

Optimization of Analytical Procedure for Hydrogen Cyanide in Mainstream Smoke

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ABSTRACT : Hydrogen cyanide(HCN), formed from pyrolysis of various nitrogenous compounds such as protein, amino acids and nitrate in tobacco, is present in both the particulate phase and vapor phase of cigarette smoke. Typically the determination of HCN in cigarette smoke has been done through colorimetric and electrochemical techniques, such as fluorescence spectrometry, UV-spectrophotometry (UV), continuous flow analyzer (CFA), capillary GC-ECD and ion chromatography (IC). Most of these techniques are known to be time-consuming and some of them lack specificity or sensitivity. The available results from both our laboratory and reported literatures for 2R4F Kentucky reference cigarette, smoked under ISO condition, show a relatively wide variation ranging from 100 to 120 ug/cig of HCN. Especially, the precision and accuracy of the analytical results of HCN tend to get worse in low tar cigarettes and under intense smoking condition.

In this paper, a more optimized analytical methods than previous ones are suggested. This method shows lower detection limit and has improved precision and accuracy, so it is applicable for wide tar level cigarettes under intense smoking condition as well as under ISO smoking condition. Important features of this method are improved sample collection and quantification systems such as the number of trapping units, volume, temperature and type of trapping solution. To avoid volatilization loss of HCN in analyzing mainstream smoke, it is highly recommended that pH values of trapping solutions should be maintained over 11 and cold traps should be used in collecting mainstream smoke.

Key words : Hydrogen cyanide, UV-spectrophotometry, continuous flow analyzer, capillary GC-ECD, mainstream smoke

Cigarette smoke is composed of more than 4,800 compounds and some of them are identified as toxic components. Especially, cyanide is one of the 44 harmful substances on Hoffmann's list which inhibits several respiratory enzymes, including cytochrome oxidase and is known to be a major ciliotoxic agent in cigarette smoke (Brunnemann *et al.*, 1997).

Cyanide refers to the anion radical. Compounds capable of releasing cyanide may be inorganic or organic in nature. Inorganic compounds may be simple (e.g. AgCN and KCN) or complex (e.g. $A[CN]_y$, $A[M]_x[CN]_y$). Organic compounds may be glycosides or nitriles. HCN is present in both the particulate and vapor phase in cigarette smoke. Total HCN can be measured by adding the HCN

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from the vapor phase and particulate phase (Rickert *et al.*, 1979; Collins *et al.*, 1973).

Collection techniques for vapor phase HCN include use of adsorbent materials such as silica gel or impingers filled with a suitable solvent. The pressure drop across the trapping units must be carefully controlled because it may significantly alter the puff profile and the analytical results. A common procedure for the determination of HCN in the smoke solution is based on a colorimetric reaction starting with the formation of cyanogens chloride obtained from HCN and chloramine T, followed by reaction with pyridine and then with pyrazolone to form a colored complex. The colorimetric detection techniques used for HCN determination, although it is relatively sensitive, must be carefully monitored for good reproducibility. Brunneemann used a gas chromatographic method based on the conversion of HCN to cyanogens chloride with chloramine T. The cyanogen chloride was extracted into hexane and analyzed by a gas chromatograph equipped with an electron capture detector (ECD).

In this paper, a more optimized analytical methods than previous ones are suggested. This method shows lower detection limit and improved precision and accuracy, so it is applicable for wide tar level cigarettes under intense smoking condition as well as ISO smoking condition.

MATERIALS AND METHODS

Reagents

All chemical reagents in this study were obtained commercially and used without further purification. Potassium cyanide, potassium phosphate monobasic, sodium phosphate dibasic and chloramine T were purchased from Aldrich Chemical Co. (USA). 3-Methyl-1-phenyl-2-pyrazolin-5-one was purchased from Fisher Scientific (USA). Bispyrazolone was purchased

from Fluka Chemical Co. (USA). Other solvents were products of Merck (USA). All reagents and chemicals used were analytical grade.

Preparation of cyanide standard solutions and other reagents

To prepare a primary stock solution equivalent to 250 ug/mL cyanogen ion, 60.2 mg of potassium cyanide was made up to 100 mL with 0.1 N NaOH solution. Aliquots of this solution were diluted to 50 mL with 0.1 N NaOH solution so as to be equivalents of 0.5, 1.0 and 8.0 ug/mL HCN. Chloramine T solution was prepared by dissolving 2.0 g of Chloramine T in 500 mL deionized water and the solution was kept refrigerated in an amber container. Saturated pyrazolone solution was prepared by stirring 5 g of 3-methyl-1-phenyl-2-pyrazolin-5-one with 2 L in deionized water for five hours, using a magnetic stirrer and stirring bar. Pyridine-pyrazolone solution was prepared by dissolving 0.08 g of bispyrazolone in 80 mL of pyridine in an amber bottle and mixing on magnetic stirrer for 30 minute. After bispyrazolone was dissolved completely, 400 mL filtered saturated pyrazolone solution was added to that solution and mixed well. The buffer solution was prepared by dissolving 13.6 g of potassium dihydrogen orthophosphate and 0.28 g of disodium hydrogen orthophosphate in deionized water and diluted to 1 L (Tob. Cont. Programme, 1999).

Instruments and their operating conditions

Chromatography was performed with Agilent technologies series 6890N equipment including a 7683 automated liquid sample-injection system, a split/splitless injector, a 30 m × 320 μm × 0.25 μm nominal HP-5 (5 %-phenylmethyl siloxane) capillary column and a ECD detector controlled by the Agilent Chemstation software. The carrier gas was helium at a constant flow rate of 0.8 mL/min and make-up gas was nitrogen at 30

mL/min. The capillary split/spiltless inlet was maintained at 100 °C. A split injection mode was used with a 1.0 µL injection volume and a split ratio of 20 : 1. The column oven and detector temperatures were 55 °C and 260 °C respectively (Juan X. *et al.*, 2006).

CFA of Bran Luebbe Analyzer II used for this study consists of an automated sampler, a proportioning peristaltic pump, colorimeter and a data processing system. The autosampler is operated at a sampling rate of 20 per hour with 2 : 1 ratio of sample to wash. Sufficient time should be allowed for the system to become stable with the reagent being pumped and the samples undergo on-line dilution.

A Jasco UV-560 double beam UV-visible spectrophotometer with a fixed bandwidth and data processing capacity was used for this experiment. The zero-order adsorption spectra were recorded over the wavelength range of 200~800 nm, against a solvent blank, in quartz cuvette with 1 cm diameter. The reactions of HCN for the determination by CFA and UV are based on the cyanogen chloride obtained from the reaction of chloramine T with HCN. It reacts with pyridine to produce glutamic aldehyde which in turn forms a colored complex with pyrazolone. A colored complex of HCN was measured at 540 nm.

Smoke collection and sample preparation

A Cerulean 20-channel smoking machine (Model SM 450) was set up to smoke sample cigarettes under ISO and intense smoking condition. Characteristics of regular cigarettes with wide tar levels and Ky2R4F analyzed in this study are shown in Table 1. The cigarette was conditioned at a relative humidity of 60 ± 5 % at 22 ± 2 °C for 48 hrs before smoking. The cigarettes smoking conditions were as follows. One puff per minute; puff duration 2 s; puff volume 35 mL filter ventilation blocking ratio 0 %

in ISO smoking condition, and one puff per 30 s puff duration 2 s; puff volume 55 mL filter ventilation blocking ratio 100 % in intense smoking condition. Also, in all experiments 5 cigarettes were smoked under ISO smoking condition and 3 cigarettes under intense smoking conditions. The machine generated smoke was led through a 44 mm Cambridge filter pad (CFP) to collect the particulate phase and then passed through several trapping units which contained 0.1 N NaOH solution or methanol to collect the vapor phase. These trapping units were connected serially to the smoking machine. After smoking, CFP was extracted with 40 mL of 0.1 N NaOH solution or methanol and then HCN in particulate phase and vapor phase were analyzed separately.

Table 1. Characteristics of sample cigarettes used in this study

Tar levels (mg/cig)	0.5	1.0	3.0	5.5	9.7*
Ventilation (%)	83	80	65	55	29
Filter type	Carbon dual	Carbon dual	Carbon dual	Carbon dual	Mono acetate

* Ky2R4F

RESULTS AND DISCUSSION

Colorimetry is being widely used for detecting cyanide and exploits its selective ability to form colored complex with various coupling reagents. Cyanide undergoes a series of reactions with chloramine T, pyridine and pyrazolone to produce a colored complex as shown in Figure 1. To set up the most suitable analytical procedure for HCN, analytical results by colorimetric measurements (UV, CFA) were compared with those by GC-ECD.

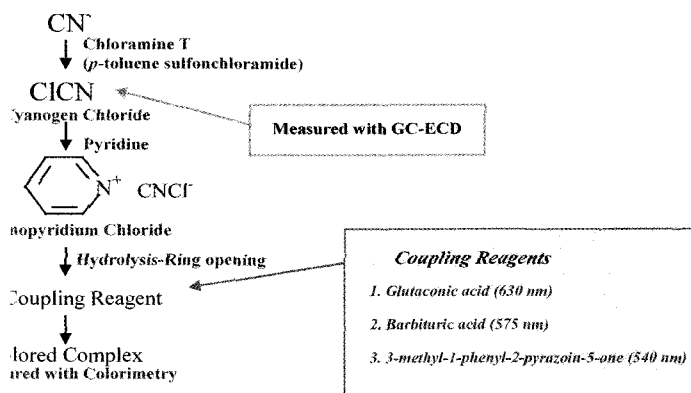


Fig. 1. Reaction scheme of CN^- to produce a colored complex for colorimetry.

The trapping system of total HCN significantly affected the trapping efficiency. The various differences between trapping systems were type of trapping unit, number of connected trapping units, volume of trapping solution, temperature of trapping solution and so on. In order to set up the most suitable trapping system, the trapping efficiency of total HCN according to type and number of trapping units was investigated. As shown in the Figure 2(a), the trapping efficiency of HCN was reduced according to the decrease in the pore size of the frit attached to trapping

unit. The amount of HCN in vapor phase of type B was slightly larger than that of type A. However, standard deviation from repeated measurements of HCN in vapor phase was 15.35 ug/cig in type B, larger than 7.43 ug/cig in type A. As shown in the Figure 2(b), trapping efficiency of the second and third trapping units was significantly lower than that of the first trapping unit when two or three trapping units were connected serially to the smoking machine. These results imply that the resistance by trapping units and change in the smoke

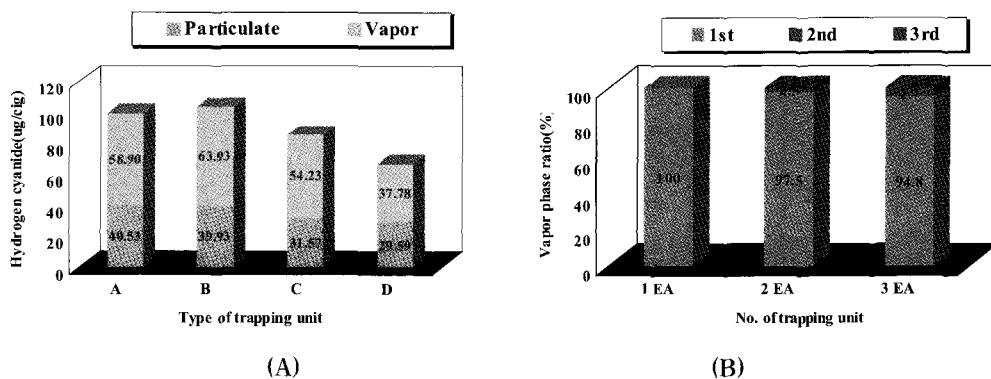


Fig. 2. HCN concentrations of Ky2R4F by trapping units of various sintered glass frit(a) and the number of trapping units(b) using 0.1 N NaOH solution.

(a) A: Without frit(Bubbler), B: 170 ~ 200 μm C: 40 ~ 60 μm , D: 10 ~ 15 μm

(b) Each impinger contains 20 mL of 0.1 N NaOH solution

Optimization of analytical procedure for hydrogen cyanide in mainstream smoke

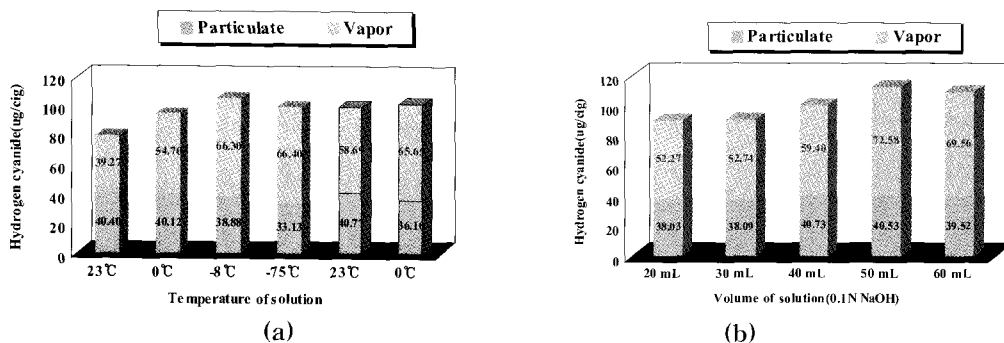


Fig. 3. HCN concentration of Ky2R4F according to temperature(a) and volume(b) of trapping solution.

(a) Without frame: methanol, with frame: 0.1 N NaOH solution

composition and puff profile may occur.

Figure 3 illustrates the change of HCN trapping according to the temperature and the volume of trapping solutions. In case of methanol, it is shown that as the temperature of trapping solution decreases, HCN trapping by the solution increases. However, the amount of HCN in the particulate phase decreases slightly as the temperature of the trapping solution decreases. When 0.1 N NaOH solution was used as trapping solution, the total amount of HCN was similar to that in methanol trapping solution maintained at very low temperatures. From Figure 3(b), we can

see that as the volume of trapping solutions increases to 50 mL, trapping efficiency for vapor phase HCN increases also. From these results, the ideal trapping system for the vapor phase HCN seems to be one bubbler filled with 50 mL of 0.1 N NaOH solution at room temperature.

Three methods of measuring HCN in cigarette smoke were tested and their results are illustrated in Figure 4. The ultra low tar cigarette with nominal tar level less than 1 mg/cig, produced very small amount of HCN in mainstream smoke under ISO condition. So it could not be detected by UV, but by CFA and

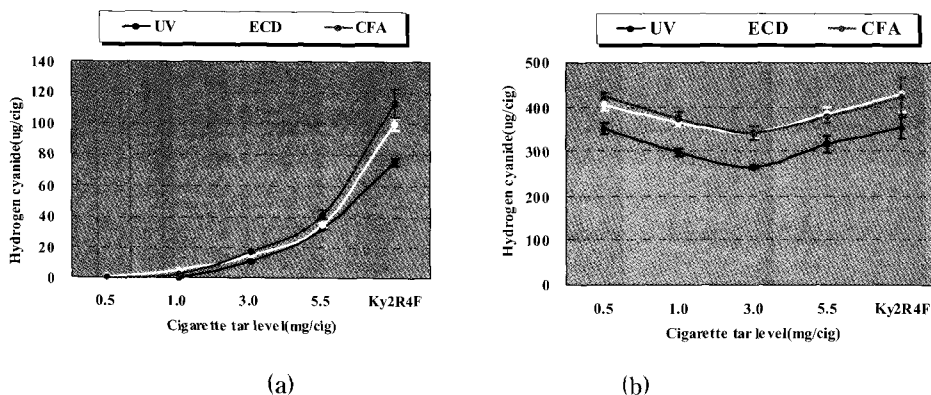


Fig. 4. HCN concentration of sample cigarettes under ISO and intense smoking conditions.

(a) ISO smoking condition (b) Intense smoking condition

Table 2. Validated data of the optimized analytical method for HCN in cigarette smoke*

Measurement method	R ²	LOD (ug/mL)	LOQ (ug/cig)	Recovery (%)	HCN of Ky2R4F in total HCN mainstream smoke			
					This work		Literature values**	
					AVE (ug/cig)	SD (ug/cig)	AVE (ug/cig)	SD (ug/cig)
UV	0.9999	0.41	3.25	90.0	75.75	12.4	109.2	9.0
GC-ECD	0.9923	0.01	0.30	91.4	100.32	4.9		
CFA	0.9999	0.09	0.69	102.1	113.78	8.9		

* Based on 10 observations of Ky2R4F (5 cigarettes/run)

** Beitr. Tabakforschung, Volume 20, No. 7, 448 ~ 458

GC-ECD. HCN contents measured by GC-ECD and CFA were very similar, although the standard deviation in CFA measurement is larger than that in GC-ECD. Under intense smoking condition, HCN contents measured by UV were constantly lower than those measured by CFA and GC-ECD. These results imply that measured of HCN by UV is not suitable for analysis of cigarette smoke.

Validated data according to measuring instruments employed in this study were shown in Table 2. Compared with relatively high limit of quantitation (LOQ) of UV measurement, measurements by CFA and GC-ECD showed about 5 to ten times lower LOQs. Recovery data of all measuring instruments were in the range of 90.0 ~ 102.1 %. The analytical results of Ky2R4F by GC-ECD and CFA under ISO smoking condition were in good agreement with published values.

CONCLUSIONS

Trapping efficiency for total HCN in cigarette smoke needs to be checked because HCN exists in vapor and particulate phase simultaneously. Therefore, an optimized analytical procedure for HCN in cigarette smoke has been set up and tested in this work. The results are as follows:

The most effective system for HCN trapping was shown to be 1 bubbler filled with 50 mL of 0.1 N NaOH solution at room temperature. As the number of trapping unit increased, trapping efficiency of total HCN decreased. However, as the volume and temperature of trapping solutions increased, trapping efficiency of vapor phase also increased. On the other hand, trapping efficiency of particulate phase decreased under the same conditions. Compared with UV measurement, GC-ECD and CFA showed lower limit of quantitations (LOQs) and broader linear range, and needed simpler sample preparation procedure. So, they could be applicable to ultra low tar cigarettes under various smoking conditions. Particularly, HCN measurement by GC-ECD seems to be the most suitable choice in the analysis of ultra low tar cigarettes under ISO smoking condition.

REFERENCES

- Brunnemann, K. D., Yu, L. and Hoffmann, D. (1977) Chemical studies on tobacco smoke: Gas chromatographic determination of hydrogen cyanide and cyanogens in tobacco smoke. *J. Anal. Toxicol.* 1(1): 38~42.
- Collins, P. F., Sarji, N. M. and William, J. F. (1973) A trapping system for the combined

determination of total HCN and total gas phase aldehydes in cigarette smoke. *Beitr. Tobakforsch. Int.* 7(2): 73~78.

- Juan, X., Hongwu, T., Xiangyang, Y., Shan, D., Zhongda, Y. and Shaomin, L. (2006) Sensitive determination of cyanide in cigarette smoke by capillary GC with a microECD. *Chromatographia*, 64(9): 609-612.
- P. X. Chen, S. C. and Moldoveanu (2003) Mainstream smoke chemical analyses for 2R4F Kentucky reference cigarette. *Beitr. Tobakforsch Int*, 20(7): 448~458.
- Rickert, W. S. and Stockwell, P. B. (1979) Automated determination of hydrogen cyanide, acrolein and total aldehydes in the gas phase of tobacco smoke. *J. Automat. Chem.* 1: 152~154.
- Tobacco Control Programme (1999) Health Canada, <http://www.hc-sc.gc.ca>.