

תאריך: 03.07.2016

אל: יונה גבריאלי – Allied Bionutrition Corporation

מאת: יגאל גוזלן - אנליטיקה מיוחדת - FCAD

תמי – מכון מחקר ופיתוח

ת.ד. 10140, מפרץ חיפה 26111

טל: 04-8469572, פקס: 04-8469320

דו"ח אנליטי מס' 14-BIN0001

עבור: אנליזה כמותית של גלברידין וגליצריזין בתוספי תזונה

JM169A/16	מספר הזמנת רכש:
98202	מספר הזמנת תמי:
TS-01.00735	מספר WBS:
קפסולות	אפיון דוגמאות:
3	מספר דוגמאות:
02.06.16	תאריך הגעת הדוגמאות:
16.06.16	תאריך הגעת הסטנדרטים:
19-27.06.16	תאריכי ביצוע:
LC/UV/MS יוליה קילשטוק, כרמלה כרמי	מבצע אנליזה:
יוליה קילשטוק, כרמלה כרמי	כותב דו"ח:

***הצהרה:** תוצאות האנליזה מתייחסות אך ורק לדוגמאות שנמסרו לבדיקה.

Analytical Report

Food additives samples were analyzed by LC/UV/MS in order to quantify Glycyrrhizic acid (GLYC) and Glabridin (GLAB) versus standards supplied by Sigma aldrich (table 1).

The GLYC was determined according to the MS detector and the GLAB was determined according to the UV detector (table 2).

Calculations were also carried out for the average actual amount of GLYC and GLAB in one capsule (table 3).

Picture of the three samples packages (dissolved in MeOH) are appeared in appendix.

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(C=0.3mg/ml, m/z=823.4111 and λ =252nm)

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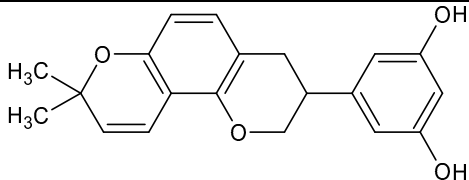
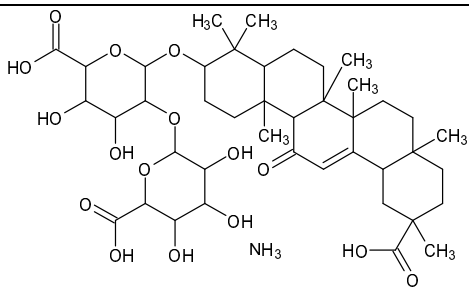
Fig. 3: LC/MS (ESI+) chromatograms of **Glycyrrhizic acid** (m/z=823.4111) in samples

Fig. 4: LC/UV/MS (ESI+) representative chromatograms of **Glabridin** STD
(C=0.04mg/ml, m/z=325.1434 and λ =280nm)

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Table 1: Standards information

<u>No.</u>	<u>Sample name</u> <u>(description)</u>	<u>TAMI</u> <u>sample</u> <u>name</u>	<u>Molecular structure</u>	LC/MS Q-TOF Data File name: LMS4564\ ESI_Pos_MS.D
1	Glabridin (GLAB), 99.3% STD - Sigma Product#G9548-25MG Batch#016M4741V (light pink powder)	LMS4574 2016-98202/4	 Molecular Formula: $C_{20}H_{20}O_4$ Monoisotopic Mass: 324.136159 Da	25, 42-43, 72
2	Glycyrrhizic acid ammonium salt (from Glycyrrhiza root) (GLYC), 99.4% STD - Sigma Product#50531-10GR Batch#BCBR3181V (white powder)	LMS4575 2016-98202/5	 Molecular Formula: $C_{42}H_{65}NO_{16}$ Monoisotopic Mass: 839.430335 Da	26, 45-46

Sub directory at LC/MS Q-TOF (critic#32-150-08): D:\MassHunter\DATA\LMS4564\ESI_Pos_MS

Table 2: Samples information and LC/UV/MS quantitative results of **Glycyrrhizic acid** and **Glabridin**

<u>Sample No.</u>	<u>Sample name</u>	<u>Glycyrrhizic acid Assay (by MS)</u>	<u>Glabridin Assay (by UV)</u>	<u>TAMI sample name</u>		LC/MS Q-TOF Data File name: LMS4564\ESI_Pos_MS.D
1	BN1 LicoRich Lot#16040091 SUPP Exp. 04/2019 Each capsule weight 555mg	0.34%	0.82%	LMS4564	2016-98202/1	8, 48-49, 71
2	BN2 THE123 LICORICH+ Lot#B1214K16 Exp. 12/2016 Each capsule weight 620mg	0.47%	<1.0 ppm	LMS4565	2016-98202/2	12, 36-37
3	BN3 DEGLYCYRRHIZED LICORICH Root extract – SOLGAR Batch#956106-03 Exp. 09/2018 Each capsule weight 583mg	0.40%	0.072%	LMS4566	2016-98202/3	16, 51-52, 70

Sub directory at LC/MS Q-TOF (critic#32-150-08): D:\MassHunter\DATA\LMS4564\ESI_Pos_MS

Table 3: Samples information and calculated results of **Glycyrrhizic acid** and **Glabridin** in one capsule

<u>Sample No.</u>	<u>Sample name</u>	Number of opened capsules	Total content weight (gr)	Average weight of one capsule (mg)	Glycyrrhizic acid calculated of average amount in one capsule (mg)	Glabridin calculated of average amount in one capsule (mg)
1	BN1 Each capsule weight 555mg	14*	6.08972	435.0	1.5	-
		14**	6.26647	447.6	-	3.6
2	BN2 Each capsule weight 620mg	12*	5.95109	495.9	2.3	-
3	BN3 Each capsule weight 583mg	12*	5.88463	490.4	2.0	-
		12**	6.14738	512.3	-	0.37

* preparation one, ** preparation two

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Fig. 1: LC/UV/MS (ESI+) representative chromatograms of **Glycyrrhizic acid** STD
(C=0.3mg/ml, m/z=823.4111 and λ =252nm)

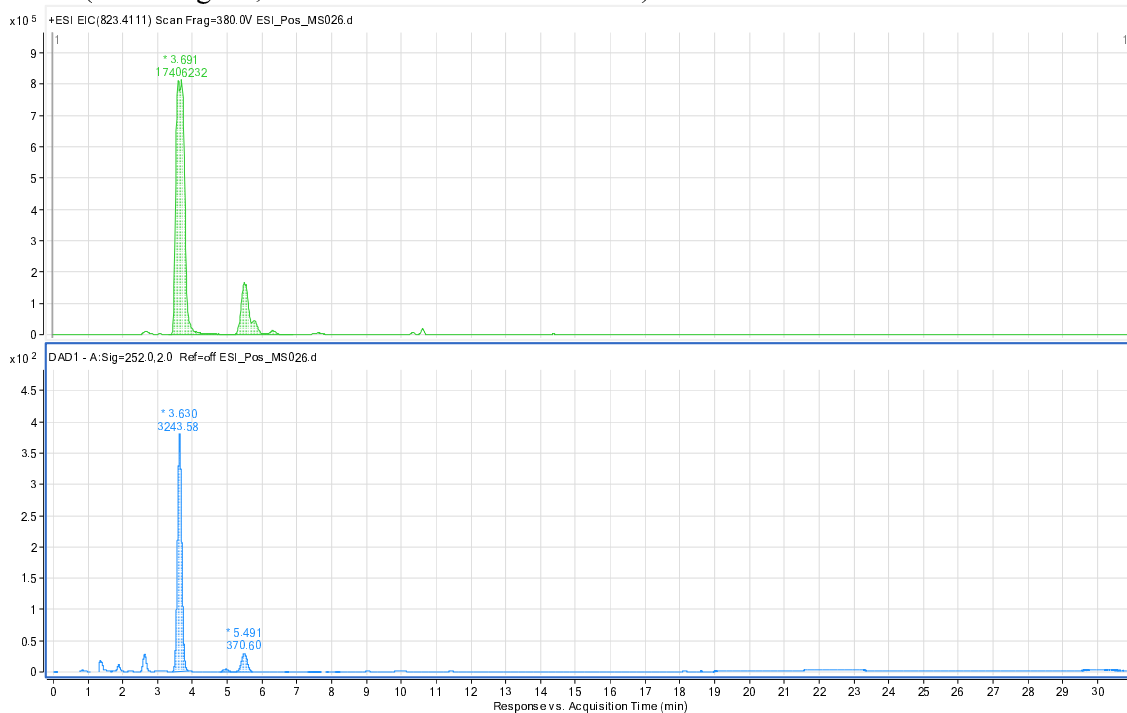


Fig. 2: LC/MS (ESI+) spectrum of **Glycyrrhizic acid**

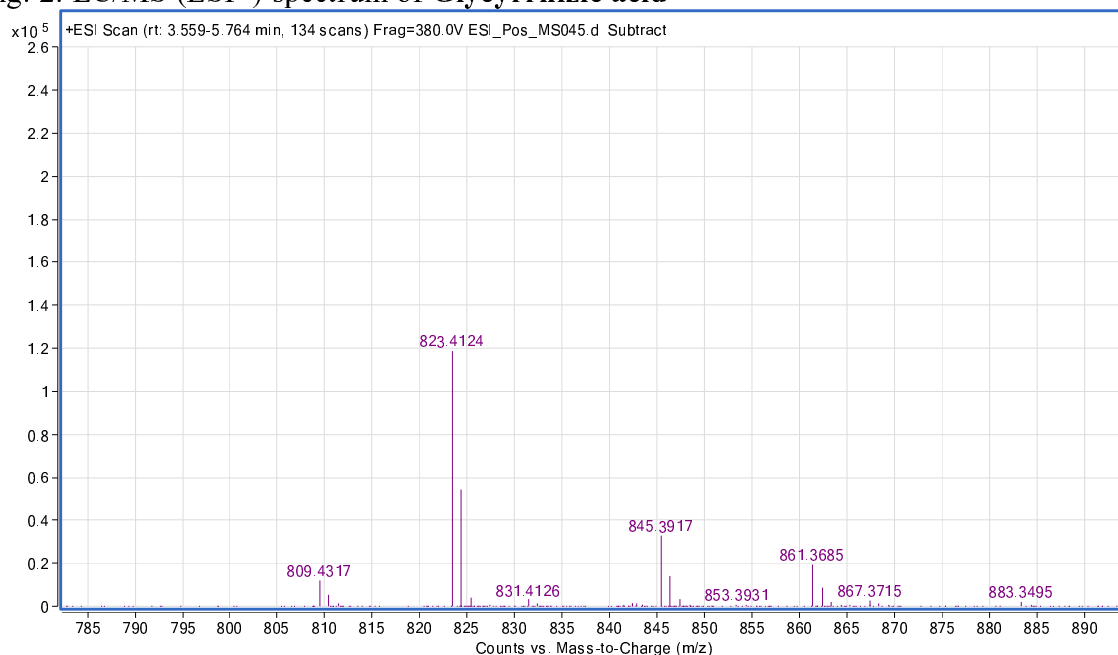
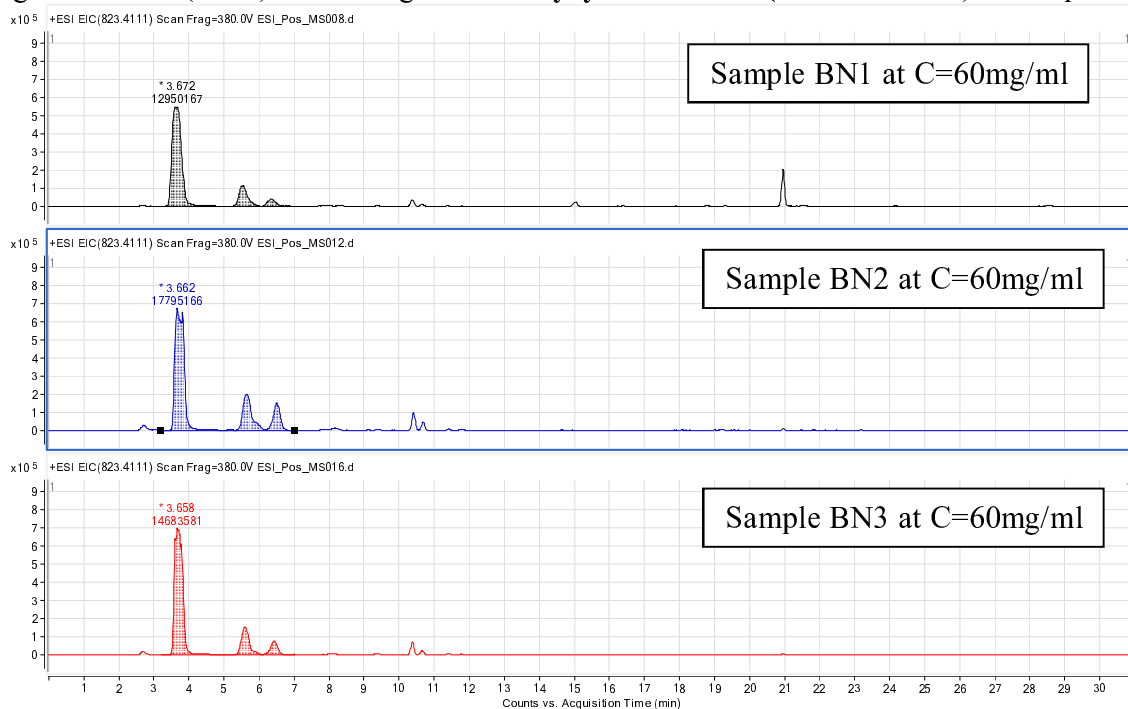


Fig. 3: LC/MS (ESI+) chromatograms of **Glycyrrhizic acid** ($m/z=823.4111$) in samples



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Fig. 4: LC/UV/MS (ESI+) representative chromatograms of **Glabridin** STD
(C=0.04mg/ml, m/z=325.1434 and λ =280nm)

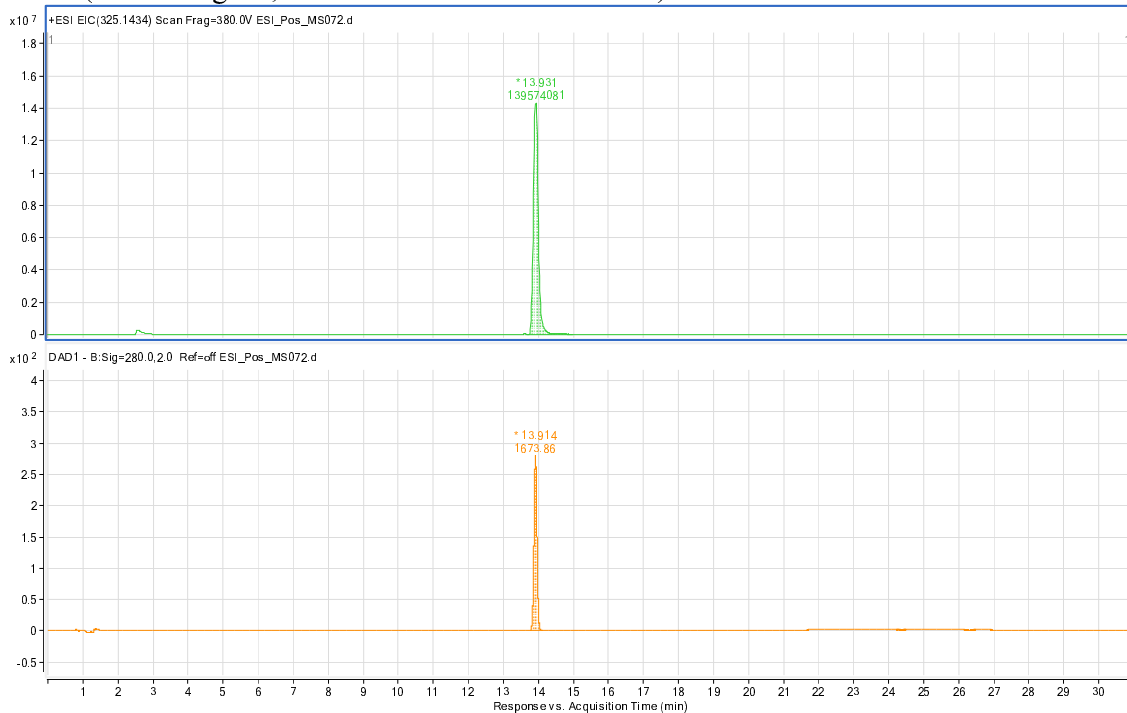


Fig. 5: LC/MS (ESI+) spectrum of **Glabridin**

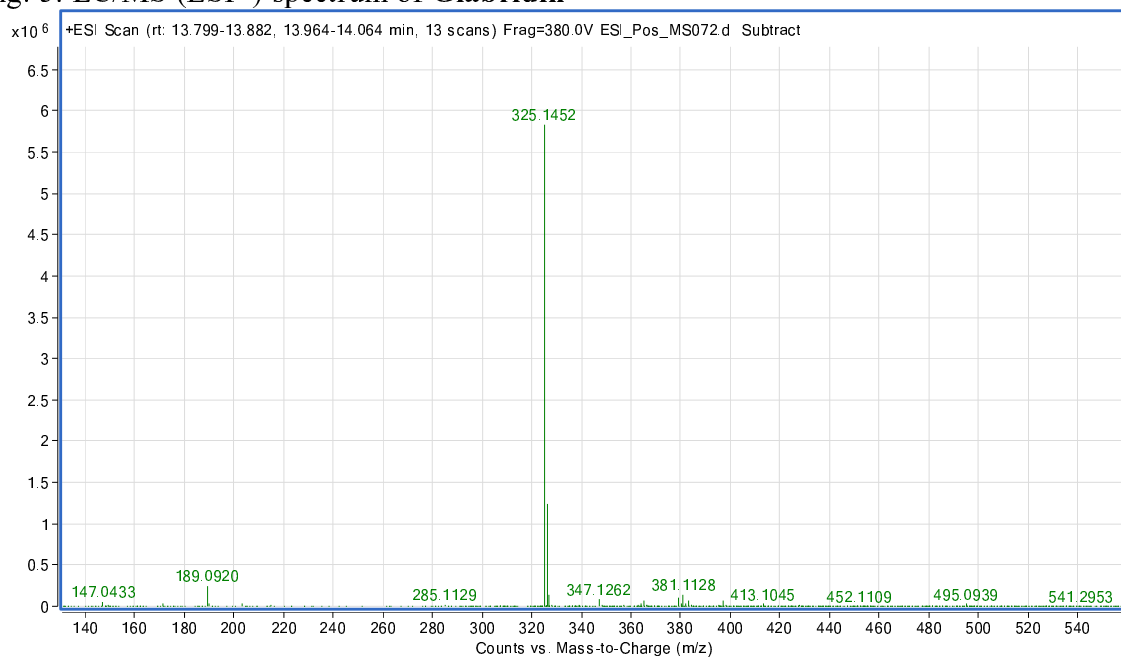
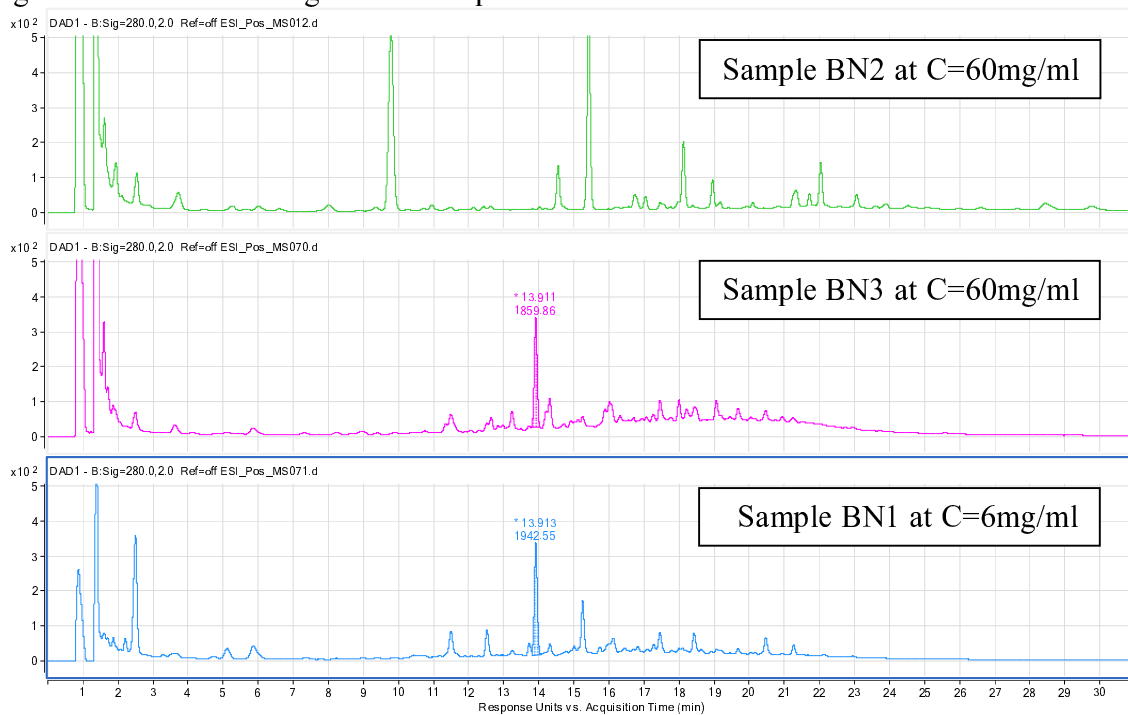


Fig. 6: LC/UV chromatograms of samples at $\lambda=280$ nm



Appendix

Picture of the three samples packages (dissolved in MeOH)



I. Gozlan Ph.D. – Head of Analytical Instrumentation Department

Signature: 