Rapid and sensitive method to analyse β-carbolines, norharman and harman in cigarette smoke

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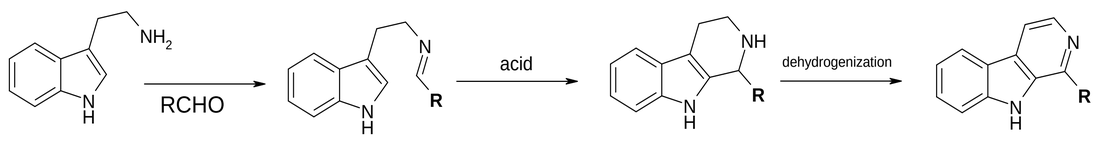
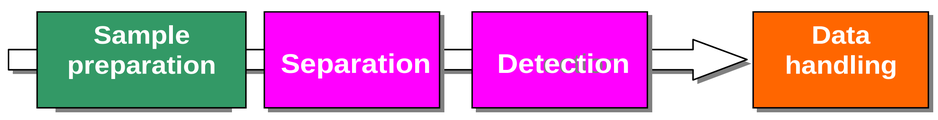
The β-carboline compounds, harman (1-methyl-9H-pyrido[3,4-b]indole) and norharman (9H-pyrido[3,4-b]indole), occur in medicinal plants and in variety of food, alcoholic beverages, industrial waste and tobacco smoke. These compounds are synthesized by the reaction of indoleamine with some aldehydes under an oxidative condition. Concerning the tobacco smoke, norharman and harman are formed during the combustion by Pictet-Spengler condensation of tryptophan with formaldehyde or acetaldehyde. These β-carbolines alkaloids exhibit a diverse range of pharmaceutical and biochemical activities.

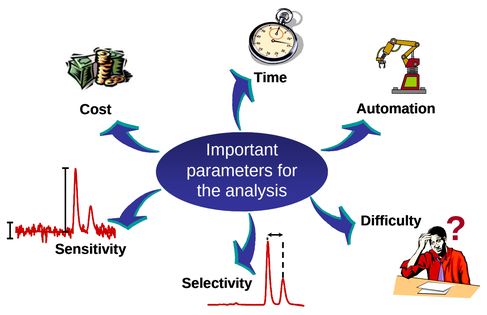


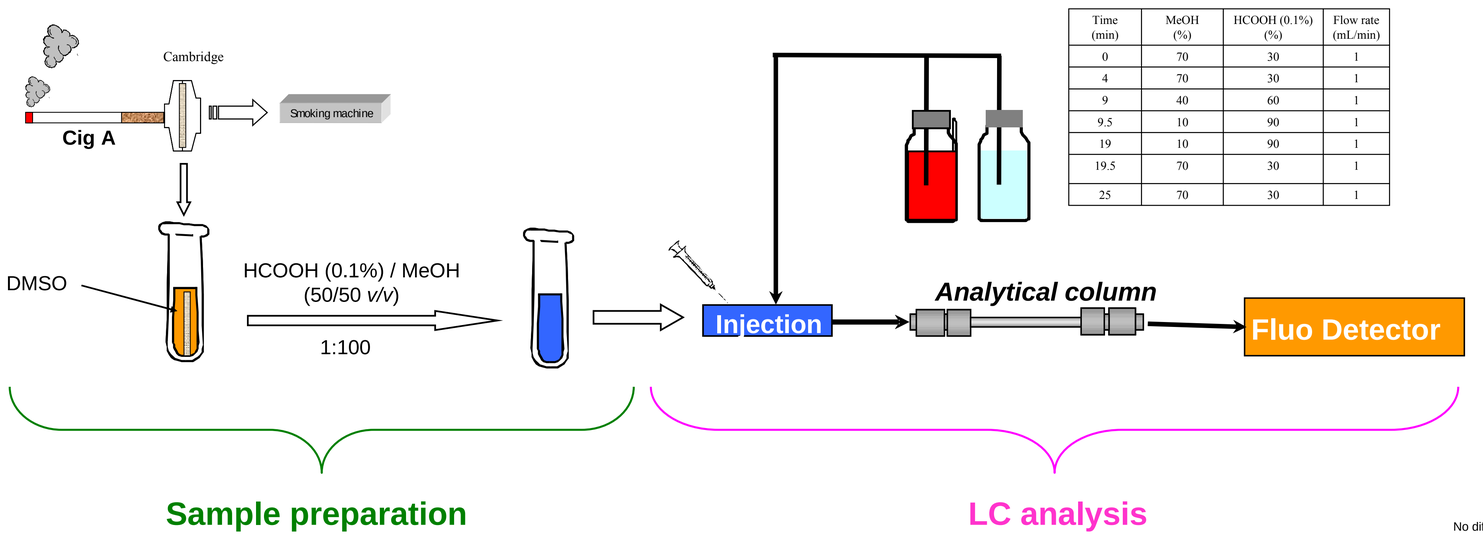
R = H : norharman
R = CH3 : harman

Here, we have reported the development of a rapid and sensitive method to analyse harmane and norharmane in cigarette smoke condensates. In order to minimize sample handling and to reduce analysis time, the method has been developed for the direct injection of smoke condensate solution. For it, the analyses were carried out by liquid chromatography using fluorescence detection. Several chromatographic parameters have been optimized, including the nature and the percentage of the organic solvent and acid modifier as well as column temperature. In-line sample preparation has also been tested and dimethylsulfoxyde appears to be the best solvent for a complete recovery. Moreover, this solvent is compatible with different in-vitro tests such as, the AMES test.

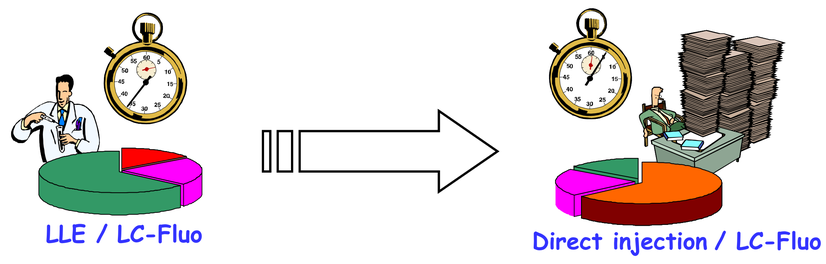
Analytical process





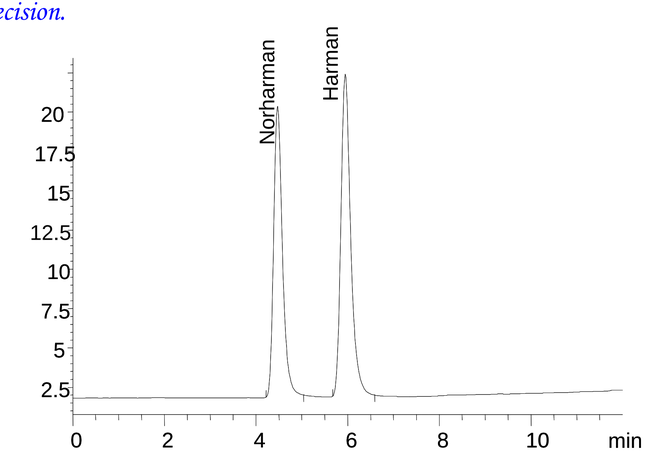


Time (min)	MeOH (%)	HCOOH (0.1%) (%)	Flow rate (mL/min)
0	70	30	1
4	70	30	1
9	40	60	1
9.5	10	90	1
19	10	90	1
19.5	70	30	1
25	70	30	1



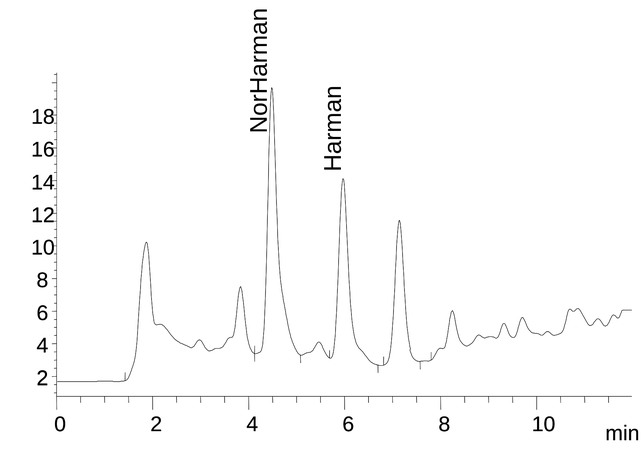
No difference

The optimal conditions give a separation of harman and norharman in less than 10 minutes with a good resolution. In order to validate the method, precision was determined measuring the repeatability, accuracy and intermediate precision.



Séparation of studied β-carbolines by LC-Fluo

Experimental conditions :
LC column : Hypersil Hypurity C18, 150 x 4.6mm, 5µm
Elution : acide formique 0.1% / Méthanol.
Flow rate : 1 ml / minute.
Column temperature : 30°C.
Injection volume : 10 µL
Fluometric detection: 260 et 440 nm.



LC-Fluo analysis of the selectec β-carbolines in smoke condensates

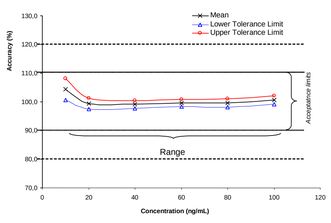
Experimental conditions :
Liquid chromatographic conditions are the same as Figure 3

Method precision was determined measuring the repeatability, accuracy and intermediate precision (between-day precision) of peak area of harman and norharman

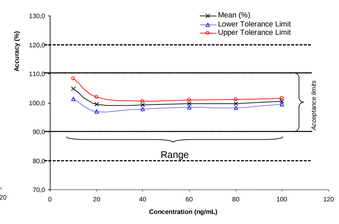
Solute	Day	Model	Coefficient correlation	slope	Intercept
Harmane	1	Linear	0.9998	14.89	29.46
Harmane	2	Linear	0.9998	14.73	31.50
Harmane	3	Linear	0.9998	14.69	34.70
NorHarmane	1	Linear	0.9998	9.54	21.32
NorHarmane	2	Linear	0.9999	9.55	21.41
NorHarmane	3	Linear	0.9999	9.49	21.70
				No difference	No difference

Accuracy Profiles

Harman



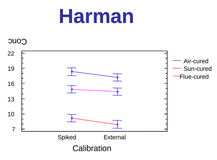
Norharman



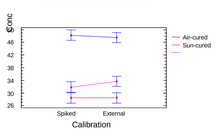
Matix effect

Solute	Matrix	Day	Measured concentration (ng/mL) External calibration method	Measured concentration (ng/mL) Spiked matrix method
Harman	Air-Cured	1	16,9	19,1
		2	17,6	18,2
		3	17	17,7
	Flue-cured	1	14,7	15
		2	15	15,2
		3	13,6	14,4
	Sun-cured	1	7,6	8,4
		2	8,4	9,5
		3	7,9	9,7
NorHarman	Air-Cured	1	46,7	50,7
		2	48	47,6
		3	47,8	46,5
	Flue-cured	1	32,8	31,3
		2	33,7	31,7
		3	34,5	32,5
	Sun-cured	1	27,7	26,9
		2	28,1	28,2
		3	29,5	30,3

Harman



NorHarman



Repetability

Injection	Harman Concentration (ng/mL)	Harman Per cigarette (ng/Cig)	Norharman Concentration (ng/mL)	Norharman Per cigarette (ng/Cig)
1	11.1	1909	18.9	3251
2	11.1	1909	19.0	3268
3	11.2	1926	19.2	3302
4	11.2	1926	19.2	3302
5	11.2	1926	19.2	3302
6	11.1	1909	19.1	3285
Mean	11.2	1918	19.1	3285
SD	0.05	9.31	0.13	21.5
RSD (%)	0.5	0.5	0.7	0.7

Reproducibility

Day (≠ smoking)	Harman Concentration (ng/mL)	Harman Per cigarette (ng/Cig)	Norharman Concentration (ng/mL)	Norharman Per cigarette (ng/Cig)
1	11.1	1909	18.9	3251
2	11.9	1952	20.6	3378
3	10.7	1883	19.4	3414
4	10.8	1966	19.5	3549
5	11.5	1886	20.3	3329
Mean	11.2	1919	19.7	3384
SD	0.50	38.0	0.69	111
RSD (%)	4.5	2.0	3.5	3.3

Harman	ng/mL	r = 0.01 ng/mL R = 0.70 ng/mL	RSD= 0.03 % RSD= 2.2 %
	ng/cig	r = 1.5 ng/cig R = 96.9 ng/cig	RSD= 0.03 % RSD= 1.8 %
Norharman	ng/mL	r = 0.06 ng/mL R = 1.98 ng/mL	RSD= 0.1 % RSD= 3.5 %
	ng/cig	r = 9.7 ng/cig R = 340.3 ng/cig	RSD= 0.1 % RSD= 3.6 %

Finally, the described method was applied to the quantitative analysis of harman and norharman in several cigarette smoke condensates.

Codification of analysed cigarettes

Cigarettes	Blend	Filter	Tar (mg/cig)	Nic (mg/cig)	CO (mg/cig)
1	American Blend	Cellulose acetate	10	0.8	10
2	American Blend	Cellulose acetate	7	0.6	9
3	American Blend	Cellulose acetate	4	0.4	5
4	American Blend	Cellulose acetate	2	0.2	4
5	American Blend	Cellulose acetate	10	0.8	10
6	American Blend	Cellulose acetate	8	0.6	9
7	UK blend	Cellulose acetate	10	0.9	10
8	American Blend	Cellulose acetate	10	0.8	10
9	American Blend	Cellulose acetate	8	0.6	9