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DETECTION AND ASSAY OF MEDICINAL NICOTINIC ACID UTILIZING ISOCRATIC HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

Ronald Bartzatt*, Alyna Nguyen, Anna Nguyen and Carolyn Fochek

*University of Nebraska Durham Science Center 6001 Dodge Street Omaha, Nebraska 68182 U.S.A.

***Corresponding Author: Ronald Bartzatt**

University of Nebraska Durham Science Center 6001 Dodge Street Omaha, Nebraska 68182 U.S.A.

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ABSTRACT

Medicinal nicotinic acid is identified and assayed by use of high performance liquid chromatography (HPLC) from aqueous based mixtures, these including mixtures: 1) Water; 2) Normal saline (0.9 grams/100 mL) mixtures; and 3) Aqueous mixtures having cellulose and/or starch as tablet excipients. Medicinal nicotinic acid lowers LDL cholesterol, increase HDL cholesterol, and boosts brain function. The standard curve ranged from 3.2854×10^{-5} molar nicotinic acid to 1.36411×10^{-3} molar nicotinic acid (about 42-fold range in concentration). The dead volume for this instrumentation is 1.5 mL. The dead time is 1.5 minutes, with nicotinic acid eluting at 2.6 minutes. The limit of quantitation (LOQ) is 4.5616×10^{-5} molar, with limit of detection (LOD) of 1.3685×10^{-5} molar nicotinic acid. Nicotinic acid was successfully identified from all aqueous samples, those assayed from normal saline mixtures, and aqueous dissolved mixtures having cellulose and starch present as excipients for tablet and capsule pharmaceutical preparations. The assay of nicotinic acid using isocratic high performance liquid chromatography will be useful for the quality control monitoring within pharmaceutical manufacturing, follow and verify patient compliance, confirmation of drug presence in other medicinal formulations, and as an assay method for averting adulterants.

KEYWORDS: Nicotinic acid, HPLC, isocratic, niacin.

INTRODUCTION

Nicotinic acid is an essential human nutrient. It is also known as pyridine-3-carboxylic acid, niacin, bionic, and vitamin B3.[1] It is used to treat and prevent niacin deficiency (known as pellagra), either from diet deficiency, alcohol abuse, malabsorption syndrome, or use of isoniazid.[1] It is also effective and utilized to reduce cholesterol levels, lower your risk of heart disease, lower LDL cholesterol, increase HDL cholesterol, and lower triglycerides.[1] When administered for medicinal purposes it can be available in various different formulations (i.e. for immediate or sustained release).[1]

Similarly, to all B vitamins, nicotinic acid helps convert food into needed energy by aiding the associated enzymes.[1]

Nicotinic acid is a major component of NAD (nicotinamide adenine dinucleotide) and NADP (nicotinamide adenine dinucleotide phosphate), which are two enzymes involved in cellular metabolism.[1] This is in addition to playing a role in cell signaling and the making/repairing of DNA through its action as an antioxidant.[1] This vitamin is distributed in all tissues and is absorbed through all portions of the intestinal tract and from parenteral sites of administration.[1] Only after administration with very high doses of nicotinic acid, does this vitamin appear in the urine unchanged.[1]

Niacin is the generic descriptor for two vitamers, nicotinic acid and nicotinamide, that give rise to coenzymes NAD and NADP.[2] In the central nervous system, this vitamin is known to be a key mediator of neuronal development and survival.[2] Nicotinic acid has had a resurgence of use for the treatment of abnormal blood lipids and pathology associated with that condition.[3] The efficacy of niacin are known to be wide-ranging and studies have shown that beneficial effects to be associated to its activity in cell signaling pathways.[3] Nicotinic acid is the most effective medicinal agent that is presently available for increasing the desirable high density lipoprotein-cholesterol.[3]

Other methodologies for the assay of niacin have been published. These include a method for the fluorimetric determination of niacin in various foods with the use of high performance liquid chromatography with a technique of post-column derivatization.[5] Other methods include a simultaneous determination of niacin, niacinamide, and nicotinuric acid.[6] A double instrument method for assay from various foods utilizes liquid chromatography with isotope dilution mass spectrometry.[7]

The vitamin niacin is delivered in various modalities. Although there have been successful formulation of orally based capsule and tablet delivery systems, patient compliance to niacin therapy is still adversely affected by events such as the induced flushing.[8] Ease of use is still the primary advantage of orally dosed formulations, however, delivery options such as transdermal delivery or polymeric micro-nanoparticle encapsulation for oral administration are showing promise in niacin reformulation.[8] More in-depth investigation is required.[8]

The methodology presented in this study utilizes isocratic high performance liquid chromatography (HPLC) with an aqueous based column solvent. The assay of nicotinic acid using isocratic high performance liquid chromatography will be useful for quality control monitoring, verify patient compliance, and confirmation of drug presence in other medicinal formulations.

MATERIALS AND METHODS

Reagents and instrumentation

All solvents were analytical grade and obtained from Sigma-Aldrich (St. Louis MO 63178 USA). The nicotinic acid compound for use as standards and preparation of samples was obtained from ACROS (New Jersey, USA). The high performance liquid chromatography analysis, utilized an Alltech 426 HPLC Pump and Linear UVS 200 detector with the reversed-phase isocratic conditions for analysis of all mixtures (Deerfield, Illinois 60015-1899USA). The HPLC Alltech instrumentation is controlled by computer interface.

Instrument settings, solvents, samples

For analysis by HPLC, a reversed-phase C-18 octadecylsilyl ($C_{18}H_{37}$) bonded phase column packing was utilized. The nicotinic acid analyte elutes at approximately 2.6 minutes. Detection by ultraviolet detector set to 265 nm, rise time 0.1, range AUFS set to 1.0. The HPLC pump was set to about 1400 psig and one milliliter per minute flow rate. The column utilized here is a 150 mm × 4.6 mm column.

For the mobile phase column solvent the working concentrations were 10% (v/v) ethanol, 1% acetic acid (v/v)(or 0.174 molar acetic acid), and 89% distilled water (v/v). Stock standard of nicotinic acid prepared in distilled water was 3.9425×10^{-2} molar. Solvent used for solubilizing nicotinic acid in various test samples, include; saline solvent type samples (utilized in clinical application for intramuscular or intravenous injection), were nicotinic acid prepared in aqueous solvent as 0.9 grams NaCl per 100 mL water, at various concentrations. Tablet/solid samples were the nicotinic acid prepared in various known amounts and in combinations of the excipients cellulose and starch.

To evaluate assay of nicotinic acid from mass produced commercial tablets, a commercial product of extended release formulation having 500 milligrams of nicotinic acid was dissolved in two liters of distilled water. Then aliquots taken for assay according to the methodology presented in this study.

Mixtures having nicotinic acid with excipients cellulose and/or starch were allowed to settle for 48 hours prior to HPLC analysis. If filtering of mixtures having cellulose and/or starch excipients where necessary, this was accomplished by Whatman 6900-2502 GD/X 25 Sterile Syringe Filter, 25mm, 0.2 Micron, PVDF Filtration Medium, with suitable plastic syringe.

Statistical Analysis and Molecular Properties

The calculation of correlation and 95% ellipses are determined by PAST version 2.06 (copyright Hammer and Harper 1999-2011). Microsoft EXCEL (copyright 2010 Microsoft Corporation, Microsoft Office Professional Plus 2010) and PAST v. 2.06 were used for summary statistical analysis. The determination of linearity for the standard curve, correlation coefficient, and standard deviation of residuals was accomplished by GraphPad Instat version 3.06 (copyright © 1992-2003 by GraphPad software, Inc).

Molecular properties of nicotinic acid were determined utilizing Molinspiration Cheminformatics http://www.molinspiration.com/ (Molinspiration Cheminformatics, Nova ulica, SK-900 26 Slovensky Grob, Slovak Republic). Outliers were identified using Grubb's test (GraphPad QuickCalcs, https: //www.graphpad. com/ quickcalcs/).

RESULTS AND DISCUSSION

Nicotinic acid, or niacin, is an essential vitamin that has attributed to it a number of important uses [1]: 1) Lowers LDL cholesterol (Niacin has been used since the 1950s to treat high cholesterol); 2) Increases HDL Cholesterol (raises HDL levels by 15–35%); 3) Lowers triglycerides; 4) Reduce risk to heart disease; 5) Reduce risk of Type 1 Diabetes; 6) Boost brain function; 7) Improve skin function; 8) Treat pellagra. These and other reasons are sufficient to continue the biological study of this vitamin and pursue methods of assay from various compositions.

The form of this vitamin that incurs the desired medicinal activity is shown in Fig. 1. The molecule has a Log P value of 0.27 and polar surface area of 50.19 Angstroms^2, and has water soluble at one gram in 60 mL of water.[1] Injections of this vitamin, when required, are prepared in aqueous solution.[1] The molecular structure contains a pyridine ring and a carboxylic acid functional group (-C(=O)OH) (see Fig. 1).

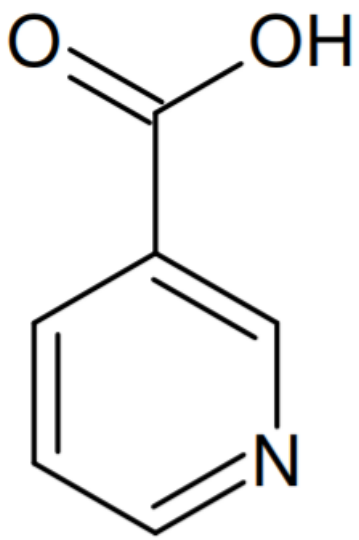

Figure 1: The molecular structure of nicotinic acid. Alternate names include pyridine-3- carboxylic acid, 3-pyridinecarboxylic acid, vitamin B3, and niacin. The SMILES notation O=C(O)c1cccnc1 and has molar mass of 123.11 grams/mole. The structure contains a pyridine ring and carboxylic acid functional group.

To ascertain the wavelength to set the ultraviolet detector for identification of nicotinic acid from injections into the HPLC, an aqueous mixture was prepared at 3.9425 x 10^{-2} molar and injected with different wavelengths settings. The outcome is shown in Fig. 2, for a range of 240 nm to 300 nm at 10 nm increments with peak area (uV.Min) tracking at each wavelength. The optimal wavelength was achieved at 265 nm (see Fig. 2) and 265 nm will be the setting for following assays by HPLC.

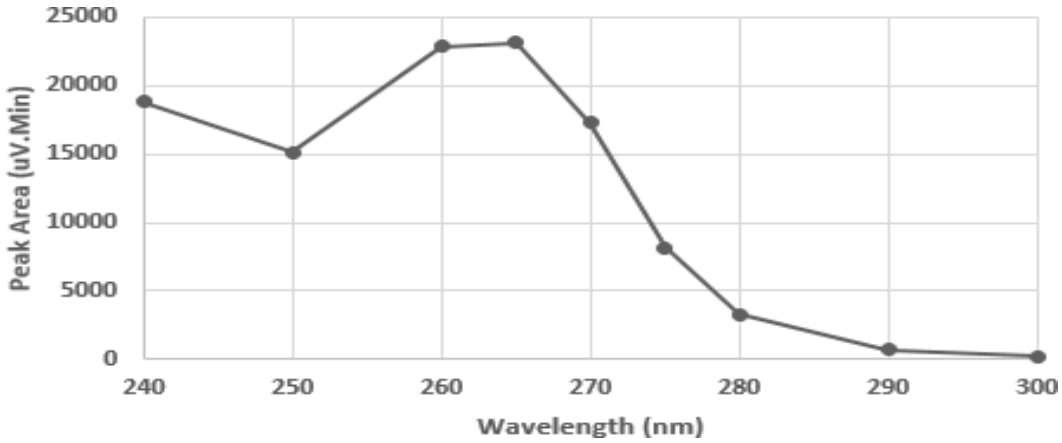

Figure 2: 2-way plot of peak area at elution with corresponding wavelength of ultraviolet detection. The optimum peak for detection of nicotinic acid elution is at 265 nm. Nicotinic acid is at concentration of 3.9425 x 10^{-2} molar in distilled water.

Following the identification of the optimal wavelength then the mobile phase for column solvent was determined. The mobile phase in reversed phase HPLC usually consists of water/aqueous solution (commonly an aqueous buffer) and an organic modifier.[9] Since water is most polar, it repels the hydrophobic analytes into the stationary phase more than any other solvent.[9] The organic modifier (ethanol in this study) is added (usually one modifier type at a time), and because these are less polar, any hydrophobic analyte is no longer as strongly repelled into the stationary phase and spends less time in the stationary phase (and therefore elutes earlier).[9] Ethanol is the organic modifier used here, and added to 1% (v/v) in the mobile phase brought about an elution time of about 2.6 minutes. The acetic acid at 0.174 molar acetic acid is used to the improve the chromatographic peak shape and to provide a source of protons in reverse phase.[9]

The standard curve developed is shown in Fig. 3 for concentration (molar) versus peak area (uV.Min). The equation of the line is: y = 57370741.61 uV.Min/molar (x) + 211.32 uV.Min, with slope equal to 57370741.61 uV.Min/molar and y-axis intercept at 211.32 uV.Min. The standard curve ranges from 3.2854 x 10^{-5} molar nicotinic acid to 1.36411 x 10^{-3} molar nicotinic acid. Coefficient of determination, denoted R^2 is the proportion of the variance in the dependent variable (peak area) that is predictable from the independent variable (molar concentration) is R^2 = 0.9990 for this plot. The Pearson r correlation coefficient is 0.9995, which is a very strong positive correlation.

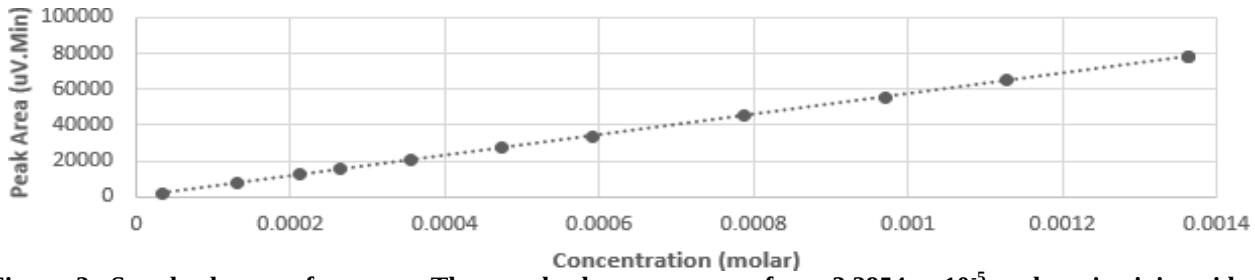

Figure 3: Standard curve for assay. The standard curve ranges from 3.2854 x 10^{-5} molar nicotinic acid to 1.36411 x 10^{-3} molar nicotinic acid (about 42-fold range in concentration). The equation for this line is: y = 57370741.61 uV.Min/molar (x) + 211.32 uV.Min. The Pearson r correlation coefficient is 0.9995. The coefficient of determination is 0.9990.

The column dead time represents the time it takes something to go through the LC column that does not interact with the column.[10] The dead time of eluting non-retained species for this instrumentation is 1.5 minutes and calculated based on relationship, dead time= to = volume/flow rate = 1.5 mL/1.0 mL/min.[10] Dead volume, Vm, is the total volume of the liquid phase in the chromatographic column.[10] Thus, for the column utilized here a 150 mm × 4.6 mm column, dead volume= Vm = 0.01 (length) = 0.01 × 150 mm = 1.5 mL. The dead volume is 1.5 milliliter.

In general, the limit of detection (LOD) is taken as the lowest concentration of an analyte in a sample that can be detected, but not necessarily quantified.[11,12] The limit of quantitation (LOQ) is the lowest concentration of an analyte in a sample that can be determined with acceptable precision and accuracy.[11,12] Therefore, the LOD and LOQ can be expressed as $LOD=3S_a/b$, $LOQ=10S_a/b$, where S_a can be taken as the standard deviation of the y-residuals and b is the slope of the standard curve. The standard deviation of the y-residuals is determined to be 261.70 for this study. Therefore, LOD is 1.3685 x 10^{-5} molar, and LOQ is 4.5616 x 10^{-5} molar.

The standard curve can be further diagnosed by presentation as confidence ellipses.[13] Standard curve is represented as 95% ellipses in Fig. 4. This plot shows that the data applied for the standard curve falls within 95% of the data points. All points of the standard curve are contained in the ellipses. A 95% confidence ellipse is an algorithm with the following property: if you were to replicate your sampling from the underlying distribution many times and each time calculate a confidence ellipse, then 95% of the ellipses so constructed would contain the underlying mean.[13]

A typical elution chromatogram for nicotinic acid utilizing this instrumentation is shown in Fig. 5. Here is seen a sharp peak at about 2.6 minutes. The concentration of nicotinic acid is at a concentration of 2.6283 x 10^{-4} molar.

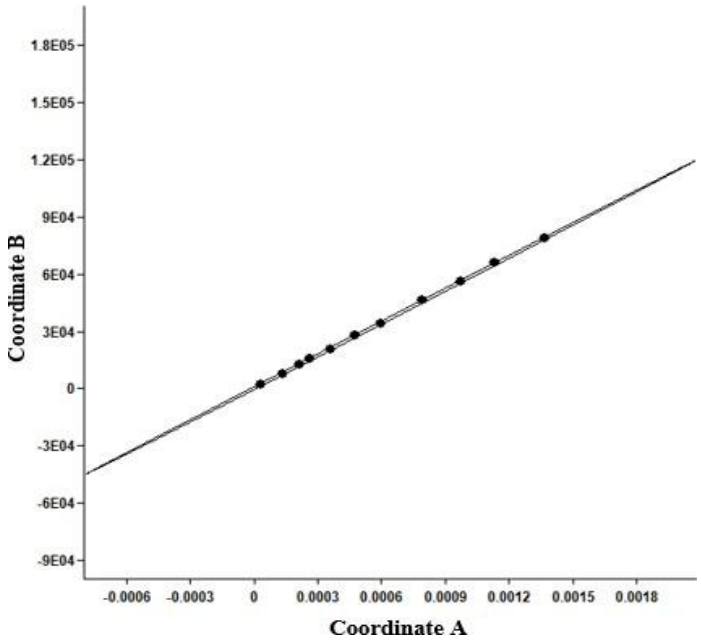

FIGURE 4: 95% ellipses for standard curve shown in Fig. 3. Coordinate A based on molar concentration and Coordinate B based on Peak Area (uV.Min).

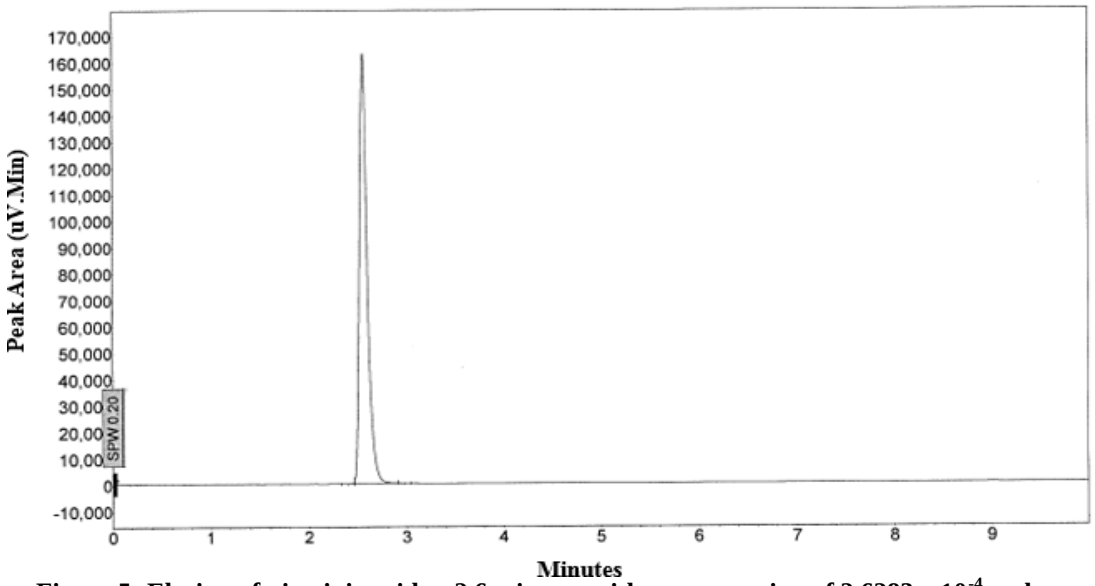

Figure 5: Elution of nicotinic acid at 2.6 minutes with concentration of 2.6283 x 10^{-4} molar.

Nicotinic acid (vitamin B3) is experiencing a resurgence in popularity as a treatment for abnormal blood lipids and the associated pathology.[3] When administered as a medicinal form using injections, it is in the form of an aqueous mixture.[1,8] The percent recovery of nicotinic acid by this methodology form aqueous mixtures is shown in Table 1. From aqueous samples, the average percent recovery is a very successful 99.3%, with standard deviation of 2.5%. The 95% confidence interval for the standard deviation is 2.0% to 3.5%.[13,14] The actual mean of percent recovery (99.3%) is not statistically different from the hypothetical mean of 100% (P=.17, two-tailed P value).[13,14] This outcome indicates that assay for nicotinic acid from aqueous solution is efficient, reproducible, and accurate for the determination of nicotinic acid.

Table 1: Percent Recovery Nicotinic Acid Assay from Aqueous Mixtures.

RUN	Molar Concentration	Molar Concentration (HPLC Assay)	Percent Recovery
1	1.9712 x 10^{-4}	1.8588 x 10^{-4}	94.3
2	3.9425 x 10^{-4}	3.8200 x 10^{-4}	96.9
3	9.8562 x 10^{-4}	9.6816 x 10^{-4}	98.2
4	1.1827 x 10^{-3}	1.1648 x 10^{-3}	98.5
5	2.3655 x 10^{-4}	2.3116 x 10^{-4}	97.7
6	3.1540 x 10^{-4}	3.1433 x 10^{-4}	99.7
7	8.6735 x 10^{-4}	8.8665 x 10^{-4}	102
8	1.1039 x 10^{-3}	1.1161 x 10^{-3}	101
9	2.7597 x 10^{-4}	2.6497 x 10^{-4}	96.0
10	5.1252 x 10^{-4}	4.8817 x 10^{-4}	95.2
11	8.6735 x 10^{-4}	8.3927 x 10^{-4}	96.8
12	7.8850 x 10^{-5}	7.4688 x 10^{-5}	94.7
13	6.0714 x 10^{-4}	6.1595 x 10^{-4}	101
14	7.6484 x 10^{-4}	7.7266 x 10^{-4}	101
15	9.2254 x 10^{-4}	9.4395 x 10^{-4}	102
16	1.0014 x 10^{-3}	1.0169 x 10^{-3}	102
17	1.1827 x 10^{-4}	1.1499 x 10^{-4}	97.2
18	2.3655 x 10^{-4}	2.3603 x 10^{-4}	99.8
19	2.7616 x 10^{-4}	2.7855 x 10^{-4}	101
20	4.3367 x 10^{-4}	4.4070 x 10^{-4}	102
21	8.9101 x 10^{-4}	8.9853 x 10^{-4}	101
22	1.0487 x 10^{-3}	1.0439 x 10^{-3}	99.5
23	1.2852 x 10^{-3}	1.2750 x 10^{-3}	99.2
24	3.2854 x 10^{-4}	3.3063 x 10^{-4}	101
25	3.9425 x 10^{-4}	3.9788 x 10^{-4}	101
26	4.5996 x 10^{-4}	4.6931 x 10^{-4}	102

Nicotinic acid in medicinal form can be delivered in saline solution for injection, etc.[1,8] Percent recovery of nicotinic acid by isocratic HPLC assay is shown in Table 2. The average percent recovery is 100%, with standard deviation of 1.6%. The 95% confidence interval for the mean is 99.2% to 100%. The minimum percent recovery is 98.3%, with the maximum percent recovery at 106%. The 95% confidence interval for the standard deviation is 1.2% to 2.4%.

There is no statistical difference between the hypothetical percent recovery of 100% and the actual mean of 100% (P=1.00, two-tailed P value).[13,14] This outcome indicates that assay for nicotinic acid from aqueous solution is efficient and reproducible.

TABLE 2: Percent Recovery From Saline Mixtures.

RUN	Molar Concentration	Molar Concentration (HPLC Assay)	Percent Recovery
1	5.5195×10^{-5}	6.1081×10^{-5}	106
2	3.8637×10^{-4}	3.8708×10^{-4}	100
3	7.3331×10^{-4}	7.3350×10^{-4}	100
4	8.9101×10^{-4}	8.8732×10^{-4}	99.6
5	9.6986×10^{-4}	9.7816×10^{-4}	101
6	4.4945×10^{-4}	4.5038×10^{-4}	100
7	4.9676×10^{-4}	4.9961×10^{-4}	101
8	6.0715×10^{-4}	6.1331×10^{-4}	101
9	2.1290×10^{-4}	2.1076×10^{-4}	99.0
10	2.6021×10^{-4}	2.5572×10^{-4}	98.3
11	4.9676×10^{-4}	4.9582×10^{-4}	99.6
12	6.8600×10^{-4}	6.8802×10^{-4}	100
13	8.1216×10^{-4}	8.1732×10^{-4}	101
14	9.3832×10^{-4}	9.4708×10^{-4}	101
15	6.5446×10^{-4}	6.6348×10^{-4}	101
16	7.3331×10^{-4}	7.4388×10^{-4}	101
17	9.2255×10^{-4}	9.4389×10^{-4}	102

Pharmaceutical Excipients are crucial for drug delivery within the body.[1,15] Usually, an excipient has no medicinal properties. Its standard purpose is to ultimately facilitate physiological absorption of the drug.[15] Excipients might aid in lubricity, flowability, disintegration, taste and may confer some form of antimicrobial function.[1,15] Cellulose (tablet disintegrant, diluent) and starch (diluent and disintegrant) are common excipients for pharmaceutical formulations.[1] Nicotinic acid was assayed from mixtures of this vitamin along with designated amounts of cellulose and/or starch present (see Table 3), and after solubilizing in water. After tablet mixtures with nicotinic acid were mixed in water, they were allowed to settle for 48 hours, followed by HPLC analysis. If filtering was necessary it was accomplished using Whatman 6900-2502 GD/X 25 Sterile Syringe Filter.

The average percent recovery is 101%, with standard deviation of 1.6%. The minimum percent recovery is 99.0%, with the maximum percent recovery at 104%. The 95% confidence interval for the mean is 100% to 101%. The 95% confidence interval for the standard deviation is 1.2% to 2.3%.[13,14] The percent recovery is excellent with high levels of reproducibility and accuracy.

Table 3: Percent Recovery From Excipients Starch and Cellulose.

RUN	Cellulose (grams)	Starch (grams)	Molar Concentration	Molar Concentration (HPLC Assay)	Percent Recovery
1	0.0153	-	2.3655×10^{-4}	2.3475×10^{-4}	99.0
2	0.0105	0.0069	2.3655×10^{-4}	2.3480×10^{-4}	99.1
3	0.0068	0.0151	7.0965×10^{-4}	7.2523×10^{-4}	102
4	0.0071	-	7.0965×10^{-4}	7.2238×10^{-4}	102
5	0.0114	0.0051	5.9138×10^{-4}	6.0182×10^{-4}	102
6	0.0017	0.0195	5.9138×10^{-4}	6.0414×10^{-4}	102
7	-	0.0252	5.9138×10^{-4}	6.0746×10^{-4}	103
8	-	0.0115	3.9425×10^{-4}	4.0315×10^{-4}	102
9	0.0054	0.0126	3.9425×10^{-4}	4.0115×10^{-4}	102
10	0.0091	-	3.9425×10^{-4}	4.0333×10^{-4}	102
11	0.0061	-	5.5195×10^{-4}	5.7623×10^{-4}	104
12	0.0086	-	5.5195×10^{-4}	5.7360×10^{-4}	104
13	-	0.0141	5.5195×10^{-4}	5.7144×10^{-4}	104
14	0.0024	0.0107	4.7310×10^{-4}	4.9137×10^{-4}	104
15	0.0033	-	4.7310×10^{-4}	4.8790×10^{-4}	103
16	-	0.0110	4.7310×10^{-4}	4.8781×10^{-4}	103
17	0.0053	-	6.3080×10^{-4}	6.3172×10^{-4}	100
18	0.0078	0.0108	6.3080×10^{-4}	6.3884×10^{-4}	101
19	-	0.0139	6.3080×10^{-4}	6.3555×10^{-4}	101
20	-	0.0270	3.1540×10^{-4}	3.1635×10^{-4}	100
21	0.0104	-	3.1540×10^{-4}	3.1750×10^{-4}	101
22	0.0120	0.0159	3.1540×10^{-4}	3.1475×10^{-4}	99.8

Nicotinic acid is available in tablet form either as extended release tablet or quick dissolve tablets. Recovery of nicotinic acid from commercial 500 milligram extended release tablet is shown in Table 4. The hypothetical molarity of a 500 milligram portion of a mass produced tablet form is 2.031×10^{-3} molar in two liters of aqueous solution. The percent recovery from numerous assays are presented in Table 4.

The average percent recovery is 104%, with standard deviation of 4.6%. The 95% confidence interval for the mean is 99.7% to 108%. The minimum percent recovery is 94.0%, with the maximum percent recovery at 107%. The 95% confidence interval for the standard deviation is 3.0% to 10%. There is no statistical difference between the hypothetical percent recovery of 100% and the actual mean of 104% (P=.06, two-tailed P value).[13,14]

Table 4. Percent Recovery From Commercial 500 milligram Tablets in Aqueous Solution.

RUN	Molarity of Commercial Tablet (500 mg)	Molarity Determined by HPLC	Percent Recovery
1	2.031×10^{-3}	2.1029×10^{-3}	104
2	2.031×10^{-3}	2.1104×10^{-3}	104
3	2.031×10^{-3}	2.1266×10^{-3}	105
4	2.031×10^{-3}	1.9020×10^{-3}	94.0
5	2.031×10^{-3}	2.1900×10^{-3}	107
6	2.031×10^{-3}	2.1767×10^{-3}	107
7	2.031×10^{-3}	2.1849×10^{-3}	107

The methodology presented here will be useful for assuring the presence of nicotinic acid in aqueous, saline, solid forms of pharmaceutical and/or commercial products, and to recognize presence of adulterants. Analysis by HPLC will continue to permit confidence in the quality of pharmaceutical products made use of, in health care facilities. The instrumental analysis of pharmaceutical products meant for human consumption is a vital function of analytical chemistry.

CONCLUSION

This study demonstrates the efficacy of isocratic HPLC determination of nicotinic acid, an important drug for the treatment of pellagra, lower LDL cholesterol, increase

HDL cholesterol, and lower triglycerides. This study shows that nicotinic acid can be assayed from aqueous mixtures, saline mixtures, as well as solid formulations having cellulose and starch. Detection is consistent, accurate, and over a broad range of concentrations. Percent recovery of analyte is very high in all modalities. The standard curve is extremely linear and has extremely high positive correlation with peak areas and molar concentration. The LOD is 1.3685 x 10^{-5} molar, and LOQ is 4.5616 x 10^{-5} molar. This methodology will be useful for quality control in industrial manufacturing and confirmation of nicotinic acid when applied as a medicinal agent in aqueous, saline, or tablet formulations.

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