Optimasi dan validasi metode analisis O6-Metilguanin dan N7-Metilguanin secara kromatografi cair kinerja ultra tinggi-tandem spektrometri massa = Analytical method optimization and validation of O6-Methyguanine and N7-Methylguanine by ultra high performance liquid chromatography tandem mass spectrometry

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Abstrak

O6-Metilguanin dan N7-metilguanin merupakan isomer DNA adduct. N7-Metilguanin memberikan efek sitotoksik, sedangkan O6-metilguanin memberikan efek mutagenik yang dapat memicu timbulnya kanker sekunder. Kedua senyawa tersebut sering ditemukan dalam kadar yang sangat rendah pada pasien kanker yang memperoleh agen pengalkilasi sebagai terapi antikankernya. Oleh karena itu untuk menganalisis kedua senyawa tersebut dibutuhkan metode analisis yang memiliki selektivitas dan sensitivitas yang sangat tinggi. Pada penelitian ini dilakukan optimasi dan validasi metode analisis O6-metilguanin dan N7- metilguanin secara kromatografi cair kinerja ultra tinggi - tandem spektrometri massa. Kondisi analisis yang optimal diperoleh dengan sistem kromatografi: kolom C18 Acquity BEH (1,7 µm, 100 mm × 2,1 mm); fase gerak larutan asam asetat 0,05% - asetonitril (95:5 v/v); laju alir 0,3 mL/menit; dan deteksi diatur pada m/z 166,10 > 149,10 dan 166,10 > 134,10 untuk O6-metilguanin, serta m/z" 166,10 > 149,10 dan 166,10 > 96,10 untuk N7-metilguanin. Metode yang diperoleh valid dengan hasil kurva kalibrasi yang linier (r > 0,999) baik untuk O6- metilguanin dan N7-metilguanin; presisi dengan nilai koefisien variasi (KV) sebesar < 6,54% untuk O6-metilguanin dan < 3,17% untuk N7-metilguanin; serta akurat dengan nilai perolehan kembali pada empat konsentrasi sebesar 90,52-109,65% untuk O6-metilguanin dan 93,77%-106,65% untuk N7-metilguanin. Nilai LLOQ untuk O6-metilguanin dan N7-metilguanin berturut-turut sebesar 0,5 ng/mL dan 1,0 ng/mL. Nilai LLOQ tersebut menunjukkan bahwa metode ini sangat sensitif.
<hr>O6-Methylguanine and N7-methylguanine are isomer of DNA adduct. N7- Methylguanine has cytotoxic effect, whereas O6-methylguanine has mutagenic effect which vulnerably leads to secondary cancer. The compounds were commonly found at very low concentration in cancer patients who had been receiving alkylating agent as their anticancer therapy. Therefore, the very selective and sensitive analytical method is needed to analyze those compounds. In this research, the optimization and validation of analytical method for analysis of O6-methylguanine and N7-methylguanine by ultra high performance liquid chromatography - tandem mass spectrometry was performed. Optimal analytical condition was obtained by system of chromatography: Acquity BEH C18 column (1.7 µm, 100 mm × 2.1 mm); mobile phase of 0.05% acetic acid - acetonitrile (95:5 v/v); flow rate of 0.3 mL/min; and detection was set at m/z 166.10 > 149.10 and 166.10 > 134.10 for O6-methylguanine, and at m/z 166.10 > 149.10 and" 166.10 > 96.10 for N7-methylguanine. The method is valid by the calibration curve with good linearity (r > 0.999) for both of O6-methylguanine and N7- methylguanine; precision by the coefficient of variation (CV) was < 6.54% for O6-methylguanine and < 3.12% for N7-methylguanine; and accurate by the recovery for four concentrations ranged from 90.52-109.65% for O6- methylguanine and 93.77-106.65% for N7-methylguanine. LLOQ values for O6- methylguanine and N7-methylguanine were found to be 0.5 ng/mL and 1.0 ng/mL, respectively. Those LLOQ values indicated that the method was very sensitive."