Advances in Analytical Methods for Pyridoxine Hydrochloride and Combination Therapies: A Comprehensive Survey

[1*]Judy Jays. [2]Pradan Jain,
[1]Associate Professor, [2]MPharm student,
[1]Department of Pharmaceutical Chemistry
[1]Faculty of pharmacy, M S Ramaiah University of Applied sciences, Bengaluru, India
[1]judyjays.py.ph@msruas.ac.in, [2] pradanpjain2000@gmail.com

Abstract

Pyridoxine Hydrochloride plays a crucial role in numerous physiological processes, and its deficiency can lead to various health complications. The development of precise analytical techniques is essential for ensuring the quality, safety, and efficacy of Pyridoxine Hydrochloride formulations, both as standalone supplements and as components of combination therapies. This study presents a comprehensive overview of current advancements in analytical methodologies used for the measurement and evaluation of pyridoxine hydrochloride and its combination therapy. Understanding the shifting landscape of analytical methods is becoming more essential as the importance of pyridoxine hydrochloride expands from solo formulations to complicated combination medicines. The utilization of various analytical methods, such as chromatographic conditions for method development and validation parameters in ensuring the quality, safety, and efficacy of these pharmaceutical formulations are reviewed. Furthermore, the challenges, future prospects, and potential research directions in the field of analytical chemistry for Pyridoxine Hydrochloride and combination therapies are discussed.

Key words: HPLC, RP-HPLC, Validation, Pyridoxine Hydrochloride, Method Development.

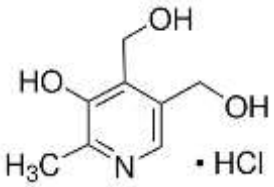
INTRODUCTION

Vitamin B, a group of water-soluble vitamins, is essential for various physiological functions within the human body, including energy metabolism, nervous system function, and red blood cell formation. Among the various B vitamins, vitamin B6, also known as pyridoxine, stands out for its diverse roles in maintaining health and well-being. The three different forms of Vitamin B6 are Pyridoxine, Pyridoxal and Pyridoxamine in which pyridoxine is found in natural sources. Structure of these three forms depend upon the substituent at fourth position of the pyridine molecule [1]. Body transforms all three derivatives of vitamin B6 into pyridoxal phosphate. The primary physiologically active form of pyridoxine or vitamin B6, is pyridoxal 5-phosphate [2]. A co-factor in a number of enzymatic processes related to amino acid metabolism is pyridoxine. In order to carry out the various metabolic transformations of amino acids the pyridoxal phosphate form is essential. Moreover, it plays a role in the generation of neurotransmitters like norepinephrine and serotonin and the transformation of stored glycogen into glucose for energy. Commercially accessible free vitamers of B6 are crystalline hydrochlorides such as pyridoxine hydrochloride and pyridoxamine dihydrochloride. Because of its superior stability than Pyridoxal and Pyridoxamine, pyridoxine hydrochloride is used as standard and the sole form utilized in food fortification and pharmaceutical formulations [3]. Pyridoxine is well acknowledged to be vital in human nutrition. It has been established that a patients will be deficient to vitamin B6 if deficiencies in other group of the B vitamin family are found. As a result, pyridoxine therapy can be combined with other vitamins' therapy. However, cutaneous and central nervous system abnormalities are notably related with pyridoxine insufficiency.

Pyridoxine is found in a variety of foods such as poultry, nuts, meat, fish, whole grains and fortified cereals. It is also available as a nutritional supplement in the form of pyridoxine hydrochloride. Daily intake of vitamin B6 will change based on a number of variables, including age, sex, and stage of life. Vitamin B6 is frequently incorporated in combination medications. Healthcare professionals hope to address various aspects of the problem by combining pyridoxine hydrochloride with other medications, resulting in a more comprehensive and successful treatment plan [4,5].

Analytical methods are fundamental tools in scientific research, industry, and various fields of applied science. These methods encompass a wide range of techniques designed to identify, quantify, and characterize substances or components within a sample. From pharmaceutical development to environmental monitoring, analytical methods play a crucial role in ensuring product quality, safety, and compliance with regulatory standards. The choice of analytical method depends on various factors, including the nature of the sample, the analyte of interest, the required sensitivity and selectivity, and the desired level of precision and accuracy. Analytical methods can be broadly categorized into qualitative and quantitative techniques. Qualitative methods are used to identify the presence or absence of specific substances or components, while quantitative methods provide information about the concentration or amount of a particular analyte in a sample. Among the plethora of analytical techniques available, reversed-phase high-performance liquid chromatography (RP-HPLC) stands out as one of the most versatile and widely utilized methods for the separation, identification, and quantification of compounds in complex mixtures [6].

Table 1 Drug Profile

Name	Pyridoxine Hydrochloride
Molecular formula	$C_8H_{11}NO_3$
Molecular weight	169.18g
IUPAC name	4,5 - bis(hydroxymethyl)-2-methylpyridin -3-ol
Solubility	Soluble in Water, DMSO, methanol, ethanol and acetone
Melting point	162°C
Structure	

METHODOLOGY

The following keywords were used in a literature search across several database sources, including PubMed and Google Scholar: HPLC, RP-HPLC, Validation, and Pyridoxine Hydrochloride. By using the suitable filter, the search was tailored to find the articles that were most relevant to this review.

ANALYTICAL METHODS

Tadvi Shabnam bi Gafoor Kha, et.al in 2023 developed a Reverse phase-HPLC methods for estimating simultaneously Melatonin and Pyridoxine in pharmaceutical formulation. Agilent with an auto sampler Gradient System DAD (Diode Array Detector) with a C18 column (250 mm X 4.6 mm) with a particle size of 5µm used for the RP-HPLC process. The mobile phase was acetonitrile 40%:60% water with 0.1% TEA (PH 6.2 with OPA) at a flow rate of 1.0 mL/min and the detecting wavelength was 265 nm. Melatonin and pyridoxine were shown to have retention of 2.008 and 3.042 min, accordingly. ICH guidelines were followed in the validation of the developed approach. The ICH guidelines limitations were met by the linearity, accuracy, range, and robustness. The approach was therefore determined to be straightforward, accurate, precise, affordable, and repeatable. [7]

A. Chavan, et.al in 2023 developed a RP-HPLC technique to estimate and validate impurity analysis of pyridoxine in a large quantities and formulation. Quality by design (QbD) strategy was used to fine-tune the method, assuring its durability and dependability. RP-HPLC method was carried out using mobile phase composed of acetonitrile: water (90:10 v/v) at a flow rate of 1mL/min and column with dimension (4.6 x 250mm, 5µm) was employed for the chromatographic separation which was maintained at 30°C. UV (DAD) detector was used to measure the absorbance of effluent at 281nm. The retention period of pyridoxine was 6.3 min and the validation parameters such as accuracy, linearity and precision were evaluated attentively as critical performance measures. The method exhibited remarkable precision with a relative standard deviation (RSD) of less than 2%, great recovery rates (100.2 and 101.2%), and a robust correlation coefficient of 0.999. The method can be employed for impurity profiling and can be employed for prolonged stability testing as it shows stability. [8]

Dr. A.Y. Ghodke, et.al in 2023 for the purpose of quantitatively determining methyl cobalamin, alpha lipoic acid, pyridoxine HCl and folic acid in bulk and commercial dosages, an accurate, reproducible and specific Reverse phase-HPLC method was proposed and the method was validated by using different parameters as per ICH guidelines. The method was performed by using column Agilent Zorbax Bonus RP (250 mm x 4.6 mm) with 5µm particle size. Mobile phase containing 0.1% Ortho-phosphoric acid and methanol in the ratio of 50:50 was used at a flow rate of 0.8ml/min for efficient separation and detected at 270nm. Retention duration was estimated to be 6.0 minutes with 10–30µg/mL linearity range, 0.9980 was the correlation coefficient and the recovery rate was found to be 99.8% accordingly. [9]

A. El-Hadi, et.al in 2022 developed RP-HPLC method containing diode array detector (DAD) to measure doxylamine succinate (DOX) and pyridoxine HCl (PYR) in the existence of DOX oxidation degradation product (DOX DEG). The analysis was performed on Xterra C18 column with 100mm x 4.6 mm dimensions using 0.01M phosphate buffer and ethanol in the ratio of 90:10 v/v at pH 5 and flowed at a rate 1mL/min with 10min run time. Absorbance was measured at 254nm by using DAD. Pyridoxine HCl, doxylamine succinate and DOX-DEG were found to have retention time of 1.41min, 5.59min and 7.36 min, accordingly. Validation was done using different parameters following ICH guidance, the linearity found in the level of 10-120µg/mL (PYR) and 5-100µg/mL (DOX)

with correlation of 0.9997 and 0.9999, respectively and exhibited excellent recovery rates (100.41% and 100.04%). Hence, the developed method could be practical and safer substitute for routine examination of the drugs under study. [10]

Marique Elizabeth Aucamp, et.al in 2021 The most effective, simultaneously separating and identifying of Terizidone (TZD) and Pyridoxine (PDX) were accomplished employing an isocratic solvent system that included ultrapure water and acetonitrile (30:70% v/v) with 1 mL glacial acetic acid. A 5 µm column measuring 150 x 6 mm used, with a 260 nm detection wavelength. The method's linearity, accuracy, precision, robustness, specificity, limit of detection (LOD), limit of quantification (LOQ) and stability of the solution were all validated and validation demonstrated that this approach was appropriate and dependable for the accurate simultaneous detection and measurement of TZD and PDX. Percentage recovery obtained for TZD and PDX is 98%. [11]

Saini, et.al in 2021 developed a Reverse phase– HPLC technique for validating and estimating Methionine, Nicotinamide and Pyridoxine hydrochloride simultaneously in combination dosage form. Methanol and 0.01M sodium acetate were used as a mobile phase in the ratio of 600:400(v/v) at a pH 5.2 and a flow rate of 1mL/min. Inertsil-ODS (Octadecyl Silica) C18 column (250mm × 4.6mm) with 5µm particle size was used for separation which was maintained at 25°C and the eluent was measured at 247nm. Methionine, Pyridoxine hydrochloride and Nicotinamide were found to have retention time of 1.4min, 2.2min and 4.4 min respectively, where the correlation coefficient was 0.999 with concentration of 10-30µg/mL. Recovery studies were conducted in order to assess the accuracy. Methionine, pyridoxine hydrochloride, and nicotinamide were found to have recovery rates of 99.9%, 98.95%, and 99.8%. [12]

Patel, et.al in 2021 reported that use of derivatizing agents improves the chromatographic behaviour. Leucine was converted into Fmoc-Leucine derivative as a derivatizing agent in order to reduce volatility and improve chromatographic behaviour of an analyte. For the determination of Fmoc-Leucine and Pyridoxine HCl simultaneously, RP-HPLC method and UV-visible spectroscopic method were developed using solvent system composed of 0.1% OPA with ACN: water (50:50% v/v) at a flow rate of 1mL/min. The column used was Zorbex C18 (250mm × 4.6mm) and maintained at 40°C, but the analysis was accomplished at ambient temperature of 25 °C and the detection of effluent was done at 285nm. The retention period of Pyridoxine HCl as well as Fmoc-Leucine obtained at 3.4 and 14.3 minutes with a run time of 10min. The methods were validated using several parameters like accuracy, precision, linearity, LOD and LOQ, robustness according to ICH guidelines. Pyridoxine hydrochloride and Fmoc-Leucine were shown to have a correlation coefficient of 0.999:0.997 and 0.999:0.995 and with percentage recovery of 98.6%, 98.7%, 99.2%, 99.3% accordingly. [13]

Bachmann, et.al in 2020 for the purpose of quantifying each B6 vitamins such as pyridoxamine (PM), pyridoxine (PN), pyridoxal (PL), pyridoxamine phosphate (PMP) and pyridoxine glycoside (PNG) in grains, fruits and vegetables LC-MS/MS and stable isotope dilution assay (SIDA) was developed. The R_t of each B-group vitamins was found to be 1.9min for PN, 1.03min for PM, 1.57min for PL, 1.06min for PMP and 2.12 min for PNG. Validation was done by using starch matrices and for each analyte, the limits of quantification and detection were 0.0085 to 0.059 mg/kg; 0.0028 to 0.02 mg/kg, accordingly. The percentage recoveries ranged from 92-111%. Additionally, using LC-MS/MS and SIDA, the first quantification of PNG was carried out without the need for enzymatic processes or inconsistent internal standards. [14]

T. Benjamin, et.al in 2019 A novel isocratic reverse phase- HPLC technique was identified for the quantification of isoniazid and pyridoxine tablets simultaneously. Thermo's Hypersil BDS column (250 x 4.6 mm, 5µ) was used to perform the separation. The mobile phase was composed of acetonitrile and phosphate buffer (pH 4) in a 75:25 v/v ratio. During the 6-minute runtime, injection volume was 10 µL, column oven temperature was 30°C, flow rate was 1.0 mL/min, and a photodiode array detector (PDA) was used for detection at 267 nm. Isoniazid and pyridoxine were found to have retention times of 3.5 and 4.3 minutes respectively, which suggests a comparatively shorter analysis time. The procedure was verified in accordance with ICH standards. The study provides a novel, precise, repeatable, and reliable technique for the simultaneous estimations of the two drugs. [15]

H. Hashem, et.al in 2018 developed RP-HPLC method by utilizing QbD strategy for the estimating Levetiracetam and Pyridoxine HCl in tablet dosages. The drugs were given together to prevent the pyridoxine HCl deficit which was brought on by antiepileptic levetiracetam. BDS Hypersil C8 column of (250mm × 4.6mm) dimensions was employed for the separation. Methanol and buffer (KH2PO4) were used as isocratic mobile phase in the ratio of 38.4: 61.6(v/v) with pH 3 and 0.8mL/min rate of flow. Detection of the effluent was done at 214nm. Validation was done as per ICH guidelines, the linearity range of levetiracetam was determined to be 1.56-100µg/mL and 0.39-100µg/mL for pyridoxine hcl, accordingly and were shown correlation coefficient of 0.99. The proposed method exhibited significant predictability and robustness and showed percentage recovery of 95.46% and 101.14%, respectively. [16]

Padmaja, et.al in 2018 reported a RP-HPLC method for the simultaneous determination of methyl cobalamin, alpha-lipoic acid, pyridoxine hydrochloride, and folic acid drugs. The column preferred was Inertsil C18 with dimensions 250mm × 4.6mm and particle size of 5µm. The mobile phase was made up of acetonitrile: buffer mixture (5.05 g of hexane-1-sulfonic acid in 1000 mL of distilled water) for efficient separation of sample at a rate of flow of1ml/min. The analysis was carried out at contexture temperature and detected at 285nm. It was found that the average retention time for methyl cobalamin, pyridoxine hydrochloride, alpha-lipoic acid, and folic acid were 3.5, 6.7, 8.5, and 9.3 min with correlation coefficient of 0.99 of all the drugs where the concentration ranging from 0-2130 µg/mL (methyl cobalamin), 0-142.5µg/mL (alpha-lipoic acid), 0-4.54µg/mL (pyridoxine HCl) and 0-2µg/mL (folic acid) respectively. The recovery rate of the developed method was found to be 98-100%. [17]

N.M. Habib, et.al in 2017 in order to determine a pair of mixed compounds containing pyridoxine HCl (PYH) with either meclizine HCl (MEH) or cyclizine HCl (CYH), HPLC-DAD and TLC-densitometric techniques were developed. Chromatographic separation of the three medications under study was performed in the devised TLC-densitometric method on silica gel 60 F254 plates using a mobile phase that contained methylene chloride: acetone: methanol (7: 1: 0.5, v/v/v) while the devised HPLC-DAD method relied on column (Zorbax Eclipse C18) with mobile phase (methanol and 0.05M KH2PO4 in 90:10 v/v; pH 5) at a flow rate of 1mL/min where

it was measured at 220nm. PYH, CYH and MEH were found to have R_t of 2.52, 4.4 and 7.65min accordingly. The linearity range was determined to be 10-50µg/mL (PYH), 10-50µg/mL (CYH) and 7-50µg/mL (MEH) respectively, with excellent correlation of 0.9999. The method showed remarkable percentage recovery (100% and 99%) and also used effectively to identify the above-mentioned drugs in their formulations. [18]

Ronald Bartzatt, et.al in 2016 utilized HPLC method in an isocratic environment and detected at 290 nm by UV to examine vit-B6 in commercialized nutrient drinks and solid tablets. C-18 column was used and the samples were prepared using 81% distilled water and 19% ethanol. After solubilization in aqueous state with ethanol ranging from 10-20% (v/v), vit-B6 was identified at 290 nm. A constant elution peak of B6 vitamin was obtained at 1.6 min at rate of flow of 1.0 mL/minute when the column pressure was set at 1900 psi. The findings showed that vitamin B6 levels were highly sensitive at 290 nm, ranging from $4.4029x10^{-5}$ molar to $7.8081x10^{-4}$ molar. With a coefficient of determination of 0.9948 and an extremely positive correlation coefficient of 0.9974, standard curves showed good linearity in the range of 0 to $7.8081x10^{-4}$ molar (y =112,521,145.5x + 2,818.6) and the percentage recovery of vitamin B6 varied from 95% to 105%. [19]

Kamepalli Sujana, et.al in 2016 developed and validated a straightforward, reliable, focused, and more precise UV spectroscopy technique for determining Pyridoxine HCl, Folic acid, and Mecobalamin in bulk pharmaceuticals and marketed formulations (capsules) employing the simultaneous equation approach. The technique entails solving simultaneous equations by taking solvent 0.1N NaOH. The absorbance maxima (λmax) of pyridoxine HCl, folic acid, and mecobalamin was obtained at 218nm, 256nm, and 220nm, accordingly. The technique was validated in accordance with ICH guidelines, and the calibration curves for all three drugs showed high linearity, as demonstrated by the correlation coefficients (r) of 0.999. Pyridoxine HCl, folic acid, and mecobalamin were recovered in percentages of 97.8%, 97.7%, and 91.76%, respectively. [20]

Adam, et.al in 2015 developed reverse phase HPLC which was quick, cost-effective, reliable and specific for estimating pyridoxine hydrochloride and its broken-down metabolites. The analysis was carried out using solvent system containing 0.015M KH2PO4: methanol (70:30 v/v, pH- 3) and performed on Thermo-hypersil GOLD C18 column with a flow rate of 1mL/min for efficient sample separation, detection was carried out at 254nm. At 3.5 minutes ± 0.02 the separation was finished, and no interference from any excipients was seen. The method was estimated to be linear in the concentration ranged from 10-50µg/ml with correlation of 0.9996 and percentage recovery ranged from 98.8 to 100.86%. The developed method was effectively used for estimating the above-mentioned drugs and in their combined form. [21]

Khateeb, et.al in 2015 developed a precise, effective and specific spectrophotometric method for an estimation of B-group vitamins such as vit B3 (nicotinamide) and vit B6 (pyridoxine HCl) in pharmaceutical products and in their pure form. The technique relies on the tri-iodide ions which were formed when vitamins react with an amalgam of potassium iodide and iodate at 25°C in an aqueous solution and detected for vit B6 at 290 and 335nm and at 288 and 350nm for vit B3 respectively. The proposed method was validated in terms of linearity, accuracy, precision, LOD and LOQ. The results were found to be linear in the concentration range of 0.5-20µg/mL for vit B3 and vit B6 with strong correlation coefficient (r^2= 0.998:0.9949, 0.9995:0.9974) and with percentage recovery ranges between 96% to 102.25% respectively, indicating that the excipients in formulation did not interfere with the vitamins. [22]

Khanage SG and coworkers in 2014 used a RP-HPLC method for the estimation of Prochlorperazine Maleate (PCM) and Pyridoxine hydrochloride (PDH) in pharmaceutical products. Mobile phase used was 40:60 v/v of methanol: water at pH 7 with a rate of flow of1 mL/min, HPLC was performed on a C18 column and effluent was measured at 272 nm. It was found that the retention times for PDH and PCM were 3.48 and 6.28 minutes respectively and the linearity was determined in the range of 5-25 µg/ml. This approach effectively analysed tablet dosage forms, with no chromatographic interferences from formulation excipients. The analysis of PCM and PDH in tablets quantitatively was effectively conducted in this study using the HPLC technique, which is easy to use, quick, free from interference from formulation excipients and doesn't require a separation step for each medication. [23]

T. Jeyalakshmi, et.al in 2014 identified accurate, easy to use, and sensitive reverse phase-HPLC method for simultaneously estimating vitamin B1 (Thiamine hydrochloride), vitamin B3 (Nicotinamide), vitamin B5 (Dexpanthenol) and vitamin B6(pyridoxine hydrochloride). The sample separation was carried out on Water C18 column (250mm × 4.6mm, 5µm). The mobile phase composed of buffer and methanol (95:5v/v, pH 3.5) was used for efficient separation at a flow rate of 1.5mL/min and the mobile were allowed to settle in the column for 15min and measured at 210nm. Thiamine HCl, Nicotinamide, Dexpanthenol and Pyridoxine HCl were found to have retention time of 2.492min, 6.748min, 20.08min, 4.077min and showed linearity in the range of 10-200µg/ml, 40-800µg/ml, 8-160µg/ml with coefficient correlation of 0.9992, 0.9994, 0.9993 and 0.9991 accordingly. The precision percentage relative to the standard deviation emerged was below 2% and found the approach was specific that could be used for routine analyses of the drug products. [24]

Shinde Prashanti, et.al in 2014 A simple, selective, rapid, precise and economical RP-HPLC method has been developed for the determination of DL-Methionine and Pyridoxine Hydrochloride in tablet formulation. Potassium dihydrogen orthophosphate was used as the mobile phase at a flow rate of 1 mL/min on an isocratic that included an Agilent 1200 HPLC system with a variable wavelength UV detector with a Peerless basic C18 (4.6 mm x 15 cm, 5 µm) column at 210 nm. Pyridoxine HCl and DL-methionine were found to have the retention time 14 min and 3.5 min and percentage recovery found ranging from 98% to 102%. The established approach was appropriate for regular drug analysis in tablet form of dosage since it was more accurate, straightforward and reproducible. [25]

G. Nagamallika, et.al in 2013 For the purpose of quantitatively determining the levels of thiamine hcl, nicotinamide, riboflavin-5′-phosphate sodium, pyridoxine hydrochloride, caffeine, D (+)-Panthenol, and preservatives such as methylparaben and propylparaben in multivitamin syrup formulation, a new, sensitive, and highly selective gradient reverse phase UPLC technique was identified and validated. The separation was obtained by utilizing solvent A (0.1% TFA in water) and solvent B (a combination of 50% acetonitrile and 50% methanol with 1.0 mL/ min), on an HSS T3 (50mm × 2.1mm, 1.7µm) column by maintaining temperature of the column at 48°C and detected at 200, 254 and 290 nm. For thiamine hcl, pyridoxine hydrochloride, caffeine, Nicotinamide, riboflavin-5′-

phosphate sodium, D (+) Panthenol, methylparaben, and propylparaben, the correlation coefficient was 0.99. The product was subjected to stressful conditions including oxidative, acidic, basic, hydrolytic, thermal, and photolytic degradation in order to demonstrate the method's stability-indicating capabilities. Thiamine hydrochloride, pyridoxine hydrochloride, D (+)-Panthenol, Nicotinamide, riboflavin-5′-phosphate sodium, caffeine, methylparaben, and propylparaben were among the breakdown products that could be clearly separated. The method's specificity, linearity, accuracy, precision, and robustness were all validated in accordance with ICH guidelines. [26]

Reema, et.al in 2013 developed a precise, accurate and effective RP-HPLC method for estimating and validating vitamin B drugs such as pyridoxine hydrochloride (B6), folic acid (B9), methyl cobalamin (B12) and atorvastatin. Due to the low concentration of methyl cobalamin (B12) in tablets, its determination was done independently. The separation was achieved using mobile phase 100% methanol for vit-B12 while phosphate buffer and 100% acetonitrile for estimating vit-B6, B9 and atorvastatin (50:50v/v, pH-3). Inertsil ODS C18 column with dimension (4.6 x 250mm) and 5µm particle size packing was used for the sample separation with a run time of 25min and detected at 265nm and 254nm. The retention time was determined to be 2.358min for vit B9, 2.516min for vit B12, 1.952min for vit B6 and 10.837min for atorvastatin. In the concentration range of 25–250 ppm (ATO), 50–500 ppm (vitamin B6), 10-100 ppm (vitamin B9), and 2–50 ppm (vitamin B12), the results appeared to be linear with correlation of 0.999 respectively. The rate of recovery was determined to be 101.14, 101.02, 101.22, 101.66 accordingly, which made the method more accurate and specific. [27]

Nayak, et.al in 2013 in order to estimate PYR (pyridoxine HCl) and DOX (doxylamine succinate) simultaneously in a tablet dose form, an easy-to-use, quick UV spectrophotometric technique was developed. Research on solubility was done to find a good solvent that would dissolve both PYR and DOX. Subsequently, the medications were dissolved separately and in combination in the solvent and the absorbances of PYR and DOX were determined at 260 nm and 290 nm, correspondingly and followed Beer-Lambert's law within the concentration that ranged from 4-20µg/ml. Validation was done in accordance with ICH guidelines using variuos parameters. The proposed technique showed excellent accuracy with recovery rates ranging from 99.41% to 100.18%, a strong correlation coefficient of 0.999 and possessed low LOD and LOQ values indicating the technique was highly sensitive towards determining the cited drugs in a large quantity and in tablet dose form. [28]

Marcin Leszek Marszall, et.al in 2005 reported a technique employing high performance liquid chromatography (HPLC) with coulometric electrochemical and UV detector for the simultaneously measuring thiamine hydrochloride, pyridoxine hydrochloride, and cyanocobalamin. Vitamin retention times were regularly measured by isocratic elution using a Supelco LC 18 column measuring 5 µm (25 cm×4.6 mm) and a mobile phase of 0.05M phosphate buffer, 10% methanol and 0.018M trimethylamine (1mL/min, pH 3.55). Retention characteristics, coulometric electrochemical analysis, and ultraviolet detection all proved the method's specificity. The detection limits of the cited drugs (pyridoxine, thiamine, and cyanocobalamin detection limits were 2.7, 9.2, and 0.08 ng/ml, correspondingly. Large concentration range, high sensitivity, and adequate accuracy (99.6–102.7%) were further characteristics of this approach. When the method's repeatability was assessed at various vitamin concentrations, the relative standard deviation was less than 4.5%. The technique has been effectively used to quantify the levels of vitamins like B1, B6, and B12 in dietary supplements and medicinal formulations. [29]

CONCLUSION

In this comprehensive survey, we have reviewed a wide array of analytical methods employed for the measurement and evaluation of pyridoxine hydrochloride (vitamin B6) and its combination therapies. Pyridoxine hydrochloride plays a crucial role in various enzymatic pathways involved in amino acid metabolism, neurotransmitter synthesis, and energy production. As its significance expands from solo formulations to complex combination therapies, it becomes imperative to employ advanced analytical techniques to ensure the quality, safety, and efficacy of pharmaceutical formulations. A number of studies have investigated the use of various analytical techniques, including RP-HPLC, LC-MS/MS, UV spectroscopy, and high-performance liquid chromatography (HPLC), to quantify pyridoxine hydrochloride alone or in combination with other medications. These techniques provide excellent sensitivity, specificity, accuracy, and precision for figuring out how much pyridoxine hydrochloride is present in various drug formulations. Furthermore, these techniques acceptability and dependability for regular analysis in pharmaceutical labs are guaranteed by their validation in accordance with International Conference on Harmonization (ICH) requirements. Several dosage forms, including tablets, nutritional beverages, and multivitamin syrups, have been successfully analysed using these techniques, proving their adaptability and suitability for use in a variety of formulations.

In conclusion, these methodologies not only contribute to the development of high-quality pharmaceutical products but also facilitate the effective management of various health conditions where pyridoxine hydrochloride supplementation is warranted. Future research in this field should focus on further optimization and validation of analytical methods to meet the evolving needs of the pharmaceutical industry and healthcare practitioners.

REFERENCES

[1] S. Saini, S. Mandal, and D. Agarwal, "Simultaneous determination by RP-HPLC of Pyridoxine hydrochloride, DL methionine and nicotinamide in tablets", Int. J. All. Res. Wr, vol. 2, pp. 21, July 2020.

[2] M.E. Swartz, and I.S. Krull, "Analytical method validation and Development", CRC press

[3] C.M. Riley, and T.W. Rosanske, "Development and validation of analytical methods, Elsevier, 1st ed, vol. 3, May 1996.

[4] S. Sharma, S. Goyal, and K. Chauhan, "A review on analytical method development and validation", Int. J. Appl. Pharm, vol.10, pp.8-15, September 2018.

[5] S. Sharma, N. singh, A.D. Ankalgi, A. Rana, and M.S. Ashawat, "Modern trends in analytical techniques for method development and validation of pharmaceuticals: a review", J. Drug Deliver. Therapeu, vol. 11, pp.121-130, February 2021.

[6] P. Aruna, "Key aspects of analytical method validation and linearity evaluation", J. Chromatogr. B, vol.877, pp.2224-2234, August 2009.

[7] T.S.B. Gafoor Kha, B.Y. Patil, and P.R. Patil, "Development and validation of RP-HPLC method for simultaneous estimation of Melatonin and Pyridoxine in bulk and pharmaceutical dosage form", Indo. Amer. J. Pharm. Sci, vol. 10, pp. 194-206, August 2023.

[8] A. Chavan, R. Gandhimathi, "A QbD based RP-HPLC method for stability indicating impurity profiling of Pyridoxine: Method development, validation, and application", Int. J. Pharm. Qual. Ass, vol. 14, pp. 701-706, March 2023.

[9] Dr. A.Y. Ghodke, V. Giri, B.N. Poul, S.S. Patil, and C.V. Panchal, "Development and validation for simultaneous estimation of Methyl cobalamin, Alpha Lipoic acid, Pyridoxine and Folic acid by RP-HPLC in bulk and marketed dosage form", Int. J. Nov. Res & Dev, vol. 8, pp. 526-610, August 2023.

[10] H.R. Abd El-Hadi, B.M. Eltanany, H.E. Zaazaa, and M.S. Eissa, "HPLC-DAD approach for determination of pyridoxine HCl and doxylamine succinate in pure and pharmaceutical dosage forms: a green stability-indicating assay method", Microchem. J, vol. 172, pp. 106982, January 2022.

[11] N.Y. Musafili, H. Samsodien, and M.E. Aucamp, "A validated RP-HPLC method for the simultaneous detection and quantification of pyridoxine and terizidone in pharmaceutical formulations", Analytica, vol. 2, pp. 206-216, December 2021.

[12] S. Saini, S. Mandal, and D. Agarwal, ". Development and validation of RP-HPLC method for the simultaneous estimation of Methionine, Pyridoxine Hydrochloride and Nicotinamide in combined dosage form", Asian. J. Pharm. Res & Dev, vol. 9, pp. 95-101, February 2021.

[13] C. patel, P. Jain, S. Shinde, and A. Shirkhedkar, "Simultaneous estimation of Pyridoxine HCl and FMOC-Leucine using derivative and chromatographic approach in pharmaceutical dosage form", *J.* Chem. Metrol, vol. 15, pp. 124-134, December 2021.

[14] T. Bachmann, A. Maurer, and M. Rychlik, "Development of a LC-MS/MS method using stable isotope dilution for the quantification of individual B 6 vitamers in fruits, vegetables, and cereals", Anal & Bioanal Chem, vol. 412, pp. 7237-7252, August 2020.

[15] T. Benjamin, and D. Ramachandran, "Development and Validation of RP-HPLC Method for Simultaneous Estimation of Isoniazid & Pyridoxine in Bulk and It Pharmaceutical Formulations", Amer. J. PharmTech. Res, vol. 9, pp. 215-225, February 2019.

[16] H. Hashem, and H.M. El-Sayed, "Quality by design approach for development and validation of a RP-HPLC method for simultaneous determination of co-administered levetiracetam and pyridoxine HCl in prepared tablets", Microchem. J, vol. 143, pp. 55-63, December 2018.

[17] V. Padmaja, M. Prashanthi, and P. Mayuri, "Stability-indicating Reversed-Phase High-Performance Liquid Chromatography method for simultaneous estimation of Methyl cobalamin, Alpha-Lipoic acid, Pyridoxine HCL, AND Folic acid in bulk and combined dosage form", Asian. J. Pharm. Clin. Res, vol 1, pp. 302-307, August 2018.

[18] N.M. Habib, M.M. Abdelrahman, N.S. Abdelwhab, and N.W. Ali, "Validated chromatographic methods for the analysis of two binary mixtures containing pyridoxine hydrochloride. J. AOAC. Int, vol. 100, pp.414-421, March 2017.

[19] R. Bartzatt, P. Gajmer, M. Nguyen, and A. Tran, "Detection and Assay of Vitamin B6 (Pyridoxine Hydrochloride) Utilizing Isocratic High Performance Liquid Chromatography. J. Sci. Res. & Rep, vol.12, pp. 1-12, January 2016.

[20] Kamepalli Sujana, T. Pavani priyanka, P. Iswarya, and K. Sai Tejaswini, "Simultaneous Estimation of Pyridoxine HCl, Folic Acid and Mecobalamine in Bulk Drugs and Marketed Formulation by Vierdott's Method. Int. J. Pharm. Sci. Rev. Res, vol.38, pp.54-58, May 2016.

[21] E.H.K. Adam, and R.I. Al-Shdefat, "Development and validation of a RP-HPLC method for simultaneous estimation of pyridoxine hydrochloride (Vitamin B6) and its degraded products formed under effect of different solvents. J. Chem. Pharm. Sci, vol.8, pp.239-245, June 2015.

[22] M. Khateeb, B. Elias, and F.A. Rahal, "Validated spectrophotometric method to Assay of B6 and B3 vitamins in pharmaceutical forms using potassium iodide and potassium iodate. Intl. Lett. Chem. Phys. Astronom, vol. 60, pp.113-119, September 2015.

[23] S.G. Khanage, P.B. Mohite, M.R. Dudhade, and V.K. Deshmukh, "Separation and Determination of Prochlorperazine Maleate and Pyridoxine Hydrochloride in Tablet Formulation by RP-HPLC", Austin. J. Anal. Pharm. Chem, vol.1, pp.1-5, August 2014

[24] T. Jeyalakshmi, A. Chenthilnathan, and A. Vairamani, "Development and Validation of RP-HPLC Method for Simultaneous Estimation of Vitamins B1, B3, B5 and B6 in Multi Vitamin Injection" Int. J. Pharm. Phytopharmacol. Res, vol.4, pp.89-94, October 2014.

[25] S. Prashanti, M. Aruna, P. Mahesh, and K. Swapna, "Simultaneous estimation of DL-methionine and pyridoxine hydrochloride in tablet dosage form by RP-HPLC", Int. J. Pharm & Chem. Sci, vol.3, pp.105-109, January 2014.

[26] G. Nagamallika, and M.A. Devi, "A validated stability indicating RP-UPLC method for simultaneous determination of water-soluble vitamins, caffeine and preservatives in pharmaceutical formulations", Int. J. Res. Pharm & Chem, vol.3, pp.456-463, August 2013.

[27] K. reema, V. Itishree, N. Shantaram, and G. Jagadish, "Method development and validation for the simultaneous estimation of b-group vitamins and atorvastatin in pharmaceutical solid dosage form by RP-HPLC", Int. J. Pharm. Chem & Biolog. Sci, vol.3, pp.330-335, 2013.

[28] S.C. Nayak, P.V. Kulkarni, V. Bhaskar, and V. Chavhan, "Development and validation of UV spectrophotometric method for simultaneous estimation of doxylamine succinate and pyridoxine hydrochloride in bulk and tablet dosage form", Int. J. Pharm. Sci, vol.5, pp. 390-393, January 2013.

[29] M.L. Marszall, A. Lebiedzinska, W. Czarnowski, and P. Szefer, "High-performance liquid chromatography method for the simultaneous determination of thiamine hydrochloride, pyridoxine hydrochloride and cyanocobalamin in pharmaceutical formulations using coulometric electrochemical and ultraviolet detection", J. Chromatogr. A, vol.1094, pp.91-98, November 2005.