Draft New monograph for Comments and Inclusion in The Indian Pharmacopoeia

DRAFT NEW MONOGRAPH FOR COMMENTS

This draft new monograph contain text for inclusion in the Indian Pharmacopoeia (IP). The content of this draft document is not final, and the text may be subject to further revisions prior to publication in the IP. This draft does not necessarily represent the decisions or the stated policy of the IP or Indian Pharmacopoeia Commission (IPC).

Manufacturers, regulatory authorities, health authorities, researchers, and other stakeholders are invited to provide their feedback and comments on this draft proposal. Comments received after the last date will not be considered by the IPC before finalizing the monograph.

Please send any comments you may have on this draft document to lab.ipc@gov.in/ biologics-ipc@gov.in **before the last date for comments.**

Document History and Schedule for the Adoption Process

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Further follow-up action as required.	

Draft Revisions for Comments (Unofficial Text)

3-*O*-DESACYL-4′-MONOPHOSPHORYL LIPID A

DEFINITION

3-*O*-Desacyl-4′-monophosphoryl lipid A is a detoxified derivative of the lipopolysaccharide (LPS) of *Salmonella minnesota*, strain R595, which retains the immunostimulatory activities of the parent LPS. It consists of a mixture of congeners, all containing a backbone of β1′→6-linked disaccharide of 2-deoxy-2-aminoglucose phosphorylated at the 4′-position, but differing in the fatty acid substitutions at the 2, 2′ and 3′ positions. The immunostimulatory activities of 3-*O*-desacyl-4′-monophosphoryl lipid A combined with the vaccine include up-regulation of co-stimulatory molecules on antigen-presenting cells and secretion of pro-inflammatory cytokines, resulting in an enhanced immune response of the Th1-type against the antigens. 3-*O*-desacyl-4′-monophosphoryl lipid A is a lyophilised powder or a sterile liquid.

Requirements given in the sections up to and including the section Triethylamine salt of 3-*O*-desacyl-4′-monophosphoryl lipid A also apply to formulations that do not proceed to the 3-*O*-desacyl-4′-monophosphoryl lipid A liquid bulk.

PRODUCTION

GENERAL PROVISIONS

The production method shall have been shown to yield consistently a 3-*O*-desacyl-4′-monophosphoryl lipid A comparable in structure and function with a preparation of 3-*O*-desacyl-4′-monophosphoryl lipid A used as adjuvant in the particular vaccine of proven clinical efficacy and safety in man.

During development studies, and wherever revalidation is necessary, a test for residual endotoxin activity is carried out by injecting intravenously 12-day-old embryonated hens' eggs with 0.1 ml of dilutions of the test sample (8 eggs per dilution) of 3-*O*-desacyl-4′-monophosphoryl lipid A. Eggs are candled and read for mortality at 18-24 hours post-inoculation and the chick embryo 50 per cent lethal dose ($CELD_{50}$) is calculated. The residual endotoxin activity of the 3-*O*-desacyl-4′-monophosphoryl lipid A is acceptable if the $CELD_{50}$ is more than 100 µg.

An endotoxin standard of *Salmonella typhimurium* is prepared and selected dilutions are injected into each group of 8 eggs.

For a test to be valid, the $CELD_{50}$ of the endotoxin standard must not be more than 0.05 µg.

Reference preparation: a batch of 3-*O*-desacyl-4′-monophosphoryl lipid A shown to be comparable in structure and function with a preparation of 3-*O*-desacyl-4′-monophosphoryl lipid A used as adjuvant in the particular vaccine of proven clinical efficacy and safety in man or a batch representative thereof.

BACTERIAL SEED LOTS

The bacterial strain used for master seed lots shall be identified by historical records that include information on its origin and the tests used to characterize the strain, in particular genotypic and phenotypic information. Only a working seed lot that complies with the following requirements may be used.

Identification

The working seed lot is identified by suitable methods such as Gram staining and fatty acid profiling

Microbial Purity

Each seed lot complies with the requirements for absence of contaminating organisms. Purity of bacterial cultures is verified by methods of suitable sensitivity and specificity.

PROPAGATION AND HARVEST

The bacteria is grown using a suitable liquid medium. At the end of cultivation, the culture is tested for purity and yield. The culture medium is separated from the bacterial mass by a suitable method, for example filtration. Only a harvest that is consistent with respect to the profiles for growth rate, pH, and O_2-consumption may be used for the extraction of LPS.

TRIETHYLAMINE SALT OF 3-O-DESACYL-4′-MONOPHOSPHORYL LIPID A

LPS is extracted from the bacterial cells by successive alcohol and chloroform-methanol extractions and is then converted to 3-*O*-desacyl-4′-monophosphoryl lipid A by hydrolysis, then purified and salified by triethanolamine before freeze-drying. The freeze-dried triethylamine salt of 3-*O*-desacyl-4′-monophosphoryl lipid A must comply with the following requirements.

Appearance. A visual description of the particular preparation after freeze-drying is established and approved by the competent authority; each batch of freeze-dried triethylamine salt of 3-*O*-desacyl-4′-monophosphoryl lipid A must comply with this description.

Protein: less than 0.5 per cent *m/m*, determined using a suitable method, for example a reversed-phase HPLC method for amino acid analysis (2.2.19). The total amino acid content in micrograms is calculated by comparison to amino acid standards and is equal to the protein concentration.

Nucleic acid: maximum 0.3 per cent *m/m*, determined using a suitable method. For example, a fluorimetric method may be used where nucleic acids are extracted from the freeze-dried triethylamine salt of 3-*O*-desacyl-4′-monophosphoryl lipid A, using a solution containing NH_4OH and a suitable non-ionic detergent, and stained by a suitable fluorescent dye. The nucleic acid content in the test sample is interpolated from a calibration curve.

Hexosamine

1000 nmol/mg to 1450 nmol/mg.

Phosphorus

0.5 µmol/mg to 0.8 µmol/mg.

Congener distribution. The relative amount of tetraacyl, pentaacyl, hexaacyl and heptaacyl congener groups are determined by a suitable method, for example reversed-phase HPLC analysis (2.4.14).

The relative amount of each congener group in the triethylamine salt of 3-*O*-desacyl-4′-monophosphoryl lipid A is:

–tetraacyl: 15 per cent to 35 per cent;

–pentaacyl: 35 per cent to 60 per cent;

–hexaacyl: 20 per cent to 40 per cent;

–heptaacyl: less than 0.5 per cent.

Triethylamine: 4.2 to 5.8 per cent *m/m*, determined by a suitable method, for example gas chromatography (2.4.12).

Water (2.3.43). maximum 6.7 per cent *m/m*.

Free fatty acids: maximum 2.6 per cent *m/m*, determined by a suitable method, for example reversed-phase HPLC analysis

2-Keto-3-deoxyoctonate: less than 0.5 per cent *m/m*, determined by a suitable method. For example, a colorimetric method may be used where 2-keto-3-deoxyoctonate is released by hydrolysis (0.2 N H_2SO_4 at 100 °C for 30 min), oxidised by periodic acid, and reacted with sodium arsenite to yield β-formylpyruvic acid, which subsequently is coupled to thiobarbituric acid to give a red coloured chromophore with absorption maximum at 550 nm. The amount of 2-keto-3-deoxyoctonate is interpolated from a calibration curve.

Identity. The test for congener distribution also serves to identify the product.

Microbial contamination

TAMC: acceptance criterion 10^1 CFU/10 mg (2.2.9).

Pyrogens (2.2.8). The triethylamine salt of 3-*O*-desacyl-4′-monophosphoryl lipid A complies with the test for pyrogens. Inject into each rabbit per kilogram of body mass 3 mL of a solution containing 2.5 µg of 3-*O*-desacyl-4′-monophosphoryl lipid A.

3-O-DESACYL-4′-MONOPHOSPHORYL LIPID A LIQUID BULK

The triethylamine salt of 3-*O*-desacyl-4′-monophosphoryl lipid A is dispersed in a liquid suitable for the subsequent processing steps at a defined target concentration. If the salt is not soluble in Water, a microfluidisation step is necessary to prepare a stable aqueous suspension.

The liquid bulk is sterilised by filtration through a bacteria-retentive filter.

Only a 3-*O*-desacyl-4′-monophosphoryl lipid A liquid bulk that complies with the requirements given below under Identification, Tests and Assay and that is within the limits approved for the particular product may be used for the preparation of 3-*O*-desacyl-4′-monophosphoryl lipid A in the final lots.

CHARACTERS

When dispersed in an aqueous solution: slightly turbid suspension.

When dissolved in an organic solvent: a description of its appearance is established and approved by the competent authority; the 3-*O*-desacyl-4′-monophosphoryl lipid A liquid bulk complies with this description.

IDENTIFICATION

Congener distribution (see Tests).

TESTS

Particle size. Where applicable, the particle size in the microfluidised liquid bulk is determined by a suitable method, for example dynamic light scattering. The particle size for each batch of liquid bulk is within the limits approved for the particular product.

Sterility

(2.2.11). It complies with the test, carried out using 10 ml for each medium.

Congener distribution. The relative amount of tetraacyl, pentaacyl, hexaacyl and heptaacyl congener groups are determined by a suitable method, for example reversed-phase HPLC analysis (2.4.14).

The relative amount of each congener group in the 3-*O*-desacyl-4′-monophosphoryl lipid A liquid bulk is:

–tetraacyl: 15 per cent to 35 per cent;

–pentaacyl: 35 per cent to 60 per cent;

–hexaacyl: 20 per cent to 40 per cent;

–heptaacyl: less than 0.5 per cent.

ASSAY

The 3-*O*-desacyl-4′-monophosphoryl lipid A content is determined by a suitable method, for example gas chromatographic quantification (2.4.13) of trifluoroacetic anhydride derivatised fatty acid methyl esters of the 3-*O*-desacyl-4′-monophosphoryl lipid A fatty acids dodecanoic acid (C12:0), tetradecanoic acid (C14:0), 3-hydroxy tetradecanoic acid (3-OH-C14:0) and hexadecanoic acid (C16:0) obtained by hydrolysis of 3-*O*-desacyl-4′-monophosphoryl lipid A in an aqueous/methanol (50:50 *V/V*) solution, containing 5 per cent of sodium hydroxide. To the test sample, a reference sample and the dilutions of the calibration curve, pentadecanoic acid (C15:0) is added as an internal standard. The temperature gradient applied must allow the separation of the fatty acid methyl esters in about 40 min.

The sum of the ratios between the area for each individual fatty acid methyl ester (C12:0, C14:0, 3-OH-C14:0 and C16:0) and the area of the internal standard (ratio = area C_x / area C15:0) is calculated. The 3-*O*-desacyl-4′-monophosphoryl lipid A quantity corresponding to the sum ratio value on the calibration curve, established with the dilutions of the 3-*O*-desacyl-4′-monophosphoryl lipid A standard, is reported.

The content of 3-*O*-desacyl-4′-monophosphoryl lipid A is not less than 80 per cent and not greater than 120 per cent of the estimated content.