ESTIMATION OF MICROBIAL LIMITS IN OILS

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ABSTRACT
Oil is an important source of fat and is important in human diet. The microbial limit tests are designed to perform the qualitative and quantitative estimations of specific viable microorganisms present in pharmaceutical substances or in the samples. The present paper deals with the various procedures in estimation of microbial limits in oils.

Key-words: Oil, microbial limits, methods, media

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INTRODUCTION

Oil is an important source of fat and is important in human diet. Beside this, oil plays a vital role in maintaining the agricultural economy of the country. The necessity for providing more oil and fat to the human society has been the incentive for intensive cultivation of crops. Scientists are working in this field to search out natural sources of oil crop's containing various drugs of medicinal importance. Their sole aim has been to have better yield. Later, as a result of more and more appreciation of oil in our daily food, they have unavoidable place in man's economy. As a matter of facts, the interest in production of oil crops has increased tremendously in recent years.

The microbial limit tests are designed to perform the qualitative and quantitative estimations of specific viable microorganisms present in pharmaceutical substances or in the samples. It includes tests for total viable count (bacteria and fungi) and specified microbial species (*Escherichia coli, Salmonellla, Pseudomonas aeruginosa* and *Staphylococcus aureus*). It must be carried out under conditions designed to avoid accidental microbial contamination of the preparation during the test. When the test specimens have antimicrobial activity or contain antimicrobial substances must be eliminated by means of procedure such as dilution, filtration, neutrilization or inactivation. For the test, use a mixture of several portions selected random from the bulk or from the contents of a sufficient number of containers. If test specimens are diluted with fluid medium, the test should be performed quickly. In performing the test, precautions must be taken to prevent biohazard. According to USP the test is designed to determine total aerobic microbial count and yeast and mould count. This test demonstrates that product is free from *Staphylococcus aureus, E. coli, Pseudomonas aeruginosa, C. albicans* and *A. niger*. The methods given are invalid unless it is demonstrated that the test specimens to which they are applied do not of themselves inhibit the multiplication under the test conditions of microorganism that can be present (Eisenhart and Wilson, 1943; Ashby, 1976; Palmerm *et al*., 1976; Claus,1989).

METHODS

The various methods adopted to determine the presence of micro-organism in the pharmaceutical herbal products are described below (Geldreich *et al*., 1975; Green, *et al*., 1980; Sartory, 1980; Pagel *et al*., 1982; Heggers and Robson, 1991; Greenberg *et al*., 1992; Madigan *et al*., 1997)

Membrane Filtration Method

This method is applied to the sample, which contains antimicrobial substances. Use membrane filters of an appropriate material with a pore size of 0.45 µm or less. Filters about 50 mm across are recommended, but other sizes may be used. Sterilize the filters, filtration apparatus, media, and other apparatus used. Usually, measure two test fluids of 10 ml each; pass each sample through a separate filter. Dilute the pretreated test fluid if the bacteria concentration is high, so that 10 100 colonies can develop per filter. After filtration, wash each filter three times or more with an appropriate liquid such as phosphate buffer, sodium chloride-peptone buffer, or fluid medium. The volume of the washings should be about 100 ml each. If the filter used is not about 50 mm in diameter, use an appropriate volume of washing, depending on the size of the filter. If the sample includes lipid, polysorbate 80 or an appropriate emulsifier may be added to the washings. After filtration, for bacteria detection, place the two filters on a plate of soybean-casein digest agar medium, and for fungi detection, add an antibiotic to the medium and place them on a plate of one of Sabouraud glucose agar, potato-dextrose agar, or GP agar media. Incubate the plates at least for 5 days at 30-35º for bacteria detection and at 20-25º ºfor fungi detection, and count the number of colonies. If counts obtained are considered to be reliable in shorter incubation time than 5 days, these counts may be adopted for calculation of the viable count.

Pour Plate Method

Use Petri dishes 9-10 cm in diameter. Use at least 2 agar media for each dilution. Take 1 ml of the test fluid or its dilution into each Petri dish aseptically, add to each dish 15º 20 ml of sterilized agar medium, previously melted and kept below 45º ºand mix. For bacteria detection, use soybean-casein digest agar medium and for fungi detection, use one of Sabouraud glucose agar, potato-dextrose agar, and GP agar media, to which antibiotic has previously been added. After the agar solidifies, incubate at least for 5 days at 30º 35º for bacteria detection and at 20º 25º ºfor fungi detection. If a large number of colonies develop, calculate viable counts obtained from plates with not more than 300 colonies per plate for bacteria detection and from plates with not more than 100 colonies per plate for fungi detection. If counts are considered to be reliable in a shorter incubation time than 5 days, these counts may be adopted.

Spread Plate Method

Place 0.05-0.2 ml of the test fluid on the solidified and dried surface of the agar medium and spread it uniformly using a spreader. Proceed under the same conditions as for the Pour Plate Method, especially about Petri dishes, agar media, incubation temperature and time, and calculation method.

Serial Dilution Method

Use 12 test tubes: 9 containing 9 ml of soybean-casein digest medium each and 3 containing 10 ml of the same medium each control. Prepare dilutions using the 9 tubes. First, add 1 ml of the test fluid to each of three test tubes and mix to make 10-times dilutions. Second, add 1 ml of each of the 10-times dilutions to each of another three test tubes and mix to make 100-times dilutions. Third, add 1 ml of each of the 100-times dilutions to each of the remaining three test tubes and mix to make 1,000-times dilutions. Incubate all 12 test tubes for at least 5 days at 30-35º ºNo microbial growth should be observed for the control test tubes. If the determination of the result is difficult or if the result is not reliable, take a 0.1 ml fluid from each of the 9 test tubes and place it to an agar medium or fluid medium, incubate all media for 24 to72 hours at 30º 35º ºand check them for the absence or presence of microbial growth.

MEDIA

Media or Culture media is very essential for the growth of specific micro-organism and also, specific culture media are used. They may be prepared or dehydrated culture media may be used provided that, when reconstituted as directed by the manufacturer, they have similar ingredients and/or yield media comparable to those obtained from the formula given below. Where agar is specified in a formula, use agar that has moisture content of not more than 15%. Water is called for in a formula, use purified water. Unless otherwise indicated, the media should be sterilized by heating in an autoclave at 115ºC for 30 minutes. In preparing media, dissolve the soluble solids in the water, using heat if necessary, to effect complete solution and add solutions of hydrochloric acid or sodium hydroxide in quantities sufficient to yield the required pH in the medium when it is ready for use. Determine the pH at 25º ± 2º. The different types of culture media are soybean-casein digest agar medium, antibiotics-added sabouraud glucose agar medium, BGLB (brilliant green lactose bile) medium, macconkey agar medium, fluid selenite-cystine medium, XLD (xylose-lysine-desoxycholate) agar medium, cetrimide agar medium, pseudomonas agar medium, vogel-johnson agar medium, baird-parker agar medium etc. (Dwivedi and Dhuria, 2008).

CONCLUSION

The microbial limit tests are designed to perform the qualitative and quantitative estimations of specific viable microorganisms present in oils. It includes tests for total viable count (bacteria and fungi) and specified microbial species (*Escherichia coli, Salmonellla, Pseudomonas aeruginosa* and *Staphylococcus aureus*). It must be carried out under conditions designed to avoid accidental microbial contamination of the preparation during the test.

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