Analisis Akrilamida dan Glisidamida sebagai Biomarker dalam Dried Blood Spot Perokok secara Kromatografi Cair Kinerja Ultra Tinggi Tandem Spektrometri Massa = Analysis of Acrylamide and Glycidamide as Biomarker in Dried Blood Spot Smoker by Ultra High Performance Liquid Chromatography-Tandem Mass Spectrometry

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Abstrak

Asap rokok adalah sumber utama paparan akrilamida setelah makanan. Akrilamida diklasifikasikan sebagai senyawa berpotensi karsinogenik pada manusia (Grup 2A). Akrilamida dimetabolisme oleh enzim CYP2E1 menjadi glisidamida yang sangat reaktif terhadap DNA dan dapat membentuk DNA adduct sehingga menyebabkan efek karsinogenik pada manusia. Kadar akrilamida dalam asap rokok berkisar 1,000–7,991 g/rokok yang dipengaruhi perbedaan merek rokok. Paparan akrilamida pada perokok 2,2–4,6 kali lebih tinggi daripada non perokok dan glisidamida 1,1–3,8 kali lebih tinggi daripada non perokok. Kadar akrilamida dan glisidamida dalam sampel Dried Blood Spot (DBS) belum diketahui. Keunggulan DBS yaitu nyaman bagi subjek, volume sampel kecil, analit lebih stabil, penyimpanan dan transportasi mudah, serta preparasi sampel sederhana. Aplikasi volumetrik menggunakan pipet volumetrik atau perangkat modern seperti microfluidic DBS dapat mengatasi efek hematokrit darah. Metode KCKUT-SM/SM dapat mengkuantifikasi sejumlah kecil analit karena menghasilkan pemisahan yang baik, waktu retensi cepat, sensitivitas dan selektivitas tinggi. Validasi metode serta analisis akrilamida dan glisidamida dalam sampel DBS perokok secara KCKUT-SM/SM penting dilakukan untuk mengukur risiko paparan akrilamida melalui asap rokok. Metode analisis menggunakan KCKUT-SM/SM dengan kolom Acquity® UPLC BEH C18; fase gerak asam formiat 0,1% dalam air-asetonitril (40:60 v/v) dengan elusi gradien; laju alir 0,20 mL/menit; deteksi massa menggunakan penganalisis massa triple quadrupole dengan mode Electrospray Source Ionization (ESI) positif tipe Multiple Reaction Monitoring (MRM); nilai m/z 71,99>55,0; 87,9>44,2; 75>58,0 untuk akrilamida, glisidamida, dan d3-akrilamida. Preparasi sampel dengan pengendapan protein menggunakan larutan pengekstraksi metanol.

.....Cigarette smoke is the major source of acrylamide exposure after food. Acrylamide is classified as a probably carcinogenic to humans (Group 2A). Acrylamide is metabolized by CYP2E1 enzyme into glycidamide that very reactive to DNA and can form DNA adducts causing carcinogenic effects in humans. Acrylamide level in cigarette smoke is around 1.000–7.991 g/cigarette caused by different cigarette brands. Acrylamide exposure in smokers is 2.2–4.6 times higher than non-smokers and glycidamide exposure 1.1–3.8 times higher than non-smokers. The levels of acrylamide and glycidamide in Dried Blood Spot (DBS) sample are still unknown. Advantages of DBS are convenient for subjects, small sample volumes, analytes are more stable, easy storage and transport, and simple sample preparation. Volumetric application by using volumetric pipettes or modern devices such as microfluidic DBS are used to overcome blood hematocrit effect. UHPLC-MS/MS method can quantify small amounts of analytes due to good separation, fast retention time, high sensitivity and selectivity. Method validation and analysis of acrylamide and glycidamide in DBS smokers by UHPLC-MS/MS is important to measure the risk of acrylamide exposure through cigarette smoke. Analysis method using UHPLC-MS/MS with Acquity® UPLC BEH C18 column;

mobile phase was 0.1% of formic acid in water and acetonitrile (40:60 v/v) with gradien elution; flow rate was 0.20 mL/min; mass detection using triple quadrupole mass analyzer with positive Electrospray Source Ionization (ESI) and Multiple Reaction Monitoring (MRM) type; m/z values were 71.99>55.0; 87.9>44.2; 75>58.0 for acrylamide, glycidamide, and acrylamide-d3. Sample preparation with protein precipitation using methanol as extraction solvent.