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Review

Chromatographic and capillary electrophoretic methods for the analysis of nicotinic acid and its metabolites

Masahiro Iwaki, Etsuko Murakami, Kazuaki Kakehi*

Faculty of Pharmaceutical Sciences, Kinki University, 3-4-1 Kowakae, Higashi-osaka, Osaka 577-8502, Japan

Abstract

Methods for the assay of nicotinic acid (NiAc) and its metabolites in biological fluids using high-performance liquid chromatography (HPLC) and capillary electrophoresis (CE) are reviewed. Most of the references cited in this review concern HPLC methods. A few CE methods that have been recently reported are also included. As these compounds are relatively polar and have a wide range of physico-chemical properties, the sample pre-treatment or clean-up process prior to analysis is included. Most HPLC methods using an isocratic elution system allow determination of a single or few metabolites, but gradient HPLC methods enable simultaneous determination of five to eight compounds. Simultaneous determination of NiAc including many metabolites in a single run can be achieved by CE. We also discuss the pharmacokinetics of NiAc and some of its metabolites.

Keywords: Reviews; Nicotinic acid; Nicotinamide

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1. Introduction

Nicotinic acid (niacin, NiAc) belongs to the hydrophilic vitamin B complex and is a component of nicotinamide adenine dinucleotide (NAD), which is biosynthesized through nicotinamide ($NiNH_2$). Although NiAc and $NiNH_2$ are similar in their function as vitamins, the pharmacological effect of NiAc differs from that of $NiNH_2$. NiAc is a hypolipidemic agent and possesses vasodilating and fibrinolytic properties [1–3], while $NiNH_2$ has no effect on plasma lipids [3]. A high dose of NiAc (2–6 g/day)

*Corresponding author. Tel.: +81-6-6721-2332 (Ext. 3822); fax: +81-6-6730-1394.

E-mail address: k_kakehi@phar.kindai.ac.jp (K. Kakehi).

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shows hypolipidemic activity and is widely used for treatment of hyperlipidemia [4–6]. However, use of NiAc often causes adverse effects such as an intense cutaneous flush involving the face and the upper part of the body. Gastrointestinal disturbances, diarrhoea, and dyspepsia are also common, and peptic ulceration has been reported. Abnormality of the hepatic function, which occurs in patients taking large doses of NiAc, is reversible when the drug is discontinued. Sustained-release preparations of NiAc frequently cause gastrointestinal irritation and hepatic toxicities compared with regular preparations [3]. As administration of $NiNH_2$ results in fewer incidences of flushing [7], these side effects after NiAc intake may be ascribed to NiAc itself and/or formed nicotinuric acid (NiUAc).

The metabolic pathways of NiAc are well documented. As shown in Fig. 1, NiAc is metabolized via two pathways: the first is the metabolic route to NiUAc through nicotinyl CoA by glycine conjugation, and the second is that to $NiNH_2$, which is utilized in NAD synthesis. The latter metabolite is further transformed to *N*-methylnicotinamide (MNA), nicotinamide *N*-oxide (NiOx) and two pyridone derivatives [*N*-methyl-4-pyridone-3-carboxamide (4-Pyr) and *N*-methyl-2-pyridone-5-carboxamide (2-Pyr)] through methylation and/or oxidation. The pyridones are considered to be the end products of NiAc and $NiNH_2$ metabolism in mammals [8].

When NiAc or $NiNH_2$ are ingested as a vitamin in microgram or miligram amounts per day, their plasma concentrations are extremely low. Thus, their urinary metabolites are commonly determined to evaluate niacin status. Niacin status is usually determined by the analysis of two major urinary metabolites, 2-Pyr and MNA. The weight ratio of 2-Pyr to MNA is regarded as an important index of NiAc status.

There have been extensive investigations on the pharmacological effectiveness of NiAc in lipid metabolism and the wide use of NiAc for treatment of hyperlipidemia, but few pharmacokinetic studies for this compound have been published. To study the hypolipidemic action and determine the unknown mechanism for the side-effects of NiAc, it is necessary to establish an easy assay method to measure NiAc and its metabolites in biological samples such as plasma and urine, because some of the metabolites of NiAc may have some pharmacological and toxicological effects.

In the early stages of NiAc determination, a wide variety of techniques including microbiological and colorimetric methods, paper chromatography, thin-layer chromatography (TLC), conventional column chromatography, and gas–liquid chromatography

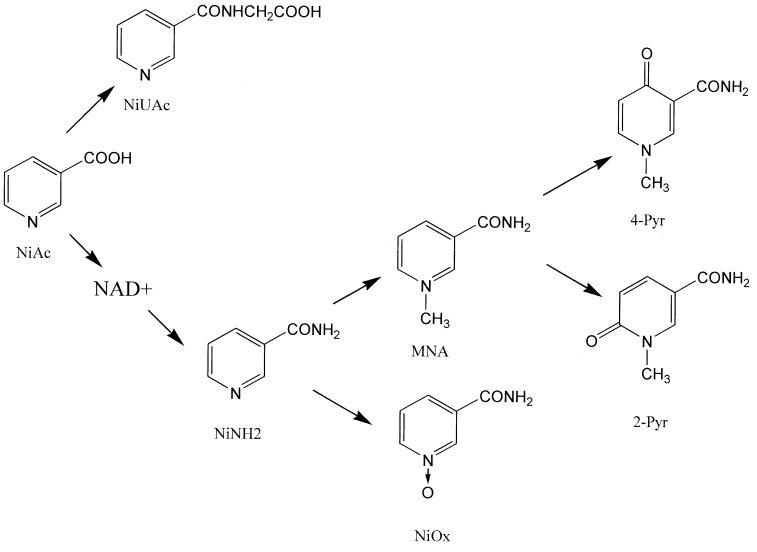

Fig. 1. Possible metabolic pathways of nicotinic acid.

(GLC) were used. Microbiological methods [9,10] are sensitive but not selective. The colorimetric method based on the König reaction [11] suffers from interference from $NiNH_2$ and other NiAc metabolites. Carlson [12] improved this photometric method by the pre-separation of NiAc using TLC, although the sensitivity was not high. Paper chromatography [13], TLC [14] and liquid chromatography using an anion-exchange column [15] are time-consuming for routine analysis. GLC requires derivatization of NiAc to a volatile compound [16,17].

Over the past two decades, HPLC methods have been most commonly employed to determine NiAc and its analogous compounds in food [18,19], infant milk [20], compound feed [18,21–23], meats [17], coffee [24] and cigarette tobacco [25], as well as in pharmaceutical and multivitamin products [26–31]. Capillary electrophoresis (CE) has been recently developed for simultaneous determination of NiAc, $NiNH_2$ and their related compounds in pharmaceutical formulations [32].

Both HPLC and CE make it possible to determine NiAc and $NiNH_2$ and their analogous compounds simultaneously after a relatively simple clean-up procedure. Any clean-up procedure for the determination of NiAc and its metabolites in biological samples faces some important problems. Liquid–liquid extraction is not appropriate due to hydrophilicity of the analytes at all pHs. Additionally, the related compounds of NiAc (see Fig. 1) include acids (NiAc and NiUAc), bases ($NiNH_2$) and neutral compounds (NiOx and both pyridones). These differences in physico-chemical properties as well as their polarity make it difficult to clean up the biological samples and to separate them using a single mode of separation. It should also be noted that the absorption spectra and pK_as of the metabolites vary and their extinction coefficients depend on the pH of the media (Table 1), suggesting that the sensitivity for these compounds will change with the pH of the mobile phase or background electrolyte.

This paper reviews the HPLC and CE methods for the measurement of NiAc and its related compounds in biological fluids as sample matrices, and the pharmacokinetic properties of NiAc after pharmacological dosing, published in the literature since the early 1980s. The reviewed references were selected by on-line searching of the Medline database and Chemical Abstract services. Only papers written in English, except for a few Japanese papers, are considered. Standard abbreviations in this Journal are used in the review. All abbreviations used are listed in Section 5.

2. Chromatographic analyses for NiAc and its metabolites in biological fluids

2.1. Sample pre-treatment

In the quantitative assay for drugs or metabolites in biological fluids, sample pre-treatment is commonly required especially in plasma or serum samples. In CE analyses, sample pre-treatment often can be avoided, but simplifies the washing processes of capillaries between electrophoretic runs, improves the reproducibility and prevents peak broadening in some cases [33].

When NiAc and its metabolites in biological samples are analyzed by HPLC, polar interfering

Table 1
Extinction coefficients of pyridine derivatives in various media

Compound	Water		0.1 *M* NaOH		1 *M* HCl	
	λ_{max} (nm)	ε_{max} ($\times 10^3$)	λ_{max} (nm)	ε_{max} ($\times 10^3$)	λ_{max} (nm)	ε_{max} ($\times 10^3$)
NiAc[a]	214, 260	na[c]	na	na	na	na
2-Pyr[b]	261	12.3	261	11.6	261	11.9
4-Pyr[b]	259	12.6	259	12.2	242	8.01
6OH–NiAc[b]	254	13.0	266	15.3	262	14.5

[a] Data from Ref. [59].
[b] Data from Ref. [66].
[c] Not available.

Table 2
Summary of published HPLC methods for biological samples

Compound	Sample matrix	Pretreatment	Column	Mobile phase	Detection method	Ref.
NiAc, NiUAc	Plasma, urine	Deproteinization with acetone followed by $CHCl_3$ washing	ODS (300×4 mm, 5 μm) ion-paired (two columns in series for urine)	water–MeOH (9:1) with TBAp (50 mmol/l)	UV 254 nm	[43]
$NiNH_2$	Plasma, urine	SPE (Sep-Pak C_{18})	ODS (250×4 mm, 10 μm) ion-paired	4.446 g sodium dioctylsulfosuccinate (pH 2.5)–MeOH (1450:1050)	UV 254 nm	[51]
NiAc, NiUAc, $NiNH_2$	Plasma	Deproteinization with acetone followed by $CHCl_3$ washing	SCX (500×2.1 mm, 25–37 μm) (cation-exchange)	0.02 *M* phosphate buffer (pH 2.6 for NiAc and pH 2.8 for metabolites)	UV 260 nm	[45]
NiAc, $NiNH_2$, NiUAc, MNA, 2-Pyr	Urine	Filtration	ODS (150×4.6 mm), linear gradient ion-paired	solvent A: 10 m*M* of PSA, TMA, KH_2PO_4 solvent B: PSA, TMA, ACN	UV 254 nm	[46]
2-Pyr, MNA	Urine	SPE (ion-exchange)	ODS (150×4 mm, 5 μm), ion paired	buffer (containing 0.15 *M* acetate, pH 5.0 and 0.01 *M* sodium heptanesulfonate)–MeOH (91:9)	UV 254 nm	[54]
NiAc	Serum	Deproteinization with acetone followed by derivatization (DCCI ester)	ODS (150×4 mm, 5 μm), ion-paired	ACN–water (4:6) containing 5 m*M* sodium 1-hexanesulphonate	FL: Ex 325 nm, Em 395 nm	[38]
MNA	Urine	Derivatization (1-methyl-7-phenyl-1,5-dihydro -5-oxo-1,6-naphthyridine)	SCX (150×4.6 mm, 7-8 μm), (cation-exchange)	0.04 *M* ammonium phosphate, monobasic–ACN (1:3)	FL: Ex 382 nm, Em 440 nm	[56]
MNA	Urine	Derivatization (1-methyl-7-phenyl-1,5-dihydro -5-oxo-1,6-naphthyridine)	SCX (150×4.6 mm, 7 μm)	25 m*M* KH_2PO_4 containing 20% ACN (pH 4.5)	FL: Ex 382 nm, Em 440 nm	[57]
2-Pyr, $NiNH_2$	Tissue, blood	Extraction with diethyl ether at alkaline condition	ODS (150×4.6 mm, 5 μm)	10 m*M* potassium dihydrogenphosphate –ACN (96:4), pH 4.5	UV 260 nm	[8,36]
NiAc, NiUAc	Blood, urine	Deproteinization with PCA	ODS (150×4.6 mm, 5 μm)	10 m*M* phosphate buffer containing TBAb (pH 7.0)–ACN (100:9)	UV 260 nm	[42]
$NiNH_2$, 2-Pyr, 4-Pyr	Urine	Extraction with diethyl ether at alkaline condition	ODS (250×4.6 mm, 7 μm)	10 m*M* potassium dihydrogenphosphate –ACN (96:4), pH 3.0	UV 260 nm	[35]

NiAc (as metabolite of esters)	Tissue homogenates	SPE (phenyl–silica)	ODS (250×4 mm, 5 μm) ion-paired	MPS buffer (8.6×10^{-3} *M*, pH 7.4) containing 10% ACN and 9.3×10^{-4} *M* *n*-decylamine	UV 262 nm	[53]
NiAc, $NiNH_2$	Gastrointestinal contents, blood	SPE (NH_2 and C_{18} in series)	ODS (250×4.6 mm, 5 μm)	–		[34,48]
MNA	Plasma, urine	Derivatization (1-methyl-7-phenyl-1,5-dihydro -5-oxo-1,6-naphthyridine)	ODS (150×4.6 mm, 5 μm)	0.01 *M* heptanesulfonic acid, 0.5% triethylamine and 22% ACN in water (pH 3.2)	FL: Ex 366 nm, Em 418 nm	[55]
$NiNH_2$, NiOX, 6OH-$NiNH_2$, NiAc, NiUAc, MNA, 2-Pyr, 4-Pyr	Plasma, urine	Deproteinization with MeOH	base-deactivated reversed-phase (250×4.6 mm, 5 μm) linear gradient	Solvent A: 7 m*M* heptanesulphonic acid, 5 m*M* orthophophoric acid, 2 m*M* trimethylamine, 5 m*M* potassiumdihydrogen orthophosphate, Solvent B: 55% ACN, 25% MeOH, 20% water for plasma or 75%ACN and 25% water for urine	UV 260 nm	[41]
NiAc	Plasma	Deproteinization with ACN	CN (150×4.6 mm, 10 μm)	ACN–MeOH–H_2O –acetic acid (700:150:150:1)	UV 263 nm	[40]
NiAc, $NiNH_2$, NiUAc	Plasma	SPE (Bakerbond spe 7090-03)	ODS (150×6 mm, 5 μm) ion-paired	10 m*M* phosphate buffer (pH 2.1) containing 10 m*M* octanesulfonic acid –ACN (97:3)	UV 254 nm	[52]
NiAc, NiUAc	Urine	SPE (cation-exchange)	ODS (250×4.6 mm, 5 μm) ion-paired	10 m*M* potassium phosphate buffer [containing 5 m*M* TBAp (pH 7.0)] /ACN (90:10)	UV 254 nm	[49]
NiAc	Plasma	Deproteinization with acetone–H_2O (2:1, v/v) and evaporated	ODS (250×4 mm, 5 μm) ion-paired	10 m*M* MOPS buffer (pH 5.4) –ACN (25:75) containing 1 m*M* *n*-dodecylamine	UV 262 nm	[39]
NiAc, $NiNH_2$	Intestinal and liver tissue	Post-column derivatization	ODS (250×4.6 mm, 5 μm) linear gradient ion-paired	Solven A: 5 m*M* TBAp Solvent B: MeOH, 90:10–70:30	UV 260 nm	[58]
NiAc, $NiNH_2$	Biological fluids	SPE (C_{18})	ODS (250×4.0 mm, 5 μm) linear gradient	0.05 *M* CH_3COONH_4 –MeOH (5:95–30:70)	UV 270 nm	[50]

components may show overlapping peaks. Due to the low solubility of these compounds in hydrophobic solvents, liquid–liquid extraction is not applied to separate the analytes from the interfering endogenous components in plasma and urine. Kollenkirchen and Harmeyer [34] indicated that NiAc is virtually unextracted with chloroform or methylene chloride at both pH 2 and 10, whereas 47% of $NiNH_2$ is recovered by extraction with chloroform at pH 10. The mixture of diethyl ether–EtOH (83:17, v/v) can extract 56% of NiAc at pH 2 and 65% of $NiNH_2$ at pH 10. Shibata et al. [35] showed that 4-Pyr and 2-Pyr, as well as $NiNH_2$, are soluble in diethyl ether under alkaline conditions, and employed diethyl ether as the solvent for extraction of $NiNH_2$, 2-Pyr, and 4-Pyr from urine [8,35,36]. Hirayama et al. [37] employed ethyl acetate for the analysis of $NiNH_2$ in urine.

Table 2 summarizes the conditions of HPLC analyses that are reviewed in this article. In the third column of the table, methods for sample pre-treatment are briefly mentioned. Deproteinization is often used for plasma and serum. Most methods for NiAc in plasma or serum samples advocate deproteinization with acetone or acetone–water [38,39], acetonitrile (ACN) [40], methanol (MeOH) [41] or perchloric acid (PCA) [42]. In some methods [38,43,44], deproteinization with acetone followed by extraction with chloroform is used to remove lipophilic components from the aqueous layer containing NiAc, although the method seems time-consuming. Takikawa et al. [45] pointed out that the aqueous layer obtained by these procedures was not sufficiently cleaned up for the HPLC analysis of NiAc. They suggested that the aqueous layer should be acidified to change the carboxyl group of NiAc into the unionized form.

Urine commonly includes small amounts of protein, and the drug and metabolites of interest sometimes show sufficiently high concentration for analysis, especially after drug administration. This sometimes allows direct injection of urine sample when endogenous substances do not interfere with the peaks of interest. The urine aliquots filtered through a membrane (0.2–0.4 μm pore size) were directly analyzed only after dilution. The methods were applied to the analysis of $NiNH_2$ and metabolic products by HPLC [46], and to the analysis of NiAc and its metabolites by CE [47].

For the pre-treatment of biological fluids, solid-phase extraction (SPE) was applied to isolate the compounds from the sample matrices. Kollenkirchen and Harmeyer [48] studied the recovery of NiAc and $NiNH_2$ from gastrointestinal contents after passage through various SPE columns. They found that Sep-Pak C_{18} and BondElut NH_2 provided the higher recovery (more than 80% for both compounds) compared with that using strong cation-exchange and carbonic acid phases, whilst BondElut C_8 provided the best recovery (97%) for $NiNH_2$. They used Sep-Pak C_{18} and BondElut NH_2, mounted in series for further clean-up, and achieved recovery of ca. 75% for both compounds. BondElut SCX was used to extract NiAc and NiUAc from urine [49], this being sufficiently effective to clean up urine samples (see Fig. 2a). Other SPE techniques using Sep-Pak C_{18} were reported for the analysis of NiAc and $NiNH_2$ in pharmaceutical preparations and biological fluids [50], and MNA in plasma and urine [51]. Bakerbond spe 7090-03 was used for the analysis of NiAc, NiUAc and $NiNH_2$ in plasma [52]. Phenyl bonded silica was used for NiAc [53], and an ion-exchange column for 2-Pyr and MNA in urine [54]. However, SPE techniques seem difficult to apply overall to metabolites, because the metabolites are polar and have different charges at different pHs. Multi-SPE cartridges mounted in series will be an alternative for extraction of all NiAc metabolites from sample matrices.

2.2. High-performance liquid chromatography

Many HPLC methods for the determination of NiAc and its related compounds using either UV absorbance and fluorimetric detection have been published. A summary of the HPLC methods reviewed in this article is also shown in Table 2. Of the 22 methods, seven were devoted specifically to urine, seven were to blood or plasma/serum, six to both specimen types, one to tissue homogenate and one to tissue and blood. Because NiAc and its metabolites have different pK_as and hydrophobic properties, it is difficult to analyse for all of the analytes simultaneously by HPLC using a single

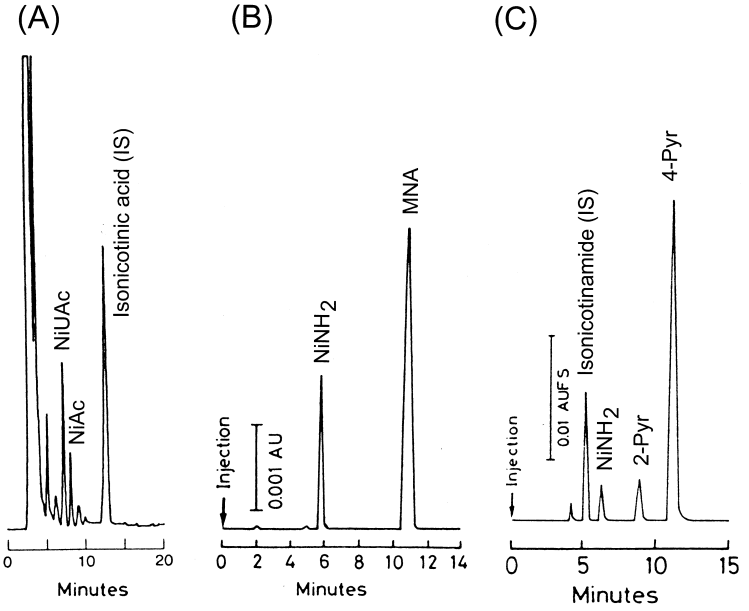

Fig. 2. (A) Representative chromatogram of a rat urine sample drawn 2–3 h after intravenous administration of 15 mg/kg of NiAc. Conditions: column, Inertsil ODS-2 (250×4.6 mm I.D., 5 μm); mobile phase, 10 m*M* potassium phosphate buffer containing 5 m*M* TBAp (pH 7.0)–ACN (90:10, v/v); flow-rate, 1.0 ml/min; detection, UV 254 nm; column temperature, 35°C. From Ref. [49]. (B) Representative chromatogram of the extract of rat liver sample containing 447 pmol of $NiNH_2$ and 1460 pmol of MNA. Conditions: column, 5-ODS-H (150×4.6 mm I.D., 5 μm); mobile phase, 10 m*M* potassium dihyrogenephospahte–ACN (94:6, v/v, pH 4.5); flow-rate, 1.0 ml/min; detection, UV 260 nm; column temperature, 25°C. From Ref. [36] with permission. (C) Representative chromatogram of the extract of rat urine sample containing 1.88 pmol of $NiNH_2$, 0.31 pmol of 2-Pyr and 4.27 pmol of 4-Pyr. Conditions: column, 7-ODS-L (250×4.6 mm I.D., 7 μm); mobile phase, 10 m*M* potassium dihyrogenphospahte–ACN (94:6, v/v, pH 3.0); flow-rate, 1.0 ml/min; detection, UV 260 nm; column temperature, 25°C. From Ref. [35] with permission.

mode of separation in a single run. Therefore, a combination of several modes of separation is required to investigate the metabolism or pharmacokinetics of NiAc, $NiNH_2$, and their metabolites (see Fig. 2). Furthermore, NiAc and all of its metabolites contain at least one nitrogen atom in their molecule, which often causes severe tailing in the reversed-phase separation mode. The low hydrophilicity of these compounds results in very fast elution from a reversed-phase column. Therefore, the ion-pair mode is usually employed. The mechanism for separation of the acids or the amides in the ODS column is based on the formation of ion-pairs between the acids and tetrabutylammonium [42,43,49], *n*-dodecylamine [39], *n*-decylamine [53] or 1-hexanesulphonate [38] and between the amides and octanesulfonate [52], dioctylsulfosuccinate [51] or heptanesulfonate [54,55].

Generally, it is difficult to separate NiAc and its analogous compounds using an isocratic elution system. Although there are several papers on the analysis of NiAc and its metabolites, two HPLC methods using linear gradient elution show it is possible to determine five (NiAc, $NiNH_2$, NiUAc, MNA and 2-Pyr) [46] and eight compounds (NiAc, $NiNH_2$, NiUAc, MNA, NiOx, 2-Pyr, 4-Pyr and 6OH–$NiNH_2$) [41] simultaneously in biological samples. However, Shibata [42] reported that the former method was not appropriate for the analysis of biological samples due to the presence of interfering peaks. Stratford and Dennis [41] suggested that the limiting factor in the separation was NiOx, because of its high polarity combined with zero net charge. NiOx could not be analyzed in an ion-pairing mode as it is only slightly retained.

The ion-pair mode in combination with reversed-

phase ODS-bonded columns has been commonly used. However, Pelzer et al. [40] reported that separation was improved using a CN-bonded column with mobile phase composed of AcCN–MeOH–water–acetic acid (700:150:150:1, v/v). Strong cation-exchange columns were also successfully used [45,56,57].

UV absorption detection has been generally employed. The detection limit for NiAc was around 0.1 μg/ml using 0.5 ml of plasma [39] and 0.1 ml of urine [49], respectively. The sensitivity is enough high for therapeutic drug monitoring after administration of pharmacological doses of NiAc. An increase in the sample volume or reduction in the final reconstitution volume somewhat improve sensitivity; however, such procedures may result in the appearance of interfering peaks. To determine the concentrations of endogenous NiAc, $NiNH_2$, and their metabolites in plasma and urine, fluorometric detection after fluorescent labelling has been developed. Tsuruta et al. [38] found that *N*,*N*′-dicyclohexyl-*O*-(7-methoxycoumarin-4-yl)methylisourea (DCCI) reacted with NiAc in acetone at high-temperature to yield a fluorescent ester that could be separated by HPLC, and provided a sensitive method for the NiAc assay in a small amount of serum using reversed-phase HPLC. The detection limit in serum was 0.2 nmol (ca. 20 ng/ml) using 0.1 ml of serum. Stein et al. [58] pointed out that the method reported by Tsuruta et al. [38] was still not suitable for the determination of NiAc in physiological fluids. Thus, they developed a double post-column derivatization technique in ion-pair reversed-phase mode using gradient elution to improve the sensitivity, where NiAc and $NiNH_2$ were converted to highly UV absorbing derivatives by the modified König reaction. This method was applied to the analysis of NiAc and $NiNH_2$ in rat intestinal tissue. MNA was converted to 1-methyl-7-phenyl-1,5-dihydro-5-oxo-1,6-naphthyridine [55–57] to achieve higher sensitivity. The derivatization procedure is based on the reaction of *N*-alkylpyridinium compounds with ketones in alkaline media. The methods developed by Hirayama et al. and Shibata [56,57] were used for the analysis of MNA in urine using cation-exchange chromatography. Somogyi et al. [55] pointed out that separation in this mode resulted in broad peaks and required high concentrations of MeOH and ACN as the eluent. Thus, they solved this problem by using ion-pair chromatography coupled with a reversed-phase ODS column, resulting in a sensitive and specific assay for endogenous MNA in both plasma and urine.

2.3. Capillary electrophoresis

CE is an attractive technique for the determination of drugs and/or their metabolites in complicated sample matrices such as plasma, serum or urine. Analysis is sometimes performed using a diluted sample solution without any clean-up procedure. NiAc is metabolized to at least seven metabolites, including the parent drug. To determine these metabolites in plasma and/or urine, CE seems an especially powerful technique, since the metabolites of NiAc and $NiNH_2$, which have different charges and physico-chemical properties, can be analyzed simultaneously. Over the last several years a couple of papers [39,47] concerning simultaneous determination of NiAc and its metabolites in biological fluids by CE as well as a paper [59] for total NiAc determination in concentrated yeast spreads have appeared.

Simple zone electrophoresis was employed for the analysis of NiAc and its metabolites in plasma samples after deproteinization [39]. Micellar electrokinetic chromatography (MEKC) for urine samples has also been reported [47]. In simple zone electrophoresis using 10 m*M* sodium borate solution, the separation among NiAc, NiUAc and OH–NiAcs is satisfactory; however, no separation is observed among $NiNH_2$, NiOx and MNA. The change of this background electrolyte to ACN–10 m*M* potassium dihydrogenphosphate buffer, pH 2.5 (1:9, v/v) produced improvement in the separation between NiAc and its metabolites, though the migration times of NiOx and 6OH–NiAc were longer (21.1 and 27.0 min, respectively) (see Fig. 3a). We attempted to separate eight possible metabolites of NiAc in urine [47]. A simple zone electrophoretic mode based on the difference in basicity of the nitrogen atom in the pyridine ring of NiAc and its metabolites failed to separate NiOx, 2-Pyr and 2OH–NiAc. On the other hand, MEKC in alkaline borate buffer (50 m*M*, pH 9.0) containing 150 m*M* SDS allowed a simultaneous determination for NiAc, $NiNH_2$, and their six

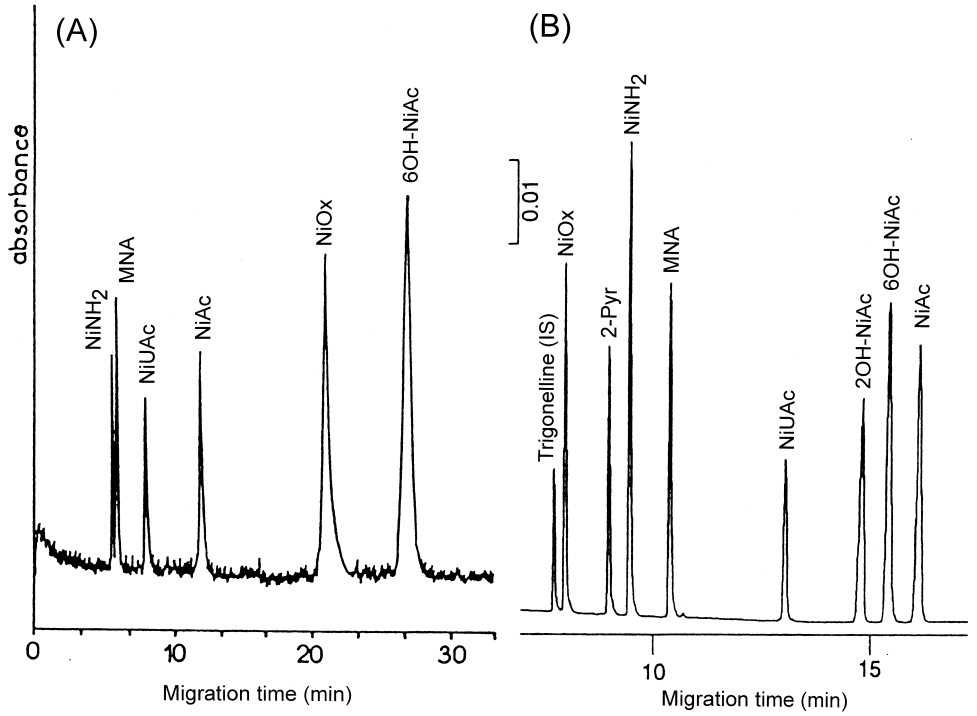

Fig. 3. (A) Electropherogram of a standard solution containing 20 μg/ml of each compound. Conditions: capillary, fused-silica (57 cm×50 μm I.D.); running buffer, ACN–10 m*M* potassium dihydrogenephosphate (pH 2.50); applied voltage, 25 kV; detection, UV 254 nm; injection, 7 s (hydrostatic method); temperature, 25°C. From Ref. [39] with permission. (B) Electropherogram of a standard solution containing 100 μg/ml of each compound by MEKC. Conditions: capillary, fused-silica (60 cm×75 μm I.D.); running buffer, 50 m*M* sodium tetraborate buffer (pH 9.0) containing 150 m*M* SDS; applied voltage, 15 kV; detection, UV 214 nm; injection, 30 s (hydrostatic method); temperature, 37°C. From Ref. [47].

possible metabolites in urine after only dilution (see Fig. 3b). The detection limits of both CE and MEKC methods were about 1 μg/ml for NiAc and $NiNH_2$. At present, CE methods are not sufficiently sensitive for the determination of endogenous levels of these compounds, but quite useful for the routine analysis in drug monitoring of NiAc such as for the treatment of hyperlipidemia.

3. Pharmacokinetics of nicotinic acid and its metabolites

Despite the wide use of NiAc for treatment of hyperlipidemia and the detailed investigation on metabolic pathways of NiAc, few pharmacokinetic studies for this compound have been published. After NiAc ingestion in humans [13,15] and in animals [13,60–62] NiUAc is recovered as a major metabolite from urine. The qualitative and quantitative metabolism of NiAc depends on the species and the dose, though NiUAc is a prominent metabolite at relatively low doses in most species. Biotransformation of NiAc to NiUAc is proposed as the saturable process [62]. Although early studies on NiAc disposition [5,6,61,63] have used rather nonspecific and insensitive measurement methods, using a specific HPLC method [43], Ding et al. [64] reported that NiAc was subject to nonlinear elimination kinetics in man. Very few attempts have been made to perform pharmacokinetic studies on NiAc. We showed that the time profiles of NiAc concentrations in plasma and urine after intravenous administration to rats [62] and rabbits [65] were adequately described by the two-compartment model including the ''pooled'' Michaelis–Menten elimination process. With increas-

ing doses of NiAc in rats, the ratio for NiUAc to unchanged drug excreted in urine decreased markedly from 4.5 at 2 mg/kg to 0.37 at 45 mg/kg while the renal clearance of NiAc remained constant [62]. This clearly shows that the glycine conjugation process is saturable, as suggested by Ding et al. [64].

Some information on plasma NiUAc disposition after administration is available in rats and rabbits [62,65]. In both these animals, plasma NiUAc is subject to linear elimination kinetics and is excreted mainly as the unchanged form in urine in the dosing ranges of 3–45 mg/kg. NiUAc rapidly appears in plasma/serum after intravenous NiAc injection to rats and rabbits, but its concentration is lower than the parent drug at any dosing sizes tested [62,65]. On the other hand, in human subjects the plasma concentrations of the formed NiUAc following NiAc infusion exceed those of the parent drug. There are few pharmacokinetic studies on the metabolites other than NiUAc, although they are identified in urine. The fact that pharmacokinetic data on the metabolites other than NiUAc are not available is due to the lack of sensitive determination methods for them in plasma or blood. The plasma/serum concentration of the metabolites is expected to be several orders of magnitude lower than the urine concentrations. It has been reported that sustained-release preparations of NiAc are more toxic than regular preparations [7], but the mechanism responsible for this phenomenon is unknown. We speculate that this may be due to the nonlinearity of NiAc disposition. To clarify the mechanism of hypolipidemic action and the undesired side effects following NiAc ingestion, a more specific and sensitive analytical method has to be developed.

4. Conclusion

Reversed-phase HPLC methods have been well represented and still offer the best means for the determination of NiAc and its metabolites. Most favoured in terms of number of publications in the time period under review are HPLC techniques, although a few CE techniques have also been presented. Most HPLC methods are capable of detecting NiAc and one or more of its metabolites using UV absorbance detection, although a few HPLC methods have succeeded in the separation of many metabolites using a linear gradient system. Fluorimetric derivatization of NiAc and MNA provided improved sensitivity for the analysis of these endogenous compounds. CE will become a powerful analytical technique for sensitive and selective determination of NiAc and its metabolites. MEKC has been shown to be capable of simultaneously analysing NiAc and its analogous compounds without pretreatment of the sample in a single run. More sensitive CE methods, using highly sensitive detection such as fluorescence detection, will be required to produce precise pharmacokinetic and toxicokinetic studies.

5. List of abbreviations

2-Pyr	*N′*-Methyl-2-pyridone-5-carboxamide
2OH–NiAc	2-Hydroxynicotinic acid
4-Pyr	*N′*-Methyl-4-pyridone-3-carboxamide
6OH–NiAc	6-Hydroxynicotinic acid
ACN	Acetonitrile
CE	Capillary electorophoresis
DCCI	*N*,*N′*-Dicyclohezyl-*O*-(7-methoxycoumarin-4-yl)methylisourea
EtOH	Ethanol
FL	Fluorescence
MEKC	Micellar electrokinetic chromatography
MeOH	Methanol
MNA	*N′*-Methylnicotinamide
MOPS	3-(*N*-Morpholino)propanesulfonic acid
MPS	3-Morpholinopropane sulphonic acid
NAD	Nicotinamide adenine dinucleotide
NiAc	Nicotinic acid
$NiNH_2$	Nicotinamide
NiOx	Nicotinamide *N*-oxide
NiUAc	Nicotiniric acid
ODS	Octadecylsilanized silica gel
PCA	Perchloric acid
PSA	1-Pentansulfonic acid sodium salt
SDS	Sodium dodecyl sulfate
SPE	Solid phase extraction
TBAb	Tetrabuthylammonium bromide
TBAp	Tetrabuthylammonium phosphate
TMA	Tetramethylammonium
UV	Ultraviolet–visible

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