VALIDATED HIGH-PERFORMANCE THIN-LAYER CHROMATOGRAPHIC METHODS FOR THE DETERMINATION OF NICOTINE IN TOBACCO (*NICOTIANA TABACUM* L.) EXTRACTS

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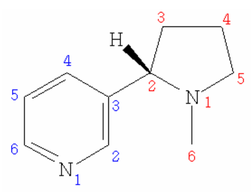
INTRODUCTION

Nicotine ((S)-3-(1-methyl-2-pyrrolidinyl)pyridine) is a chiral amine with a tobacco-like odour [1] which has been suspected to contribute to various human diseases such as stroke, reproductive disorder and renal disease [2].



Nicotine occurs in a wide variety of plant, especially in tobacco (*Nicotiana tabacum* L., *Solanaceae*) leaves [3] where it constitutes the principal alkaloid.

Mainly used in cigarettes or as rolling tobacco, tobacco leaves are also extracted in flavour and fragrance industries to be used as tobacco flavouring and sometimes in perfume compositions.

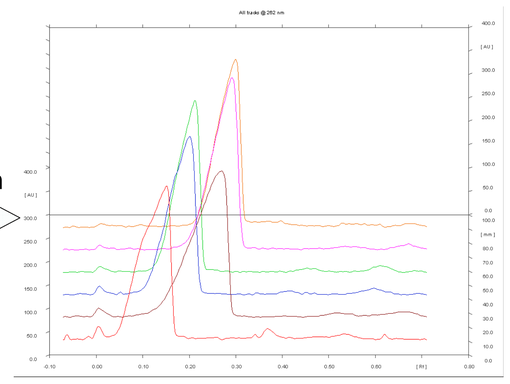
Due to its toxicity, nicotine content has to be precisely determined in tobacco extracts. Many analytical methods (GC, HPLC, UV…) have reported nicotine quantification in biological samples (blood, urine, plasma or serum) formulated products or smoke and air samples. Only few studies have reported nicotine quantification in ground tobacco leaves and no report refers to the nicotine quantification in tobacco extracts. That is why a sensitive and robust HPTLC method was developped and validated, according to ICH guidelines [4].

METHODE DEVELOPMENT

Optimum HPTLC conditions

Eluant	AcOEt (mL)	MeOH (mL)	Eau (mL)	Ammonia 2.8 % (mL)
A [5]	20	2.7	2	0
B	20	10	2	0
C	20	5	0	2
D	20	5	0	3
E	20	10	0	3
F	20	10	0	4

Scan 262 nm



Best results are obtained with **eluant F in « sandwich » mode:** ammonia is needed to stabilize nicotine under its non-protonated form and to reach a pH value higher than that of pKa_1 (11.24 vs 8.01)

Optimization of sample preparation

Solvent	Water bath (60°C, 1h); **% of nicotine**	Ultrasonic bath (42kHz ,3h); **% of nicotine**
KOH-MeOH 0.05N	7.42	7.24
KOH-MeOH 0.1N	8.70	8.20
KOH-MeOH/Water (8:2) 0.1N	8.30	8.07
MeOH/Water (8:2)	**9.08**	8.83
HCl-MeOH/Water (8:2) 0.1N	8.82	8.47

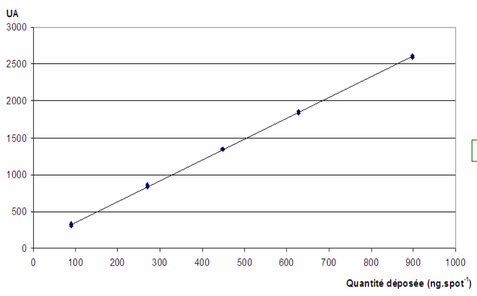
Experiment conducted on a tobacco oleoresin

The Biggest content of nicotine were found when samples are solubilized in MeOH/Water (8:2) and heated at 60°C. After parameters optimization, time preparation was shorten and the following conditions were defined: **MeOH/Water (8:2) heated 15 min. at 60°C.**

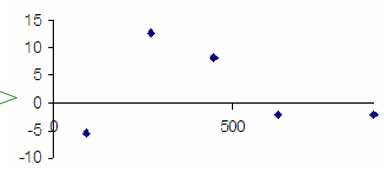
METHODE VALIDATION

Linearity and working range

5 standard solutions (90, 270, 450, 630 and 900 mg/L) were applied (5 times for lowest and highest concentration, other levels in duplicate): n = 16, k = 5



Lack of fit test



$F_{exp} < F_{th}$ (4, 11, 95 %)

Linearity is validated between 90 – 900 ng.spot^{-1}

Accuracy

Recovery studies were performed at three different levels. 50, 100 and 150 % of nicotine were added to the pre-analyzed and corresponding solutions were analyzed by the developped method.

% Recovery: 99 – 101 %

Precision

To determine intra- and inter-day precision of the method, sample preparation was done in triplicate on the same day (intra-day), on three different days (inter-day) and resulting solution were analyzed in triplicate. % R.S.D. were calculated.

% R.S.D. (Intra- and inter-day precision) < 1 %

Robustness

Robustness of the method was evaluated by introducing small changes in the method (mobile phase composition, mobile phase volume, migration distance or plate pre-washing. % R.S.D. were calculated.

% R.S.D. (Intra- and inter-day precision) < 1 %

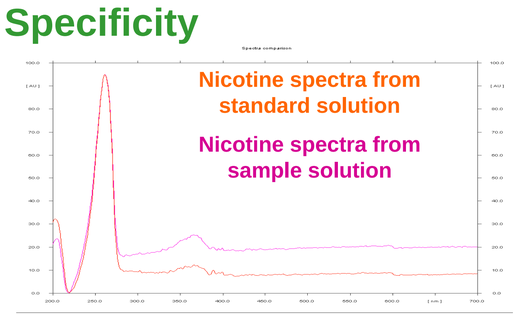
LOD/LOQ

Solution of nicotine (standard) and solvent of solubilization (blank) were spotted and analyzed; signals at $Rf_{(nicotine)}$ in blank tracks were integrated.

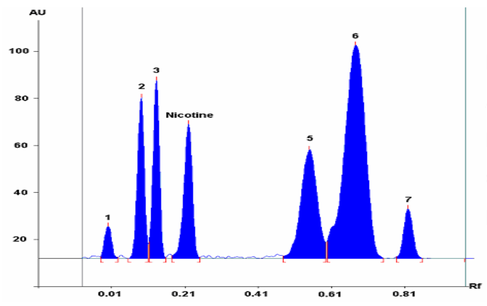
- LOD = 3 × noise
- LOQ = 10 × noise

LOD = 7 mg/L, LOQ = 24 mg/L

Specificity



R = 0.9994 for spectra correlation



D,L-nornicotine (2)
Anabasine (3)
Nicotine
Cotinine (5)
Myosmine (6)

Nicotine does not co-elute with other tobacco alkaloids

The developped method is specific for nicotine quantification.

NICOTINE QUANTIFICATION IN TOBACCO EXTRACTS

Results obtained by HPTLC were compared to these obtained by HPLC (Gémini NX 5 µm C18, 10 mM NH_4HCO_3 (pH10)/ACN: from (95:5) to (5:95), λ = 261.9 nm)

Concrete: Extraction of tobacco leaves with petroleum solvent.

Absolute: Solubilization of concrete in ethanol and filtration.

Oleoresin: Extraction of tobacco leaves ethanol.

LN extract: Oleoresin denicotinized

Extracts	HPTLC	HPLC	S.D.	% R.S.D.
Absolute (%)	37.91	36.80	0.78	**2.1**
Oleoresin (%)	2.61	2.66	0.04	**1.4**
LN extract (ppm)	228	214	10	**4.5**

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