Validation of analytical method of cyanide compounds in blood by HS-GC-NPD

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Introduction

A method is described for the qualitative and quantitative analysis of cyanide (CN), a very short-acting and powerful toxic agent. Suicidal, homicidal and accidental deaths by inhalation of hydrogen cyanide (HCN) or ingestion of CN salts are encountered in clinical and forensic science practice. Blood CN concentrations are also raised in fire victims. To determinate the rate of CN in blood, gas chromatography (GC) connected to headspace (HS) with nitrogen phosphorus detector (NPD), was used for the method validation.

Materials

When CN was extracted after its conversion into HCN by acidification, the HeadSpace (HS) gas was injected into the GC system using gas-tight syringes. For this, GC was performed on a Hewlett-Packard HP7890 series gas chromatograph interfaced with an Agilent G1888 HeadSpace. An Agilent PoraBOND U (polar stationary phase) capillary column (25m x 0.32mm x 7µm, film thickness) and an oven temperature program from 90°C (5 min) to 140°C at 10°C/min are used. The carrier gas was H2 and its flow was 2 mL/min. To detect HCN and the internal standard, a nitrogen phosphorus detector was used with an air flow of 60 mL/min. The run time was 10 min with a solvent delay of 3 min.

Validation procedure

Linearity was established over six orders of magnitude in the concentration range 0.1-5µg/mL. Calibration samples are prepared by spiking the matrix with appropriate amounts of CN: CH_3CN was used as internal standard.
Calibration curves were constructed by plotting the peak area ratio between the analyte and the IS versus CN concentrations using six calibration points per curve (0.1, 0.25, 0.5, 1, 2.5 and 5 µg/mL). (fig. 1) Regression analysis was used to construct the calibration curves. The precision of the method was calculated as the percentage of relative standard deviation of three levels in water (0.175, 0.75 and 3.75 µg/mL), obtaining five replicates at each level (intra-day precision, n=5) and over three days (inter-day precision, n=12).
The accuracy was evaluated by calculating the percentage recovery of three spiked levels in water, as follows:

$$R\% = C_{OBS}/C_{SPIKE} \times 100$$

Where COBS was the mean concentration of the fortified sample and CSPIKE was the spiked concentration. All measurements were replicated five times.

Results and discussion

Acceptance for the accuracy and reliability criteria were given in the Guidance for Industry Bioananalytical Method Validation (FDA).The total validation was done in water for three consecutive days and partial validation in the blood should be done during the day.
Method validation was carried out in terms of linearity, precision and accuracy in both aqueous solutions and blood. The limit of detection (LOD) and limit of quantification (LOQ) were determined only in aqueous solutions.

The assay was linear over six orders of magnitude (0.1-5µg/mL), and the LOD and LOQ in water were 0.01µg/mL and µg/mL, respectively.
Good intra and inter-essay was obtained, always <9%. (fig. 2)

Figure 1: Chromatogram obtained for HS-GC-NPD analysis of IS and HCN gas

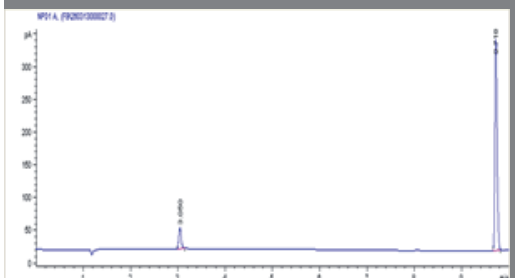

Figure 2 : Results of validation method in water during three days

Calibration point	[CN-] (µg/mL)	Accuracy (%)		Precision (%)
G1	0.10	6.0		114.1
G2	0.25	1.0		93.7
G3	0.50	4.9		96.8
G4	1.00	4.4		96.1
G5	2.50	2.2		96.4
G6	5.00	1.2		102.9
CQB	0.175	3.2	9.4	106.0
CQM	0.75	2.4	2.6	100.4
CQH	3.75	5.9	5.6	106.0

Conclusion

The method is simple, fast and sensitive enough for the rapid diagnosis of cyanide intoxication in clinical and forensic toxicology.

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