CHAPTER IV

CONCLUSIONS

The main purpose of this research is to apply MIPs as a solid sorbent in solid phase extraction (SPE) for NVP sample pre-treatment, therefore MIPs selective to NVP were investigated. Generally, MIPs are prepared by using the target analtye as a template molecule. After imprinting by polymerization of monomer with cross-linker in a presence of templae, the obtained polymers is washed to remove the template molecule from the polymer matrix. However, leakage of a trace amount of template from MIPs when used in SPE can cause error in the analysis. In this study, NVP-structurally related compounds were used as dummy templates instead of NVP to prepared MIPs. The best performing MIP was selected to apply as a solid sorbent in SPE of NVP and its efficiency in NVP sample clean-up and preconcentration were investigated.

In a primary study, four MIPs were synthesized using NVP, NAM, BZM and BZP as template molecules to investigated their binding capacity with the template itself and with NVP. P(NVP) was used as a positive control, while P(BZP) structurally unrelated to NVP was used as a negative control. All MIPs were synthesized by precipitation polymerization in THF:MeOH:water (5:4:1,v/v) using MAA and TRIM (1:1) as the functional monomer and cross-linker, respectively.

Equilibrium binding performances of MIPs with their corresponding templates were evaluated using UV-Vis spectrophotometric technique. It was found that P(NVP) showed a good capacity in binding with its template, meanwhile the rest of polymers showed low binding capacity to their templates when using an equal amount of polymers. However, when the amount of polymer the binding studies was increased, the binding capacity of template of polymer was also enhanced.

To evaluate the binding performance with NVP, the above experiment was performed using P(NAM) P(BZM) and P(BZP). It was found that P(NAM) showed a high binding efficiency with NVP in comparison to P(NVP). Moreover, the highest NVP selectivity factor was also obtained from this polymer. These results indicated that P(NAM) can selectively bind with NVP efficiently. The competitive binding study was also investigated to study the effect of NAM concentration on the binding of NVP to P(NAM). When the amount of NAM was varied from 0.2 mM to 2mM, it was found that the binding capacity of NVP was gradually decreased. However, the amount of NAM generally found in biological sample was lower than 0.2 mM, therefore, the corresponding amount of NAM should not affect the capacity of P(NAM) in MISPE experiment for NVP.

Due to the performance of P(NAM), this polymer was applied as a sorbent in MISPE for extraction of NVP from aqueous samples. Equal amounts of NVP were loaded into MISPE cartridge and the extraction conditions in MISPE were investigated including washing and eluting conditions. The washing conditions were optimized by varying pH and the amount of organic solvent used in phosphate buffer. The results showed that without adding organic solvent, high percentage of NVP was retained in MISPE column at the pH ranging from 4 to 10.5. When increasing

amount of organic solvent in the washing solution to 20%, most of NVP were washed out of the column. Therefore the phosphate buffer pH 7 was selected as the washing solvent to avoid the leakage of NVP in further study.

In the eluting step, acetonitrile was selected to be used as eluting solvent for the ease of sample preparation for further HPLC analysis. The eluting conditions were compared using neutral, basic and acidic solvent. When sample was eluted with acidic solvent (1% formic acid or 1% acetic acid in acetonitrile), higher recovery of NVP was obtained than eluting with neutral solvent (pure acetonitrile). Meanwhile the basic condition (1%TEA in acetonitrile) gave the lowest NVP recovery. This may be due to the basic property of NVP molecule. At low pH, the binding sites in MIPs were protonated, therefore NVP can be easily washed off. However, at high pH, those binding sites were deprotonated causing an enhancement in template-polymer binding interaction via ionic bond. Nevertheless, recovery of NVP was not affected much with pH, therefore pure acetonitrile was selected to be used as the eluting solvent.

After extraction conditions were optimized, the selected protocol was applied with the real plasma samples spiked with NVP at concentration range 0.5-100 μg/ml. From the results, the developed MISPE protocol can efficiently remove impurities from the plasma matrix. After pre-concentration, NVP can be obtained in high percentage recovery.

In summary, MISPE was developed for sample clean-up and preconcentraction of NVP using polymer imprinted with NAM. The MISPE technique showed high capacity of sample clean-up and the high percent recovery of the target analyte. This method was validated with linearity, recovery, accuracy and

precision. Since P(NAM) is more selective than other commercial sorbent, less complicate solvent mixture can be used with this MISPE protocol. Moreover, the MISPE method is less time-consuming and require less amount of organic solvent in comparison with LLE, therefore this method can be used for selective extraction of NVP from biological samples which can facilitate the screening of NVP in infected patients.

In continuation of the work described in this study, the following suggestions for further work are made:

1.) The internal standard should be employed through the analysis to improve reproducibility of the MISPE-HPLC analysis.

2.) Combination of NVP with other anti-HIV drugs should be tested for simultaneous analysis of the drug level in real infected patient samples.