Published in final edited form as:

J Chromatogr A. 2015 October 2; 1414: 158–162. doi:10.1016/j.chroma.2015.08.050.

A Direct and Rapid Method to Determine Cyanide in Urine by Capillary Electrophoresis

Qiyang Zhang, Naveen Maddukuri, and Maojun Gong*

Department of Chemistry, Wichita State University, Wichita, Kansas, 67260, USA

Abstract

Cyanides are poisonous chemicals that widely exist in nature and industrial processes as well as accidental fires. Rapid and accurate determination of cyanide exposure would facilitate forensic investigation, medical diagnosis, and chronic cyanide monitoring. Here, a rapid and direct method was developed for the determination of cyanide ions in urinary samples. This technique was based on an integrated capillary electrophoresis system coupled with laser-induced fluorescence (LIF) detection. Cyanide ions were derivatized with naphthalene-2,3-dicarboxaldehyde (NDA) and a primary amine (glycine) for LIF detection. Three separate reagents, NDA, glycine, and cyanide sample, were mixed online, which secured uniform conditions between samples for cyanide derivatization and reduced the risk of precipitation formation of mixtures. Conditions were optimized; the derivatization was completed in 2-4 minutes, and the separation was observed in 25 s. The limit of detection (LOD) was 4.0 nM at 3-fold signal-to-noise ratio for standard cyanide in buffer. The cyanide levels in urine samples from smokers and non-smokers were determined by using the method of standard addition, which demonstrated significant difference of cyanide levels in urinary samples from the two groups of people. The developed method was rapid and accurate, and is anticipated to be applicable to cyanide detection in waste water with appropriate modification.

Keywords

Cyanide; Urine; Naphthalene-2,3-dicarboxaldehyde; Glycine; Capillary electrophoresis; Fluorescence

1. Introduction

Cyanides in the form of hydrogen cyanide (HCN) and its alkali metal salts (such as NaCN and KCN etc.) are extremely toxic chemicals which widely exist in nature and industrial processes [1,2]. Deliberate or accidental cyanide poisoning is rare but may be fatal to involved humans or animals [3]. The most common accidental cyanide exposure is the inhalation of smoke from residential and industrial fires that produce cyanides through

*Corresponding Author, Tel: (316) 978-7381. Fax: (316) 978-3431. maojun.gong@wichita.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

nitrogen-containing combustibles [4]. The natural sources of cyanides mainly attribute to the nitrogen metabolic products of bacteria, fungi and algae and to the degradation of cyanogenic glycosides in some plants including bitter almonds, cassava roots, and whole sorghum[5]. Tobacco smoking also produces cyanide thus increasing cyanide levels in blood and urine of smokers but rarely intoxicating. On the other hand, cyanides have important industrial applications including pesticides, electroplating, mineral refinery, and polymer synthesis [1,6], in which cyanides are possibly released into environments. Therefore, cyanide exposure is difficult to avoid, and thus quick identification of potential cyanide intoxication is valuable for forensic lab testing, determining chronic low-level cyanide exposure, and chemical warfare monitoring.

Various methods have been developed and reviewed for cyanide detection[5,7-9]. These methods may be based on spectrophotometry, potentiometry, or fluorometry[7]. Involved instrumentation can be HPLC (high-performance liquid chromatography), CE (capillary electrophoresis), GC (gas chromatography), UV/V is absorbance, or sensors. Although most of these techniques meet application requirements, there are operational limitations such as complexity and multiple steps of sample pretreatment. Therefore, simple and rapid methods are highly desired to facilitate the quick clues of cyanide exposure so that involved patients could be timely and appropriately treated. The indicators of cyanide intoxication can be biological markers (thiocyanate and 2-aminothiazoline-4-carboxylic acid (ATCA)) or the direct measurement of cyanide ions (CN^-)[8]. The requirement of the detection sensitivity depends on the sources of biological fluids. For instance, cyanide ion concentrations are in micromolar ranges in blood while in nanomolar in urine due to the fast metabolic processes that convert cyanide mainly to thiocyanate (~80%) and other species[8]. The typical half-life of CN^- is approximately 20-60 min[8], which further suggests that appropriate cyanide conservation methods or quick measurements be performed.

Tobacco smokers expose themselves to cyanide and would have elevated levels of cyanide in blood and urine. Rapid and accurate determination of the cyanide levels would help to investigate the effect of smoking between smokers and non-smokers. Here we report a direct electrophoretic method for rapid determination of cyanide in urine. In this method, urine samples were mixed online with fluorogenic reagents, NDA (naphthalene-2,3-dicarboxaldehyde) and glycine, to produce fluorescent derivatives. The mixture was then injected for electrophoretic separations. The separation was completed in 25 s and successive injections could be performed. Cyanide concentrations were rapidly measured by using the method of standard addition.

2. Experimental

2.1. Materials and reagents

Sodium tetraborate and all amino acids were purchased from Sigma (St. Louis, MO, USA). Potassium cyanide, acetonitrile (ACN), dimethylsulfoxide (DMSO), dimethylformamide (DMF), ethylenediaminetetraacetic acid (EDTA), creatinine, creatine, urea, and sodium hydroxide were purchased from Fisher Scientific (Chicago, IL, USA). NDA was ordered from Invitrogen (Eugene, OR, USA). Glycine amide [5-(aminoacetamido)fluorescein] (FL-

Gly) was purchased from Setareh Biotech (Eugene, OR, USA). Fused silica capillaries were purchased from Polymicro Technologies (Phoenix, AZ, USA).

Aqueous solutions were prepared in deionized (DI) water, and the NDA stock solution was in DMF. The stock solution of KCN (100 mM) was prepared in DI water. Cyanide standard solutions were prepared by diluting the stock KCN solution. Buffer solutions with pH 9.2 were prepared in DI water by using sodium tetraborate ($\text{Na}_2\text{B}_4\text{H}_7 \cdot 10\text{H}_2\text{O}$). Amine solutions (glycine, alanine, glutamine, and glutamate) were prepared in DI water, and were diluted to appropriate concentrations with DI water or borate buffer

2.2. Instrumentation

The microfluidic detection system has been described elsewhere [10]. Briefly, a 442-nm laser (Laser glow Technologies, Toronto, ON, Canada) beam was focused on the separation capillary through a 40 $\times$ oil-immersion objective (Carl Zeiss Microscopy, Thornwood, NY, USA), and the fluorescence was collected by using the same objective and then transported to a photomultiplier tube (Hamamatsu Photonics, Japan) after emission filtration (482 ± 17 nm). Sample injection and separation was performed by using a high-voltage power supply (Model CZE1000R) purchased from Spellman High Voltage Electronics Corporation (Hauppauge, NY, USA). A sample plug was electrokinetically injected via the flow gated injection method, and then separated. This procedure was controlled by a Lab VIEW program. A 4-syringe pump (Chemyx Inc., Stafford, TX, USA) was used to supply sample and derivatization reagents through individual gas-tight Hamilton syringes (Reno, Nevada, USA). The flow gate and other interfaces were fabricated with poly(dimethylsiloxane) (PDMS) as described in the reference [10].

A 17-cm long capillary with the effective length of 10 cm, ID 10 μm , and OD 360 μm was used to perform separation and detection. The flow-gated injection was run at $-5 \text{ kV} \times 0.5 \text{ s}$ with the high voltage applied to the outlet side of the capillary, and the inlet side was grounded. Separations were performed under -23 kV , and the separation buffer was 20-mM tetraborate with pH 9.2.

2.3. Derivatization of cyanide

The derivatization was performed through offline or online mixing of three solutions: NDA in DMF/water at 50/50 by volume, glycine in buffer with EDTA (5.0 mM), and KCN in buffer or in urine at room temperature. The mixing ratio of NDA/glycine/samples was 1:1:1 or 1:1:2 by volume as indicated in Figure captions and/or the text. The concentrations of NDA, Gly and CN^- indicate original concentrations in three separate solutions before mixing. The urinary samples were donated by three male regular smokers and three male non-smokers in their ages of 20-30 years old. No personal information was recorded except that they were smokers or non-smokers. Urine samples were collected fresh on the experimental day and diluted by adding NaOH solution (final 15 mM in urine) to conserve cyanides. The samples with standard additions were prepared via KCN standard solutions; and the final volume of each sample consisted of 95% urine plus NaOH (15 mM) and standard KCN (various concentrations).

2.4. Standard addition and data analysis

Standard cyanide (KCN) was prepared in DI water, and was diluted to appropriate concentrations. The concentrations added to the urine samples were 0, 100, 200, 300, 400, and 500 nM before mixing with the derivatization reagents. The cyanide possibly existing in water and/or DMF was subtracted by using the background signal obtained via replacing the urine sample branch with NaCl solution with an equivalent conductivity to the urine sample. After separations, the cyanide-derivative peaks were integrated and the peak areas were recorded and averaged (>5 repeated injections). The calibration curves were constructed and the cyanide levels in urine were calculated as the absolute values of x-axis intercepts.

3. Results and Discussion

3.1. Fluorogenic derivatization of cyanide ions

NDA was first developed to efficiently derivatize primary amines at the existence of cyanide ions in 1986 [11]. Recent years have seen wide application of NDA-derivatization of amino acid neurotransmitters for sensitive laser-induced fluorescence (LIF) detection [12-14]. To measure amines in a sample, excessive NDA and CN^- (typically in the mM range) were often used to derivatize amines to the greatest extent. On the other hand, this derivatization strategy has also been employed to measure cyanide ions by using high concentrations of NDA and an amine compound such as glycine and taurine [15-19]. Similarly, excessive NDA and amine were used to facilitate the derivatization of cyanide ions thus quantitatively determining the levels of cyanide in waste water, blood, and urine.

However, NDA and the primary amine reacted to produce imines. The resulted water-insoluble species caused the solution faintly yellow, brown, and finally black as shown in Fig. 1a. NDA at 5.0 mM and glycine or taurine at 20 mM were mixed at 1:1 by volume at pH 9.2; the mixture changed color rapidly (~ 2 min). Experiments also showed that higher concentrations of amines and/or NDA accelerated the color change, which indicated that the reaction rate was amine- and/or NDA concentration dependent. Jackson et al. observed the similar phenomenon when they mixed NDA and taurine [1], and the formation of NDA-ditaaurine species was proposed considering the NDA molecule had two aldehyde groups which could react with primary amines. When the reaction mixture was analyzed with CE, capillary clogging might occur due to the precipitation and should be avoided. To confirm the reaction between NDA and primary amines, FL-Gly and NDA were mixed and then separated with CE, and the signal was detected with LIF at 491-nm excitation and 520-nm emission. As shown in Fig. 1b, the derivative imines of FL-Gly and NDA were observed as a wide peak with fronting, which might include derivatives of NDA with bi-glycine and/or mono-glycine. Higher concentrations of NDA (such as 80 μM versus 20 μM of FL-Gly) produced the maximum NDA-FL-Gly peak while the FL-Gly peak disappeared. Further increased concentrations (0.5 or 1.0 mM) of NDA did not change the derivative signal magnitude and peak shapes, which indicated that the derivative of NDA and FL-Gly was independent of NDA concentration when the concentration of NDA was enough to derivatize all FL-Gly in the mixture. It was anticipated that glycine, similar to FL-Gly, reacted with NDA to form dark precipitate (Fig. 1a). Therefore, it was proposed to mix Gly and NDA online.

To enhance the analytical throughput, the online mixing of the derivatization reagents and samples was performed by using three syringes for liquid delivery, respectively, to a 4-way mixer prepared with PDMS[10]. This format was able to avoid sequential pipetting involved in offline mixing, could maintain derivatization conditions, especially the reaction time (time from the mixer to the flow gate), and could reduce the risk of capillary clogging by precipitation. A reaction capillary of 100 μ m (ID) $\times$ 30 cm was used to have 2- 4 min for the derivatization reaction with the total flow rate in the range of 600-1200 nL/min. A new sample in an alternative syringe could be switched rapidly while maintaining the derivatization reagents.

The NDA stock solution could be prepared in methanol or acetonitrile [1,13,16]. However, acetonitrile was unsuitable because it contained micro-amount of hydrogen cyanide as shown in Fig. 2, which was also observed by Sano et al. [20]. The solubility of NDA in methanol was insufficient, and the presence of methanol often promoted bubble formation because of its volatility. These bubbles might interrupt sample injection and separation. To efficiently dissolve NDA but to tolerate salt contents when mixing, polar solvents were potential options such as DMSO and DMF. However, DMSO, like acetonitrile, contained hydrogen cyanide as shown in Fig. 2. Therefore, DMF was finally selected as the solvent to prepare NDA stock solutions and was used for the following experiments.

3.2. Optimization of conditions

To make experiments convenient, three working solutions were prepared: (1) NDA, NDA at specific concentrations prepared in DMF/water at 50/50 by volume; (2) Gly, glycine at appropriate concentrations prepared in borate buffer with EDTA (5.0 mM) added; and (3) sample, standard KCN dissolved in borate buffer or urinary sample with/without standard additions. NDA was slightly soluble in water and its work solution was prepared in DMF/water at 50/50 by volume. A concentration of NDA at 2.0-10.0 mM was usually used for the detection of primary amines [14]. To perform online mixing and derivatization, the reaction time was manipulated by selecting the combination of a flow rate and a length of the reaction capillary (100 μ m ID). Additionally, the appropriate combination of NDA and amine concentrations was optimized at the selected flow rates (800 nL/min in total) and the reaction capillary (100 μ m ID $\times$ 30 cm long). The mixing ratio of NDA/Gly/sample was 1:1:1 or 1:1:2 by volume which resulted in equivalent concentrations of [NDA]/3, [Gly]/3, and [CN⁻]/3, or [NDA]/4, [Gly]/4, and [CN⁻]/2 in the reaction mixture, respectively (Note: without special notation, concentrations indicate before mixing). To study the matrix effect on the derivatization kinetics, the variation of peak area with time was recorded for KCN dissolved in borate buffer and in urine. The mixture obtained by mixing the three solutions of 2.0mM NDA, 80.0mMGly and 5.0 μ M CN⁻ in borate buffer reached the fluorescence plateau in 3.0 min (data not shown), while it took 11 min for the mixture prepared with CN⁻ in urinary matrix (Fig. 3a, [NDA]=2.0 mM). Apparently, the urinary matrix slowed down the reaction kinetics. To accelerate the reaction, the NDA concentration was increased to 8.0 mM, and the reaction time was shortened to 3.0 min (Fig. 3a, [NDA]=8.0 mM). Therefore, 8.0 mM NDA was selected for online mixing when using 1:1:2 mixing ratio which produced the final reaction NDA concentration at 2.0 mM. Similarly, the concentration of Gly was optimized while maintaining the concentration of NDA at 8.0 mM and cyanide at 5.0 μ M in

urine. As can be seen in Fig. 3b, 80 mM of Gly produced the optimal signal. Therefore, 80 mM Gly was used for 1:1:2 mixing ratio.

The target sample is the biological fluid of urine which contains inorganic ions (Na^+ , K^+ , Cl^- , Mg^{2+} , Ca^{2+} , NH_4^+ , SO_4^{2-} , and PO_4^{3-} , etc.), amino acids, urea, creatinine, and creatine, etc. [21]. The compositional complexity of urine might have matrix effect on CN^- measurement. For example, amino acids in urine may have competition with standard Gly for the limited amount of cyanide ions, which would reduce the analyte peak size related to Gly. However, typical levels of most urinary amino acids in healthy adults are low (<0.5 mM)[21,22]. In addition, relative large concentrations of urea (~ 150 mM) and creatinine (~ 5 mM) exist in urine[22]. Fortunately, both compounds have conjugated amines that rarely react with NDA at the existence of cyanide ions thus lacking effect on cyanide detection. Glycine was selected because former research has proved that Gly has fast reaction kinetics over others, and urinary Gly inherent concentration is large over other amino acids[11,19,21,23]; and taurine may also be a promising option that has been reported [17]. The concentration of Gly on the one hand affects the derivatization kinetics, and on the other hand it reduces the effect from competitive primary amines inherent in urine. As shown in Fig.3b, an increase in Gly concentration could increase the signal of CN^- -related fluorescent derivatives until it reached the maximum at 80 mM. However, high concentrations of Gly also promoted the reaction between NDA and Gly which formed imines or precipitate thus leading to experimental concerns (Fig. 1). Therefore, 80 mM Gly (20 mM after mixing) was selected together with 8.0 mM NDA which achieved CN^- recovery of $\sim 88\%$ in urine.

3.3. Potential interferences

Besides urine matrix effect, other potential interferences include thiocyanate (SCN^-), thiols (R-SH), sulphide (S^{2-}), and sulphite (SO_3^{2-}) [9,24]. As shown in Fig.4, these potential interferences at a 3-fold concentration of CN^- slightly decreased the CN^- derivative peak height. S^{2-} , thiol, and SCN^- decreased the CN^- derivative signal by 10% while SO_3^{2-} effect was trivial. On the other hand, no additional peaks were observed, which indicates that the potential interfering species did not react with NDA/Gly or their products were non-fluorescent at the selected excitation/emission wavelengths.

Considering the matrix effect and potential interferences existing in urinary samples, the method of standard addition was employed to quantitatively determine the CN^- levels in urine. The compositional levels from different individuals at various times may also vary, so the matrix effect on CN^- varies, which can be observed on the various slopes of calibration curves as seen in Table 1.

3.4. Cyanide measurements of smokers and nonsmokers

The optimized conditions and the method of standard addition were applied to CN^- detection in urinary samples from smokers and non-smokers. The samples were collected fresh with 15 mM NaOH added. A series of urine sample solutions was prepared by adding standard KCN solution in water, and the original urine took 95% in volume. Three solutions: NDA, Gly, and urine sample, were delivered through three individual gas-tight syringes at the volume ratio of 1:1:2 which was performed by using a Hamilton syringe pump. The

three flows mixed at a PDMS mixer with a reaction capillary of 100 μm ID $\times$ 30 cm in length. The total flow rate in the reaction capillary was 800 nL/min which secured the reaction time $\sim$ 3min. As shown in Fig.5, CN^- derivative peak height increased with the concentration of standard additions. The electropherograms in Fig. 5 also demonstrate additional signals before and after the derivative peaks, which convinced that electrophoretic separations were necessary although the major peak was the CN^- derivative. These unidentified additional peaks probably were due to the by-products of glycine and NDA reaction. For the determination of CN^- in urine samples, each series of samples produced a calibration curve, and the results are summarized in Table 1. The urinary samples were donated by three male regular smokers and three male non-smokers. The smokers got urinary CN^- levels of $518 \pm 123 \text{ nM}$, while nonsmokers had $217 \pm 6 \text{ nM}$. These results were in fair agreement with published results [8,25] presumably due to the living environmental differences. Student's t test suggested that the level difference of smokers and nonsmokers was significant.

Note that peak areas were preferred for the quantification of CN^- in urine. Urinary matrix contained large quantities of electrolytes which rendered conductivity mismatch between the derivatization mixture and the separation buffer. This mismatch resulted in peak broadening and peak height variation non-proportional to the concentration of standard additions. Peak area correction was not performed since the migration time of each series of standard additions had %RSD at $<1.3\%$.

Concluding Remarks

The reported method takes advantage of the property of CN^- fluorogenic derivatization with NDA at the existence of a primary amine. The strategy of online mixing and derivatization with three separate syringes for reagent and sample delivery simplifies and accelerates the analysis procedure. The reaction takes about 2-4 min for derivatization, and the rapid separation facilitates analytical throughput which is important when early results are desired. This strategy also avoids the oxidative loss of cyanide ions in the sample with the online mixing, and the LIF detection is sensitive (LOD 4.0 nM at 3-fold S/N for standard cyanide in buffer). The method of standard addition with multiple points might weaken the benefits of fast separations in 25 s, but the alternative two-point standard addition may be employed to estimate the cyanide levels in urine in minutes. It is also anticipated that the developed method could be potentially extended to the detection of cyanide ions in blood samples after appropriate sample pretreatment and condition optimization.

Acknowledgments

The authors thank Dr. Susan M. Lunte (University of Kansas) for her constructive mentoring and valuable suggestions. This research was financially supported by the National Institute of General Medical Sciences with the grant number of P20 GM103418.

References

1. Jackson R, Oda RP, Bhandari RK, Mahon SB, Brenner M, Rockwood GA, Logue BA. Development of a Fluorescence-Based Sensor for Rapid Diagnosis of Cyanide Exposure. *Anal. Chem.* 2014; 86:1845–1852. [PubMed: 24383576]

2. Ma JA, Dasgupta PK, Blackledge W, Boss GR. Cobinamide-Based Cyanide Analysis by Multiwavelength Spectrometry in a Liquid Core Waveguide. *Anal. Chem.* 2010; 82:6244–6250. [PubMed: 20560532]
3. Reade MC, Davies SR, Morley PT, Dennett J, Jacobs IC. Review article: Management of cyanide poisoning. *Emerg. Med. Australas.* 2012; 24:225–238. [PubMed: 22672162]
4. Logue BA, Kirschten NP, Petrikovics I, Moser MA, Rockwood GA, Baskin SI. Determination of the cyanide metabolite 2-aminothiazoline-4-carboxylic acid in urine and plasma by gas chromatography-mass spectrometry. *J. Chromatogr. B.* 2005; 819:237–244.
5. Xu Z, Chen X, Kim HN, Yoon J. Sensors for the optical detection of cyanide ion. *Chem. Soc. Rev.* 2010; 39:127–137. [PubMed: 20023843]
6. Tian Y, Dasgupta PK, Mahon SB, Ma J, Brenner M, Jian H, Boss GR. A disposable blood cyanide sensor. *Anal. Chim. Acta.* 2013; 768:129–135. [PubMed: 23473259]
7. Ma JA, Dasgupta PK. Recent developments in cyanide detection: A review. *Anal. Chim. Acta.* 2010; 673:117–125. [PubMed: 20599024]
8. Logue BA, Hinkens DM, Baskin SI, Rockwood GA. The Analysis of Cyanide and its Breakdown Products in Biological Samples. *Crit. Rev. Anal. Chem.* 2010; 40:122–147.
9. Lindsay AE, Greenbaum AR, O'Hare D. Analytical techniques for cyanide in blood and published blood cyanide concentrations from healthy subjects and fire victims. *Anal. Chim. Acta.* 2004; 511:185–195.
10. Zhang Q, Gong M. Prototyping of poly(dimethylsiloxane) interfaces for flow gating, reagent mixing, and tubing connection in capillary electrophoresis. *J. Chromatogr. A.* 2014; 1324:231–237. [PubMed: 24331370]
11. De Montigny P, Stobaugh JF, Givens RS, Carlson RG, Srinivasachar K, Sternson LA, Higuchi T. Naphthalene-2,3-dicarboxyaldehyde/cyanide ion: a rationally designed fluorogenic reagent for primary amines. *Anal. Chem.* 1987; 59:1096–1101.
12. Nandi P, Scott DE, Desai D, Lunte SM. Development and optimization of an integrated PDMS based-microdialysis microchip electrophoresis device with on-chip derivatization for continuous monitoring of primary amines. *Electrophoresis.* 2013; 34:895–902. [PubMed: 23335091]
13. Wang M, Roman GT, Schultz K, Jennings C, Kennedy RT. Improved Temporal Resolution for in Vivo Microdialysis by Using Segmented Flow. *Anal. Chem.* 2008; 80:5607–5615. [PubMed: 18547059]
14. Shou M, Ferrario CR, Schultz KN, Robinson TE, Kennedy RT. Monitoring Dopamine in Vivo by Microdialysis Sampling and On-Line CE-Laser-Induced Fluorescence. *Anal. Chem.* 2006; 78:6717–6725. [PubMed: 17007489]
15. Akiyama H, Toida T, Sakai S, Amakura Y, Kondo K, Sugita-Konishi Y, Maitani T. Determination of cyanide and thiocyanate in Sugihiratake mushroom using HPLC method with fluorometric detection. *J. Health Sci.* 2006; 52:73–77.
16. Lu Q, Collins GE, Evans T, Hammond M, Wang J, Mulchandani A. Vapor and liquid phase detection of cyanide on a microchip. *Electrophoresis.* 2004; 25:116–122. [PubMed: 14730575]
17. Chinaka S, Tanaka S, Takayama N, Tsuji N, Takou S, Ueda K. High-Sensitivity Analysis of Cyanide by Capillary Electrophoresis with Fluorescence Detection. *Anal. Sciences.* 2001; 17:649–652.
18. Chinaka S, Takayama N, Michigami Y, Ueda K. Simultaneous determination of cyanide and thiocyanate in blood by ion chromatography with fluorescence and ultraviolet detection. *J Chromatogr. B.* 1998; 713:353–359.
19. Gamoh K, Sawamoto H. Determination of cyanide ion by high-performance liquid-chromatography with fluorometric detection. *Anal. Sciences.* 1988; 4:665–666.
20. Sano A, Takezawa M, Takitani S. High-performance liquid-chromatography determination of cyanide in urine by pre-column fluorescence derivatization. *Biomed. Chromatogr.* 1989; 3:209–212. [PubMed: 2804427]
21. Tan IK, Gajra B. Plasma and urine amino acid profiles in a healthy adult population of Singapore. *Ann. Acad. Med. Singap.* 2006; 35:468–475. [PubMed: 16902722]
22. Brooks T, Keevil CW. A simple artificial urine for the growth of urinary pathogens. *Lett. Appl. Microbiol.* 1997; 24:203–206. [PubMed: 9080700]

23. Carlson RG, Srinivasachar K, Givens RS, Matuszewski BK. New derivatizing agents for amino acids and peptides. 1. Facile synthesis of N-substituted 1-cyanobenz[f]isoindoles and their spectroscopic properties. *J. Org. Chem.* 1986; 51:3978–3983.
24. Sano A, Takezawa M, Takitani S. Spectrofluorimetric determination of cyanide in blood and urine with naphthalene-2,3-dialdehyde and taurine. *Anal. Chim. Acta.* 1989; 225:351–358.
25. Jermak S, Pranaityte B, Padarauskas A. Headspace single-drop microextraction with in-drop derivatization and capillary electrophoretic determination for free cyanide analysis. *Electrophoresis.* 2006; 27:4538–4544. [PubMed: 17058310]

Fig. 1.

(a) Images of dark color for taurine (20.0 mM) and glycine (20.0 mM) mixing with NDA (5.0 mM) after 2.0 min. (b) Electropherograms of FL-Gly (20.0 μM) at various concentrations of NDA added. Buffer 20.0 mM borate at pH 9.2.

Fig. 2.

Peak height of the derivative by NDA, glycine and CN^- . NDA (5.0 mM) was dissolved in organic solvent/water at 50/50 by volume; glycine was dissolved in 60.0mM borate buffer pH9.2; and DI water. The three were mixed offline at 1:1:1 ratio by volume. CN^- was contributed by the organic solvent and/or water.

Fig. 3.

(a) Peak area change with time at two different NDA concentrations. Glycine at 80.0 mM.

Note: There was 60 s before the first injection. (b) Peak area (maximum) at various concentrations of glycine. NDA at 8.0 mM. For both, NDA in DMF/water (50/50 by volume), glycine in borate buffer (80.0 mM, pH 9.2) and EDTA (5.0 mM), and KCN (5.0 μ M) in urine/water (95/5 by volume). Offline mixing ratio was 1:1:2 (2 for KCN) by volume.

Fig. 4. Interference effect on CN⁻ derivative peak height. NDA (5.0 mM) in DMF/water (50/50 by volume), glycine (80.0 mM) in borate buffer (80.0 mM, pH 9.2), and KCN (1.0 μM in DI water) only, KCN (1.0 μM) plus Na₂S, Na₂SO₃, mercaptoethanol, and KSCN at 3 μM, respectively. Offline mixing ratio was 1:1:2 (2 for KCN) by volume.

Fig. 5.

Electropherograms of standard additions with urine from a non-smoker. The KCN concentrations added are indicated. The major peak indicates the derivative of NDA, KCN and glycine. NDA (8.0 mM) in DMF/water (50/50 by volume), glycine in borate buffer (80.0 mM, pH 9.2) and EDTA (5.0 mM), and KCN in urine/water (95/5 by volume). Online mixing ratio was 1:1:2 (2 for KCN) by flow rate.

Table 1

Summarized results of smokers and non-smokers.

	Calibration Curve	R ²	CN ⁻ (nM) in Urine *	Ave±s
Non-smoker 1	$y = 0.606x + 129$	0.9943	224	217±6
Non-smoker 2	$y = 0.556x + 112$	0.9981	212	
Non-smoker 3	$y = 0.624x + 127$	0.9998	214	
Smoker 1	$y = 0.709x + 300$	0.9970	445	518±123
Smoker 2	$y = 0.687x + 294$	0.9972	450	
Smoker 3	$y = 1.058x + 663$	0.9973	660	

* CN⁻ concentrations were calculated for urine of 95% sample volumes.