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NEW VALIDATED RP-HPLC METHOD FOR THE SIMULTANEOUS ESTIMATION OF NIACIN AND ITS METABOLITE 1-METHYLNICOTINAMIDE IN RAT PLASMA

Nagi Reddy N[1*,2], *Venkateshwara Rao J*[1]

[1*]**Talla Padmavathi College of pharmacy, Orus, Karimabad, Warangal, Andhra Pradesh, India**
[2]**Department of pharmacy, acharyanagarjuna University,** Guntur,Andhra Pradesh, India
***Corresponding Author Email:** nagireddy_81@yahoo.co.in**

Research Article

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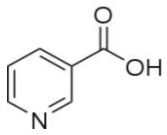
ABSTRACT

A simple, accurate and precise HPLC method for simultaneous analysis of Nicotine (N) and 1-Methylnicotinamide (1-MN) in plasma has been developed and validated. Separation was done by using 25×4.6 cm i.d, 5um particle, Hichrome KR 100 column with 70:30 (Acetonitrile & 10%KH_2PO_4) as mobile phase, at flow rate of 1ml/min and UV-detection at 275nm. Total run time was 20 mins: N & 1-MN were eluted with retention times of 13.9 min & 8.4mins respectively. This increase in retention time can avoid the interference of plasma peaks. This method was validated for linearity, accuracy, precision and specificity as per ICH guide lines. The high recovery and low coefficients of variation confirm the suitability of the method for simultaneous analysis of two drugs in plasma.

KEYWORDS: *Niacin, HPLC, 1-Methylnicotinamide*

INTRODUCTION



NIACIN

Niacin is a neutraceutical substance, which is highly polar and having low molecular weight. Niacin is a type of B vitamin. It is water soluble, which means it is not stored in the body Water soluble vitamins dissolve in water. Leftover amounts of the vitamin leave the body through the urine [6].

Function

Niacin assists in the functioning of the digestive system, skin and nerves. It is also important for the conversion of food to energy.

Food Sources

Niacin (also known as vitamin B_3) is found in dairy products, poultry, fish, lean meats, nuts, and eggs. Legumes and enriched breads and cereals also supply some niacin.

Side Effects

A deficiency of niacin causes pellagra. The symptoms include inflamed skin, digestive problems and mental impairment.

Recommendations

Recommended daily allowances (RDAs) are defined as the levels of intake of essential nutrients that the Food and Nutrition Board at the Institute of Medicine has found to be adequate to meet the known nutrient needs of most healthy persons. The Food and Nutrition

Board at the Institute of Medicine recommends the following dietary intake for niacin:

Infants

0 - 6 months: 2 milligrams per day (mg/day), 7 - 12 months: 4 mg/day

Children

1 - 3 years: 6 mg/day, 4 - 8 years: 8 mg/day, 9 - 13 years: 12 mg/day

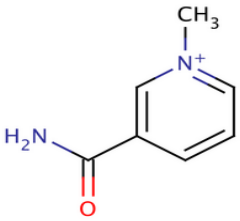
Adolescents and Adults

Males age 14 and older: 16 mg/day, Females age 14 and older: 14 mg/day

Specific recommendations depend on age, gender, and other factors (such as pregnancy). Women who are pregnant or producing breast milk (lactating) need higher amounts. Ask your health care provider which amount is best for you.

The best way to get the daily requirement of essential vitamins is to eat a balanced diet that contains a variety of foods from the food guide pyramid.

Large doses of niacin can cause liver damage, peptic ulcers, and skin rashes. Even normal doses can be associated with skin flushing [8]. It can be prescribed as a treatment for elevated total cholesterol and other types of lipid disorders, but it should only be used with medical supervision due to its potential for severe side effects.



1-METHYLNICOTINAMIDE

1-Methylnicotinamide(1-MN), a major metabolite of nicotinamide (N), has been shown recently to act *in vivo* as a very efficient anti-inflammatory age. After topical application of 1-MN, a remarkable therapeutic effect was observed in such inflammatory skin diseases as rosacea and acne vulgaris as well as in skin burns and wound healing [5]. On the other hand, 1-MN therapy was not effective in cases with accompanying bacterial infection, which indicates that 1-MN's therapeutic effectiveness is related to its anti-inflammatory, but not anti-bacterial, properties. Moreover, it was demonstrated that 1-MN displays an anti-thrombotic potential. All these observations contradict the common view that 1-MN is a biologically inactive metabolite of N. However, in contrast to N, the mechanism of 1-MN's anti-inflammatory activity is still unclear. It remains to be examined whether 1-MN is able to inhibit the functions of immune cells, especially those involved in an inflammatory response.

MATERIALS AND METHODS

Niacin is a gift sample from symbiotic pharma lab limited, Indore (Batch number: BCD 07003, Percent purity: 99.86, Molecular weight: 123.11, Storage: 2-8^{o}C). 1-Methylnicotinamide is a gift sample from symbiotic pharma lab limited, Indore (Batch number: ACZC0452, Percent purity: 98.86, Storage: 2-8^{o}C). Methanol and Acetonitrile HPLC grade (Rankem, Mumbai, India). Ethyl acetate HPLC grade (Merck, Mumbai, India), KH_2PO_4 AR grade (Merck, Mumbai, India) Milli-Q water (NISHKA scientific and reference labs, Hyderabad) and DMSO GC grade (spectrochem pvt. Ltd.,Mumbai, India).

HPLC: shimadzu, prominence, LC-20AT, SIL-20AC.

Initial chromatographic conditions:

To achieve minimum acceptable separations the chromatographic conditions used are mobile phase prepared by taking HPLC grade water

(1000 ml) in clean and dry beaker and mixed with 13.6 gm of KH_2PO_4 and P^H is adjusted to 3.2 with orthophosphoric acid [5]. In another beaker HPLC grade acetonitrile (1000 ml) was taken and both the contents are filtered through 0.45 micron membrane and sonicated for 5 mins. Initial condition was optimized to mobile phase ratio 10:90 (10%KH_2PO_4:90% Acetonitrile).Wavelength was adjusted to 275 nm, flow rate adjusted to 1ml/min and the column used are Hichrome-KR100, 4.6×250mm and 5 µm [2].

Preparation of standard stock solution:

Definite quantity of standard drug of Niacin and 1-Methylnicotinamide was weighed and transferred into volumetric flask. Initially these substances are dissolved in small quantity of DMSO and finally the volume was made up to the mark with methanol to get 5mg/ml solution [4]. Further dilutions were done with methanol to get concentration10mcg/ml.

Preparation of sample solution:

Drug extraction from the plasma was carried out by precipitation method. Methanol double the volume was added as precipitating agent and vortexed for 5mins then centrifuged for 15mins at 5000 rpm. The supernatant solution was separated and filtered through 0.45µm and 50µl of the solution was injected into HPLC [7]

Method validation [1]:

Linearity & Range: by analyzing standard solution of 80-120% test concentrations.

Accuracy: by recovery studies

Precision: by analyzing five replicates of standard solution

Specificity: by injecting placebo solution

System Suitability: by injecting six subsequent injections

Robustness: Robust

RESULTS AND DISCUSSION

The typical chromatograms are presented in figures 1 & 2, initially the retention time for Niacin and 1-Methylnicotinamide was found to be 5.5 min and 2.5 min respectively. This run time very early for the analytes, plasma peak interference may elute in the early run time of chromatogram. Plasma interference avoided by increasing the retention time of Niacin and 1-Methylnicotinamide to 13.9 and 8.4 mins respectively. The retention time has been increased by changing in the mobile phase ratio 30:70 (10%KH_2PO_4: 90% Acetonitrile). For the evaluation of linearity, five different concentrations of standard solutions were prepared in the concentration range of 80-120% with correlation coefficient of 0.96 for Niacin and 0.98 for 1-MN. Accuracy of the method ascertained by recovery studies (Table-1) and found to be accurate. Precision of method (Table-2) ascertained by % RSD (0.98) N & (1.8)1-MN. Method robustness was checked with the system suitability after six subsequent injections coefficient of variation of the both analytes are 2.8 for Niacin and 2.1 for 1-Methylnicotinamide.

CONCLUSION

The proposed HPLC method is simple, linear, accurate, precise, sensitive and reproducible. Thus the developed method can be easily used for the pharmacokinetic studies, bioavailability studies, drug interaction studies of Niacin and 1-Methylnicotinamide in biological fluids within a short analysis.

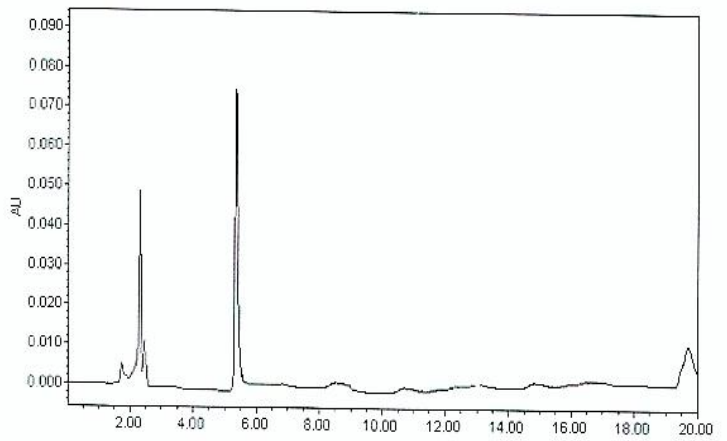


Fig- 1 HPLC chromatogram for Niacin (RT 5.5 min) and its metabolite 1-Methyl nicotinamide (RT 2.5 min)

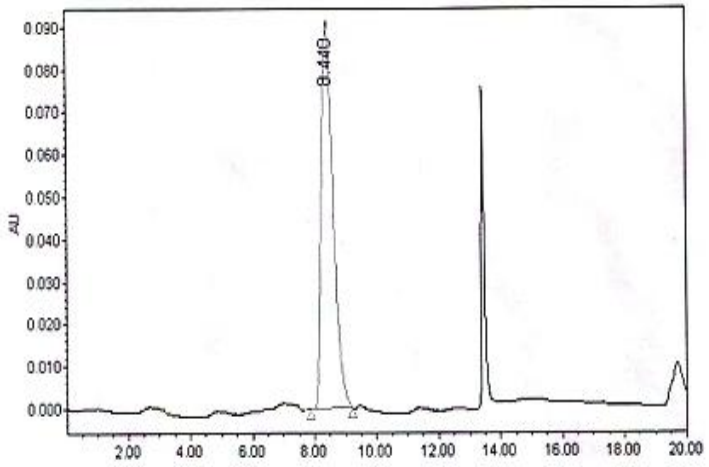


Fig-2 HPLC chromatogram for Niacin (RT 13.9 min) and its metabolite 1-Methyl nicotinamide (RT 8.4 min)

Table-1: Accuracy (Percentage recovery studies)

Amount Added (%)	% Recovery of Niacin	% Recovery of 1-MN
80	100.58	100.21
100	99.830	100.06
120	99.960	99.990

Table-2: Precision

	Niacin (n=5)	1-MN (n=5)	Acceptance
Mean	1444.69	969.68	--
%RSD	0.98	1.80	NMT 2

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*** Corresponding Author**
N.Nagi Reddy
Asst.Professor, Dept.Of Pharmaceutical Analysis
Tallapadmavathi College of pharmacy
Orus, Warangal, Andhra Pradesh-506002, India