Heterocyclic aromatic amines in cigarette smoke, chemical and biological assessments

B. Teillet, N. Blin, O. Sevestre, V. Couillard, S. Achard, V. Troude.

ALTADIS Research Centre, 4 rue André Dessaux, 45400 Fleury les Aubrais, France

Objectives: Developing biological and chemical analyses in order to evaluate Heterocyclic Aromatic Amines (HAA) individual toxicity combined with inhibitory effect of mainstream cigarette smoke condensate mutagenicity.

Chemical Assessment - Abstract

Chemical assessment consists in the development of liquid chromatography tandem mass spectrometry (LC-MS/MS) for the screening of HAA (Acrylamide, PhIP, IQ, Trp-P, Glu-P, 5-AFC, 6-PGF) in mainstream cigarette smoke condensate by fly-2R4F reference cigarettes.

For the first time, HPLC/MS/MS/MS is applied to Heterocyclic Aromatic Amines analysis in mainstream cigarette smoke condensate. A purification step is required and consists in Blue Rayon following by Molecular Imprinted Polymer NNLAL solid phase extraction. Screening step needs further investigations for Glu-P and 5-AFC identification. The quantification of Acu, MeAc, Tp-P, 3-P and Trp-P-2 is performed by internal standard calibration which holds the reference to an identical extraction. Acu, MeAc, Tp-P and Trp-P-2 by 2R4F contents are assessed respectively to 28, 2.9, 5.7, and 3.5 pg/µg.

HAA, MeIQ, and PhIP contents seem to be lower than 0.3 ng/µg as formulated cigarettes at this level have been detected. The high toxicity of individual compounds especially IQ and MeIQ will involve method adjustment to enhance detection sensitivities.

As a result, the HPLC/MS/MS method is performing to achieve HAA screening for biological assessment correlation.

Biological Assessment - Abstract

The effect of HAA on the mutagenic activity of 2R4F Smoke condensate is assessed by Ames test using Salmonella typhimurium TA98 in presence of metabolic activation.

The HAA tested are IQ, MeIQ, and PhIP. Two Ames procedure have been compared:
- Direct Incorporation
- Direct Pre-Induction

In both conditions, MeIQ is more mutagenic than IQ when the HAA are tested alone.

When HAA at 0.03 to 30 ng/µg are combined with 2R4F Condensate (CSC) of 0.25µg/plate, a strong interaction between HAA and CSC was observed.

Mutagenicity results lead to two further questions:
- Are the interactions due to a toxic effect of the HAA?
- Do the HAA or their metabolites react with the CSC in order to reduce the mutagenic activity?

Ky-2R4F Screening by liquid chromatography tandem mass spectrometry

Liquid chromatography tandem mass spectrometry (LC-MS/MS) has been regarded to take over the first position factor in HAA analysis [1]. The full and accurate detection of HAA based on standards responses.

Figure 1: Chemical structure of Heterocyclic aromatic amines

Applied to Cigarette Smoke Condensate acid extract, a liquid chromatography tandem mass spectrometry detection appears to be specific enough against matrix smoke interferences. However, the highest contents, as HAA properties very heterogeneous (polarity, polarity), purification is focused on molecular recognition. Blue Rayon followed by Molecular Imprinted Polymer (MIP) NNLAL, solid phase extractions. Blue Rayon allows to eliminate the non target compounds and MIP NNLAL, seems to be a downstream support against resulted matrix interferences.

All HAA preparations are very heterogeneous. Polarity, polarity, purification is focused on molecular recognition. Blue Rayon followed by Molecular Imprinted Polymer (MIP) NNLAL, solid phase extractions. Blue Rayon allows to eliminate the non target compounds and MIP NNLAL, seems to be a downstream support against resulted matrix interferences.

The HPLC/MS/MS method is promoting to achieve HAA screening for biological assessment correlation.

Chemical assessment consists in the development of liquid chromatography tandem mass spectrometry (LC-MS/MS) for the screening of HAA (Acrylamide, PhIP, IQ, Trp-P, Glu-P, 5-AFC, 6-PGF) in mainstream cigarette smoke condensate by fly-2R4F reference cigarettes.

For the first time, HPLC/MS/MS/MS is applied to Heterocyclic Aromatic Amines analysis in mainstream cigarette smoke condensate. A purification step is required and consists in Blue Rayon following by Molecular Imprinted Polymer NNLAL solid phase extraction. Screening step needs further investigations for Glu-P and 5-AFC identification. The quantification of Acu, MeAc, Tp-P, 3-P and Trp-P-2 is performed by internal standard calibration which holds the reference to an identical extraction. Acu, MeAc, Tp-P and Trp-P-2 by 2R4F contents are assessed respectively to 28, 2.9, 5.7, and 3.5 pg/µg.

HAA, MeIQ, and PhIP contents seem to be lower than 0.3 ng/µg as formulated cigarettes at this level have been detected. The high toxicity of individual compounds especially IQ and MeIQ will involve method adjustment to enhance detection sensitivities.

As a result, the HPLC/MS/MS method is performing to achieve HAA screening for biological assessment correlation.

Mutagenicity assessment with Ames test

Cigarette smoke condensate (CSC) extracted from the cigarette, 2R4F is dissolved in DMSO to make stock solution (10 mg/L) which were stored at -80°C prior to use.

AMES TEST METHODOLOGY in presence of S9 : Mutagenicity was assayed by the Ames assay [2]. The bacterial used (TA98) are histidine requirement mutants of Salmonella typhimurium.

In presence of mutagen, the bacteria revert to a wild-type phenotype and restore the capacity to synthesize histidine (forward). The revertants are detected by their ability to grow in the medium without histidine.

The number of revertants is related to the mutagenic activity of the tested product.

MUTAGENICITY OF EACH COMPOUND ASSESSED INDIVIDUALLY:

Mutagenic potentiality for 2R4F condensate was assessed in dose ranging from 0.05 to 0.3 ng/µg.

Mutagenic potentiality for both HAA were assessed in dose ranging from 0.03 to 30 ng/µg.

EFFECT OF HAA ASSOCIATED TO 2R4F CONDENSATE:

2R4F CSC of the dose 0.25 µg/plate is combined with two doses of each HAA : 0.3 and 3 ng/µg.

Theoretical values were calculated by adding the number of revertants induced by 0.3 or 3 ng/µg of each HAA to the number of revertants induced by the CSC of 0.25 ng/µg.

In both conditions, a strong interaction is observed between CSC and the two HAA at dose 3 ng/µg (the observed mutagenicity is lower than the theoretical value).

In direct incorporation condition, this effect is also observed with the MeIQ at a lower dose (0.1 ng/µg).

These results lead to two further questions:
- Are the interactions due to a toxic effect of the HAA?
- Do the HAA or their metabolites react with the CSC in order to reduce the mutagenic activity?

References: