

KAJIAN KANDUNGAN ASAM FOLAT PADA OBAT IKAN SEDIAAN PREMIKS MENGGUNAKAN METODE *HIGH PERFORMANCE LIQUID* *CHROMATOGRAPHY*

INTISARI

Asam folat (B9) merupakan vitamin larut air yang berperan sebagai substrat pada sejumlah reaksi enzimatik yang terlibat dalam sintesis asam amino dan metabolisme protein. Suplementasi asam folat pada ikan dalam bentuk sediaan obat ikan diperlukan untuk memaksimalkan pertumbuhan dan imunitas sehingga dapat meningkatkan kualitas produksi perikanan budidaya. Metode standar untuk penetapan kadar asam folat pada obat ikan tidak dapat digunakan untuk menetapkan vitamin larut air lainnya secara simultan sehingga dikembangkan metode analisis yang dapat melakukan pemisahan campuran vitamin larut air dan menetapkan kadar asam folat dalam obat ikan melalui optimasi dan validasi metode analisis.

Optimasi metode analisis penetapan kadar asam folat dilakukan dengan RP-HPLC dan gradien fase gerak yaitu campuran NaH_2PO_4 , *hexane-1-sulfonic acid sodium salt*, air, metanol, dan *acetonitrile*, pada panjang gelombang 282 nm. Optimasi dan kemudian validasi metode analisis dilakukan dengan *spiking* standar asam folat pada matriks berupa laktosa. Metode analisis divalidasi dan diuji kesesuaian sistemnya menggunakan acuan USP dan *guideline* ICH.

Hasil analisis parameter validasi meliputi akurasi, presisi, spesifisitas, LOD, LOQ, dan linearitas telah semua memenuhi standar penerimaan yaitu akurasi dengan *recovery* 101,12% (90-107%), presisi dengan $\% \text{RSD} < \frac{2}{3} \times \% \text{CV}$ Horwitz, spesifisitas dengan tidak terdapatnya gangguan saat pengukuran blanko, LOD dan LOQ yaitu 2 mg/L dan 7 mg/L, serta linearitas ditunjukkan melalui koefisien korelasi (r) 0,9994. Dilakukan uji kesesuaian sistem dengan parameter resolusi, *repeatability*, *theoretical plates*, *tailing factor*, faktor kapasitas, dan *signal-to-noise ratio* yang semua juga telah memenuhi standar penerimaan. Metode yang dikembangkan kemudian diterapkan untuk uji penetapan kadar asam folat dalam sampel obat ikan dengan hasil memenuhi standar mutu obat ikan berdasarkan peraturan yang berlaku.

Kata kunci: asam folat, premiks, *high performance liquid chromatography*, validasi

***STUDY OF FOLIC ACID CONTENT IN FISH MEDICINE PREMIX
PREPARATIONS USING THE HIGH PERFORMANCE LIQUID
CHROMATOGRAPHY METHOD***

ABSTRACT

Folic acid (B9) is a water-soluble vitamin that acts as a substrate in a number of enzymatic reactions involved in amino acid synthesis and protein metabolism. Supplementation of folic acid in fish in the form of fish medicine is needed to maximize growth and immunity so that it can improve the quality of aquaculture production. The standard method for determining folic acid levels in fish medicine cannot be used to determine other water-soluble vitamins simultaneously, therefore an analysis method that can separate a mixture of water-soluble vitamins and determine folic acid levels in fish medicine was developed through optimization and validation of the analysis method.

Optimization of the analysis method for determining folic acid levels was carried out using RP-HPLC and a gradient mobile phase, namely a mixture of NaH₂PO₄, hexane-1-sulfonic acid sodium salt, water, methanol, and acetonitrile, at a wavelength of 282 nm. Optimization and then validation of the analysis method were carried out by spiking the folic acid standard on a lactose matrix. The analysis method was validated and tested for system suitability using USP references and ICH guidelines.

The results of the validation parameter analysis including accuracy, precision, specificity, LOD, LOQ, and linearity have all met the acceptance standards, namely accuracy with recovery of 101.12% (90-107%), precision with $\% RSD < \frac{2}{3} \times \% CV$ Horwitz, specificity with no interference during blank measurement, LOD and LOQ of 2 mg/L and 7 mg/L, and linearity is shown through a correlation coefficient (r) of 0.9994. A system suitability test was carried out with resolution parameters, repeatability, theoretical plates, tailing factor, capacity factor, and signal-to-noise ratio, all of which have also met the acceptance standards. The developed method was then applied to the test for determining folic acid levels in fish medicine samples with the results meeting the quality standards for fish medicine based on applicable regulations.

Keywords: folic acid, premix, high performance liquid chromatography, validation