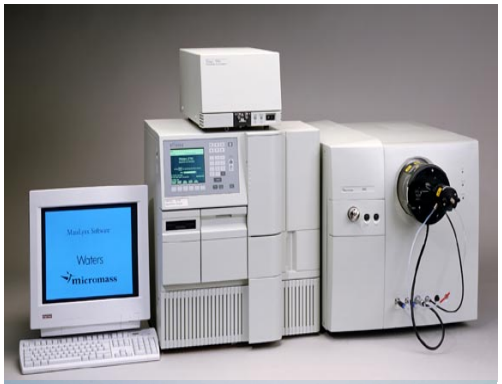
A SPE Method Development for Two Hydrophobic, Water-insoluble Compounds

Optimizing the existing Oasis® HLB generic method

Jianmei Ding, Waters Corporation, 34 Maple Street, Milford, MA



Key Words:
Oasis® HLB SPE method, Quantitation, hydrophobic pharmaceutical compounds, LC-MS

Most pharmaceutical drugs and metabolites from biological matrices can be selectively extracted with Oasis® HLB solid phase extraction (SPE) sorbent (in the format of cartridges or plates) following a generic method (1). However, there are some instances that the generic method does not yield acceptable results. In this example, two hydrophobic, water-insoluble compounds from a large pharmaceutical company were encountered. Following the generic method using 100% methanol as an elution solvent, the recoveries for both the analyte and the internal standard were low and inconsistent. The results indicate that the compounds might not be eluted off the Oasis® HLB plate effectively due to the relatively low elution strength of methanol. Therefore, we experimented with different types of organic solvents, considering their elution strength and selectivity. Solvents such as acetonitrile, mixtures of methylene chloride and methanol and mixtures of acetonitrile and methanol were investigated. We observed that pure acetonitrile did not yield acceptable recoveries, mixtures of methylene chloride and methanol were not ideal solvent for subsequent LC-MS analysis (2). However, a mixture of 70:30 acetonitrile and methanol resulted in high recoveries for both compounds (Table 1). The modified SPE procedure is described in Figure 1.

During the study, the two compounds were initially dissolved in methanol and then diluted with phosphate buffer saline solution. Subsequently, a linear calibration curve was established by LC-MS with a correlation coefficient of 0.999, indicating that the solubility of the compounds in the saline solution was acceptable (Figure 2).

The results indicate that these two compounds are not only hydrophobic, but may also have some polar groups which provide hydrogen-bonding interaction with the hydrophilic moiety N-vinylpyrrolidone in the Oasis® HLB copolymer material. Therefore, some percentage of methanol is required to assist in the breakage of the H-bonding.

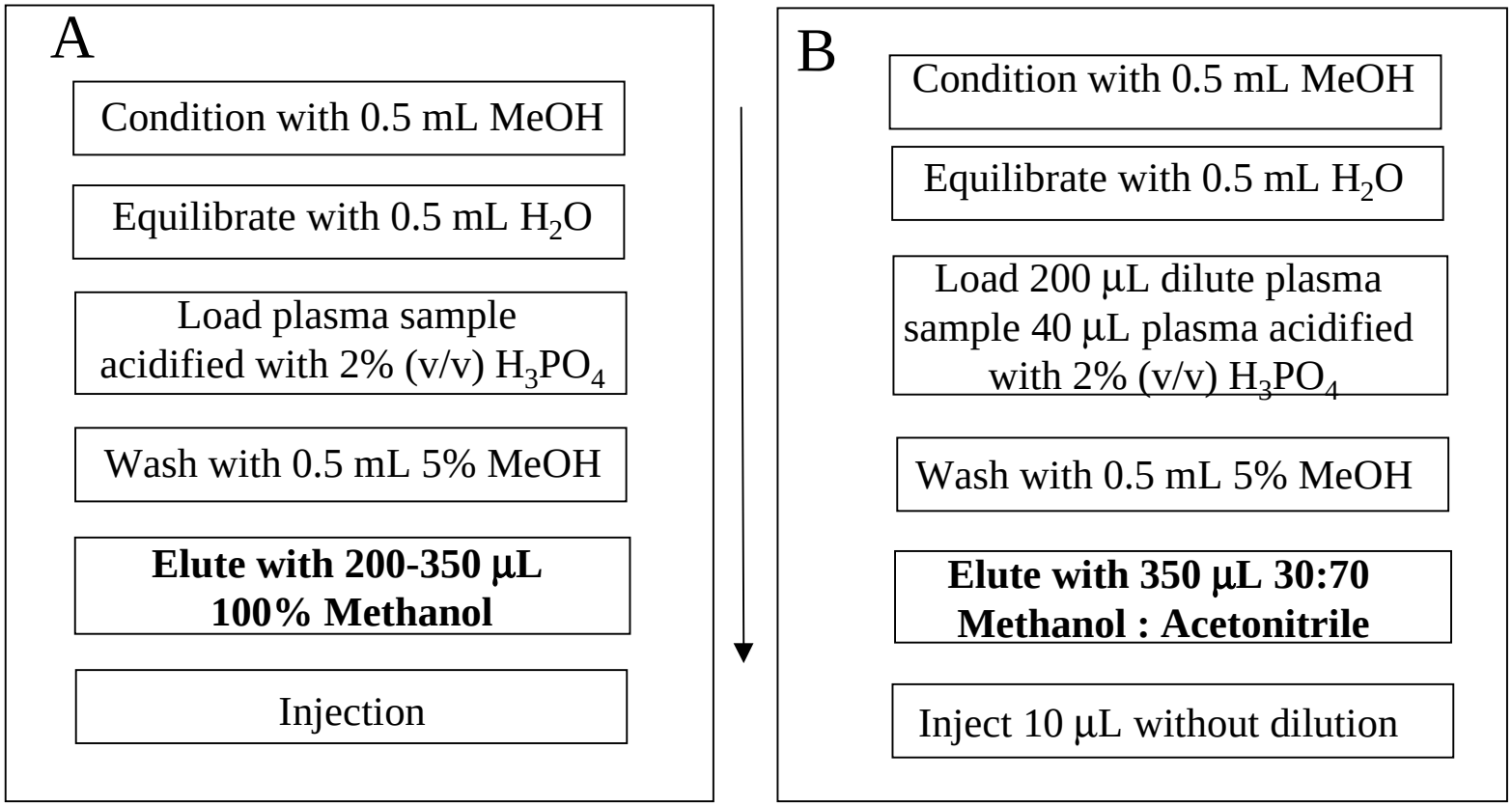


Figure 1 SPE procedures. (A) the generic method and (B), the optimized method for the two hydrophobic compounds. A 96-well (10 mg per well) Oasis® HLB extraction plate was used in this study.

Table 1 Recoveries for both compounds 1 and 2 at different concentration levels

Compounds	Conc. (ng/mL)	Recovery (%)	RSD (%)
Compound 1	50	98	11
Compound 1	500	90	3
Compound 2	1000	96	5
Compound 2	500	91	4

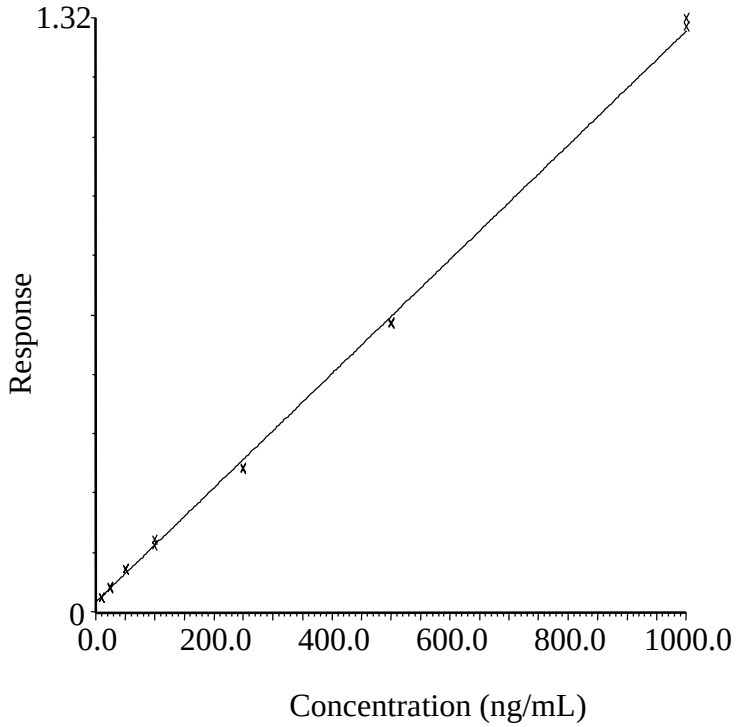


Figure 2 A calibration curve for compound 1 before and after the recovery study (24 injections). Compound 2 was used as the internal standard. The correlation coefficient is 0.999 using 1/x as a weighting factor.

References:
(1) Waters Oasis® HLB application notebook
(2) Ding, J. Waters Corporation

