Acta Scientific Pharmaceutical Sciences

Volume 2 Issue 3 March 2018

Research Article

Development and Validation of First Derivative Spectroscopic Method for Simultaneous Estimation of Pyridoxine Hydrochloride with Total and Discrete Citrate in Formulations Used for the Prevention and Recurrence of Nephrolith

Kajal Gandhi and Rajashree Mashru*

Faculty of Pharmacy, The Maharaja Sayajirao University of Baroda, Vadodara, India

***Corresponding Author:** Rajashree Mashru, Faculty of Pharmacy, The Maharaja Sayajirao University of Baroda, Vadodara, India.

Received: December 30, 2017; **Published:** February 24, 2018

Abstract

Present study deals with the standardization of formulations containing potassium magnesium citrate with Vit.B6. It is used to prevent kidney stones. It is freely available over the counter. So, its estimation becomes important. As it contains inorganic ions as well as Vit.B6, standardization was done using different methods. For estimation of Total Citrate and Vit.B6 simple, rapid and accurate new method is described as Vit.B6 interferes in citrate determination by already reported methods. This method was characterized by excellent precision and accuracy; the coefficient of variation in each case is below the maximal permissible value (%RSD < 2). The assay of the method was in good agreement with standards and enables the method to be adapted for routine analysis of the both substances in combined dosage forms. For estimation of magnesium reported colorimetric method by titan yellow was used as it gives results in acceptance limit ensuring that interference of others does not affect and passes validation. For estimation discrete citrate and potassium ion mathematical back calculation was used as it gave satisfactory results. Hence, proposed methods for standardization of kidney stone formulations were successfully applied on marketed products and found to be simple, rapid and accurate.

Keywords: Pyridoxine Hydrochloride; Total Citrate; Discrete Citrate; Nephrolith Formulations

Abbreviations

PYR: Pyridoxine Hydrochloride; CA: Citric Acid; Vit.B6: Vitamin B6; Conc.: Concentration; Mg: Magnesium; Pot: Potassium; HCl: Hydrochloride; D1: First Derivative; SD: Standard Deviation; Abs.: Absorbance; RSD: Relative Standard Deviation

Introduction

Nephrolith is a medical term for kidney stones. This is one of the oldest and most common problems of urinary system. In this condition a hard, crystalline mineral material forms within the kidney and urinary track. Almost 9in 100 people are expected to get kidney stone in their lifetime. Treatment of this depends on the type of stone, its position and duration in body [1]. Potassium-magnesium citrate successfully avoids repetitive calcium oxalate stones. Potassium Magnesium citrate is a nutritious supplement and battles kidney stones in various ways. Every particle has its own particular move against kidney stones. The potassium gave by the supplement raises the pH of the pee, making it more basic, in this manner diminishing the measure of calcium that the pee can break down. At the point when there is less calcium in the pee, less calcium kidney stones can frame. The magnesium in the supplement lessens the measure of calcium the kidneys draw out of the body and into the pee. What's more, the citrate in the supplement keeps oxalates broke down in the pee so they are flushed away before they can shape kidney stones [2]. Vitamin B6 insufficiency is one of the reasons for stone formation in any case. Thus, potassium magnesium citrate with Vit.B6 turns out to be more successful against kidney stones [3].

Formulation containing potassium magnesium citrate with Vit. B6 used for prevention and recurrence of nephrolith can be estimated in three stages.

1. Simultaneous estimation of PYR (Vit.B6) and total citrate
2. Determination of Magnesium content by colorimetric method using Titan yellow and
3. Determination of potassium content by back calculation method.

Several analytical methods have been reported for the determination of Vit.B6 or Citric acid/citrate either alone or in combination with other drugs or ions. As per I.P., PYR in formulation is evaluated by UV spectroscopy using absorptivity value [4]. CA is also official in U.S.P [5]. There is no published literature dealing with simultaneous quantification of PYR and CA in bulk material and pharmaceutical preparations. Therefore, the objective of this study was to develop a simple first ordered derivatized simultaneous UV spectroscopic method for rapid and accurate estimation of individual drug content from the formulation.

Other components of formulation like magnesium can be estimated by reported colorimetric method which includes use of Titan yellow [6,7]. Basis of this method is that magnesium in presence of Titan yellow (or Clayton yellow) produces deep orange to red color. Alkaline environment is provided by addition of 4N sodium hydroxide solution along with 0.1% PVA solution to stabilize the coloration and/or precipitation [8].

Potassium content of formulation can be evaluated by various colorimetric methods [9-11] and by flame photometry [12], however, to save time and making estimation economic it can likewise be figured by the back-computation strategy as different parts are assessed effectively by said methods.

Thus, the present work deals with the UV spectrophotometric technique and mathematical calculations for the estimation/standardization of formulations containing potassium magnesium citrate with Vit.B6.

Experimental Section

Whole experiment is divided in four parts:

- Determination of Pyridoxine Hydrochloride (Vit.B6) and Total Citrate by first ordered derivatised simultaneous equation method.
- Colorimetric determination of Magnesium by Titan Yellow method.
- Mathematical determination of Potassium and Discrete Citrate by using back calculation method.
- Standardization of Formulations.

Instrumentation

UV-VIS spectrophotometer, UV-1700 pharmaspec model, from Shimadzu, Japan. This spectrophotometer is adequate and equipped with quartz cuvettes having the optical path of 10 mm.

Materials and Reagents

All materials and reagents were of analytical-reagent grade. Formulations (Ston1B6, Kmac B6, Alkamax-MB6, Potrate-MB6, Alkaston-B6 and Noculi B6) used for analysis were purchased from local market.

Determination of pyridoxine hydrochloride (Vit.B6) and total citrate by first ordered derivatised simultaneous equation method

Preparation of Standard stock and working standard solutions

For preparation of Standard Citrate Solution various active ingredients such as magnesium citrate, potassium citrate, tri Potassium citrate and citric acid were tried. Citric acid being the representative of the whole citrate group and also gave optimal results in the experimental range was selected as a Standard Citrate Solution.

Accurately about 100 mg of Pyridoxine hydrochloride (PYR) and Anhydrous Citric acid (CA) were weighed and dissolved in 0.3N HCl in two separate 100 ml volumetric flasks. The volume was made up to 100 ml with 0.3N HCl to obtain standard stock solutions (1000 µg/ml). For CA standard stock solution was used as working stock solution of CA. For PYR 4 ml solution was withdrawn from standard stock solution and diluted up to 100 ml with 0.3N HCl (40 µg/ml) and used as working stock solution of PYR.

Selection of Analytical wavelength

Working standard solutions were scanned separately in the range of 200 - 400 nm. The overlain zero order spectra of PYR and CA (Figure 1) showed that at absorption maxima of CA, PYR exhibits substantial absorbance. This clearly indicates the existence of spectral interference in estimation of PYR and CA. To overcome this, spectra of both drugs were derivatised to first order between 200 - 400 nm with $\Delta\lambda$ = 5 nm and scaling factor = 1 and Simultaneous equations on derivative spectra were derived at selected wavelengths. The overlain first derivative spectra of PYR and CA (Figure 2) revealed that PYR concentration was proportional to the first derivative signals at 302.5 nm and CA can be estimated at 227.5 nm.

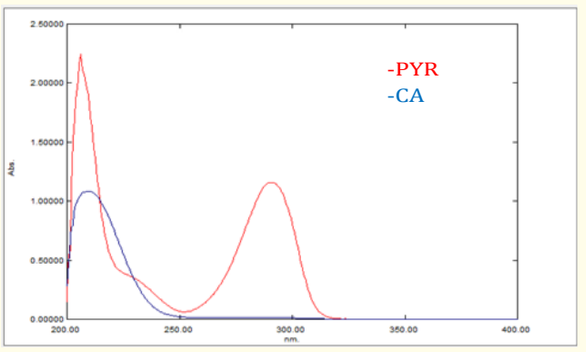


Figure 1: Overlain zero order spectra of PYR and CA.

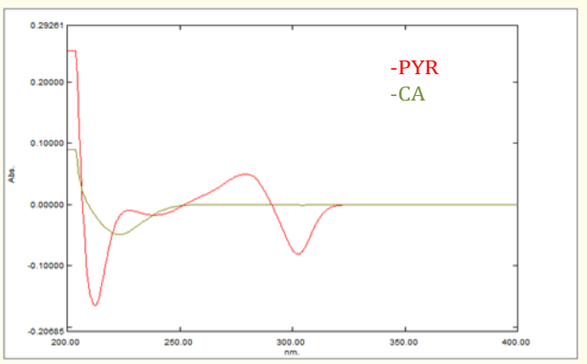
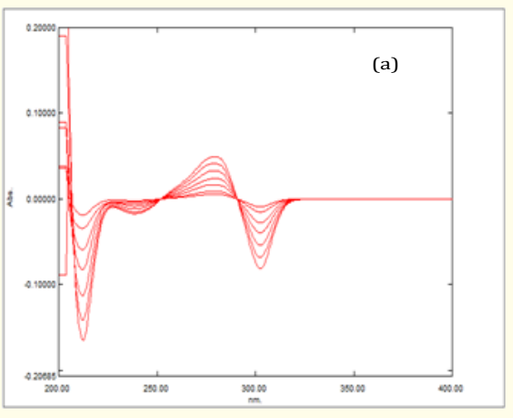


Figure 2: Overlain first derivative spectra of PYR and CA.

Preparation of Calibration curve

From working stock solutions appropriate dilutions were made to obtain concentration range of 2, 4, 8, 12, 16, 20 and 24 µg/ml for PYR and 50, 100, 200, 400, 600, 800 and 1000 µg/ml for CA. the spectra were recorded and derivatised to first ordered, absorbance were measured at 302.5 nm and 227.5 nm for PYR and CA respectively and calibration curves were plotted. First derivative spectra of both Calibration curves (PYR and CA) showed in figure 3.



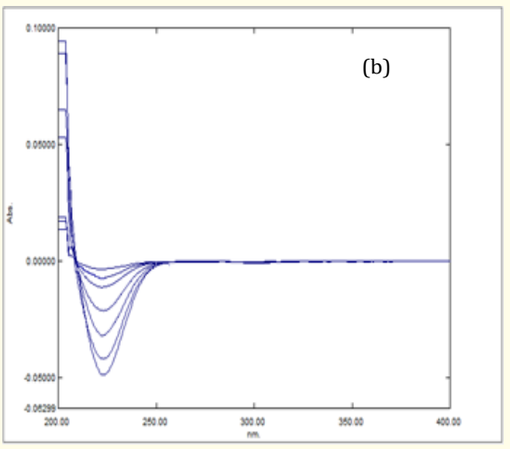


Figure 3: (a) First ordered derivatised Calibration curve of PYR. (b) First ordered derivatised Calibration curve of CA.

Sample preparation

For Tablets, average weight of 20 tablets was determined. Powder equivalent to 661.85 mg citrate ion (CA) and 15 mg PYR was weighed accurately and transferred to 100 ml volumetric flask. The drugs were extracted into 20 ml of 0.3N HCl by sonication, filtered and the residue was washed with 0.3N HCl and the volume was adjusted to 100 ml with 0.3N HCL. From this, further appropriate dilutions were made for estimation of drug content.

For Syrups, syrup equivalent to 945.49 mg citrate ion (CA) and 20 mg PYR was pipetted out and transferred to 50 ml volumetric flask and was diluted to 20 ml with 0.3N HCl. It was mixed well by sonication and volume was made up to 50 ml with 0.3N HCl. From this, further appropriate dilutions were made for estimation of drug content.

Simultaneous Equation

The stock solutions of both drugs were further diluted separately with 0.3N HCL to get a series of standard solutions of 2 - 24 µg/ml concentration of PYR and 50 - 1000 µg/ml concentration of CA and scanned between 200 - 400 nm. The obtained spectra of the above solutions were derivatised to first order and absorbances as well as absorptivity values of all solutions were measured at both the wavelengths 302.5 nm and 227.5 nm respectively and substituted in the following equation:

$$C_X = (DA_2 da_{y1} - DA_1 da_{y2}) / (da_{x2} da_{y1} - da_{x1} da_{y2})$$

$$C_Y = (DA_1 da_{x2} - DA2 da_{x1}) / (da_{x2} da_{y1} - da_{x1} da_{y2})$$

Where, C_X = concentration of PYR. C_Y = concentration of CA.

DA_1=first derivatised absorbance of samples at 302.5 nm.

DA_2= first derivatised absorbance of samples at 227.5 nm.

da_{x1} is the first derivatised absorptivity of PYR at 302.5 nm.

da_{x2} is the first derivatised absorptivity of PYR at 227.5 nm.

da_{y1} is the first derivatised absorptivity of CA at 302.5 nm.

da_{y2} is the first derivatised absorptivity of CA at 227.5 nm.

Method Validation

The proposed method was validated according to the International Conference on Harmonization (ICH) guidelines [13].

Linearity

The calibration curves were constructed with concentrations ranging from 2 - 24 µg/ml for PYR and 50 - 1000 µg/ml for CA. The first derivatised absorbance of the drug was considered for plotting the graph (Figure 5). The linearity was evaluated by linear regression analysis which was calculated by the least square regression method.

Precision

The measures of precision, i.e. repeatability (intra-day precision) and intermediate precision (inter-day precision), were determined for three standard solutions. For each concentration, the absorbance measurement was repeated six times (n = 6) on the same day (repeatability examination). This procedure was continued over three consecutive days (intermediate precision examination).

Accuracy

The accuracy of the method was determined through percentage recovery studies. The pre-analyzed sample was spiked with three different levels (80%, 100% and 120%) of reference standard solution and the absorbance was measured in triplicate (n = 3). The percentage recovery was calculated individually for both drugs.

Limit of Detection and Quantification

The Limit of detection (LOD) and Limit of Quantification (LOQ) were calculated from standard deviation of response and slope.

Assay

The proposed method was applied to the determination of PYR and CA in commercial products. Each product was prepared by procedure given in sample preparation and analyzed in six replications (n = 6).

Colorimetric determination of magnesium by titan yellow method [6,7]

Materials and Reagents

1. P.V.A solution (0.1% W/V): 1g of polyvinyl alcohol is stirred into cold water and dissolved by gentle heating and stirring. The solution is then made up to 1000 ml and stored for use.
2. Titan Yellow solution (0.05% W/V): 100 mg dissolved in water, made to 200 ml and filtered. The solution should be kept in a brown bottle and should be freshly prepared about every fortnight.
3. Sodium Hydroxide: 4N.

Preparation of Standard stock and working standard solution

Standard stock solution of Magnesium: 10.131 g $MgSO_4.7H_2O$ are dissolved in distilled water, 0.5 ml chloroform added, and the volume made to 1 l with distilled water (1 ml standard stock solution contains 1 mg Mg).

Working standard solutions: 2 ml of standard stock solution is diluted to 100 ml (1 ml working standard contains 0.02 mg Mg).

Sample Preparation

For tablets, average weight of 20 tablets was determined. Powder equivalent to 42.53 mg Magnesium Ion was weighed accurately and transferred to 100 ml volumetric flask. Mg was extracted into 20 ml of distilled water by sonication, filtered and the residue was washed with distilled water and the volume was adjusted to 100 ml with distilled water. From this, further appropriate dilutions were made for estimation of Mg content.

For Syrups, syrup equivalent to 7.29 mg Magnesium Ion was pipetted out and transferred to 50 ml volumetric flask and was diluted to 20 ml with distilled water. It was mixed well by sonication and volume was made up to 50 ml with distilled water. From this, further appropriate dilutions were made for estimation of drug content.

Procedure

In 10 ml volumetric flasks, appropriate aliquot of working standard for calibration curve ranging from (0.2 - 6.4 µg/ml) were taken and 4 ml distilled water, 1 ml 0.1% PVA, 1.5 ml Titan yellow, 2 ml NaOH were added. The volume was made up to 10 ml with distilled water.

A blank solution was prepared by taking 5 ml distilled water, adding 1 ml 0.1% PVA, 1.5 ml Titan yellow, 2 ml NaOH and volume was made up to 10 ml with distilled water.

For sample estimation, appropriate aliquots from sample solutions were withdrawn and reagents were added as per standard solutions.

Solutions were scanned separately in the range of400-800 nm on the UV-Vis spectrophotometer. Maximum absorbance obtained at 541 nm (Figure 4).

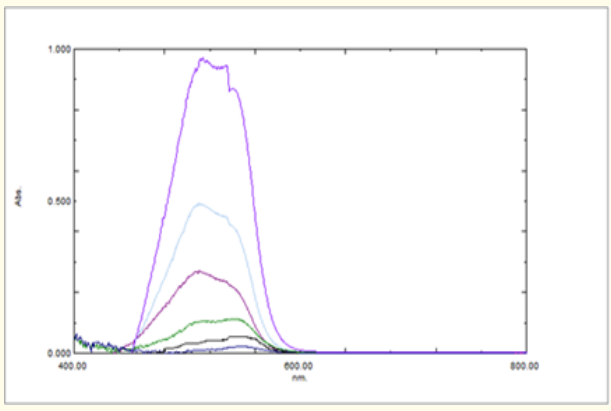


Figure 4: Calibration curve for Magnesium Ion.

Method Validation

The proposed method was validated according to the International Conference on Harmonization (ICH) guidelines [13].

Linearity

The calibration curve was constructed with concentrations ranging from 0.2 - 6.4 µg/ml for Magnesium ion. The absorbance of Magnesium ion against Concentration was considered for plotting the graph (Figure 6). The linearity was evaluated by linear regression analysis which was calculated by the least square regression method.

Precision

The measures of precision, i.e. repeatability (intra-day precision) and intermediate precision (inter-day precision), were determined for three standard solutions. For each concentration, the absorbance measurement was repeated six times (n = 6) on the same day (repeatability examination). This procedure was continued over three consecutive days (intermediate precision examination).

Accuracy

The accuracy of the method was determined through percentage recovery studies. The pre-analyzed sample was spiked with three different levels (80%, 100% and 120%) of reference standard solution and the absorbance was measured in triplicate (n = 3). The percentage recovery was calculated.

Limit of Detection and Quantification

The Limit of detection (LOD) and Limit of Quantification (LOQ) were calculated from standard deviation of response and slope.

Assay

The proposed method was applied to the determination of Magnesium ion in formulations. Each product was prepared by procedure given in sample preparation and analyzed in six replications (n = 6).

Mathematical determination of potassium and discrete citrate by using back calculation method

In this mathematical method, any ion, atom/molecule from compound can be calculated by using label claim and molecular weight.

Back calculation method for Discrete Citrate

Here, Formulations contain two types of Discrete Citrates: Citrate of Magnesium and Citrate of Potassium. Both were calculated by using the calculation method. The Concentration of both discrete citrates calculated in µg/ml by using molecular weight and Concentration of Total Citrate (Obtained as per developed method (Part.1)). Hence, the concentrations of discrete citrates can be calculated (Table 13 and 14) by using the following formulas:

Conc. of Citrate (Mg) = (Obtained conc. of Mg * Labeled conc. of citrate (Mg))/(Labeled conc. of Mg)

Conc. of Citrate (pot.) =Obtained conc. of total citrate – Obtained conc. of citrate (Mg)

Back calculation method for Potassium

Here, Potassium ion from Potassium citrate was calculated by using the calculation method. The concentration of Potassium citrate and the concentration of Potassium ion as per claim calculated in µg/ml by using molecular weight. Hence, the concentration and of Potassium ions can be calculated by using the following formulas and % assay values were also obtained (Table 15):

Conc. of pot. = Obtained conc. of potassium citrate – Obtained conc. of citrate (pot).

Where, Obtained conc. of potassium citrate = (Obtained conc. of citrate (pot).* Labeled conc. of pot.citrate)/(Labeled conc. of citrate (pot).)

Standardization of formulations by back calculation method

Here, Formulations used for nephrolith mainly contain Potassium Citrate, Magnesium Citrate and Pyridoxine Hydrochloride (Vit. B6). Concentrations of these were obtained by different methods mentioned in chart 1 and their % assay values were also obtained (Table 16, 17 and 18).

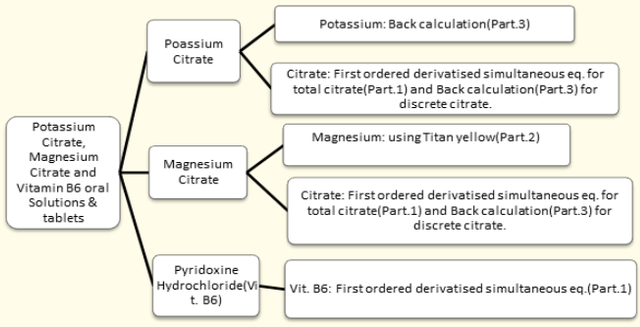


Chart 1: Methods used for standardization of Formulations.

Results and Discussion

Determination of pyridoxine hydrochloride (Vit.B6) and total citrate by first ordered derivatised simultaneous equation method

Simultaneous Equation

Simultaneous equations were developed in order to determine conc. Of PYR and CA. upon substitution of Absorptivity values, the equations were further simplified to:

Concentration of PYR in g/100 ml = (DA_2 (-0.0000017) – DA_1 (0.00005))/0.00000018065 (Equation 1)

Concentration of CA in g/ 100 ml = (DA_1(-0.00050) - DA_2 (-0.00363))/0.00000018065 (Equation 2)

Where, DA_1 is the first derivatised absorbance of samples at 302.5 nm. DA_2 is the first derivatised absorbance of samples at 227.5 nm.

Method Validation

Linearity

The linearity of calibration curves in pure solution was checked over the concentration range of about 2 - 24 µg/ml for PYR and 50 - 1000 µg/ml for CA (Figure 5). The represented data was shown in table 1.

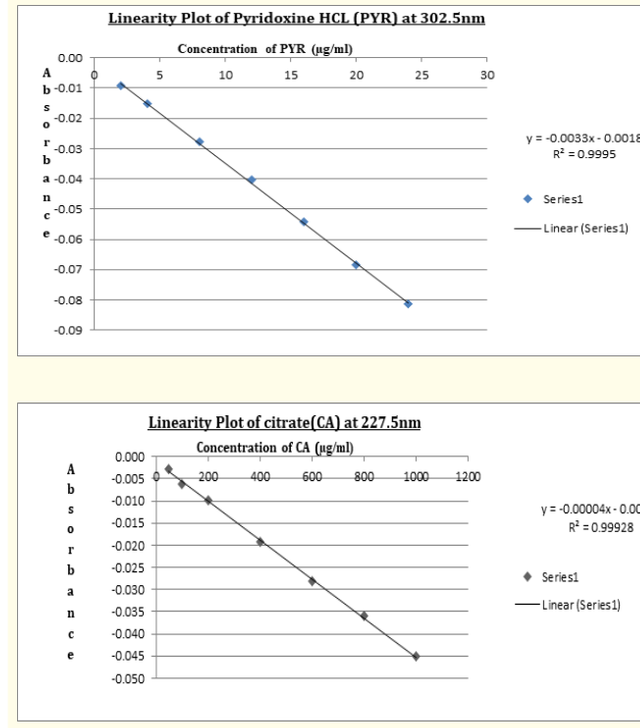


Figure 5: Linearity plots of PYR and CA.

Parameters	PYR (D^1)	CA (D^1)
λ_{max}, nm	302.5	227.5
Calibration range, µg/ml	2 - 24	50 - 1000
Regression Equation (y = bx + c)	y = -0.0033x – 0.0018	y = -0.00004x – 0.00128
Slope (b ± SD)	0.0033 ± 5.8 × 10^{-6}	0.00004 ± 1×10^{-7}
Intercept (c ± SD)	0.0018 ± 4.4 × 10^{-5}	0.00128 ± 3.5×10^{-5}
Regression Coefficient (r^2)	0.9995	0.9993
LOD, µg/ml	0.044	2.64
LOQ, µg/ml	0.132	7.99

Table 1: Characteristic Parameters for the regression equations of the proposed UV method for determination of PYR and CA.

Precision

The results obtained from the intra-day and inter-day precision study were less than 2% (i.e. in the range of 0.079 - 0.962 and 0.084 - 0.754% for intra and inter day for proposed methods, respectively) indicating that the proposed methods were sufficiently precise for the analysis of drug. The results are summarized in table 2.

Conc. (µg/ml)	Intraday precision		Inter day precision	
	Abs. (mean ± SD) n = 6	% RSD	Abs. (mean ± SD) n = 6	% RSD
PYR:				
2	$-0.00911 \pm 3.88 \times 10^{-5}$	0.426	$-0.00914 \pm 3.39 \times 10^{-5}$	0.371
12	$-0.04042 \pm 5.35 \times 10^{-5}$	0.132	$-0.04042 \pm 6.19 \times 10^{-5}$	0.153
24	$-0.08134 \pm 6.39 \times 10^{-5}$	0.079	$-0.08137 \pm 6.83 \times 10^{-5}$	0.084
CA:				
50	$-0.00289 \pm 2.79 \times 10^{-5}$	0.962	$-0.00288 \pm 2.17 \times 10^{-5}$	0.754
400	$-0.01914 \pm 5.75 \times 10^{-5}$	0.301	$-0.01913 \pm 5.18 \times 10^{-5}$	0.271
1000	$-0.04510 \pm 5.32 \times 10^{-5}$	0.118	$-0.04510 \pm 6.74 \times 10^{-5}$	0.149

Table 2: Precision studies of PYR and CA.

Accuracy

The accuracy of the method was determined by calculating percentage recovery of three levels in triplicate. The results are shown in table 3. The result lies within the prescribed limit of 98 - 102%, showing that both methods are free from interference from excipients.

LOD and LOQ

From the standard deviation (SD) of response and slope curve, it was possible to calculate the detection and quantification limits. The LOD and LOQ for both drugs were shown in table 4. These low values indicated the good sensitivity of the methods proposed.

Sr. no	Sample Name	Primary Conc. (µg/ml)	Enrichment (%)	PYR		CA	
				% Recovery (mean ± SD) n = 6	% RSD	% Recovery (mean ± SD) n = 6	% RSD
1	Ston1B6	198.66	80	100.60 ± 0.34	0.334	99.12 ± 0.41	0.413
			100	100.81 ± 0.74	0.735	98.18 ± 0.47	0.475
			120	100.82 ± 0.57	0.561	99.14 ± 0.62	0.623
2	KmacB6	198.66	80	99.30 ± 0.13	0.132	99.48 ± 0.88	0.884
			100	98.99 ± 0.35	0.349	100.15 ± 0.79	0.790
			120	99.01 ± 0.05	0.053	99.12 ± 0.24	0.239
3	Alkamax-MB6	198.66	80	99.65 ± 0.18	0.179	100.52 ± 0.42	0.419
			100	100.53 ± 0.32	0.314	99.08 ± 0.46	0.466
			120	99.95 ± 0.14	0.140	100.48 ± 0.23	0.232
4	Potrate-MB6	264.75	80	99.51 ± 0.45	0.454	100.31 ± 0.25	0.253
			100	100.21 ± 0.38	0.382	100.58 ± 0.17	0.168
			120	100.58 ± 0.23	0.225	99.02 ± 0.27	0.268
5	Alkaston-B6	198.66	80	100.64 ± 0.52	0.515	100.21 ± 0.25	0.246
			100	101.03 ± 0.47	0.467	100.08 ± 0.31	0.305
			120	99.60 ± 0.31	0.315	101.04 ± 0.23	0.232
6	NoculiB6	198.66	80	100.12 ± 0.34	0.336	99.22 ± 0.59	0.596
			100	99.11 ± 0.54	0.546	100.28 ± 0.29	0.285
			120	100.29 ± 0.37	0.372	99.04 ± 0.26	0.259

Table 3: Recovery studies of PYR and CA.

	Intercept (mean ± SD) n = 3	Slope (mean ± SD) n = 3	LOD (µg/ml)	LOQ (µg/ml)
PYR	0.0018 ± 4.4 × 10^{-5}	0.0033 ± 5.8 × 10^{-6}	0.0436	0.1322
CA	0.00128 ± 3.5×10^{-5}	0.00004 ± 1×10^{-7}	2.6399	7.9997

Table 4: LOD and LOQ for proposed method.

Assay

The proposed method was applied to the determination of PYR and CA in commercially available marketed products. Satisfactory results were obtained for both drugs and were in a good agreement with the label claims and indicate that there is no interference from the excipients used in the formulation (Table 5 and 6).

Sr. no.	Sample Name	PYR			
		Label Claim (mg)	Conc. found (mean ± SD) n = 6, (mg)	% Assay (mean ± SD) n = 6	% RSD
1	Ston1B6	20	21.11 ± 0.03	105.56 ± 0.17	0.162
2	KmacB6	20	20.10 ± 0.02	100.50 ± 0.12	0.116
3	Alkamax-MB6	20	20.41 ± 0.03	102.03 ± 0.17	0.167
4	Potrate-MB6	15	14.48 ± 0.01	96.56 ± 0.08	0.086
5	Alkaston-B6	20	20.12 ± 0.03	100.62 ± 0.15	0.150
6	NoculiB6	20	19.88 ± 0.04	99.40 ± 0.19	0.194

Table 5: Assay of PYR.

Sr. no.	Sample Name	CA			
		Label Claim (mg)	Conc. found (mean ± SD) n = 6, (mg)	% Assay (mean ± SD) n = 6	% RSD
1	Ston1B6	993.31	1043.29 ± 3.80	105.03 ± 0.38	0.364
2	KmacB6	993.31	1017.75 ± 2.64	102.46 ± 0.27	0.260
3	Alkamax-MB6	993.31	1000.61 ± 2.43	100.73 ± 0.24	0.242
4	Potrate-MB6	661.87	686.22 ± 2.32	103.68 ± 0.35	0.338
5	Alkas-ton-B6	993.31	962.27 ± 8.61	96.87 ± 0.87	0.891
6	NoculiB6	993.31	978.12 ± 3.33	98.47 ± 0.34	0.340

Table 6: Assay of CA.

Statistical comparison of developed method for PYR with Pharmacopoeial (IP) method

The results obtained by using proposed method were statistically compared with the pharmacopoeial method (IP method) [4] using student t- and F- test (at 95% confidence level). The results (Table 7) show that the calculated t- and F- values were less than the tabulated ones, indicating no significant difference between the proposed and reported methods.

	Mean of % Assay (n = 6)	Mean SD	t- test	F- test
Developed method	100.78	2.97	0.1197	1.3618
IP method	100.11	3.47		
Tabulated t- value at 95% confidence level:				2.228
Tabulated F- value at 95% confidence level:				5.05
Degree of Freedom				10

Table 7: Statistical comparison between results of proposed and pharmacopoeial method.

Colorimetric determination of magnesium by titan yellow method

Method Validation

Linearity

Standard solutions of Mg were prepared, and the absorbance was measured at 541 nm (Figure 6). The linear regression parameters for Mg are summarized in table 8.

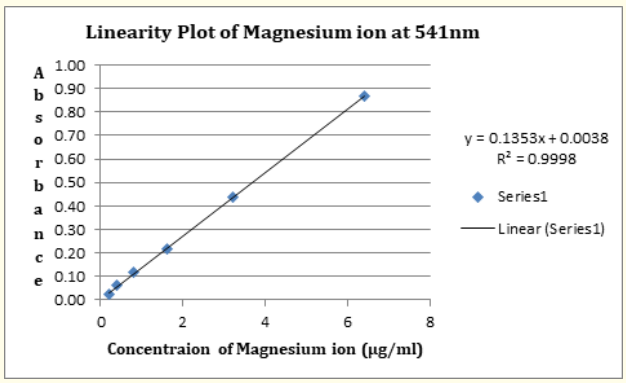


Figure 6: Linearity plot of Magnesium Ion.

Parameters	Mg
λ_{max}, nm	541
Calibration range, µg/ml	0.2 - 6.4
Regression Equation (y = bx + c)	y = 0.1353x + 0.0038
Slope (b ± SD)	$0.1353 \pm 2.1 \times 10^{-4}$
Intercept (c ± SD)	$0.0038 \pm 4.0 \times 10^{-4}$
Regression Coefficient (r^2)	0.9998
LOD, µg/ml	0.00986
LOQ, µg/ml	0.02988

Table 8: Characteristic Parameters for regression equations for determination of Mg.

Precision

The repeatability and intermediate precision are expressed in % RSD which was obtained < 2%. The results are summarized in table 9.

Conc. of Mg (µg/ml)	Intraday precision		Inter day precision	
	Abs. (mean ± D) n = 6	% RSD	Abs. (mean ± SD) n = 6	% RSD
0.2	$0.02459 \pm 3.1 \times 10^{-4}$	1.240	$0.02466 \pm 2.1 \times 10^{-4}$	0.883
1.6	$0.21582 \pm 5.6 \times 10^{-4}$	0.259	$0.21629 \pm 9.6 \times 10^{-4}$	0.445
6.4	$0.87024 \pm 1.8 \times 10^{-3}$	0.207	$0.87045 \pm 6.0 \times 10^{-5}$	0.007

Table 9: Precision studies of Mg.

Accuracy

Sr. no.	Sample Name	Primary Conc. (µg/ml)	Enrich-ment (%)	Magnesium	
				% Recovery (mean ± SD) n = 6	% RSD
1	Ston1B6	1.45	80	102.06 ± 0.25	0.243
			100	99.95 ± 0.26	0.262
			120	99.88 ± 0.15	0.152
2	KmacB6	1.45	80	100.61 ± 0.25	0.248
			100	101.70 ± 0.16	0.160
			120	101.86 ± 0.17	0.169
3	Alkamax-MB6	1.45	80	99.16 ± 0.18	0.178
			100	100.24 ± 0.23	0.224
			120	101.03 ± 0.13	0.130
4	Potrate-MB6	1.0	80	98.99 ± 0.16	0.160
			100	100.22 ± 0.30	0.294
			120	99.60 ± 0.18	0.183
5	Alkas-ton-B6	1.45	80	98.20 ± 0.15	0.149
			100	98.20 ± 0.31	0.133
			120	100.60 ± 0.14	0.141
6	NoculiB6	1.45	80	101.25 ± 0.21	0.208
			100	100.84 ± 0.13	0.125
			120	101.70 ± 0.14	0.135

Table 10: Recovery studies of Mg.

LOD and LOQ

	Intercept (mean ± SD) n = 3	Slope (mean ± SD) n = 3	LOD (µg/ml)	LOQ (µg/ml)
Mg	$0.0038 \pm 4.0 \times 10^{-4}$	$0.1353 \pm 2.1 \times 10^{-4}$	0.00986	0.02988

Table 11: LOD and LOQ for Mg determination.

Assay

Sr no.	Sample	Magnesium			
		Label Claim (mg)	Conc. found (mean ± SD) n = 6, (mg)	% Assay (mean ± SD) n = 6	% RSD
1	Ston1B6	60.61	65.57 ± 0.02	108.19 ± 0.20	0.036
2	KmacB6	60.61	55.68 ± 0.04	91.86 ± 0.32	0.069
3	Alkamax-MB6	60.61	62.50 ± 0.01	103.11 ± 0.10	0.020
4	Potrate-MB6	42.53	43.40 ± 0.38	102.07 ± 0.89	0.875
5	Alkaston-B6	60.61	57.97 ± 0.02	95.64 ± 0.13	0.026
6	NoculiB6	60.61	60.15 ± 0.05	99.23 ± 0.38	0.076

Table 12: Assay of Mg.

Mathematical determination of potassium and discrete citrate by using back calculation method

Assay of discrete citrates

Sr no.	Sample	Discrete Citrate of Magnesium			
		Label Claim (mg)	Conc. found (mean ± SD) n = 6, (mg)	% Assay (mean ± SD) n = 6	% RSD
1	Ston1B6	314.39	340.14 ± 0.62	108.19 ± 0.20	0.1822
2	KmacB6	314.39	288.81 ± 0.99	91.86 ± 0.32	0.3438
3	Alkamax-MB6	314.39	324.20 ± 0.33	103.11 ± 0.10	0.1013
4	Potrate-MB6	220.57	225.10 ± 1.97	102.07 ± 0.89	0.8747
5	Alkaston-B6	314.39	300.71 ± 0.39	95.64 ± 0.13	0.1313
6	NoculiB6	314.39	311.99 ± 1.18	99.23 ± 0.38	0.381

Table 13: Assay of discrete citrate (Mg).

Sr. no.	Sample	Discrete Citrate of Potassium			
		Label Claim (mg)	Conc. found (mean ± SD) n = 6, (mg)	% Assay (mean ± SD) n = 6	% RSD
1	Ston1B6	678.92	703.15 ± 3.5	103.57 ± 0.52	0.497
2	KmacB6	678.92	728.94 ± 2.42	107.37 ± 0.36	0.332
3	Alkamax-MB6	678.92	676.41 ± 2.21	99.63 ± 0.32	0.328
4	Potrate-MB6	441.3	461.12 ± 3.40	104.49 ± 0.77	0.738
5	Alkaston-B6	678.92	661.56 ± 8.85	97.44 ± 1.30	1.338
6	NoculiB6	678.92	666.14 ± 3.40	98.12 ± 0.50	0.511

Table 14: Assay of discrete citrate (pot).

Assay of Potassium

Sr. no.	Sample	Potassium			
		Label Claim (mg)	Conc. found (mean ± SD) n = 6, (mg)	% Assay (mean ± SD) n = 6	% RSD
1	Ston1B6	421.08	436.11 ± 2.17	103.57 ± 0.52	0.497
2	KmacB6	421.08	452.10 ± 1.5	107.37 ± 0.36	0.332
3	Alkamax-MB6	421.08	419.52 ± 1.38	99.63 ± 0.32	0.328
4	Potrate-MB6	273.6	285.89 ± 2.11	104.49 ± 0.77	0.738
5	Alkaston-B6	421.08	410.31 ± 5.49	97.44 ± 1.30	1.338
6	NoculiB6	421.08	413.15 ± 2.11	98.12 ± 0.50	0.511

Table 15: Assay of potassium.

Standardization of formulations

Assay of formulations

Sr. no.	Sample Name	Type	Potassium Citrate (%Limit: 95-105) [5]		
			Label Claim (mg)	% Assay (mean ± SD) n = 6	% RSD
1	Ston1B6	Syrup	1100	103.57 ± 0.52	0.498
2	KmacB6	Syrup	1100	101.37 ± 0.36	0.332
3	Alkamax-MB6	Syrup	1100	99.63 ± 0.33	0.328
4	Potrate-MB6	Tablet	714.9	104.49 ± 0.77	0.739
5	Alkaston-B6	Syrup	1100	97.44 ± 1.30	1.338
6	NoculiB6	Syrup	1100	98.12 ± 0.50	0.511

Table 16: Assay of Potassium citrate.

Sr. no.	Sample Name	Type	Magnesium Citrate (%Limit: 90-110) [5]		
			Label Claim (mg)	% Assay (mean ± SD) n = 6	% RSD
1	Ston1B6	Syrup	375	108.19 ± 0.20	0.182
2	KmacB6	Syrup	375	91.87 ± 0.32	0.344
3	Alkamax-MB6	Syrup	375	103.12 ± 0.10	0.101
4	Potrate-MB6	Tablet	263.1	102.06 ± 0.89	0.875
5	Alkaston-B6	Syrup	375	95.65 ± 0.13	0.131
6	NoculiB6	Syrup	375	99.24 ± 0.38	0.381

Table 17: Assay of Magnesium citrate.

Sr. no.	Sample Name	Type	PYR (%Limit: 90 - 110) [4]		
			Label Claim (mg)	% Assay (mean ± SD) n = 6	% RSD
1	Ston1B6	Syrup	20	105.56 ± 0.17	0.162
2	KmacB6	Syrup	20	100.50 ± 0.12	0.116
3	Alkamax-MB6	Syrup	20	102.03 ± 0.17	0.167
4	Potrate-MB6	Tablet	15	96.56 ± 0.08	0.086
5	Alkaston-B6	Syrup	20	100.62 ± 0.15	0.150
6	NoculiB6	Syrup	20	99.40 ± 0.19	0.194

Table 18: Assay of Pyridoxine Hydrochloride.

Conclusion

The developed and validated analytical method for pyridoxine hydrochloride and total citrate demonstrates good sensitivity and accuracy as there is no difference between the developed and IP method for PYR. This shows comparative pharmacopoeial standard of the developed method. The paper concludes estimation of PYR and total citrate by first derivative simultaneous equation method and discrete citrate by application of mathematical calculation methods used first time for estimation of nephrolith formulations which is applicable to both tablet as well as syrup formulations, is an added advantage.

Bibliography

1. "Kidney stones: A patient guide". Urology Care Foundation: The official foundation of the American Urological Association.
2. Juicing The Rainbow: Juicing for Health with Nature's Bounty. "Potassium Magnesium Citrate and Kidney Stones".
3. Easy Immune System Health. "Preventing Kidney Stones: Just takes understanding the Cause".
4. Indian Pharmacopoeia. Volume 3. New Delhi: Government of India, The Controller of Publications (2007): 1010.

5. The United States Pharmacopoeia XXIX The National Formulary. NF 24. United States. Pharmacopoeial Convention, Washington.

6. Garner R. "Colorimetric determination of magnesium in plasma or serum by means of titan yellow". *Biochemical Journal* 40.5-6 (1946): 828-831.

7. Heaton F. "Determination of magnesium by the Titan yellow and ammonium phosphate methods". *Journal of Clinical Pathology* 13.4 (1960): 358-360.

8. VOGEL's textbook of quantitative chemical analysis, 5th addition, GH Jeffery, J Bassett, J Mendham, RC Denny: 699-701.

9. Abul-Fadl M. "Colorimetric determination of potassium by Folin-Ciocalteu phenol reagent". *Biochemical Journal* 44.3 (1949): 282-285.

10. Schmidt V. "A Quick Method for Colorimetric Determination of Potassium". *Scandinavian Journal of Clinical and Laboratory Investigation* 3.3 (1951): 252-254.

11. Faber R and Dirkse TP. "Colorimetric Determination of Potassium with Dipicrylamine". *Analytical Chemistry* 25.5 (1953): 808-810.

12. Berry JW., *et al*. "Improved method of flame photometry". *Industrial and Engineering Chemistry Analytical Edition* 18.1 (1946): 19-24.

13. International Conference on Harmonisation, ICH Harmonised Tripartite Guideline- Validation of Analytical Procedures: Methodology. Federal Register 62 (1997): 27463.

Volume 2 Issue 3 March 2018