114.02.08 修

建立動物用單株抗體藥物有效性檢驗分析技術

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摘要

單株抗體藥物是一種可針對特定抗原作用，利用抗體和抗原的高度專一性，可治療多種疾病，如癌症、自體免疫性疾病及病毒感染等。第一個人用單株抗體獲得美國食品藥物管理局(FDA)核准上市為Muromommb-CD3（Orthoclone OK3®），於 1986 年在美國正式推出，主要用於治療腎移植患者之急性排斥反應。其後有眾多針對癌症及自體免疫疾病治療之單株抗體藥物上市，現今單株抗體已成為人類疾病治療的重要選擇。因應未來動物用單株抗體藥物上市需求，建立動物用單株抗體藥物有效性檢驗分析技術。單株抗體有效性檢驗包括含量、純度、鑑別及效價。含量檢驗以紫外光光譜法(ultraviolet spectroscopy)，利用蛋白質於波長 280 nm 附近有最大吸光度，計算蛋白質含量。檢測結果各濃度樣品重複性相對標準偏差(relative standard deviation, RSD)為 1.3-5.2%。純度以分子篩高效液相層析法(size-exclusion high-performance liquid chromatography，SE-HPLC)檢驗，透過分子大小和形狀的差異來分離單株抗體中的免疫球蛋白組分，計算樣品中聚集體(Aggregates)、單體(Monomers)及片段

(Fragments)的比例。檢驗結果標準品重複性相對標準偏差(RSD)為1.92%。鑑別及效價以酵素結合免疫分析法(enzyme-linked immunosorbent assay, ELISA)檢驗技術，基於抗原及抗體相互作用原理鑑定並量化單株抗體藥物效價。檢驗結果標準品檢量線線性範圍為0.78-4.70ng/mL，線性迴歸決定係數(R^2)為 0.9997，各濃度樣品重複性相對標準偏差(RSD)為 0.79-6.02%。

114.02.08 修

Establishment of Analytical Techniques for Evaluating the Efficacy of Monoclonal Antibody Drugs in Animals

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Abstract

Monoclonal antibody (mAb) drugs are highly specific therapeutic agents that act on particular antigens through precise antibody-antigen interactions. These drugs have been employed in the treatment of diverse diseases, including cancer, autoimmune disorders, and viral infections. The Muromonab-CD3 (Orthoclone OKT3®) was the first monoclonal antibody drug which had been approved by the U.S. Food and Drug Administration (FDA) for clinical use in humans, then that became available in 1986 as a treatment for acute rejection in kidney transplant patients. Following this milestone, numerous monoclonal antibodies targeting cancers and autoimmune diseases have been introduced, and these therapies have now become integral to treatment options for human diseases. To meet the growing demand for veterinary monoclonal antibody drugs, analytical methods were developed to assess their efficacy in animal applications. These evaluations encompass the determination of quantity, purity, identity, and potency. Quantity was determined by using ultraviolet (UV) spectroscopy, leveraging the characteristic maximum absorbance of proteins near 280 nm. The repeatability of the measurements was evaluated across various concentrations, with relative standard deviation (RSD) values ranging from 1.3% to 5.2%. Purity was assessed by using size-exclusion high-performance liquid chromatography (SE-HPLC), which separates immunoglobulin components based on differences in molecular size and shape. The proportions of aggregates, monomers, and fragments were calculated, and the RSD for repeatability of the reference standard was determined to be 1.92%. The identity and potency of the monoclonal antibody were analyzed by using enzyme-linked immunosorbent assay (ELISA), a technique grounded in the interaction between antigens and antibodies. The calibration curve demonstrated a linear range of 0.78–4.70 ng/mL, with a regression coefficient (R^2) of 0.9997. The repeatability of measurements across different concentrations showed RSD values ranging from 0.79% to 6.02%.