preparation. Accurately weigh 1 gram of the standard homosalate sunscreen preparation into a 100-milliliter volumetric flask. Add 50 milliliters of the assay solvent. Heat on a steam bath and mix well. Cool the solution to room temperature (15 to 30 °C). Then dilute the solution to volume with the assay solvent and mix well to make a 1-percent solution.

(3) Preparation of the test solution (1:50 dilution of the 1-percent solution). Filter a portion of the 1-percent solution through number 1 filter paper. Discard the first 10 to 15 milliliters of the filtrate. Collect the next 20 milliliters of the filtrate (second collection). Add 1 milliliter of the second collection of the filtrate to a 50-milliliter volumetric flask. Dilute this solution to volume with assay solvent and mix well. This is the test solution (1:50 dilution of the 1-percent solution).

(4) Spectrophotometric determination. The absorbance of the test solution is measured in a suitable double beam spectrophotometer with the assay solvent and reference beam at a wavelength near 306 nanometers.

(5) Calculation of the concentration of homosalate. The concentration of homosalate is determined by the following formula which takes into consideration the absorbance of the sample of the test solution, the dilution of the 1-percent solution (1:50), the weight of the sample of the standard homosalate sunscreen preparation (1 gram), and the standard absorbance value (172) of homosalate as determined by averaging the absorbance of a large number of batches of raw homosalate:

\[
\text{Concentration of homosalate} = \frac{\text{absorbance} \times 50 \times 100 \times 172}{\text{percent concentration by weight}}
\]

§ 352.72 General testing procedures.

(a) Selection of test subjects (male and female). (1) Only fair-skin subjects with skin types I, II, and III using the following guidelines shall be selected:

Selection of Fair-skin Subjects

Skin Type and Sunburn and Tanning History
(Based on first 30 to 45 minutes sun exposure after a winter season of no sun exposure.)

I—Always burns easily; never tans (sensitive).
II—Always burns easily; tans minimally (sensitive).
III—Burns moderately; tans gradually (light brown) (normal).
IV—Burns minimally; always tans well (moderate brown) (normal).
V—Rarely burns; tans profusely (dark brown) (insensitive).
VI—Never burns; deeply pigmented (insensitive).

(2) A medical history shall be obtained from all subjects with emphasis on the effects of sunlight on their skin. Ascertain the general health of the individual, the individual’s skin type (I, II, or III), whether the individual is taking medication (topical or systemic) that is known to produce abnormal sunlight responses, and whether the individual is subject to any abnormal responses to sunlight, such as a phototoxic or photoallergic response.

(b) Test site inspection. The physical examination shall determine the presence of sunburn, suntan, scars, active dermal lesions, and uneven skin tones on the areas of the back to be tested. The presence of nevi, blemishes, or moles will be acceptable if in the physician’s judgment they will not interfere with the study results. Excess hair on the back is acceptable if the hair is clipped or shaved.