建立貓ω型干擾素含量檢驗分析技術

動物用藥品檢定分所

林文華 副研究員兼系主任

摘要

干擾素(Interferon，IFN)是一種廣效性抗病毒、抗腫瘤及具有免疫調節作用之活性醣蛋白(Glycoproteins)。第一個動物用干擾素，為1994 年由日本東麗(Toray)公司利用家蠶核多角體病毒(Nucleopolyhedrovirus，NPV)為載體，研發治療貓卡希利病毒(Calicivirus)干擾素產品。為因應未來動物用干擾素產品上市需求，以貓ω型干擾素(Feline-Interferon Omega，FeIFN-ω)為例，建立動物用干擾素含量試驗分析方法。含量活性試驗方法，以 *Felis catus* 9 (Fc9)細胞株與干擾素感作後，加入水泡性口炎病毒(Vesicular stomatitis virus)，藉由干擾素於細胞內抑制病毒生長，以半數存活細胞有效劑量(concentration for 50% of maximal effect，EC50)計算含量活性，並進行含量活性試驗方法確效，其重複性相對標準偏差為 6.39%，精確度相對標準偏差為 3.18%，符合生物確效標準。另建立高效能液相層析含量試驗分析方法，樣品與標準品以去離子水稀釋與過濾後，以 Vydac® C4 (5μm，內徑 4.6mm×25cm)液相層析管柱，0.1%三氟乙酸溶液及 0.1%三氟乙酸/90%乙腈溶液作為移動相梯度條件，依流速

0.5mL/min 以高效液相層析儀配合光二極體陣列偵測器於波長 214 nm 進行檢測，其檢量線線性範圍為 14-72μg/mL，線性迴歸決定係數(R^2)為 0.9952，重複性相對標準偏差為 1.91%，精確度相對標準偏差為 2.92%，符合分析確效標準。

Development of Methods for the Analysis of Feline-Interferon Omega Activity, Quantity and Quality

Wen-Hua Lin

Abstract

Type I Interferons (IFNs) are well known cytokines that function as key components of the host immune response with broad and strong antiviral, antitumor, and immunomodulatory effects. The first animal interferon product was developed by Toray Industries Inc. of Japan in 1994 using nucleopolyhedrovirus (NPV) as a carrier to treat feline calicivirus. Since market demand for interferon products for the treatment of animals will increase in the future, we used Feline-Interferon Omega (FeIFN-ω) as a proof-of-concept example for the establishment of interferon analysis techniques and protocols. To test for interferon activity, vesicular stomatitis virus was added to *Felis catus* 9 (Fc9) cell lines induced with FeIFN-ω, . The interferon suppressed viral growth in the cell lines and interferon activity was calculated based on half of the maximal effective dose (EC50). For the calculated EC50, the relative standard deviation of repeatability was 6.39% and the relative standard deviation of accuracy was 3.18%, which meet the standards for biological validation. In addition, we developed a high-performance liquid chromatography analytical method for the determination of was established for the quantification of inteferons as well as for the determination of sample contents (closely related protein variants, degradation products, and other high-molecular weight substances). In this method, samples and standards are diluted and filtered by deionized water, loaded onto a Vydac® C4 (5μm, inner diameter 4.6×250mm) liquid chromatography column, with a 0.1% trifluoroacetic acid/90% acetonitrile solution used as a mobile phase gradient, and a photodiode array detector was used for detection at a wavelength of 214 nm and a flow rate of 0.5mL/min. The linear range for the analysis is 14 - 72μg/mL, (R^2 = 0.9952), while the relative standard deviation of repeatability and the relative standard deviation of precision were 1.91% and2.92%, respectively, which meet the standards for analytical validation.