R. 45 Quantitation of cigarette mainstream smoke cyanide by LC-MS/MS

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Introduction

Cigarette smoke is a highly complex aerosol composed of several thousand chemical substances distributed between the gas and particulate phases. Numerous chemical classes are represented in cigarette smoke including saturated and unsaturated hydrocarbons, alcohols, aldehydes, ketones, carboxylic acids, esters, phenols, nitriles, terpenoids, and alkaloids. The enormous complexity of cigarette smoke is a consequence of multiple thermolytic processes (e.g., distillation, pyrolysis, and combustion).
Hydrogen cyanide is known to be present in cigarette smoke, and is thought to be generated by the pyrolysis or pyrosynthesis of tobacco leafs' components.

A method was developed to quantify cigarette mainstream smoke cyanide by LC-(ESI)-MS/MS, with the purpose of obtaining a selective, robust and reliable method. This method is based on a previous report of quantitation of blood cyanide by LC-MS after derivatisation[1].

Results & Discussion

The derivatisation reaction was found to be very quick, less than 5 minutes, for the standard as well as the samples. However, an excess of derivatising agents was necessary for the samples.

A 7-point calibration curve with a concentration range of 1.99 to 398.5 µg/cig was established with a correlation coefficient of $r^2 \geq 0.9995$.
The limit of quantitation was calculated as 0.013 µg/cig and the limit of detection as 0.004 µg/cig based on the variance of the calculated amount for the standard with the lowest concentration.
The average yield of HCN in the University of Kentucky reference cigarettes 1RF5F and 2R4F was of 28.95 µg/cig (SD: 2.32, RSD: 8.32%, n=4) and 103.90 µg/cig (SD: 3.75, RSD: 3.6% n=4) respectively.

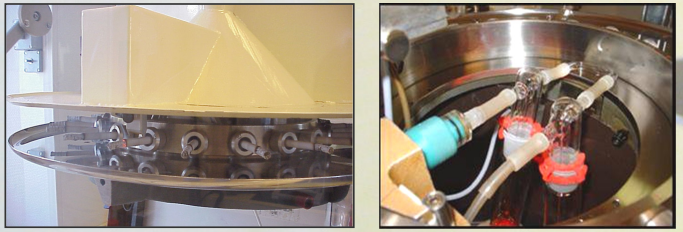
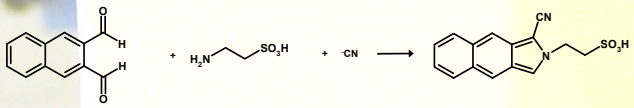


Fig. 3: The 20-port rotary smoking machine (left) and the two wash bottles connected in series (right)

Instrument repeatability was checked by ten injections of a 1R5F and ten of a 2R4F. The standard deviation obtained for each of them was of 0.32 µg/cig and 0.51 µg/cig respectively.



naphthalene-2,3-dicarboxaldehyde (NDA) | 2-aminoethane sulfonic acid (Taurine) | cyanide | 1-cyano-2-(ethyl-2-sulfate)-benz[f]isoindole (CBI)

Fig. 1: Schematic illustration of the reaction[2]

The derivatisation procedure was also tested: two 2R4F smoke extracts were derivatised 5 times each and then injected. The standard deviation obtained for the 1st was of 2.4 µg/cig and for the 2nd 4.5 µg/cig.

One of the causes of variability with the results was the smoking process. To obtain a better repeatability, the internal diameter of the plunger of the wash bottles used for smoke collection was narrowed from 5 to 2 mm. Thus, smaller bubbles were generated, which increased the contact surface with the solution and resulted in a three-fold decrease in the standard deviation for the 2R4F cigarette.

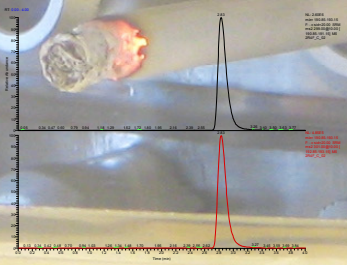


Fig. 3: Example of a chromatogram obtained with a 2R4F sample with sample (top) and ISTD (bottom)

Conclusion

A new, simple, fast, sensitive and reliable method for quantification of cyanide in cigarette mainstream smoke by LC-MS/MS was developed and validated.
This method requires the derivatisation of cyanide and uses $K^{13}C^{15}N$ as an internal standard. The reaction time is quick, less than 5 minutes, and the LC run under 4 min.

Experimental

Sample collection:

Prior to smoking, cigarettes were conditioned according to ISO conditions.
10 cigarettes per brand were smoked on a 20-port rotary smoking machine equipped with two wash bottles containing 40 mL each of an aqueous 0.1 M NaOH solution. The mainstream smoke was generated according to the ISO 3308 rule (35 mL puff volume and a 2 sec. puff duration every minute)[3].

Derivatisation procedure:

For the standards: KCN solutions were prepared in the appropriate concentration range in NaOH 0.1 M. 10 µL was pipetted into a vial containing 10 µL each of NDA (10 mM), Taurine (50 mM) and the ISTD. The solution was diluted to 1 mL with water.
For the samples: smoke samples were filtered through a 0.45 µm membrane and 20 µL added to a vial containing 50 µL each of NDA (10 mM) and Taurine (50 mM) and 10 µL of the ISTD. The solution was diluted to 1 mL with water.

LC-MS/MS analysis:

Column: RP-C18 Gravity, 70 x 4.6 mm, 3 µm, Macherey-Nagel
Mobile Phase: 60% NH_4COOH buffer (2 mM, adujsted to pH 3) and 40% Acetonitrile
Run: Isocratic
Flow rate: 800 µL/min
Injection vol.: 10µL
Retention time: 2.80 min

Detection was done by Multiple Reaction Monitoring (MRM) in the negative ion mode on a triple quad mass detector (TSQ Quantum, ThermoElectron) equipped with an ESI interface.
MS parameters: Spray voltage: 3 kV, Capillary °T: 350°C, CID source: 20, Sheath gas: 20
Monitored transitions: for the samples: 299.0 → 191.0 m/z
for the ISTD: 301.0 → 193.0 m/z

References:

[1]Determination of blood cyanide by HPLC-MS, Tracqui A., Raul J.S., Géraut A., Berthelon L., Ludes B., *Journal of Analytical Toxicology*, vol. 26 (2002)
[2]New derivatizing agents for amino acids and peptides. 1. Facile synthesis of N-substituted 1-cyanobenz[f]isoindoles and their spectroscopic properties; Carlson R.G., Srinivachasar K., Givens R.S., Matuszewski B.K., *Journal of Organic Chemistry*, vol. 51, n° 21 (1986)
[3]International Standard ISO 3308 1991. Routine Analytical Cigarette-smoking Machine - Definitions and Standard Conditions ISO (International Organization for Standardization) (3rd edn). ISO: Geneva