INTISARI

Biotin (BI) dan kalsium pantotenat (KP) merupakan bahan aktif yang umum digunakan dalam formula obat ikan sediaan premiks untuk mendukung metabolisme dan pertumbuhan ikan. Sebelum obat ikan diedarkan, perlu dilakukan pengujian mutu untuk menjamin kualitasnya. Laboratorium Obat dan Residu BPKIL Serang menggunakan metode pengujian HPLC untuk penetapan kadar BI dan KP dalam sediaan premiks secara sendiri-sendiri. Namun, penetapan kadar secara simultan belum pernah dilakukan sehingga diperlukan pengembangan, optimasi, dan validasi metode guna mendapatkan hasil yang akurat dan presisi.

Pengembangan metode dilakukan berdasarkan metode analisis *dexpanthenol* oleh Mahboubi (2019), kemudian dievaluasi dari segi keandalan dan efisiensi, dioptimasi dengan variasi pH buffer, temperatur kolom, dan komposisi fase gerak, serta divalidasi. Parameter pengembangan dan optimasi metode meliputi parameter *system suitability* yang mencakup jumlah lempeng teoritis, resolusi, faktor *tailing,* dan *repeatability* waktu retensi. Parameter validasi metode meliputi spesifisitas, akurasi, presisi (*repeatability* dan *intermediate precision*), sensitivitas (LOD dan LOQ) serta linearitas dan rentang.

Kondisi optimum diperoleh dengan kolom C18 (5 µm, 250 mm × 4,6 mm), detektor UV-Vis pada panjang gelombang 205 nm, temperatur kolom 50°C, laju alir 1 mL/menit, volume injeksi 50 µL, serta fase gerak buffer fosfat pH 3,5 dan metanol dengan perbandingan 7:3 v/v. Selanjutnya, seluruh parameter uji kesesuaian sistem terhadap BI dan KP secara simultan memenuhi standar penerimaan. Hasil validasi metode menunjukkan seluruh parameter telah memenuhi standar penerimaan yaitu akurasi (*Average recovery* BI 99,275-100,551% , KP 97,319-99,994%), presisi (RSD BI 0,278-0,752, KP 0,508-0,696), *intermediate precision* (*sig*. (2-*tailed*) > 0,05), spesifisitas (tidak terdapat gangguan), LOD (BI 0,417 mg/L, KP 0,404 mg/L), LOQ (BI2,083 mg/L, KP 1,516 mg/L), serta linearitas (BI r = 0.9997, KP r = 0.9998) pada rentang 50-150% baik pada BI maupun KP. Metode yang dikembangkan kemudian diaplikasikan pada penetapan kadar BI dan KP secara simultan dalam premiks obat ikan untuk uji mutu obat ikan berdasarkan peraturan yang berlaku.

Kata kunci : biotin, kalsium pantotenat, validasi, HPLC

ABSTRACT

Biotin (BI) and calcium pantothenate (KP) are active ingredients commonly used in premix fish medicine formulations to support fish metabolism and optimal growth. Before distribution, fish medicines must undergo quality testing to ensure their quality. The Laboratory of Drugs and Residues at BPKIL Serang uses HPLC testing methods to determine the content of BI and KP in premix formulations separately. However, simultaneous determination has not yet been conducted, thus the development, optimization, and validation of a method are required to obtain accurate and precise results.

The method development was based on the analysis method of dexpanthenol by Mahboubi (2019), followed by evaluation of reliability and efficiency, optimization with variations in buffer pH, column temperature, and mobile phase composition, and subsequent validation. The parameters for method development and optimization included system suitability parameters, such as the number of theoretical plates, resolution, tailing factor, and retention time repeatability. Method validation parameters included specificity, accuracy, precision (repeatability and intermediate precision), sensitivity (LOD and LOQ), linearity, and range.

The optimum conditions were obtained using a C18 column (5 μm, 250 mm × 4.6 mm), UV-Vis detector at a wavelength of 205 nm, column temperature of 50°C, flow rate of 1 mL/min, injection volume of 50 μL, and a mobile phase of phosphate buffer pH 3.5 and methanol in a 7:3 v/v ratio. All system suitability test parameters for simultaneous determination of BI and KP met the acceptance criteria. Method validation results showed that all parameters complied with the acceptance standards: accuracy (average recovery of BI 99.275–100.551%, KP 97.319–99.994%), precision (BI RSD 0.278–0.752, KP 0.508–0.696), intermediate precision (sig. (2-tailed) > 0.05), specificity (no interference), LOD (BI 0.417 mg/L, KP 0.404 mg/L), LOQ (BI 2.083 mg/L, KP 1.516 mg/L), and linearity (BI r = 0.9997, KP r = 0.9998) within the range of 50–150% for both BI and KP. The developed method was then applied for simultaneous determination of BI and KP in fish medicine premix for quality testing in accordance with applicable regulations.

Keywords: biotin, calcium pantothenate, validation, HPLC