建立動物用疫苗防腐劑硫柳汞及酚含量分析方法確效

動物用藥品檢定分所

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摘要

動物用疫苗在製程與多劑量注射過程中為防止微生物污染，允許加入適量防腐劑，由於防腐劑具有毒性，在各國藥典與法規均規範最低有效安全濃度。依據「動物用藥品檢驗標準」第三章各節檢驗標準，不同種類動物用疫苗規範硫柳汞及酚防腐劑含有量限量值分別為 0.02-0.01 %以下及 0.5-0.1 %以下。現行藥典與公定書規範藥品中防腐劑硫柳汞及酚檢驗方法，以滴定法及紫外光/可見光光譜儀為主，但以上方法對於複雜基質疫苗缺少特異性，透過高效能液相層析儀對於基質與分析物有效分離作用，可提高檢驗精確度。本研究分析方法開發建立，依據國際動物用藥品檢驗登記技術資料協和會(International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products, VICH) GL02 分析確效作業指導手冊進行分析方法確效。在動物用疫苗前處理液液萃取方法方面，酚萃取條件以四氫呋喃做為萃取液，而硫柳汞萃取條件以二氯甲烷溶解脂溶性物質後，以去離子水進行萃取。在分析條件方面，酚分析以乙腈-去離子水(55:45, v/v)為移動相，分析管柱 Waters Symmetry® C18 (4.6 mm× 25 0mm, 5 μm)，偵測波長 217 nm 進行分析；硫柳汞分

析以甲醇-0.02 M 醋酸銨 (35:65, v/v)為移動相，分析管柱 Hypersil™ GOLD (4.6 mm× 250 mm, 5 μm)，偵測波長 215 nm 進行分析。分析方法確效執行項目包括準確度、精密度、選擇性、耐變性、最低檢測濃度、最低定量濃度、線性及範圍等項目，其結果均符合指導手冊標準要求，故此檢驗技術可應用於動物用疫苗國家檢定上，以確保國內疫苗品質。

Establishment of Analytical Methods Validation for Thimerosal and Phenol Content in Preservatives for Animal Vaccines

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Abstract

In order to prevent microbial contamination during the manufacturing process and multi-dose injection, it is permitted to add appropriate amounts of preservatives to vaccines for animal use. Since the preservatives are toxic, the pharmacopoeia and regulations of each country specify the minimum effective safety concentration. According to the test standards established in Chapter 3 of the Test Standards for Veterinary Drugs, the preservative content limits for thimerosal and phenol in different types of animal vaccines are 0.02-0.01 % or less and 0.5-0.1% or less, respectively. In the current pharmacopoeia and official compendium, the methods for testing the preservatives thimerosal and phenol mainly include titration and UV/VIS spectrophotometer. However, these methods lack specificity for complex matrix vaccines. By using high performance liquid chromatography (HPLC) to effectively separate the matrix from the analyte, the accuracy of the test can be improved. The analytical methods developed and established in this study were validated in accordance with the GL02 Analytical Validation Guideline published by the International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products (VICH). For the liquid extraction method of animal vaccine pretreatment solution, Tetrahydrofuran was used as the extraction solution for phenol extraction, while Dichloromethane was used for thimerosal extraction and deionized water was used for extraction of fat-soluble substances. For phenol analysis, Acetonitrile-deionized water (55:45, v/v) was used as the mobile phase and a Waters Symmetry® C18 (4.6 × 250 mm, 5 μm) column with a detection wavelength of 217 nm was used for analysis. Thimerosal analysis was performed using Methanol-0.02 M ammonium acetate (35:65, v/v) as mobile phase and Hypersil™ GOLD (4.6 × 250 mm, 5 μm) column with a detection wavelength of 215 nm. The items required for validation of analytical methods include accuracy, precision, selectivity, robustness, limit of detection (LOD), limit of quantification (LOQ), linearity, and range. The results are in accordance with the standard requirements of the guideline, and this testing technique can be applied to the national validation of animal vaccines to ensure the quality of animal vaccines.