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Journal of Chromatography A, 985 (2003) 303–312
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

Analysis of pyridines in mainstream cigarette smoke

N.P. Kulshreshtha, S.C. Moldoveanu*
Brown & Williamson Tobacco Corp., 2600 Weaver Road, Macon, GA 31217, USA

Abstract

A new technique has been developed for the quantitative analysis of pyridines in mainstream cigarette smoke using a GC–MS technique. For analysis, 10 cigarettes are smoked using conditions based on US Federal Trade Commission recommendations. The smoke is collected in a water trap and analyzed using a GC–MS technique. A standard or a fast GC separation can be applied for the analysis. The standard separation was followed by MS detection using selected ion monitoring (SIM) acquisition on a quadrupole instrument. The fast GC was followed by MS detection with total ion acquisition on a time-of-flight instrument. The levels of pyridine depend on the type of cigarette: for a full flavor cigarette pyridine is as high as 18.0 μg/cigarette (cig.), and for an ultra light cigarette is about 3.0 μg/cig. Substituted pyridines vary between 5.0 μg/cig. to 0.1 μg/cig. for a full flavor cigarette, and between 0.2 μg/cig. and a few ng/cig. for an ultra light cigarette. The reproducibility of the technique is very good, with less than 7–8% RSD in both separation procedures for most of the analyzed compounds.

Keywords: Cigarette smoke; Pyridines

1. Introduction

Pyridine and a number of substituted pyridines are present in tobacco smoke and they play an important role in the sensory properties of cigarette smoke. For this reason, the determination of the levels of various pyridines in cigarette smoke is of interest. A number of procedures for the determination of pyridines in cigarette smoke or in environmental tobacco smoke are reported in the literature [1–6]. Most of these techniques use the collection of particulate phase smoke on a pad followed by collection of the vapor phase in a cryogenic trap containing a solvent. Two collection steps are usually needed since pyridine and C_1–C_3 substituted pyridines are present in both particulate phase and vapor phase smoke. Therefore, the collection of particulate phase smoke alone (total particulate phase or TPM) does not completely capture the pyridines due to their volatility. The smoke condensate on the pad is usually dissolved in a solvent which is then combined with the contents of the trap. Single-step collection techniques for cigarette smoke such as electrostatic precipitation [1] can be utilized, but this procedure does not account for vapor phase pyridine. Solvent trap collection alone also can be utilized for pyridine collection. After the smoke collection, most procedures use gas chromatography (GC) or GC–mass spectrometry (MS) directly for analysis, without special treatment of the sample [1]. Typically, only pyridine and 3-vinylpyridine are determined in smoke, while the level of other substituted pyridines are only estimated [5]. A sample preparation procedure is necessary when pyridine and substituted pyridines are analyzed in very complex samples containing numer-

*Corresponding author.
E-mail address: smoldov@aol.com (S.C. Moldoveanu).

0021-9673/02/$ – see front matter
PII: S0021-9673(02)01472-3

ous other nitrogenous compounds like alkylquinolines, alkylbenzoquinolines, alkylcarbazoles, etc. This is, for example, the case of analysis of alkylpyridines in matrices such as coal distillates [7], deasphalted heavy oils [8], shale-derived fuels [9], etc.

The procedure described in this report does not use a pad, instead, two traps collect both particulate and vapor phase smoke. The analysis of pyridines further uses a cleanup step before the analysis. This step allows the removal of many interferences and significantly improves method sensitivity. The analysis of the processed sample is done by a GC–MS procedure with good reproducibility even at very low levels of pyridine or substituted pyridines typically present in the smoke of ultra light cigarettes.

2. Experimental

For analysis, 10 cigarettes are smoked on a Borgwaldt RM 20/CS rotary machine using a puff of 35 ml with 2.4 s duration every 60 s [modified US Federal Trade Commission (FTC) conditions]. The modification of the smoking conditions to 2.4 s puff duration from 2.0 s puff duration as recommended by the FTC for smoking through a smoke pad, was performed to compensate for the pressure drop in the collecting traps. The smoke is passed through two traps fitted with extra coarse frit, each trap containing 100 ml of DI water. The two volumes of water are combined and a 100-ml aliquot is acidified with 0.5 ml of 3 *M* HCl solution. To this solution 50 μl of 2000 ppm ^{15}N labeled pyridine as internal standard is added. The choice of ^{15}N-pyridine as an internal standard instead of, for example, $^{2}H_5$-pyridine was made to minimize the modification in time of the concentration of a deuterated standard, due to isotopic exchange. The sample solution is extracted twice with 5 ml CH_2Cl_2. The non-aqueous phase is discarded. The aqueous phase is treated with 0.5 ml of 20% NaOH and than extracted twice with 2.5 ml CH_2Cl_2. The non-aqueous phases are combined and analyzed by GC–MS. A flow chart showing this cleanup procedure is shown in Fig. 1.

Two procedures were applied for the GC separation, one regular and the other using fast GC, both with He as a carrier gas. The chromatographic conditions applied for the two separations are described in Table 1. The detection for the regular separation was done using a HP 5971 (Agilent) mass spectrometer working in the selected ion monitoring (SIM) mode. A typical chromatogram containing standards at 1 μg/ml concentration and obtained in the conditions described for the regular separation and SIM acquisition is shown in Fig. 2. The detection for the fast separation was done using a Pegasus II TOF instrument from Leco acquiring 5 scan/s. A typical chromatogram containing standards at 1 μg/ml concentration and obtained in the

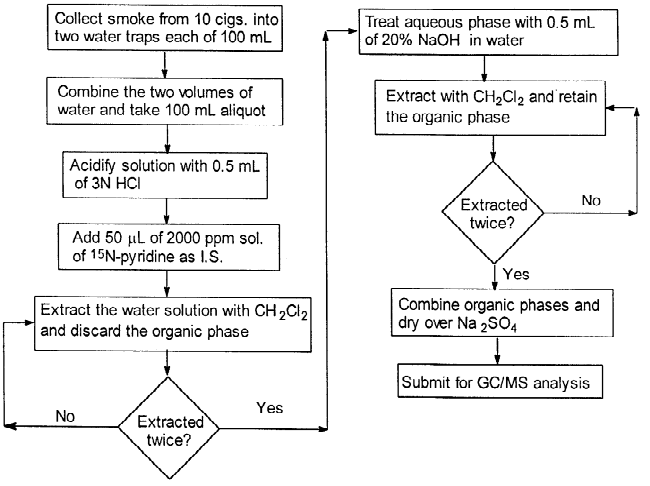

Fig. 1. Flow chart showing the cleanup procedure for a smoke sample.

Table 1
Chromatographic conditions for the separation of pyridines

Condition	Regular	Fast
Column type	DB WAX	Rtx-Wax
Column length	30 m	20 m
Column I.D.	0.25 mm	0.1 mm
Column film thickness	0.5 μm	0.2 μm
Injection temperature	280 °C	280 °C
Initial oven temperature	40 °C	100 °C
Initial hold time	5.0 min	3.5 min
Rate of temperature program	4 °C/min	20 °C/min
Hold oven temperature	110 °C	165 °C
Hold time	0.0 min	0.0 min
Rate of temperature program	10 °C/min	40 °C/min
Final oven temperature	230 °C	240 °C
Final hold time	5.5 min	3.0 min.
Gas velocity (He constant flow)	2.0 ml/min	2.0 ml/min

conditions described for the fast separation and total ion acquisition is shown in Fig. 3. The comparison of Fig. 2 and Fig. 3 shows that the two procedures lead to equivalently good separations. Table 2 gives the names, the retention times for the regular and for the fast chromatographic separation, as well as the masses detected in SIM mode for the pyridines analyzed in this study.

Both techniques were further applied for the quantitation of pyridine and substituted pyridines in mainstream cigarette smoke. The quantitation for pyridine was needed at a higher accuracy than for the substituted pyridines and has been done using the standard addition technique. Standard addition can be used to analyze an unknown sample of concentration c_x only if it can be assumed that the dependence of the peak area A_i of the concentration c_i is linear and the line passes through the origin. In other words, the dependence between the concentration of the injected sample and the chromatographic peak area is assumed of the form:

$$c_i = bA_i$$

Using only one addition, and assuming that the addition of the standard does not dilute the sample the unknown concentration c_x can be obtained from an expression of the form:

$$c_x = \frac{c_1 A_0}{A_1 - A_0}$$

where c_1 is the contribution to the concentration of the solution to be analyzed from the added amount of analyte, A_0 is the area of the peak corresponding to c_x, and A_1 the area of the peak after the addition of the standard. Better accuracy is obtained if a set of known amounts of analyte $\{q_i\}_{j=0,1,2\ldots n}$ with $q_0 = 0$

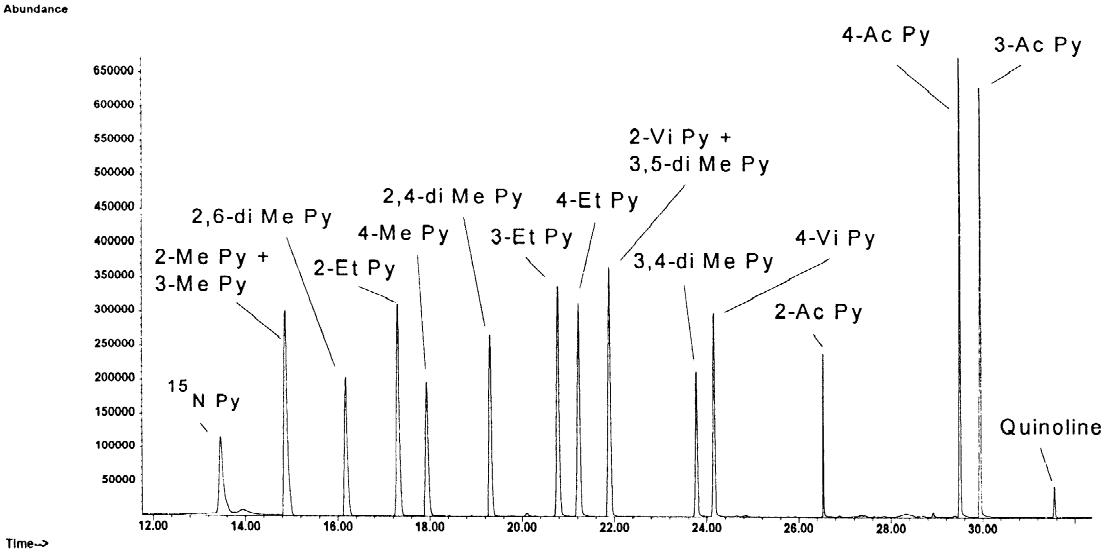

Fig. 2. Typical chromatogram containing standards at 1 μg/ml concentration and obtained under the conditions described for the regular separation and SIM acquisition. (Pyridine and 3-vinylpyridine are not added in the standard, but quinoline is also included in the mixture).

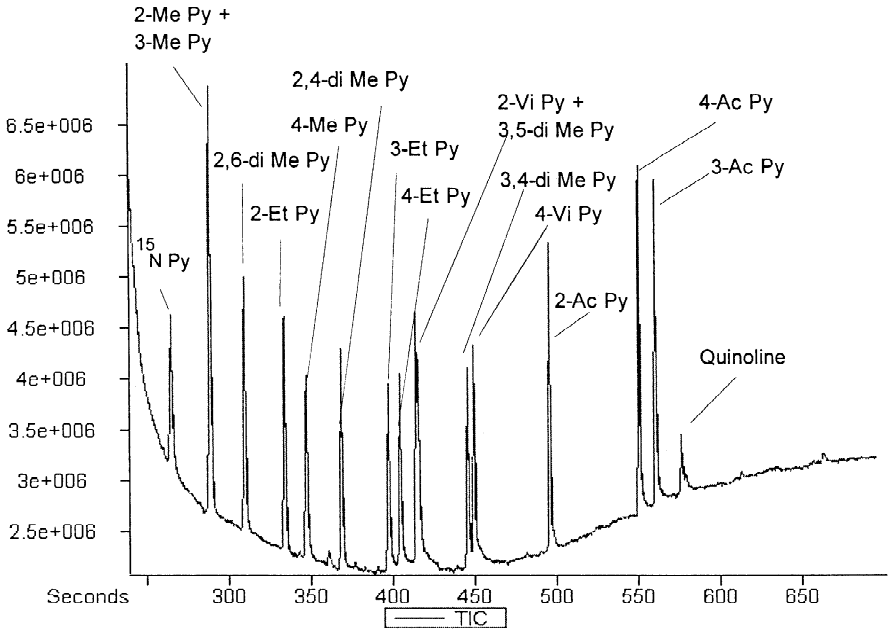

Fig. 3. Typical chromatogram containing standards at 1 μg/ml concentration and obtained under the conditions described for the fast separation and total ion acquisition. (Pyridine and 3-vinylpyridine are not added in the standard, but quinoline is also included in the mixture).

are added to the unknown sample, leading to the concentrations $c_i = (q_x + q_i)/(V_x + V_i)$ where V_x is the known volume of the sample to be analyzed, V_i is the volume of the added solution with the "i" standard and $c_x = c_0 = q_x/V_x$. The values for $c_0 = c_x$ and b (as parameters) can be obtained from the added amounts and peak area measurements $\{q_j, A_j\}_{j=0,1\ldots N}$ using, for example, least-square fitting. Only pyridine was

Table 2
Compounds, their retention times in regular (SIM) separation, in fast separation, and the masses detected in SIM mode

Compound	Retention time in SIM (min)	Retention time in fast (s)	SIM masses
N-Pyridine	13.50	264.9	80, 81
Pyridine	13.50	264.9	79, 52
2+3-Methylpyridine	14.92	288.5	66, 93
2,6-Dimethylpyridine	16.21	309.7	92, 106, 107
2-Ethylpyridine	17.35	333.9	79, 106, 107
4-Methylpyridine	17.96	347.1	66, 93
2,4-Dimethylpyridine	19.35	369.5	92, 106, 107
3-Ethylpyridine	20.83	397.3	92, 106, 107
4-Ethylpyridine	21.27	404.3	92, 106, 107
2 Vinylpyridine	21.92	414.3	104, 105, 107
3,5-Dimethylpyridine	21.95	415.5	104, 105, 107
3-Vinylpyridine	23.76	444.5	104, 105
3,4-Dimethylpyridine	23.80	445.5	106, 107
4-Vinylpyridine	24.23	449.3	104, 105
2-Acetylpyridine	26.53	495.3	79, 93, 121
4-Acetylpyridine	29.51	550.3	78, 106, 121
3-Acetylpyridine	29.96	560.5	78, 106, 121

analyzed by this procedure, and only in the regular separation with SIM detection. The cigarettes were smoked as previously described and known amounts of pyridine solution were added to the smoke solution prior to extraction. The peak area of the ion m/z 79 for quantitation were used for the measurements. For this peak, the condition of zero response for no pyridine is not achieved, because pyridine elutes together with ^{15}N-pyridine and the ion m/z 79 is present in the mass spectrum of this compound. The ion with m/z 79 represents 12% of the base peak with m/z 80. Because in each sample there are 20 μg/ml ^{15}N-pyridine, the quantitation of pyridine based on ion m/z 79 will give 2.4 μg/ml pyridine which corresponds to 1.2 μg/cigarette (cig.) for a blank sample. The concentration of added pyridine and resulting concentrations expressed as μg/cig. are shown in Table 3. The use of least-square fitting between added concentration and calculated concentration for each added pyridine level leads to the regression equation $y=0.982x+9.2$. The resulting content of pyridine is 8.17 μg/cig., which is obtained from the value for $y=0$ in the regression line, and subtraction of 1.2 μg/cig. that represents the background for a blank sample. The regression line shows an $R^2=0.9024$. This indicated a good linear correlation between the added and calculated values.

The standard addition procedure applied for the analysis of pyridine is, however, rather time consuming and requires at least two chromatographic runs for each sample. Other simpler procedures can also be used for quantitation. One of these is based solely on the peak area ratios for two compounds. For this procedure, a response factor F_x must be obtained initially. This response factor using an internal standard is calculated from the peak area $A_{15N\text{-}pyr}$ of the internal standard and the peak area A_{pyr} of the compound to be analyzed, both added to a blank sample at equal amounts (concentration). The ratio of the two areas, usually obtained as an average of several measurements, gives the response factor:

$$F_x = A_{15N\text{-}pyr}/A_{pyr}$$

Ideally, the value for F_x remains constant for an interval of values for the pair of concentrations of the standard and the sample. The concentration c_x of the unknown is then obtained by measuring in the same run the peak area A_x of the compound to be analyzed (at unknown concentration) and peak area A_{is} of the standard using the formula:

$$c_x = F_x \cdot (A_x/A_{is}) \cdot c_{is}$$

where c_{is} is the concentration of the standard. This type of quantitation procedure has been used for pyridine and for substituted pyridines. The response factors used for the quantitation using the regular separation and SIM acquisition are given in Table 4. The ion m/z 80 for ^{15}N-pyridine was used as an internal standard. One problem with this choice is that 5.5% of the base peak 79 of pyridine is the ion with m/z 80. This indicates that the internal standard

Table 3
Added pyridine and calculated pyridine concentrations in smoke of a 1R4F Kentucky reference cigarette

No.	Added concentration (μg/cig.)	Calculated concentration from each addition (μg/cig.)
1	0	7.73
2	3	11.28
3	5	12.51
4	10	20.08

Table 4
The m/z value for the ion used for quantitation, and the response factors F_x for various pyridines (averages of three runs) for the SIM detection in the regular separation and for TI detection in the fast chromatographic separation

Compound	Quantitation mass	F_x SIM	F_x fast
Pyridine	79	1.00	1.00
2+3-Methylpyridine	93	1.02	1.08
2,6-Dimethylpyridine	107	0.95	1.06
2-Ethylpyridine	106	0.75	0.84
4-Methylpyridine	93	0.94	1.19
2,4-Dimethylpyridine	107	0.89	1.18
3-Ethylpyridine	92	1.11	1.45
4-Ethylpyridine	107	1.27	1.69
2 Vinylpyridine	105	1.15	1.47
3,5-Dimethylpyridine	107	1.00	0.98
3-Vinylpyridine	105	1.08	1.36
3,4-Dimethylpyridine	107	0.97	1.39
4-Vinylpyridine	105	1.08	1.36
2-Acetylpyridine	79	1.28	1.32
4-Acetylpyridine	106	1.39	1.47
3-Acetylpyridine	106	1.02	1.11

The ion m/z 80 for ^{15}N-pyridine was used to monitor the internal standard.

may have some interference from the pyridine present in cigarette smoke. However, for 5 μg/cig. pyridine the increase in the peak area of the ion with m/z 80 is about 2.7%. This increase was negligible, and because ^{15}N-pyridine behaves in the analytical procedure similar to the other pyridine, its choice as an internal standard was considered acceptable.

For the fast chromatographic separation the quantitation was done using the response factor procedure for all analytes including pyridine. The peak area used for this purpose was that of the extracted ions identical to those used for SIM quantitation. The response factors for fast chromatographic separation are also given in Table 4. The data were obtained as an average of three measurements for solutions containing 1 μg/ml of each component. As seen in Table 4, the response factors for SIM acquisition and that for fast chromatography and total ion (TI) acquisition are not identical, but they follow the same trend.

3. Results and discussion

The technique for the analysis of pyridines has been evaluated regarding typical parameters used for a validation, such as specificity/selectivity, precision, reproducibility, accuracy, linearity range, limit of detection, recovery efficiency, robustness, and stability. The lack of interferences in the quantitation of every pyridine is rather difficult to prove. The level of background generated by the internal standard itself is discussed in the experimental part. Other interferences from the sample matrix were verified for each peak. A typical case is pictured in Fig. 4 where the spectrum for the peak corresponding to 2-acetylpyridine, the library hit for the spectrum and the spectra differences are displayed.

The selectivity of the procedure was very good for most components. As an example, the peaks of the ion m/z 107 used for the quantitation of various dimethylpyridines and ethylpyridines are shown for the standard mixture in Fig. 5, and for a sample of smoke from the 1R4F cigarette in Fig. 6, for the separation obtained using fast chromatography. Except for 3,4-dimethylpyridine where the peak contains an interference probably from *N*,*N*′-dimethylbenzylamine, the other C_2-pyridines are clean and can be easily quantitated.

The results for the pyridines on a 1R4F Kentucky reference cigarette analyzed using the regular separation and SIM detection, and the quantitation using the response factor procedure are given in Table 5 as averages of five replicates. As seen from Table 5, the

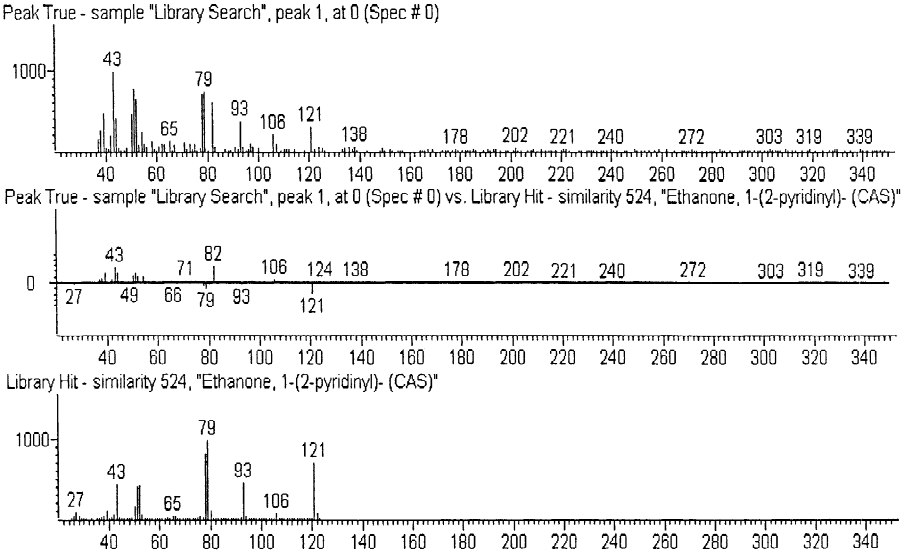

Fig. 4. The spectrum for the peak corresponding to 2-acetylpyridine in the smoke sample for a 1R4F cigarette, the library hit for the spectrum and the spectra differences.

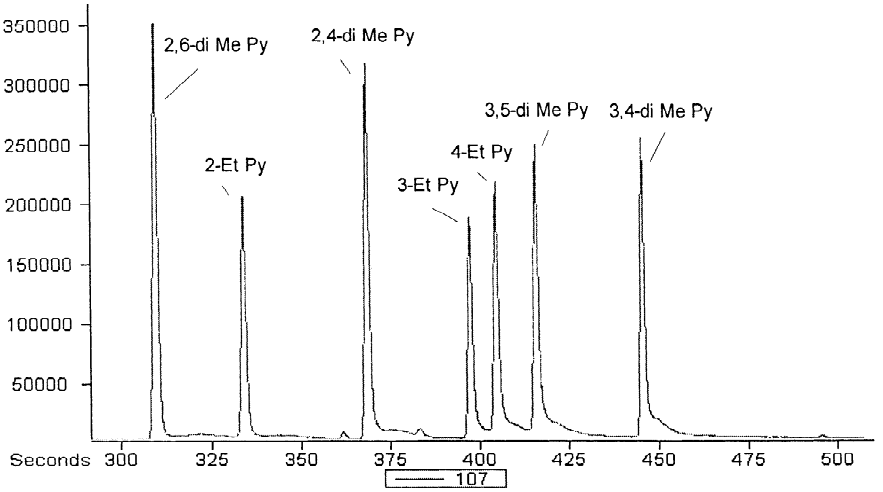

Fig. 5. Extracted ion m/z 107 characteristic for C_2-pyridines for a standard containing 1 μg/ml pyridines.

level of pyridine obtained using the response factor procedure is 7.82 μg/cig. as compared to that using the standard addition procedure that is 8.17 μg/cig. This is a very good agreement, considering that the smoking of the cigarettes may also produce certain variability from sample to sample. The standard deviation of the for various pyridines is lower than 6–7% except for 3,5-dimethylpyridine and for 4-vinylpyridine (which is present at a very low level in smoke). This indicates good precision for the procedure.

Accuracy is difficult to assess for the analysis of pyridines in smoke. Some results are reported for pyridine in mainstream smoke, but no data were found reporting the levels of substituted pyridines. Also, the data reported in the literature for pyridine level in smoke are not in good agreement. A level of 3.28 ± 0.60 μg/cig. was reported for electrostatic precipitation collection for 1R4F cigarette [1]. Electrostatic precipitation is likely to collect only particulate phase pyridine and not account for vapor phase pyridine. A different report [10] indicated 2.1

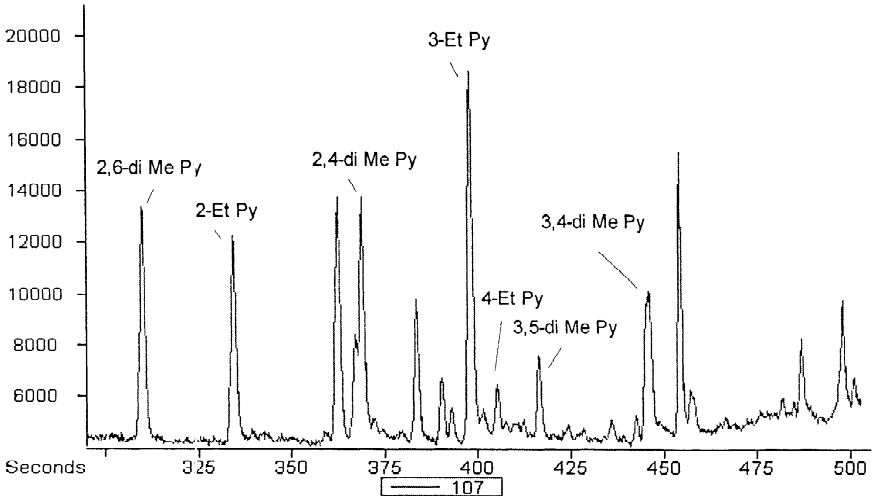

Fig. 6. Extracted ion m/z 107 for a smoke sample of 1R4F cigarette.

Table 5
Levels of pyridines in μg/cig. for 1R4F Kentucky reference cigarette analyzed using the regular separation and SIM detection, and the quantitation using the response factor procedure

Compound	μg/cig.	SD μg/cig.	RSD (%)
Pyridine	7.82	0.062	0.79
2+3-Methylpyridine	2.62	0.062	2.37
2,6-Dimethylpyridine	0.45	0.032	7.11
2-Ethylpyridine	0.66	0.010	1.52
4-Methylpyridine	0.51	0.024	4.71
2,4-Dimethylpyridine	0.38	0.006	1.58
3-Ethylpyridine	1.04	0.016	1.54
4-Ethylpyridine	0.13	0.005	3.85
2 Vinylpyridine	0.30	0.011	3.67
3,5-Dimethylpyridine	0.39	0.057	14.74
3-Vinylpyridine	2.51	0.041	1.63
3,4-Dimethylpyridine	0.15	0.013	8.67
4-Vinylpyridine	0.03	0.004	13.33
2-Acetylpyridine	0.17	0.015	8.82
4-Acetylpyridine	0.11	0.008	7.27
3-Acetylpyridine	0.28	0.012	4.29

Note: Standard addition procedure gives 8.17 μg/cig. pyridine.

Table 7
Variation of the response factor F_x for various concentrations of the internal standard ^{15}N-pyridine and of various pyridines

Compound	Concentration (μg/ml)				
	10.0	5.0	1.0	0.1	0.01
Pyridine	1.00	0.98	1.00	1.16	1.02
2+3-Methylpyridine	1.02	1.00	1.02	1.02	0.70
2,6-Dimethylpyridine	0.95	0.94	0.95	1.02	1.14
2-Ethylpyridine	0.75	0.75	0.75	0.81	0.85
4-Methylpyridine	0.94	0.94	0.94	0.85	1.11
2,4-Dimethylpyridine	0.88	0.89	0.89	0.87	0.85
3-Ethylpyridine	1.10	1.12	1.11	1.08	1.25
4-Ethylpyridine	1.27	1.25	1.27	1.19	1.41
2 Vinylpyridine	1.15	1.14	1.15	1.15	1.49
3,5-Dimethylpyridine	1.00	0.99	1.00	1.02	1.19
3-Vinylpyridine	1.15	1.14	1.08	1.15	1.49
3,4-Dimethylpyridine	0.97	0.97	0.97	0.99	1.14
4-Vinylpyridine	1.09	1.08	1.08	1.05	1.18
2-Acetylpyridine	1.28	1.28	1.28	5.26	1.85
4-Acetylpyridine	1.37	1.39	1.39	1.39	1.67
3-Acetylpyridine	1.03	1.01	1.02	1.02	1.18

μg/cig. pyridine for 1R4F cigarette. On the other hand, the range of pyridine levels in mainstream smoke was reported to be between 16 and 46 μg/cig. for plain cigarettes [11], 12.1, 11.8, 11.1, and 5.5 μg/cig. (in different laboratories) for a cigarette with 12 mg TPM, which is similar to 1R4F cigarette [12], and about 8.4±1.4 μg/cig. using a GC–MS determination for the 1R4F cigarette [13]. For this reason, accuracy of the present technique cannot be really determined. A proof indicating good results was obtained by using two different chromatographic types of analysis (regular separation with SIM detection, and fast separation with total ion detection), although the sample preparation procedure was

Table 6
Levels of pyridines in μg/cig. for the 1R4F Kentucky reference cigarette analyzed using the fast separation with total ion detection and the difference from the regular separation with SIM detection

Compound	Fast μg/cig.	SD μg/cig.	RSD (%)	Difference from SIM (%)
Pyridine	7.86	0.068	0.87	0.51
2+3-Methylpyridine	3.49	0.065	1.86	28.48
2,6-Dimethylpyridine	0.55	0.030	5.45	20.00
2-Ethylpyridine	0.75	0.015	2.00	12.77
4-Methylpyridine	0.60	0.034	5.67	16.22
2,4-Dimethylpyridine	0.44	0.016	3.64	14.63
3-Ethylpyridine	1.08	0.018	1.67	3.77
4-Ethylpyridine	0.15	0.012	8.00	14.29
2 Vinylpyridine	0.36	0.015	4.17	18.18
3,5-Dimethylpyridine	0.41	0.063	15.37	5.00
3-Vinylpyridine	2.59	0.046	1.78	3.14
3,4-Dimethylpyridine	0.24	0.018	7.50	46.15
4-Vinylpyridine	0.06	0.014	23.33	66.67
2-Acetylpyridine	0.18	0.011	6.11	5.71
4-Acetylpyridine	0.12	0.006	5.00	8.70
3-Acetylpyridine	0.30	0.011	3.67	6.90

Table 8
The level in μg/cig. of various pyridines in several Full Flavor (FF) and Lights (Lts) commercial cigarettes and total particulate matter (TPM) values in mg/cig.

	Cig. 1, FF		Cig. 2, FF		Cig. 1, Lts		Cig. 2, Lts		Cig. 3, Lts	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
TPM (mg/cig.)	17.2		19.1		12.7		12.8		13.0	
Pyridine	16.37	0.87	19.47	1.23	10.64	0.75	8.30	0.11	6.07	0.38
2+3-Methylpyridine	6.47	0.67	7.06	0.46	3.19	0.33	0.32	0.05	0.17	0.03
2,6-Dimethylpyridine	1.13	0.18	1.27	0.10	0.56	0.03	0.07	0.01	0.06	0.01
2-Ethylpyridine	1.71	0.16	1.85	0.15	1.02	0.05	0.60	0.03	0.32	0.03
4-Methylpyridine	1.19	0.13	1.12	0.08	0.60	0.10	0.19	0.03	0.14	0.03
2,4-Dimethylpyridine	1.15	0.19	1.08	0.10	0.62	0.06	0.04	0.01	0.04	0.01
3-Ethylpyridine	1.59	0.19	1.45	0.15	1.03	0.11	0.64	0.05	0.38	0.02
4-Ethylpyridine	0.22	0.03	0.20	0.02	0.09	0.05	0.08	0.00	0.05	0.00
2 Vinylpyridine	0.32	0.05	0.33	0.03	0.18	0.06	0.20	0.07	0.09	0.02
3,5-Dimethylpyridine	0.40	0.06	0.36	0.04	0.19	0.04	0.14	0.02	0.10	0.01
3-Vinylpyridine	4.57	0.56	4.54	0.49	3.05	0.23	2.13	0.11	1.29	0.05
3,4-Dimethylpyridine	0.35	0.05	0.27	0.02	0.30	0.06	0.18	0.01	0.12	0.01
4-Vinylpyridine	0.08	0.04	0.06	0.01	0.00	0.00	0.02	0.00	0.01	0.00
2-Acetylpyridine	0.59	0.21	0.65	0.12	0.29	0.18	0.11	0.03	0.13	0.03
4-Acetylpyridine	0.15	0.03	0.14	0.02	0.11	0.02	0.12	0.02	0.14	0.00
3-Acetylpyridine	0.55	0.08	0.43	0.04	0.33	0.06	0.18	0.02	0.24	0.01

identical for the two techniques. A comparison of the quantitation for pyridines in 1R4F reference cigarette by the two procedures is shown in Table 6. As shown in Table 6, the results obtained by the two procedures are very similar. The pyridine determination was also done using the standard addition (indicating 8.17 μg/cig.) and based on the response factor indicating 7.12 and 7.16 μg/cig. for SIM and TIC detection modes, respectively. Similar range of errors was also obtained using the two procedures. Although reproducibility typically refers to similar precision between different laboratories, the agree-

Table 9
The levels in μg/cig. of various pyridines in several Ultra Lights (Ult), Super Lights (Slt), and 1 mg TPM (1 mg) commercial cigarettes and total particulate matter (TPM) values in mg/cig.

	Cig. 1, Ult		Cig. 2, Ult		Cig. 1, Slt		Cig. 1, 1 mg		Cig. 2, 1 mg	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
TPM (mg/cig.)	7.5		–		6.7		1.4		1.3	
Pyridine	6.97	0.25	3.19	0.09	3.80	0.48	3.68	0.54	3.33	0.38
2+3-Methylpyridine	1.76	0.08	0.22	0.02	0.69	0.29	0.37	0.29	0.24	0.20
2,6-Dimethylpyridine	0.28	0.02	0.03	0.01	0.06	0.04	0.06	0.04	0.05	0.03
2-Ethylpyridine	0.51	0.02	0.06	0.01	0.11	0.06	0.10	0.07	0.07	0.05
4-Methylpyridine	0.28	0.02	0.04	0.01	0.07	0.03	0.06	0.03	0.04	0.02
2,4-Dimethylpyridine	0.27	0.01	0.03	0.01	0.06	0.02	0.04	0.02	0.03	0.01
3-Ethylpyridine	0.47	0.02	0.06	0.01	0.10	0.03	0.06	0.03	0.05	0.02
4-Ethylpyridine	0.07	0.00	0.01	0.00	0.02	0.01	0.01	0.00	0.01	0.00
2 Vinylpyridine	0.08	0.00	0.01	0.00	0.02	0.01	0.02	0.01	0.00	0.00
3,5-Dimethylpyridine	0.10	0.01	0.01	0.00	0.03	0.01	0.01	0.00	0.01	0.00
3-Vinylpyridine	1.21	0.06	0.13	0.04	0.36	0.16	0.14	0.05	0.13	0.03
3,4-Dimethylpyridine	0.11	0.01	0.01	0.00	0.04	0.02	0.01	0.00	0.01	0.00
4-Vinylpyridine	0.03	0.01	0.02	0.00	0.04	0.01	0.02	0.01	0.02	0.01
2-Acetylpyridine	0.09	0.02	0.01	0.00	0.05	0.01	0.01	0.00	0.01	0.00
4-Acetylpyridine	0.06	0.00	0.00	0.00	0.05	0.01	0.01	0.00	0.01	0.00
3-Acetylpyridine	0.16	0.01	0.00	0.00	0.11	0.01	0.02	0.00	0.02	0.00

ment between the results shown in Table 6 also indicates good reproducibility of the results.

The linearity range and the limit of detection of the procedure described in this report can be evaluated based on the data given in Table 7. This table shows variation of the response factor F_x for various concentrations of the internal standard ^{15}N-pyridine and of various pyridines. As seen in Table 7, the response factor remains relatively constant for various concentrations, indicating that as little as 10 ng/ml (5 ng/cig.) of each pyridine can be analyzed without a significant loss of accuracy using this procedure.

The quantitation based on the standard addition provided indirect indication that the recovery for pyridine is good. No direct recovery study was conducted for pyridine or substituted pyridines. The technique has been used for a large number of samples, and over a period of several months, indicating robustness, and good stability for both versions of the technique. Some results regarding the levels of pyridines in the mainstream smoke of various cigarette types are given in Tables 8 and 9. All these results were obtained using the response factor procedure for the regular separation and SIM detection. As seen in Tables 8 and 9, pyridine deliveries were as high as 18.0 μg/cig. for a full flavor cigarette, while ultra light cigarette delivered about 3.0 μg/cig. Substituted pyridines levels varied between 5.0 and 0.1 μg/cig. for full flavor cigarettes, and between 0.2 μg/cig. and a few ng/cig. for ultra light cigarettes. The reproducibility of the technique is very good, with less than 7–8% RSD in both separation procedures for most of the analyzed compounds.

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