# 4. DISCUSSION AND CONCLUSIONS

## 4.1 Discussion

In this study, the GC-MS method was employed to determine amounts of amphetamine, methamphetamine, and ephedrine in urine samples. In order to obtain the appropriate condition for GC-MS analysis, it was necessary to optimize the GC-MS conditions to ensure adequate chromatographic resolution for amphetamine, methamphetamine, and ephedrine. The parameters used to optimize the GC conditions in the GC-MS system were injection temperature, initial temperature, final temperature, ramp rate and flow rate of carrier gas, as shown in Tables 3.1-3.11 and Figures 3.1-3.11.

For the underivatized drugs, data in Tables 3.1-3.5 and chromatograms in Figures 3.1-3.5 were taken into consideration. Optimal GC parameters were intended to achieve minimum GC analysis time together with reasonable sensitivity and good peak shapes. As a compromise for these, the optimal GC parameters appeared to be the injector temperature at 250 °C, initial temperature at 50 °C (held for 1min prior to temperature programming at 20 °C/min to the final temperature at 180 °C, held for 2 min) and the helium carrier gas flow rate of 1.5 ml/min. However, Figure 3.6, of which the data are listed in Table 3.6, did suggest that the initial temperature at 100 °C would be the best temperature to choose as the analysis time was minimal and all peaks, including the internal standard peak (phenylpropanolamine), were adequately separated. The initial temperature employed in the GC runs of amphetamine, methamphetamine, ephedrine and phenylpropanolamine was thus 100 °C with the other parameters kept as above.

For the HFBA derivatives, of which the data are listed in Tables 3.7-3.11 and chromatograms shown in Figures 3.7-3.11, selection of the optimal GC conditions was also based on the above criteria. As the HFBA derivatives are more volatile than their parent compounds, the GC conditions can be expected to be milder. The GC

parameters chosen for these HFBA derivatives thus included the following: the injector temperature at 190 °C, initial temperature at 50 °C (held for 1 min prior to temperature programming at 10 °C/min to the final temperature at 170 °C, held for 2 min) and the helium carrier gas flow rate of 1.2 ml/min.

The parameters used to optimize the MS conditions were the trap manifold and ion trap temperature as shown in Tables 3.12-3.13. The suitable conditions due to the reasonable retention time, peak area and good peak shapes are described in Sections 3.1.1-3.1.2. The overall optimized GC-MS conditions established in this work are summarized in Tables 3.14-3.15. It should be noted that the GC-MS conditions chosen for the analysis of HFBA derivatives, as listed in Table 3.15, were not the same as those reported elsewhere [19, 24, 28-30, 32, 35, 44], mainly because the column and its dimensions in this work were different. The column reported in the literature with nearest resemblance to this work was the one employed by Thurman, Pedersen, Stout and Martin [19]; it was a non-polar column (HP-5) which was the same type of column used in this work but its length was shorter and its film thickness was thicker.

Quantitative analysis of compounds by GC is most accurate and precise when using the internal standard method. Selection of an internal standard involves a variety of considerations to ensure that internal standard is as chemically similar to the analyte of interest as possible. MS allows the use of stable isotope-labeled analogues. Deuterium-labeled internal standards are widely used but are not the option, a nonisotopic internal standard can also be evaluated [39]. In this work, deuterium-labeled internal standard was difficult to obtain, so the choice had to be a compound with suitable characteristics. Ideally, the compound chosen should give a peak well separated from all other sample peaks but its retention position should be close to the analyte peak. Four drugs, namely, phenylpropanolamine, phenylephrine, chlorphenniramine, and bromhexine were employed for this purpose as already described in Section 2.2.1.4

When comparing the optimized GC-MS conditions for analysis of underivatized drugs and HFBA derivatives, one distinct difference is that the temperature required in the analysis of HFBA derivatives was lower than that for the underivatized drugs, as shown in Tables 3.14-3.15. This is because HFBA derivatives are more volatile compounds than their corresponding parent compounds. Furthermore, drugs of amphetamine groups often require derivatization prior to determination by GC-MS. This is to eliminate peak tailing and to improve chromatographic peak shape and thus improve sensitivity [25]. Figures 3.12 shows a chromatogram of underivatized drugs yielding peak tailing whereas Figures 3.13 shows that this problem was solved with HFBA derivatizing.

Figures 3.12-3.13 show that the order of elution of underivatized drugs is different from that of HFBA derivatives. For underivatized drugs, phenylpropanolamine was eluted just before ephedrine. But for HFBA derivatives, phenylpropanolamine was eluted after amphetamine. It can be seen that the order of elution of underivatized drugs coincides with the increasing molecular weight. It should be noted that the molecular weights of amphetamine, methamphetamine, phenylpropanolamine and ephedrine are 135.2, 149.2, 151.2 and 165.2, respectively. As the column used was a DB-5MS fused silica column containing 95%dimethyl-5%diphenylpolysiloxane, it was a relatively non-polar column which usually separates compounds on the basis of boiling point. In terms of molecular mass and hence boiling point, ephedrine > phenylpropanolamine > methamphetamine > amphetamine. That is why the order of elution of the underivatized drugs was amphetamine, methamphetamine, phenylpropanolamine and ephedrine. The base peaks of underivatized amphetamine, methamphetamine, phenylpropanolamine, and ephedrine are 44, 58, 44 and 58, respectively.

For the HFBA derivatives, of which the structures together with the corresponding mass spectra are shown in Appendix B, the relative molecular masses of

amphetamine-HFBA, methamphetamine-HFBA, phenylpropanolamine-2HFBA and ephedrine-2HFBA are 331, 345, 543 and 557, respectively. But the order of elution on the DB-5MS GC column was found to be slightly different from the order of molecular mass. While amphetamine-HFBA was found to be the first peak with ephedrine-2HFBA as the last peak, as might be expected, phenylpropanolamine-2HFBA ( M.W. 543) was found to have been eluted before methamphetamine-HFBA (M.W.345) but after the amphetamine-HFBA peak. This is possibly due to the preferential retention of methamphetamine-HFBA by the relatively non-polar column which perceived methyl groups in this derivative as something similar to its non-polar nature and the molecular mass was not a key factor in this case.

For HFBA derivatives, the order of elution is followed by the order of base peak. For amphetamine-HFBA, phenylpropanolamine-2HFBA, methamphetamine-HFBA, and ephedrine-2HFBA, the base peaks were found to be 240, 240, 254 and 254 , respectively. When considering the pattern of mass spectra, underivatized drugs have the pattern similar to that of the NIST Library (see Appendix A), while HFBA derivatives have the pattern similar to that in literature [19] (see Appendix B).

The retention times and principal ions used for the identification are shown in Tables 3.16-3.17. Both amphetamine and phenylpropanolamine have the 44 amu ion for the underivatized drugs and the 240 amu ion for the HFBA derivatives as their base peak. The results of the fragmentation of amphetamine and phenylpropanolamine are shown in Figures 3.14 and 3.17. Although amphetamine and phenylpropanolamine have the same base peak but amphetamine has a reference spectrum and its retention time was seen to be different from that of phenylpropanolamine. Thus, one can distinguish the fragmentation ions for amphetamine from phenylpropanolamine. In the same way, methamphetamine and ephedrine have the same base peak but they have different reference spectra and retention times too. The results of the fragmentation of methamphetamine and ephedrine are shown in Figures 3.15- 3.16.

For amphetamine, methamphetamine and ephedrine, the LOD of each of these underivatized drugs was 2 ug/ml with mean S/N values of 11.0, 62.2 and 10.2, respectively. As for HFBA derivatives, the LOD values were 100, 50 and 100 ng/ml with mean S/N values of 6.6, 10.8 and 5.6 in the same order (Table 3.18). From these LOD values, it can be seen that the use of HFBA derivatizing agent improved the sensitivity of GC-MS analysis. The linearity ranges of the underivatized drugs with correlation coefficient of the straight line better than 0.990 for amphetamine, methamphetamine and ephedrine were 2 - 160, 2 - 160 and 2 - 80 ug/ml, respectively, and the linearity ranges of the HFBA derivatives with correlation coefficient of the straight line better than 0.991 were 0.2 - 40, 0.2 - 40 and 0.2 - 30 ug/ml in the same order (Tables 3.19 - 3.20, Figures 3.18 - 3.23). The repeatability and reproducibility were found to be good for both the underivatized drugs and the HFBA derivatives, with the RSD values found to be between 3.1 - 7.0 % of repeatability and 2.7 - 9.0 % of reproducibility (Tables 3.21-3.24).

In determining amounts of amphetamine, methamphetamine and ephedrine in a biological matrix, extraction or isolation prior to quantitation is generally required. SPE is a popular technique for the preparation of samples for analysis. In this work, the $C_{18}$ adsorbent was used to extract amphetamine, methamphetamine and ephedrine from urine samples. The influence of the parameters on extracting by SPE depended on the pH of the samples, rinse solution and elution solution. The effect of pH on the retention of analytes on an adsorbent altered the recovery of the extraction. It was necessary to adjust the pH of the urine sample to ensure that amphetamine, methamphetamine and ephedrine were in the appropriate forms to achieve efficient retention by the adsorbent. The best results were obtained with extraction at a pH value of 5 (Table 3.25). Once the analyte was retained on the sorbent, the sorbent was usually rinsed with a suitable solvent to wash off undesirable compounds. To trace the effect of rinse solution, the pH of urine was adjusted to the value that gave the best results. A solvent mixture of EtOH

: $NH_4OH$ : $H_2O$ (50:10:40 v/v) gave the best results (Table 3.26). The analytes were desorbed from the adsorbent after the rinsing step. The optimum pH value of the sample and rinse solution were used. The solvent mixture containing $CH_2Cl_2$ : *i*-propanol : $NH_4OH$ (78:20:2v/v) gave the best results (Table 3.27).

The recovery after sample pretreatment with the suitable condition of SPE was determined at concentration 500 ng/ml. The percent recoveries of amphetamine, methamphetamine and ephedrine were 55.8, 66.9 and 26.9, respectively (Table 3.28). The results revealed that the $C_{18}$ adsorbent was not good due to the low recovery. The limits of detection for the samples were found to be in the same range as these for the standards (Table 3.29).

In order to asses the practical utility of this method, it was applied to real urine samples. The results of the determination of amphetamine, methamphetamine and ephedrine in urine samples by GC-MS are summarized in Table3.30. From 19 specimens initially found to contain methamphetamine, most specimens met the 500 ng/ml cutoff requirement for methamphetamine but only 5 specimens met the 500 ng/ml cutoff for amphetamine and ephedrine was not detected in all specimens. The confirmation cutoff concentrations indicate the extent of the use of abused drugs such as 500 ng/ml for amphetamine and methamphetamine [41]. Notification of Public Health Ministry No 135 BE 2539 and Psychotropic Substance Act BE 2518 do not impose any limit of confirmation cutoff concentrations.

**4.2 Conclusion**

The optimized GC-MS method described in this work was found to be applicable to the determination of amphetamine, methamphetamine and ephedrine in urine samples. When analyzed without derivatization, peak tailing and resultant sensitivity problems were encounted. To correct these problems, the HFBA derivatives

were employed. Sample preparation by SPE was applied to sample cleanup but with recoveries lower than 70% with $C_{18}$ adsorbent.

GC-MS, as the definitive procedure for confirmation of drugs of abuse, should be further investigated to determine drugs of abuse other than amphetamine, methamphetamine and ephedrine such as methylenedioxyamphetamine (MDA) and methylenedioxymethamphetamine (MDMA, Ecstasy) since both MDA and MDMA are likely to become a problem in Thailand in the very near future.