*Journal of Chromatography*, 188 (1980) 357–362

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CHROM. 12,417

# DETERMINATION OF CYANIDE AND THIOCYANATE IN BIOLOGICAL FLUIDS BY GAS CHROMATOGRAPHY–MASS SPECTROMETRY

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(Received September 10th, 1979)

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## SUMMARY

A sensitive, specific and accurate method is described for the determination of cyanide and thiocyanate in biological fluids. Potassium cyanide containing 95% $^{15}N$ is added to the sample as internal standard and both endogenous cyanide and standard are separated by gas-phase diffusion into sodium hydroxide solution. Cyanogen chloride is prepared by reaction with sodium *p*-toluenesulphonchloramide and extracted into *n*-heptane. Quantitative analysis is carried out by gas chromatography–mass spectrometry (GC–MS) with selective ion monitoring of the molecular ions at *m/e* 61 and *m/e* 62. Thiocyanate levels are determined by a similar procedure in which $^{15}N$-labelled sodium thiocyanate is used as internal standard. The method is applicable to a wide range of biological samples and is free from interference by other sample components.

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## INTRODUCTION

A study of the role of smoke and fire gases in causing fire fatalities has indicated that hydrogen cyanide, produced by the pyrolysis and combustion of both natural and synthetic nitrogen-containing polymers[1–3], may be an important toxic factor in at least 5% of fire deaths[4]. The proportion of casualties due to inhalation of smoke and toxic gases has increased threefold over the last twenty years[5], in parallel with the widespread introduction of modern synthetic materials for the furnishings and construction of both domestic and industrial buildings. While no direct connection between these changes has been proved, one possible explanation of the rising mortality rate in U.K. fires lies in an increase in the toxicity of the fire atmosphere caused by the combustion of these new materials. In our study of fire deaths it was necessary to develop an analytical method for cyanide which was sensitive, accurate and specific.

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Cyanide is a normal constituent of blood usually present at concentrations below 12.0 μmol/l although in smokers it may rise to 20.0 μmol/l[5,6]. Thiocyanate is the principal metabolite of cyanide and plasma thiocyanate may be used as a measure of transformed cyanide[7,8]. Previous methods for the determination of the two species in biological fluids used visible or ultraviolet spectrophotometry[6,7,9,10]. In general, these methods lack sensitivity and specificity, both of which can substantially be improved by using gas chromatographic procedures especially where electron capture detection is used[11–14].

A gas chromatographic–mass spectrometric (GC–MS) method is now reported for the determination of cyanide and thiocyanate in biological fluids, in which $^{15}N$-labelled analogues are used as internal standards.

## EXPERIMENTAL

All reagents were of analytical grade and *n*-heptane (BDH, Poole, Great Britain) was redistilled before use through a 1-m fractionating column packed with glass spirals. Sodium *p*-toluene sulphonchloramide (chloramine-T) (Hopkins and Williams, Chadwick Heath, Great Britain) was recrystallised from methanol–water. A buffered solution of chloramine-T was prepared immediately before use by mixing 3 parts 1 *M* $NaH_2PO_4$ and 1 part 0.25% (w/v) chloramine-T in distilled water. Potassium cyanide containing 95% $^{15}N$ and sodium thiocyanate containing 95.2% $^{15}N$ were obtained from Prochem, B.O.C., London, Great Britain. Standard cyanide solutions were prepared in 0.1 *M* NaOH solution, to minimise loss of HCN, and were determined by titration against $AgNO_3$, using *p*-dimethylaminobenzylidenerhodanine as indicator.

### *Methods*

Blood samples were processed immediately after arrival through the first stage of microdiffusion and the resulting hydroxide solutions were stored at 4° until analysis[15]. If this was not possible the blood samples were stored at 4° until microdiffusion could be performed. Plasma samples were subjected to protein precipitation and the resulting deproteinised plasma stored at −28° until analysis.

*Cyanide.* Whole blood, or other biological fluid (2 ml) and standard solution of $KC^{15}N$ (10–50 μ*M* in $KC^{15}N$, 1 ml) were placed in the outer ring of a Conway Microdiffusion dish and mixed gently. A 1-ml volume of 0.1 *M* NaOH was then placed in the inner ring and 2 ml of 3 *M* $H_2SO_4$ added to the outer ring. The dish was covered immediately, swirled gently and microdiffusion allowed to proceed for 2 h.

The NaOH solution was then transferred to a 5-ml septum vial containing 0.1 ml *n*-heptane and 1 ml of freshly prepared chloramine-T solution. After mixing of the contents, the vial was placed in an ice bath for 10 min. The vial was then removed and reshaken. When the layers had separated, 1–5 μl of the heptane layer containing the dissolved ClCN were used for GC–MS. Standard solutions of $KC^{14}N$ (10–50 μ*M*) were similarly treated.

*Thiocyanate.* Plasma was obtained by centrifugation of whole blood at 1500 *g* for 5 min. To 1 ml of plasma was added 1 ml of a standard $^{15}N$-sodium thiocyanate solution (50 μ*M*, in distilled water). Proteins were removed by the addition of 10% (w/v) trichloroacetic acid solution (4 ml) and repeating the centrifugation. A 1-ml

aliquot of the supernatant was transferred to a septum vial containing 1 ml of freshly prepared chloramine-T solution, 0.1 ml of 0.25% (w/v) $FeCl_3$ and 0.1 ml *n*-heptane. The method then proceeded as for the cyanide analysis. Standard solutions of KSCN (50 $\mu M$, in distilled water) were treated similarly.

*Gas chromatography–mass spectrometry*

A Pye 104 gas chromatograph interfaced to a V.G. Micromass 16F mass spectrometer was used for the analysis. Gas–liquid chromatography (GLC) was carried out at 80° with a glass column (9 ft. × ¼ in. I.D.) packed with 7% Hallcomid M-18 on 80–100 mesh Chromosorb W AW DMCS. The helium carrier gas flow-rate was 30 ml/min. Under these conditions ClCN and solvent had retention times of 1.8 min and 4 min, respectively (Fig. 1). The column was clear for injection of the next sample after 10 min. The mass spectrometer was operated in the selective ion monitoring (SIM) mode at 70 eV ionising energy, filament current 200 $\mu$A and multiplier voltage 3.0 kV. The source and interface temperatures were 220° and 100°, respectively. A four-channel peak selection unit was used to monitor the ions at *m/e* 61 and *m/e* 62, corresponding to the molecular ions of $^{35}ClC^{14}N$ and $^{35}ClC^{15}N$, respectively, and a reference background ion at *m/e* 60. With a recorder voltage of 100 mV, a 2-ml blood sample 10 $\mu M$ in $CN^-$ would typically give a deflection of 50% of full scale on injecting 1.0 $\mu$l of heptane.

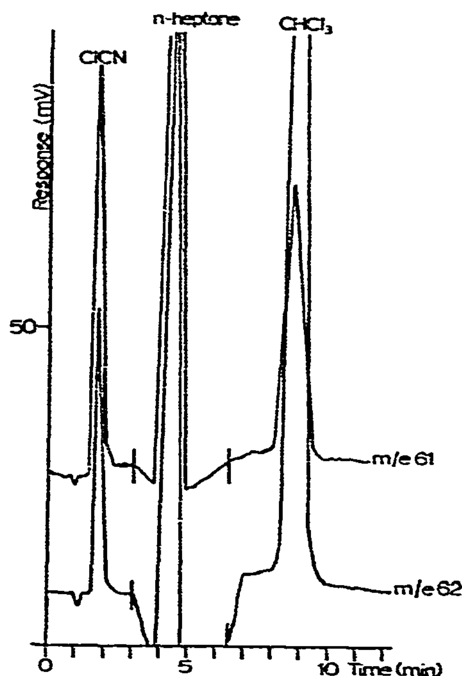


Fig. 1. Chromatograms obtained during the analysis of cyanide by SIM in a blood sample from a fire fatality. $C^{15}N^-$ is used as the internal standard and, following conversion to cyanogen chloride, the corresponding molecular ions are monitored at *m/e* 61 and *m/e* 62. The mass spectrometer dump valve is closed between 3 and 6.5 min during the elution of the solvent, *n*-heptane. Chloroform, an impurity in the solvent, elutes at 8.5 min.

## RESULTS

The GC–MS method was evaluated using aqueous standards of cyanide and thiocyanate and found to be linear over a range of concentrations from 5–100 μmol/l (Figs. 2 and 3). Four replicate analyses were carried out for each of five standard solutions with concentrations in the range 5–100 μmol/l. The standard deviation was less than 3.5% for cyanide analyses or 5.4% for thiocyanate analyses. The reproducibility of the method for blood analysis was determined by analysing eight replicate samples from blood with a low cyanide level, blood with a high cyanide level and plasma with a high thiocyanate concentration. The results are summarised in Table I.

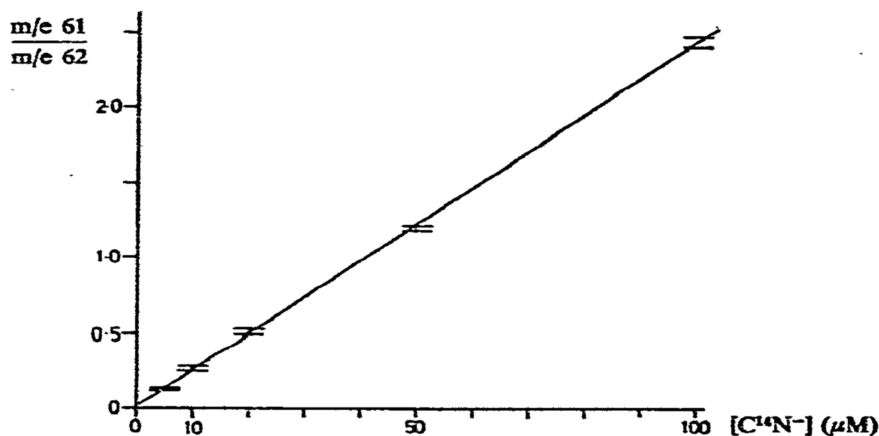


Fig. 2. Calibration curve determined with aqueous solutions of cyanide in the range 5–100 μmol/l, using a 50 μmol/l solution of $KC^{15}N$ as internal standard. The ratio of the peak heights of ClCN at *m/e* 61 and *m/z* is plotted against the $C^{14}N^-$ concentration (μmol/l).

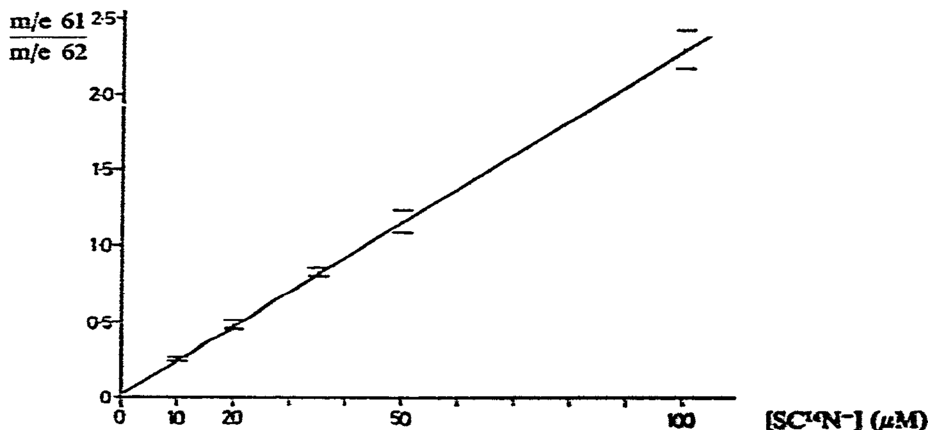


Fig. 3. Calibration curve determined with aqueous solutions of thiocyanate in the range 10–100 μmol/l using a 50 μmol/l solution of $NaSC^{15}N$ as internal standard. The ratio of the peak heights of ClCN at *m/e* 61 and *m/e* 62 is plotted against the $SC^{14}N^-$, concentration (μmol/l).

TABLE I

REPRODUCIBILITY OF THE GC–MS METHOD FOR WHOLE-BLOOD CYANIDE AND PLASMA THIOCYANATE

Number of samples: 8.

| *Species* | *Mean level (μmol/l)* | *Range (μmol/l)* | *S.D. (μmol/l)* |
|---|---|---|---|
| $CN^-$ | 3.0 | 2.7– 3.3 | ± 0.2 |
| $CN^-$ | 121.7 | 118.2–131.0 | ± 4.8 |
| $SCN^-$ | 181.5 | 167.7–194.1 | ± 7.9 |

Addition of $^{15}$N-labelled KCN to whole blood and then subsequent plasma thiocyanate analysis has shown that cyanide does not interfere in the thiocyanate method. Analyses of the cyanide content of blood to which $^{15}$N-labelled NaSCN has been added have shown that, at most, about 3% of the available thiocyanate has been converted to cyanide.

## DISCUSSION

Conventional colourimetric and GLC methods have been used for some time in the Department of Forensic Medicine for the analysis of small quantities of cyanide and thiocyanate in body fluids. Both techniques involve separation of cyanide from blood by microdiffusion, with subsequent trapping in alkali, and both depend on the absence of significant quantities of cyanide in plasma to permit isolation of thiocyanate. The ratio of $SCN^-$ to $CN^-$ in normal human plasma is usually 80:1 (ref. 7). Colourimetric procedures have several disadvantages including a lack of sensitivity, low stability of the coloured dyestuffs formed which are usually stable over a period of a few minutes only, the involvement of carcinogenic reagents, the inapplicability of internal standards and the possibility of negative errors and interference arising from the presence of sulphide[15]. The latter problem can be extremely important in the analysis of post-mortem blood samples.

Existing chromatographic methods offer considerable advantages in terms of specificity and sensitivity. Internal standards can be used but normally these may be added only at the final stage of the analysis.

The GC–MS method reported here was developed from a GLC procedure reported by Valentour *et al.*[12], in which cyanide is converted to cyanogen chloride, a volatile gas readily detectable by electron-capture GLC. Modifications were introduced to permit the use of $^{15}$N-labelled cyanide as the internal standard which is added directly to the sample, thereby compensating for incomplete recovery and conversion. Analyses are carried out by SIM using the molecular ions of $^{35}ClC^{14}N$ and $^{35}ClC^{15}N$ at *m/e* 61 and *m/e* 62. The stationary phase selected for the GLC column, Hallcomid M-18, gives a very low background at these ions and, as the solvent is eluted after the ClCN, large sample volumes up to 10 μl can be injected without interference in the analysis.

Most samples encountered in the course of our work have cyanide and thiocyanate levels in the range 0–100 μmol/l, within which the method was found to give a linear response (Figs. 2 and 3). A wide variety of biological fluids have been analysed using the method, including fresh blood from humans and experimental animals, old

blood samples, often putrified, from post-mortem dissections, plasma, serum, urine, dialysis fluid and stomach contents. No problems have been encountered as a result of interference by other sample constituents, although when interpreting the levels of cyanide observed, it must be borne in mind that artefactual changes occur during *in vitro* storage of blood samples and also, in some cases, thiocyanate may be converted to cyanide[16,17].

ACKNOWLEDGEMENTS

This work was supported by a contract from the Building Research Establishment, Fire Research Station, Borehamwood, Herts (No. FRO/28/059) and forms part of the work of the Fire Research Station. The authors gratefully acknowledge the assistance of Professor W. A. Harland, Dr. A. A. Watson and Dr. I. S. Symington in providing blood samples. It is contributed by permission of the Director of Building Research Station.

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