

# Pengembangan dan validasi metode analisis siklofosamid dan 4-hidroksisiklofosamid dalam volumetric absorptive microsampling (VAMS) menggunakan KCKUT-SM/SM = Development and Validation of Cyclophosphamide and 4-hydroxycyclophosphamide quantification Method in volumetric absorptive microsampling (VAMS) by Liquid chromatography - tandem mass spectrometry

Steven, author

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## Abstrak

Development and Validation of Cyclophosphamide and 4-Hydroxycyclophosphamide Quantification Method in Volumetric Absorptive Microsampling (VAMS) by Liquid Chromatography - Tandem Mass Spectrometry

.....Cyclophosphamide is an anticancer alkylating prodrug, metabolized by CYP450 into its active metabolite, named 4-hydroxycyclophosphamide (4-OHCP). Its therapeutic effectiveness is determined by the 4-OHCP concentration. Several analytical methods using plasma and Dried Blood Spot have been developed to analyze cyclophosphamide and 4-OHCP. However, there are lots of disadvantages. Therefore, this study was conducted to develop a validated cyclophosphamide and 4-OHCP analysis method with 4-hydroxycyclophosphamide-d4 (4OHCP-d4) as the internal standard in Volumetric Absorptive Microsampling (VAMS) using Ultra-High-Performance Liquid Chromatography-Tandem Mass Spectrometry. VAMS requires small volume of sample, is not affected by hematocrit, and more efficient sampling process. Sample preparation was started by derivatization with 5 L semicarbazide hydrochloride to overcome the instability of 4-OHCP and 4-OHCP-d4, which was absorbed by VAMS. Afterwards, 25 L samples were absorbed into VAMS and extracted using the protein deposition method with methanol. The analysis was performed using a triple quadrupole Mass Spectrometry with positive electrospray ionization mode. The optimum conditions were obtained using the Acquity® UPLC BEH C18 column (2.1 x 100 mm; 1.7 m); flow rate 0.2 mL/min; mobile phase 0.01% formic acid and methanol; gradient elution mode for 6 minutes; multiple reaction monitoring detection with m/z values 260.65>140.03 for cyclophosphamide, 333.65>221.04 for 4-OHCP-SCZ, and 337.71>225.05 for 4-OHCP-d4-SCZ. The method has met the validation requirements set by the FDA (2018). Cyclophosphamide LLOQ value was 5 ng/mL and the calibration curve range was 5 - 60,000 ng/mL. Furthermore, 4-OHCP LLOQ value was 2.5 ng/mL and the calibration curve range was 2.5 - 1,000 ng/mL.