الهيئة العامة للغذاء والدواء
Saudi Food & Drug Authority

Testing of Residual Formaldehyde

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Saudi Food & Drug Authority
Drug Sector

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Saudi Food and Drug Authority

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Document Control

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1. INTRODUCTION

1.1Objective of Guideline

Many inactivated veterinary vaccines, particularly bacterins contain residual levels of formaldehyde. It is important to determine the residual level of formaldehyde to:

a) help assure product safety,
b) assure the product will not inactivate other products used in combination,
c) help assure the product remains active throughout its shelf life, and
d) assure any clostridial toxoids will be antigenic and safe.

This document provides a guideline for the general requirements for residual formaldehyde testing. The guideline leaves the flexibility for other testing methods based on the specific scientific situations or characteristics of the target material. These variations must be stated in the manufacturers production method and include equivalence data.

1.2 Scope of Guideline

This guideline applies to final product testing for all formaldehyde-containing new veterinary vaccines.

1.3 Background

A number of assays are available for the determination of residual free formaldehyde in inactivated vaccines, including acetyl acetone titration, ferric chloride titration and the basic fuchsin test. The ferric chloride method was selected since it has shown to be compatible with products neutralized with sodium bisulphite[1].
Residual formaldehyde will be reported as g/L, a conversion table is provided as Appendix1.

1.4 General Principle

Total formaldehyde is determined based on the reaction of formaldehyde with Methylbenzothiazolone hydrazone hydrochloride (MBTH). The method involves (a) the combination of MBTH and formaldehyde to give one product, (b) the oxidation of excess MBTH to give another product and (c) the combination of these two to give a blue chromophore which is measured at 628 nm.[2]

2. FERRIC CHLORIDE METHOD

1. Reagents

1.1 Ferric chloride-sulphamic acid reagent. A solution containing 10 g/L of ferric chloride and 16 g/L of sulphamic acid.

1.2 Methylbenzothiazolone hydrazone hydrochloride reagent. (MW 233.7). [CAS 149022-15-1]. 3-Methylbenzothiazol-2(3H)one hydrazone hydrochloride monohydrate. An almost white or yellowish crystalline powder. mp: about 270 °C. A solution containing 0.5 g/L L (WARNING: This solution is not stable and should be prepare fresh daily).

1.3 Suitability for determination of aldehydes. To 2 ml of aldehyde-free methanol add 60 µl of a 1 g/L solution of propionaldehyde in aldehyde-free methanol and 5 ml of a 4 g/L solution of methylbenzothiazolone hydrazone hydrochloride. Mix, allow to stand for 30 min. Prepare a blank omitting the propionaldehyde solution. Add 25.0 ml of a 2 g/L solution of ferric chloride to the test solution and to the blank, dilute to 100.0 ml with acetone R and mix. Measure absorbance of the test solution on a spectrophotometer at 660 nm in a 1 cm cell using the blank as compensation liquid. The absorbance of the test solution must be greater than or equal to 0.62 absorbance units.

1.4 Formaldehyde solution, containing not less than 34.5 percent w/v and not more than 38.0 percent w/v of formaldehyde (CH2O)

1.5 isopropyl myristate, analytical grade

1.6 hydrochloric acid (1 M), analytical grade

1.7 chloroform, analytical grade

1.8 sodium chloride (9 g/L and 100 g/L aqueous solutions), analytical grade

1.9 polysorbate 20, analytical grade

2. Sample and Standards Preparation

2.1 Prepare formaldehyde standards of 0.25, 0.50, 1.00 and 2.00 g/L by diluting formaldehyde solution (1.3) with water in suitable volumetric flasks.

2.2 If vaccine to be examined is an oil emulsion, the emulsion should be broken by a suitable method. The formaldehyde concentration in the aqueous phase should be measured. The following separation techniques have been shown to be appropriate.

A. Add 1.00 ml of vaccine to 1.0 ml of isopropyl myristate and mix. To the mixture, add 1.3 ml of 1 M hydrochloric acid, 2.0 ml of chloroform and 2.7 ml of 9 g/L sodium chloride. Mix thoroughly. Centrifuge at 15,000 g for 60 min. Transfer the aqueous phase to a 10 ml volumetric flask and dilute to volume with water. Use the diluted aqueous phase for the test for formaldehyde. If this procedure described fails to separate the aqueous phase, add 100 g/L of polysorbate 20 to the sodium chloride solution and repeat the procedure, but centrifuge at 22,500 g.

B. Add 1.00 ml of vaccine to 1.0 ml of a 100 g/L solution of sodium chloride and mix. Centrifuge at 1000 g for 15 minutes. Transfer the aqueous phase to a 10 ml volumetric flask and dilute to volume with water. Use the diluted aqueous phase for the test for formaldehyde.

C. Add 1.00 ml of vaccine to 2.0 ml of a 100 g/L solution of sodium chloride and 3.0 ml of chloroform and mix. Centrifuge at 1000 g for 5 minutes. Transfer the aqueous phase to a 10 ml volumetric flask and dilute to volume with water. Use the diluted aqueous phase for the test for formaldehyde.

NOTE: Volumes used for breaking emulsions are for the purpose of illustration. Volumes may differ subject to proportional adjustment of the volumes of other reagents used in the extraction process.

3. Test Method

3.1 To 0.50 ml of a 1 in 200 dilution of the vaccine to be examined (if emulsion, use 0.50 ml of a 1 in 20 dilution of the diluted aqueous phase), and to 0.50 ml of 1 in 200 dilution of each of the formaldehyde standards, add 5.0 ml of the methylbenzothiazolone hydrazone hydrochloride reagent. Close the tubes, shake, and allow to stand for 60 min.

3.2 Add 1 ml of ferric chloride-sulphamic acid reagent and allow to stand for 15 min.

3.3 Measure absorbance of vaccines and standards on a spectrophotometer at the maximum at 628 nm in a 1 cm cell, using the reagent blank as compensation liquid.

4. Calculations and Interpretation

Calculate total formaldehyde concentration (g/L) from the standard curve using linear regression (acceptable correlation coefficient [r] equal to or greater than 0.97).

Appendix 1

Formaldehyde level conversion table

G/L formaldehyde	% w/v formaldehyde	% v/v Formaldehyde solution*	ppm formaldehyde
2.0	0.2	.5	2000
0.8	0.08	0.2	800
0.4	0.04	0.1	400
0.5	0.05	0.125	500
0.05	.005	0.0125	50
0.04	.004	0.01	40

*** based on 40% formaldehyde solution**

References

1. Chandler, M.D. & G.N. Frerichs, Journal of Biological Standardization (1980) 8, 145-149

2. Knight, H, & Tennant R.W.G. Laboratory Practice, (1973) 22, 169-173