BIOCHEMICAL FUNCTIONS AND PHARMACEUTICAL AND CLINICAL ANALYSIS OF NICOTINAMIDE

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ABSTRACT
The major biochemical role of nicotinamide (vitamin B3) is its involvement in redox reactions and energy metabolism. The biological forms of nicotinamide are nicotinamide adenine dinucleotide (NAD) and nicotinamide adenine dinucleotide phosphate (NADP) coenzymes. Nicotinamide is a component of vitamin B-complex and is used in combination with other vitamin B compounds in pharmaceutical preparations. Several analytical methods have been used for the assay of nicotinamide in commercial products. These methods include high-performance liquid chromatography (HPLC), mass spectrometry in the multiple reaction modes (LC/UV/MS/MRM), liquid chromatographic-isotope detection mass spectrometry (LC/DMS), and planar chromatography-multiple detection by electro-spray mass spectrometry (ES/MS), capillary zone electrophoresis (CZE), thin-layer chromatography (TLC)-densitometry, differential pulse polarography and cyclic voltammetry. The clinical analysis of nicotinamide involves the application of liquid chromatography-tandem mass spectrometry (LC-MS/MS), high-performance liquid chromatography (HPLC) and capillary electromicrography.

Keywords: Nicotinamide, niacinamide, biochemical functions, pharmaceutical analysis, clinical analysis.

INTRODUCTION
Nicotinamide (niacinamide, niacin, vitamin B3) was identified as a vitamin as a result of an urgent need to cure pellagra, with symptoms of diarrhea, neurological disturbances (dementia) and sun-sensitive dermatitis1-2. Pellagra may occur in AIDS patients3 and is associated with anorexia nervosa4. Low nicotinamide condition is very common in cancer patients5 and pellagra may be induced by chemotherapy6. In carcinoid cancers, high levels of serotonin are produced from tryptophan, and, therefore, these patients are at risk for the vitamin deficiency if their intake of preformed nicotinamide is low7. If poorly diagnosed nicotinamide deficient patients are supplemented with micro nutrients lacking the sufficient vitamin, they may rapidly move towards a full pellagrous dementia8. It is also likely that subclinical deficiencies of nicotinamide exists in developed countries, for example, 15% of women in Sweden had blood nucleotide pools with suboptimal vitamin intake9. Pellagra may be difficult to identify for the reason that the symptoms of dermatitis, diarrhea and dementia occur in an unpredictable order. It is often uncommon to find all three aspects until the disease is very advanced10.

Nicotinamide is used in the treatment and prevention of its deficiency. Doses of up to 500 mg daily in divided doses have been recommended11. Nicotinamide is excreted in the urine in the unchanged form. Nicotinic acid, N-methyl nicotinamide and nicoturonic acid are metabolites of vitamin12.

BIOCHEMICAL FUNCTIONS
The first established biochemical role of nicotinamide is its involvement in redox reactions and energy metabolism. The biological forms of nicotinamide adenine dinucleotide (NAD) and nicotinamide adenine dinucleotide phosphate (NADP) coenzymes. The C-4 position of the pyridine ring of the nicotinamide moiety participates in the oxidation and reduction
reactions. In view of the electronegativity of the amide group and the nitrogen at position 1 on this ring, hydride ions can readily reduce the oxidized C-4 position. This is the basis for the enzymatic hydrogen-transfer reactions in oxidative phosphorylation and biosynthetic reactions. NAD$^+$ is reduced to NADH in glycolytic reactions, oxidative decarboxylation of pyruvate, oxidation of acetate in the TCA cycle, oxidation of alcohol, $\beta$-oxidation of fatty acids, and a large number of other cellular oxidation reactions. The electrons derived from them oxidation reactions are transferred to the electron transport chain through the oxidation of NADH. The energy resulting from these transfers is used to generate ATP$^{10,13,14}$. For further biochemical reactions of NAD and NADP coenzymes, the reader is referred to advanced textbooks of biochemistry.

PHARMACEUTICAL ANALYSIS
Nicotinamide and other B vitamins (B$_1$, B$_2$, B$_3$, B$_6$, biotin, folic acid) in multi-vitamin, multi-mineral supplements have been determined by liquid chromatography with a photodiode array detector (PAD) in sequence with a triple-quad mass spectrometer in the multiple reaction mode (LC/UV/MS/MSM). The sample does not require any cleanup, pre-steps except for centrifugation and filtration of the extract$^{15}$. The content of nicotinamide, vitamin B$_1$, B$_2$, B$_3$, B$_6$ in multi-vitamin/multi-element tablets have been determined by liquid chromatographic-isotope dilution mass spectrometry (LC/DMS). LC is carried out on a C18 reversed phase column using an Agilent 1100 HPLC system and a Qualtro micro triple-quad mass spectrometer. The assay of B vitamins is conducted using is gradient LC profile in the MS/MS detection in a multiple reaction mode. The unknown vitamin concentrations are determined by a comparison of the ratio of the integrated LC peaks at different masses of the unlabelled and labelled vitamins$^{16}$.

Planner chromatography-multiple detection by electrospray ionization mass spectrometry (ES/MS) has been employed for the simultaneous assay of nicotinamide, riboflavin, pyridoxine, caffeine and taurine in energy drinks. The overall recoveries of these vitamins are 81 and 106% at there concentration levels with a RSD of 0.8-1.5%. High-performance liquid chromatography (HPLC) has been extensively used for the assay of vitamin mixtures including nicotinamide in pharmaceutical preparations. The simultaneous liquid chromatographic assay of nicotinamide and other B vitamins has been carried out by ion-pair chromatography using a RP C18 column and mobile phase of methanol: water (15:85, v/v) with 0.5% triethylamline at pH 3.6. Nicotinamide is measured at 280 nm. The recoveries of the vitamins are in the range of 98.2-102.0%. Six water-soluble vitamins (B$_1$, B$_2$, B$_3$, B$_5$, B$_6$ and B$_{12}$) including nicotinamide in vitamin supplements has been determined by capillary zone electrophoresis. The RSD of the method ranges from 1.08-3.68% (intra-day precision) and 1.26-3.5% (inter-day precision). The method is fast accurate and simple for the assay of B-vitamins$^{17}$. A competitive capillary zone electrophoretic (CZE) method for the quality control analysis of multivitamin preparations of B-group vitamins containing nicotinamide has been developed with diode array detector at 214 nm. The method has been validated for various analytical parameters$^{18}$. A sensitive reversed phase UV-HPLC stability indicating method has been used for the potential impurities of nicotinamide active pharmaceutical ingredients. Forced degradation studies confirmed that the newly developed method is specific and selective for the degradation products including niacinamide N-oxide. The method has been validated according to ICH guidelines with respect to specificity, precision, linearity and accuracy. Regression analysis showed correlation coefficient values greater than 0.999 for nicotinamide and its six impurities. The detection limit of impurities is in the range of 0.003-0.005% indicating the high sensitivity of the method. Accuracy of the method has been established on the basis of the recovery obtained between 93.35 and 130.0% for all impurities$^{19}$. The reduced nicotinamide adenine dinucleotide (NAD) phosphate has been determined using HPLC with fluorescence detection.
The ratio of the reduced form was taken as a biomarker of oxidative stress. A thin-layer chromatography (TLC)-densitometric method has been developed to determine N-(hydroxymethyl) nicotinamide in tablets using silica gel F254 plates and chloroform-ethanol (2:2, v/v) as mobile phase. The densitometric observations were made at 260 nm. The recovery ranged from 97.60 to 100.82% with a RSD of 2.37%. The limit of detection was 0.1 mcg/sport while the linearity range was from 0.2 to 1.75 mcg/sport.

The behavior of nicotinamide has been studied by differential pulse polarography and cyclic voltammetry in the presence of ionic and non-ionic surfactants including cetyltrimethylammonium bromide (CTAB), sodium dodecylsulfate (SDS) and Triton X-100 (TX-100). The cathodic peak potential (E(p(c))) and peak current (I(p(c))) of nicotinamide were found to be remarkably dependent on the charge and concentration of the surfactant. A sharp peak with more than two-fold increase in current has been used to determine the limit of detection and linear working range using the differential pulse polarographic technique. The present method has successfully been used for the simultaneous determination of nicotinamide and pyridoxine hydrochloride and for the determination of nicotinamide in multivitamin pharmaceutical preparations.

An enzyme inhibition biosensor has been used for the analysis of nicotinamide. It consisted of a H2O2-amperometric electrode coupled to a functionalized nylon membrane chemically bonding the enzymes, a butyrylcholine standard solution in glycine buffer acted as substrate. The response of the system to the inhibitor was characterized completely and the analyses of several formulations containing nicotinamide are nicotinic acid has been performed.

**CLINICAL ANALYSIS**

High-performance liquid chromatography (HPLC) has extensively being used determination of nicotinamide and derivatives in biological fluids. N1-Methylnicotinamide and N1-Ethynicotinamide are reacted with acetophenone to form fluorescent derivatives. These derivatives are separated by HPLC on a C18 reverse-phase column using a mobile phase of acetonitrile-triethylamine and 0.01 M heptanesulfonic acid adjusted to pH 3.2. Fluorescence detection is achieved at 418 nm emission using 366 nm excitation. The limit of quantification is 2ng/ml. The method has been used to determine the concentration of endogenous N1-Methylnicotinamide in the plasma of 36 subjects with various pathology. The main concentration was 18 ng/ml and the range was 6.2-116.7 ng/ml. A HPLC method has been developed for the determination of nicotinamide and eight of its metabolites in human plasma and urine. Calibration curves were linear up to 2 mumol/ml for nicotinamide and 200 nmol/ml for the metabolites; both the intra- and inter-assay relative standard deviations ranged between 1 and 8%.

A liquid chromatography-tandem mass spectrometry LC-MS/MS method for the simultaneous quantization of niacin and its metabolites, i.e. nicotinamide in human plasma has been developed and validated using nevirapine as an internal standard (IS). Extraction of the niacin and its metabolites i.e. nicotinamide along with the IS from human plasma is accomplished and chromatographic separation of these compounds is achieved on a Hypersil-BDS column using a mobile phase consisting of 0.1% formic acid; acetonitrile (20/80, v/v) at a flow rate of 1 ml/min. The method is sensitive, specific, precise, accurate and suitable for bioequivalence and pharmacokinetic studies in humans. Nicotinamide-containing cofactors are ubiquitous in biological systems. A HPLC method has been developed for the resolution of oxidized and reduced cofactors (NADPH).

A simple method for the separation of vitamin B analytes including nicotinamide has been developed by capillary electrophromatography using methacrylate-based monolithic column. The method was successfully applied to the assay of nicotinamide and other vitamin B analytes in human urine samples.

**DISCUSSION**

Nicotinamide is a component of vitamin B-complex
and is used in the treatment and prevention of pellagra. Its symptoms include diarrhea, neurological disturbances (dementia) and the sun-sensitive dermatitis. Nicotinamide plays its biological role in the form of nicotinamide adenine dinucleotide (NAD) and nicotinamide adenine dinucleotide phosphate (NADP). These are oxidizing coenzymes for more than 200 dehydrogenases. These coenzymes are involved in metabolic oxidation and reduction. The various methods used for the determination of nicotinamide in pharmaceutical preparations and clinical samples have been reviewed. The analysis of nicotinamide in commercial products requires highly sensitive and specific methods. This is particularly necessary for the analysis of nicotinamide in complex systems such as those containing vitamin mixtures and biological fluids. These need the application of advanced techniques and also their combinations to achieve accurate and reliable results. The techniques used by analysts for this purpose include high-performance liquid chromatography, mass spectrometry and their combinations. In addition to these TLC-densitometry, capillary zone electrophoresis, capillary electrochromatography have also been used in clinical and pharmaceutical analysis of the vitamin.

REFERENCES

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