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THERMOGRAVIMETRY AND VAPOR PRESSURE MOISTURE
Applications to determination of residual moisture in BCG vaccine

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Thermogravimetry (TG), thermogravimetry/mass spectrometry (TG/MS), and loss-on-drying methodology are used to provide residual moisture results for freeze-dried biological products regulated by the US Food and Drug Administration. Residual moisture specifications must be met in order to ensure freeze-dried biological product potency and stability throughout the licensed product's shelf life. TG, TG/MS, loss-on-drying and vapor pressure moisture measurements are compared for a BCG Vaccine. Comparisons are made between residual moisture data for the freeze-dried cake and vapor pressure moisture determinations in the space above the freeze-dried cake in the final container. Vapor pressure moisture precision data is presented for α-interferon and BCG vaccine. Impact of residual moisture and vapor pressure moisture upon product stability is presented.

Keywords: *BCG vaccine, lyophilization, residual moisture, thermogravimetry*

Introduction

This paper describes the TG, TG/MS, and loss-on-drying residual moisture results for BCG vaccine. These methods measure residual moisture in the freeze-dried vaccine cake in the final container vial. The accurate determination of residual moisture in freeze-dried biological products is necessary to meet regulatory requirements [1–3]. Vapor pressure moisture measurements (VPM) in the space above the BCG vaccine cake in the final container vial are also described and compared to the residual moisture values. Stability data is accumulated and results for biological products in glass vials with elastomeric container closures are studied. BCG vaccine (freeze-dried) for intracutaneous administration is made from a culture of an attenuated strain of living bovine tubercle bacillus (Bacilllus Calmette–Guerin) [4]. The manufacturing and testing procedures for this vaccine comply with WHO recommendations [5]. This product is used for the vaccination of tuberculin negative individuals to prevent tuberculosis [6]. The data presented and conclusions made are research findings.

Experimental

Samples and control materials

Samples of BCG vaccine (BCG live) were obtained from Aventis Pasteur, Ltd. (Toronto, Canada). This freeze-dried product has a shelf life of 6 months at 2–8°C.

Samples of α-interferon (Interferon Alfa-2b, Recombinant) were obtained from Schering Corporation (Kenilworth, NJ). Under certain conditions the shelf life of this freeze-dried product was stated to be a maximum of three years at 2–8°C.

Sodium tartrate dihydrate was obtained from Fisher Scientific (Fair Lawn, NJ). This was used as a control material for the determination of residual moisture. The two waters of hydration in this compound were determined to an accuracy of 1.23% [7] using thermogravimetry (TG).

Methods

Loss-on-drying (LOD)

Loss-on-drying, also known as the gravimetric method, measures the maximum loss of mass of a weighed sample equilibrated over anhydrous phosphorus pentoxide at a pressure of not more than one mm of mercury and at a temperature of 20 to 30°C for a length of time that has been established as sufficient to result in a constant mass [8].

Thermogravimetry (TG), thermogravimetry/mass spectrometry (TG/MS)

Thermogravimetric measurements were made with a TA Instruments (Wilmington, DE) Thermal Analyst 51 in a Plexiglass dry box maintained at a low humidity by phosphorus pentoxide. Humidity in the dry box is monitored by a portable hygrometer

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(Bacharach, Pittsburgh, PA). This unit was used with the Thermal Analyst 2200 (TA Instruments) and an IBM Laser Printer 10 by Lexmark.

TG/MS measurements were made with a Netzsch STA 409PC Luxx (Selb, Germany) thermogravimetric analyzer interfaced to a Pfeiffer Vacuum Technology (Asslar, Germany) ThermoStar mass spectrometer.

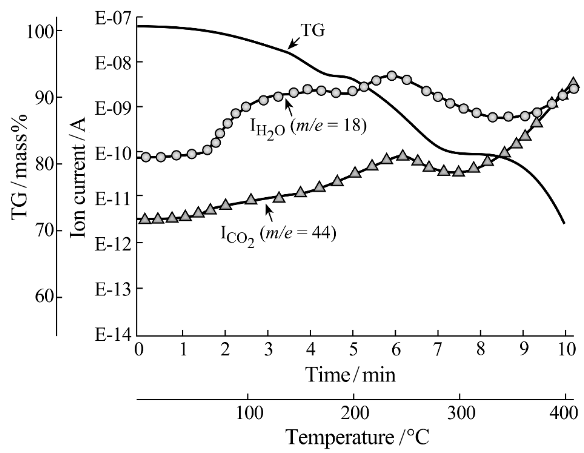

Fig. 1 TG curve and mass spectral ion intensities (I) for H_2O (m/e=18) and CO_2 (m/e=44) *vs*. time and temperature for BCG vaccine

Vapor pressure moisture methodology

Vapor pressure moisture (VPM) measurements were carried out with a vapor pressure moisture instrument devised [9] by Professor Louis Rey and collaborators. This instrument uses an electro-optical dew point measurement to obtain the moisture content in the space above the freeze-dried cake (headspace) in a final container vial. The condensation temperature of the moisture in the head space is determined. The moisture content in micrograms is calculated using the Ideal Gas Law.

Table 1 LOD and TG residual moisture data for freeze dried BCG vaccine

Sample	LOD[a]	TG[b]
	H_2O/mass/mass%	H_2O/mass/mass%
BCG vaccine, Lot A (vial)	1.43	1.30±0.01
BCG vaccine, Lot C (vial)	1.23	1.25±0.01

[a]Loss-on-drying or gravimetric method – pool of 100–200 mg from several sample vials; [b]arithmetic mean and standard deviation of two sample test results

Table 2 Vapor pressure moisture precision data for several lots of α-interferon and BCG vaccine showing repeat assay results on the same vial and different vials

Product type	Lot	Vial	Condensation T/°C	μg H_2O/vial	
				Value	Standard deviation
α-interferon	A	1	–24.3	2.02	±0.04
α-interferon	A	1	–24.6		
α-interferon	B	1	–11.8	6.67	±0.00
α-interferon	B	1	–11.8		
α-interferon	C	1	–15.0	4.76	±0.00
α-interferon	C	1	–15.0		
BCG vaccine	B	1	–42.0	0.24	±0.003
BCG vaccine	B	2	–41.9		

Table 3 Residual moisture and vapor pressure moisture stability data for BCG vaccine and α-interferon lots

Sample	Initial (0 month)		Final (25 month)	
	Residual moisture[a]/%	Vapor pressure moisture[b]/mg H_2O/vial	Residual moisture[a]/%	Vapor pressure moisture[b]/μg H_2O/vial
α-interferon, Lot C (vial)	1.28	4.76	0.78	2.06
BCG vaccine, Lot A (vial)	1.43	0.48	1.37	0.10
BCG vaccine, Lot B (vial)	1.19	0.32	1.00	0.10

[a]Gravimetric (Loss-on-drying) method – each test on a pool of samples from several vials; [b]measurements on one vial

Results and discussion

Figure 1 shows the TG and TG/MS data obtained for BCG vaccine. In the TG/MS data the ion abundances of mass peaks m/e=18 and m/e=44 show that the evolution of water from sample decomposition begins below 100°C.

TG moisture contents of 1.30 and 1.25% (Table 1) , respectively, were calculated for lots A and C of BCG vaccine using the transition indicated by the TG/MS data for residual moisture near 80°C. The TG results for Lot A and Lot C differed from the LOD results by 9.15% and 1.6%, respectively.

Table 2 shows the high precision attainable in measuring both the condensation temperatures and the subsequent calculation of the micrograms of water in the vial headspace by the vapor pressure moisture methodology using repeat assays on the same vial and different vials.

Table 3 shows 25-month stability data for both residual moisture and vapor pressure moisture in two lots of BCG vaccine and one lot of α-interferon. Both of these products are freeze-dried in glass vials with elastomeric container closures. The stability data shows the same trend for both product types, i.e., the cake residual moisture decreases after 25 months and the moisture in the headspace decreases after 25 months. In these cases this could indicate absorption of water by the closure over time indicating changes in moisture content in the various constituents (freeze-dried cake, vial headspace, vial closure) of the final container vial over time. Crist [10] studied lyophilized pharmaceuticals sealed under reduced pressure and illustrated that vial pressure primarily is caused by desorption of water vapor from the stopper into the headspace of the vial. The presence of hydrophilic substances in the freeze-dried cake decreases the rate of pressure rise, and pressure increases faster in smaller vials. Both products listed in Table 3 show a relatively dry environment in the vials with no excessive moisture in the cake or the headspace initially and no excessive moisture migrating from the closure to the cake or headspace over time.

The data in Table 1 shows that comparable results are obtained by both loss-on-drying (gravimetric) and TG methods. The thermogravimetric method has the advantage of requiring only about 5 mg of sample for accurate analysis whereas the loss-on-drying method requires 100 to 200 mg of sample per test. Both methods are used by manufacturers of biological products to determine moisture content. Vapor pressure moisture data provides additional information on the state of dryness of the head space of the final container vial. This data complements residual moisture results obtained for the freeze-dried cake.

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