Evaluation of novel analyte protectants to improve the accuracy of determination of 7 minor alkaloids in mainstream cigarette smoke by GC-MS

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Report Contents

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1 Research Background

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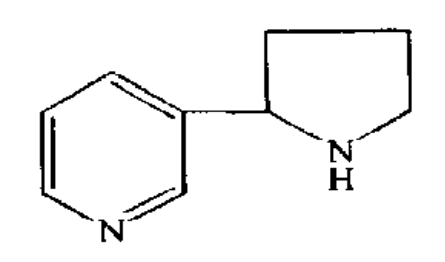
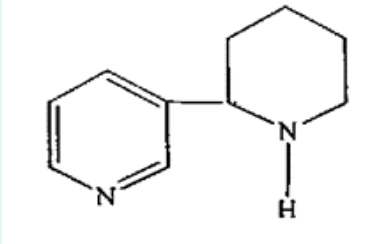
- Minor tobacco alkaloids in cigarette smoke play important roles in some sensory properties such as harshness and bitter taste.
- Two minor alkaloids are listed in " Harmful and potentially harmful constituents in tobacco products and tobacco smoke: established list"(FDA 2012).



Nornicotine



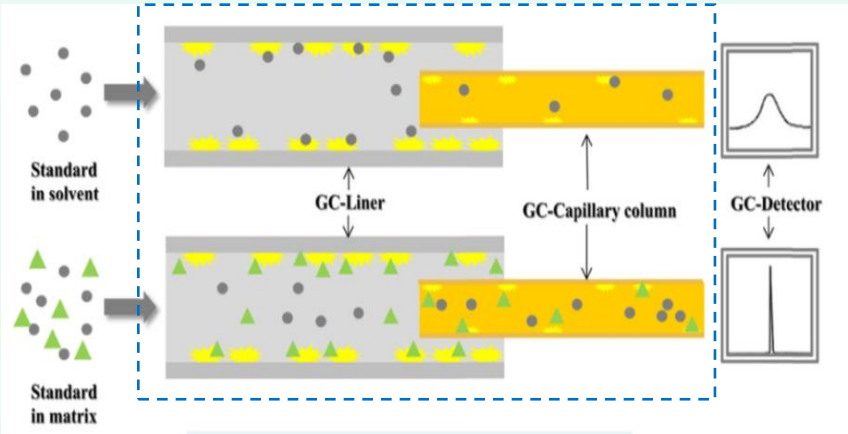
Anabasine

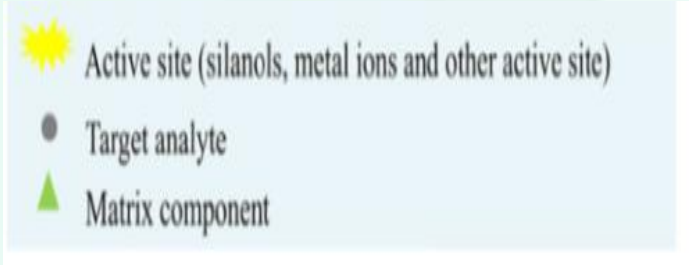
Therefore, their accurate quantifications are of great significance.

1 Research Background

➢ The main cause of poor accuracy in quantification of minor alkaloids by GC/MS is “matrix effect ”(ME).

GC/MS flow path





The ME is observed because of the presence of active sites in various parts of the GC-MS system such as the inlet, column head, and ion-source. In addition, the matrix of the sample competes with target analytes for the active sites. The adsorption behavior of the same analyte in the standard solution and the actual sample are widely different, which directly leads to the ME, making accurate quantification difficult.

1 Research Background

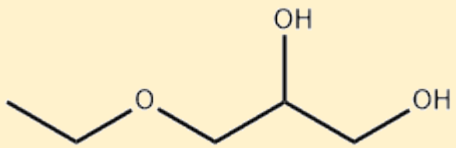
- The major methods to overcome matrix effects of GC:

A	B	C	D
Use of matrix matched standards	Matrix purification	Use of isotopically labeled internal standards	Use of analyte protectents (APs)
Not applicable	Not applicable	Helpful	Efficient

APs are added to the sample extracts and solvent standards to block the active sites dynamically during the run, inducing an even-response enhancement.

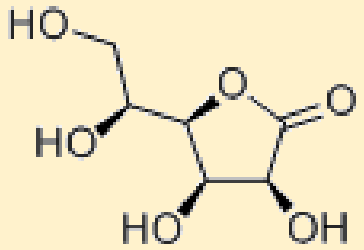
1 Research Background

- APs widely used in pesticide analysis



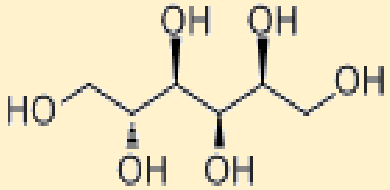
3-ethoxy-1,2-propanediol

+



L-Gulonic acid-γ-lactone

+



D-Sorbitol

- Limitations of the classical APs above
  - ✓ Only applicable for polar solvent system such as ACN, acetone, methanol.

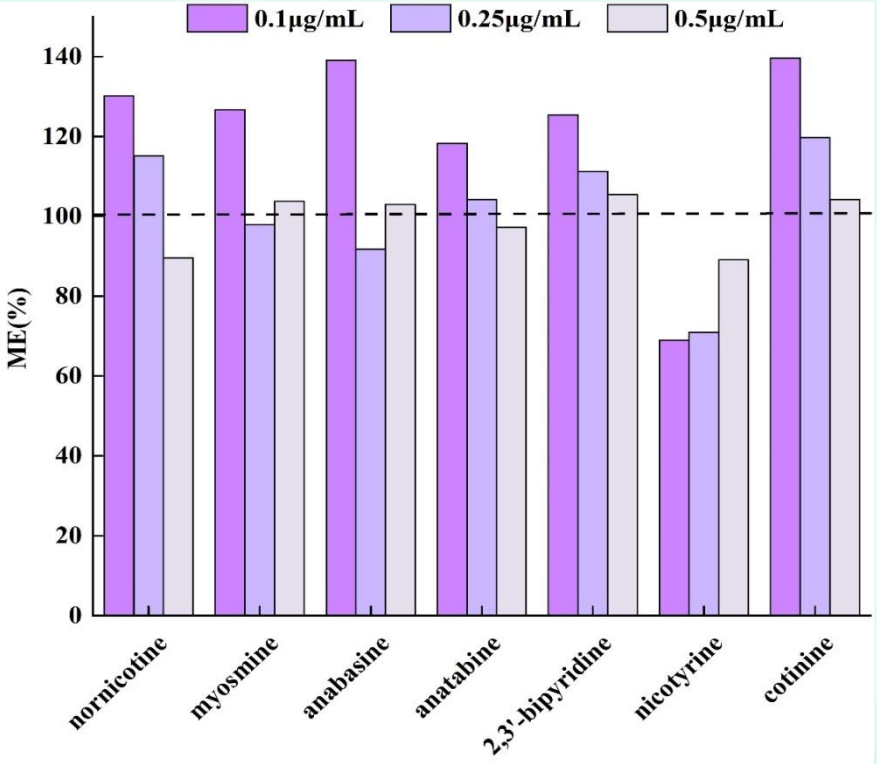
- Very limited attention was paid to overcome the ME outside the field of pesticide analysis

2 Research Content

- Evaluation of “matrix effect” (do not use AP)

$$ME\ (\%) = B/A \times 100$$

where *A* is the peak area of the minor alkaloid in dichloromethane, and *B* is the peak area increment of the smoke matrix at the same spiked concentration



- ✓ The lower the concentration, the stronger the ME.
- ✓ Most minor tobacco alkaloids showed significant matrix induced enhancement (ME > 120%) at low spiked levels (0.1 μg/mL).

2 Research Content

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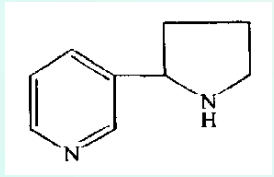
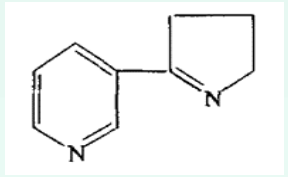
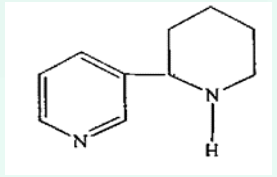
This work aims to find appropriate AP(s) to compensate for the ME to improve the long-term accuracy and repeatability of the test method for determination of 7 minor alkaloids in cigarette smoke.



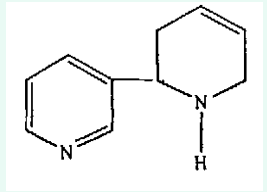
Nornicotine



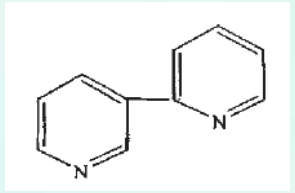
Myosmine



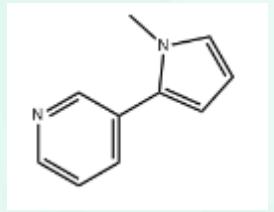
Anabasine



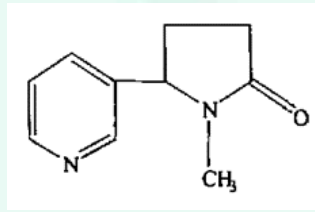
Anatabine



2,3'-Bipyridine



Nicotyrine



cotinine

IS1:
Nornicotine-d_4

IS2:
2,3'-Bipyridine-d_8

IS3:
Benzophenone-d_{10}

2 Research Content

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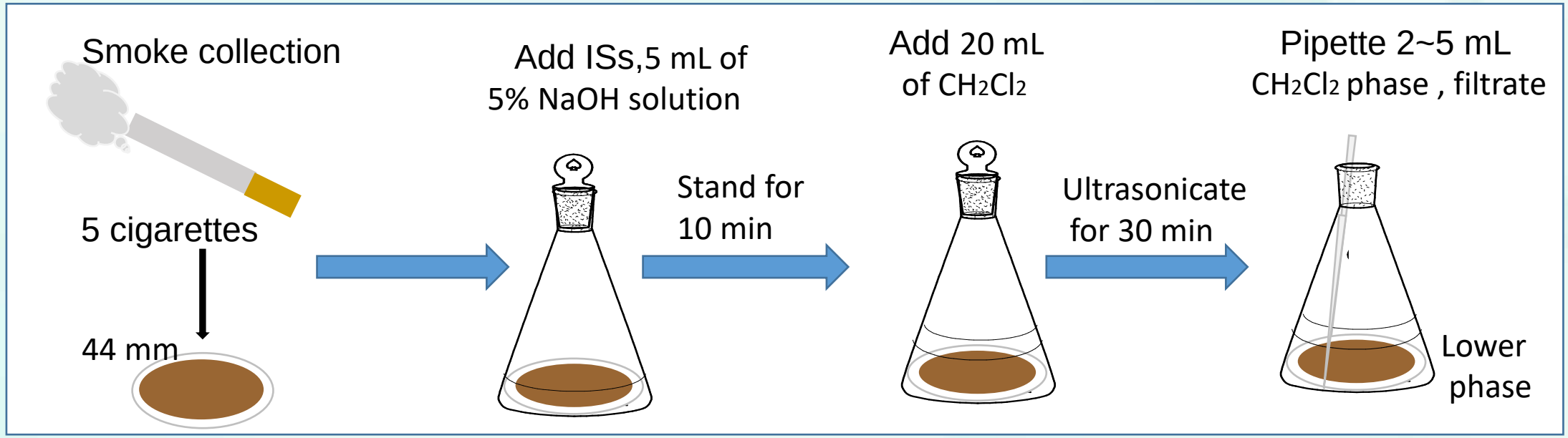
Sample preparation



1mL CH_2Cl_2 extract solution
+50 μL AP solution

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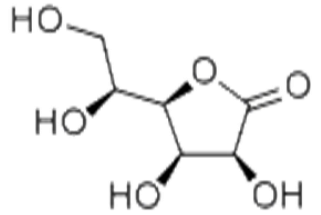
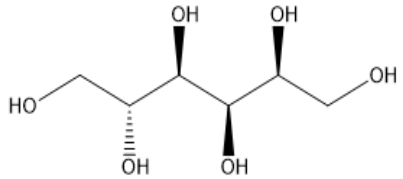
➢ Selection of AP

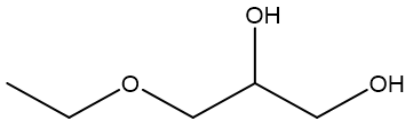
APs often used in pesticide analysis



L-Gulonic acid-γ-lactone



D-Sorbitol



3-ethoxy-1,2-propanediol

Vicinal diol used in our previous work

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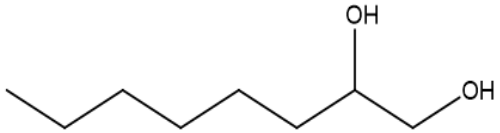
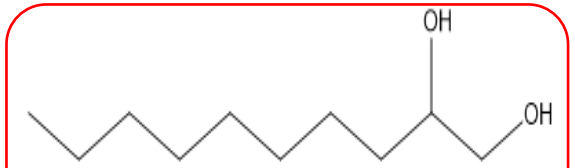


1,2-Octanediol



1,2-Decanediol

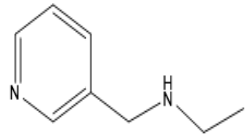
1,2-Tetradecanediol

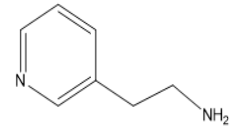
compounds containing nitrogen based adsorption group

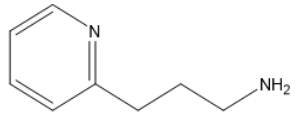
Dodecanamine 

Tridecanamine 

Tetradecanamine 

4-(Ethylaminomethyl)pyridine 

2-Pyridylethylamine 

3-Pyridin-2-yl-propylamine 

2 Research Content

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We compared the compensation effect of these APs based on the increment ratio of the peak heights after the addition of each AP to a 0.2 ppm mixed standard solution prepared in CH_2Cl_2.

Code	Compound	RT /min	Percentage increase in height/%						
			nornicotine	myosmine	anabasine	anatabine	2,3'-bipyridine	nicotyrine	cotinine
C	3-Ethoxy-1,2-propanediol	/	–	8	–	–	–	13	7
D	1,2-Octanediol	13.48	–	59	37	43	61	interference	36
E	1,2-Decanediol	16.32	20	84	62	67	83	70	61
F	1,2-Tetradecanediol	25.1	–	3	17	–	27	98	129
G	Dodecanamine	15.46	65	–	18	60	3	44	21
H	Tridecanamine	16.98	180	18	40	37	–	37	4
I	Tetradecanamine	18.91	173	22	63	109	–	73	21
J	4-(Ethylaminomethyl)pyridine	13.75	interference	interference	interference	6	39	71	68
K	2-Pyridylethylamine	12.68	224	58	48	188	70	371	76
L	3-Pyridin-2-yl-propylamine	14.25	interference	48	64	115	30	134	40

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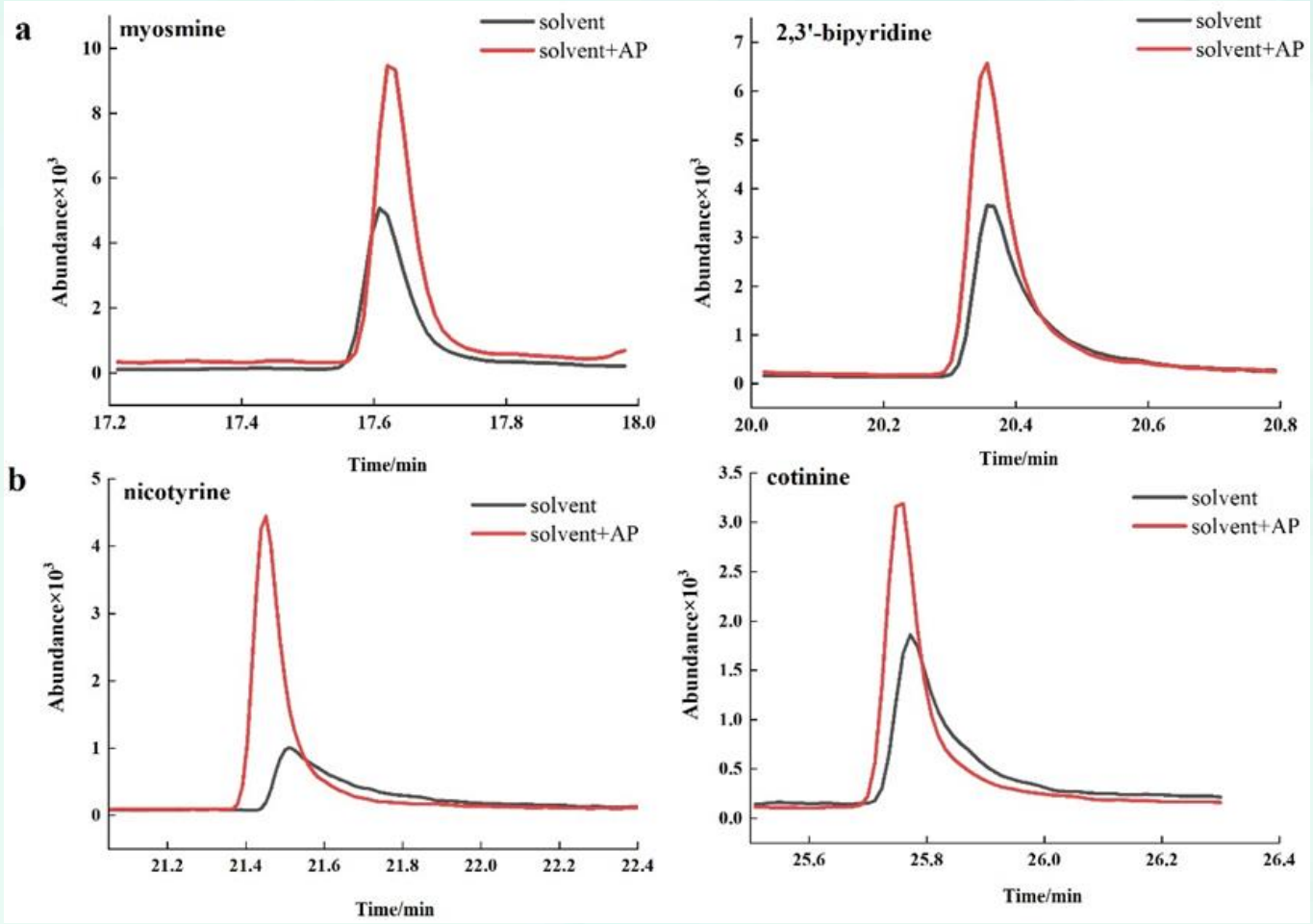
Add 1,2-decanediol (1mg/mL)

Add 2-pyridylethylamine (1mg/mL)

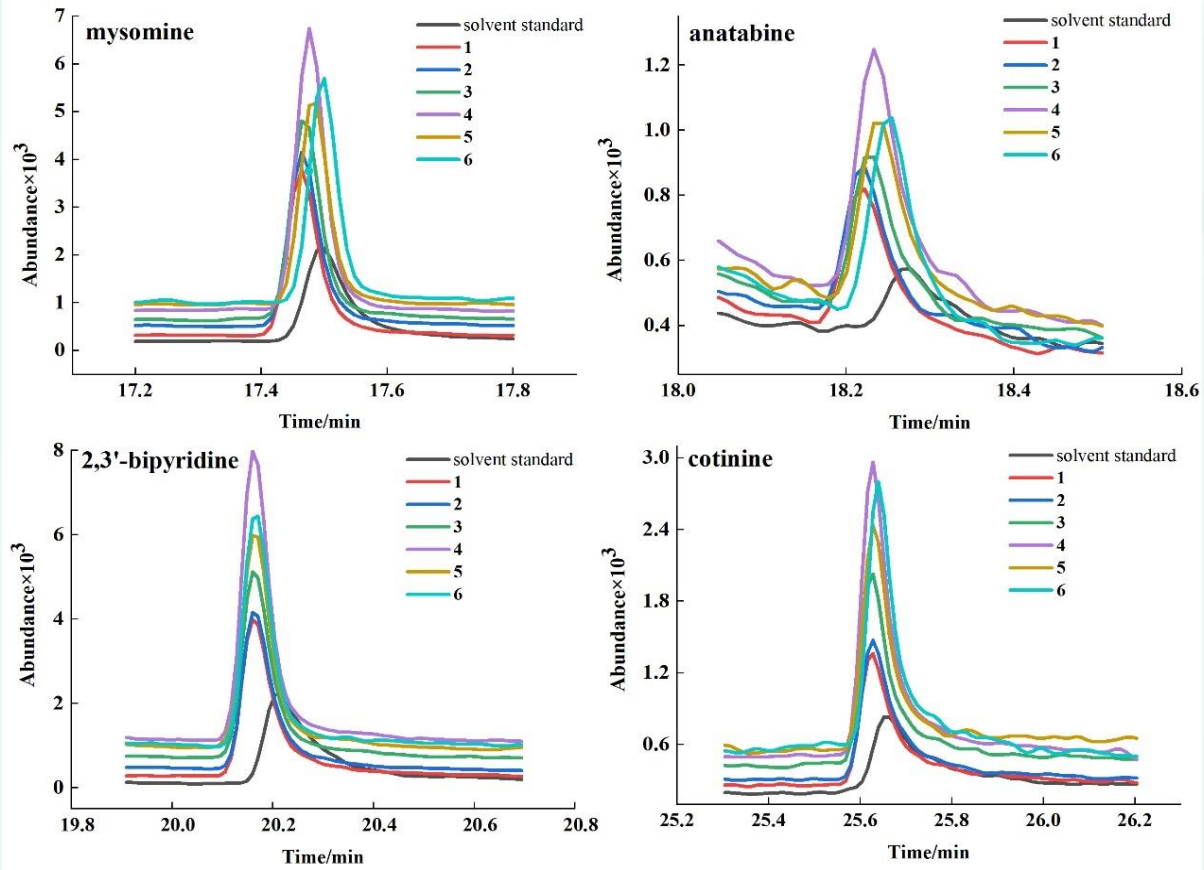


Chromatographic peaks of standard solution

2 Research Content

- Effects of AP combination on the peak height and shape of analytes

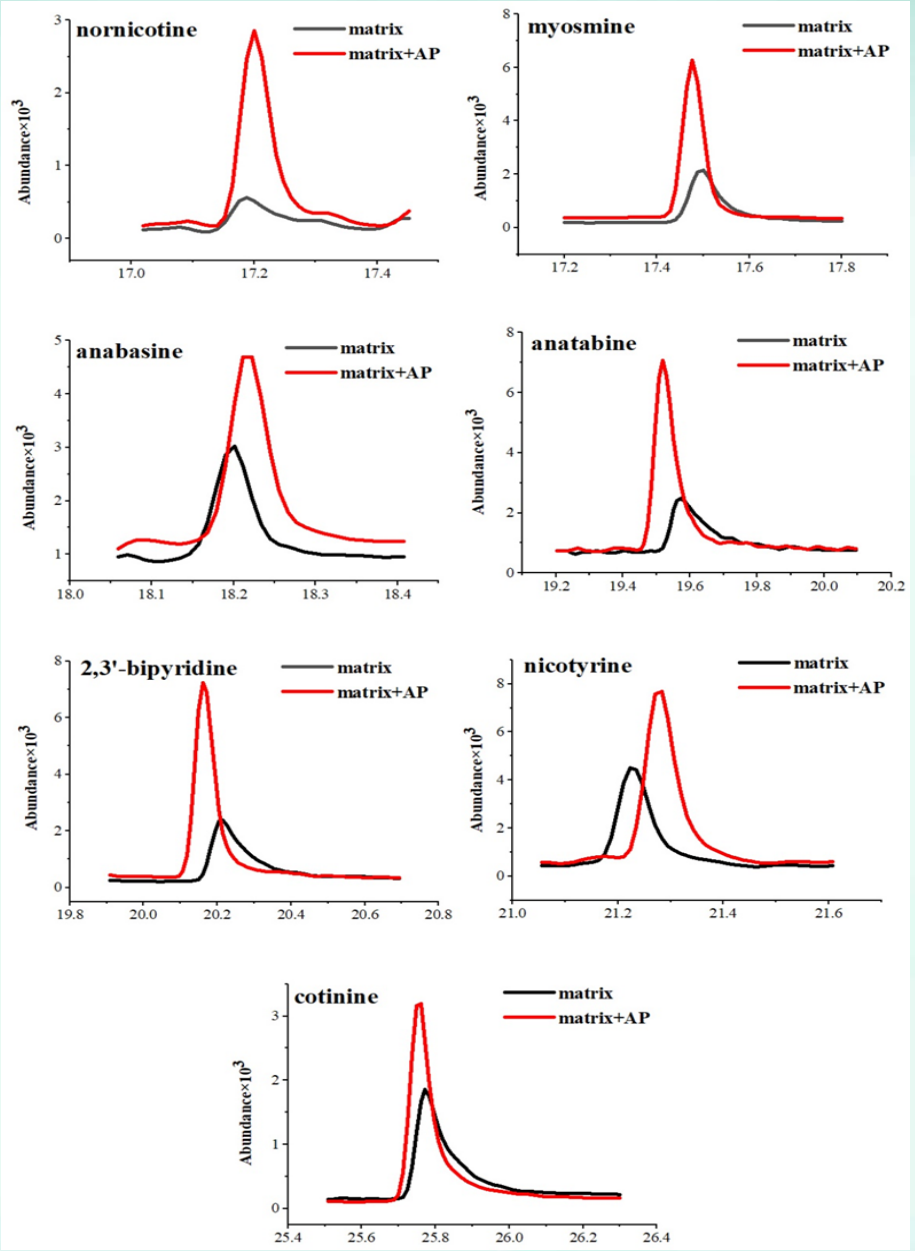
1,2- decanediol (1 mg/mL) + 2-pyridylethylamine (2 mg/mL)



2 Research Content

- The effect of adding AP to smoke matrix

- ✓ The minor alkaloids in smoke matrix solution also suffered serious adsorption in GC/MS system.
- ✓ So it was necessary to add APs to both the smoke matrix and the standard solution.



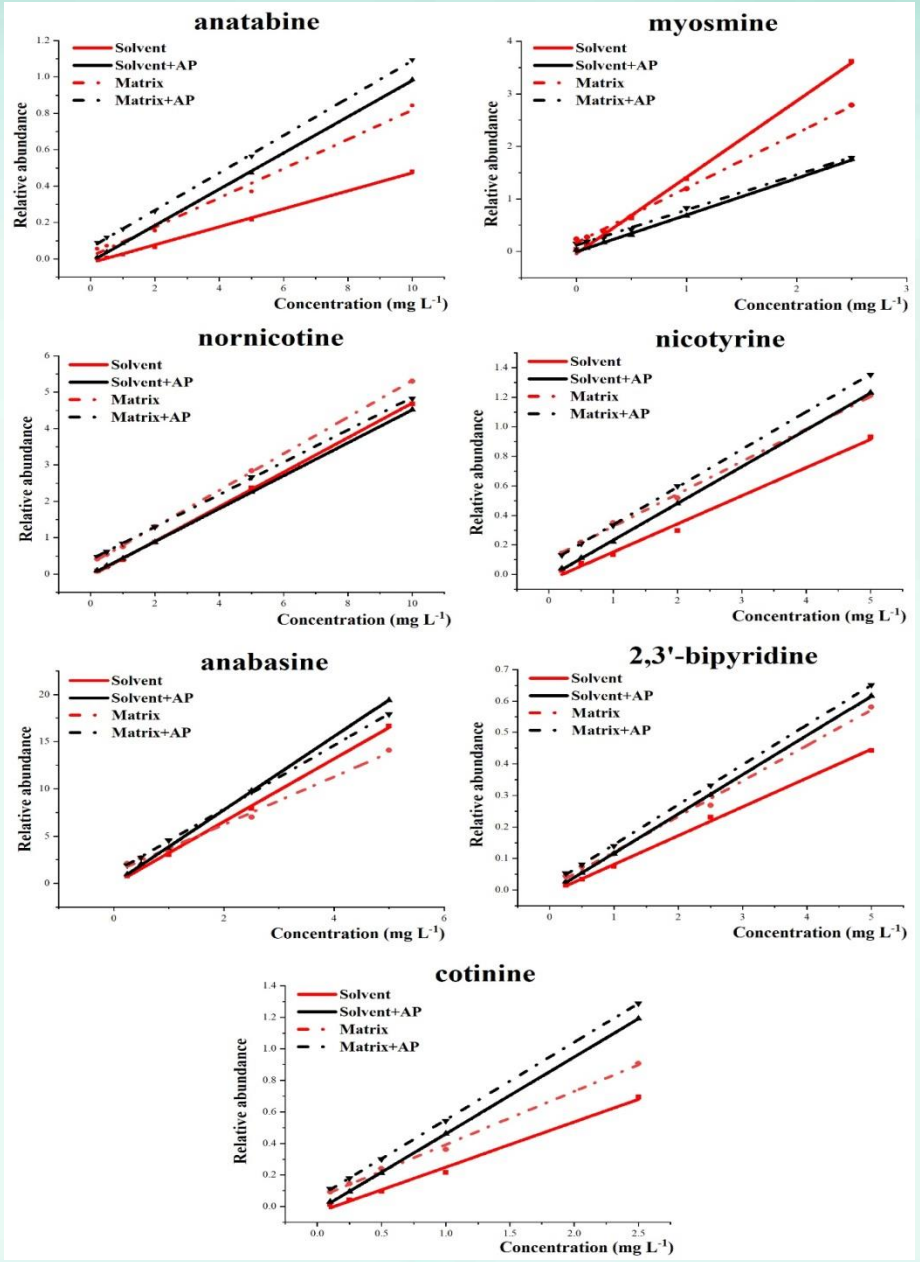
2 Research Content

- Validation of the method

✓ Without the addition of this AP combination, slope ratios of calibration curves for calibration solutions in pure CH_2Cl_2 (red straight line) vs. matrix-matched calibration solutions (red dotted line) were 71.4–159.8% for the seven target chemicals.

✓ **With the addition of AP**, the slope ratios of for calibration solutions in CH_2Cl_2(**blank straight line**) vs matrix-matched calibration solutions (**black dotted line**) became **87.4–105.6%.**

The AP combination provides the same degree of protection, regardless of whether the solution contains a matrix.



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2 Research Content

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➢ Validation of the method

Analyte	r^2				Recovery (%)						Intraday precision (%)	Interday precision (%)
					Low		Medium		High			
	Solvent	Solvent+AP	Matrix	Matrix+AP	Solvent	Solvent+AP	Solvent	Solvent+AP	Solvent	Solvent+AP		
Nornicotine	0.9970	0.9996	0.9882	0.9997	130.2	104.2	115.1	91.4	105.7	93.4	7.5	5.6
Myosmine	0.9979	0.9990	0.9965	0.9990	55.1	94.7	63.2	83.7	72.3	80.9	9.5	8.5
Anabasine	0.9977	0.9993	0.9920	0.9995	130.5	102.3	126.3	106.4	121.9	100.7	1.5	5.5
Anatabine	0.9834	0.9995	0.9805	0.9991	181.1	100.4	159.5	103.6	148.8	102.0	3.8	3.7
2,3′-Bipyritine	0.9984	0.9997	0.9999	0.9998	111.6	111.4	98.9	109.6	94.0	99.3	3.8	3.1
Nicotyrine	0.9855	0.9996	0.9945	0.9996	122.8	96.2	126.4	103.8	122.3	103.7	4.5	7.6
Cotinine	0.9917	0.9994	0.9971	0.9997	188.8	119.6	161.6	111.1	154.1	108.5	3.7	6.1

- ✓ When AP is added, calibration solutions prepared in CH_2Cl_2 showed good linearity: $r^2 > 0.999$
- ✓ Without use of APs: recoveries are 55.1-188.8%
- ✓ With the addition of APs: recoveries are 80.9-119.6%
- ✓ Intra- day precisions: 1.5%-9.5%
- ✓ Inter-day precisions: 3.1% - 8.5%

2 Research Content

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- Performance of the proposed method in different experimental scenarios

(1) A dirty state after 300 consecutive injection of smoke sample;
(2) After ion sources were cleaned and new liner, spacers, column was replaced;
(3) After 100 injections under condition (2), and the instrument was tuned;
(4) After replacement of the chromatographic column.

The order of the injections under each instrument state are the same :
(1–7) calibration standards in solvent without APs;
(8–16) smoke matrix of samples A, B and C without AP, each in three replicates;
(17–19) three repeat injections of the mixed solvent solution of 0.8 mg/L without AP;
(20–26) calibration standards in solvent with APs;
(27–35) smoke matrix of samples A, B and C with APs, each in three replicates;
(36–38) three repeat injections of the mixed solvent solution with APs of 0.8 mg/L.

2 Research Content

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- Performance of the proposed method in different experimental scenarios

Analyte	Sample A				Sample B				Sample C				0.8 mg/L solvent standards	
	Release/(μg·cig^{-1})		RSD (n = 4)/%		Release/(μg·cig^{-1})		RSD (n = 4)%		Release/(μg·cig^{-1})		RSD (n = 4)%		RSD (n = 4)%	
	Without AP	With AP	Without AP	With AP	Without AP	With AP	Without AP	With AP	Without AP	With AP	Without AP	With AP	Without AP	With AP
Nornicotine	1.92	1.76	12.9	2.9	2.07	1.98	13.6	3.7	1.95	1.86	14.5	4.0	7.6	2.2
Myosmine	1.57	1.57	22.7	4.7	1.81	2.08	25.4	4.1	2.42	3.07	22.4	5.5	21.7	1.1
Anabasine	1.16	1.30	19.4	4.5	1.67	1.69	12.9	3.5	1.53	1.49	16.0	2.2	11.3	2.1
Anatabine	4.13	4.33	21.2	4.0	6.35	5.83	23.1	2.5	4.67	4.32	19.7	2.3	22.5	2.2
2,3′-Bipyritine	6.06	6.12	3.5	2.6	6.38	6.55	4.1	2.7	6.60	7.04	4.2	2.0	3.2	1.4
Nicotyrine	1.20	1.14	21.8	2.9	1.36	1.04	21.6	4.8	1.47	1.12	20.5	5.2	16.8	3.0
Cotinine	3.65	3.44	11.2	4.9	4.31	4.45	6.2	3.0	5.35	5.83	11.7	6.7	15.6	2.1

- When APs were not added : the RSDs of the quantification results under the four different experimental scenarios for cigarette samples A, B, and C were 3.5–22.7%, 4.1–25.4%, and 4.2–22.4%.
- When APs were added: the RSDs of the test results of the three cigarettes significantly improved to 2.6–4.9%, 2.5–4.8%, and 2.0–6.7%, respectively.
- The RSDs of 0.8 mg/L monitoring mixture standards were 3.2–22.5% and 1.1–3.0%, without and with AP, respectively.

3 Conclusions

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- In this study, 12 APs were tested to observe their matrix compensation effect on seven minor tobacco alkaloids, and the combination and concentration of APs were optimized.
- Using standard solutions prepared in dichloromethane and added with APs, the validation results demonstrated that this method exhibited good linearity($r2 > 0.999$), recovery (87.4–119.6%), and intra- and inter-day precision (1.5–9.5% and 3.1–8.5%, respectively).
- Moreover, the use of selected APs could significantly improve the reproducibility and accuracy of the quantification results under different instrument contamination condition.

APs developed in this research have prospects for practical applications.

Thank you for your attention!