# DEVELOPMENT AND VALIDATION OF HPLC-UV METHOD FOR SIMULTANEOUS ANALYSIS OF ACRYLAMIDE AND GLYCIDAMIDE IN VOLUMETRIC ABSORPTIVE MICROSAMPLING

**MUHAMMAD IKHSAN[1], YAHDIANA HARAHAP[1,2], FADLINA CHANY SAPUTRI[2]**

**[1]Faculty of Military Pharmacy, Republic of Indonesia Defense University, Bogor, West Java, Indonesia**

**[2]Faculty of Pharmacy, Universitas Indonesia, Depok, West Java, Indonesia**

**Email: m.ikhsan.c@gmail.com**

## ABSTRACT

Acrylamide is a carcinogenic compound that can be found in commonly consumed foods and cigarette smoke. This compound is metabolized by cytochrome P450 in the human body to a more reactive metabolite, glycidamide. This study aimed to optimize and validate a sensitive HPLC-UV method for determining acrylamide and glycidamide simultaneously in the volumetric absorptive microsampling (VAMS) sample. Isoniazid as an internal standard was added to the VAMS sample containing acrylamide and glycidamide prior to protein precipitation. The analytes and internal standard were separated using reversed-phase chromatography with the C18 Sunfire$^{TM}$ Waters® column (5 µm; 250 mm x 4.6 mm) and an ultraviolet detector. The optimum chromatographic condition was eluted at a column temperature of 30 $^{o}$C with a mobile phase of 6 mM potassium dihydrogen phosphate pH 3.5 – methanol (96:4 v/v) using a flow rate of 0.50 ml/min and was detected at 210 nm. The LLOQ was obtained at 1.0 µg/mL for both acrylamide and glycidamide. The calibration curve was linear over the concentration range of 1.0-100.0 µg/ml. The developed bioanalytical method was valid based on US FDA Guideline for Bioanalytical Method Validation 2018.

**Keywords:** Acrylamide; Glycidamide; HPLC-UV; Volumetric Absorptive Microsampling; Validation