SHORT COMMUNICATION

RESIDUAL FORMALDEHYDE IN BACTERIAL VACCINE PRODUCTS

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ABSTRACT. This study is to determine the residual level of formaldehyde to ensure product safety. A number of assays are available for the determination of residual free formaldehyde in inactivated vaccine, including acetyl acetone titration, ferric chloride titration and the basic fuchsin test. In the current study, ferric chloride quantitative method was used to evaluate formaldehyde residue on 4 types of vaccine produced in Veterinary Research Institute (VRI), Ipoh. Four types of vaccine tested were haemorrhagic septicaemia oil (HS oil), haemorrhagic septicaemia alum (HS alum) vaccine, duck vaccine (DV) and sheep and goat pasteurellosis vaccine (SGP). Results revealed formaldehyde residues in vaccines tested were between 0.873 g/L to 1.385 g/L. Continuous work towards reduction of formaldehyde level should be addressed to fulfil GMP requirements.

Keywords: Vaccine, formaldehyde residue, ferric chloride

INTRODUCTION

Formaldehyde is being used as a preservative for human and veterinary drugs and biological materials, which contain 0.05% formalin as an inactivating agent. It is a colourless chemical available as 37% aqueous solution and commonly referred to as formalin (IARC, 1995).

Inactivation process using formaldehyde or formalin is practised for many vaccines in the world such as polio and diphtheria. Other than formalin, phenol also is being used for inactivation process (CDC, 2020). According to the United States Food and Drug Association (US, FDA) the amount of formaldehyde present in some vaccines is so small compared to the concentration that occurs naturally in the body that it does not pose a safety concern.

Ferric chloride method for quantitative determination of total formaldehyde residue has been adopted by many agencies such as Canadian Food Inspection Agency, United States Department of Agriculture, Animal and Plant Health Inspection Service (USDA, APHIS), United States Code of Federal and European Agency for the Evaluation of Medicinal Products.

Determination of formaldehyde spectrophotometrically is a simple, sensitive method (Ross *et al.*, 2002) and can be measured by visually and by spectrophotometric methods. In this study, ferric chloride method was used for quantitative determination of total formaldehyde residue as recommended by The International Cooperation on Harmonization of Technical Requirements for the Registration of Veterinary Medicinal

Products (VICH, 2019). Previous study by Nurulaini *et al.*, 2019 also adopted this method for determination of formaldehyde residues in inactivated vaccine products.

Veterinary Research Institute (VRI), Ipoh produces vaccines for livestock and poultry such as fowl cholera vaccine, haemorrhagic septicaemia oil (HS oil), haemorrhagic septicaemia alum (HS alum) vaccine, duck vaccine (DV) and sheep and goat pasteurellosis vaccine (SGP). This study involves determination of residual formaldehyde in the vaccine products which are currently produced and sold by VRI, DVS.

MATERIALS AND METHOD

In this study, formalin residue in vaccines is tested according to the guideline by European Agency for the Evaluation of Medicinal Products (European Pharmacopoeia, 2014) and VICH (2002). Total formaldehyde is determined based on the reaction of formaldehyde with methylbenzothiazolone hydrazine hydrochloride (MBTH). The absorbance was measured at 628 nm using a UV-visible spectrophotometer (ThermoScientific, USA). Isopropyl myristate, hydrochloric acid, chloroform and chloride-sulphamic acid were obtained from Merck, Germany while sodium chloride was obtained from Sigma Aldrich, USA.

Four types of vaccines tested were HS oil, HS alum, DV and SGP. All the vaccine products are being sold and distributed for the livestock industry in Malaysia.

Sample and standards preparation

Formaldehyde standards of 0.25, 0.50, 1.00 and 2.00 g/L were prepared by diluting formaldehyde solution with water in suitable volumetric flasks. Oil emulsion vaccine was separated by a suitable separation method according to European Ph., VICH (2002). A volume of 1.00 ml vaccine was added to isopropyl myristate and mixed thoroughly by a vortex mixer. Then, 1.3 ml of 1M hydrochloric acid, 2.0 ml chloroform and 2.7 ml of 9 g/L sodium chloride was added, mixed thoroughly and centrifuged at 15,000 $\times g$ for 60 minutes. The aqueous phase was then transferred to a 10 ml volumetric flask and diluted with water. The diluted phase was used for the test of residual formaldehyde in the vaccine sample.

Sample analysis

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 aqueous phase), and to 0.50 ml of 1 in 200 dilution of each of the formaldehyde standards, 5 ml of MBTH was added. Each of the tested samples were done in triplicate to reduce errors. The tubes were shaken and allowed to stand for 60 minutes. Then 1 ml of ferric chloride-sulphamic acid reagent was added and allowed to stand for 15 minutes. Absorbance of vaccine and standards was measured on a spectrophotometer at 628 nm in a 1 cm cell.

Formaldehyde concentration (g/L) and their absorbance (OD) were recorded. Then, the standard curve of different formaldehyde concentration (g/L) and their absorbance was plotted.

Table 1. Residual formaldehyde concentration on the vaccines tested.

Types of Vaccine	Residual Formaldehyde (g/L)
HS OIL	1.369
HS ALUM	1.385
DV	1.064
SGP	0.873

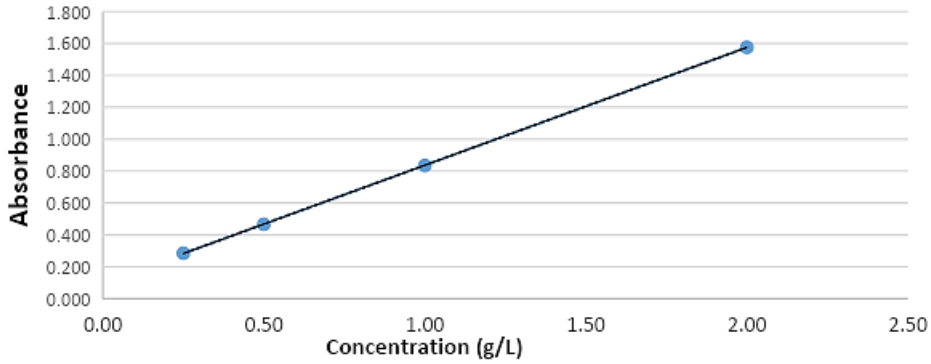

Figure 1. Standard curve of different formaldehyde concentration (g/L) and their absorbance by using ferric chloride method.

Calculations and interpretations

Total formaldehyde concentration (g/L) was calculated from the standard curve using linear regression (acceptable correlation coefficient (R) equal to or greater than 0.97).

RESULTS AND DISCUSSION

Figure 1 shows a relationship between formaldehyde concentration (g/L) and absorbance (OD) accordingly. A standard curve using linear regression of correlation coefficient (R) equal to or greater than 0.97 was used for determination of formaldehyde in samples. Obtained results showed that residual formaldehyde concentration tested was between 0.873 g/L to 1.385 g/L. Residual formaldehyde values for each vaccine were 1.369 g/l for HS oil, 1.385 g/L for HS alum, 1.064 g/L for DV and 0.873 g/L for SGV as in Table 1.

Ferric chloride quantitative method suitable due to its simple operation, inexpensive equipment (using spectrophotometer) and higher sensitivity as supported by Abeer and Abd El-Hakim (2015). The ferric chloride method is based on the reaction of formaldehyde with MBTH which combined with formaldehyde to give one product. The oxidation of excess MBTH gives another product and these

two products are combined to give a blue chromophore which is measured at 628 nm against known formaldehyde standard solutions. Their absorbance ranged between 0.285 to 1.576 from the standard curve using linear regression with correlation coefficient, ρ=1 as shown in Figure 1. Ross *et al.* (2002) conducted international collaborative study of quantitative colourimetric method for determination of formaldehyde in veterinary vaccines products by 15 laboratories in North America, Europe and Japan to harmonise and provide guideline standard method for residual formaldehyde.

According to European Pharmacopeia, the concentration of free formaldehyde should not be greater than 0.5 g/L while the United States Code of Federal stated free formaldehyde must not exceed 0.74 g/L. Therefore, laboratory animal batch safety testing (USDA, 2000 and VICH, 2019) has been conducted for each batch of vaccine products and only considered safe when more than 80% of test mice showed no reaction after being injected subcutaneously with 0.5 ml of the vaccine during 21 days of observation.

CONCLUSION

The formaldehyde values detected in this study were slightly higher than the recommended amount. However, if a higher amount was detected other tests should be conducted in order to ensure vaccine safety. Nevertheless, extensive study to reduce the amount of formaldehyde residue in accordance to recommended value in vaccines should be conducted in future.

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