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Headspace sampling and gas chromatographic–mass spectrometric determination of amphetamine and methamphetamine in betel

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Abstract

A hot headspace sampling (HS) and gas chromatographic–mass spectrometric method (GC–MS) for the determination of amphetamine (AP) and methamphetamine (MA) in betel is described. The method uses potassium carbonate to alkalize the aqueous matrix and to salt-out the analytes prior to HS. The complicated matrices necessitate the separate analysis of betel nut, piper betel, and red slaked lime, which are the three major parts of a finished betel product. Qualitative identification is aided by the mass spectrum provided by the proposed method. The limits of quantitation vary from 0.02 to 1.20 μg/ml. Precisions calculated for the 5 and 50 μg/ml concentration range are ca. 20%. The method is simple, rapid, solventless, and requires only a small amount of sample. It may serve as a screening protocol for the determination of AP and MA in betel.

1. Introduction

Betel originally grown in Malay is now widely planted in the tropical zone and part of the subtropical area. An edible finished betel product is generally prepared by cutting an unripe betel nut into two halves and sandwiching between the two halves with piper betel and red slaked lime (consisting mostly of oyster shell powder and orange rind). Upon chewing, betel could generate effects such as cooling, cold-protection, and stimulating saliva production [1]. These effects have drawn tens of millions of betel lovers widespread in southeast Asia. In Taiwan, there are about two million betel-chewing people among a population of twenty one million. With the recent increase of drug abuse, the merchants were reportedly resorting to the addition of amphetamine (AP) and methamphetamine (MA) to betel to maintain the loyalty of their customers [2]. The need for analytical methods to detect AP and MA in betel becomes apparent. The headspace sampling (HS) technique has traditionally been used for the analysis of gaseous and volatile analytes [3–7]; however, it has also been reported for the analysis of non-volatile organic compounds [8]. The HS technique is currently being used to determine trace amounts of the semi-volatile compounds AP and MA in urine samples [9–11]. From these studies, HS sampling has shown to be a simple, rapid, solventless, and reliable technique. The

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aim of the present study is to develop a similar HS technique in conjunction with gas chromatography–mass spectrometry (GC–MS) to detect AP and MA in betel. We use MS as the detector to aid qualitative identification, since, from the regulatory point of view, positive confirmation is of prime importance.

2. Experimental

2.1. Materials

Racemic D,L-amphetamine sulfate (AP-H_2SO_4) and D,L-methamphetamine hydrochloride (MA-HCl) were purchased from Sigma Chemical (St. Louis, MN, USA), benzyl alcohol from Merck Chemical (Darmstadt, Germany), potassium carbonate (K_2CO_3) from Janssen Chemica (Geel, Belgium), ethyl acetate from Fisher Chemical (Fair Lawn, NJ, USA), and directly used without distillation. A 1 mg/l standard solution was prepared by dissolving 100.0 mg of AP-H_2SO_4 and 100.0 mg of MA-HCl in 100 ml deionized water. The three components of a finished betel product (betel nut, piper betel, and red slaked lime) used for fortified studies were obtained from a local betel vendor and found to be free of AP and MA. Fortified samples were prepared by soaking each individual component overnight in 20 ml aqueous solution containing an appropriate amount of AP-H_2SO_4 and MA-HCl. An edible finished betel product was prepared by cutting a betel nut into two halves and sandwiching between the halves with piper betel and red slaked lime. Fortified and real samples were stored in a 4°C refrigerator before analysis.

2.2. Headspace sampling

The three components of a finished betel product were analyzed separately. A 1.5-g portion of betel nut (0.3 g piper betel or 0.3 g red slaked lime) was crushed (unnecessary for piper betel and red slaked lime) and placed in a 15-ml HS vial. An appropriate amount (5, 25, 50, 125, or 250 μl) of standard solution was added. After 5 min, a 3.5-g portion of K_2CO_3 was added. The total volume of the vial contents was adjusted to 5 ml by adding deionized water. The vial was sealed with an aluminum hole cap–Teflon-faced septum, then sufficiently shaken, and placed in a 80°C oven for 20 min. Meanwhile, a 1-μl aliquot of 1:50 (v/v) benzyl alcohol in ethyl acetate, used as internal standard (I.S.), was sealed in another empty HS sampling vial. The I.S. vial was also placed in the 80°C oven for 20 min. The I.S. was allowed to vaporize completely using the "full evaporation technique (FET)" [12]. Afterwards, 0.7 ml of the analyte vapor or 0.1 ml of the I.S. vapor was simultaneously injected onto the GC system using a 1.0-ml gas-tight syringe and determined by a mass spectrometer operated in the full scan mode.

2.3. Apparatus

GC–MS analyses were carried out using a Hewlett-Packard HP-5890 Series II gas chromatograph coupled to a HP-5971 Series mass selective detector (MSD) operated in full scan mode. The column used was a Hewlett-Packard HP-5 capillary column (25 m × 0.2 mm I.D., 0.33 μm film thickness). The GC was operated in the splitless mode with the injector temperature at 250°C. Helium was used as the carrier gas at a flow-rate of 1 ml/min. The column temperature was initially held at 60°C for 2 min, then programmed at 10°C/min to 120°C, than at 18°C/min to 250°C, and held for 4.78 min. Effluents from the GC column were transferred via a transfer line held at 280°C and fed into a 70-eV electron impact (EI) ionization source.

The MS analyses were performed using the full scan mode accompanied by extracting ion chromatograms. The m/z values used for AP, MA, and I.S. were 44, 58, and 108, respectively. The calibration curves were produced by plotting the peak-area ratio (analyte: benzyl alcohol) against the effective concentration (in μg/ml, the denominator was the total volume of all materials placed in the HS vial including the added deionized water, i.e., 5 ml) of the appropriate analyte in the fortified samples. The peak-

area ratio used was the mean of three replicate analyses.

3. Results and discussion

3.1. Matrix effects and full evaporation technique

A finished betel product consisting of betel nut, piper betel, and red slaked lime fortified with AP and MA is not an ideal solution. Each part would show different and possibly cross-interacting matrix effects on the evaporization of the analytes. Fig. 1 shows the differences in matrix effects when the three parts of a betel were analyzed together (Fig. 1A) and separately (Figs. 1B–D). The peaks at t_R (retention time) 7.21, 8.74, and 9.48 min in Fig. 1A of the total-ion chromatogram (TIC) of a finished betel product are from the I.S., AP, and MA, respectively. They are further confirmed by the exact match of their mass spectra to those stored in the library. The intense peaks at $t_R = 9.93$ and 10.64 min are from the matrix and are tentatively assigned as 3-pyridinecarboxylic acid [1] and 5-(2-propenyl)-1,3-benzodioxole, respectively. Comparing Fig. 1A to Figs. 1B–D, it is evident that the former peak comes from the betel nut only and the latter is from both piper betel and red slaked lime. The coherence of the t_Rs of the same analyte in Fig. 1 demonstrates that the method could yield analytically reproducible t_Rs.

The success of this method depends heavily on the use of an appropriate internal standard. We used benzyl alcohol, as suggested in the literature [13]. However, the correlation coefficients of the calibration curves prepared by adding I.S. directly into the HS vials containing betel samples are not satisfactory, i.e., generally less than 0.85. This is because the HS technique used in this study performs best when sampling and analyzing part of the analytes in their vapor phase to achieve the total-amount quantitation. According to Henry's law, the partial vapor pressure of a volatile solute in an ideal solution is equal to the vapor pressure of the pure solute multiplied by its mole fraction in the ideal solution. Therefore, the analytical results are expressed in units of μg/ml rather than the traditional units of μg/g. Although similar amounts of I.S. were added into each HS vial, the mole fractions of I.S. in aqueous betel samples were not necessarily the same because of the differences in matrices. Consequently, the partial pressure of I.S. in the headspace of each vial might vary. This problem was solved by using the FET technique to add I.S. into another empty HS sampling vial to achieve complete evaporization. The linearity of the calibration curves was significantly improved, with correlation coefficients generally greater than 0.98. In addition, the similar intensity of the peaks at $t_R = 7.21$ min in all four TICs shown in Fig. 1 illustrates the appropriateness of the selection of benzyl alcohol as the I.S. and the success of the FET technique.

Table 1 indicates that both AP and MA in the individual betel parts (Figs. 1B–D) show considerably higher intensity than in the finished betel product (Fig. 1A) provided the same amount (50 μl of 1 mg/ml standard solution) of analytes was added. This difference in intensity is ascribed to the slow transfer of AP and MA from the aqueous layer to the headspace due to the competitive transfer of matrix components. The relatively smaller r_{AP} and r_{MA} in the finished betel product indicate the presence of a more serious matrix effect in a finished betel. The smaller r_{AP} and r_{MA} in the betel nut are presumably due to the adsorption of AP and MA by the cellulose component in betel nut. On the other hand, the largest r_{AP} and r_{MA} values–found in the red slaked lime–are tentatively ascribed to the extra alkalizing and salting-out effects generated by the $Ca(OH)_2$ in the oyster shell powder which is the major component of the red slaked lime.

3.2. Endogenous alkalizing and salting-out effects of red slaked lime

The red slaked lime was thought to have endogenous alkalizing and salting-out effects on the detection of the concealed AP and MA. To explore this phenomenon, three portions (each

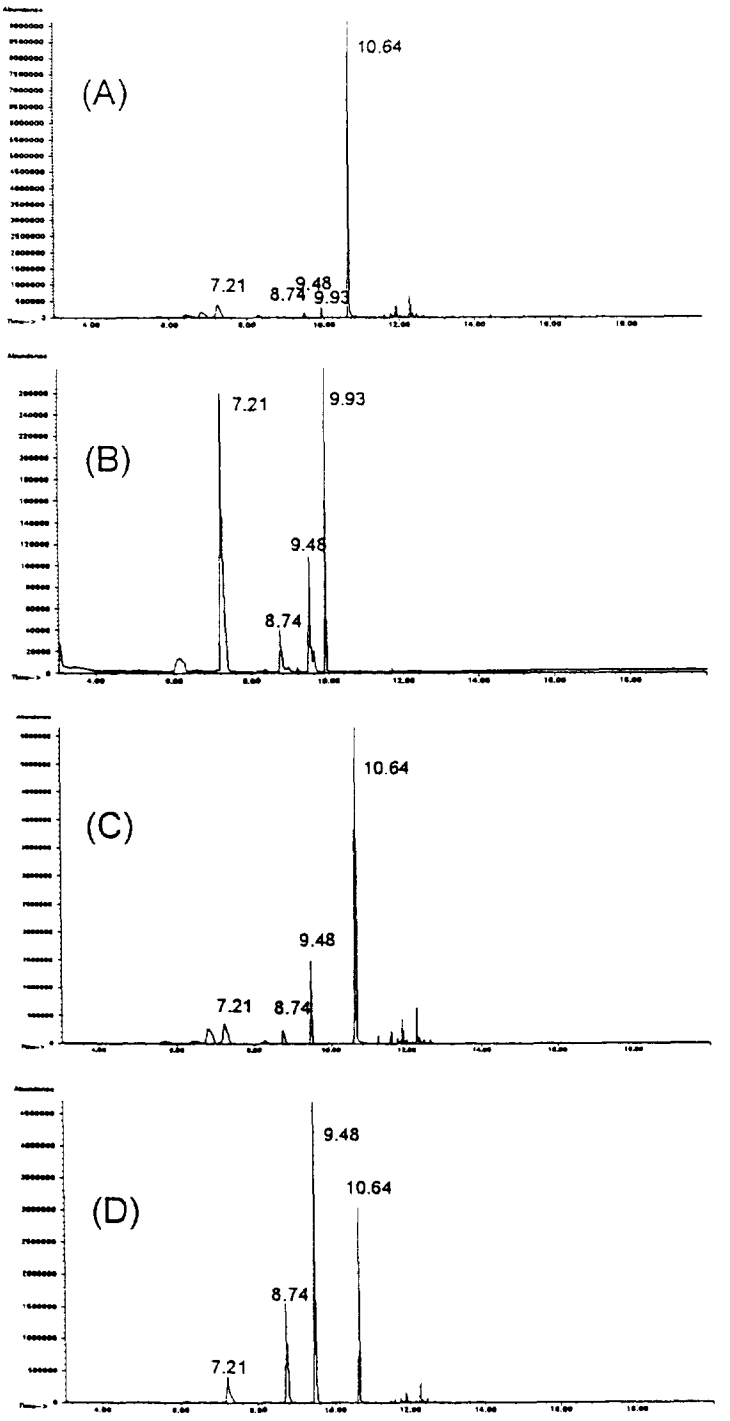

Fig. 1. Total ion chromatograms of samples fortified with amphetamine and methamphetamine: (A) finished betel product, (B) betel nut, (C) piper betel, and (D) red slaked lime.

Table 1
Matrix effects on the detection of amphetamine and methamphetamine in finished betel product, betel nut, piper betel, and red slaked lime

Matrix	r_{AP}[a]	r_{MA}[b]	r_{M1}[c]	r_{M2}[d]
Finished betel product	0.02	0.14	0.19	5.74
Betel nut	0.12	0.28	0.44	–
Piper betel	0.36	1.37	–	4.06
Red slaked lime	2.51	5.66	–	2.36

[a] Peak-area ratio of AP/benzyl alcohol (I.S.).
[b] Peak-area ratio of MA/I.S.
[c] Peak-area ratio of matrix peak at $t_R = 9.93$ min/I.S.
[d] Peak-area ratio of matrix peak at $t_R = 10.64$ min/I.S.

weighing 0.3 g) of red slaked lime were each mixed with 25 μl of 1 mg/ml standard solution. The foregoing HS procedure was followed except that (a) vial A was left at ambient temperature for 20 min with no K_2CO_3 added, (b) vial B was heated in an 80°C oven for 20 min with no K_2CO_3 added, and (c) vial C was heated in an 80°C oven for 20 min with 3.5 g of K_2CO_3 added. The resulting TICs are shown in Fig. 2. Fig. 2A indicates that without an elevated temperature no detectable amounts of the analytes were found in the vapor phase. Fig. 2B indicates that at elevated temperature the endogenous alkalizing and salting-out effects of the red slaked lime give a small amount of the analytes in the vapor phase. Fig. 2C clearly indicates that the added K_2CO_3 assists the conversion of AP-H_2SO_4 and MA-HCl into their free state. The corresponding increase in ionic strength generates a stronger salting-out effect. The combined effects lead to a 6.5 and 8.5 times higher peak intensity for AP and MA, respectively. The endogenous alkalizing and salting-out effects caused the matrix peak at $t_R = 10.64$ min to increase proportionally.

3.3. Quantitation

The fortification levels of 1–50 μg/ml used in this study were selected because they cover the ranges over which these dopes generate sensational effects [14]. Fig. 3 shows the six calibration curves for AP and MA in the three parts of betel. The correlation coefficients are better than 0.98. The slopes of the calibration curves of MA are generally larger than those of AP. This higher sensitivity of MA is attributed to its higher volatility and its higher MS response. Of the three matrices, red slaked lime shows the highest sensitivity, benefiting from the extra endogenous alkalizing and salting-out effects; betel nut gives the lowest sensitivity, presumably due to the adsorption of AP and MA by the cellulose component.

The limit of detection, LOD (and of quantitation, LOQ), is defined as the analyte concentration giving a peak in the extracted ion chromatogram with a height equal to the mean + $N \times$ standard deviation (where $N = 3$ for the LOD and 10 for the LOQ) [15]. The mean (standard deviation) is the measured average (fluctuations) taken from a baseline region located far away from the analyte peak using the 5 mg/l fortified sample. The LODs and LOQs using fortified betel nut, piper betel, and red slaked lime are summarized in Table 2. The LOQs for AP vary from 0.55 μg/ml in red slaked lime to 1.59 μg/ml in piper betel, whereas for MA, they vary from 0.02 μg/ml in betel nut to 0.04 μg/ml in piper betel. The precision is about 20% for the data shown in Fig. 3. Most of this uncertainty is attributed the manual-injection error.

The proposed HS-GC–MS method was applied to the determination of AP and MA in real betel. Among the samples found positive of AP and MA, the AP and MA residues in the betel nut are significantly higher (ranging from 30 to >50 μg/ml) as compared to those found in the piper betel and red slaked lime matrices (ranging from 1 to 3 μg/ml). This is most likely due to the transfer of analytes from the betel nut to the other matrices.

4. Conclusion

The determination of AP and MA in betel can be achieved using the proposed headspace sampling and GC–MS method. The method is simple, rapid, solventless, and requires only a small amount of sample. Qualitative identification is

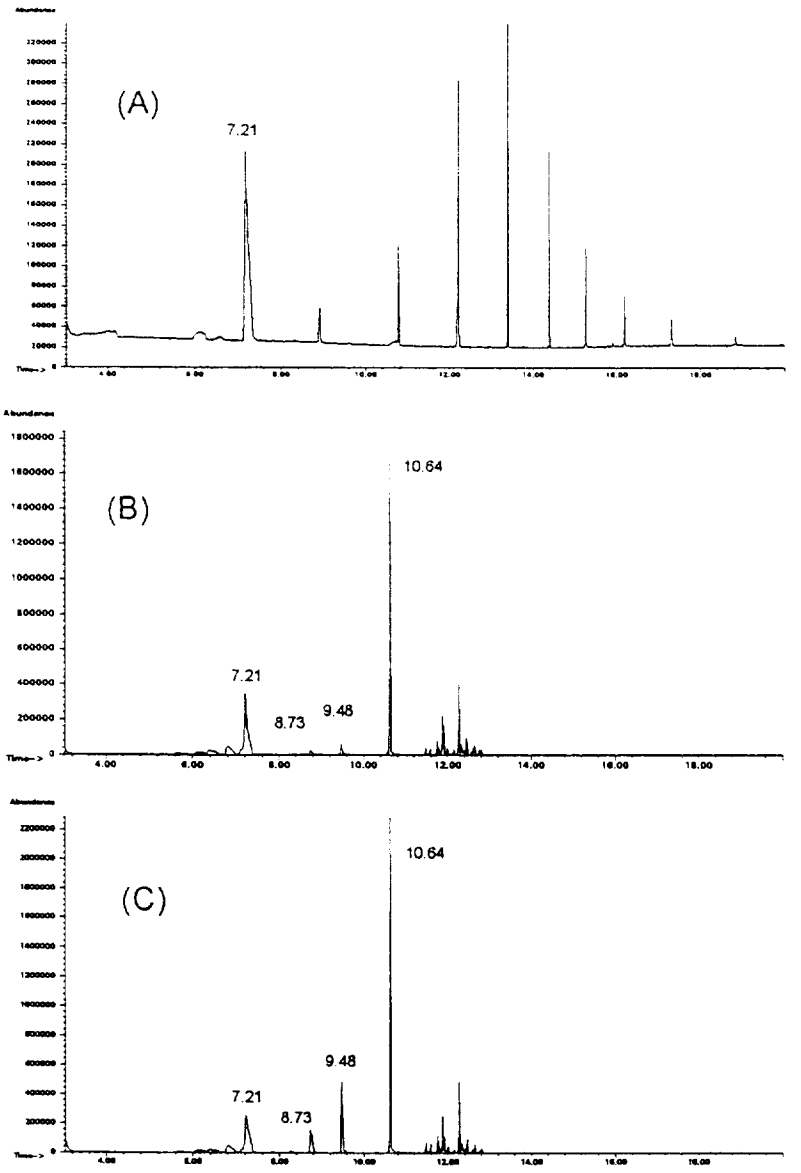

Fig. 2. Total ion chromatograms of 0.3 g red slaked lime containing 25 μg each of AP-H_2SO_4 and MA-HCl under different HS conditions: (A) at ambient temperature for 20 min with no K_2CO_3 added, (B) at 80°C for 20 min with no K_2CO_3 added, and (C) at 80°C for 20 min with 3.5 g of K_2CO_3 added.

aided by the mass spectrum provided by the proposed method. The limits of quantitation are low enough to detect real samples doped with AP and MA.

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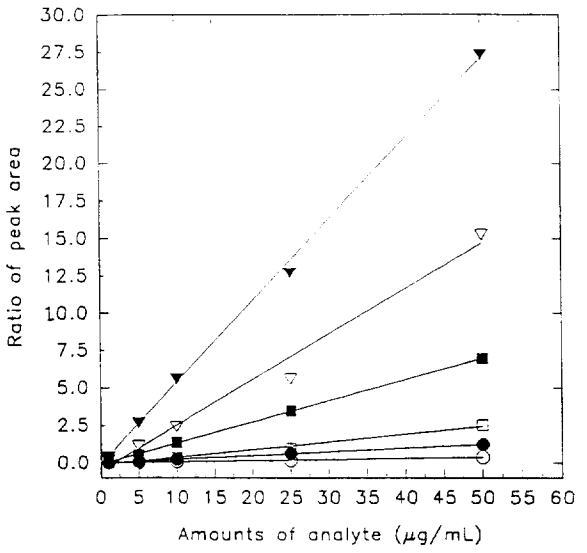

Fig. 3. Calibration curves for AP and MA in the three parts of betel. (○) AP in betel nut, (●) MA in betel nut, (□) AP in piper betel, (■) MA in piper betel, (▽) AP in red slaked lime, (▼) MA in red slaked lime.

Table 2
Detection limits of amphetamine and methampetamine in betel nut, piper betel, and red slaked lime

Matrix	Analyte	LOD[a]	LOQ[b]
Betel nut	AP[c]	0.85	1.20
	MA[c]	0.01	0.02
Piper betel	AP	1.13	1.59
	MA	0.02	0.04
Red slaked lime	AP	0.40	0.55
	MA	0.01	0.03

[a] Limit of detection, in μg/ml.
[b] Limit of quantitation, in μg/ml.
[c] AP: amphetamine; MA: methamphetamine.

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References

[1] J.L. Huang and M.J. McLeish, J. Chromatogr., 475 (1989) 447.
[2] United Daily News, Taiwan, ROC, February 25, 1994.
[3] A. Bianchi and M.S. Varney, Analyst, 114 (1987) 47.
[4] B. Kolb and M. Ander, Fresenius J. Anal. Chem., 336 (1990) 291.
[5] B. Kolb and M. Ander, Fresenius J. Anal. Chem., 336 (1990) 297.
[6] K. Pekari, M.L. Piekkola and A. Aitio, J. Chromatogr., 491 (1989) 309.
[7] P. Lansens, C. Meuleman, M. Leermakers and W. Baexens, Anal. Chim. Acta, 234 (1990) 417.
[8] S. Heitefuss, A. Heine and H.S.H. Seifert, J. Chromatogr., 532 (1990) 374.
[9] H. Tsuchihashi, K. Nakajima and M. Nishikawa, J. Chromatogr., 467 (1989) 227.
[10] H. Tsuchihashi, K. Nakajima, M. Nishikawa, S. Suzuki, K. Shiomi and S. Takahashi, Anal. Sci., 7 (1991) 19.
[11] S.-M. Wang, L.-C. Tsai, Y.-S. Giang and Y.-C. Ling, Chemistry (Chin. Chem. Soc. Taiwan, ROC), 51 (1993) 261.
[12] M. Markelov and J.P. Guzowski, Jr., Anal. Chim. Acta, 276 (1993) 235.
[13] H. Tsuchihashi, K. Nakajima, M. Nishikawa, S. Suzuki, Y. Oka and K. Otsuke, Forensic Sci. Int., 45 (1990) 181.
[14] T.H. Shih, Forensic Sci., 32 (1991) 45.
[15] Analytical Methods Committee, Analyst, 112 (1987) 199.