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ANALYTICAL METHOD VALIDATION FOR DOCOSAHEXAENOIC ACID CONTENT IN SURBEX PREGNANCY CAPSULES BY GAS CHROMATOGRAPHY

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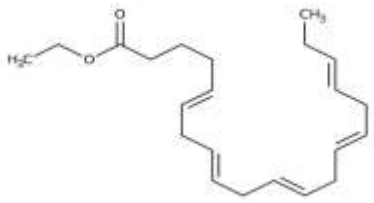
ABSTRACT

Fatty acid content in Surbex pregnancy capsules has frequently been measured using gas chromatography with flame ionization detection (GC-FID). The typical method for calculating the amount of docosahexaenoic acid (Omega-3-fatty acid) in Surbex Pregnancy Capsules was validated in this study employing GC-FID. Capillary, DB-23 (300X0.25X0.25)-Agilent was used to separate the DHA, and the entire run duration was 30.75 minutes. According to ICH criteria, the method's linearity, precision, accuracy, specificity, and sensitivity were validated. Finally, this technique successfully and precisely identified and quantified omega-3 and fatty acids, and it may be applied to routine examination of Surbex Preganacy Capsules.

KEYWORDS: Docosahexaenoic acid, GC Validation, Capillary, DB-23 column ICH guidelines.

INTRODUCTION

An omega-3 fatty acid called docosahexaenoic acid, or DHA, is a major structural element of the human brain, skin, retina, and cerebral cortex. It can be directly absorbed from breast milk or manufactured from alpha-linolenic acid. Fish oil, fatty fish, Or oil from algae. Commercial DHA is also produced from two types of microalgae, one belonging to the genus Schizochytrium and the other to the crypthecodinium-cohnii. Breast milk contains DHA, which is beneficial to an infant's development. A significant fatty acid in the retina and phospholipids of the brain is DHA. During the third trimester and the first 18 months of life, DHA is especially crucial for the development of the brain and retina in fetuses.



MATERIALS AND METHODS

Chemicals

Docosahexaenoic Acid, Iso-octane.

Instrumentation

Analysis was performed on Agilent GC software: Openlabs, ultra sonicator – Pci analytics, Analytical balance – Shimadzu, Micro balance – RadWag.

Chromatographic Conditions

Capillary, DB-23 (300X0.25X0.25): column were used for chromatographic separation using Nitrogen as carrier gas: Iso-Octane as diluent : The split ratio of l : 100 with a flow rate of 1.0 ml / min, Injection volume of 1.0µl : Run time of about 30.75 mins: Using Flame ionization detector and the retention time and peak shape is good.

VALIDATION

System suitability parameters: The preparation of a standard solution consisting Docosahexaenoic acid injection was used for determining the system suitability characteristics. It is recommended that the percentage RSD for the area of six standard injection results not exceed 2%.

Specificity: Checking for interference in the best approach. When using this technique to measure the retention durations of these medication, we shouldn't observe any conflicting peaks in the blank and placebo. It was stated that this approach was specific.

Precision

Preparation of Standard solution: Weigh about 200 mg of DHA (Docosahexaenoic acid)in a 50 mL volumetric flask, add 25 mL of Iso-octane shake well and sonicate for 10 minutes to dissolve. Then make upto 50 mL with Iso-Octane.

Preparation of Sample solution: Cut and open 20 capsules and collect the medicament in a clean petridish, weigh about 700 mg of medicament (Equivalent to 200 mg of DHA) in a 50mL volumetric flask, add 25 mL of Iso-Octane shake well and sonicate for 10 minutes to dissolve. Then make upto its volume with Iso-Octane and filter through 0.45µ filter and inject.

Linearity

Preparation of Linearity Standard stock solution

Weigh about 2500 mg (Equivalent to 1000mg of DHA) of Fish oil(10X40) in a 25 mL volumetric flask, add 15 mL of Iso-Octane shake well and sonicate for 10 minutes to dissolve.

Then make up to 25 mL with Iso-Octane.

Preparation of Linearity Solutions

1) 50.0% solution

Pipette out 1.0 mL of above standard stock solution in to 20 mL volumetric flask and make upto volume with diluent.

2) 75.0% solution

Pipette 1.5 mL of above standard stock solution in to 20 mL volumetric flask and make up to volume with diluent.

3) 100.0% solution

Pipette out 2.0 mL of above standard stock solution in to 20 mL volumetric flask and make upto volume with diluent.

4) 125.0% solution

Pipette 2.5 mL of above standard stock solution in to 20 mL volumetric flask and make up to volume with diluent.

5) 150.0% solution

Pipette 3.0 mL of above standard stock solution in to 20 mL volumetric flask and make up to volume with diluent.

Accuracy

Preparation of Accuracy Standard stock solution (Solution-A)

Weigh about 2500.0mg of (Equivalent to 200mg of DHA) of Fish oil (10X40).
WS/RS in to a 50mL volumetric flask, add 325 mL of Iso-Octane, shake for 10 minutes to dissolve and make up to 100mL with Iso-Octane.

Accuracy 50% solution

Weight about 200.0 mg of placebo and 5 mL of solution-A into a 50 mL volumetric flask, add 25 mL of Iso-Octane shake well and sonicate for 10 minutes to dissolve. Then make up to its volume with Iso-Octane and filter through 0.45µ filter and inject.

Accuracy 100% solution

Weight about 200.0 mg of placebo and 10 mL of solution-A into a 50 mL volumetric flask, add 25 mL of Iso-Octane shake well and sonicate for 10 minutes to dissolve. Then make up to its volume with Iso-Octane and filter through 0.45µ filter and inject.

Accuracy 150% solution

Weight about 200.0 mg of placebo and 15 mL of solution-A into a 50 mL volumetric flask, add 25 mL of Iso-Octane shake well and sonicate for 10 minutes to dissolve. Then make up to its volume with Iso-Octane and filter through 0.45µ filter and inject.

Procedure

The injections included a standard solution, the accuracy -50%, the accuracy -100%, and the accuracy -150% solutions. The amounts added and discovered for the mean recovery values of docosahexaenoic acid have been calculated and the results were compiled.

Robustness

The robustness of the analytical method for Content of DHA (Docosahexaenoic acid) in "Surbex Pregnancy Capsules" is demonstrated with small but deliberate variations in Column Oven Temperature (158ºC, 160°C and 162ºC), flow rate(0.8mL/min, 1.0 mL/min and 1.2mL/min) and Injector Oven Temperature (258ºC, 260°C and 262ºC).

Filter validation

Divide the sample solution into three portions. Centrifuge one portion of the sample for 15 minutes at 2500 rpm in a centrifuger, filter the other portion of sample through 0.45µ nylon filter as per method and filter the third portion through Whatman filter No.42.

Stability of analytical solutions

Stability of standard and sample solution will be demonstrated by injecting standard and sample solution with different time interval from the time of injection. Solutions shall be injected once in 12 hours upto 48 hours. The stability of solution shall be decided based on the area obtained at different time interval. If the results are not meeting the acceptance criteria within the time interval specified, discontinue the test and report the hours upto which the solution is found to be stable.

RESULTS

The results obtained by each parameters are shown below,

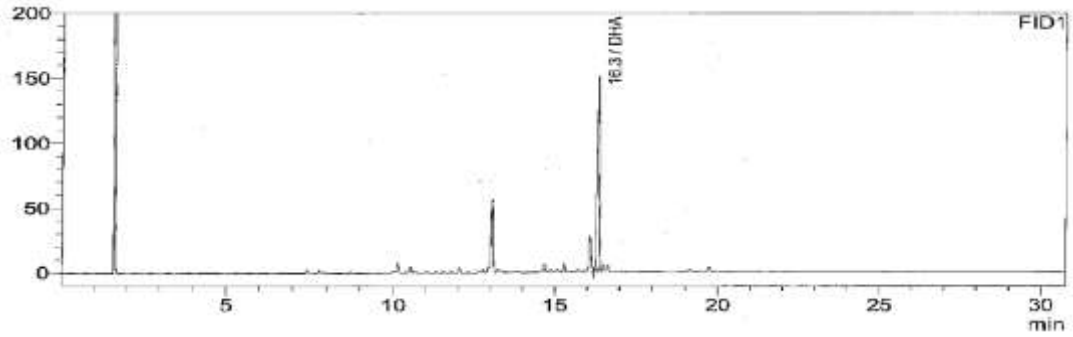


Figure 1: Chromatogram For System Suitability.

Table 1: Linearity average area response for each concentration.

% Concentration	Average Area Response
50	297027
75	456972
100	610997
125	743367
150	966417

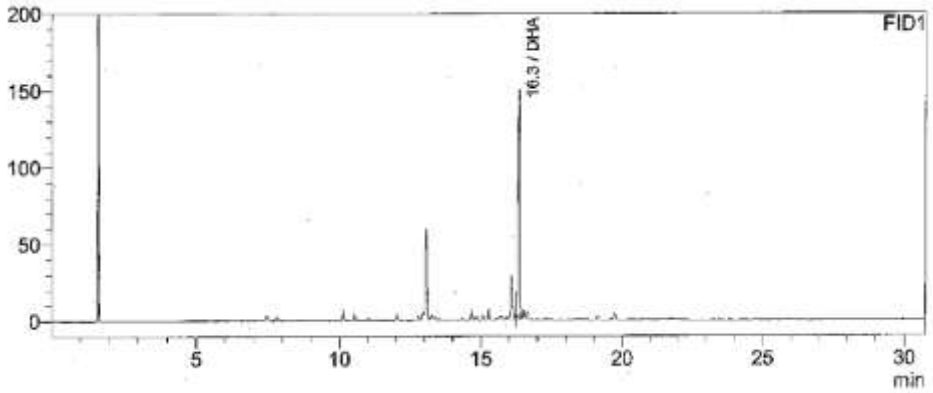


Figure 2: Chromatogram For Specificity.

Validation parameter for linearity

Table 2: Results obtained for linearity.

Concentration %	DHA (Docosahexaenoic acid) (30X20) (Stock)Solution ppm	Volume of stock solution taken in mL.	% Purity of DHA	Conc. in ppm
50	2497.94→50	1.0→20	41.5	2073.29
75		1.5→20		3109.94
100		2.0→20		4146.58
125		2.5→20		5183.23
150		3.0→20		6219.87

Table 3: Acceptance cretaria for linearity.

System Suitability Parameters	Observed value		Acceptance criteria
Correlation coefficient(r^2)	**0.99**		**NLT 0.99**
% of y-Intercept	**5.7**		**±10.0**
The % RSD for the peak area of DHA(Docosahexaenoic acid)obtained from 6 replicate standard preparations of 50 % and 150 % .	**Low**	**High**	**NMT 15.0**

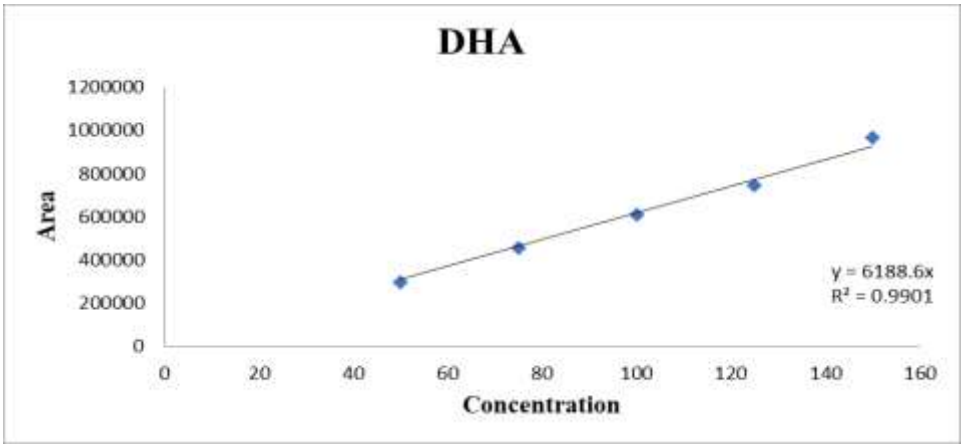


Figure 3: Linearity curve chromatogram.

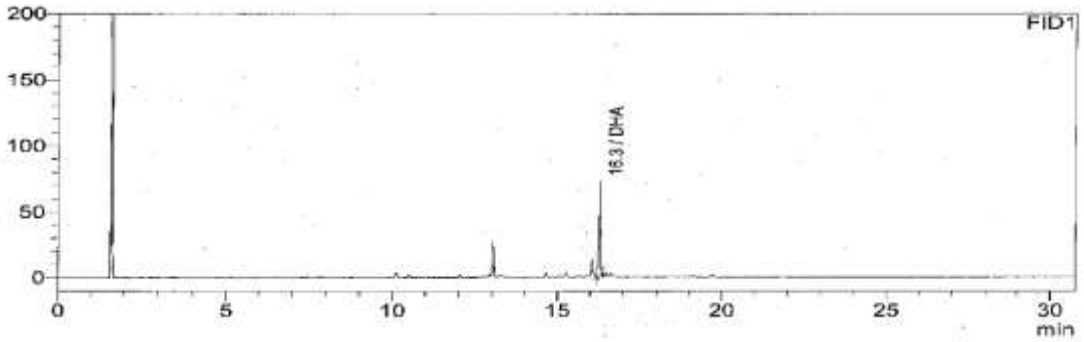


Figure 4: Chromatogram 50% Linearity.

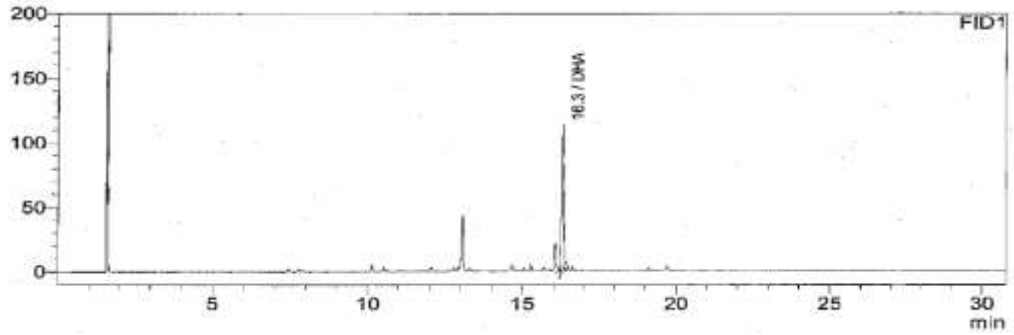


Figure 5: Chromatogram 75% Linearity.

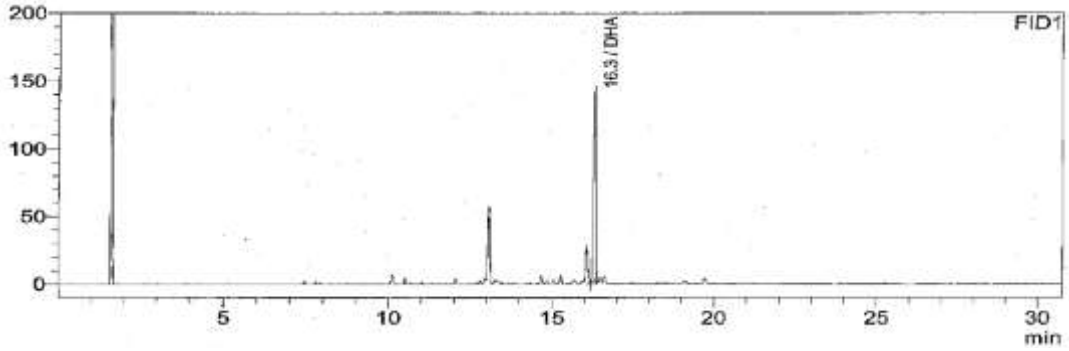


Figure 6: Chromatogram 100% Linearity.

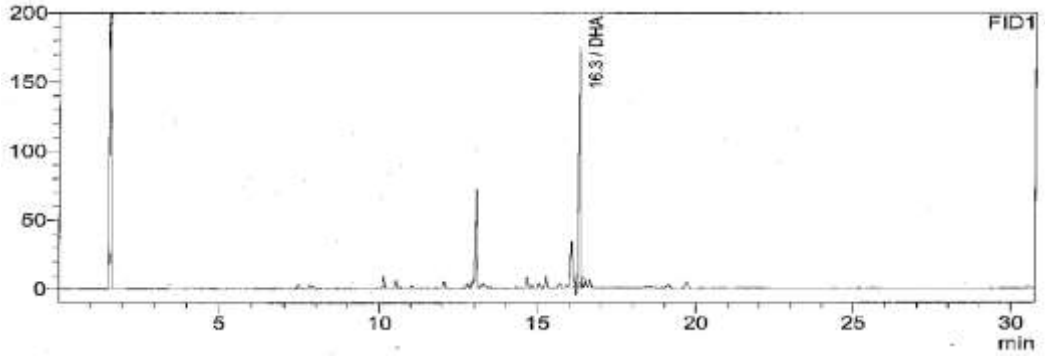


Figure 7: Chromatogram 125% Linearity.

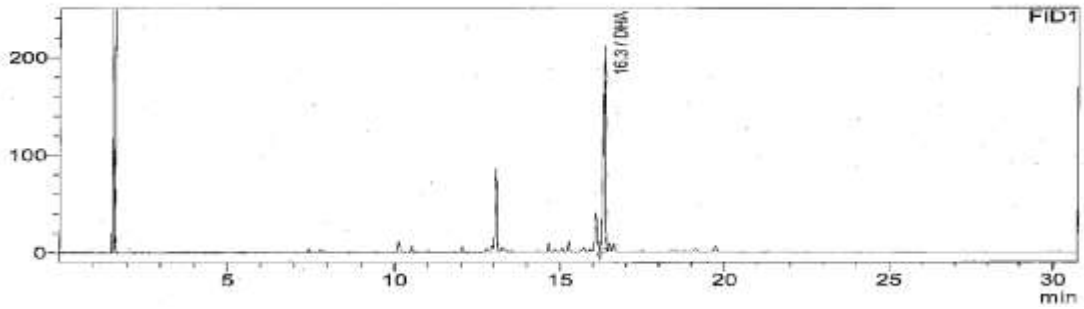


Figure 8: Chromatogram 150% Linearity.

Validation parameter for Accuracy

Table 4: Results obtained for Accuracy.

S No.	Concentration	Amount added for DHA in ppm	Amount recovered for DHA in ppm	Recovery in %	Average in %	RSD in %
01	50.0%	5001.8800	4934.0814	98.64	96.6	1.9
	50.0%		4794.4700	95.85		
	50.0%		4760.9890	95.18		
02	100.0%	10003.7600	10015.6617	100.12	99.4	0.7
	100.0%		9925.1271	99.21		
	100.0%		9876.3901	98.73		
03	150.0%	15005.6400	15539.1301	103.56	105.0	3.4
	150.0%		15344.5398	102.26		
	150.0%		16363.2954	109.05		
Average					**100.3**	**NA**
%RSD					**4.2**	

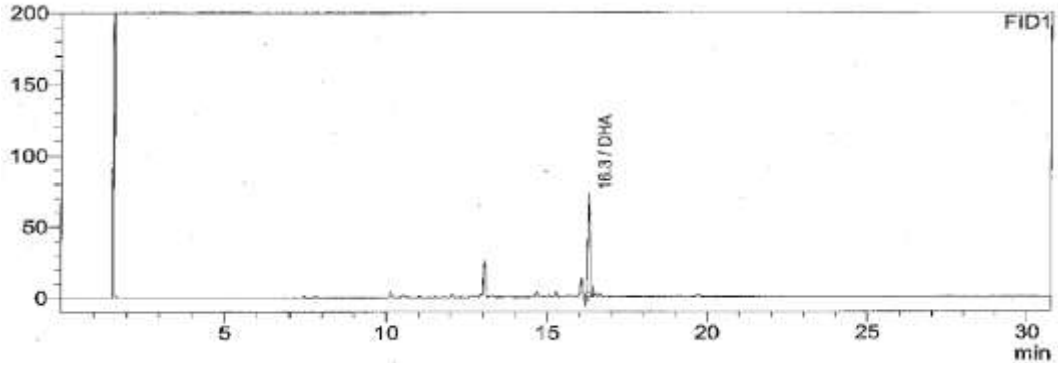


Figure 9: Chromatogram for 50% Accuracy.

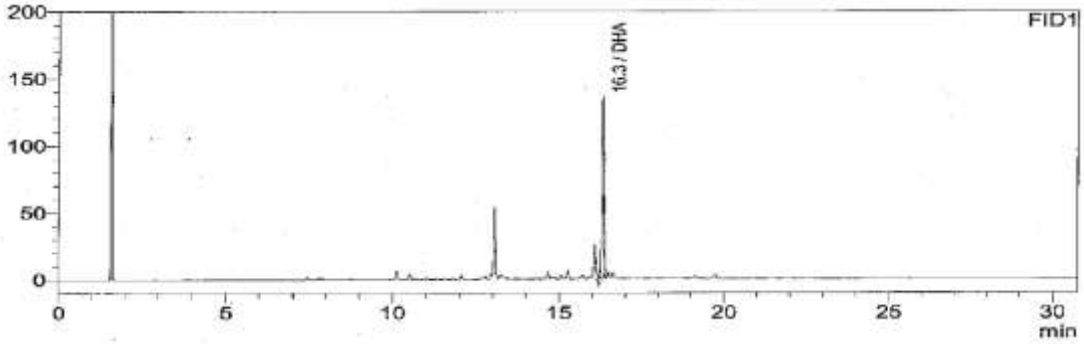


Figure 9: Chromatogram for 100% Accuracy.

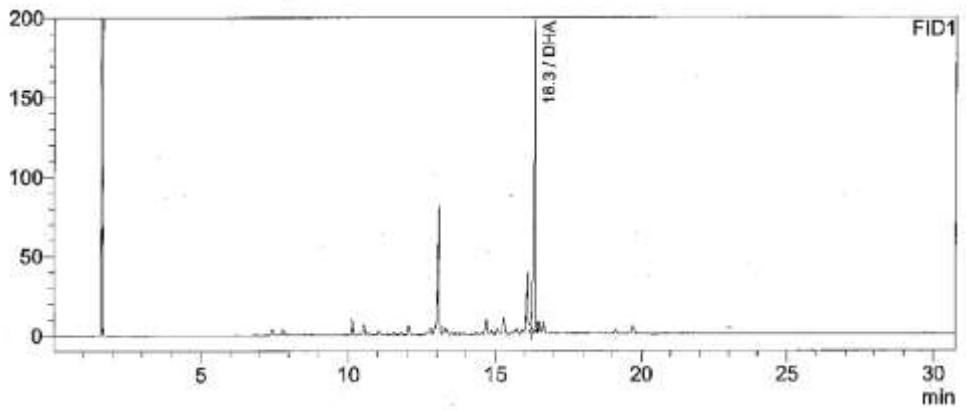


Figure 9: Chromatogram for 150% Accuracy.

Validation Parameter for Precision

Table 5: Results obtained for Method precision.

Preparation	% of content
1	102.68
2	102.79
3	100.75
4	102.19
5	101.24
6	102.04
Average	**101.9**
SD	**0.8078**
%RSD	**0.8**
Confidence limit	**0.6**

Validation parameter for Stability of analytical solution

Table 6: Results obtained for stability of analytical solution.

Time Intervals	Standard Area	Sample Area
Initial	577931	562867
12th Hour	542393	519301
24th Hour	564793	550054
36th Hour	559676	549155
48th Hour	607118	615814
Average	**570382**	**559438**
Std dev	**24164.8534**	**35336.9131**
% RSD	**4.2**	**6.3**

CONCLUSION

For the validation of docosahexaenoic acid content in surbex pregnancy capsules by gas chromatography was, accurate, and exact approach was validated. The retention times for docosahexaenoic acid were determined to be 16.3 minutes, respectively. There is no interference between blank and Placebo and having the retension time of 0.06. The

correlation coefficient of 0.99 & the % of y-intercept of 5.7. The method that was created was easy to use and cost-effective, making it suitable for routine analysis. This study of validation of docosahexaenoic acid content in surbex pregnancy capsules are validated according to the ICH guidelines.

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