Acta Poloniae Pharmaceutica – Drug Research, Vol. 69 No. 6 pp. 1364–1367, 2012 ISSN 0001-6837
Polish Pharmaceutical Society

KARL FISHER DETERMINATION OF RESIDUAL MOISTURE IN VETERINARY VACCINES – PRACTICAL IMPLEMENTATION IN MARKET MONITORING

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Abstract: Residual moisture content plays a significant role in assessing the stability of veterinary vaccines. Analysis of water amount is often a critical parameter, which determines the quality of product, its appearance as well as the expiration date. The aim of the study was to validate a coulometric Karl Fisher method for practical use in the national monitoring of veterinary vaccines market. Immunological veterinary medicinal product (ivmp) for three different animal species – cats, dogs and rabbits – were used. Automated coulometric analysis in chamber without diaphragm was used, as well as a solution for titration, which was a mixture of diethanolamine, imidazole, methanol and sulfur dioxide. The weight of a single sample was 15–100 mg. The most important concern was optimization of the way of transferring a vaccine sample into titration cell, so that atmospheric moisture would not affect baseline drift and repeatability of the results. Humidity level in lyophilized biopharmaceuticals was validated in accordance with the guidelines. The method was linear in the range of one to five percent of water content with $R^2 = 0.9998$. Repeatability for different sample types was found to be not higher than CV% = 5.9. The method was used for vaccines market monitoring in 2010 and 2011. Thirteen vaccines from the market were tested and all were found to be compliant with official EU guidelines.

Keywords: Karl Fisher, coulometric method, veterinary vaccines, monitoring

Vaccines are often produced as lyophilizates. This ensures proper stability and makes the product more resistant for transport and storage conditions (1), prevent loss of potency and decomposition of vaccines (2). Lyophilization is dehydration process, also known as freeze-drying, in which liquid material is frozen and then water sublimates in a vacuum. Therefore, under conditions of low pressure and temperature, water is removed from vaccines.

However, small amount of residual water is always present in freeze-dried vaccines. It is one of the most important parameters of vaccines lyophilization cycle (1, 3). Residual moisture content (RMC) of biopharmaceutical products depends also on ways of storage (2). In accordance with the guidelines, humidity level in lyophilized biopharmaceuticals should be 1 to 5%. It is tested in vaccines quality monitoring (4–7). RMC is a significant factor influencing stability, temperature storage conditions, long-term shelf-life and appearance of lyophilized bioproducts (1, 8, 9).

Too high residual moisture value can cause reduction of potency, e.g., degradation index of lysozyme increases at high RMC (8). It may also cause chemical stability decrease and an increase in protein's flexibility and degradation (2, 10). On the other hand, too low RMC can be a reason of bioproducts quality changes, e.g., converse antigen structure. Results of this kind of modifications are protein complex formation and antigens release profile changes, which are hard to predict (3). Proteins in solution are surrounded by multilayer of water molecules. During lyophilization process, bulk water sublimes first leaving monolayer of residual water. Thanks to this, it is possible to dry heat-labile products to low RMC under moderate conditions of temperature (2).

There are many different methods for determination of residual moisture in dried biological products, e.g., loss on drying as well as thermogravimetry, gas chromatography and near infrared spectroscopy methods. However, the most widespread

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technique of water analysis in the world is Karl Fisher titration method. It is fast repetitious method, applied in wide range of concentrations and materials and can be easily automated (2). It is the method of choice in worlds pharmacopoeias and, for example, recommended by American Society for Testing and Materials.

The aim of this study was to optimize and validate Karl Fisher method of residual moisture content determination in veterinary vaccines. The presented physicochemical method was used in assessing the quality of these products in monitoring of the Polish market in 2010 and 2011.

MATERIALS AND METHODS

Materials

Samples of three different immunological veterinary medicinal product (ivmp) were used for validation purposes: (I) for cats: anti-rabies, against calcivirus, equine viral bovine rhinotracheitis and panleucopenia; (II) for rabbits: against myxomatosis and hemorrhagic disease; (III) for dogs: against disempter and parvovirus. Analysis of certified reference material was performed to assure proper quality of results. One mg/g (0.1%) liquid water standard (Merck) for coulometric titration method and Hydranal Coulomat AD (Sigma Aldrich), which is a mixture of diethanolamine, imidazole, methanol and sulfur dioxide, were used for titration.

Apparatus

Measurements were performed on C30 Compact Karl Fischer Coulometer, with cell without diaphragm (Mettler Toledo). To determine mass of analyzed lyophilizates, analytical balance (Mettler Toledo, XS205DU) was used.

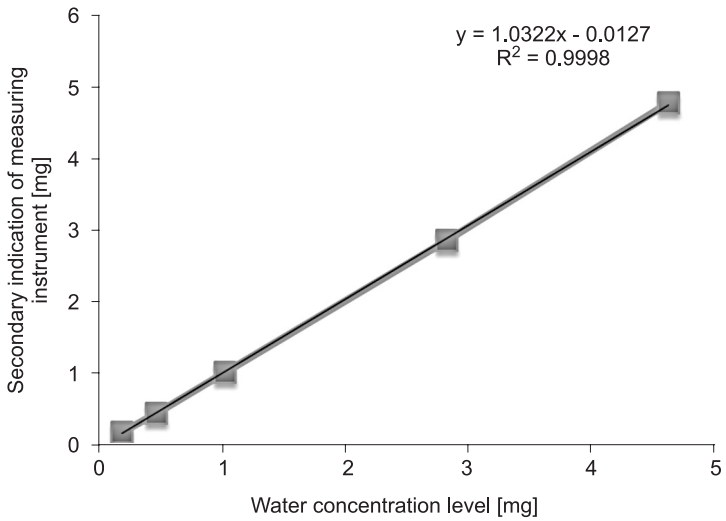

Figure 1: Linearity of Karl Fisher method

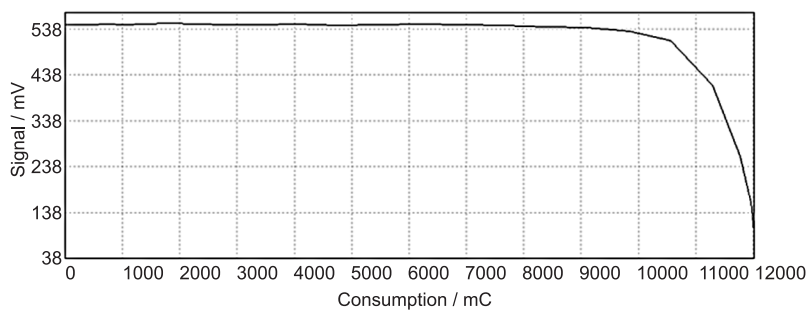

Figure 2: Correlation of electric charge (mC) consumption and voltage signal (mV) to end point of titration of vaccines

Table 1. Analytical and validation parameters of Karl Fisher method. Results of analysis for three different vaccines: (I) for cats: antirabies, against calcivirus, equine viral bovine rhinotracheitis and panleucopenia; (II) for rabbits: against myxomatosis and hemorrhagic disease; (III) for dogs: against disempter and parvovirus.

	METHOD ANALYTICAL PARAMETERS					
METHOD	Coulometric titration					
ANALYSIS TIME	5 minutes					
MAX. DRIFT	25 µg/min					
ENVIRONMENTAL CONDITIONS	18 – 25°C (temperature) 20 – 80% (humidity)					
REAGENTS	methanol: imidazole: iodine: sulfur dioxide					
SAMPLE MASS	15 – 100 mg					
	METHOD VALIDATION PARAMETERS					
	REPEATABILITY			**PRECISION**		
	I	II	III	II	II	II
$\bar{x}$	3.487	3.267	2.267	3.267	3.099	3.086
s	0.204	0.078	0.130	0.078	0.135	0.138
CV	5.858	2.399	5.736	2.399	4.363	4.46
RANGE (%)	1 – 5					
UNCERTAINTY	≤ 2					

Procedure

Plastic tubes and plugs were inserted to desiccator for minimum of one hour. A vaccine ampoule was mechanically broken, lyophilizate transferred to a test tube and closed with stopper. Tube was weighted and balance tared. Prepared sample was then introduced to titration cell. Special care was taken to tightly seal the cell inlet after sample transfer. The time between opening vial of lyophilizate and introduction of sample to measuring cell was set to be no longer than 90 s.

RESULTS AND DISCUSSION

The main challenge in this research was to eliminate an atmospheric moisture effect during sample handling. This stage has significant impact on coulometric titration results, especially when small amount of sample and low residual moisture content are taken into consideration. At the beginning of method optimization, exact amount of anhydrous methanol was added to lyophilizate in an original container and the sample was left to dissolve. Known amount of this solution was introduced to titration cell. Water content of methanol was assayed as blank. After some trials it was noted that not all freeze-dried products dissolve well in methanol, which makes the results unrepeatable.

The solution of this problem was a method based on mechanical breakdown of final container and transferring freeze-dried product to a sealed plastic tube. It was crucial to standardize constant time needed for extracting from container, weighing and finally introducing the sample to Karl Fischer titration cell. The best results were obtained when this time wasn't longer than 90 s.

The value of maximum drift was also optimized. It was determined to be less than 5 µg/min just after filling titration cell with reagent and should not exceed 25 µg/min during the analysis.

An important analytical parameter is the solution mixing time after sample introduction during Karl Fisher reaction. According to the Pharmacopoeia, coulometric titration is quantitative reaction of water with sulfur dioxide and iodine in an anhydrous medium using the principle of adequate buffering capacity. During this reaction, all water is consumed and the testing solution contains small excess of iodine, which is detected thanks to electrode polarization. When solution in measuring cell becomes yellow, it is a moment of the end point of titration (Fig. 2). In accordance with Faraday's law, an amount of generated iodine is directly proportional to amount of electric charge, which is again proportional to the amount of water in tested substance. And this mathematical relationship is simple: 10.712 mC = 1 µg of water (11).

Different times of sample mixing in the titration cell were tested. Sixty seconds time allowed to obtain reproducible results for water standard.

However, lyophylized vaccines, being complex material, are difficult to dissolve. On the basis of experiments it was determined that maximum time needed to dissolve the sample is 5 min. Thus, in the final procedure, mixing time for the liquid and vaccines was standardized to be 5 min.

Low values of residual moisture in vaccines (1–5%) was the reason to use coulometric titrator. Due to a lack of solid residual moisture reference material, in investigated range of water content liquid form of standard was used. Optimized method was validated on three different types of vaccines. The method is linear in the given range with R^2 = 0.9998 (Fig. 1). All validation parameters are shown in Table 1.

CONCLUSIONS

Residual moisture is one of the most important quality parameters of lyophilized vaccines and is officially controlled in Poland during the market monitoring research. The above-described method was set up, validated and used in this monitoring in years 2010 and 2011. Test specimens were randomly sampled from the market by provincial officers of pharmacovigilance from various pharmaceutical wholesalers across the country. Bioproducts were intended to different animal species vaccination – pigs, cattle, poultry, cats, dogs and rabbits.

A total of thirteen vaccines were analyzed for residual moisture content. Based on these analyses, the average residual moisture level was 2.16%. All results of water determination were found to be compliant with official EU guidelines.

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