

VALIDASI METODE PENETAPAN KADAR INOSITOL MENGUNAKAN *HIGH PERFORMANCE LIQUID CHROMATOGRAPHY* (HPLC) DAN APLIKASINYA PADA OBAT IKAN SEDIAAN PREMIKS

INTISARI

Inositol merupakan salah satu contoh zat dalam premiks obat ikan yang dapat membantu metabolisme lemak, membantu pertumbuhan, mempengaruhi kadar antioksidan dan status oksidatif sel pada ikan. *High Performance Liquid Chromatography* (HPLC) merupakan salah satu metode analisis senyawa yang sensitif dan selektif. Penelitian ini bertujuan untuk melakukan validasi metode penetapan kadar inositol pada obat ikan. Optimasi metode dilakukan dengan uji coba beberapa variasi fase gerak asetonitril : air (30:70, 20:80, 10:90, dan 0:100) dan suhu kolom (60 °C, 70 °C, dan 80 °C). Pemilihan metode yang optimal didasarkan pada evaluasi parameter uji kesesuaian sistem. Kondisi kromatografi yang optimal pada penelitian ini adalah menggunakan kolom Hi-Plex Ca (300 mm x 7,7 mm) dengan fase gerak air 100%, suhu kolom 80 °C, laju alir 0,6 mL/menit, dan detektor indeks bias. Metode penetapan kadar yang digunakan dievaluasi menggunakan parameter-parameter metode analisis meliputi akurasi, presisi (*repeatability* dan *intermediate precision*), *limit of detection* (LoD), *limit of quantification* (LoQ), linearitas, rentang, dan stabilitas. Hasil penelitian menunjukkan metode akurat dan teliti yang ditunjukkan dengan nilai rata-rata % *recovery* sebesar 99,67 % dan nilai $RSD < 2/3 \times CV \text{ Horwitz } (\%)$ serta presisi antara tidak berbeda signifikan ($p\text{-value} > 0,05$). Metode ini menghasilkan linearitas yang baik dengan nilai R^2 0,999 pada rentang 2000 - 10000 mg/L. Nilai LoD sebesar 95 mg/L dan nilai LoQ 125 mg/L. Pada uji stabilitas, metode memberikan presisi dan akurasi yang baik (stabilitas yang baik). Hasil aplikasi metode yang telah teroptimasi dan tervalidasi dari pengujian 6 sampel obat ikan, sebanyak 6 sampel telah memenuhi syarat nilai perolehan kembali.

Kata kunci: inositol, laktosa, HPLC, validasi metode, penetapan kadar

VALIDATION OF INOSITOL LEVEL DETERMINATION METHOD USING HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC) AND ITS APPLICATION IN FISH MEDICINE PREMIX PREPARATIONS

ABSTRACT

Inositol is one example of substance in fish medicine premix that can help fat metabolism, help growth, affect antioxidant levels and cell oxidative status in fish. High Performance Liquid Chromatography (HPLC) is one of the sensitive and selective compound analysis methods. This study aims to validate the method for determining inositol levels in fish medicine. Method optimization was carried out by testing several variations of acetonitrile: water as mobile phase (30:70, 20:80, 10:90, and 0:100) and column temperature (60 °C, 70 °C, and 80 °C). The selection of the optimal method is based on the evaluation of system suitability test parameters. The optimal chromatography conditions in this study were to use a Hi-Plex Ca column (300 mm x 7.7 mm) with a 100% water mobile phase, column temperature of 80 °C, flow rate of 0.6 mL/min, and refractive index detector. The optimum method used was evaluated using analysis method parameters including accuracy, precision (repeatability and intermediate precision), limit of detection (LoD), limit of quantification (LoQ), linearity, range, and stability. The results showed an accurate and precise method as indicated by an average recovery value of 99.67 % and an RSD value of $< \frac{2}{3} \times \text{CV Horwitz (\%)}$ and the intermediate precision was not significantly different ($p\text{-value} > 0.05$). This method produces good linearity with an R^2 value of 0.999 in the range of 2000 - 10000 mg/L. The LoD value is 95 mg/L and the LoQ value is 125 mg/L. In the stability test, the method provides good precision and accuracy (good stability). The results of the application of the optimized and validated method from testing 6 fish medicine samples have met the recovery value requirements.

Keywords: inositol, lactose, HPLC, method validation, determination of levels