Short term Stability of Water Soluble Vitamins in Solution with Respect to pH

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Abstract

ROBERT C THOMPSON, Albion Advanced Nutrition, Clearfield, Utah: Short term Stability of Water Soluble Vitamins in Solution with Respect to pH. With the modern chromatographic tools available to today's chemist, it is possible to test for many different vitamins simultaneously. Current AOAC methodology does not address the simultaneous analysis of water soluble vitamins. Many different challenges exist with the extraction, stability, and quantification of multiple water soluble vitamin analytes. Solubility and stability can be affected by the pH of the extraction solvent used. With the simultaneous analysis of multiple water soluble vitamins, one must be aware of the different solubility and stability factors that will influence the overall accuracy and precision of the analytical procedure in question. The simultaneous analysis of water soluble vitamins by HPLC was studied. As a first step in the development of an assay for the analysis of multiple water soluble vitamins, we have studied the short term stability of several water soluble vitamins at various pH levels in a Na H_2PO_4 buffer. The analysis of eight water soluble vitamins was carried out using a reverse phase C18, paired ion HPLC gradient separation with photodiode array detection. Analytes were dissolved into a 25mM NaOH solution, and titrated to various pH levels with a 100mM solution of H_3PO_4. The various samples were then assayed at regular intervals for content over a nine day period. We found that riboflavin, for instance, exists in three crystal structures and is only sparingly soluble in neutral or acidic solutions. Riboflavin is much more soluble but less stable in an alkaline solution. (Merck Index, 13th ed.) While Folic acid is reported as being only slightly soluble in acidic solutions, it is much more soluble and stable in somewhat basic solutions. If the standards and samples are dissolved in a solution in which they are not completely soluble and given the short term stability, the overall accuracy and precision of the analytical procedure will suffer as a result. We conclude that when the pH of the extraction is controlled, it is possible to combine several water soluble vitamins in a single chromatographic procedure.

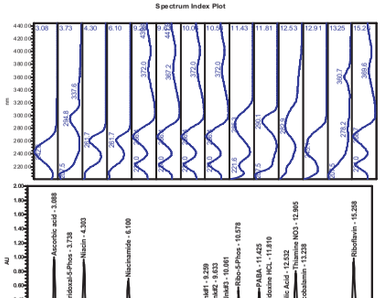
Introduction

Modern chromatography gives today's scientist the ability to analyze many different complex chemical matricies. Advances in instrumentation, columns and detection systems, have given us the ability to separate very complex mixtures in relatively short run time periods. Water soluble vitamins are currently sold in a wide variety of formulas as dietary supplements. These formulas contain everything from single B-vitamins to formulas that contain many different water soluble vitamins in complex matricies such herbal blends. The current AOAC methodology for the analysis of water soluble vitamin does not address the ability to analyze multiple water soluble vitamin analytes simultaneously. As a first step in the development of the simultaneous analysis of multiple water soluble vitamins, we have studied the short term stability and solubility of 11 water soluble vitamins in a sodium phosphate buffer.

HPLC Procedure

Water Soluble Vitamin

HPLC Conditions for C18 Paired Ion Separation Method



Instrument: Waters 2695 Separations Module
Column: Phenomenex LUNA 3µ C18(2) 4.6 x 150mm Part# OOF-4251-E0
Injection Volume : 10µl
Mobile Phase : %C Acetonitrile
%D 25mM Ammonium Phosphate mono-basic , 5mM Na Hexanesulfonate pH 2.5
Flow rate: 0.65 ml/min
Detector: Waters 2996 Photodiode array

Detection: PDA 200nm - 450nm

Analytical Wavelengths
Ascorbic Acid - 243nm
Pyridoxal-5-phosphate - 290nm
Niacin - 262nm
Niacinamide - 262nm
Riboflavin-5-phosphate - 262nm
Paraminobenzoic Acid - 290nm
Pyridoxine - 290nm
Folic Acid - 290nm
Thiamine - 243nm
Cyanocobalamin - 361nm
Riboflavin - 262nm

W2690/5 Gradient Table

	Time	Flow	%A	%B	%C	%D	Curve
1	-	0.65	0.0	0.0	4.0	96.0	-
2	4.00	0.65	0.0	0.0	4.0	96.0	1
3	6.00	0.65	0.0	0.0	13.0	87.0	6
4	19.00	0.65	0.0	0.0	19.0	81.0	6
5	19.00	0.65	0.0	0.0	40.0	60.0	6
6	20.00	0.65	0.0	0.0	4.0	96.0	2
7	30.00	0.65	0.0	0.0	4.0	96.0	1

Procedure

In order to study the decomposition of multiple water soluble vitamins in solution, we first needed an analytical method suitable for this purpose. We chose C18 HPLC (high performance liquid chromatography) with an ion pairing mobile phase for this analysis. (see Water Soluble Vitamin)

With reverse phase paired ion chromatography we generated a 9 point standard curve of the following range concentrations, which generated the corresponding correlation coefficients. (Table 1) All 11 analytes were dissolved in 25Mm NaOH with the pH of the solution adjusted to 6.5 with H_3PO_4 prior to the HPLC analysis.

Standard Curve Data

Analyte	Supplier	CAS#	Standard Curve Range in µg/ml	Correlation Coefficient
Ascorbic Acid	Sigma	50-81-7	3.07 - 787	0.9977
Pyridoxal-5-phosphate • H_2O	Acros	54-47-7	0.789 - 202	0.9993
Niacin	Sigma	59-67-6	1.69 - 434	0.9997
Niacinamide	Sigma	98-92-0	1.59 - 407	0.9996
Riboflavin-5-phosphate	ICN	130-40-5	0.766 - 196	0.9994
4-Aminobenzoic Acid	Sigma	150-13-0	0.816 - 209	0.9996
Pyridoxine Hydrochloride	Sigma	58-56-0	1.60 - 409	0.9983
Folic Acid	Sigma	59-30-3	0.402 - 103	0.9996
Thiamin mono-nitrate	ICN	532-43-4	1.60 - 409	0.9988
Cyanocobalamin	Roche	68-19-9	0.605 - 155	0.9996
Riboflavin	ICN	83-88-5	0.785 - 201	0.9997

Table 1

With Standard curves in place we then dissolved all 11 analytes in 1000mL of 25mM NaOH at the following concentrations (Table 2). Seven 100mL aliquots were taken from the 1000mL water soluble vitamin mixture. Each of the 100ml aliquots was then titrated with 100mM H_3PO_4 to one of seven specific pH values. (2.20, 3.49, 5.46, 7.00, 8.53, 10.47, and 11.90) Each of these aliquots was filtered using a 4.5 micron filter into an HPLC vial and kept at 20 °C in the dark during the 9 day analysis via the refrigeration compartment of the Waters 2695 Alliance separations module. In order to compensate for dilutions made during titration, all sample recovery data was presented in milligrams recovered per 100mL sample titrated.

Sample Data

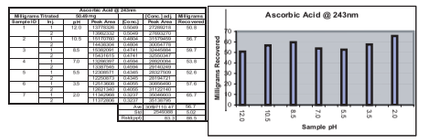
Analyte	Supplier	Milligrams Titrated Per 100ml Aliquot
Ascorbic Acid	Sigma	50.5
Pyridoxal-5-phosphate • H_2O	Acros	30.2
Niacin	Sigma	20.4
Niacinamide	Sigma	20.3
Riboflavin-5-phosphate	ICN	10.4
4-Aminobenzoic Acid	Sigma	10.3
Pyridoxine	Sigma	20.1
Folic Acid	Sigma	5.97
Thiamin mono-nitrate	ICN	20.2
Cyanocobalamin	Roche	9.81
Riboflavin	ICN	10.2

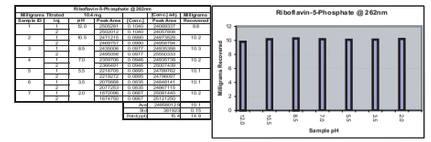
Table 2

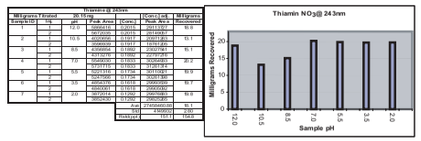
Conclusion

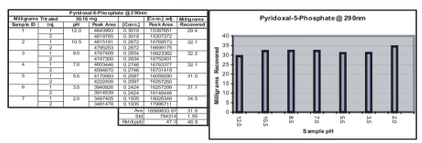
The simultaneous analysis of multiple water soluble analytes can be a very complex task in today's world. The short term stability of multiple water soluble analytes in a single matrix is crucial to the development of an accurate analytical method for this determination. Based on our analysis of 11 water soluble vitamins in a sodium phosphate buffer over a nine day period at various pH's, we believe that if the pH is controlled during extraction, accurate results can be obtained for the analysis of most of the vitamins tested. This procedure is not well suited for ascorbic acid or pyridoxal-5-phosphate, due to the rapid decomposition of these two compounds over the entire pH range tested in the first 24 hours. From our analytical data we believe that the optimum pH for this analysis is 7.0. Both the solubility and stability of water soluble vitamins can be affected by pH. The solubility of several of the water soluble vitamins tested is affected by the pH of the extraction solvent. Ascorbic acid, thiamin, riboflavin and cyanocobalamin show the greatest affect on solubility at different pH values. At a high pH we see the decomposition of niacinamide to niacin, and at a low pH we see the precipitation of folic acid out of solution. In conclusion our data shows that if pH is not buffered in both of the sample and standard matrix, the overall accuracy of any analytical results will be negatively affected.

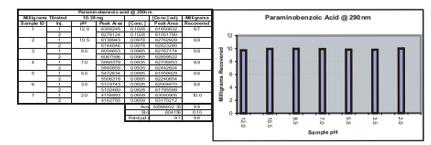
Solubility

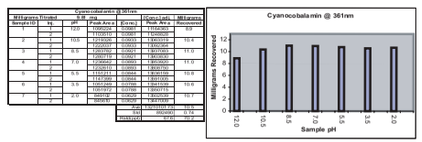


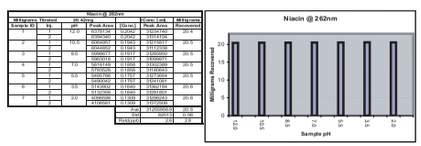


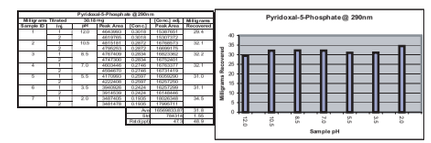


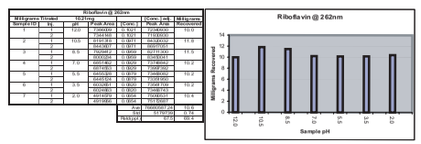


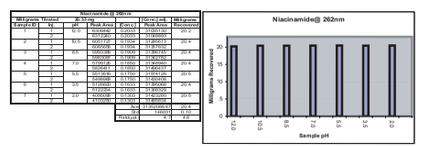


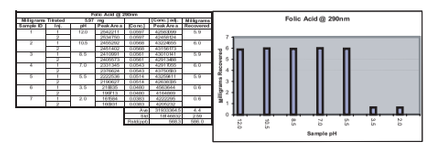




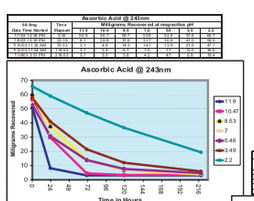


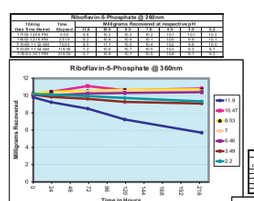


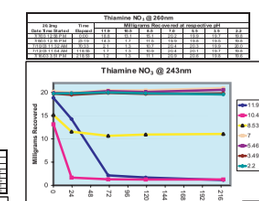


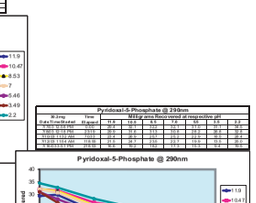


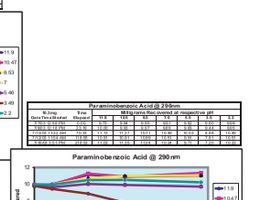
Stability

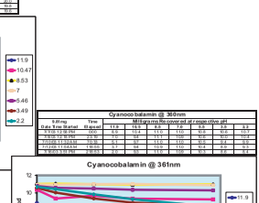


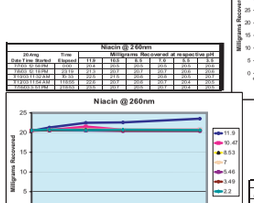


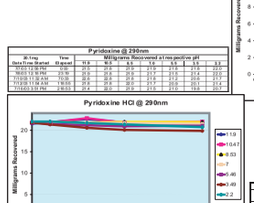


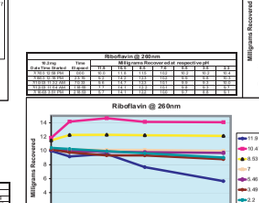


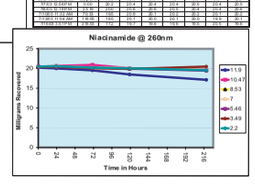


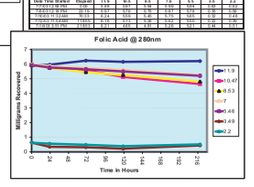












Acknowledgement

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