Test Methods of Indole -3- Carbinol by HPLC

Note: Control Techniques developed, tested by independent laboratories, reference to the model of analytical equipment are not subject to the conditions of the analysis.

Description. Crystalline powder from white to light yellow or light brown in color, with a characteristic odor. The test is performed organoleptically.
Solubility. Easily soluble in 95% alcohol and acetone, sparingly soluble in chloroform, practically insoluble in water.

Authenticity.
HPLC. Retention time of the main peak in the chromatogram of the test solution must correspond to the retention time of the principal peak in the chromatogram of solution SB indole-3-carabinol.

Quantitation. Approximately 0.025 g (accurately weighed) of the drug is placed into a volumetric flask with 25 ml capacity, add 10 ml of mobile phase B (PF V), stirred until dissolved sample is adjusted in volume of PD solution to the mark and mixed. 1 ml of the resulting solution was placed in a volumetric flask of 25 ml, the volume is adjusted in the PD solution to the mark and mixed. (Test solution). The solution is used freshly prepared.

Chromatographic conditions.
Column - 25 x 0.46 cm Luna C18 (5 microns); or equivalent;
Mobile phase - PF k phosphorus-ammonia buffer solution having a pH of 7.0;
The PF: acetonitrile - ammonium phosphate-buffered saline at pH 7.0 (70:30);
The elution in a linear gradient mode:

Time, minutes	PF*A*, %	PF*B*, %
0 —–> 8	91	9
8 —–> 20	0	100
20 —–> 25	0	100

Flow rate - 2,0 ml/min;
Detector - Spectrophotometry, 280 nm;
Sample volume - 20 мкл;
Registration time of chromatogram - 25 minutes.

Consistently chromatography PF B (control chromatography), the test solution and CO indole-3-carabinol solution.
C9H9NO content in the product (X) as a percentage based on dry matter was calculated by the formula:

$$Y = \frac{S1 \times a0 \times 25 \times P \times 100 \times 100}{S0 \times a1 \times 25 \times 25 \times 100 \times (100 - W)} = \frac{S1 \times a0 \times P \times 100}{S0 \times a1 \times (100 - W)}$$

Where:
S1 - indole-3-carabinol peak area in the chromatogram of the test solution;
S0 - the peak area in the chromatogram indole-3-carabinol CO solution;
a1 - preparation weighed in grams;
a0 - Hitch COindole-3-carabinol in grams;
P - the actual content of C_9H_9NO in the COindole-3-carabinol as a percentage;
W - loss on drying of the preparation, as a percentage.

Results of the analysis are considered valid if the following test requirements "Checking the suitability of the chromatographic system."
Preparation of solutions.

1. Preparation of phosphate-buffered ammoniacal solution with a pH of 7.0. To 950 ml of water is added 3 ml of concentrated phosphoric acid, stirred, adjusted to pH 7,0 ± 0,1 (potentiometrically) with concentrated ammonia solution, water solution volume was adjusted to 1000 ml and stirred. The solution is used freshly prepared.

2. Preparation of the solution SO indole-3-carabinol. Approximately 0.025 g (accurately weighed) placed in a CO indole-3-carabinol volumetric flask of 25 mL, add 10 mL of the PF B, sample mixed until dissolved, the volume was adjusted PD solution B to the mark and mixed. 1 ml of the resulting solution was placed in a volumetric flask of 25 ml, the volume is adjusted in the PD solution to the mark and mixed. The solution is used freshly prepared.

3. Preparation of the solution for system suitability test. 0.025 g of indole-3-carabinol (Fluka, № 57190) was dissolved in 25 ml of PF B. To 5 ml of this solution was added 15 ml of solution indole-3-carabinolCO, PF in 5 mL and mixed.

The solution is used freshly prepared.
4. Check the suitability of the chromatographic system. Chromatograph the solution for system suitability test. The order of elution of the components: indole-3-carabinol. The resolution (R) between the peak and the peak indole-3-carabinolshould be not less than 2.0.
Chromatograph CO indole-3-carabinol solution. The effectiveness of the column (of N), calculated from peak indole-3-carabinol shall be not less than 1500 theoretical plates, the asymmetry factor (T) for indole-3-carabinol peak should be not more than 1.5. The relative standard deviation of the area of the peak and the retention time in five consecutive indole-3-carabinolinjection should not exceed 2.0%.