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Quantitation of the niacin metabolites 1-methylnicotinamide and l-methyl-2-pyridone-5-carboxamide in random spot urine samples, by ion-pairing reverse-phase HPLC with UV detection, and the implications for the use of spot urine samples in the assessment of niacin status

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Abstract

A simple ion-pairing reverse-phase HPLC method, with UV diode array detection, was developed and validated for quantitation of the urinary niacin metabolites 1-methylnicotinamide and l-methyl-2-pyridone-5-carboxamide in a single run. Urine samples were purified using a polymer-based mixed mode anion exchange reverse-phase cartridge. Analysis was performed on a reverse-phase C18 column, using a methanol gradient elution system, containing phosphate buffer pH 7.0, 1-heptanesulphonic acid as the ion-pairing agent and trimethylamine as a modifier. The assay was applied to the measurement of the niacin status of two subjects using spot urine samples. The samples were collected over 4 consecutive days and at four time points during 1 day. Status, expressed as the concentration ratios (2-PYR or 1-MN)/creatinine and 2-PYR/l-MN, varied within and between days and was least for fasting samples. This work illustrates the potential of spot urine sampling for niacin status assessment, but highlights the need for further validation prior to its use in field nutritional surveys.

Keywords: Niacin; Metabolism; Human; Urine; Spot sampling; 1-Methylnicotinamide; l-Methyl-2-pyridone-5-carboxamide; HPLC

1. Introduction

Pellagra is a disease caused by a diet deficient in niacin (the generic name for nicotinic acid and nicotinamide) and tryptophan, or by an inability to absorb and process these nutrients. Metabolism of dietary tryptophan, via the kynurenine pathway [1], leads to the formation of niacin. The process is not very efficient (a conversion rate of 60 mg tryptophan to 1 mg niacin is usually assumed), however given the amounts of tryptophan found in some foods; it can be an important source. Zinc, iron, riboflavin and vitamin B6 dependent enzymes are involved in the pathway and deficiencies in these may also contribute to pellagra. Although pellagra is rare nowadays, mainly due to awareness and food fortification, it remains an issue in situations where food is not fortified and diets are restricted, for example in disaster situations [2,3]. In addition, there is increasing interest in niacin status due to its possible association with HIV related conditions [4] and cancer [5]. Clinical signs are only apparent when deficiency is well advanced, are initially difficult to identify, and include changes to the skin, gastrointestinal tract and nervous system. As a consequence, when individuals with pellagra are identified in a population, they usually represent a far greater underlying public health problem and prompt action is required. In order to determine the extent and severity of deficiency, suitable biochemical methods are required to survey at-risk populations. Niacin status is currently assessed by quantification of the major urinary metabolites 1-methylnicotinamide (1-

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MN) and l-methyl-2-pyridone-5-carboxamide (2-PYR) and sometimes the more minor metabolite 1-methyl-4-pyridone-3-carboxamide [3,6,7]. Status is usually expressed as the concentration ratio 2-PYR/l-MN or as the concentrations of the individual metabolites relative to creatinine. Both 2 and 24 h urine collections [8] have been used, however in field studies these are often not practical due to compliance issues, the difficulty of re-finding individuals and households, and the costs associated with solving these problems. Random spot urine sampling, together with the measurement of 1-MN and 2-PYR concentrations, has been suggested as an alternative, as it avoids these issues, and would provide a guide to status [2,7]. However, no studies have actually used random spot sampling for niacin status assessment in populations. In order to investigate the utility of spot urine sampling, a simple HPLC method was developed and validated for the analysis of 2-PYR and 1-MN in urine. The literature contains a number of methods for the analysis of the separate niacin metabolites; however there are very few straightforward assays in which both metabolites are analysed in the same run. The advantages of this assay are that it is simple, uses commercially available purification cartridges and gives baseline resolution for both metabolites. The method was applied to the analysis of spot urine samples collected from two subjects on consecutive days and at regular intervals during 1 day. The relationship between the metabolites and niacin status is discussed.

2. Experimental

2.1. Reagents and standards

Standard 1-methylnicotinamide chloride and 1-methylnicotinamide iodide were purchased from Sigma–Aldrich Company Ltd. (Poole, UK). Standard 2-PYR, was synthesized (Appendix B) by the oxidation of 1-methylnicotinamide iodide [9]. Ion-pairing agent, 1-heptanesulphonic acid sodium salt, was purchased from VWR Int. Ltd. (Lutterworth, UK). All other chemicals were of analytical grade and were purchased from a variety of suppliers.

2.2. Instrumentation and chromatographic conditions

HPLC was performed on a Jasco LC-1500 series system [Jasco (UK) Ltd., Great Dunmow, Essex, UK] incorporating two PU-1580 pumps, an AS-1555-10 autosampler, a CO-1560 column thermostat, a HG-980-30 high pressure mixer, a MD-1510 diode array detector and an in-line DG-980-50 degasser. Analysis was performed using a Luna C18(2) (particle size 5 μm, pore diameter 100 Å) 150 mm × 4.6 mm column, protected with a C18 (ODS) 4(L) × 3.0(D) mm guard cartridge (Phenomenex UK Ltd., Macclesfield, UK) and maintained at 25 °C. All buffers were filtered under reduced pressure, through a 0.2 μm filter (Anodisc 47, Whatman, Maidstone, UK), prior to use. Purified urine sample or standard (25 μL) was injected onto the column. Elution was performed at a flow rate of 1 mL/min using a binary system consisting of solution (A) (10 mmol/L pH 7.0 sodium phosphate buffer, containing 7.00 mmol/L sodium 1-heptanesulphonic acid and 4.00 mmol/L trimethylamine hydrochloride) and solution (B) [20%, v/v methanol in solution (A)]. The column was eluted isocratically with 5% B for 10 min and with a linear gradient from 5 to 40% B over a further 15 min. The column was cleaned by increasing B to 100% over 5 min and re-equilibrated by decreasing B to 0% over 5 min and holding at 0% B for 10 min. The total run time was 45 min. Eluate was monitored at 195–650 nm and integrated at 265 nm.

2.3. Standard curve preparation

Concentrated standard solutions of 2-PYR (6.6 mmol/L) and 1-methylnicotinamide chloride (1-MNC1; 5.8 mmol/L) were prepared in water, stored at −80 °C, and diluted in mobile phase (A) prior to analysis. Standard curves were prepared for 1-MN and 2-PYR over the range from 0.3 to 579 μmol/L and 0.3 to 657 μmol/L, respectively. Curves were fitted using the Lorentzian robust minimization (Table Curve 2D, Systat Software Inc., Richmond, CA, USA) procedure.

2.4. Urine sample collection

Urine samples were provided by subjects in good health, who consumed western style omnivorous diets and some of whom took vitamin supplements. Samples were collected in mid-flow, in sterile containers without preservative, aliquoted and stored frozen at −80 °C. A pooled urine sample from seven subjects was used for developmental and validation studies. The pool was generated by combining equal volumes of urine from each subject. Stability studies were conducted with urine samples from three subjects. To monitor changes in the output of niacin metabolites with time, urine samples were collected from two subjects over 4 consecutive days. On each day, an overnight fasting urine sample was collected early in the morning and on day 4, additional non-fasting urine samples were collected at 3-h intervals over 9 h (total of three additional samples). Any use of vitamin supplements was noted.

2.5. Purification of urine samples for analysis

Each urine sample was vortex mixed, an aliquot (1.00 mL) taken and adjusted to pH 7.4–8.5 by addition of microlitre aliquots of 1 mol/L NaOH. Following each addition, the sample was remixed and the pH measured by addition of 5 μL to a pH indicator strip (VWR International Ltd., Lutterworth, Leicestershire, UK). The sample was transferred to a preconditioned [methanol (2 mL) and water (3 mL)] mixed-mode anion exchange-reversed phase 3 mL 60 mg Oasis MAX extraction cartridge (Waters Ltd., Elstree, Herts, UK), eluted (0.5 mL/min) under reduced pressure and the eluate collected. The cartridge was eluted with water (1 mL), to ensure that all

of the sample had passed onto the solid phase, and a further aliquot of water (5 mL). The water eluates were combined with the sample eluate, frozen at −80 °C, and lyophilised. The lyophilised material was dissolved in mobile phase (A) (0.5 mL) and the solution added to a 1 mL volumetric flask. A further aliquot of mobile phase A (0.4 mL) was used to wash out the container and this was also added to the volumetric flask. The sample solution was made up to 1 mL and syringe filtered (Puradisc, 0.2 μm, 13 mm, nylon filter media, Whatman, Maidstone, UK) into an injection vial. Samples were maintained at 9 °C in the auto-sampler until analysis.

2.6. Intra and intersample precision

Nine aliquots of a pooled urine sample were prepared for analysis as described above. One aliquot was analysed 10 times to give a measure of intrasample precision and the remaining eight aliquots were analysed once to measure intersample precision.

2.7. Measurement of recovery from urine and artificial urine

Aliquots (1.00 mL) of pooled urine were spiked with either 0, 10, 25 or 50 μL aliquots of a solution of a mixture of 1-MNCl (1170 μmol/L) and 2-PYR (1328 μmol/L) to give samples, containing an additional 0, 12, 29, 58 μmol/L and 0, 13, 33, 66 μmol/L, respectively, of each metabolite. Each concentration of spiked urine was prepared in triplicate, purified and analysed as described.

Artificial urine [10] was spiked with 2-PYR (6.57 mmol/L) and 1-MNCl (5.79 mmol/L) and serially diluted to give samples, containing 657, 329, 164, 82, 41, 21, and 10 μmol/L and 579, 290, 145, 72, 36, 18, and 9 μmol/L, respectively, of each metabolite. Each concentration of spiked artificial urine was prepared in triplicate, purified and analysed as described.

2.8. Limits of detection and quantification

Limits of detection and quantification were measured using standards diluted in mobile phase (A). Limits of detection were calculated on the basis of three times the baseline noise. Limits of quantification for 2-PYR and 1-MNCl, were calculated from precision profiles (% CV versus analyte concentration) constructed from eight standard curves, containing 3–12 replicates for each analyte concentration, and setting a maximum acceptable CV of 5%. Limits were confirmed by the measurement of standards (five replicates per concentration) at concentrations of 0.1 and 0.5 μmol/L for 2-PYR, and 0.4 and 2.6 μmol/L for 1-MN.

2.9. Stability studies of 1-MN and 2-PYR in urine

To test the stability of 1-MN and 2-PYR in urine samples, single aliquots (1 mL) of urine samples from three subjects were stored in the dark at 37, 23 (room temperature), 2–8 and −20 °C for 0, 24 and 48 h. After storage for the required time, samples were purified and lyophilised. The lyophilised material was stored at −80 °C and analysed as above.

2.10. Effect on recoveries of volume of urine loaded onto extraction cartridge

To confirm that the extraction cartridges were not overloaded by the volume of urine used, a pooled urine sample was adjusted to pH 8.1 by the addition of 1 mol/L NaOH and single aliquots of 0.25, 0.5, 0.75, 1.0, 1.25, 1.50 and 2.0 mL were purified and analysed as above.

2.11. Range of linearity between volume of purified urine injected and peak area

To confirm that the volume of purified urine injected onto the column came within the range over which the peak areas for 1-MN and 2-PYR were directly proportional to the volume loaded, a purified pooled urine sample was analysed using injection volumes of 5, 10, 35, 45, 55, 75 and 100 μL.

2.12. Analysis of urine samples from inter and intra-day niacin metabolite excretion study

Urine samples were analysed for 2-PYR and 1-MN as above, and for creatinine by Camelia Botna Laboratories, Great Ormond Street, London WC1N 3JH, using Vitros CREA slides.

3. Results

3.1. HPLC analysis of 2-PYR and 1-MN in urine

A typical chromatogram is shown in Fig. 1. Baseline resolution was obtained for the 2-PYR and the 1-MN peaks. Peak identification and purity were based on photodiode array spectroscopic data, retention time and co-elution with standards. To measure the recoveries from urine, a pooled urine sample, containing 2-PYR (132 μmol/L) and 1-MNCl (29 μmol/L), was spiked with 2-PYR at 0, 13, 33, 66 μmol/L and 1-MNCl at 0, 12, 29, 58 μmol/L. Each spiked solution was prepared in triplicate and following analysis gave recoveries of ≥98%. Artificial urine [10] was also prepared and spiked with a range of concentrations of 2-PYR (10–657 μmol/L) and 1-MNCl (9–579 μmol/L). The solutions were prepared in triplicate and following purification and analysis, gave recoveries of 94–99% for 2-PYR and 104–111% for 1-MN. Intersample variation was assessed by the analysis of 9 aliquots of a pooled urine sample and was 4.4 and 4.6% for 2-PYR and 1-MN, respectively. Intrasample variation was measured by the analysis of a single aliquot of purified urine 10 times and was 1.5 and 1.9% for 2-PYR and 1-MN, respectively. Limits of detection (LOD) and quan-

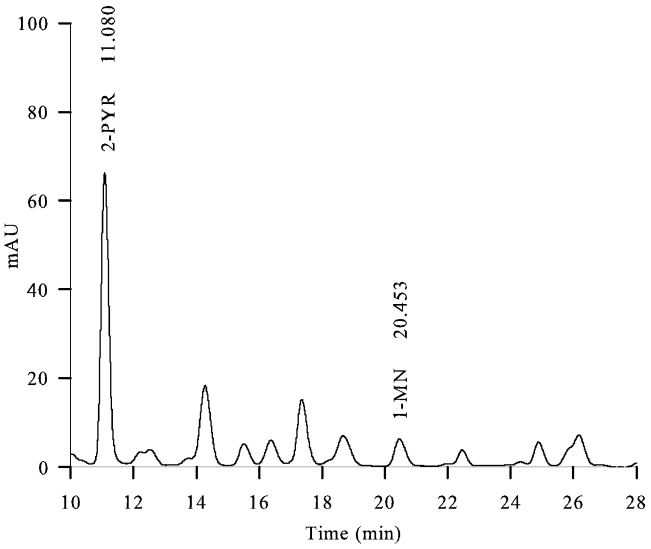

Fig. 1. Typical HPLC chromatogram of a purified urine sample.

tification (LOQ) were calculated for each analyte, and were confirmed by measuring standards prepared at these concentrations (five replicate measurements per standard). For 2-PYR, LOD and LOQ were 0.1 (9.5% CV) and 0.5 (3.6% CV) μmol/L, respectively, and for 1-MN, were 0.4 (16.4% CV) and 2.6 (1.0% CV) μmol/L, respectively. To investigate the effect of urine volume on recovery from the extraction cartridges, a range of urine volumes (0.25–2.0 mL) were loaded. Quantities of 2-PYR and 1-MN measured were directly proportional ($r^2 = 0.999$) to the volume of urine loaded. Measured concentrations of 2-PYR and 1-MN were also directly proportional ($r^2 = 0.999$) to sample injection volume over the range 5–75 μL. Standard curves were constructed for 1-MNCl and 2-PYR using an injection volume of 25 μL and were linear from the limit of quantification to 579 and 657 μmol/L, respectively. Standard curve fit data is summarized in Tables 1 and 2.

Stability of 2-PYR and 1-MN in urine (with no added preservative) from 3 subjects was studied over 48 h at 37, 23 (room temperature), 2–8 and −20 °C. There were no changes in the concentrations of 2-PYR and 1-MN with time or temperature.

3.2. Inter and intra-day variation in urinary niacin metabolites

Fig. 2 shows the results for the analysis of urine samples collected from two subjects to investigate inter and intra-day variations in 2-PYR and 1-MN concentrations. Subject 1 took multivitamin supplements, containing 18 mg (147 μmol) nicotinamide, each morning following collection of the fasting urine sample and subject two consumed niacin-fortified breakfast cereals. Concentrations (relative to creatinine) of 2-PYR and 1-MN, and 2-PYR/l-MN, show considerable inter and intra-day variation for each individual. For example, the intra-day range for 2-PYR/l-MN was 1.6–8.1 and 3.1–7.7 for subjects 1 and 2, respectively. These fluctuations were greatest following the consumption of a mul-

Table 1
Data for 2-PYR standard curves

Standard curve	Range (μmol/L)	No points	Slope	Intercept	Standard error	r^2
1	0.32–329	11	16613	228	12627	1.0000
2	10–329	6	16566	−870	12964	1.0000
3	10–657	7	16527	1066	14742	1.0000
4	0.32–657	10	16835	767	15362	1.0000
5	0.32–657	10	16373	1537	29140	0.9999
6	0.32–657	10	16658	314	22609	1.0000
7	0.32–657	10	16454	879	31700	0.9999
Mean			16575	560		
S.D.			149	772		
%CV			0.9	138		

Table 2
Data for 1-MN standard curves

Standard curve	Range (μmol/L)	No points	Slope	Intercept	Standard error	r^2
1	0.28–290	11	4935	94	3049	1.0000
2	9.05–290	6	4967	−1473	3626	1.0000
3	9.05–579	7	4926	1531	8892	0.9999
4	0.28–579	10	4966	786	7107	0.9999
5	0.28–579	10	4896	561	12494	0.9998
6	0.28–579	10	4916	439	12955	0.9998
7	0.28–579	10	4866	601	18079	0.9997
Mean			4924	363		
S.D.			37	921		
%CV			0.7	254		

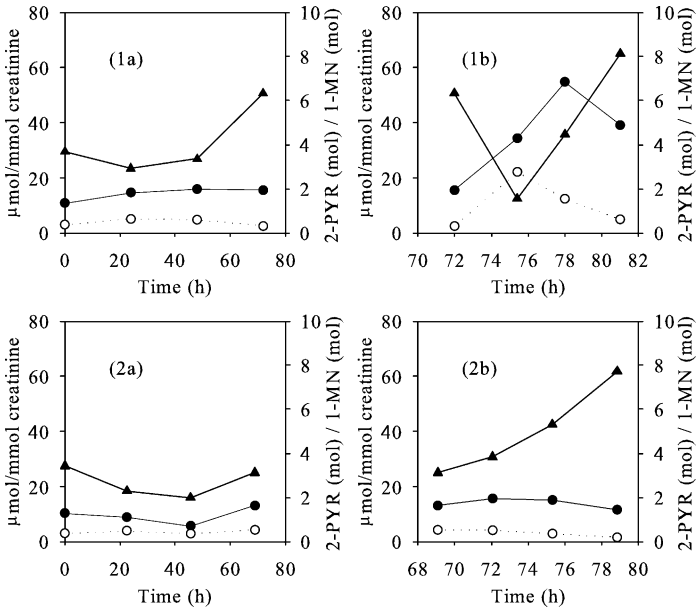

Fig. 2. Inter-day (a) and intra-day (b) excretion profiles for 2-PYR and 1-MN in two subjects 1 and 2. (◯) 1-MN µmol/mmol creatinine, (●) 2-PYR µmol/mmol creatinine, (▲) 2-PYR (mol)/l-MN (mol).

tivitamin supplement by subject 1 (Fig. 2, profile lb). Concentrations of 1-MN and 2-PYR increased rapidly in subsequent urine samples and reached maxima after 3 and 6 h, respectively. As a consequence of the different maximal excretion times, 2-PYR/l-MN decreased rapidly from 6.3 to 1.6, and subsequently, increased to 8.1. In subject 2 (Fig. 2, profile 2b), 2-PYR/l-MN increased steadily during the test day whilst the concentrations of both metabolites, relative to creatinine, slowly fell. With both subjects, the inter-day fasting concentrations of 2-PYR (subject 1, 10.9–15.9 µmol/mmol creatinine; subject 2, 5.7–13.0 µmol/mmol creatinine), 1-MN (subject 1, 2.4–5.0 µmol/mmol creatinine; subject 2, 2.8–4.2 µmol/mmol creatinine) and 2-PYR/l-MN (subject 1, 2.9–6.3; subject 2, 2.0–3.4) remained relatively stable.

4. Discussion

4.1. HPLC analysis of 2-PYR and 1-MN in urine

Analysis of urine by HPLC, for 2-PYR and 1-MN, has been performed using a number of methods and these have been reviewed [11]. The majority use a prior purification step, with an anion exchange resin [12], a mixture of anion and cation exchange resins [13], reversed phase extraction [14] or solvent extraction [15,16]. Analysis has also been performed following enzymatic treatment [17] and with no prior purification [18,19]. UV detection and reversed phase columns with ion-pairing agents, or ion exchange columns [15], have mainly been used. Analysis of 1-MN has also been performed using prior derivatization, with ketones [20] under alkaline conditions, to yield a fluorescent product, which was quantified using a fluorescent detector.

In our approach, development of the HPLC method was based on a literature precedent [17] in which nicotinamide and three metabolites were analysed in a single run, in (β-glucuronidase and sulfatase treated urine samples, using ion-pairing conditions and a base deactivated reverse-phase column. In our hands, even after enzymatic treatment, it was not possible to analyse urine without prior purification due to the number of interfering compounds. Urine samples were purified using a polymer-based mixed mode anion exchange reverse-phase cartridge (chloride form), which retained interfering compounds. In literature examples [12], where an anion exchange resin was used in this manner, the resin was converted to the more active hydroxide form prior to use. In this case, however, the use of the hydroxide form resulted in the hydrolysis of 1-MN in urine samples and as a result was discontinued. Hydrolysis occurred because salts within the urine displaced hydroxide ions and generated a high pH (>11) in the sample front. Following purification, samples were lyophilised and reconstituted back to their original volume in mobile phase. Analysis was performed on a Cl8 reversed phase column using a gradient elution system. A methanol gradient was established using solution (A) (phosphate buffer at pH 7.0, containing 1-heptanesulphonic acid as the ion-pairing agent and trimethylamine as a modifier) and solution (B) (solution (A), containing 20%, v/v methanol). A column cleanup step was used at the end of each run to ensure that there was no carry over.

Validation showed the procedure to be repeatable, robust and to give good recoveries. Transfer of the procedure to a new column, of a different manufacturing batch, gave very similar results. Detection limits were comparable with other similar HPLC assays [18] in which 2-PYR and 1-MN were analysed in a single run. The advantages of this assay were:

Table 3
Guidelines for the interpretation of urinary niacin metabolite concentrations [2,7]

Status	2-PYR μmol/mmol creatinine	1-MN μmol/mmol creatinine	2-PYR/1-MN
Deficient	<1.5	<0.4[a]	<0.5
Low	1.5–2.9	0.4–1.31[a]	<1.0
Acceptable	>3.0	1.32–3.54[a]	1.0–4.0

[a] Adults (males and non-pregnant, 1st trimester pregnancy and non-lactating females).

that it allowed both metabolites to be quantified in a single run with baseline resolution, was relatively simple to perform and made use of commercially available purification cartridges. Urine samples were stable for 48 h at −20 to 37 °C, temperatures similar to those likely to be found in field studies, without the need for added preservative. In a related study [13], it was shown that urine samples, containing 0.16 mol/L boric acid as a preservative, were stable for 7 days at 30, 4 and −20 °C.

4.2. Establishment of niacin status using spot urine sampling

This study was carried out to investigate the suggestion that spot urine samples could be used to measure niacin status in situations where 2 and 24 h urine collections were not feasible. Such situations are frequently encountered in field surveys, especially in developing countries and disaster-affected populations. The results are shown in Fig. 2. Table 3 gives the current guidelines [2,7] for the interpretation of urinary niacin metabolite data collected using 2 and 24 h urine collections. A comparison with the data in Fig. 2 shows that both subjects have a high niacin status and although there is considerable variability, at no time is there any overlap with a lower status.

As with many other static measures of nutrient status, the detected levels of urinary niacin metabolites probably reflect components of recent intake and longer term nutritional status. It has been shown in human subjects on controlled niacin diets (using 24 h urine collections) that changes in 1-MN and 2-PYR concentrations, but not 2-PYR/l-MN, correlated with dietary niacin intake [21]. Concentrations of 1-MN also reflect concentrations of erythrocyte NAD [22]. NAD and NADP are the active anabolic products of niacin and as a result are in theory a more direct measure of functional niacin status. Urinary 1-MN concentrations respond rapidly to changes in niacin dietary intake [22], whereas erythrocyte NAD concentrations respond more slowly and NADP concentrations remain constant. Concentrations of 2-PYR react to dietary niacin in a similar manner to 1-MN concentrations, however the magnitude of the change is greater and as a result the ratio of 2-PYR/l-MN is often used as an indicator of status. A dose response study [21], in which nicotinamide was given in a drink, showed that urinary excretion of 1-MN and 2-PYR was rapid and reached a maximum after 1 h. Excretion of 1-MN declined after 1 h, whereas 2-PYR excretion plateaued for the remaining 3 h of the study. The amount of 2-PYR excreted was niacin status dependent, the higher the status, the greater the amount of 2-PYR excreted. In this study, following ingestion of a supplement, maximal excretion concentrations occurred at different times for each metabolite, 3 h for 1-MN and 6 h for 2-PYR. It is speculated that the pronounced phasing of excretion occurs as a result of differences in clearance rates and enzymatic activity in the metabolic pathway. These differences lead in turn, to a highly unstable intra-day metabolite ratio that would be important if spot sampling, rather than 24 h sampling, were performed. In subject 2, who had consumed niacin-fortified cereal, 2-PYR/l-MN increased through the day, whereas the metabolite/creatinine ratio remained relatively stable.

In the fasting urine samples, the metabolite/creatinine and 2-PYR/l-MN ratios were more stable. Normalisation of urinary excretion, by expressing metabolite concentrations relative to creatinine, appeared in this study to give more stable results. However, when applied to pellagrous patients [3,23] (24 h urine collection) with low creatinine output, the metabolite/creatinine ratio suggested a higher niacin status than the 2-PYR/l-MN ratio and agreed less well with clinical signs. This contradiction was attributed to protein malnutrition, which reduced urinary creatinine concentrations relative to the niacin metabolites and elevated the apparent niacin status. As a result, where protein malnutrition may be important, such as in emergency affected populations or in people suffering from diseases, such as AIDS, alternative methods for expressing the level of metabolite excretion, should be investigated. This could include the use of specific gravity to normalise urine volumes.

In conclusion, from our data and supporting data in the literature, spot urine samples are likely to provide an indication of both longer term niacin status and current dietary intake at the individual and population level. However, these preliminary data indicate that further validation of the use of spot urine sampling is required prior to its use in the field. The data also suggests that the use of fasting, early morning urine samples may reduce the impact of recent dietary intake, and therefore, be more indicative of longer term status. Field-testing of the method will need to be performed and a comparison made with the more traditional 24 h urine collections and clinical signs of deficiency. Guidelines will need to be developed for the interpretation of the results, especially where there is a risk of protein malnutrition.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jchromb.2004.12.012.

References

[1] T.W. Stone, Prog. Neurobiol. 64 (2001) 185.
[2] WHO, The Management of Nutrition in Major Emergencies, WHO, Geneva, 2000.
[3] J.C. Dillon, P. Malfait, G. Demaux, C. Foldi-Hope, Ann. Nutr. Metab. 36 (1992) 181.
[4] M.F. Murray, Clin. Infect. Dis. 36 (2003) 453.
[5] E.L. Jacobson, P.U. Giacomoni, M.J. Roberts, G.T. Wondrak, M.K. Jacobson, J. Photochem. Photobiol. B 63 (2001) 141.
[6] H. Okamoto, A. Ishikawa, Y. Yoshitake, N. Kodama, M. Nishimuta, T. Fukuwatari, K. Shibata, Am. J. Clin. Nutr. 77 (2003) 406.
[7] H.E. Sauberlich, Laboratory Tests for the Assessment of Nutritional Status, second ed., CRC Press LLC, Boca Raton, FL, 1999.
[8] D.J. De Lange, C.P. Joubert, Am. J. Clin. Nutr. 15 (1964) 169.
[9] M.E. Pullman, S.P. Colowick, J. Biol. Chem. 206 (1954) 121.
[10] J.R. Burns, B. Finlavson, Invest. Urol. 18 (1980) 167.
[11] M. Iwaki, E. Murakami, K. Kakehi, J. Chromatogr. B Biomed. Sci. Appl. 747 (2000) 229.
[12] E.G. Carter, Am. J. Clin. Nutr. 36 (1982) 926.
[13] R.C. Terry, M. Simon, J. Chromatogr. 232 (1982) 261.
[14] W.P. Moore, C.H. Bolton, L. Downs, H.A. Gilmor, E.A. Gale, Biomed. Chromatogr. 14 (2000) 69.
[15] J.S. Sandhu, D.R. Fraser, Int. J. Vitam. Nutr. Res. 51 (1981) 139.
[16] K. Shibata, T. Kawada, K. Iwai, J. Chromatogr. 424 (1988) 23.
[17] K. Aoyama, K. Matsubara, K. Okada, S. Fukushima, K. Shimizu, S. Yamaguchi, T. Uezono, M. Satomi, N. Hayase, S. Ohta, H. Shiono, S. Kobayashi, J. Neural Transm. 107 (2000) 985.
[18] R.W. McKee, Y.A. Kang-Lee, M. Panaqua, M.E. Swendseid, J. Chromatogr. 230 (1982) 309.
[19] M.A. Kutnink, H. Vannucchi, H.E. Sauberlich, J. Liq. Chromatogr. 7 (1984) 969.
[20] R. Pumpo, G. Sarnelli, A. Spinella, G. Budillon, R. Cuomo, Am. J. Gastroenterol. 96 (2001) 1183.
[21] R.A. Jacob, M.E. Swendseid, R.W. McKee, C.S. Fu, R.A. Clemens, J. Nutr. 119 (1989) 591.
[22] C.S. Fu, M.E. Swendseid, RA. Jacob, R.W. McKee, J. Nutr. 119 (1989) 1949.
[23] J.G. Prinsloo, J.P. Du Plessis, H. Kruger, D.J. De Lange, L.S. De Villiers, Am. J. Clin. Nutr. 21 (1968) 98.