Comparison of Solvent Extraction and Static-Headspace Analysis for Determination of Cyanide in Cigarette Smoke by Gas Chromatography Electron Capture Detector

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Two typical pretreatment methods including liquid-liquid extraction and static-headspace analysis had been optimized to determine cyanide in cigarette smoke by gas chromatography equipped with a micro electron capture detector. Within optimization of the extractions, both methods were compared including linearity, limit of detection, repeatability and recovery. Several commercial products were also analyzed and compared. As a conclusion, liquid-liquid extraction possessed higher sensitive while static-headspace analysis was green and broader linearity ranges. Both methods were accuracy and suitable for determine of cyanide in cigarette smoke.

Key Words: Liquid-liquid extraction, Static-headspace analysis, Cyanide, Cigarette smoke, Gas chromatography.

INTRODUCTION

Cyanide, a chemical asphyxiant, was a rapidly acting and powerful poison. Cyanide exerted its toxic effects by reacting with the trivalent iron of cytochrome oxidase, thus inhibiting electron transport and preventing the cells from using oxygen (hypoxia), which resulted in a rapid impairment of vital functions. Most of the cyanide in blood concentrated in the erythrocytes presumably bounded to methemoglobin, an oxidized form of hemoglobin, which acted as a "cyanidesink"[1].

Since its powerful toxicity, cyanide was list on the famous "Hoffmann's list" as one of the 44 types of harmful substances in cigarette smoke. Cyanide in cigarette smoke was mostly produced by the conversion of protein, amino acid and nitrate, especially by the pyrolysis of glycine, proline and dibasic amino acid[2]. The amount of cyanide in cigarette smoke might directly influent people's health[3].

However, to our best of knowledge a standard method for the determination of cyanide in cigarette smoke had not been established to date. Known methods were based on potentiometry, amperometry, fluorescence spectrometry, ion chromatography, spectrophotometry and segmented flow-injection analyzer (FIA)[4]. In these methods, ubiquitous problems were carcinogenic or mephitical reagents adopted as to pollute the environment to some extent. The determination was commonly interfered by other ions and not sufficiently sensitive to determine the amount of cyanide in cigarette samples, especially for "less harmful cigarettes" or "potential reduced exposure

products" (PREPs). Furthermore, the processes were used to be as complicated and time-consuming as to achieve automated analysis to meet the need of the factory.

The analysis of volatile compounds was normally carried out by gas chromatography (GC) after previous extraction and concentration. However, the extraction and concentration of volatile components from a complex system such as cigarette smoke, prior to their analysis, constituted a problem that had still not been satisfactorily resolved yet even today. Classical analytical methods, such as liquid-liquid extraction (LLE)[5], static-headspace analysis (SHS)[6], purge and trap (P & T)[7-10], simultaneous distillation-solvent extraction (SDE)[11], solid-phase extraction (SPE)[12], solid-phase micro extraction (SPME)[13] and so on had been widely used.

In spite of this great variety of analytical methods, liquid-liquid extraction method continued to be the most common technique for the extraction of volatile compounds. In this sense, a liquid-liquid extraction method with *n*-hexane in previous studies carried out by our team[14] was optimized here and a static-headspace method also had been adopted to determine the cyanide in cigarette smoke.

The purpose of this study was to compare the analytical result of cyanide from cigarette smoke by two methods coming from different classes: conventional solvent extraction and static-headspace methods. The choice of methods was made base on the accuracy, simplicity and practicality for analysis. The sample analysis was done using a conventional gas chromatography electron capture detector technique. In this

paper, the optimizations of liquid-liquid extraction and static-headspace analysis method for analysis of cyanide in cigarette smoke had been carried out. Then, both analytical methodologies had been validated and comparatively applied to the determination of cyanide in different brands of cigarettes. The analytical and procedural advantages and disadvantages of these two methods had been evaluated.

EXPERIMENTAL

Potassium cyanide (analytical grade) and *n*-hexane (spectrograde) were obtained from Dikma (USA). Chloramine-T, sodium hydroxide, potassium dihydrogen phosphate, disodium hydrogen phosphate and other chemicals and reagents were all analytical grade obtained from Shanghai No.3 Chemical Reagents Company, China. Deionized water was used.

The 92 mm diameter Cambridge filters were from Phipps and Bird (Richmond, VA, USA). Cigarette samples were supplied by Technology Center of Hefei Cigarette Factory, China.

Preparation of cyanide standards and other reagents: Prepared a primary stock solution equivalent to 500 μg mL^{-1} cyanide ion (60.2 mg of potassium cyanide made up to 50 mL with 0.1 mol L^{-1} sodium hydroxide). Diluted 0.1, 1 and 10 mL aliquots of this solution each to 50 mL with 0.1 mol L^{-1} sodium hydroxide as to be equivalent to 1, 10 and 100 μg mL^{-1} cyanide ion. These standards should be prepared fresh weekly[15].

A series of standard solution were obtained by dilution of the stock potassium cyanide standard with 0.025 mol L^{-1} sodium hydroxide.

Chloramine -T solution was prepared by dissolving 0.7743 g in 50 mL deionized water and the solution was kept refrigerated in an amber container. The buffer solution (pH 6.86) 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.

Equipments and conditions: Chromatography was performed with Agilent technologies (Wilmington, DE, USA) serious 6890 N equipment including a 7683 automated liquid sample-injection system, a split/split less injector, a 30 m × 320 μm × 0.25 μm nominal HP-5 (5 % pheny methyl siloxane) capillary column (Agilent Technologies, USA) and a μ-ECD detector controlled by the Agilent Chemstation software. General operating conditions for both methods were as follows in Table-1.

TABLE-1
INSTRUMENT PARAMETER SETTINGS

Parameter	Liquid-liquid extraction	Static-headspace analysis
Split ratio	20:1	50:1
Flow rate	0.8 mL min^{-1}	0.8 mL min^{-1}
Column oven temperature	55 ºC	55 ºC
Inlet temperature	100 ºC	100 ºC
Detector temperature	260 ºC	260 ºC
Transfer line temperature	-	90 ºC
Loop temperature	-	90 ºC
Vial equilibration time	-	25 min
Shaking (mixing) speed	-	Low
Loop fill time	-	0.5 min
Loop equilibration time	-	0.1 min

For static-headspace analysis, the analyte was transferred into 20 mL flat base headspace vials capped with 20 mm aluminum silver seals with PTFE gray butyl rubber septa (Agilent Company), other parameters were set as listed in Table-1.

Smoke collection and pretreatment: Seven brands of cigarettes were analyzed in present study. The cigarettes were stored for at least 24 h in a laboratory maintained at a relative humidity of 60 ± 5 % at 22 ± 2 ºC and were then selected by weight (± 20 mg of average weight of 200 cigarettes) and by draw resistance (± 7 % of average draw resistance of 50 cigarettes selected by weight). The cigarette smoking conditions were one puff per minute; puff duration 2 s; puff volume 35 mL.

Cigarettes were smoked with a Phipps and Bird 20-port smoking machine (Bargwaldt Technik RM 20/cs, Germany). The smoke was led through a Cambridge filter holder containing a 92 mm fiber glass filter disc to collect the particulate phase and then through several trap tubes each one contained with 20 mL of 0.1 mol L^{-1} sodium hydroxide solution in series connected to the smoke machine to collect cyanide in the gas phase. After smoking, mixed all sodium hydroxide solution and washed each tube with 5.0 mL of 0.1 mol L^{-1} sodium hydroxide, consolidated all trap solutions, totally there was 75 mL trap solution for smoke of each sample. The pad was collected and extracted with 50 mL of 0.1 mol L^{-1} sodium hydroxide on a wrist action shaken for 0.5 h. After these, the gas phase and particulate phase trap solutions were obtained for both methods.

For liquid-liquid extraction, the analyte solution was prepared in 25 mL cell. Firstly 10 mL of trap solution was piped into the cell with 2 drops of phenolphthalein, then 2.0 mol L^{-1} acetic acid until colour disappeared, at last 5 mL buffer solution and 0.30 mL chloramine-T solution added orderly into the cell. After these, the cell was immediately sealed and placed for 10 min, then 3 mL of *n*-hexane be used as extraction solvent to extract cyanogen chloride, briefly shaken and placed until the organic phase separated. 1 μL sample was injected into the gas chromatography. Reaction time and dosage used in the chloramine-T were selected based on studies performed.

For static-headspace analysis, the analyte solution was prepared by transferring 5 mL of trap solution with 2 drops of phenolphthalein, then 2 mol L^{-1} acetic acid until colour disappeared, after this, 5 mL buffer solution and 0.30 mL chloramine-T solution added orderly into a 20 mL headspace vial and immediately sealed with a PTFE-lined septum and an aluminum crimp cap (Agilent). Equilibrated for 25 min at sampling temperature (50 ºC) and, after this, using a 3 mL headspace syringe (Agilent Technologies, Wilmington, DE, USA), 1 mL volume of the headspace vapour was injected into the GC inlet.

The same treatment was adopted to treat the gas phase and particulate phase trap solutions of both methods.

RESULTS AND DISCUSSION

Optimization of sampling conditions: Both of the two pretreatment methods were based on the transformation of cyanide to cyanogen chloride by chloramine-T in suitable

buffer solution. Concentration of cyanide in analytes was obtained by determination of cyanogen chloride with GC-μECD after pretreatment. Since the chemical compositions of cigarette smoke were significantly complex and special[16], whether the sampling conditions used in usual methods would be suitable for cigarette sample were needed to be validate detailed. Considering this, sampling conditions had been optimized in this study.

It was necessary to ensure that all, or at least a very high proportion, of the analyte to be measured was successfully trapped. It was well known that a number of traps placed in series containing a smaller volume of solvent were more efficient than one trap containing a larger volume. Experiments were undertaken using different traps in series[17]. Each trap contained 20 mL of sodium hydroxide solution. The number of trap tubes significantly affected the collecting efficiency. In order to ensure all cyanide in cigarette smoke was trapped, the number of tubes was investigated. Different collecting efficiency was obtained with 1, 2, 3 and 4 tubes and the results indicated that for cyanide the trap efficiency with one tube and two tubes was respectively only 26.3 % and 64.8 % of that of three tubes. This might be caused by characteristics of cigarette smoke. Penetration factor of the smoke was strong and smoke flow rate was rapid, thus, even two tubes could not sufficiently trap all cyanide. On contrast, when more than four tubes were used, the resistance increased as to reduce the yield of smoke, the trap efficiency and even to change the composition of smoke. A good compromise between these two factors was achieved when three trap tubes were used.

The dosage of chloramine-T and acid salt tolerance of buffer solution could significantly influence the yield of cyanogen chloride. The used level of chloramine-T was significant concerned with the yield of cyanogen chloride. If not sufficient, the yields of cyanogen chloride would be less than the exact amount, on contrast, superfluous might cause the decomposition of cyanide since excessive oxidant. To study the effect of chloramine-T, validated with standard solution at two concentration levels (0.50 μg mL^{-1} and 40 μg mL^{-1}). The results indicated that the value was almost reached balance within 0.015 mL and 0.30 mL of chloramine-T for 0.50 μg mL^{-1} solution, while the maximum was obtained with 0.30 mL chloramine-T for 40 μg mL^{-1} solution. Considering the concentration of samples, 0.30 mL chloramine-T was ideal.

Although buffer solution did not directly enter into any of the reactions, it was perhaps one of the most important parameters that could directly influence the amount of cyanogen chloride. Optimization of pH for buffer solution was done here. The effect of pH was investigated within 5.91-7.38 (all used potassium dihydrogen orthophosphate and disodium hydrogen orthophosphate), pH 6.86 was the most suitable for further analysis.

The reaction time of cyanide with chloramine-T for liquid-liquid extraction was investigated in this study. It was obvious that as soon as the reagent was added the formation of cyanogen chloride was almost reached the maximum, within 7 min and 12 min, the value was kept. All of these indicated that the reaction was rapid and thorough. Finally, 10 min was chosen. Furthermore, the stability of cyanogen chloride was also investigated to ensure the experimental result was creditable.

The stability of extraction solution was investigated by storage of stock solution at 5 ºC, over different times (0, 1, 2, 3, 4, 6 h). It was found to be stable for 4 h[18]. The response value was trend to decrease beyond 4 h. This might be caused by the complexity of cigarette smoke. Further study would be done later.

Optimization of static-headspace analysis method: A static-headspace gas chromatographic analysis had been developed for the determination of cyanide in cigarette smoke samples. Taking into account a considerable number of variables were involved in static-headspace analysis performance, the optimization had been carried out in order to reduce the time and achieve the optimum working conditions. All these studies were carried out in triplicate and the optimization basically consisted of a factorial design, plus several replicates and statistical validity of results, must be carried out.

To study the dependence of the amount of analytes as a function of equilibrium temperature, experimental conditions were studied increasing the range of temperature. The effect of sample temperature values 40, 45, 50, 55 and 60 ºC was examined. The temperature 50 ºC gives the optimum response. Since one side the disturbance of water peak would be strengthen with the increase of temperature, other side, various low molecular weight compounds such as volatile acetaldehyde and ketone in cigarette smoke might get into the column and interfere in the separation of chromatographic peaks. Under the optimized conditions, ideal chromatograms were obtained and listed in Fig. 1.
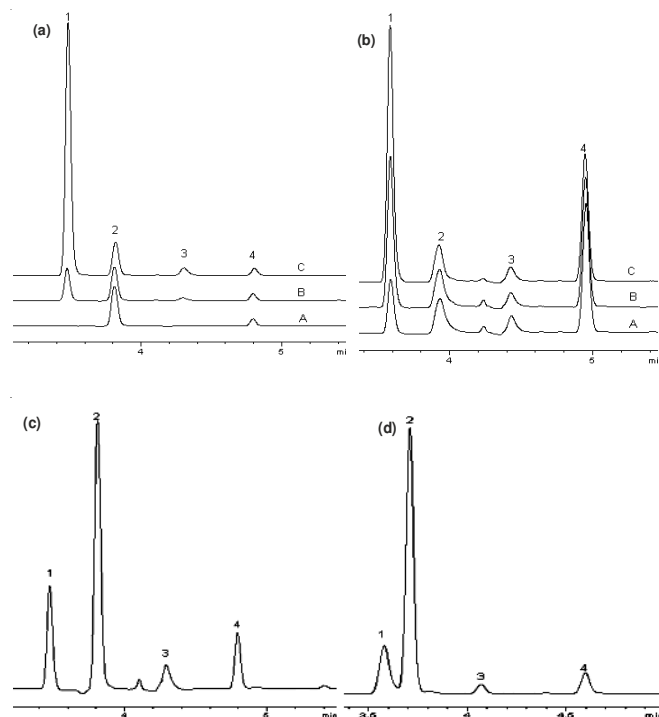

Fig. 1.   Chromatograms obtained from a standard solution; (1a for liquid-liquid extraction, 1b for static-headspace analysis) and cigarette sample (1c for liquid-liquid extraction, 1d for static-headspace analysis); In 1a, A = Reagent blank, B = 0.20 μg mL^{-1}, C = 0.60 μg mL^{-1}; in 1b, A = 0.20 μg mL^{-1}, B = 0.40 μg mL^{-1}, C = 0.80 μg mL^{-1}. In the four figures, peak 1 was cyanogen chloride, peak 2 in 1a and 1c was n-hexane, peak 2, 3 and 4 in the four figures were also observed in reagent blank and thought to be an impurity. They were not identified

TABLE-2
CALIBRATION CURVES RANGE OBTAINED IN THIS STUDY

Method	Linearity range	Linear equation	Correlation coefficient (r^2)
Liquid-liquid extraction	25.0 ng mL^{-1} - 15.0 µg mL^{-1}	y = 1107.80x - 57.96	0.9996
Static-headspace analysis	5.0 ng mL^{-1} - 50.0 µg mL^{-1}	y = 7258.85·x + 1800.74	0.9997

The effect of equilibrium time on the extraction efficiency was studied. Values of time of 10, 20, 25, 30 and 40 min of equilibrium time were applied. The results indicated that the response value increased with equilibrium time and the equilibrium state was reached at 25 min, while the response value had a trend to decrease for longer equilibrium time. The cost and time-consuming would also be increased with unnecessarily long equilibrium time.

The sensitivity of static-headspace analysis method was firmly concerned with the volume ratio of headspace to liquid phase. The two parameters were inverse relation. Different volume ratios had been optimized as 3:7, 5:5 and 7:3. Considering the sensitivity and the volatile of cyanogen chloride, the ratio 5:5 was final choice.

Other parameters setting had also been optimized such as the split ratio, carrier gas flow rate, *etc*. The best response factors were given in Table-1.

Optimization of liquid-liquid extraction method: The most popular technique for determination volatile chemicals involved some liquid-liquid extraction. The type of extractant was very important for achieving efficient analyte preconcentration. As we known, three kinds of extractants as diethyl ether[19], *n*-hexane[20] and methyl *tert*-butyl ether (MtBE)[21] had been used to extract cyanogen chloride.

In this study, cyclohexane had also been evaluated to determine the extraction efficiency of cyanogen chloride besides diethyl ether and *n*-hexane. As a result, cyclohexane could not be separated from cyanogen chloride, diethyl ether and *n*-hexane both were satisfied. The *n*-hexane response served as an accurate, relative measure of the volume of the cyanogens chloride extract injected, as an internal standard[20]. However, relative standard deviation (RSD) of determination results for parallel samples extracted with aether was not acceptable (> 5 %). This might be caused by the intense volatility of diethyl ether. Thus, *n*-hexane was our final choice. Other determination conditions were shown in Table-1.

Comparison of two methods: Two kinds of methods had been stabled for the determination of cyanide in cigarette smoke. Some differences were found between both analytical methods. Specific comparisons were made especially to show the advantages and disadvantages of two methods. Some typical chromatograms had been given in Fig. 1.

Linearity: Good linearity was obtained for both methods with correlation coefficient (r^2) greater than 0.9997 for static-headspace analysis and 0.9996 for liquid-liquid extraction. The curve ranges were 5 ng mL^{-1} to 50 µg mL^{-1} for static-headspace analysis and 25 ng mL^{-1} to 15 µg mL^{-1} for liquid-liquid extraction (Table-2). The values obtained were, in general, low enough to permit the determination of cyanide in real cigarette samples, although, as could be expected, those obtained by liquid-liquid extraction were lower than those obtained by static-headspace analysis. While much larger curve ranges was obtained by static-headspace analysis than that of liquid-liquid

extraction. The difference might be caused by the dosage of extractant used in liquid-liquid extraction method. 2 mL *n*-hexane might be relative absent when the concentration of analytes was beyond 15 µg mL^{-1}. However, just simply increasing the dosage of extractants would damage the solvent concentration. Taking account of this point, static-headspace analysis might have an advantage over liquid-liquid extraction.

Limit of detection and sensitivity: The limit of detection (LOD) was calculated from the signal-to-noise ratio (S/N) of the lowest detectable concentration and the value was 0.57 ng mL^{-1} for liquid-liquid extraction, while for static-headspace analysis, LOD was 2.3 ng mL^{-1}. The slope of the straight calibration lines was a measure of method sensitivity and depended on both extraction efficiency and detector response for each compound. For liquid-liquid extraction, the distribution of an analyte between two unmixed phases was an equilibrium phenomenon that depended on two groups of factors, one derived from the analytical process (volume ratio, ionic strength of the medium, *etc*.) and the other related to the characteristics of each compound, such as polarity, molecular structure and solubility in the aqueous matrix. In general, higher slopes were obtained for static-headspace analysis while lower LOD was for liquid-liquid extraction.

Repeatability and recovery: The repeatability had been evaluated by means of three sets of five extractions of a global standard solution. The measurements were found to be repeatable with RSD values of 2-6 % for static-headspace analysis and 0.5-5 % for liquid-liquid extraction.

To study recovery, two different kinds of cigarette smoke solutions were used for each method. Each sample solution was divided into four parts; one had no standard solution added, the other three were mixed with standard solutions in the approximate content rations 2:1, 1:1 and 1:2. The recovery, calculated with five replicates, was in the range of 91.4-104.3 % for liquid-liquid extraction method and 92.4-102.7 % for static-headspace analysis method (Table-3).

Comparison of results of cigarette samples: Both analytical methods were used to analyze six brands of cigarettes supplied by Hefei Cigarette Factory. The results obtained for these samples were shown in Table-4. As could be seen, the values obtained for these compounds by static-headspace analysis were similar to those obtained from liquid-liquid extraction.

Methods comparison with spectrophotometry: The two methods were validated by comparison with a spectrophotometry method which had been widely used for detecting cyanide and exploited for its selective ability to form coloured adducts with various assays[4,15]. The results obtained using the three methods for two samples were listed in Table-5. Analysis of the data showed that the results obtained from the three analytical methods had no significant difference (P > 0.05)[14], which demonstrated that there were good agreement between them.

TABLE-3
RESULTS OF RECOVERY EXPERIMENT

Sample No.	Static-headspace analysis				Liquid-liquid extraction			
	Cyanide added (μg)	Cyanide found (μg)	Recovery (%)	Average (%)	Cyanide added (μg)	Cyanide found (μg)	Recovery (%)	Average (%)
1	0.0	3.52	-	102.7	0.0	1.07	-	91.4
	1.5	5.08	104.0		0.5	1.58	102.0	
	3.0	6.78	108.7		1.0	1.93	86.0	
	6.0	9.24	95.3		2.0	2.80	86.5	
2	0.0	4.31	-	92.4	0.0	1.03	-	104.3
	1.5	5.75	96.0		0.5	1.55	104.0	
	3.0	7.04	91.0		1.0	2.19	116.0	
	6.0	9.73	90.3		2.0	2.89	93.0	

TABLE-4
CYANIDE CONTENT OF DIFFERENT CIGARETTE SAMPLES ($\mu g\ cig^{-1}$)

Methods	Sample no.					
	1	2	3	4	5	6
Liquid-liquid extraction	129.6	152.3	178.8	126.4	98.4	145.7
Static-headspace analysis	125.0	139.6	169.5	112.1	98.5	154.2

TABLE-5
RESULTS FROM COMPARISON OF THREE ANALYTICAL METHODS FOR DETERMINATION OF CYANIDE IN CIGARETTE SMOKE

Sample no.	Cyanide found [a] ($\mu g\ cig^{-1}$)			t (P = 0.95)	
	Liquid-liquid extraction	Static-headspace analysis	Spectrophotometry	Liquid-liquid extraction	Static-headspace analysis
1	88.1	88.5	90.2	0.84	0.81
2	87.4	-	85.6	0.39	-

[a]Not include cyanide in pad, for each kind of cigarette, five parallel samples were determined (n = 5, P = 0.95, $t_{0.05, 4}$ = 2.78)

Some comparisons between the two methods had also been done. The whole time consumed in liquid-liquid extraction method was less than static-headspace analysis while more manual intervention than that of static-headspace analysis. Taking account of the cost and ability of batch processing, liquid-liquid extraction was prior to static-headspace analysis. While the requirement of large quantities of expensive, toxic solvents that could be harmful to the environment was one of the biggest concerns with liquid-liquid extraction[22]. Static-headspace extraction was an attractive replacement for liquid-liquid extraction since it was faster and was a solvent-free sample preparation technique so it minimized the cost of high-purity solvents, it was easy to use and fast and very small sample volumes were necessary for the analysis.

Conclusion

A novel static-headspace analysis with capillary column GC-μECD for determination of cyanide in cigarette smoke had been established in this study for the first time. Further and detailed comparisons had also been done with traditional solvent extraction studied by our team before. The liquid-liquid extraction showed higher sensitivities than static-headspace analysis while static-headspace analysis with broader linearity ranges. The liquid-liquid extraction procedure provided the possibility of simultaneous extraction of several samples. However, the procedure itself was time-consuming and often requires pre-concentration of the extract prior to analysis. The requirement of large quantities of expensive, toxic solvents that could be harmful to the environment was one of the biggest concerns with this method. While static-headspace analysis was a solvent-free method presenting major advantages, such as small sample volume, sampling could be done rapidly, directly and could be easily automated sample preparation technique, higher sensitivity and simplicity. It constituted a good alternative to other commonly used pretreatment methods. As to the determination of real cigarette samples, both methods were adequate and sensitive and the results were demonstrated to be in good agreement.

As could be seen, in present case, both methods generated repeatable results, met the need of factory's batch processing and could be widely adopted to determine the cyanide in cigarette smoke.

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