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Determination of exogenous methamphetamine in betel by purge-and-trap gas chromatography-mass spectrometry

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ABSTRACT

A self-made off-line purge-and-trap device using ethyl acetate as absorbent solvent is described as a sample preparation method for the fast gas chromatographic-mass spectrometric analysis of illegally added methamphetamine hydrochloride in betel. To both alkalize the aqueous matrix and salt-out the free-base analyte prior to the formal purge-and-trap sampling procedure, an appropriate amount of potassium carbonate was routinely added to the purging vial containing betel and water. The calibration curve of methamphetamine in betel was established by plotting six peak area ratios of analyte to internal standard (benzyl alcohol) against the absolute weights of analyte in the six serial spikes, and showed excellent lineality within the range of 0.1~100 μg (of MA · HCl) with the correlation coefficient being 0.997. The detection limit of the method is lower than 0.1 μg. More details concerning the advantages and limitations of the method are discussed.

Keywords: Methamphetamine, Purge and Trap, Gas Chromatography-Mass Spectrometry, Betel

Introduction

Betel originally grows in Malay and is now widely planted in the tropical zone and part of the subtropical area. An unripe betel nut cut in two halves with some piper betel and red slaked lime (consisting mostly of the powder of oyster shell and orange rind) sandwiched in between yields such effects as cooling, cold-protecting, stimulating and saliva-creating [1], which in Southeast Asia have drawn tens of millions of betel lovers widespread in all walks of life throughout the year. In Taiwan, the betel-chewing population is getting close to four million in contrast to the total population of twenty three million. With the recent increase of drug abuse, the perpetrators and merchants have been reportedly resorting to the addition of amphetamines to betel (probably by solution injection, powder mixing, solution soaking, etc.) to create a mass of amphetamines consumers while obscuring the identifiable features of the original drugs.

A thorough literature search indicates that most of the previous studies on betel have been associated with the relationship between betel chewing and oral cavity carcinoma as well as the functions of the endogenous alkaloids and the added slaked lime while chewing betel [2,3], but not with the analytical methodologies towards the added abused drugs. So far as the analyses of amphetamines are concerned, several reports that employ various techniques with various matrices [4-15] have appeared in the literature. In spite of the fact that purge-and-trap preconcentration followed by thermal desorption or solvent extraction (several other names, e.g., adsorption-elution technique, dynamic headspace

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sampling, etc., have been prevalent in different areas) has been extensively used for the sampling of volatile and semi-volatile organic compounds from aqueous or non-aqueous specimens [16-20], little attention, however, has been paid to the recovery of free base amphetamines from their commonly occurring salt forms using this simple, speedy, economic, reliable, robust, and solvent-saving technique or its variations. On the other hand, our previous work using headspace gas chromatography-mass spectrometry to deal with the same analyte in the same matrix has shown the static gas-sampling approach to be feasible with amphetamines originally in salt form [21,22]. It was that success that prompted us to assemble an ever-the-simplest dynamic counterpart, i.e., the purge-and-trap preconcentrator, of our own, and use it in tandem, but in an off-line fashion, with a gas chromatograph-mass spectrometer operated in a trivial mode to fast analyze intentionally added methamphetamine hydrochloride in betel.

Experimental

Materials

The racemic *d*, *l*-methamphetamine hydrochloride (MA·HCl) to be tested, the benzyl alcohol used as internal standard (IS) and the potassium carbonate (K_2CO_3) used to both alkalize the aqueous matrix and salt-out the analyte were all reagent grade and purchased from Sigma Chemical Co., Merck Chemical Co. and Janssen Chemica, respectively. Ethyl acetate (EA; Fisher Chemical Co.) was analytical grade and directly used without distillation. The three major parts of betel, i.e. betel nuts, piper betel and red slaked lime, were bought unmixed from a common betel vender in Taipei.

Purge-and-trap preconcentration (P&T)

The flow pathway of the self-made purge-and-trap device used in preconcentrating the MA from betel-water matrix is shown in Fig.1, and is in the following sequence: (1) high purity nitrogen gas cylinder; (2) gas flow rate regulator; (3) gas purifier; (4) 2 ft long ×1/8 in O.D. Teflon tubing with its outlet-end in the purging vial being under the water level; (5) 50-mL test tube (as purging vial); (6) 1/2 ft long ×/8 in. O.D. Teflon tubing with its head in the purging vial being over the water level; (7) drying tube filled with molecular sieves 3 A; (8) 1/2 ft long × 1/8 in. O.D. Teflon tubing; (9) micro-concentration tube (as sample collector) containing 5 mL of EA (as absorbent solvent), with foregoing Teflon tubing's outlet-end immersed in the solvent. The general procedure of purge-and-trap is as follows: A whole bead of betel nut was weighed, crushed and placed in the purging vial, to which about 0.3 g each of piper betel and red slaked lime, and an appropriate amount (1, 10 μL for 100 ppm solution; 25, 50, 75, 100 μL for 1000 ppm solution) of MA·HCl (in D.I. water) were added. This would make the corresponding spikes contain 0.1, 1, 25, 50, 75 and 100 μg, respectively, of MA ·HCl. About 5 min later, a 5-g portion of K_2CO_3 was added. To the contents of the purging vial was added more D.I. water until the total volume reaches 20 mL. The P&T was then carried out under an 80℃ (water bath) purge temp. for 1 hr with the purge gas (N_2) flow rate controlled at 25 mL/min. The resulting EA trap was further purged with N_2 until the volume of the MA extract became less than 1 mL. After adding 10 μL of 1000-ppm benzyl alcohol in EA, more EA was added until making up a 1-mL sample solution. A 1- μL aliquot of this solution was injected onto the GC-MS for analysis.

Gas chromatography-mass spectrometry (GC-EIMS EIC)

An HP 5890 Series II Gas Chromatograph interfaced with a Finnigan INCOS 50 Mass Spectrometer was used to analyze the trapped methamphetamine free base. The GC separation was performed on a HP-5 MS capillary column (cross-linked 5% phenyl methyl siloxane, 30 m by 0.25 mm i.d., 0.25 μm film thickness). The injection of sample was carried out in the splitless mode, but the splitter was turned on 1 min after injection. Injector temp. was maintained at 250℃. Oven temperature was programmed as follows: initial temp, 60℃; initial hold, 2 min; ramp 1, 10℃/min to 120℃; ramp 2, 18℃/min to 250℃; final hold, 4.8 min; total running time, 20 min. Helium was used as carrier gas at a flow rate of 1mL/min (controlled by an electronic pressure control, EPC). Ion source was maintained at 180℃ under the electron impact (EI) of 70 eV. The MS analysis was performed using the full scan mode accompanied by extracted ion chromatograms (EIC), with the analyte underivatized. The m/z values used for MA and IS were 58 and 108, respectively. The calibration curve was produced by plotting six peak area ratios of MA·HCl to IS (i.e., instrumental response factor, γ) against the absolute weights of MA · HCl (in μg) in the six serial spikes. Each of the six peak area ratios was, in turn, the means of three independently obtained values. In fo-

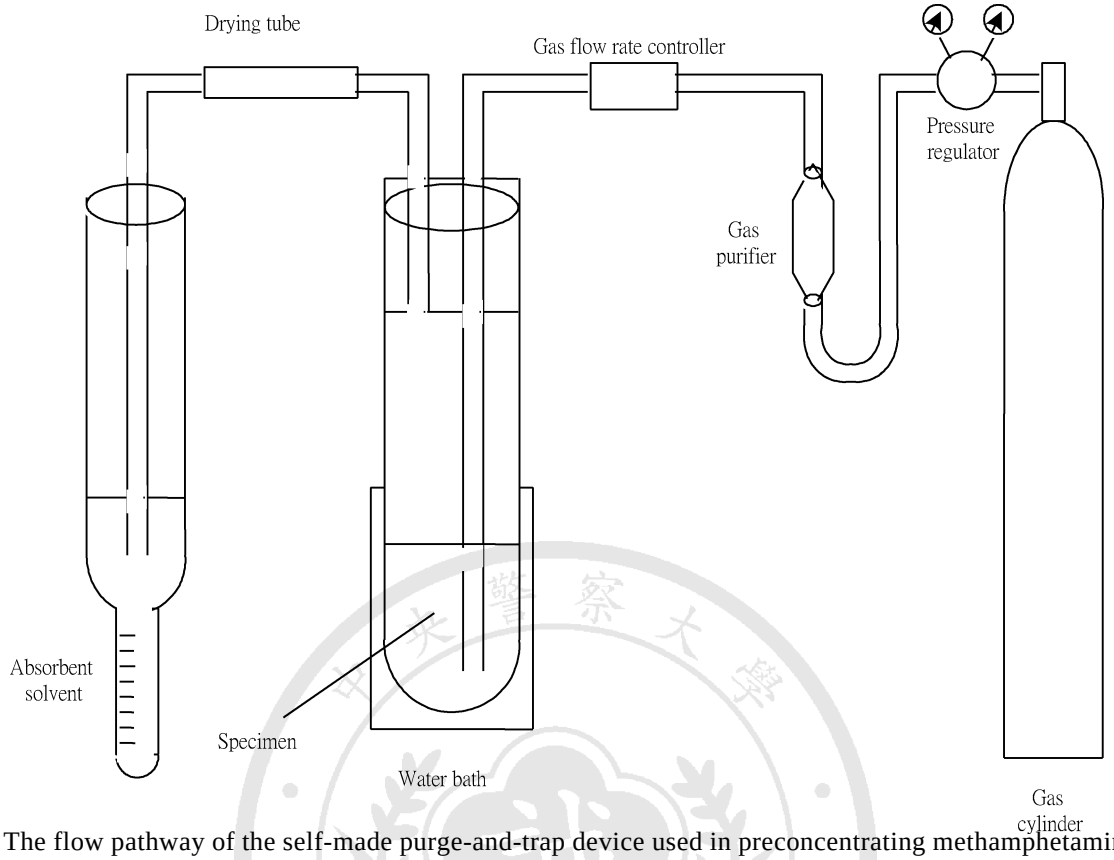

Fig. 1 The flow pathway of the self-made purge-and-trap device used in preconcentrating methamphetamine from betel-water matrix.

rensic practice, the data to be reported for a specific betel exemplar may contrast the found absolute weight of MA·HCl with the original weight of the whole betel exemplar, or the final unit may be in ppm (w/w) or the like.

Results and Discussion

Total-amount analysis and analyte confirmation

A parallel study directed toward the matrix effects on the headspace GC-MS analyses of amphetamines in betel has previously been conducted in our laboratory [22]. Although headspace GC-MS shows a much poorer sensitivity (by the order of about 10^{-2}) than P&T GC-MS, some of the conclusions drawn in that study are still in agreement with what have been observed with the present method. For instance, the endogenous extra alkalizing and salting-out effects of the red slaked lime were found not so obvious at ambient temperature as to jeopardize the normal analysis of a finished-product betel exemplar even though the sample had been stored in open air at ambient temperature for one week. Although each of the three major parts of a finished-product betel exemplar, i.e. betel nut, piper betel and red slaked lime, shows a different matrix effect on the non-total-amount static headspace analysis of the analyte [22], the present dynamic method, in contrast, is based on a total-amount analysis and allows all the three parts to be analyzed together. A typical chromatogram obtained upon the P&T GC-EIMS EIC analysis of an MA·HCl-containing betel exemplar shows the relevant peaks from benzyl alcohol (IS) and MA at RT (retention time) 6.49 and 9.11 min, respectively (Fig.2), as confirmed by the exact match of their mass spectra (obtained without chemical derivatization; Figs. 3 and 4) to those stored in the library [22]. It should be emphasized that both the RT's and the mass spectra obtained for the underivatized analytes using the proposed P&T GC-

EIMS EIC method under the optimal experimental conditions always show excellent reproducibility, presumably because the interferences from the matrices have been unserious.

Quantitation

The six-point (regression) calibration curve plotted for MA in betel (equation: y = 0.037368 x + 0.50499; Fig.5) shows excellent lineality within 0.1~100 μg (of MA·HCl), with the correlation coefficient being 0.997. The precisions (N = 3) calculated for the analyses of the six calibrator concentrations are typically smaller than 7.1%, which is significantly better than the range of 10~20% achieved by headspace GC-EIMS EIC [22]). The limit of detection, LOD (and of quantitation, LOQ), is defined as the lowest analyte levels giving a peak in the extracted ion chromatogram (EIC) with an S/N peak area ratio no less than 3 (10). The LOD and LOQ achieved using the proposed method are lower than 0.1 μg since at that level both the S/N ratio and the root mean square S/N ratio are still far larger than 10.

Optimization of experimental conditions

The operation conditions of the purge-and-trap process were crucial to the recovery of the analyte. Fortunately, a series of preliminary tests indicated that most of the factors involved here would not cross-interact too much. Therefore, the optimal operation con-

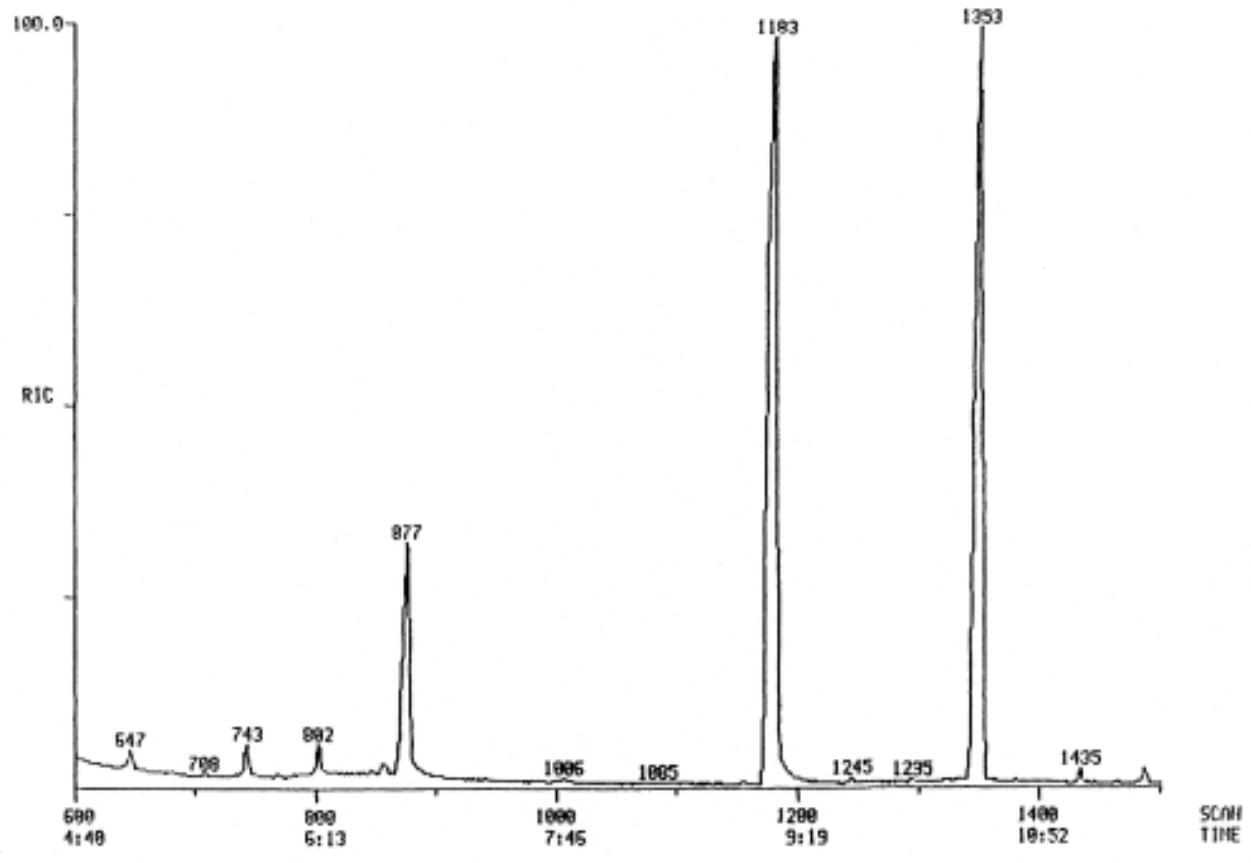

Fig. 2 A typical chromatogram obtained upon the P&T GC-EIMS EIC analysis of an MA · HCl-containing betel exemplar showing the relevant peaks from benzyl alcohol (IS) and MA at RT 6.49 and 9.11 min, respectively.

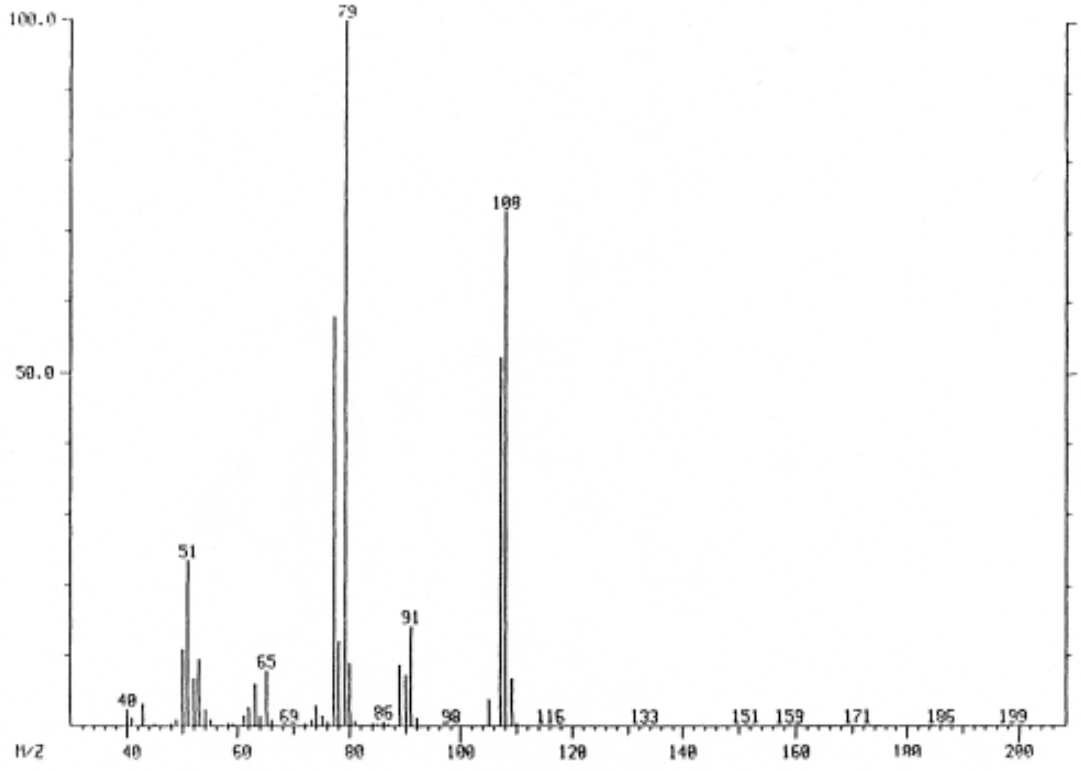

Fig. 3 The mass spectra of benzyl alcohol (IS; underivatized) obtained upon the P&T GC-EIMS EIC analysis of an MA · HCl-containing betel exemplar.

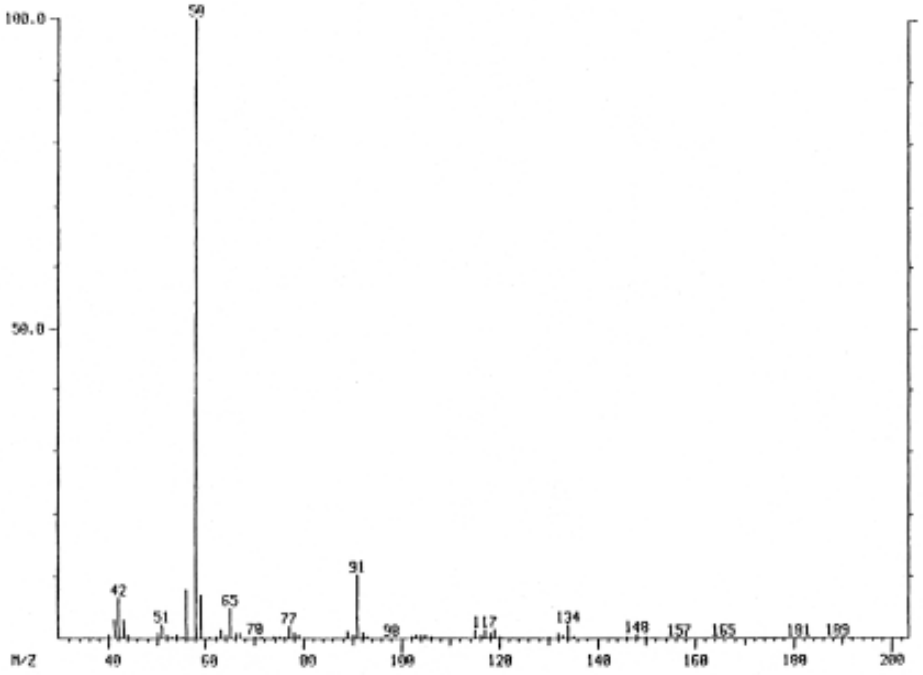

Fig. 4 The mass spectra of methamphetamine (underivatized) obtained upon the P&T GC-EIMS EIC analysis of an MA · HCl-containing betel exemplar.

ditions were determined simply by a few unifactorial experiments; that is, we altered only one factor at a time to monitor the P&T performance while keeping other factors invariant. With the above stated system and sample size, the optimal conditions were finally set to be (in the order of making variations): purge gas flow rate, 25 mL/min; purge temperature, 80℃; purge time, 60 min. Too fast a purge gas flow could result in poor trapping efficiency, whereas too slow a gas flow could lead to unacceptable purge efficiency. Too high a purge temperature could cause too much moisture to be trapped by the EA absorbent and would likely interfere with the following GC-MS analysis, whereas too low a purge temperature could gave rise to limited vaporization of the analyte. Too long a purge time could allow for the co-redesorption of the analyte from the EA adsorbent, whereas too short a purge time could show incomplete

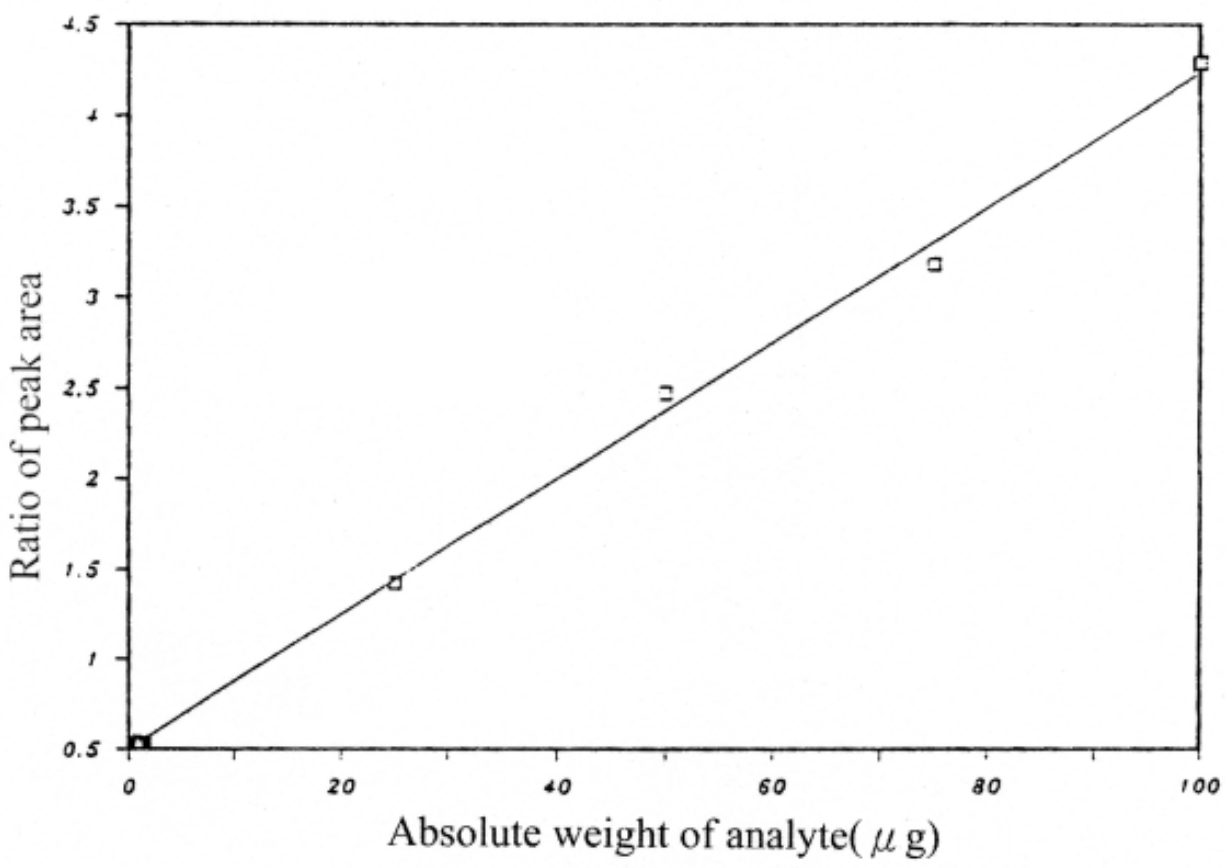

Fig. 5 Calibration curve for the P&T GC-EIMS EIC analysis of MA · HCl in betel. The 0.1- μ g point partially superimposes the 1- μ g point.

purge. As to the amount (5 g) of K_2CO_3 used for a P&T procedure, it was determined empirically, particularly based on the previous experience in doing headspace sampling [21,22], and was demonstrated to function well.

Under the optimal conditions, the method recovery of free-base MA calculated for a betel exemplar containing 25 μg of MA · HCl was 94.7 ± 2.8% based upon triplicate analyses. [Method recovery = γ_{found} / $\gamma_{theoretical}$ × 100%; where γ found is the instrumental response factor produced by the recovered sample solution after the total analysis of 25 μg of spiked MA · HCl, and γ theoretical is that produced by directly injecting 20.08 μg of free-base MA onto GC-MS.] The high recoveries and good precisions indicate that the whole analytical scheme including the sample preparation and the GC-MS analysis is effective, reliable, and robust.

It should also be pointed out that the reason why we did not choose another way of performing P&T that an environmental analyst or arson analyst would prefer to use, i.e., trapping MA with a marketed adsorbent tube followed by thermal desorption of the analyte directly onto GC-MS, was due to its lower efficiency with regard to the thermal desorption of MA, poorer resolution and reproducibility at the step of GC separation and the compatibility between P&T and GC-MS.

Conclusions

Neither is P&T GC-MS a new methodology nor salting-out a new theory; methamphetamine itself is also quite a known compound. The analysis of exogenous MA · HCl in such complicated matrix as betel using the above stated combined methods, however, has not previously been reported yet. With the recent increase of drug abuse, the situations in which amphetamines in their salt forms are intentionally added to betel are not unlikely to be encountered, especially in the tropical zone and part of the subtropical area.

The results of this study demonstrated that P&T GC-EIMS EIC is a simple, convenient, solvent-saving, low-background, robust and reliable technique for the fast analysis of exogenous methamphetamine in betel. Although the P&T device described above is a self-made and off-line one and the recovered MA may be analyzed without chemical derivatization, the whole analytical scheme including analyte trapping and chemical derivatization, if necessary, is well suited for automation. Based on essentially a total-amount analysis, the proposed method suffers far less cross-interacting matrix effects than the non-total-amount-analysis headspace method does and allows the three major parts of a finished-product betel exemplar to be analyzed in "one pot" while causing only tolerable deviations in the quantitaton of methamphetamine. The drawback of the present method is that, unlike headspace method, it cannot afford the knowledge of the relative abundances of the analyte in the three parts of betel which usually provides the investigators with valuable information with regard to the manner in which the methamphetamine has previously been added. Also, for the proposed method to be even more efficient, specific and realistic, it would need to be challenged with other similar drugs (e.g., phentermine, ketamine, etc.) and tested against more specimens with multiple types of drugs to determine the effects of possible interference from other drugs.

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