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Analysis and stability study of myristyl nicotinate in dermatological preparations by high-performance liquid chromatography

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Abstract

Myristyl nicotinate is an ester prodrug under development for delivery of nicotinic acid to skin for treatment and prevention of conditions that involve skin barrier impairment such as chronic photodamage and atopic dermatitis or for mitigating skin barrier impairment that results from therapy such as retinoids or steroids. The formulation stability of myristyl nicotinate is crucial because even small amounts of free nicotinic acid cause skin flushing, an effect that is not harmful but would severely limit tolerability. We report here reversed-phase HPLC methods for the rapid analysis of myristyl nicotinate and nicotinic acid in dermatological preparations. Because of the large differences in polarity, myristyl nicotinate and nicotinic acid were analyzed by different chromatographic conditions, but they can be rapidly extracted from cream formulations using HPLC mobile phase as a solvent followed by HPLC analysis in less than 10 min. The methods were validated in terms of linearity, precision and accuracy and mean recovery of myristyl nicotinate from topical creams ranged from 97.0–101.2%. Nicotinic acid at levels of 0.01% in the formulations could be quantified. Stability studies show that myristyl nicotinate formulations are stable at room temperature for 3 years with less than 0.05% conversion to nicotinic acid. These methods will be effective for routine analysis of myristyl nicotinate stability in dermatological formulations.

Keywords: Myristyl nicotinate; Nicotinic acid; HPLC; Dermatological formulations; Skin barrier function

1. Introduction

The skin plays many roles in maintaining homeostasis including providing barrier protection from the environment [1]. Impairment of the barrier function of skin is associated with a number of pathological conditions that include skin photodamage [2,3] and atopic dermatitis [4,5]. Additionally, front line therapeutic agents for dermatology conditions including retinoids [6] and steroids [7] can cause skin barrier impairment as a side effect of therapy. Myristyl nicotinate, a nicotinic acid derivative developed for optimal delivery of nicotinic acid to skin [8], has been shown to strongly enhance skin barrier function [9]. This agent is under development for treatment of conditions with impaired barrier function and/or to mitigate the side effects of current therapies that impair barrier function. While nicotinic acid enhances skin barrier function by multiple mechanisms, a major limitation to its use in therapy is a skin flushing effect that results from a vasodilation property that is harmless but significantly lowers its tolerability threshold [10]. Myristyl nicotinate is converted to nicotinic acid by skin esterases but avoids skin flushing by slowing release into the skin to keep nicotinic acid levels below the threshold for vasodilation [8]. However, the stability of myristyl nicotinate in dermatological formulations is crucial as conversion of even small amounts of myristyl nicotinate to nicotinic acid in the formulation can lead to skin flushing. We report here rapid methods for determination of myristyl nicotinate and nicotinic acid in dermato-

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logical preparations and application of these methods to stability studies of dermatological formulations containing myristyl nicotinate.

2. Experimental

2.1. Chemicals and reagents

Myristyl nicotinate was provided by Niadyne, Inc., (Tucson, AZ, USA). Trifluoroacetic acid (TFA) and nicotinic acid were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Acetonitrile HPLC grade was purchased from EMD Chemicals Inc. (Gibbstown, NJ, USA). Brij-58, glyceryl monostearate, cetostearyl alcohol, white petrolatum, sorbic acid, butylated hydroxytoluene, simethicone, sorbitol 70% solution, propylene glycol and polyethylene glycol were purchased from Spectrum Chemical Mfg. Corp. (Gardena, CA, USA). Double distilled deionized water was used.

2.2. HPLC instrumentation and chromatographic conditions

2.2.1. Myristyl nicotinate

An HPLC system consisting of Varian Pro-star solvent delivery system, model 230 (Varian Chromatography systems, CA, USA) was connected to a UV/Visible Spectroflow 757 absorbance detector (ABI, NJ, USA) and HP 3395 integrator (Hewlett Packard, DE, USA). The injector was fitted with a loop of 50 μ1. The chromatographic separation was carried out under isocratic reversed-phase conditions on a Phenomenex Nucleosil 5 μ C18 100A, 250 mm × 4.6 mm column (Phenomenex, CA, USA). The detection wavelength was 254 nm at 0.1 a.u.f.s. The mobile phase was a mixture of 0.01% trifluoroacetic acid (TFA) and acetonitrile (2:98 v/v) and the flow rate was 2.0 ml/min. The mobile phase was filtered through a 0.45 μm membrane filter (Advantec MFS Inc., CA, USA).

2.2.2. Nicotinic acid

Analysis of nicotinic acid was performed on the same HPLC system described above with chromatographic separation using a 10 μm C18 μBondapak, 300 mm × 3.9 mm column (Waters, CA, USA). The mobile phase used was water/acetonitrile/ trifluoroacetic acid (TFA) (1980:20:1 v/v) at a flow rate of 1.0 ml/min. The detection wavelength was 263 nm.

2.3. Cream preparations

Three different cream formulations containing 5% myristyl nicotinate were used. Formulations 1 and 2 were prepared by a contract research laboratory (Shine & Pretty, CA, USA) and used for longer-term stability studies. Formulations 1 and 2 were developed for commercial applications while Formulation 3 was developed for studies for methods development and shorter-term stability studies.

Formulation 1 contained: water, octinoxate, octisalate, zinc oxide, PPG-3-myristyl ether heptanoate, myristyl nicotinate, cetearyl alcohol, dicetyl phosphase, glycerin, ceteth-10 phosphate, propylene glycol, panthenol, steareth-10, sodium hyaluronate, methylparaben, propylparaben, DEA-oleth-3-phosphate, citric acid, cetylhydroxyethylcellulose, tocopheryl acetate, potassium sorbate, fragrance, disodium EDTA, Aloe Barbadensis extract, Diazolidinyl urea, Yellow 5, and RED 40.

Formulation 2 contained: water, myristyl nicotinate, propylene glycol, glyceryl stearate, jojoba oil, ethylhexyl palmitate, squalane, hydrogenated polydecene, shea butter oil, ethyl macadamiate, glycerin, kernel flour, tromethamine, phenoxyethanol, panthenol, sodium hyaluronate, tocopheryl acetate, carbomer, dimethicone, potassium sorbate, fragrance, disodium EDTA, methylparaben, propylparaben, ethylparaben, butylparaben, red 40, yellow 6 and yellow 5.

Formulation 3 was prepared using the following components: water, propylene glycol, sorbitol 70%, sorbic acid, butylated hydroxytoluene, simethicone, white petrolatum, cetostearyl alcohol, Brij-58, glyceryl monostearate, polyethylene glycol and myristyl nicotinate. The ratio of the oil phase to the aqueous phase was (28:72 w/w). The water phase was mixed and dissolved in one container at 65–75 °C, and the oil phase was melted and mixed in another container at 65–75 °C using a water bath. The oil phase was then added to the water phase and mixed until cream was formed using an IKA mixer model RW 20DZM (IKA-works Inc. NC, USA). The cream was cooled to room temperature while stirring.

2.4. Extraction procedures

2.4.1. Myristyl nicotinate analysis

A cream sample of 100 mg was weighed into a 50 ml conical centrifuge tube using a Mettler balance model PB-303S (Mettler–Toledo, Switzerland). The sample was dissolved in 50 ml of mobile phase (Section 2.2.1) by vortex mixing for 4 min and 1.5 ml was transferred to a microcentrifuge tube and centrifuged at 10,000 rpm for five minutes using Eppendorf microcentrifuge (Brinkmann Inst Inc., NY, USA). Samples were prepared in triplicate and 50 μ1 of the supernatant was injected directly into the HPLC.

2.4.2. Nicotinic acid analysis

A cream sample of 50 mg was weighed into a 50 ml conical centrifuge tube and dissolved in 50 ml mobile phase (Section 2.2.2) by vortex mixing for two minutes. A sample of 6 ml was filtered through a pre-equilibrated 600 mg Alltech C18 column (Alltech, IL, USA), and the fourth ml from the elute solution was collected for analysis by HPLC. Samples were prepared in triplicate and 50 μ1 of the supernatant was injected directly into the HPLC.

2.5. Quantification

2.5.1. Standard solutions

A stock solution containing 1.0 mM of myristyl nicotinate was prepared by dissolving 15.95 mg in 50 ml of mobile phase used for myristyl nicotinate analyses (Section 2.2.1). A 1.0 mM nicotinic acid stock solution was prepared by dissolving 12.3 mg

in 100 ml of mobile phase used for nicotinic acid analyses (Section 2.2.2). Serial dilutions of these standard solutions were made to construct calibration curves.

2.5.2. Calibration curves

A calibration curve was constructed by injecting samples containing myristyl nicotinate at concentrations ranging from 10 to 500 μM. The peak area was determined and plotted versus the concentration of myristyl nicotinate. For nicotinic acid, the calibration curve was constructed in the concentrations ranged from 0.5 to 80 μM.

2.6. Recovery

The recovery of myristyl nicotinate was determined as a percentage of the theoretical drug recovered from the 5% myristyl nicotinate cream in Formulations 1, 2, and 3. For nicotinic acid, the recovery was determined as a percentage of the theoretical drug recovered from 0.1, 0.25, and 0.5% nicotinic acid spiked into cream Formulation 1.

2.7. Stability studies

Portions of Formulation 3 were divided into two parts and subjected to short-term stability studies at room temperature ($22 \pm 1\,^{\circ}C$) and $40 \pm 2\,^{\circ}C$. Samples were taken in triplicate and analyzed for the concentration of myristyl nicotinate at time 0, 1,2, 3, 4, 5, and 6 months. Formulations 1 and 2 were subjected to long-term stability studies at room temperature and samples were analyzed for myristyl nicotinate and nicotinic acid at 0, 3, 6, 9, 12, 18, 24, and 36 months.

3. Results and discussion

3.1. Chromatographic conditions

Reversed-phase HPLC methods with UV detection were developed for determination of myristyl nicotinate and nicotinic acid in topical dermatological creams. Due to the great difference in polarity of myristyl nicotinate and nicotinic acid, it proved more convenient to use two different sets of chromatographic conditions to achieve analysis of both components. The mobile phase for myristyl nicotinate analysis of 0.01% trifluoroacetic acid (TFA): acetonitrile (2:98 v/v) gave a well resolved and sharp peak for myristyl nicotinate with a retention time of approximately 8.0 min with no interference from other components of the cream formulations as shown in Fig. 1. The mobile phase for nicotinic acid analysis of water: acetonitrile: TFA (1980:20:1 v/v/v) also gave a sharp peak for nicotinic acid with a retention time of approximately 3.8 min as shown in Fig. 2. Under these conditions, the limit of detection was as low as 3.2 μg/ml for myristyl nicotinate and 6.2 ng/ml for nicotinic acid.

3.2. Method validation

3.2.1. Linearity

To determine linearity, six standard solutions were injected in triplicate into the HPLC. The peak area versus concentration was determined in the concentration range from 10 to 500 μM for myristyl nicotinate and 0.5 to 80 μM for nicotinic acid. Statistical analysis using least squares regression indicated excellent linearity both for myristyl nicotinate and nicotinic acid as shown in Tables 1 and 2, respectively.

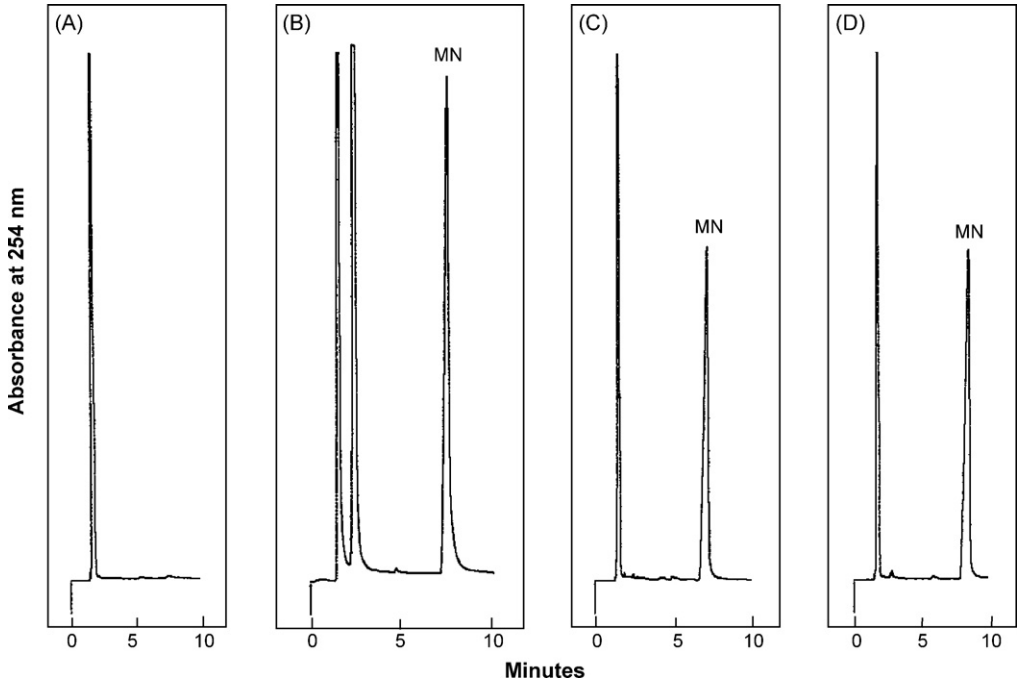

Fig. 1. HPLC chromatograms for (A) a placebo cream without myristryl nicotinate; (B) Formulation 1; (C) Formulation 2; and (D) Formulation 3. The elution position of myristyl nicotinate is shown as MN.

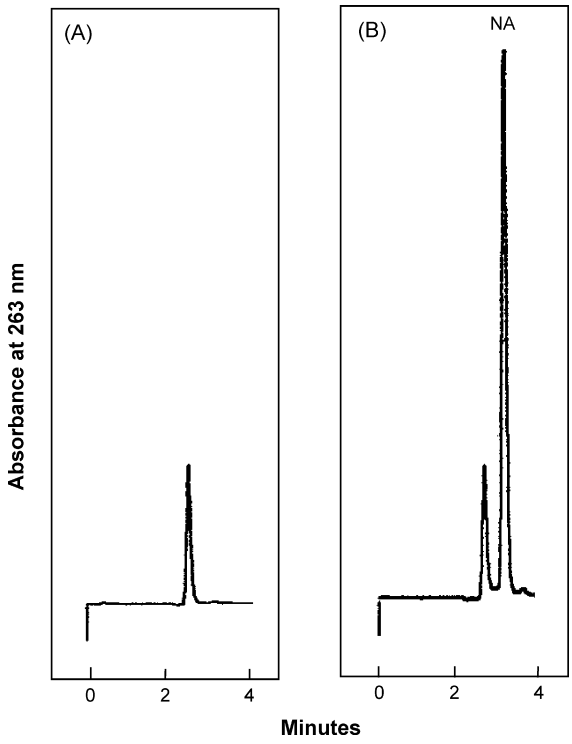

Fig. 2. HPLC chromatograms for (A) freshly prepared Formulation 1 and (B) Formulation 1 stored at room temperature for 24 months. The elution position of nicotinic acid is shown as NA.

3.2.2. Recovery

The recovery of myristyl nicotinate was calculated as percentage of the theoretical concentration recovered from cream formulations 1,2, and 3. As shown in Table 3, the mean recovery was found to be 99.5% of the theoretical concentration of myristyl nicotinate. The recovery of nicotinic acid was calculated as percentage of theoretical concentration recovered from three concentrations of nicotinic acid spiked in Formulation 1. The results shown in Table 4 shows recovery of nicotinic acid ranged from 101.0–102.6% of the theoretical concentration.

3.2.3. Accuracy and precision

The accuracy of this method was determined as the percentage of the theoretical drug concentration recovered from a 5% myristyl nicotinate cream in Formulations 1, 2, and 3 as shown in Table 3. The mean value for myristyl nicotinate recovered from the three cream formulations was found to be 4.97%, which accounts for 99.5% of the theoretical concentration of myristyl nicotinate in the cream formulations. The coefficients of variance were 3.0, 2.5, and 7.3% for Formulations 1, 2, and 3, respectively, indicating both good accuracy and precision of the method. The key issue for analysis of nicotinic acid was the ability to accurately quantify low levels of nicotinic acid that could be derived from myristyl nicotinate hydrolysis as levels of nicotinic acid as low as 0.2% can result in skin flushing [8]. The accuracy and precision of determination of low levels of nicotinic acid was evaluated by replicate analysis of cream Formulation 1 that had been spiked at 0.1, 0.25, and 0.5 g% as shown in Table 4. The values determined were very close to theoretical and the coefficient of variance of the analyzed samples ranged from 4.0–4.9%, indicating good accuracy and precision for the detection of even relatively low levels of nicotinic acid derived from myristyl nicotinate hydrolysis.

3.2.4. Specificity and selectivity

The selectivity of the assay for myristyl nicotinate is shown in the chromatograms in Fig. 1. Panel A shows Formulation 3 from which myristyl nicotinate had been omitted showing that no detectable peaks were observed at the elution position of myristyl nicotinate. Panels B, C and D show representative chromatograms of Formulations 1, 2 and 3, respectively. Fig. 2 illustrates the ability to detect nicotinic acid derived from hydrolysis of myristyl nicotinate during long-term storage of Formulation 3. Panel A shows the formulation at the time of manufacture, and panel B shows an analysis of a sample in which hydrolysis of myristyl nicotinate was observed. The peak of nicotinic acid shown represents nicotinic acid at 0.04% in the formulation.

Table 1
Statistical analysis of linear regression of myristyl nicotinate

Concentration (μM)	Area 1	Area 2	Area 3	Area 4	Area 5	Area 6
10	47998	51034	45237	46104	44962	44872
15.625	56961	60708	56320	59206	55195	57619
31.25	109503	107654	108937	110040	109895	111576
62.5	208353	207682	209145	224805	214553	207612
125	401406	406018	397096	419147	418690	429733
250	804276	791949	822583	805502	773963	823012
500	1560961	1553267	1618743	1568654	1587638	1608675
Slope	3105	3080	3225	3111	3136	3203
Intercept	14558	16501	7219	19970	12276	14050
r^2	0.9998	0.9999	0.9999	0.9997	0.9996	0.9998
95% Confidence intervals						
Slope	3062 to 3148	3052 to 3109	3181 to 3271	3053 to 3169	3061 to 3213	3148 to 3258
Y-intercept when $X = 0.0$	5192 to 23920	10290 to 22710	−2619 to 17060	7363 to 32580	−4297 to 28850	2002 to 26100
X-intercept when $Y = 0.0$	−7.776 to −1.657	−7.417 to −3.322	−5.337 to 0.8046	−10.60 to −2.338	−9.347 to 1.349	−8.243 to −0.6181

Table 2
Statistical analysis of linear regression of nicotinic acid

Concentration (μM)	Area 1	Area 2	Area 3	Area 4	Area 5	Area 6
0.5	6163	6867	6874	10603	8846	9809
1	12305	11179	11680	15830	14837	14335
2	22100	24841	26183	26970	26643	26208
4	49037	45388	44589	51178	51949	52314
8	91311	91317	89934	102456	101841	99319
10	115329	112588	119938	121003	124296	122173
40	490370	453883	445896	511782	491962	523248
80	981740	993498	978963	1014530	1003429	1006745
Slope	12300	12291	12129	12677	12479	12646
Intercept	−2669	−6162	−3830	1232	1014	1774
r^2	0.9999	0.9983	0.9990	0.9999	0.9998	0.9996
95% Confidence intervals						
Slope	12190 to 12410	11790 to 12800	11570 to 12600	12560 to 12790	12350 to 12610	12400 to 12890
Y-intercept when $X = 0.0$	−6169 to 830.8	−22250 to 9927	−20840 to 12110	−2496 to 4961	−3132 to 5162	−6177 to 9726
X-intercept when $Y = 0.0$	−0.0679 to 0.4991	−0.8270 to 1.770	−1.027 to 1.684	−0.3934 to 0.1959	−0.4161 to 0.2495	−0.7779 to 0.4831

Table 3
Accuracy, precision and relative error of HPLC analysis of myristyl nicotinate from cream formulations

Cream formulation	Theoretical concentration (g%)	Recovered concentration (g%)	S.D. (g%)	C.V. (%)	Accuracy recovery (%)	R.E. (%)
1	5.0	5.01	0.15	3.0	100.2	0.2
2	5.0	4.85	0.12	2.5	97.0	−3.0
3	5.0	5.06	0.37	7.3	101.2	1.2
Mean		4.97			99.5	

$N = 5$.

Table 4
Accuracy, precision and relative error of HPLC analysis of nicotinic acid spiked in Formulation 1

Theoretical concentration (g%)	Recovered concentration (g%)	S.D. (g%)	C.V. (%)	Accuracy recovery (%)	R.E. (%)
0.10	0.101	0.004	4.0	101.0	1.0
0.25	0.255	0.011	4.3	102.0	2.0
0.50	0.513	0.025	4.9	102.6	2.6

$N = 5$.

3.2.5. Intra-day accuracy and precision

The intra-day accuracy and precision of the assay for myristyl nicotinate was measured by analyzing five replicates of 2.5, 5.0, and 10.0 g% quality control cream samples of Formulation 1. Intra-day accuracy of the method for myristyl nicotinate ranged from 101.2 to 105.6%, while the intra-day precision ranged from 2.3 to 3.7% as shown in Table 5.

3.2.6. Inter-day accuracy and precision

The inter-day precision of the assay for myristyl nicotinate was measured by analyzing 15 replicates of 2.5, 5.0, and 10.0 g% quality control cream samples of Formulation 1 obtained from day 1 to 3. The inter-day accuracy ranged from 101.4 to 102.0%, while the inter-day precision ranged from 0.79 to 1.87% as shown in Table 6.

3.3. Stability studies

3.3.1. Shorter-term stability studies

Stability studies were performed on Formulation 3. The effect of temperature was also examined. The method was applied to analysis of myristyl nicotinate cream formula at each time point in triplicate. The results shown in Fig. 3 demonstrate that myristyl nicotinate in the cream formula-

Table 5
Intraday accuracy, precision and relative error of myristyl nicotinate in cream quality control samples of Formulation 1

Theoretical concentration (g%)	Recovery mean (g%)	S.D. (g%)	Precision as C.V. (%)	Accuracy (%)	R.E. (%)
2.5	2.64	0.10	3.7	105.6	5.6
5.0	5.06	0.12	2.4	101.2	1.2
10.0	10.14	0.24	2.3	101.4	1.4

$N = 5$.

Table 6
Inter-day accuracy, precision and relative error of myristyl nicotinate in cream quality control samples of Formulation 1

Theoretical concentration (g%)	Recovery mean (g%)	S.D. (g%)	Precision as C.V. (%)	Accuracy (%)	R.E. (%)
2.5	2.55	0.02	0.82	102.0	2.0
5.0	5.07	0.04	0.79	101.4	1.4
10.0	10.13	0.19	1.87	101.3	1.3

$N = 5$.

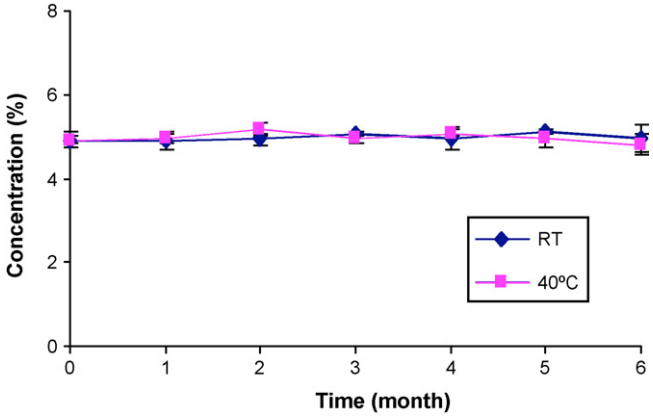

Fig. 3. Myristyl nicotinate concentration as a function of time in Formulation 3 at room temperature (RT) and at 40 °C for 6 months.

tion is stable at both at room temperature and at 40 °C for 6 months.

3.3.2. Longer-term stability studies

Formulations 1 and 2 were stored at room temperature and analyzed for both myristyl nicotinate and nicotinic acid for a period of three years. Although these formulations are very complex, the method was able to determine myristyl nicotinate without any interference from any of these constituents

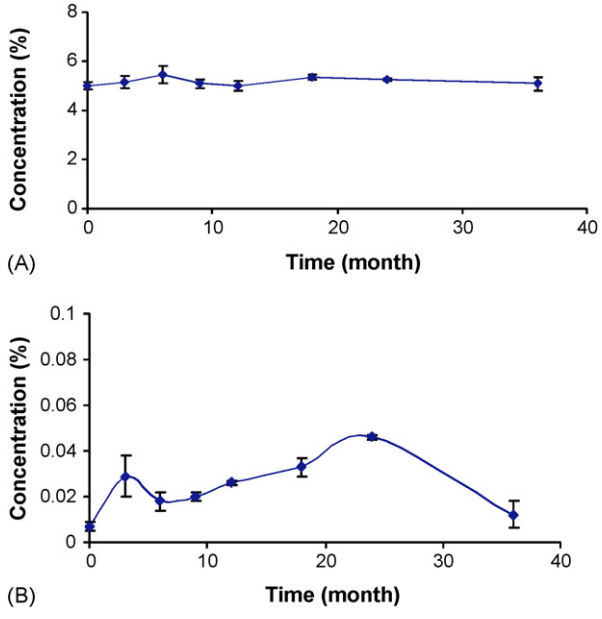

Fig. 4. Concentration of myristyl nicotinate (A) and nicotinic acid (B) as a function of time for Formulation 1 at room temperature for 36 months.

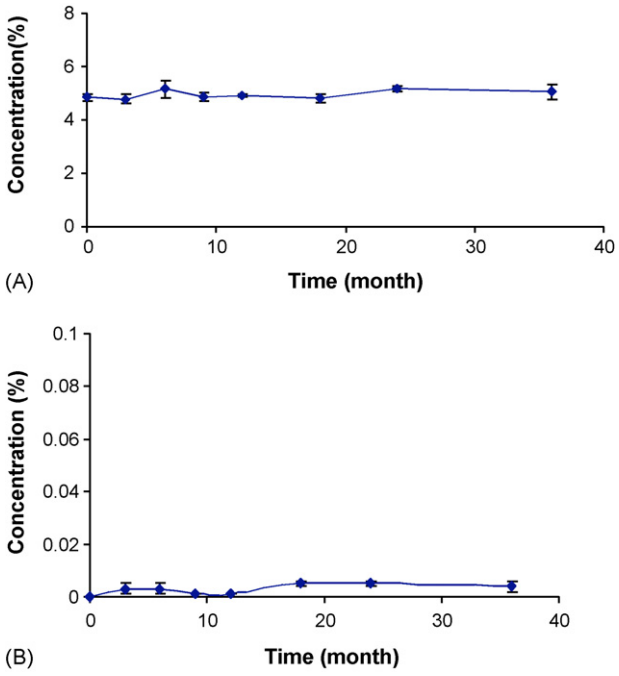

Fig. 5. Concentration of myristyl nicotinate (A) and nicotinic acid (B) as a function of time for Formulation 2 at room temperature for 36 months.

(Fig. 1). The results indicated that both Formulations 1 and 2 show excellent stability for up to three years as demonstrated by Figs. 4A and 5A. The concentration of nicotinic acid was also determined in these stability studies as shown in Figs. 4B and 5B. The results show that the formation of nicotinic acid was very low in both formulations, although slightly more nicotinic acid was observed in Formulation 1 compared to Formulation 2. The highest amount of nicotinic acid detected was 0.05% for Formulation 1 and 0.01% for Formulation 2. The variability observed for Formulation 1 in Fig. 5B can be attributed to variability between different containers of the material.

4. Conclusions

Simple and rapid methods with good selectivity, accuracy, and precision will enhance the development of myristyl nicotinate as an agent to mitigate conditions where skin barrier impairment is present. The ability to detect small amounts of hydrolysis of myristyl nicotinate to nicotinic acid is crucial to this development to avoid compromising compliance due to skin flushing. Stability studies indicate that dermatological formulations of myristyl nicotinate of acceptable stability are achievable.

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References

[1] D.J. Tobin, Chem. Soc. Rev. 35 (2006) 52–67.
[2] B.A. Gilchrest, Br. J. Dermatol. 135 (1996) 867–875.
[3] M. Robert, V. Bissonauth, G. Ross, M. Rouabhia, Int. J. Radiat. Biol. 75 (1999) 317–326.
[4] D.W. Kim, J.Y. Park, G.Y. Na, S.J. Lee, W.J. Lee, Int. J. Dermatol. 45 (2006) 698–701.
[5] B. Melnik, J. Hollmann, G. Plewig, Br. J. Dermatol. 119 (1988) 547–549.
[6] H. Tagami, T. Tadaki, M. Obata, J. Koyama, Br. J. Dermatol. 127 (1992) 470–475.
[7] J.S. Kao, J.W. Fluhr, M.Q. Man, A.J. Fowler, J.P. Hachem, D. Crumrine, S.K. Ahn, B.E. Brown, P.M. Elias, K.R. Feingold, J. Invest. Dermatol. 120 (2003) 456–464.
[8] E.L. Jacobson, H. Kim, M. Kim, G.T. Wondrak, M.K. Jacobson, in: D.S. Alberts, Hess, M. Lisa (Eds.), Fundamentals of Cancer Prevention, Springer–Verlag, Berlin Heidelberg, 2005, pp. 139–160.
[9] E.L. Jacobson, H. Kim, M. Kim, J.D. Williams, D.L. Coyle, W.R. Coyle, M.S. Stratton, G. Grove, R. Rizer, M.K. Jacobson, submitted for publication.
[10] R.H. Knopp, J. Ginsberg, J.J. Albers, C. Hoff, J.T. Ogilvie, G.R. Warnick, E. Burrows, B. Retzlaff, M. Poole, Metabolism 34 (1985) 642–650.