

**Analytical Test Report****Determination of Protodioscin in Samples Submitted by Thermolife by HPLC**

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1. OBJECTIVE

To determine the amount of protodioscin in samples submitted by Thermolife by HPLC.

2. SUMMARY

Nine samples of finished products submitted by Thermolife over a 3 month period were assayed for protodioscin using an HPLC method based on the work of Ganzera et al. (J. Pharm. Sci. 90(11) 2001, 1752-1758). UV detection at 205 nm was used, as an evaporative light scattering detector (ELSD) specified in the paper was not available. The labels on all products indicated they contained *Tribulus terrestris* extracts. The results from the analysis of these samples are presented in Table 1.

TABLE 1

Manufacturer	Product Name	Lot #	Dosage Form	Result
AST Sports Science	Trioxalon 500	202086857	Capsules	ND
EAS	EcdyMax	2G09-3	Liquid	ND
Optimum	Tribulus 625	206049	Capsules	ND
VPX Sports	Paradeca XS 357	1604	Liquid	ND
MRM	TribuPlex 750	32427	Capsules	1.72 mg/cap
MuscleTech	Anotesten	002071	Capsules	1.9 mg/cap
Sopharma	Tribestan	60202	Tablets	33.4 mg/tablet
Biotest	Tribex 500	022071	Capsules	35.7 mg/cap
Thermolife	Tribosten	7728	Tablets	73.3 mg/tablet

ND = None Detected

3. SAMPLES

1. EAS EcdyMax liquid, lot 2G09-3 (control #U1274)
2. Sopharma Tribestan tablets, lot 60202 (control #U1300)
3. Optimum Tribulus 625 capsules, lot 20649 (control #U1302)
4. VPX Sports Paradeca XS 357 liquid, lot 1604 (control #U1303)
5. Biotest Tribex 500 capsules, lot 022071 (control #U1304)
6. MuscleTech Anotesten capsules, lot 002071 (control #U1305)
7. Thermolife Tribosten tablets, lot 7728 (control #U1328)
8. Metabolic Response Modifiers (MRM) TribuPlex 750 capsules, lot 32427 (control #U1329)
9. AST Sports Science Trioxalon 500 capsules, lot 1161-0601 (control #U1375)

4. **STANDARD**

Protodioscin, ChromaDex lot #00-16310-101 (control 1340 and 1365)

5. **REAGENTS**

Acetonitrile – HPLC Grade, Pharmco lot #3001039-ACN
Water – Filtered RO/DI Water

6. **EQUIPMENT**

Dionex Summit HPLC System
Ohaus 250D Analytical Balance
Branson 3210 Ultrasonication Bath
Drucker Benchtop Centrifuge
Labline Multi-wrist Shaker
100- and 10- mL volumetric flasks
2- and 4- mL volumetric pipets
Assorted glassware
High purity C18 HPLC Column, 4.6 x 150 mm, 5 µm particle size

7. **METHODOLOGY**

Preparation of Standard

About 5 mg of protodioscin is weighed and transferred into a 10- mL volumetric flask. The standard is dissolved and diluted to volume with 10:90 acetonitrile/water (v/v). Final Stock concentration is approximately= 0.5 mg/mL.

Linearity Standard 1

4 mL of the Stock standard is pipetted into a 10- mL volumetric flask and diluted to volume with 10:90 acetonitrile/water. Linearity Standard 1 concentration is approximately 0.2 mg/mL.

Linearity Standard 2

2 mL of the Stock standard is pipetted into a 10- mL volumetric flask and diluted to volume with 10:90 acetonitrile/water. Linearity Standard 2 concentration is approximately 0.1 mg/mL.

7. **METHODOLOGY** (continued)**Preparation of Samples**Tablets

For tablet dosage forms, 20 whole tablets of each sample were weighed. The tablets were ground to a fine powder, and an amount of powdered tablet equivalent to 500 mg of tribulus extract (based on label claim) was then weighed into a 100- mL volumetric flask. About 50 mL of 10:90 acetonitrile/water was added to the flask, and the flask was shaken for 15 minutes, and then sonicated for 15 minutes. 10 mL of this solution was pipetted into a 50- mL volumetric flask and diluted to volume with 10:90 acetonitrile/water. An aliquot was centrifuged for 10 minutes, and a portion of the supernatant was transferred to an HPLC autosampler vial. The tablet sample preparation data is presented in Table 2.

TABLE 2 – Tablet Data

Name	Label Claim (mg Tribulus/Tablet)	Tablet Weight (g)	Sample Weight (g)
Sopharma Tribestan	250	0.6599	1.30891
Thermolife Tribosten	250	0.6776	1.38747

Capsules

For capsule dosage forms, 20 capsules of each sample were weighed. The contents of the capsules were then emptied and mixed, and the empty shells re-weighed. The average capsule fill weight was determined. An amount of the capsule fill material equivalent to about 500 mg of tribulus extract was then weighed into a 100- mL volumetric flask. About 50 mL of 10:90 acetonitrile/water was added the flask, and the flask was sonicated at ambient temperature for 10 minutes then diluted to volume with 10:90 acetonitrile/water. 10 mL of this solution was pipetted into a 50- mL volumetric flask and diluted to volume with 10:90 acetonitrile/water. An aliquot was centrifuged for 10 minutes, and a portion of the supernatant was transferred to an HPLC autosampler vial. The capsule sample preparation data is presented in Table 3.

TABLE 3 – Capsule Data

Name	Label Claim (mg Tribulus/cap)	Capsule Wt. (g)	Shell Wt. (g)	Fill Wt. (g)	Sample Wt. (g)
MRM TribuPlex 750	400	0.8934	0.1180	0.7754	1.03430
Optimum Tribulus 625	625	0.7902	0.1158	0.6744	0.54419
Biotest Tribex 500	250	0.7666	0.1230	0.6436	1.28718
Muscletech Anotesten	250	0.9395	0.1165	0.8230	1.64650
AST Trioxalon 500	500	0.94923	0.1127	0.8216	1.51360

Liquids

For liquid dosage forms, an amount of liquid sample equivalent to about one serving size was weighed into a 100- mL volumetric flask. About 50 mL of 10:90 acetonitrile/water was added the flask, and the flask was sonicated at ambient temperature for 10 minutes then diluted to volume with 10:90 acetonitrile/water. An aliquot was then filtered through a 0.45 µm PTFE syringe filter into an HPLC autosampler vial. The liquid sample preparation data is presented in Table 4.

TABLE 4 – Liquid Data

Name	Label Claim (mg Tribulus/dose)	Dosage Size (mL)	Sample Wt. (g)
VPX Sports Paradeca XS-357	Unknown	2	2.19055
EcdyMax	500	7.5	6.0283

HPLC

Column: High purity C18, 4.6 x 150mm, 5 µm particle size
(Zorbax Eclipse XDB-C18, Phenomenex Luna C18(2), and Nacalai
Cosmosil C₁₈-MS-II were used)

Mobile Phase: A – Water
B – Acetonitrile

Gradient: 20% B – 35% B over 15 minutes
35% B – 80% B over next 20 minutes
Hold at 80% B for 5 minutes

Flow Rate: 1.0 mL/min

Injection Volume: 25 µL

Detection: 205 nm

Column Temperature: 25° C

8. RESULTS

For each analysis, the correlation coefficient of the calibration curve was >0.9999, with an RSD of <2.0%, indicating very good linearity.

Duplicate injections of each sample solution were made. The results, in terms of % protodioscin in the samples, are listed in Table 5.

TABLE 5

Manufacturer	Product Name	Lot #	Result
AST Sports Science	Trioxalon 500	1161-0601	ND
EAS	EcdyMax	2G09-3	ND
Optimum	Tribulus 625	206049	ND
VPX Sports	Paradeca XS 357	1604	ND
MRM	TribuPlex 750	32427	1.72 mg/cap
MuscleTech	Anotesten	002071	1.9 mg/cap
Sopharma	Tribestan	60202	33.4 mg/tablet
Biotest	Tribex 500	022071	35.7 mg/cap
Thermolife	Tribosten	7728	73.3 mg/tablet

ND = None detected; estimated limit of detection is ~2 mg per capsule/tablet.

Figure 1
Protodioscin Standard Chromatogram – Stock Standard Solution

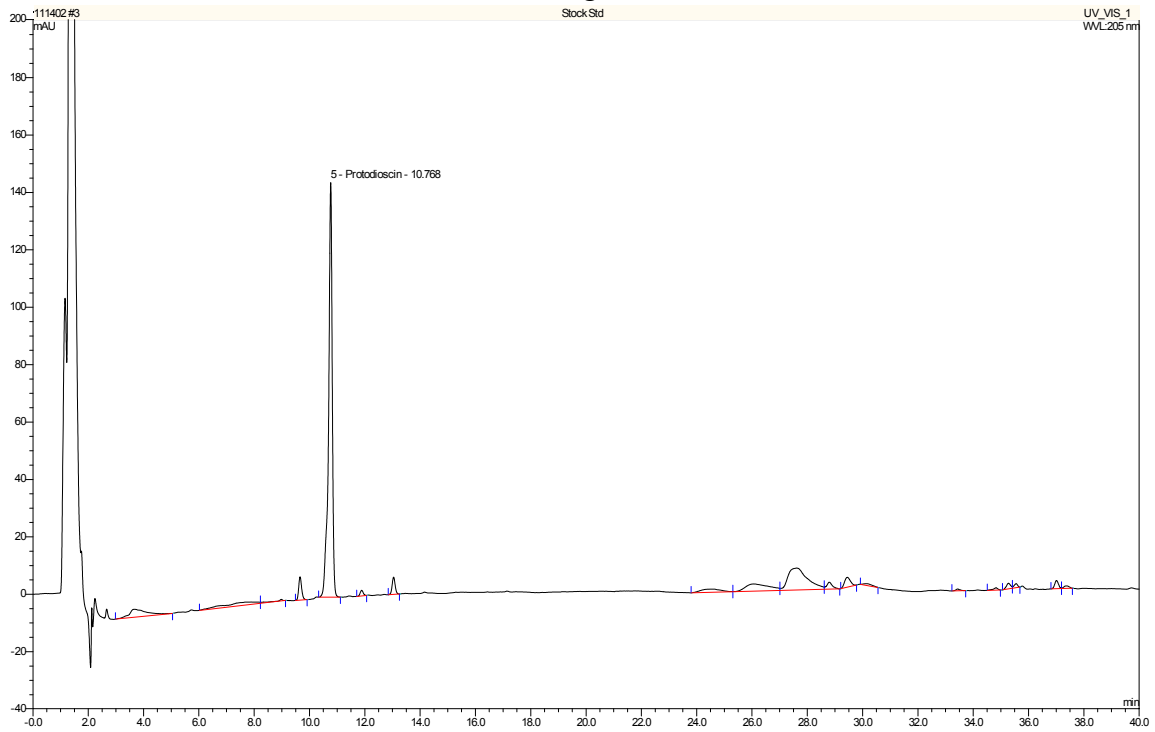


Figure 2
Sample Chromatogram – Thermolife Tribosten Lot 7728

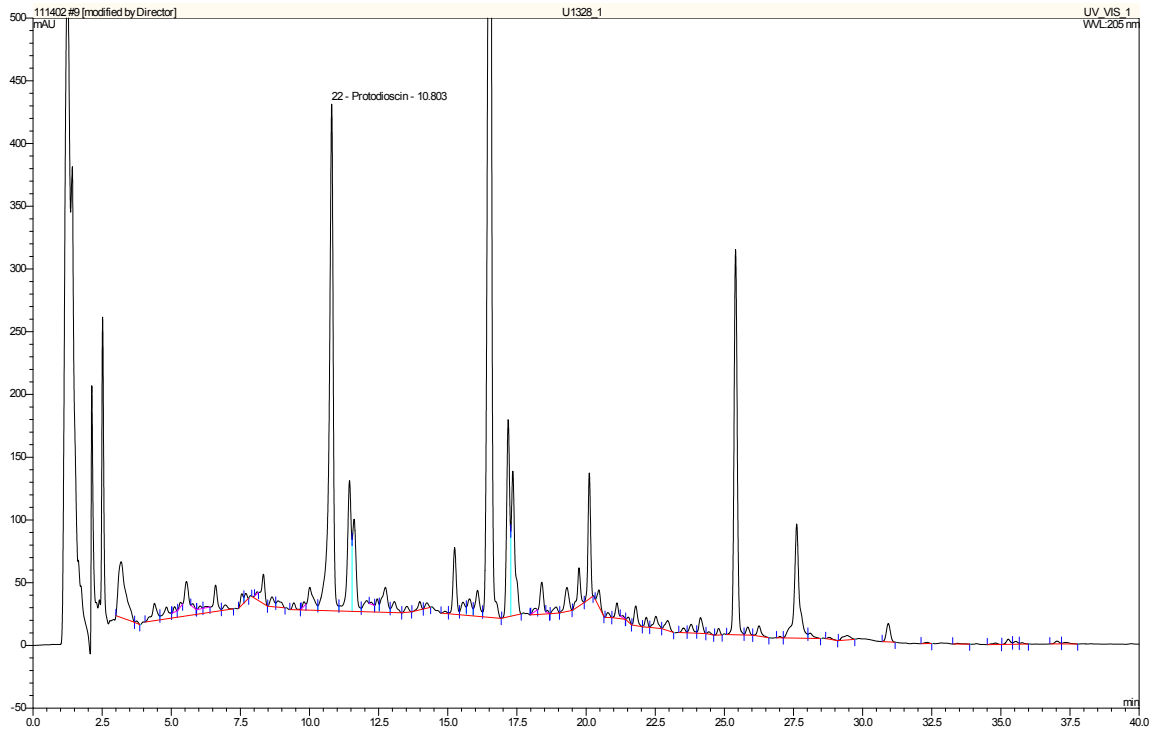


Figure 3
Sample Chromatogram – Biotest Tribex 500 Lot 022071

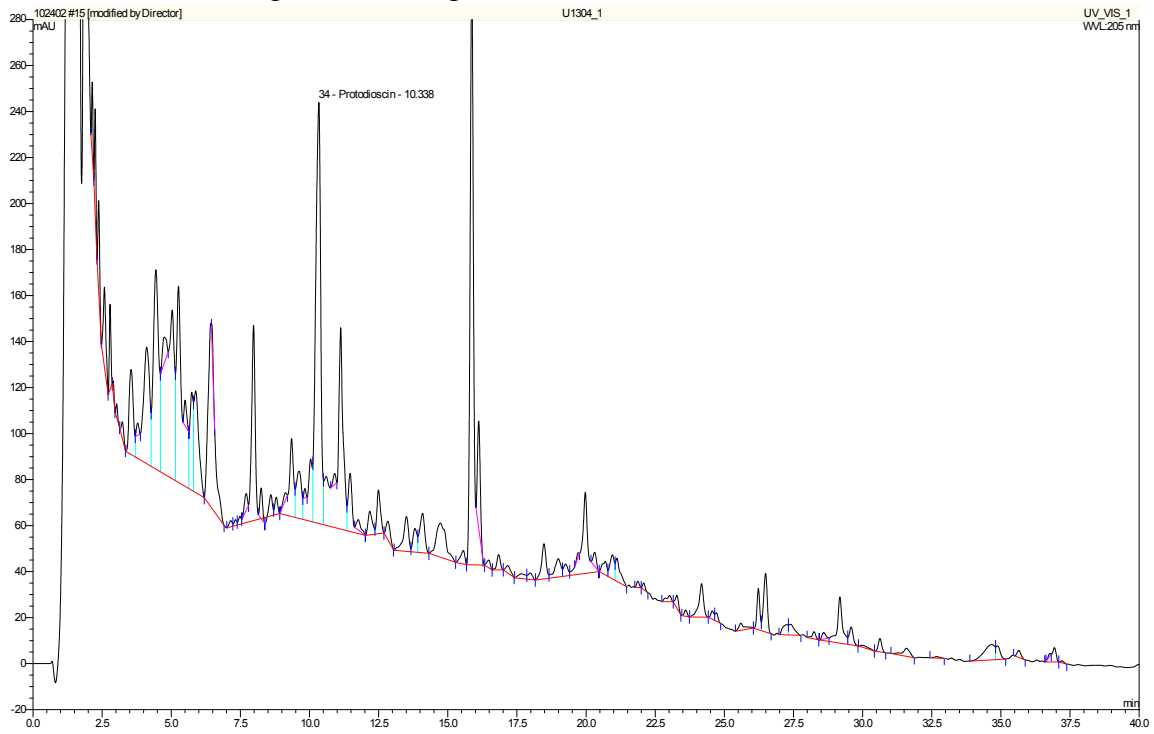


Figure 4
Sample Chromatogram – Sopharma Tribestan Lot 60202

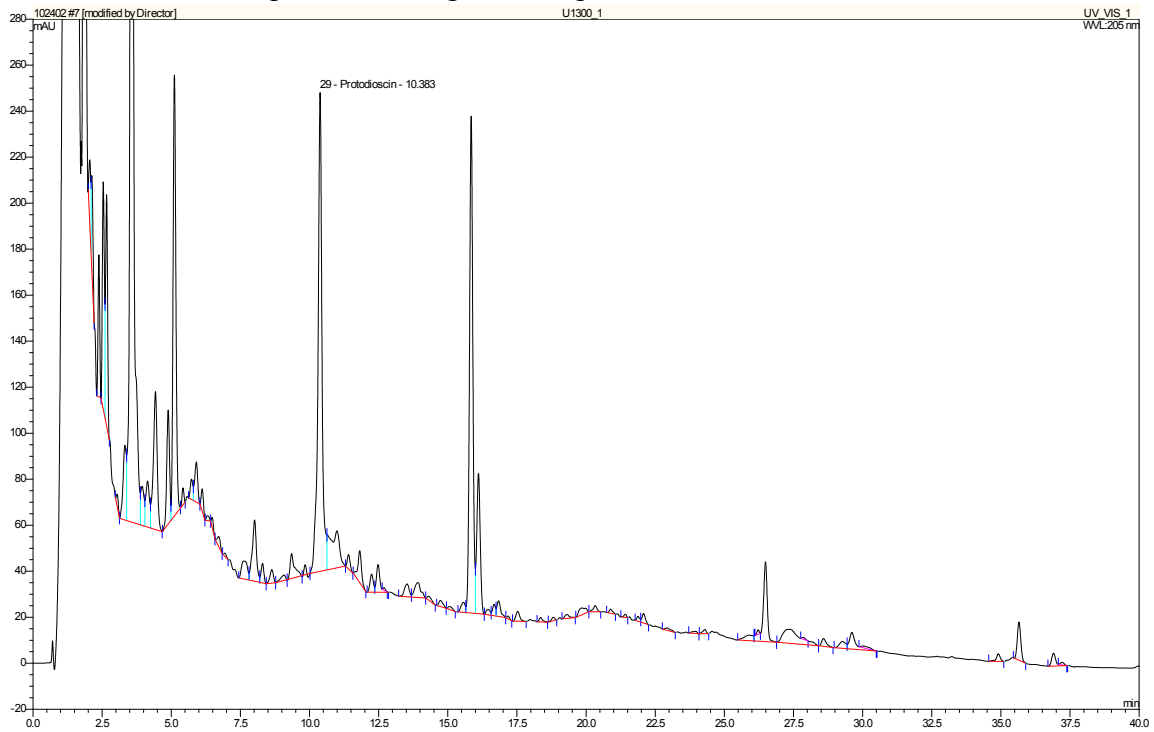


Figure 5
Sample Chromatogram – Muscletech Anotesten Lot 022071

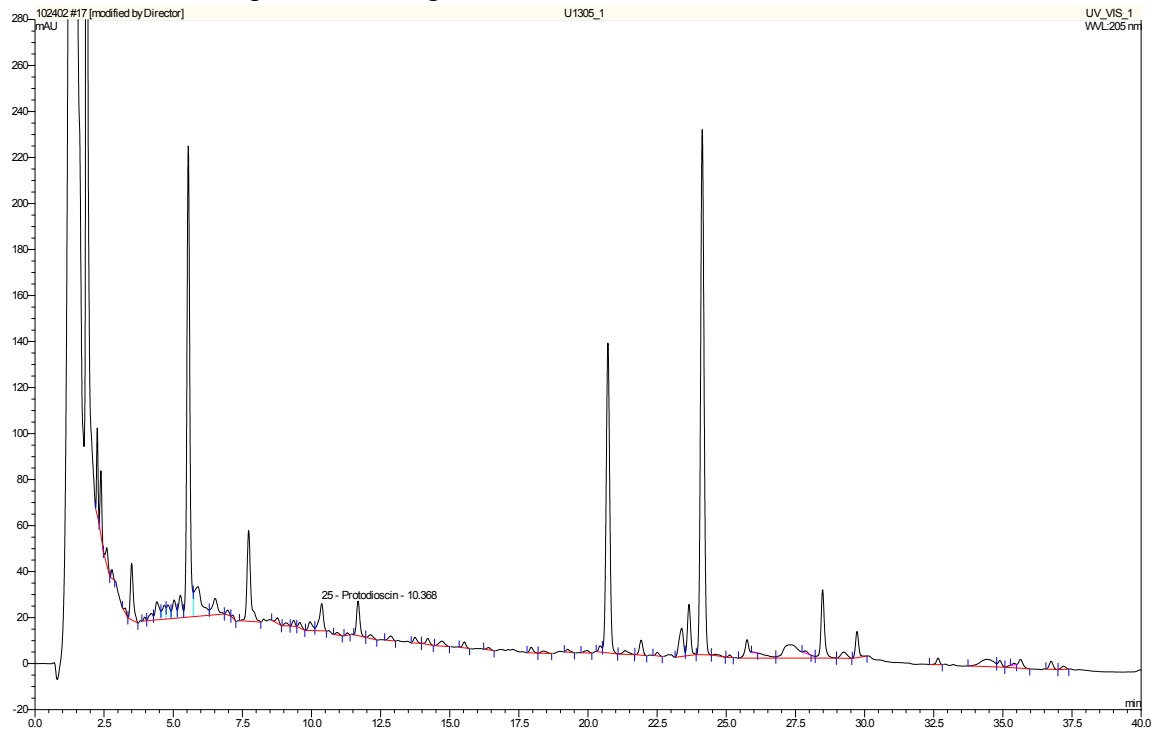


Figure 6
Sample Chromatogram – Optimum Tribulus 625 Lot 206049

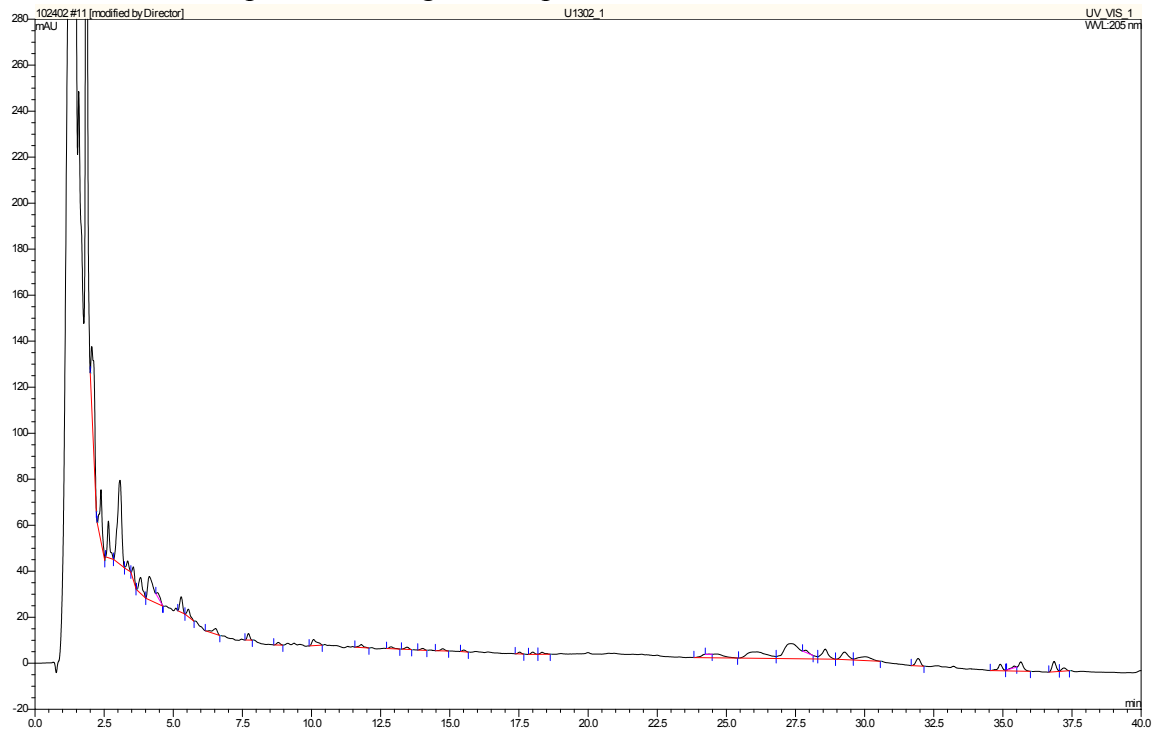


Figure 7
Sample Chromatogram – VPX Sports Paradeca XS 357 Lot 1604

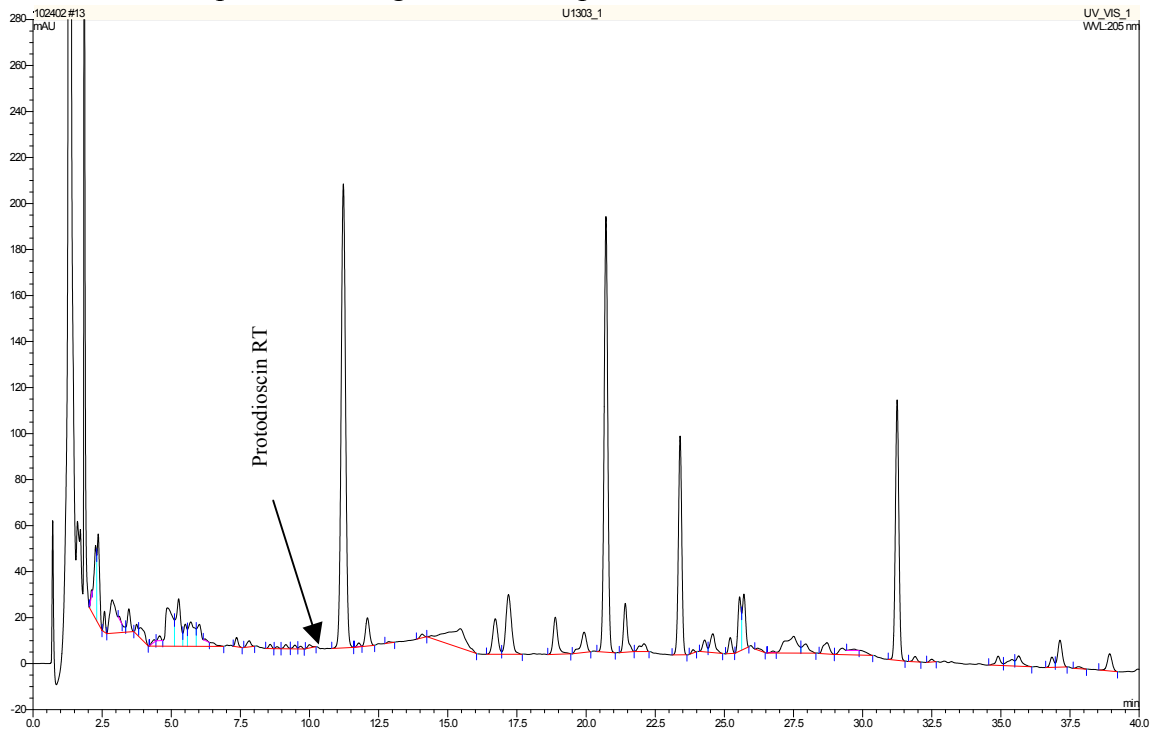


Figure 8
Sample Chromatogram – EAS Ecdymax Lot 2G09-3

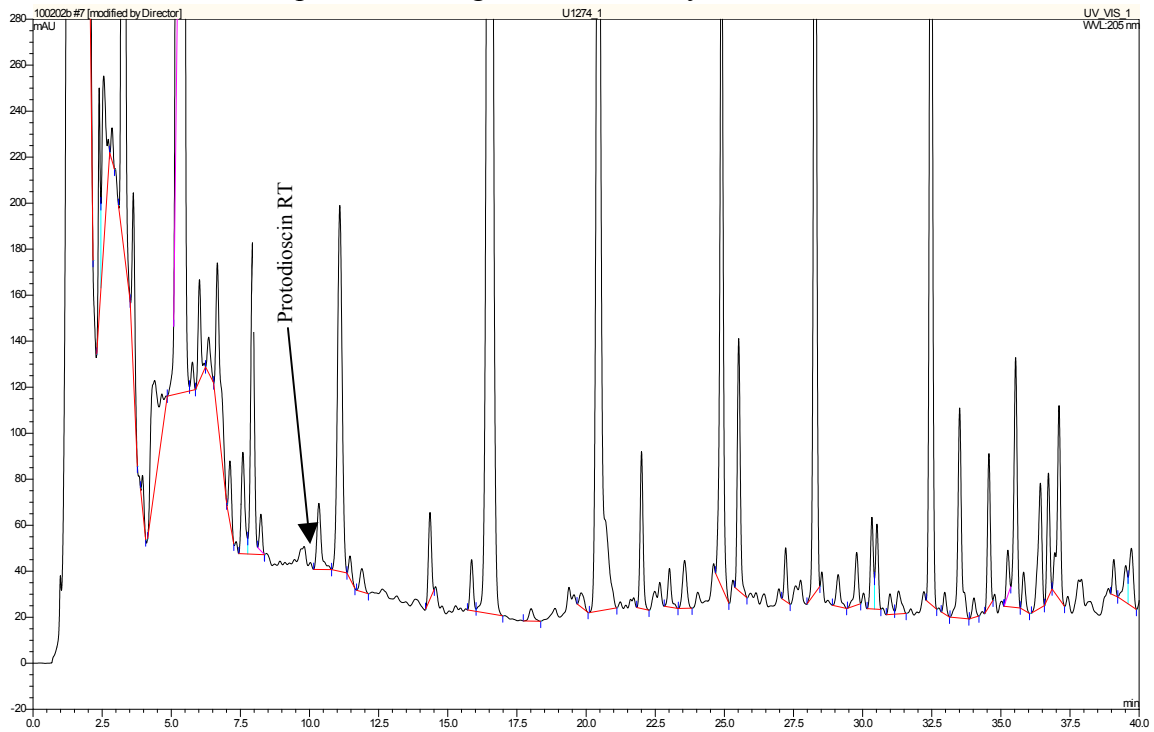


Figure 9
Sample Chromatogram – MRM TribuPlex 750 Lot 32427

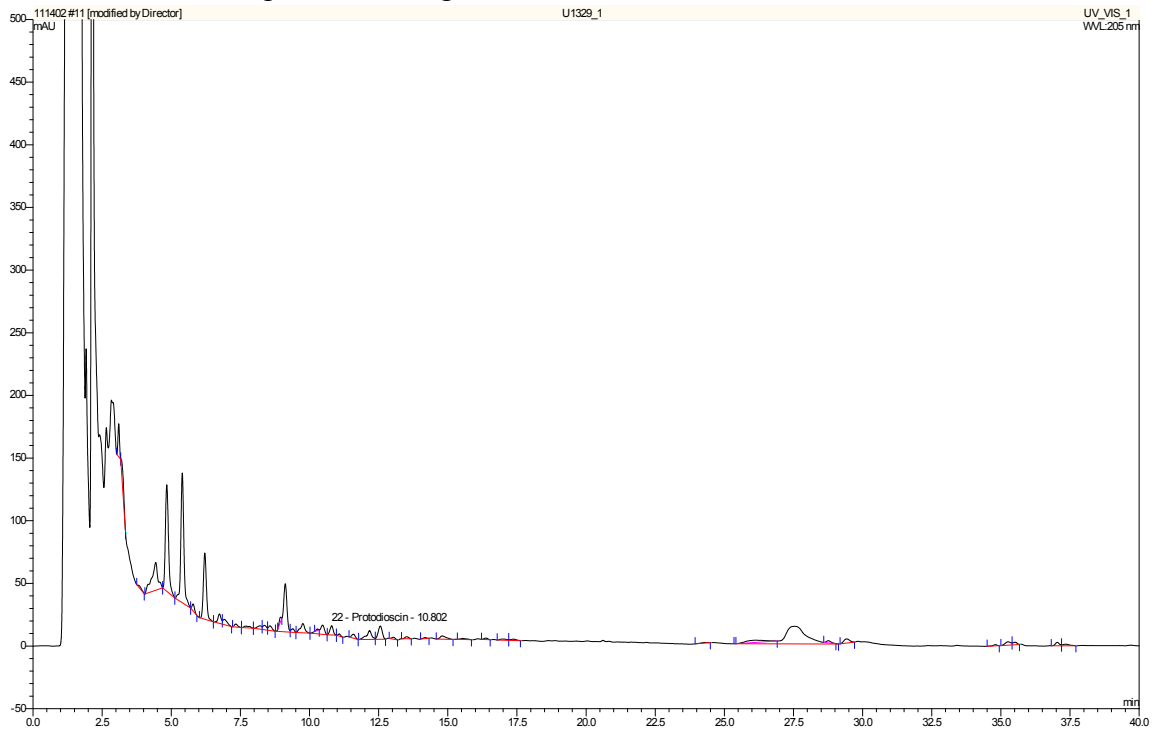
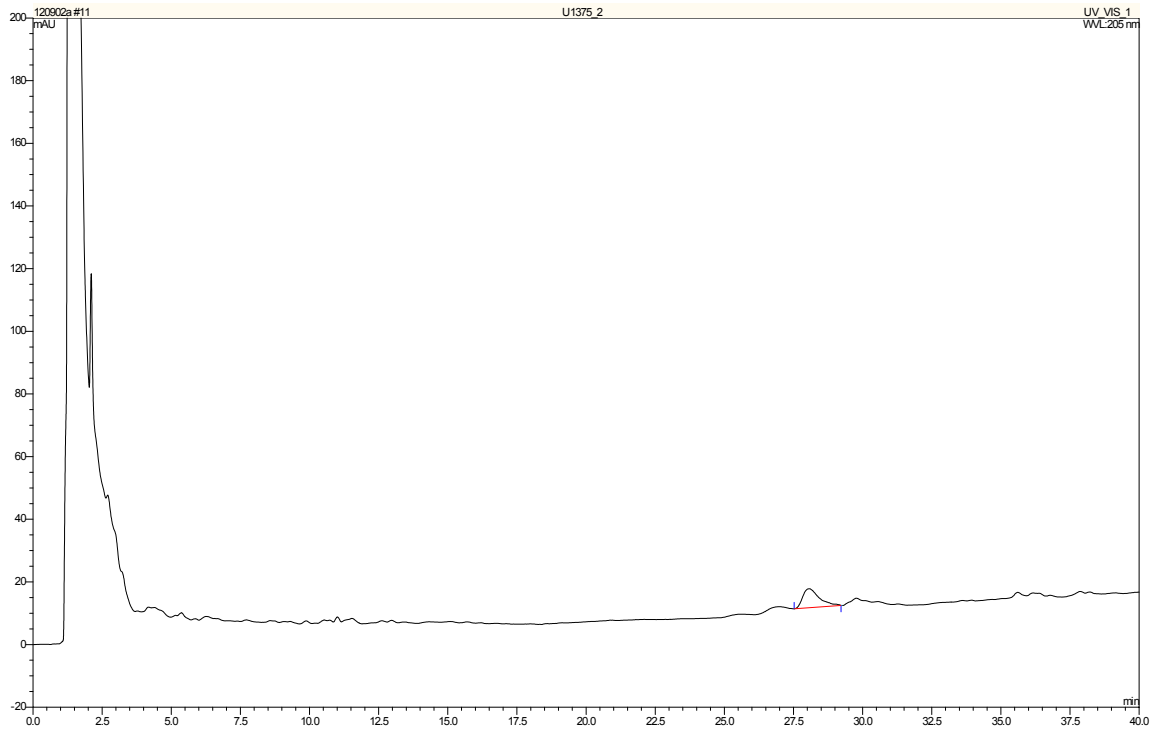


Figure 10
Sample Chromatogram – AST Trioxalon 500 Lot 1161-0601



9. CONCLUSIONS

Thermolife Tribosten tablets lot 7728 were found to contain 73.3 mg of protodioscin. Biotest Tribex 500 capsules were found to contain 35.7 mg of protodioscin. Sopharma Tribestan tablets were found to contain 33.4 mg of protodioscin. Muscletech Anotesten capsules were found to contain 1.9 mg of protodioscin. MRM TribuPlex 750 capsules lot 32427 were found to contain 1.72 mg of protodioscin, although this was near the limit of detection for the method, and the amount found may be a result of a small interference in the sample matrix. Protodioscin was not detected in AST Sports Science Trioxalon 500, Optimum Tribulus 625, VPX Sports Paradeca XS 357, and EAS EcdyMax.

10. REFERENCES

ChromaDex notebook RD007-45
ChromaDex notebook RD007-147
ChromaDex notebook RD008-39
ChromaDex notebook RD008-145

11. **HISTORY OF REVISIONS**

<u>Revision</u>	<u>Date</u>	<u>Changes</u>
00	12/10/02	New Report: CD-ATR-062-00 "Determination of Protodioscin in Samples Submitted by Thermolife by HPLC"

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