophotometric technique. The procedure is sensitive, fast, reproducible, and simple to use. High B6 recovery values ranging from 96.0–102.0 % and EF 34–45 were achieved from pharmaceutical formulations and Redbull, demonstrating the precision and accuracy of the new approach.

## Authors' declaration

- Conflicts of Interest: None.
- We hereby confirm that all figures and tables in the manuscript are ours. Furthermore, figures and images that are not ours have been included with the necessary permission for re-publication, which is attached to the manuscript.
- No animal studies are present in the manuscript
- No human studies are present in the manuscript
- Ethical Clearance: The project was approved by the local ethical committee at the University of Baghdad.

## Authors' contribution statement

S.G.A. and W.S.K. contributed to the design and implementation of the research, to the analysis of the results, and to the writing of the manuscript.

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# تقدير الضئيل لهيدروكلوريد البيريدوكسين (فيتامين ب 6) في أشكال جرعات وعينات ريدبول باستخدام تقنية الاستخلاص الدقيق للسائل والسائل مقترنة القياس الطيفي

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**الخلاصة**

يقدم البحث طريقة الاستخلاص الدقيق لتشتت سائل-سائل الى جانب القياس الطيفي بناء على تكوين معقد نقل الشحنة بين بيردوكسين هيدروكلورايد والكلورانيل باستخدام محلول قلوي pH=10.5. بعد ذلك يتم استخلاص مركب المتكون باستخدام حقن مزيج من الكلوروفورم والايثانول ,وتم قياس القطرات العضوية الناتجة عند 535 نانومتر. تم دراسة العوامل المهمة التي تؤثر على تكوين المعقد وكفاءة الاستخلاص مثل تركيز وحجم الكلورانيل, درجة الحموضة وقت التفاعل ونوع وحجم الاستخلاص والمذيبات التشتت ووقت الاستخلاص وسرعة الطرد المركزي. تحت الظروف المثلى, تم الحصول على الخطية التي تراوحت بين 0.85-0.025 مايكروغرام/مل وللشراب 0.75-0.05 مايكروغرام/مل للمحلول القياسي والعينات, مع معامل الخطية 0.9997 ,كانت حدود الكشف 0.011,0.019,0.036,0.021,0.018 مايكروغرام, الحد الكمي 0.06,0.08,0.04,0.03,0.18 مايكرو غرام على التوالي. وعامل الاسترجاع الجيد 96.4-102.09 % وعامل التخصيب 34-45 للعينات الدوائية ومشروب الطاقة على التوالي. تم تطبيق الطريقة بنجاح لتقدير البيروكسدين هيدروكلورايد في المستحضرات الصيدلانية و نموذج الريدبول بدون الحاجة الى فصل اولي.

**الكلمات المفتاحية:** الكلورانيل، طريقة الاستخلاص المايكروية تشتت سائل–سائل، المستحضرات الصيدلانية، بيروكسدين هيدروكلورايد، الريدبول، المطيافية، فيتامين ب 6.