STPOST 13

SS POST - 13

RAPID METHOD FOR RESIDUAL ANALYSIS OF BENZO(a)PYRENE IN TOBACCO AND TOBACCO PRODUCTS BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY WITH FLUORESCENCE DETECTOR

B.J.Rajesha, S.V. Dhalewadikar, S. K. MEHTA.

ITC R&D Centre, ITC Ltd,

Bangalore, India

Abstract

A Quick and simple HPLC method is developed for the determination of benzo(a)pyrene { B(a)P} residues in tobacco and tobacco products using HPLC with fluorescence detector. B(a)P is extracted from tobacco after refluxing with ethanolic KOH and liquid - liquid partition into a mixture of (cyclohexane & chloroform) and distilled water, concentrating the extract using rota evaporator and diluting with acetonitrile and subsequent chromatographic separation on LiChrospher RP- 18e (250mm x 5 micron x 4.0 mm) under gradient condition using Mobile phase A - {Acetonitrile (70%), 1% iso-propyl alcohol in distilled water(30%)} and Mobile phase B- {Acetonitrile (100%)}.

The separations are monitored by fluorescence absorbance excitation at 365 nm and emission at 425 nm. The method has been validated by standard validation protocols i.e. limit of detection, limit of quantification, recovery, repeatability and reproducibility. Minimum recovery of 83% was obtained with a linear regression coefficient of 0.9999. Limit of detection was 0.07 ppb.

Titled method provides several advantages such as sensitivity, rapid and easy sample preparation.

Introduction

Currently standard method for the determination of B(a)P in tobacco is health Canada method.It involves tedious multi step sample cleaning with the use of cartridge / column to remove matrix interference of tobacco. Therefore it was felt to develop a simple analytical method without any matrix interference of tobacco.

Literature

Few analytical methods :

a) Health Canada test method T-307. Determination of benzo(a)pyrene in whole Tobacco.

b) Coresta recommended method 58. Determination of benzo(a)pyrene in cigarette mainstream smoke by GCMS method

Sample preparation

Weigh about 2g ground tobacco in 250 ml round bottom flask, reflux for 2 hrs with alcoholic KOH solution and filter

↓

Transfer the filtrate into the separating funnel, B(a)P is extracted with the mixture of cyclohexane and chloroform

↓

Wash the organic layer 2-3 times with water collect the organic layer and rinse the water layer 3-4 times with the organic solvent mixture (Cyclohexane and chloroform mixture)

↓

Concentrate the organic solvent mixture layer with rota evaporator to 2 ml , transfer to 25 ml volumetric flask and make up volume with acetonitrile, sonicate for 10 min and filter through 0.45 um disposable syringe filter and Inject the sample to the LC system.

LC Conditions

Fluorescence detector

Excitation wavelength : 365 nm

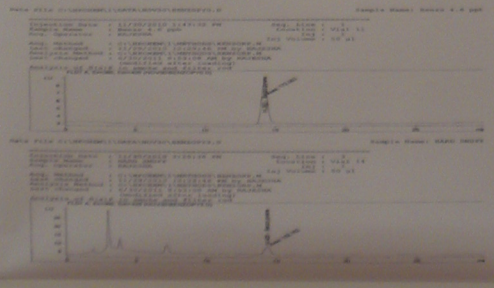
Emission wavelength : 425 nm

Mobile phase –A : 70:30 (Acetonitrile : 1% IPA in distilled water)
Mobile phase – B : 100% (Acetonitrile)

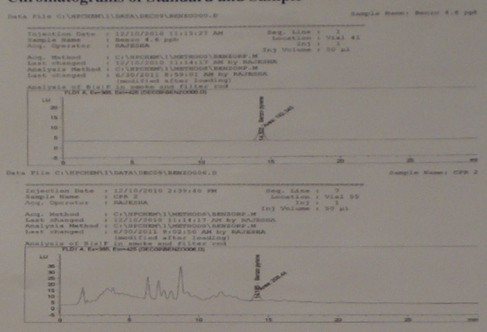
Flow rate : 1.0 ml /min
Gradient programming :

Time,Min	Pump A,Flow %	Pump B,Flow %
0	55	45
20	65	35
25	75	25
28	100	0
34	100	0
Method end Action :	100	0

Chromatograms of Standard and Sample



Chromatograms of Standard and Sample



Limit of detection and limit of quantification

Determination of signal-to-noise ratio is performed by comparing measured signals from samples with known low concentration of analyte with those of blank samples and establishing the minimum concentration at which the analyte can be detected.

1) Signal to noise ratio between 3 :1 is considered limit of detection.

2) Signal to noise ration between 10:1 is considered limit of quantification.

Inference :

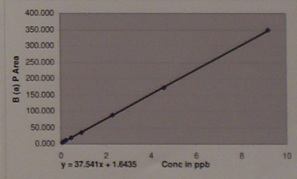
a) Limit of detection is : 0.07 ppb (S/N ratio : 3.6)

b) Limit of quantification : 0.42 ppb (S/N ratio : 13.1)

Validation : Linearity

Fig: Indicates variation of responses of benzo(a)pyrene peak against respective **responses**

Conc, ppb	B(a) P Area
0.07	4.0
0.14	6.9
0.23	11.9
0.46	19.1
0.92	35.0
2.3	88.9
4.6	171.8
9.2	348.1



Recovery

Studies conducted by adding a known amount of B(a) P to the tobacco sample

Trail No	B(a)P in ppb		Recovery%
	Spiked	Recovered	
1	1.44	1.20	83.3
2	3.68	3.31	89.9
3	182.8	161.4	88.3

Repeatability

Tobacco samples of straight grade were analyzed under repeatability condition and Rsd was well within the limit.

Trial No	Benzo(a)pyrene (ppb)
1	24.06
2	25.88
3	22.49
4	26.42
5	19.66
6	24.22
Avg	**23.79**
Sd	**2.46**
RSd%	**10.35**

Reproducibility

Same blend samples analysed under reproducible conditions and RSd % was well within the acceptable limits.

Trial No	Benzo(a)pyrene (ppb)
1	26.2
2	27.0
3	24.5
4	28.4
5	21.7
6	25.2
Avg	**25.32**
Sd	**2.29**
RSd%	**9.04**

Samples

Various tobacco products were analyzed

Sl NO	Sample	B(a) P in ppb
1	Pouch snus	2.51
2	Moist snus	62.86
3	Dry snus powder	45.14
4	Hard snuff pellet	1.86
5	Loose leaf	1.60

Conclusion

1. **This method is fast ,easy sample preparation and eliminates the use of catridges /cleaning columns.**

2. **Excellent resolution of Benzo(a)pyrene peak from tobacco impurities.**

3. **No matrix interference as evidenced from the recovery studies.**

2011_STPOST13_Mehta.pdf

SSPT2011 - Document not peer-reviewed by CORESTA