



Medicinal Plants and Drugs Research Institute

Date: January 9, 2023

Subject of analysis:

Analysis of scorpion venom of *Mesobuthus eupeus*, *Androctonus crassicauda* and *Odontobuthus doriae* was performed using high performance chromatography (HPLC) and total protein examination of *Mesobuthus eupeus* scorpion was performed using Kjedal method.

Materials and Methods:

1-Appearance characteristics of scorpions.

Table1: List of scorpions to be analyzed with appearance features.

Name	Appearance Features	Picture
<i>Mesobuthus eupeus</i>	<i>Mesobuthus eupeus</i> reach a size of 4 to 5 centimetres (1.6 to 2.0 in) in length. The entire body is yellow to yellowish brown. The dorsal segments (tergites) of the mesosoma often have longitudinal irregular stripes that are black to dark brown. They exhibit sexual dimorphism, the adult females being generally larger than males but have a lower number of pectinal teeth (16 to 23, as opposed to 22 to 28 in males)	
<i>Androctonus crassicauda</i>	<i>Androctonus crassicauda</i> is a generalist desert species, an Old World scorpion. Adults can vary in colour from a light brown to reddish to blackish-brown, to black. They can grow to over 10 centimetres (3.9 in) in length	
<i>Odontobuthus doriae</i>	<i>Odontobuthus doriae</i> can reach a length of about 65–70 millimetres (2.6–2.8 in). These medium-sized scorpions show a basic coloration ranging from yellow to pale yellow.	

2-Venom extraction.

Venom is obtained by electrical stimulation of the scorpion tarsi with standard stimulation set and cumulated in sterile glass container.

3-Keep and storage.

The venom is frozen at -80 °C and stored in chemical non effective (in contact with glass or pure metal alloy) containers, emergency alarm system.

4-Preparation of venom:

First, 3 mg of venom has prepared from each of the scorpions, and by dissolving the venom in water and 30 microliters for analysis, it has injected in the HPLC system for analysis. Also, 100 mg of *Mesobuthus eupeus* venom has tested by Kjeldal test, to determine the total protein.

5-Instrument:

- **HPLC System:**

After preparing the sample, scorpion venom peptides were finally isolated by HPLC model Knauer (Berlin, Germany) with gradient system Smartline equipped with PDA Detectors 2800. The sample was injected by autosampler, using a 100 microliter loop and injected 30 microliters of the sample. Column C18 (4.6 × 250 mm, 5 µm, 100 Å, Knauer (Berlin, Germany)) was used for separation. The gradient program consisted of solvent A (water and 0.1% trifluoroacetic acid) and solvent B (acetonitrile 0.1% trifluoroacetic acid) with a flow rate of 0.5 ml / min and chromatograms was recorded in the wavelength of 215 nm.

- **Kjeldahl device:**

The Kjeldahl method was performed according to method 981.10 of the AOAC International. Approximately 100 mg of raw material was hydrolyzed with 15 mL concentrated sulfuric acid (H₂SO₄) containing two copper catalyst tablets in a heat block (Kjeldahl system 2016 digester (Table 1), Tecator Inc., Herndon, VA, USA) at 420 °C for 2 h. After cooling, H₂O was added to the

hydrolysates before neutralization and titration. The amount of total nitrogen in the raw materials were multiplied with both the traditional conversion factor of 6.25 to determine total protein content.

Table 2: Technical features of Kjeldahl device

Measuring range	0.1~200 mgN
Nitrogen recovery	>99.5%
Sample weight	solid <5 g / liquid < 20 mL
Distillation speed	5 min/sample
Data storage capacity	1000 pieces
Cooling water consumption	1.5 L/min
Operation mode	Manual / Automatic
Display	Large LCD touch screen
Power	1.3 KW
Dimension (LxWxH)	455*391*730mm

The results:

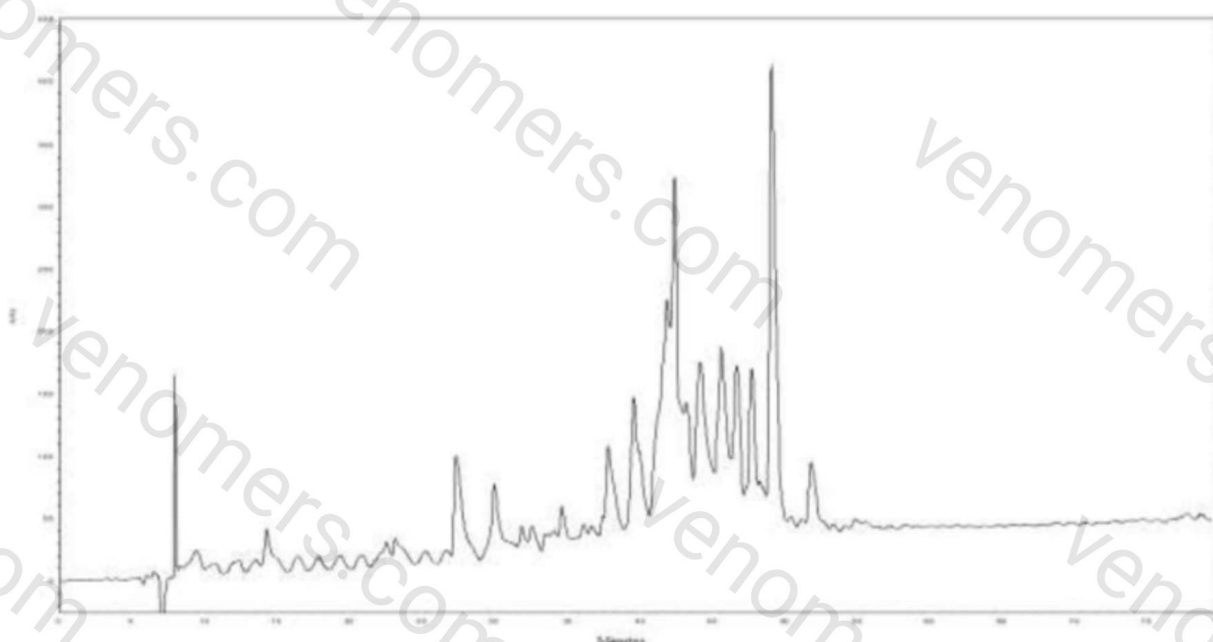


Figure 1: *Mesobuthus eupeus* scorpion HPLC chromatogram.

Table 3: Output data of *Mesobuthus eupeus* scorpion HPLC chromatogram.

Pk #	Retention Time	Area	Height	Width	Area Percent
1	0.883	121438	3513	0.80	0.065
2	1.617	325926	7071	0.83	0.174
3	2.350	491119	10444	0.80	0.261
4	3.400	691989	15379	0.82	0.368
5	3.983	871415	18025	0.82	0.464
6	5.033	1081399	23028	0.83	0.576
7	5.633	862314	26031	0.58	0.459
8	6.067	767844	30524	0.45	0.409
9	6.567	1378595	35733	0.80	0.734
10	7.600	820142	32559	0.65	0.437
11	7.983	2033888	194790	0.47	1.083
12	8.367	880780	41679	0.37	0.469
13	9.500	3933394	52133	1.47	2.094
14	10.850	3012642	40732	1.33	1.604
15	12.400	3408047	41379	1.55	1.814
16	13.583	2287986	40458	1.03	1.218
17	14.333	4633414	64298	1.83	2.467
18	16.533	2890627	39741	1.43	1.539
19	17.933	2772211	37823	1.45	1.476
20	19.417	2801130	37838	1.48	1.491
21	20.917	2540877	36228	1.38	1.353
22	22.633	3120078	44795	1.47	1.661
23	23.217	3388368	47537	1.57	1.804
24	25.350	2613366	34252	1.52	1.391
25	26.783	1830589	33436	1.03	0.975
26	27.417	6376683	108850	1.95	3.395
27	30.050	5301291	82885	1.95	2.822
28	31.117	1331633	35662	0.65	0.709
29	31.950	1650171	47140	0.70	0.878
30	32.667	2118231	46129	0.95	1.128
31	33.583	911963	39384	0.43	0.485
32	34.083	1368954	41499	0.58	0.729
33	34.650	2725846	60619	1.05	1.451
34	35.617	643821	34386	0.32	0.343
35	36.117	1742205	44142	0.75	0.927
36	36.683	1681980	42574	0.73	0.895
37	37.483	893933	49451	0.35	0.476
38	37.833	5547611	104954	1.42	2.953
39	39.600	4816258	142147	0.93	2.564
40	39.983	3325318	99243	0.77	1.770
41	41.900	12194809	218378	1.47	6.492

42	42.417	10886308	316171	0.90	5.795
43	43.233	4689243	133784	0.70	2.496
44	44.183	9980894	165325	1.45	5.313
45	45.650	8548867	176035	1.18	4.551
46	46.700	5751048	159784	0.85	3.062
47	47.750	5796596	155788	1.00	3.086
48	48.317	1979974	66054	0.55	1.054
49	49.133	13094445	396894	1.42	6.971
50	50.433	1322643	34388	0.70	0.704
51	51.217	1072158	32100	0.60	0.571
52	51.850	4158119	76782	1.65	2.214
53	53.383	983705	25591	0.72	0.524
54	54.233	852941	23468	0.65	0.454
55	54.883	1284298	27713	0.85	0.684
56	55.617	1151219	25051	0.83	0.613
57	56.433	803022	20326	0.68	0.427
58	57.233	1083602	19735	0.97	0.577
59	58.317	1125518	19689	1.02	0.599
60	59.083	796182	17754	0.77	0.424
61	60.150	960489	16874	0.97	0.511
62	60.900	828655	16167	0.88	0.441
63	61.917	837279	15218	0.95	0.446
64	62.800	734224	13919	0.92	0.391
65	63.800	683328	12662	0.93	0.364
66	64.800	693520	12045	1.00	0.369
67	65.683	687222	11623	1.05	0.366
68	66.667	607686	10520	1.03	0.324
69	67.733	459730	9441	0.85	0.245
70	68.750	612766	9495	1.17	0.326
71	69.767	448736	8478	0.95	0.239
72	70.700	401116	7448	0.98	0.214
73	71.750	344349	6597	0.95	0.183
74	72.750	396572	7147	1.10	0.211
75	73.767	206385	6300	0.60	0.110
76	74.117	139387	5936	0.42	0.074
77	74.750	291145	6116	0.97	0.155
78	75.733	198486	4673	0.93	0.106
79	76.783	155675	4613	0.78	0.083
80	77.817	395507	7151	1.32	0.211
81	78.800	213488	5952	1.03	0.114
Totals		187846812	4277676		100

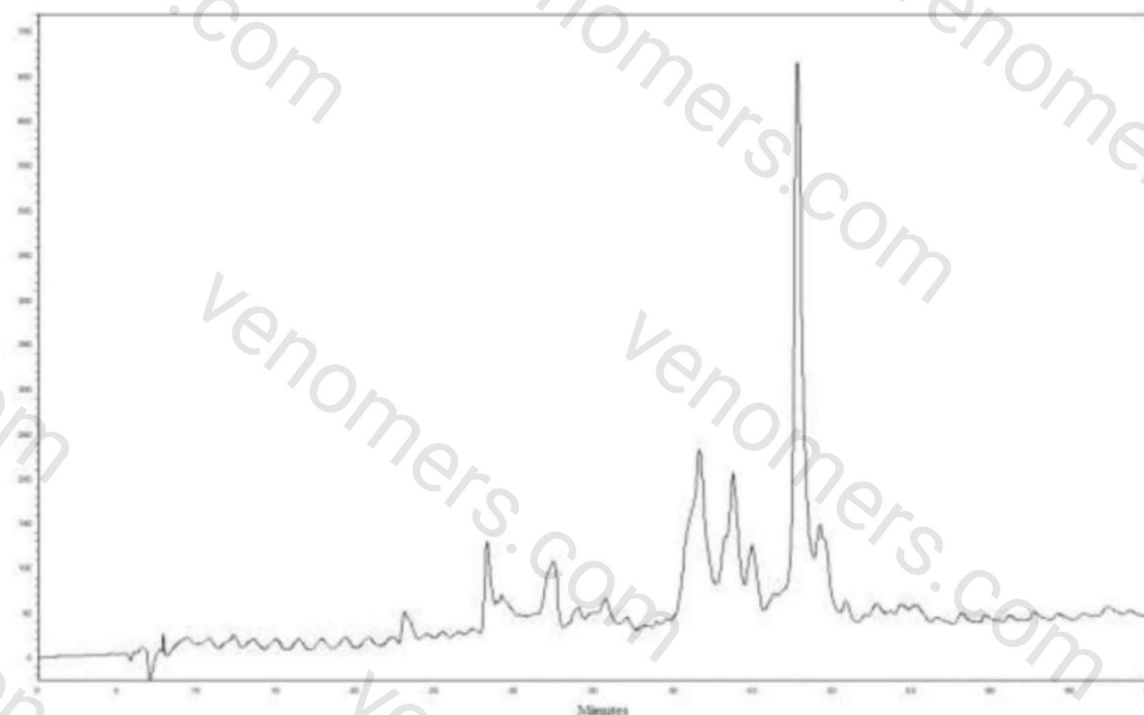


Figure 2: *Androctonus crassicauda* scorpion HPLC chromatogram.

Table 4: Output data from the device software according to the *Androctonus crassicauda* scorpion chromatogram

Pk #	Retention Time	Area	Height	Width	Area Percent
1	0.667	126709	3350	0.85	0.065
2	1.517	316157	7728	0.78	0.163
3	2.283	523596	11486	0.82	0.270
4	3.150	721279	15758	0.82	0.372
5	3.967	908069	19913	0.80	0.469
6	4.700	1209237	23350	0.88	0.624
7	5.533	1152580	27233	0.75	0.595
8	6.133	761382	31101	0.45	0.393
9	6.567	1470577	38771	0.78	0.759
10	7.750	1208932	37405	0.78	0.624
11	7.933	573477	55397	0.23	0.296
12	9.450	4816268	48731	1.92	2.486
13	10.800	3474071	46755	1.38	1.794
14	12.383	3648924	48665	1.48	1.884
15	13.633	3286395	42615	1.47	1.697
16	15.033	2882295	41150	1.37	1.488
17	16.483	2924720	39376	1.48	1.510
18	17.933	2698877	38125	1.40	1.393
19	19.400	2831903	37757	1.50	1.462
20	20.900	2781219	36823	1.50	1.436
21	22.383	2277776	35537	1.22	1.176
22	23.133	3434397	63230	1.23	1.773

23	24.600	1947087	36540	0.95	1.005
24	25.567	1997841	37303	0.97	1.031
25	26.600	1977874	36015	0.97	1.021
26	27.517	2029525	39510	0.92	1.048
27	28.350	4952728	134454	1.02	2.557
28	29.000	807227	68604	0.20	0.417
29	29.267	4500304	74237	1.18	2.323
30	30.467	1320642	51296	0.43	0.682
31	32.550	9891171	108976	2.45	5.106
32	34.100	3706515	55604	1.37	1.914
33	35.083	1986585	49174	0.70	1.026
34	35.750	4123232	63654	1.40	2.129
35	37.067	2280139	41605	1.08	1.177
36	38.267	1705915	31673	0.95	0.881
37	38.950	1095497	34638	0.57	0.566
38	41.650	21523582	223380	3.48	11.112
39	43.767	13000172	195610	1.78	6.711
40	44.950	5925674	113425	1.23	3.059
41	46.400	2696771	58571	0.88	1.392
42	47.833	29634951	650560	2.27	15.299
43	49.233	8308725	131372	1.68	4.289
44	50.883	2084630	44779	1.07	1.076
45	52.100	955073	27533	0.65	0.493
46	52.817	2198008	39781	1.10	1.135
47	53.667	1017645	30508	0.58	0.525
48	54.400	1818915	36845	0.92	0.939
49	55.233	2264889	35516	1.35	1.169
50	56.633	1463321	20527	1.50	0.755
51	58.150	1462339	23015	1.52	0.755
52	59.700	1317977	18818	1.55	0.680
53	61.233	1088161	17492	1.37	0.562
54	62.767	1387262	18918	1.72	0.716
55	64.300	1017364	15491	1.42	0.525
56	65.833	919390	13895	1.32	0.475
57	67.383	1614664	20731	1.78	0.834
58	68.767	966947	14277	1.48	0.499
59	70.383	660066	9133	1.48	0.341
60	71.950	337435	7014	0.88	0.174
61	72.317	238002	6379	0.68	0.123
62	73.417	289390	5909	0.87	0.149
63	73.883	146901	5295	0.48	0.076
64	74.317	50789	4707	0.18	0.026
65	74.967	189706	4800	0.68	0.098
66	75.567	151863	4328	0.58	0.078
67	75.900	66532	3904	0.28	0.034
68	76.500	148069	3794	0.65	0.076
69	77.267	160694	3636	0.75	0.083
70	77.433	126978	3424	0.83	0.066
71	78.917	118375	1881	1.52	0.061
Totals		193702382	3428787		100

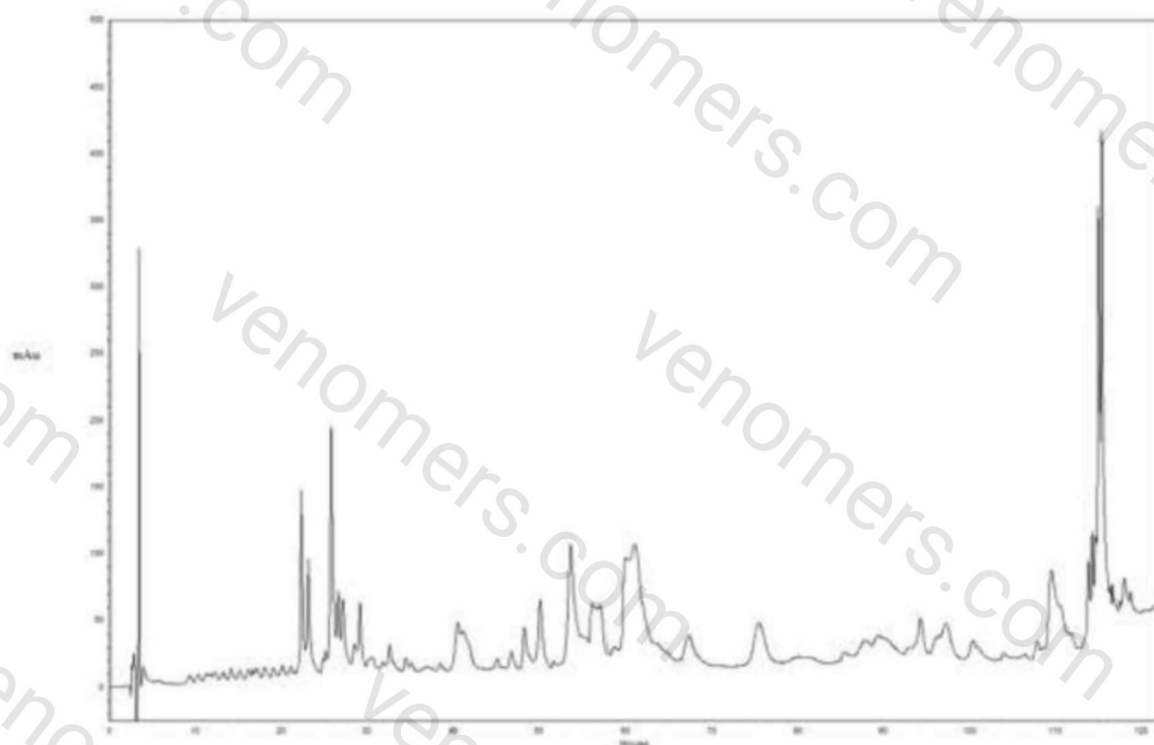


Figure 3: *Odontobuthus doriae* scorpion HPLC chromatogram.

Table 5: Output data from the device software according to the *Odontobuthus doriae* scorpion chromatogram.

Pk #	Retention Time	Area	Height	Width	Area Percent
1	0.217	6518	507	0.30	0.005
2	0.617	24585	1393	0.38	0.018
3	1.067	51625	2452	0.42	0.038
4	1.383	60056	3138	0.35	0.044
5	1.867	77758	4371	0.35	0.057
6	2.183	143437	5671	0.48	0.105
7	2.833	1971943	87353	0.60	1.443
8	3.467	2938074	435391	0.50	2.151
9	7.383	3071	440	0.22	0.002
10	7.817	6703	697	0.38	0.005
11	13.267	245322	9577	0.95	0.180
12	16.167	239940	10241	0.65	0.176
13	16.700	156416	10038	0.38	0.114
14	17.167	284754	11514	0.57	0.208
15	17.450	26016	3671	0.30	0.019
16	18.133	313877	11506	1.00	0.230
17	19.133	264852	10552	0.93	0.194

18	20.133	330271	12005	1.05	0.242
19	21.633	2384	281	0.28	0.002
20	22.350	3595610	198163	1.07	2.632
21	23.167	2541201	125676	1.25	1.860
22	25.200	83611	7829	0.32	0.061
23	25.817	4304504	221416	0.95	3.151
24	26.617	55828	11410	0.13	0.041
25	28.550	177084	10474	0.52	0.130
26	29.167	1062967	60125	0.83	0.778
27	32.600	62452	5502	0.25	0.046
28	34.550	194319	10087	0.60	0.142
29	35.167	29568	2163	0.37	0.022
30	38.517	217153	8244	0.98	0.159
31	39.200	380	149	0.10	0.000
32	40.567	507839	18922	0.93	0.372
33	45.133	281153	11116	1.55	0.206
34	45.900	9756	623	0.48	0.007
35	46.800	49303	2288	0.40	0.036
36	48.350	1315144	41937	1.40	0.963
37	50.167	697210	34036	0.73	0.510
38	56.200	95869	6233	0.28	0.070
39	57.200	129624	8443	0.43	0.095
40	61.033	63544	2713	0.52	0.047
41	64.133	33509	117	0.77	0.025
42	67.267	1983017	25236	4.27	1.451
43	70.533	5817	349	0.45	0.004
44	71.050	17650	627	0.78	0.013
45	71.750	7516	306	0.62	0.006
46	72.717	17999	805	0.62	0.013
47	73.117	21180	1121	0.37	0.016
48	75.483	4444114	41743	4.87	3.253
49	78.217	96616	5510	0.30	0.071
50	80.133	1071168	11481	2.03	0.784
51	80.517	249851	11477	0.37	0.183
52	81.050	1331821	11652	2.42	0.975
53	83.533	174696	6587	0.47	0.128
54	83.683	64716	6539	0.17	0.047
55	85.333	1797608	18557	2.55	1.316
56	87.850	3054306	28885	2.27	2.236
57	89.400	5984581	33868	3.73	4.381
58	92.800	1051177	25086	0.78	0.769
59	94.267	4051231	52243	2.13	2.965
60	97.250	7484839	51474	4.02	5.479

61	100.300	4742566	35077	3.63	3.471
62	103.333	610553	16091	0.65	0.447
63	103.967	1923640	24166	1.65	1.408
64	105.283	265476	17852	0.25	0.194
65	106.333	1987605	21154	1.73	1.455
66	107.850	1755410	35097	1.07	1.285
67	108.533	675659	27508	0.42	0.495
68	109.483	9532218	95136	2.52	6.977
69	111.400	3296674	43910	1.47	2.413
70	113.817	4393784	115398	1.40	3.216
71	114.283	2521467	137652	0.40	1.846
72	114.667	2404750	139539	0.32	1.760
73	114.967	7769678	419280	0.43	5.687
74	115.383	12367559	490196	1.05	9.053
75	116.383	1368963	94105	0.27	1.002
76	116.667	3603337	97371	0.78	2.638
77	117.450	1072588	76941	0.25	0.785
78	118.017	4956146	98829	1.02	3.628
79	118.750	5347377	80540	1.48	3.914
80	120.217	3327409	51120	1.15	2.436
81	121.350	543699	45625	0.20	0.398
82	121.567	3256066	45742	1.33	2.383
83	122.950	1469457	37756	0.77	1.076
84	123.817	973163	37169	0.52	0.712
85	124.167	514405	24581	0.43	0.377
86	124.683	373817	21384	0.45	0.274
Totals		136618599	4071229		100



Test Report

شرکت معیار دانش پارس

Laboratory Identification	Customer Identification	Manufacturer Identification
Name/Location : Meyar Danesh Pars	Name/Unit :	Name/Unit : Javad Atashi
Reception Code : 1400051374	Request Date : 2023 , 01, 09	Address : Isfahan
Report Code : 14-ENGLISH-1041	Address :	

Identification of Test Items

Name: Mezo Scorpion	Date of Manufacture:-	Test Start Date: 2021.08.12
Type: -	Sampling Date: -	Test End Date: 2021.08.14
Sample/Product Brand:	Serial/Seal No: -	Expire Date: -
Weight/Volume of Samples:-	Reception Date: 2021.08.12	
Status of Samples:-		

Test Results

No.	Test name/sample code	Measurand	Result	Unit	Uncertainty	Analysis Method	Ref. Standard	Specification Limit
1	Mezo Scorpion	Protein	91.7	g/100	-	Kjeldahl Method	-	
*	*	*	*	*	*	*	*	*
*	*	*	*	*	*	*	*	*
*	*	*	*	*	*	*	*	*
*	*	*	*	*	*	*	*	*
*	*	*	*	*	*	*	*	*
*	*	*	*	*	*	*	*	*
*	*	*	*	*	*	*	*	*
*	*	*	*	*	*	*	*	*
*	*	*	*	*	*	*	*	*

Remarks :

- 1-The results relate only to the tested items. 2-The test report shall not be reproduced except in full. 3-The sample is store in laboratory for two month.
4-The protest period for the test answer is a maximum of 10 days

Validation Unit	Analyzed By	Reviewed By	Approved By	Laboratory Stamp
Name	Atefe Vali Khani	Nilofar Nokhodian	Dr Mahdy Nabi	
Responsibility	Expert	Technical Manager	Top Manager	
Sing & Date	2021.08.15	2021.08.15	-	

page 1 of 1

دفتر مرکزی: اصفهان، خیابان جی شرقی، ارتفاعیه، بلوار دانشگاه، ساختمان پژوهشگاه دانشگاه آزاد اصفهان (خوراسگان)
تلفن: ۰۲۱-۹۵۲۴۰۸۸ - ۰۲۱-۳۵۳۵۲۱۴۶ - فکس: ۰۲۱-۳۵۳۵۴۱۵۹ - صندوق پستی: ۸۱۵۹۵ - ۱۵۸

info@meyardanesh.com

www.meyardanesh.com

www.meyarisp.com

Prof.: Alireza Ghassempour