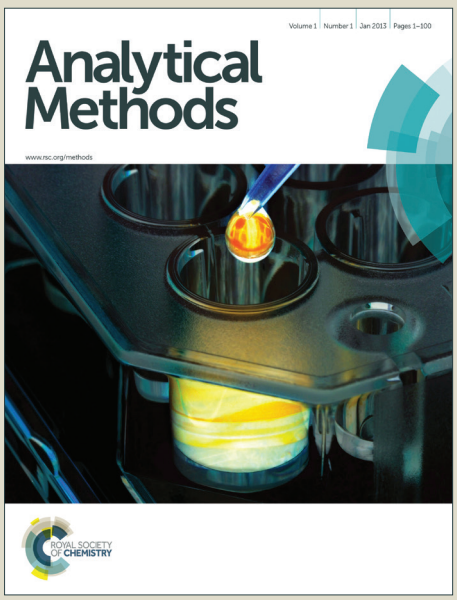
Analytical Methods

Accepted Manuscript



ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/methods

The influence of nitrogen dioxide on hydrogen cyanide level in mainstream and sidestream cigarette smoke and the improvement of the determination method by continuous flow analyzer

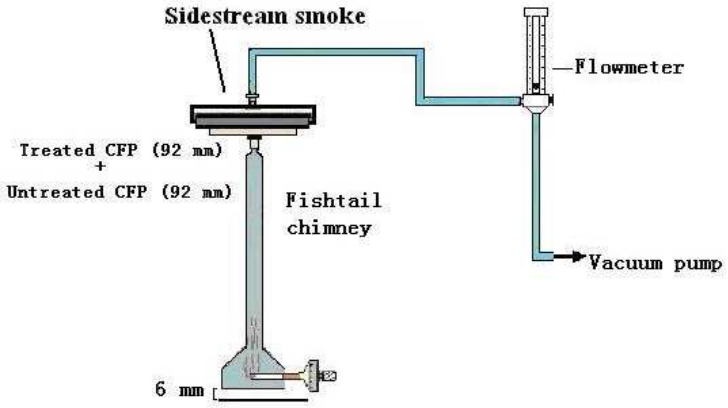
Xingyu Liu, Li Ma, Jun Zhou*, Yanjun Ma, Ruoshi Bai, Lihong Yan

Beijing Third Class Tobacco Supervision Station, No. 99, Wansheng South Street, Tongzhou District, Beijing, 101121, China

* Corresponding author. Tel.: +86 10 59028201; fax: +86 10 59028266.
E-mail address: zhoujun20130409@yahoo.cn (J. Zhou).

ABSTRACT

Hydrogen cyanide is a well-known toxic component in cigarette smoke. Accurate determination of hydrogen cyanide is of great significance to access the risk of cigarette to public health. In the conventional methods for determination of hydrogen cyanide in cigarette smoke, alkaline solution was used to collect hydrogen cyanide. In our study, nitrogen oxides in the smoke were found to dissolve in the alkaline solution, which could react with alkaline solution to produce nitrates and nitrites which can further react with cyanide to result in the underestimation of the yield of hydrogen cyanide. An improved method for the determination of hydrogen cyanide was developed in our laboratory to solve the problem, in which the hydrogen cyanide from mainstream cigarette smoke was collected using a Cambridge filter pad (CFP) treated by ethanol-water solution of sodium hydroxide and detected by continuous flow analyzer based on a coloring system with isonicotinic acid and 1,3-dimethyl barbituric acid. The collection efficiency of hydrogen cyanide for this method was significantly improved compared to conventional methods trapping hydrogen cyanide using alkaline solution. Besides, the collection and sample preparation process was simpler with higher stability of the collected hydrogen cyanide. Limit of Detection (LOD) and Limit of Quantification (LOQ) for the determination of hydrogen cyanide was less than 1.08×10^{-2} mg/L and 3.74×10^{-2} mg/L, respectively. The improved method achieved excellent recoveries with 99.13-100.37% for mainstream smoke detection and 99.67-101.96% for sidestream smoke detection. Excellent precision for hydrogen cyanide determination was obtained that intra-assay and inter-assay relative standard deviation (RSD%) for mainstream smoke detection were 3.58% and 4.44%, respectively, and intra-assay and inter-assay RSD% for sidestream smoke detection were 1.48% and 2.28%, respectively. The developed method is reliable and suitable for routine analysis of hydrogen cyanide in both mainstream and sidestream cigarette smoke.



1 Introduction

Hydrogen cyanide (HCN) is a volatile toxic substance. The chronic oral Reference Dose to human is 0.0006 mg/kg/day.[1] Hydrogen cyanide can be absorbed through skin and by inhalation. Hydrogen cyanide causes giddiness, headache, unconsciousness and convulsion with the paralysis of the respiratory centre in brain.[2-6] Cyanide ion can form stable complexes with biologically active metal ions to exhibit the inhibition to the activity of the enzymes containing metal atom. Cyanide ion inhibits the activity of numerous enzymes, including cytochrome oxidase, nitrogenase, peroxidase, catalase, and nitrite and nitrate reductase. In addition, cyanide can interact with nonmetalloenzymes such as ribulose diphosphate carboxylase. Such inhibition is thought to involve a reaction between cyanide ion and a Schiff-base intermediate to form an inhibitory compound.[1, 7-9] Hydrogen cyanide in cigarette smoke mainly derived from decomposition products through burning process of protein, amino acid, nitrate and nitrogen compounds.[10] For the hazard and cilia toxic effects, hydrogen cyanide has been included in "Toxicants list" by Hoffmann et al.[11] Furthermore, hydrogen cyanide is listed in the seven toxic chemicals contributing to the Hazard Index (HI) established by State Tobacco Monopoly Administration of China (STMA). Hydrogen cyanide delivery in cigarette smoke is required to be detected in routine analysis.[12] So, it is very important to accurately measure hydrogen cyanide in cigarette smoke.

Different methods have been adopted to trap hydrogen cyanide in cigarette smoke. Alkaline solution and Cambridge filter pad (CFP) are recommended to collect gas phase and particulate phase hydrogen cyanide, respectively. Health Canada Bureau (HC) uses sodium hydroxide solution and CFP to trap hydrogen cyanide in mainstream and sidestream cigarette smoke.[13, 14] British American Tobacco p.l.c. (BAT) adopts high concentration of alkaline solution to trap hydrogen cyanide in mainstream cigarette smoke.[15] STMA uses alkaline solution and CFP to trap hydrogen cyanide in mainstream and sidestream cigarette smoke.[16, 17] Then, the trapped hydrogen cyanide was analyzed by different instruments,[18] such as continuous flow cytometry,[19-23] selective electrode,[24, 25] chromatography,[26-31] and spectrometry.[32, 33]

In those conventional trapping systems, alkaline solution is usually employed to trap gas phase hydrogen cyanide in cigarette smoke. However, hydrogen cyanide decreases in alkaline solution. Some chemicals, such as carbonyl compounds in cigarette smoke, can affect the stability of hydrogen cyanide in alkaline solution.[21, 24] In our study, nitrogen oxides in smoke were found to react with alkaline ion to produce nitrates and nitrites which can further react with cyanide ion to result in the underestimation of the yield of hydrogen cyanide in cigarette smoke.[34] Also, those reported methods mainly focus on various analytical instruments rather than trapping systems. In fact, trapping hydrogen cyanide is the first step of hydrogen cyanide analysis and really a tough task. The use of impinger and plastic tubes brings tedious work to sample collection. In the collection of sidestream smoke analytes, drawing rate of vacuum pump should be adjusted to 3 L/min. In that case, alkaline solution in impinger will easily dash out into vacuum pump and damage the instrument. In addition, hydrogen cyanide can be absorbed by the plastic tubes used to connect cigarette holder and impingers, which was very difficult to be rinsed. All the above will result in the loss of hydrogen cyanide and underestimation of the yield of hydrogen cyanide in cigarette smoke.

Herein, we developed a simple trapping system only using CFPs pre-treated by ethanol-water solution containing sodium hydroxide to collect hydrogen cyanide in mainstream and sidestream cigarette smoke. Hydrogen cyanide extract was then detected by continuous flow analyzer based on a coloring system with isonicotinic acid and 1,3-dimethyl barbituric acid. The amount and stability of hydrogen cyanide trapped by different trapping systems were investigated in our study. The improved method was also fully evaluated and validated to be accurate and robust.

2 Experimental

2.1 Chemicals and Reagents

3R4F and 1R5F reference cigarettes were obtained from Kentucky Tobacco Research and Development Center of University of Kentucky (KY, USA). CFPs were purchased from Whatman Corp. (Maidstone, Kent, UK). Chloramine-T (99.9%) and Brij-35 (30%) were purchased from Sigma-aldrich Corp. (St. Louis, MO, USA). Potassium biphthalate (99.99%) and isonicotinic acid (99.0%) were purchased from Acros Corp. (Burlington, WA, USA). 1,3-Dimethylbarbituric acid (99.9%) was purchased from Tokyo Chemical Industry Co. Ltd. (Tokyo, Japan). Anhydrous ethanol and sodium hydroxide were obtained from Beijing Chemical Reagent Company (Beijing, China). Standard sodium cyanide was purchased from State Administration of Quality Supervision, Inspection and Quarantine (Beijing, China).

Ethanol-water solution containing sodium hydroxide was prepared by firstly dissolving sodium hydroxide with deionized water and secondly adding the same volume of ethanol to the deionised water solution (ethanol:water=1:1, V/V). Chloramine-T solution was prepared by adding 2 g of chloramine-T to 500 mL of deionised water. 2.3 g of sodium hydroxide and 20.5 g potassium biphthalate was dissolved in deionised water and diluted to 1 L to prepare buffer solution. Then 0.5 mL of Brij-35 was added to the buffer solution. Chromogenic reagent solution was made by dissolving 7.0 g sodium hydroxide, 16.8 g 1,3-dimethylbarbituric acid and 13.6 g isonicotinic acid in deionised water and then diluting to 1 L with deionised water. Then 0.5 mL of Brij-35 solution was added to chromogenic reagent solution. The pH value of the buffer solution and chromogenic reagent solution should be adjusted to 5.3. Sodium hydroxide and hydrochloric acid solution might be needed for the adjustment. Those reagents were stocked at 4 °C and prepared monthly.

2.2 Cigarette sample preparation

According to Recommended Method N° 21 of Cooperation Centre for Scientific Research Relative to Tobacco (CORESTA), 3R4F and 1R5F reference cigarettes (blended type cigarette) used in this study were conditioned in a CLIMACELL 707-comfort laboratory incubator (MMM Medcenter Einrichtungen GmbH, München, Germany) at 22 °C ± 1 °C and 60% ± 2% of relative humidity for at least 48 h.[35] The conditioned cigarettes were selected and marked according to CORESTA Recommended Method N° 24.[36]

2.3 CFP preparation

Mainstream smoke. A 44 mm CFP was treated with 2.0 mL ethanol-water solution of sodium hydroxide. After totally soaked, it was transferred to a CLIMACELL 707-comfort laboratory incubator and conditioned for 2 h at 22 °C ± 1 °C and 60% ± 2% of relative humidity.

Sidestream smoke. A 92 mm CFP was treated with 9.0 mL ethanol-water solution of sodium hydroxide. After totally soaked, it was transferred to a CLIMACELL 707-comfort laboratory incubator and conditioned for 2 h at 22 °C ± 1 °C and 60% ± 2% of relative humidity.

2.4 Cigarette smoke collection

Mainstream smoke (Fig. 1A). One treated and one untreated 44 mm CFP were placed into the cigarette holder. The treated CFP was placed in the front facing to the incoming smoke. All the rough sides of the two CPFs should face to the incoming smoke. The cigarette holder was assembled to smoking machine and the rear section of cigarette holder was directly connected to the piston of smoking machine without any impinger in between. The assembled system was examined by a leak tester (KC Automation Inc., Richmond, VA, UK) to ensure no leaking. The puff volume was adjusted to 35.0 ± 0.3 mL and examined by a puff volume tester (KC Automation Inc., Richmond, VA, UK). Cigarette smoking was performed according to method ISO 4387 with four cigarettes in one run by a modified SM 405 linear smoking machine (Cerulean Corp., Milton Keynes, UK).[37]

Sidestream smoke (Fig. 2A). One treated and one untreated 92 mm CFP were placed into the cigarette holder. The treated CFP was in the front facing to the incoming smoke. All the rough sides of the two CFPs should face to the incoming smoke. The front side of cigarette holder was attached to the top of fishtail chimney and the other side was attached to flowmeter and vacuum pump without any impinger in between. The assembled system was

examined by a leak tester to ensure no leaking. The puff volume of cigarette smoking was adjusted to 35.0 ± 0.3 mL and examined by a puff volume tester. Cigarette smoking was performed according to method ISO 20773 with two cigarettes in one run by a modified SM 405 SV linear smoking machine (Cerulean Corp., Milton Keynes, UK).[38] Drawing rate of vacuum pump was adjusted to 3 L/min.

2.5 Extraction of hydrogen cyanide sample

After collection of cigarette smoke, the two 44 mm CFPs for mainstream smoke were transferred into 150 mL triangular flask containing 100 mL of 0.1 mol/L sodium hydroxide solution. While, the two 92 mm CFPs for sidestream smoke were transferred into 500 mL triangular flask containing 200 mL of 0.1 mol/L sodium hydroxide solution. The flasks were shaken at 200 rpm for 30 min on a wrist action shaker (Wode Instrumentation Corp., Beijing, China). Filtered by 0.45 μm membrane, the extract was directly injected into a sample cup and detected by continuous flow analyzer (SEAL Analytical GmbH, Norderstedt, Germany). Hydrogen cyanide in sidestream smoke adhered to fishtail chimney was rinsed by 50 mL of 0.1 mol/L sodium hydroxide solution and detected by continuous flow analyzer.

2.6 Blank test

To ensure no exogenous hydrogen cyanide imposed on CFP, the blank tests were performed without cigarette smoking according to the above procedure from Experimental section of 2.3 to 2.5.

2.7 Hydrogen cyanide collection by different trapping systems

In HC trapping system, hydrogen cyanide collection for mainstream cigarette smoke was done according to method T-107 (Fig. 1B).[13] Hydrogen cyanide collection for sidestream cigarette smoke was done according to method T-205 (Fig. 2B).[14] In BAT trapping system, hydrogen cyanide collection for mainstream cigarette smoke was performed following BAT method as shown in Fig. 1C.[15] In STMA trapping system, hydrogen cyanide collection for sidestream cigarette smoke was carried out according to method YC/T 350 as shown in Fig. 2C.[17]

2.8 Hydrogen cyanide and NOx determination

Hydrogen cyanide in the buffer solution is converted to cyanogen chloride by an aqueous solution of chloramine-T. The cyanogen chloride then reacts with isonicotinic acid to give glutaconic aldehyde, upon reaction with 1,3-Dimethylbarbituric acid, forms a colored complex. A single channel spectrometer (600 nm) is employed to detect the colored complex, which is quantified by an external standard method. The Auto-sampler is operated at a sampling rate of 30 per hour with a 1:1 sample to wash ratio. The bubbles should flow smoothly through the instrument tubes and be uniform in shape and spacing with rounded ends. Sufficient time should be required for the system to become stable with the reagents being pumped. The manifold of continuous flow analyzer and the typical output of results are shown in the Fig. 3 and 4, respectively.

The determination of NOx was performed by the chemiluminescent method derived from Health Canada method T-110.[39] The gas phase of mainstream smoke filtered by CFP was directed into an evacuated mixing chamber puff by puff, and then NOx was determined by a NOx analyzer. NOx determination was sampled behind the cigarette holder with and without CFP, impinger, respectively.

2.9 Data analysis

Six working standard solutions of sodium cyanide (0.100，0.300，1.600，3.000，4.200，5.700 mg/L) were prepared by dissolving stock standard solution (50 mg/L) (GBW (E) 080115) into 0.1 mol/L sodium hydroxide solution. All standard and sample concentrations were determined using peak height of external standard versus peak height of the analyte. Raw data were processed using AACE 6.05 software of Auto Analyzer 3. In the article, standard deviation (SD) was equal to the square root of the arithmetic mean of the squares of the deviations from the arithmetic mean. Relative standard deviation (RSD) was calculated as SD divided by arithmetic mean. For mainstream smoke, the amount of hydrogen cyanide per cigarette (μg/cig) was calculated as 1.038 × C × 100 / 4, in which C represents the concentration (mg/L) of 44 mm CFP extraction. For sidestream smoke, the amount of

hydrogen cyanide per cigarette (μg/cig) was calculated as $1.038 \times (C_1 \times 200 + C_2 \times 50) / 2$, in which C_1 represents the concentration (mg/L) of 92 mm CFP extraction and C_2 represents the concentration (mg/L) of rinsing solution of fishtail chimney.

3 Results and Discussion

3.1 Optimization of CFP trapping system

If CFP was soaked by the pure water containing sodium hydroxide, it will take a very long time to reach a proper humidity suitable for the collection of hydrogen cyanide in cigarette smoke. Ethanol was chosen as the solvent because of the high volatility. Ethanol will quickly volatilize at 22 °C, which produce lower water retention on CFP and subsequently higher permeability to smoke. Hydrogen cyanide level in sidestream smoke is generally higher than that in mainstream smoke and the flowing mode between mainstream and sidestream smoke are different. In order to completely collect hydrogen cyanide in sidestream smoke, 92 mm CFPs were employed in our study. The procedure of treating CFP was set as follows: 2 mL and 9 mL of ethanol-water (V/V=1/1) solution containing sodium hydroxide was used to soak 44 mm and 92 mm CPFs, respectively. After CFPs were totally soaked, the treated CFP should be conditioned in a CLIMACELL 707-comfort laboratory incubator at 22 °C ± 1 °C and 60% ± 2% of relative humidity.

The trapping efficiencies were shown in Table 1. 1.0 mol/L and 4.0 mol/L of sodium hydroxide solution were favorable and enough for trapping almost all the hydrogen cyanide in mainstream (99.69%) and sidestream cigarette smoke (97.83%), respectively. Higher concentration of sodium hydroxide solution was too viscous and will result in a strong change of the puffing profile of the smoking machine. The concentration of sodium hydroxide solution was set at 1.0 and 4.0 mol/L for mainstream smoke and sidestream smoke collection, respectively.

In order to facilate the volatilization of ethanol, the treated CFP is essential to be conditioned for a period of time in the CLIMACELL 707-comfort laboratory incubator at 22 °C ± 1 °C and 60% ± 2% of relative humidity. The effects of conditioning time on hydrogen cyanide amount in CFP trapping system were investigated in our study. As shown in Table 2, conditioning time exerted no significant effects on the hydrogen cyanide amount. Considering volatilization of ethanol and saving conditioning time, 2 h was adopted in our laboratory.

3.2 The amount of hydrogen cyanide trapped by different systems

Presently, HC, BAT and STMA trapping systems are available for hydrogen cyanide collection of mainstream smoke. Due to the similarity of HC and STMA trapping sytems, only HC and BAT trapping systems were chosen to be compared with the CFP trapping system. Several points herein should be addressed: In HC trapping system, 30 mL of sodium hydroxide solution (0.1 mol/L) was loaded into the only one impinger, while in BAT trapping system 25 and 10 mL of sodium hydroxide solution (1 mol/L) was loaded into the two consecutive impingers, respectively. The impingers with sodium hydroxide solution were attached to the rear section of cigarette holder and connected by plastic tubes. As shown in Table 3, the highest level of hydrogen cyanide was obtained by CFP trapping system. BAT trapping systems produced relative lower level of hydrogen cyanide. Too many plastic pipes used in BAT trapping systems might be a cause for hydrogen cyanide loss, since hydrogen cyanide was easily absorbed in the inner walls of those plastic pipes.

As for the present hydrogen cyanide trapping systems for sidestream cigarette smoke, HC and STMA trapping systems were chosen to be compared with the CFP trapping system. Some differences are exist between HC and STMA trapping system. In HC trapping system, 90 mL of sodium hydroxide solution (0.1 mol/L) was loaded into the impinger and hydrogen cyanide in impinger, fishtail chimney and CFP were combinedly measured. For STMA method, hydrogen cyanide in both of the two impingers with 35 mL of sodium hydroxide solutions (0.1 mol/L) and fishtail chimney were measured together, though hydrogen cyanide in CFP was measured separately. The results were shown in Table 4 that the highest level of hydrogen cyanide was obtained by CFP trapping system

and significantly lower levels were obtained by HC and STMA trapping systems.

3.3 The stability of hydrogen cyanide

The stability of the trapped hydrogen cyanide was compared using CFP, HC and BAT trapping systems in our laboratory. It was found that hydrogen cyanide is strongly unstable in HC and BAT trapping system when stored at room temperature. The trapped hydrogen cyanide decreased significantly in 48 h by HC and BAT trapping system, while hydrogen cyanide changed very slightly by CFP trapping system. The results are shown in Table 5. It was interestingly found that hydrogen cyanide on CFP was very stable and hydrogen cyanide in impinger solution was much more unstable in both HC and BAT trapping systems. It can be inferred that some compounds of gas phase, not particle phase, in cigarette smoke affected the stability of hydrogen cyanide.

Fig. 5 shows that higher concentration of cyanide ion exhibits faster reduction rate in impinger solution, while the lower exhibits relatively slower reduction rate. As shown in Fig. 5, 5.45 mg/L of cyanide was reduced by 67% of control, while 1.25 mg/L cyanide was reduced by 13% of control in 24 h. Different cigarettes containing different hydrogen cyanide level would induce inconsistent decreasing rate of hydrogen cyanide. It will bring conflicting results for hydrogen cyanide analysis.

3.4 The influence of nitrogen oxides on the stability of hydrogen cyanide in cigarette smoke

A significant loss of nitrogen oxides was observed when an impinger containing sodium hydroxide solution was used in the trapping system (Table 6). Nitrogen oxides, especially nitrogen dioxide is a very strong oxidant and tends to react with hydrogen cyanide in water solution.

To further identify whether nitrogen oxides reacted with hydrogen cyanide in the impinger solution and resulted in the loss of hydrogen cyanide, we bubbled nitrogen dioxide to the impinger solution. Fig. 6 and 7 showed that the level of cyanide ion decreased with the imposing dose and time of nitrogen dioxide. Although the kinetics and thermodynamics of the reaction between nitrogen dioxide and cyanide ion was unclear, it could be inferred that nitrogen dioxide could react with alkaline solution to produce nitrates and nitrites which can further react with cyanide to result in the loss of hydrogen cyanide in the impinger solution.

3.5 Quantification of analyte

The analyte was quantified by means of calibration curves from known concentrations of standard solutions. Six calibration levels described in the Experimental section were used with five replicate tests made at each concentration. The calibration curves were found to be linear over the entire range with the values of the coefficient of determination (R^2=0.9999). The regression equation was that: A=0.079C+0.0006, where C is the concentration of the standard solution and A is absorbance value.

3.6 LOD, LOQ, Recovery and Precision

The sensitivity of the method was evaluated by LOD and LOQ. Nine repeated standard solutions at lowest level were measured and standard deviation (SD) was calculated. LOD was calculated as t-value × SD and LOQ was calculated as 10 × SD, where t-value is 2.896. Typical values of LOD and LOQ for simple trapping system are 1.08×10^{-2} mg/L and 3.74×10^{-2} mg/L, respectively. It is much lower than the actual level of hydrogen cyanide in mainstream and sidestream cigarette smoke.

Recovery of the trapping system was evaluated by adding standard solution into the blank CFP at low, middle and high concentration. The recovery was calculated as the ratio of the mean of the experimentally determined level from replicated analysis to the nominal level (Table 7). It showed excellent recovery for mainstream detection (99.13%-100.37%) and sidestream detection (99.67%-101.96%).

The precision for the trapping system was evaluated as RSD% for both inter-assay and intra-assay in our laboratory. The precision for the intra-assay samples was determined by analyzing the 1R5F sample five times on three separate days. The precision for the inter-assay samples was determined by analyzing fifteen 1R5F samples, five of which were analyzed on three separate days. As shown in Table 8, excellent precision was obtained that

intra-assay and inter-assay RSD% for mainstream smoke detection were 3.58% and 4.44%, respectively, and intra-assay and inter-assay RSD% for sidestream smoke detection were 1.48% and 2.28%, respectively.

4 Conclusions

Some substances, such as formaldehyde and nitrogen oxides in the cigarette smoke can be easily dissolved in water solution and react with HCN in the solution to result in the loss of hydrogen cyanide. Without water, the reactions are very difficult to occur. Due to this, in the trapping system, water should be used as little as possible. The CFP trapping system should be a very good choice for trapping hydrogen cyanide in both mainstream and sidestream cigarette smoke.

An improved method was developed for the determination of hydrogen cyanide in our laboratory, in which the hydrogen cyanide in cigarette mainstream smoke was collected using a 44 mm CFP treated by ethanol-water solution with sodium hydroxide and an untreated CFP and the hydrogen cyanide in cigarette sidstream smoke was collected using a 92 mm CFP treated by ethanol-water solution with sodium hydroxide and an untreated CFP, and detected by continuous flow analyzer based on the detection of the coloring system with isonicotinic acid and 1,3-dimethyl barbituric acid instead of toxic pyridine/pyrazolone reagent. The special trapping way for hydrogen cyanide is the basis of the improved method, which can greatly reduce the loss of hydrogen cyanide resulted from its reaction in water solution and the use of connecting plastic pipes. CFP trapping system is much easier to control and shows higher trapping efficiency than other trapping systems used. Compared to previous methods, this method is much simpler and easy to handle. The puffing status for smoking is more stable without using an impinger. The improved method is suitable for routine analysis of hydrogen cyanide in mainstream and sidestream cigarette smoke with excellent recovery, precision and sensitivity.

Acknowledgement

We would like to thank Zeming Wu for this critical reading of the manuscript.

Part of the research results were reported in 2011 CORESTA SSPT Study Groups' meeting in Graz, Austria.

References

1. Hydrogen Cyanide and Cyanide Salts (CASRN Various), U. S. ENVIRONMENTAL PROTECTION AGENCY, http://www.epa.gov/iris/subst/0060.htm#refunc.

2. A. A. Salkowski and D. G. Penney, *Veterinary and human toxicology*, 1994, **36**, 455-466.

3. D. C. Mathangi, R. Shyamala, R. Vijayashree, K. R. Rao, A. Ruckmani, R. Vijayaraghavan and R. Bhattacharya, *Neurochem. Res.*, 2011, **36**, 540-548.

4. H. B. Leavesley, L. Li, K. Prabhakaran, J. L. Borowitz and G. E. Isom, *Toxicological sciences: an official journal of the Society of Toxicology*, 2008, **101**, 101-111.

5. R. K. Tulsawani, M. Debnath, S. C. Pant, O. Kumar, A. O. Prakash, R. Vijayaraghavan and R. Bhattacharya, *Chem. Biol. Interact.*, 2005, **156**, 1-12.

6. Soto-Blanco, Benito, Marioka, Paulo César, Górniak and S. Lima, *Ecotoxicol. Environ. Saf.*, 2002, **53**, 37-41.

7. J. Hariharakrishnan, R. M. Satpute, G. B. K. S. Prasad and R. Bhattacharya, *Toxicol. Lett.*, 2009, **185**, 132-141.

8. M. A. Haxhiu, B. Erokwu, E. van Lunteren, N. S. Cherniack and K. P. Strohl, *J. Appl. Physiol. (Bethesda, Md. : 1985)*, 1993, **74**, 574-579.

9. J. Hariharakrishnan, R. M. Satpute and R. Bhattacharya, *Indian journal of experimental biology*, 2010, **48**, 731-736.

10. F. M. Esposito, *Journal of Free Sciences*, 1988, **6**, 195-242.

11. E. L. Wynder and D. Hoffmann, *Cancer Res.*, 1994, **54**, 5284-5295.

12. J. Xie, 2008 CORESTA Congress, Shanghai, China, 2008.

13. T-107, Determination of hydrogen cyanide in mainstream tobacco smoke, Health Canada Bureau, Ottawa, Canada, 1999.

14. T-205, Determination of hydrogen cyanide in sidestream tobacco smoke, Health Canada Bureau, Ottawa, Canada, 1999.
15. BAT Method - Determination of hydrogen cyanide in mainstream smoke, Research & Development at British American Tobacco, http://www.bat-science.com/groupms/sites/BAT_7AWFH3.nsf/vwPagesWebLive/CEDE84EE9731E0E8C125741A002E802A/$FILE/Hydrogen%20cyanide%20in%20Mainstream%20Smoke.pdf?openelement.
16. YC/T 253, Cigarette - Determination of hydrogen cyanide in cigarette mainstream smoke - Continuous flow method, China Standard Press, Beijing, China, 2008.
17. YC/T 350, Cigarette - Determination of hydrogen cyanide in cigarette sidestream smoke - Continuous flow method, China Standard Press, Beijing, China, 2010.
18. C. Pohlandt, E. A. Jones and A. F. LEE, *J. S. Afr. Inst. Min. Metall.*, 1983, **83**, 11-19.
19. P. F. Collins, N. M. Sarji and J. F. Williams, *Beiträge zur Tabakforschung International*, 1973, **7**, 73-78.
20. P. F. Van Peborgh, *Biochem. Med.*, 1972, **6**, 105-110.
21. B. Sun and B. N. Noller, *Water Res.*, 1998, **32**, 3698-3704.
22. *United States of America Pat.*, 1980.
23. Y. Jiang, N. Lu, F. Yu, Q. Li and H. Xu, *Fresenius' J. Anal. Chem.*, 1999, **364**, 786-787.
24. M. A. Koupparis, C. E. Efstathiou and T. P. Hadjiioannou, *Anal. Chim. Acta*, 1979, **107**, 91-100.
25. A. Safavi, N. Maleki and H. R. Shahbaazi, *Anal. Chim. Acta*, 2004, **503**, 213-221.
26. M. Nonomura, *Anal. Chem.*, 1987, **59**, 2073-2076.
27. M. Odoul, B. Fouillet, B. Nouri, R. Chambon and P. Chambon, *J. Anal. Toxicol.*, 1994, **18**, 205-207.
28. N. Mottier, F. Jeanneret and M. Rotach, *J. AOAC Int.*, 2010, **93**, 1032-1038.
29. S. Chinaka, N. Takayama, Y. Michigami and K. Ueda, *J. Chromatogr., B: Biomed. Sci. Appl.*, 1998, **713**, 353-359.
30. M. Shibata, K. Inoue, Y. Yoshimura, H. Nakazawa and Y. Seto, *Arch. Toxicol.*, 2004, **78**, 301-305.
31. T. T. Christison and J. S. Rohrer, *J. Chromatogr., A*, 2007, **1155**, 31-39.
32. P. Kaur, S. Upadhyay and V. K. Gupta, *Analyst*, 1987, **112**, 1681-1683.
33. J. Michal, *Fire Mater.*, 1982, **6**, 13-15.
34. L. Ma, J. Zhou, Y. Ma, R. Bai, L. Yan and J. Wang, 2011 CORESTA Congress, Graz, Austria, 2011.
35. CORESTA Recommended Method N° 21, Atmosphere for conditioning and testing tobacco and tobacco products, CORESTA, Paris, French, 1991.
36. CORESTA Recommended Methods N° 24, Cigarettes - Sampling, CORESTA, Paris, French, 1991.
37. ISO 4387, Cigarettes - Determination of total and nicotine-free dry particulate matter using a routine analytical smoking machine, International Organization for Standardization, Geneva, Switzerland, 2000.
38. ISO 20773, Cigarettes - Determination of nicotine-free dry particulate matter and nicotine in sidestream smoke - Method using a routine analytical linear smoking machine equipped with a fishtail chimney, International Organization for Standardization, Geneva, Switzerland, 2007.
39. T-110, Determination of oxides of nitrogen in mainstream tobacco smoke, Health Canada Bureau, Ottawa, Canada, 1999.

Table 1 The effects of concentration of sodium hydroxide solution on the amount of trapped hydrogen cyanide in the mainstream and sidestream smoke of 3R4F reference cigarette smoke and trapping efficiencies of CFP trapping system (n=5)

	Concentration (mol/L)	Fishtail chimney (μg/cig)	CFP (μg/cig)	Impinger (μg/cig)	Sum (μg/cig)	Trapping efficiency (%) [c]
Mainstream	0.1	/ [a]	101.99	4.28	106.28	95.97
	0.5	/ [a]	106.76	0.43	107.19	99.60
	1.0	/ [a]	107.31	0.33	107.64	99.69
	2.0	/ [a]	93.64	5.01	98.65	94.92
	4.0	/ [a]	92.69	/ [b]	92.69	-
Sidestream	1.0	15.81	113.89	6.66	136.35	95.11
	2.0	15.00	126.43	8.40	149.83	94.42
	4.0	14.61	155.06	3.77	173.44	97.83
	6.0	16.61	161.99	3.32	181.93	98.17
	8.0	13.64	160.85	2.97	177.46	98.33

[a] indicated no fishtail chimney was attached to the trapping system. [b] there is no impingers in the collection system. c Trapping efficiency is percentage of the amount of CFP to the Sum.

Table 2 The amount of hydrogen cyanide in the mainstream and sidestream smoke of 3R4F reference cigarettes influenced by conditioning time of treated CFP in the trapping system (Mean ± SD, n=5)

Time (h)	1	2	3	4	5
Mainstream (μg/cig)	103.79 ± 5.17	108.52 ± 6.57	105.64 ± 5.15	105.66 ± 6.49	103.67 ± 3.39
Sidestream (μg/cig)	169.53 ± 5.85	171.49 ± 10.62	167.22 ± 2.11	169.87 ± 3.00	170.66 ± 4.44

Table 3 The amount of hydrogen cyanide in the mainstream smoke of 3R4F reference cigarettes trapped by CFP, HC and BAT trapping systems (n=5)

Trapping system	CFP(μg/cig)	1st impinger (μg/cig)	2nd impinger (μg/cig)	Sum (μg/cig)
CFP	112.46	/ [a]	/ [a]	112.46
HC	42.31	59.80	/ [a]	102.11
BAT	/ [b]	93.34 [c]		93.34

[a] indicated no impinger was attached to the trapping system. [b] indicated no CFP was used in BAT trapping system. [c] indicated the sum of hydrogen cyanide in the two impingers.

Table 4 The amount of hydrogen cyanide in the sidestream smoke of 3R4F reference cigarettes trapped by CFP, HC and STMA trapping systems (n=5)

Trapping system	CFP (μg/cig)	Fishtail chimney (μg/cig)	Impinger (μg/cig)	Sum (μg/cig)
CFP	155.19	14.49	/ [a]	169.68
HC		144.38 [b]		144.38
STMA	12.75	145.30 [c]		158.06

[a] indicated no impinger was attached to the trapping system. [b] indicated the sum of hydrogen cyanide in CFP, fishtail chimney and impinger. [c] indicated the sum of hydrogen cyanide in fishtail chimney and the two impingers.

Table 5 The stability of hydrogen cyanide in the mainstream smoke of 3R4F reference cigarettes trapped by different systems

Time (h)	1		4		24		48	
	CFP	Impinger	CFP	Impinger	CFP	Impinger	CFP	Impinger
CFP (μg/cig)	112.46	/ [a]	109.01	/ [a]	106.89	/ [a]	101.45	/ [a]
HC (μg/cig)	46.51	43.60	45.75	31.08	44.69	7.24	42.14	1.48
BAT (μg/cig)	/ [b]	93.34	/ [b]	89.72	/ [b]	86.41	/ [b]	82.64

[a] indicated no impinger was attached to the trapping system. [b] indicated no CFP was attached to the trapping system.

Table 6 NOx and HCN delivery levels using CFP system and HC system (μg/cig)

	Control group	CFP trapping system		HC trapping system	
Repeated tests (n=5)	NOx	NOx	HCN	NOx	HCN
1	211.92	180.94	122.86	127.60	103.79
2	218.09	176.70	126.07	126.78	108.52
3	217.70	183.97	137.85	137.81	112.32
4	224.28	194.77	125.86	124.52	114.86
5	212.24	191.68	135.83	136.62	109.00
Mean value	216.85	185.61	129.69	130.67	109.70
RSD (%)	2.34	4.04	5.15	4.67	3.82

Table 7 Recoveries for the method (n=5)

	hydrogen cyanide	Determined level（mg/L）	Spiked level（mg/L）	Recovery (%)
Mainstream	Low	0.269	0.268	100.37
	Middle	3.201	3.216	99.53
	High	6.642	6.700	99.13
Sidestream	Low	0.590	0.612	99.67
	Middle	3.12	3.06	101.96
	High	5.834	5.814	100.34

Table 8 Precision for the method (μg/cig)

	Intra-assay (n=15)			Inter-assay (n=15)		
	Day 1	Day 2	Day 3	Day 1	Day 2	Day 3
Mainstream	21.31	20.67	19.82	20.15	21.01	21.36
	21.34	20.32	19.35	21.67	21.47	22.03
	21.77	20.84	20.14	21.78	20.63	20.31
	21.59	20.15	19.77	20.76	20.07	22.11
	21.25	20.37	20.05	21.21	19.23	22.83
Mean value		20.58			21.11	
RSD (%)		3.58			4.44	
Sidestream	152.07	155.16	156.32	152.59	152.74	157.28
	151.31	155.21	157.13	161.43	152.22	155.36
	151.57	155.16	157.13	152.07	155.16	158.04
	152.04	155.05	157.13	152.95	148.95	156.82
	151.55	156.14	156.89	153.55	151.99	147.81
Mean value		154.66			153.93	
RSD (%)		1.48			2.28	

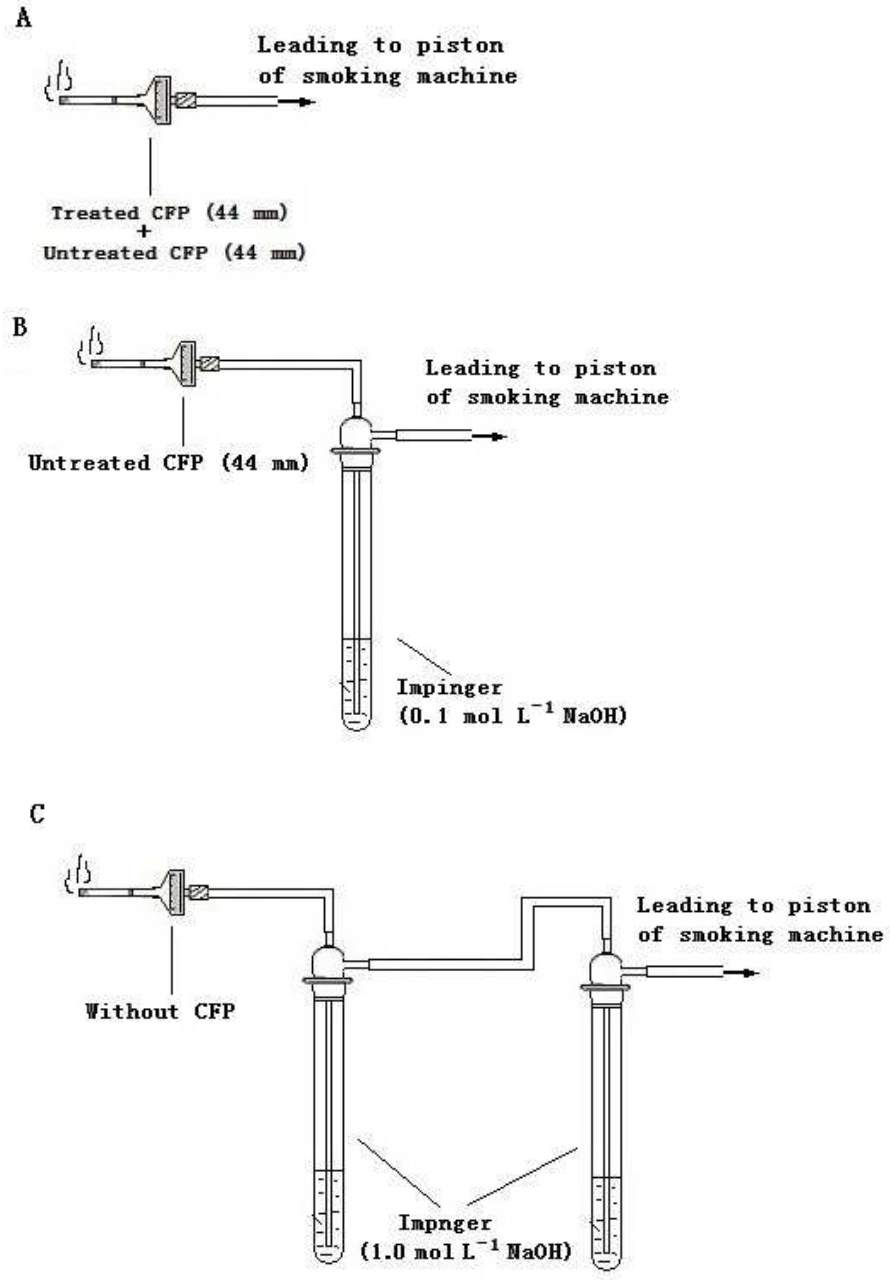


Fig. 1 Different trapping systems for hydrogen cyanide determination in mainstream cigarette smoke. (A) CFP trapping system. (B) HC trapping system. (C) BAT trapping system.

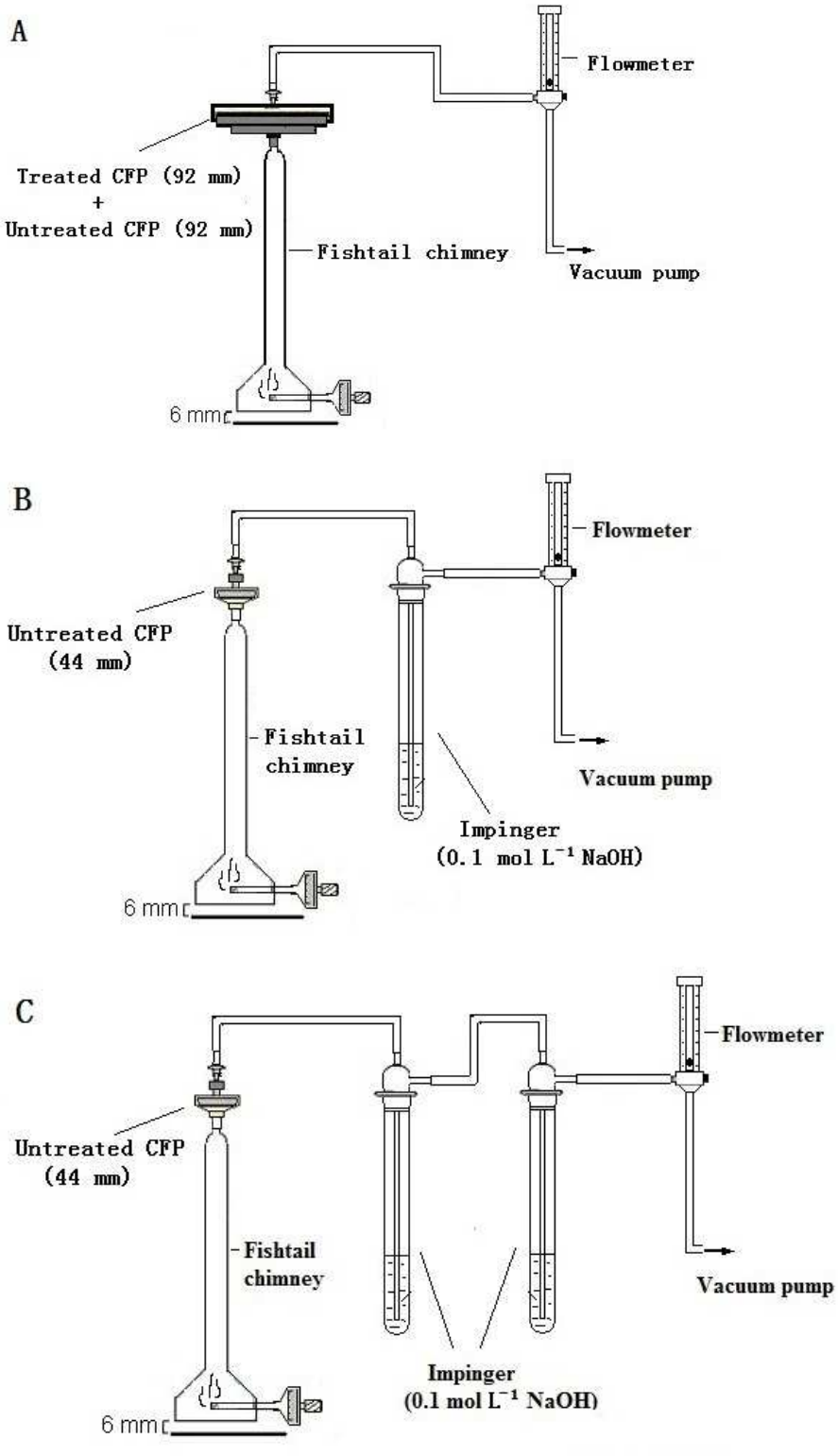


Fig. 2 Different trapping systems for hydrogen cyanide determination in sidestream cigarette smoke. (A) CFP trapping system. (B) HC trapping system. (C) STMA trapping system.

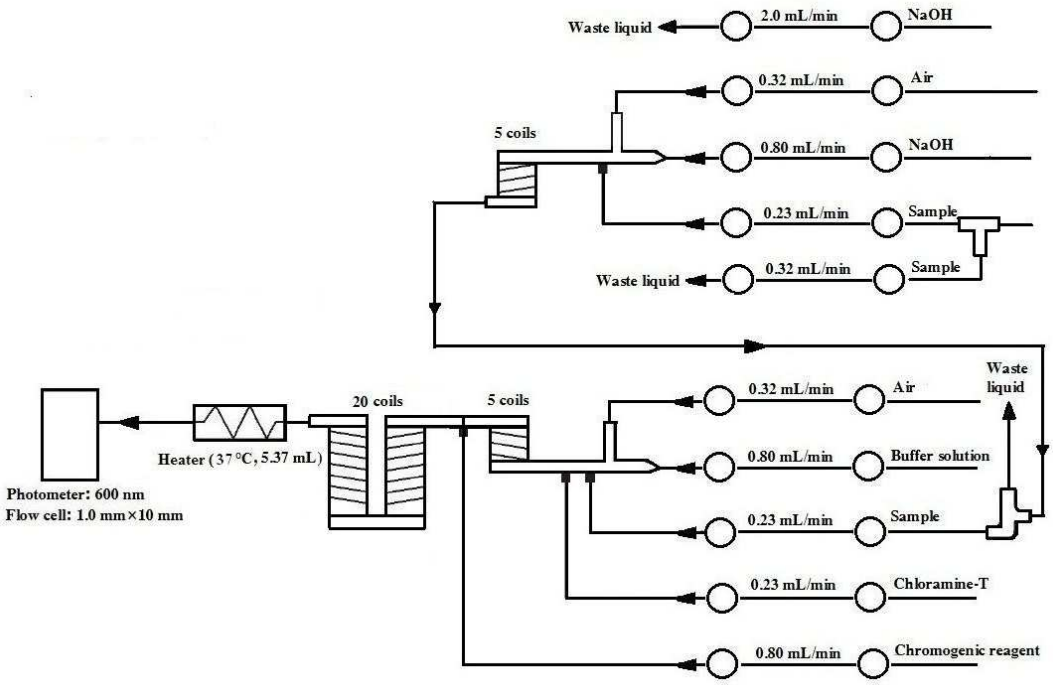


Fig. 3 The manifold of continuous flow analyzer for hydrogen cyanide determination

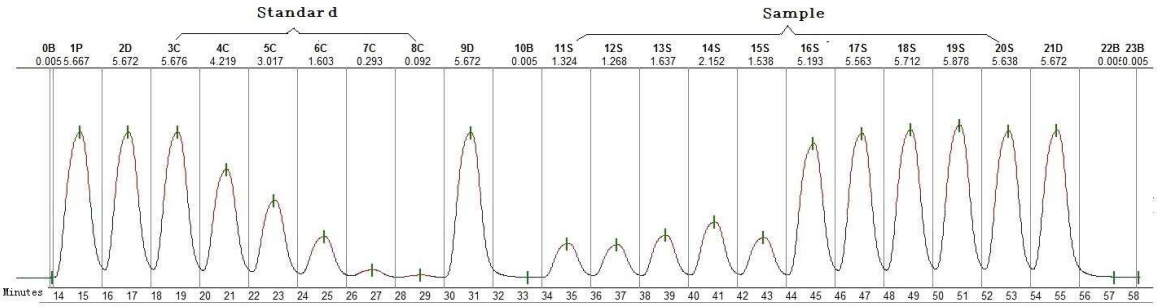


Fig. 4 The graph of cyanide ion concentration against running time (Typical output)

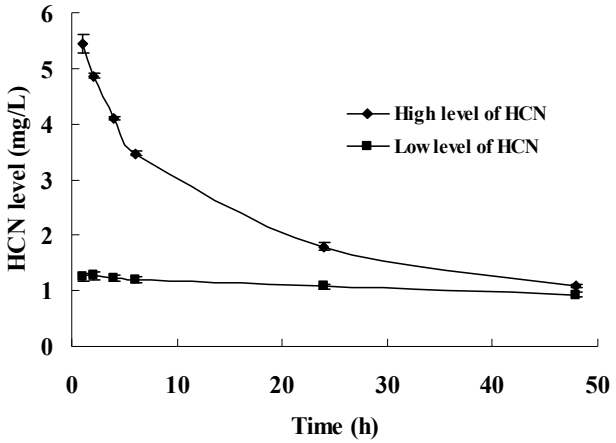


Fig. 5 The decrease of hydrogen cyanide in impinger solution (n=5).

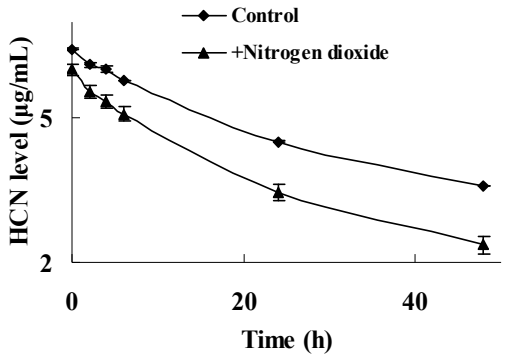


Fig. 6 Time-response of influence of nitrogen dioxide on hydrogen cyanide stability in impinger solution (n=5).

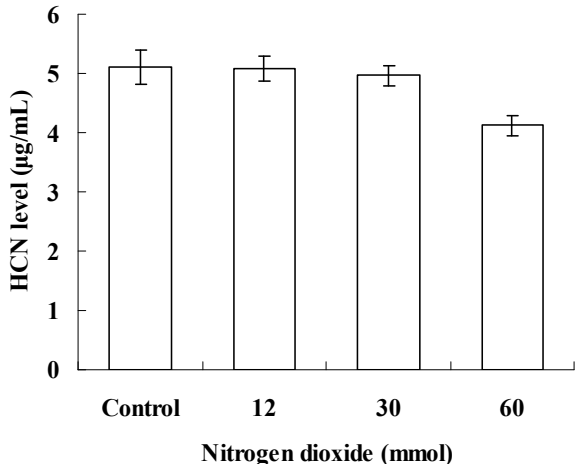


Fig. 7 Dose-response of influence of nitrogen dioxide on hydrogen cyanide stability in impinger solution (n=5).