Appendices

Appendix A: Real-time quantitative reverse transcription-PCR (RT-PCR)

A1: RNA extraction protocol

1. Disrupt samples in Lysis/Binding Solution
2. Add an equal volume of 64% Ethanol and mix
3. Draw the lysate/ethanol mixture through a Filter Cartridge
4. Wash with 700 µl Wash Solution #1
5. Wash with 2 x 500 µl Wash Solution #2/3
6. Elute RNA with 40-60 µl preheated Elution Solution
7. Elute with a second 10-60 µl aliquot of Elution Solution

A2: Reverse transcription of RNA to cDNA

Reaction Components:

Component	Volume per Reaction	Final Concentration
Complete Master Mix	4 µl	1X
RNA	_ µl*	1 µg
Nuclease free water	_ µl**	-
Total volume	20 µl	-

* Volume needed for 1 µg RNA

** Volume needed after subtracted volume of Complete Master Mix and RNA

Thermal cycler conditions
Step 1: 5 minutes at 25 ℃
Step 2: 30 minutes at 42 ℃
Step 3: 5 minutes at 85 ℃
Step 4: Hold at 4 ℃

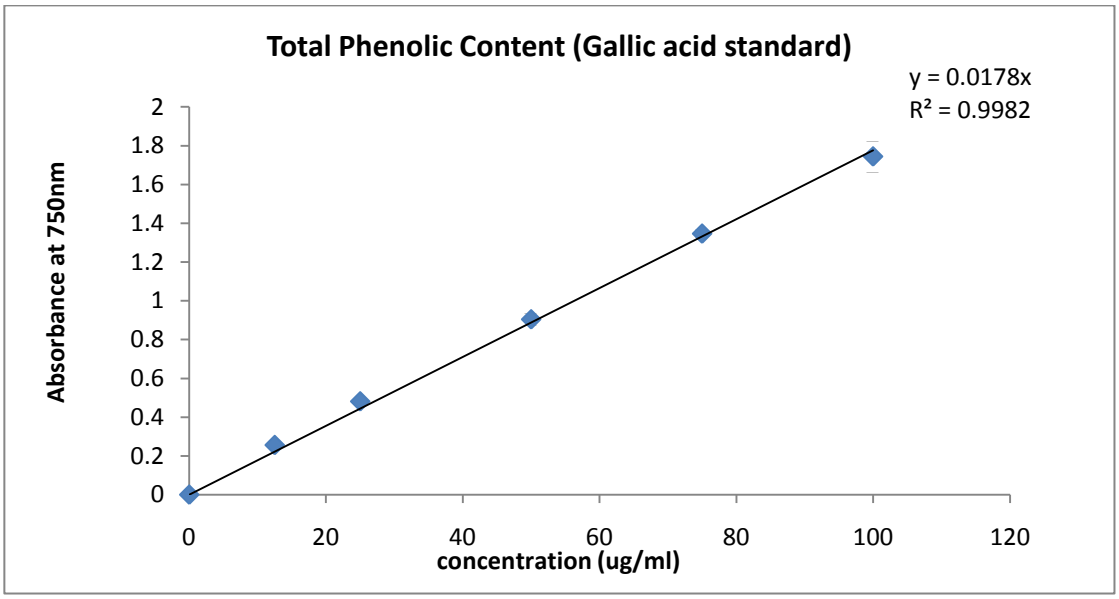
A3: Gene expression analysis

Reaction Components

Component	Volume per Reaction	Final Concentration
Taqman Gene Expression Master Mix	5 µl	1X
cDNA	1 µl	-
Nuclease free water	4 µl	-
Total volume	10 µl	-

Thermal cycler conditions
Step 1: 2 minutes at 50 ℃
Step 2: 10 minutes at 95 ℃
Step 3: 40 cycles of 15 seconds at 95 ℃ and 1 minutes at 60 ℃

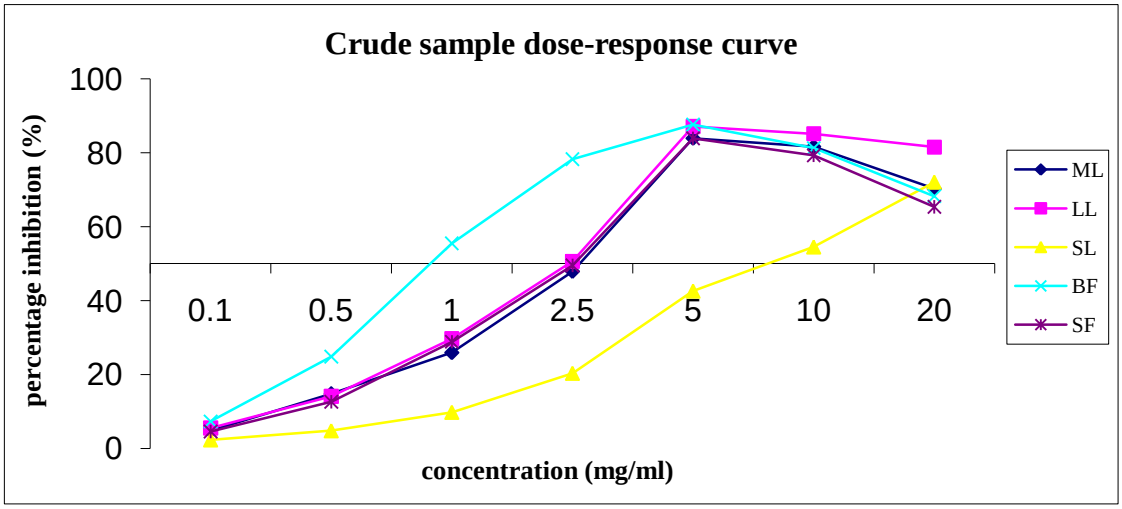
Appendix B: Graph of gallic acid standard



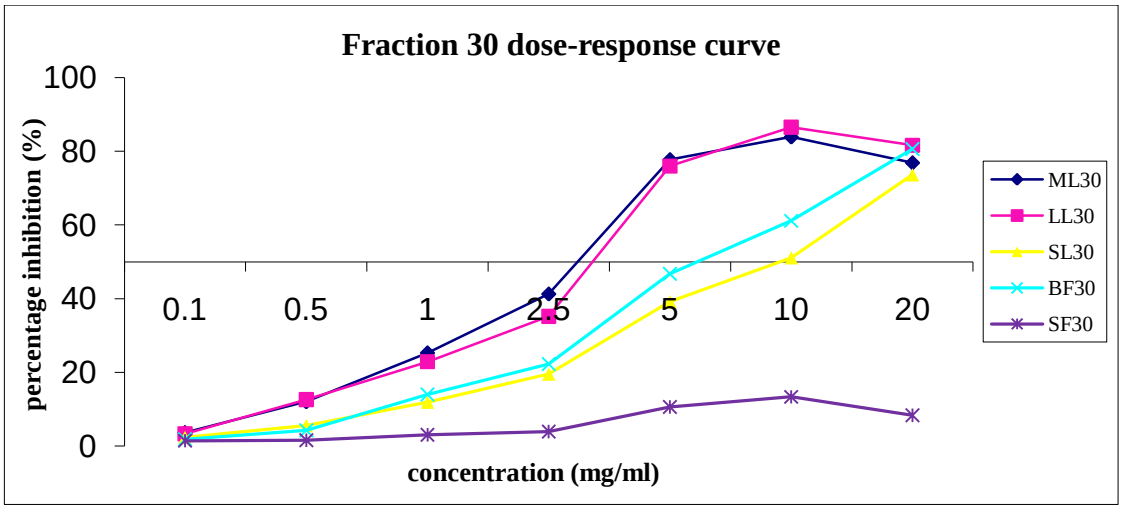
The phenolic content of F.deltoidea's extracts and its fractions were calculated based on the equation y=0.0178x.

Appendix C: Dose-response curve of samples and ascorbic acid in DPPH assays

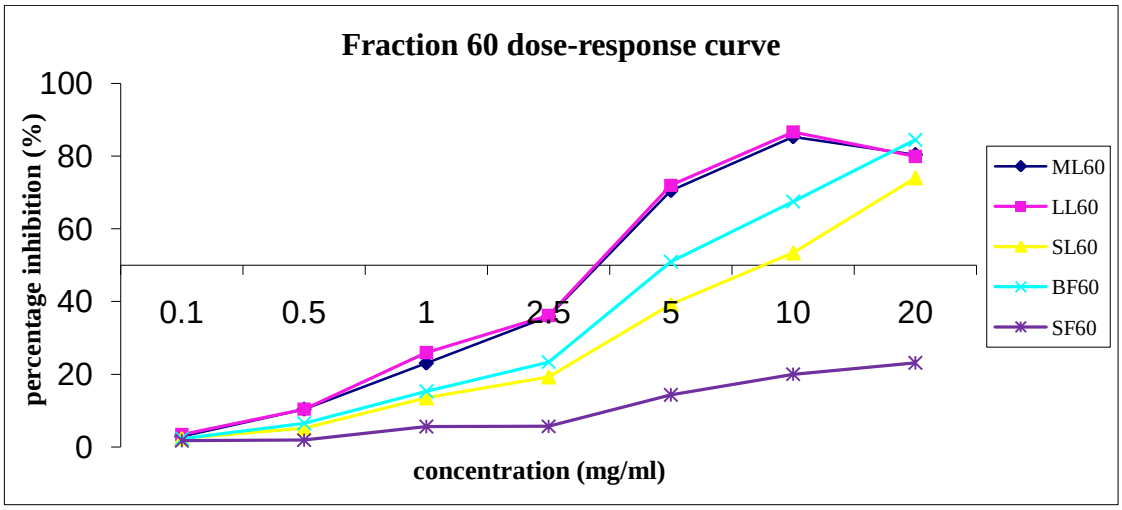
C1: Crude sample curve



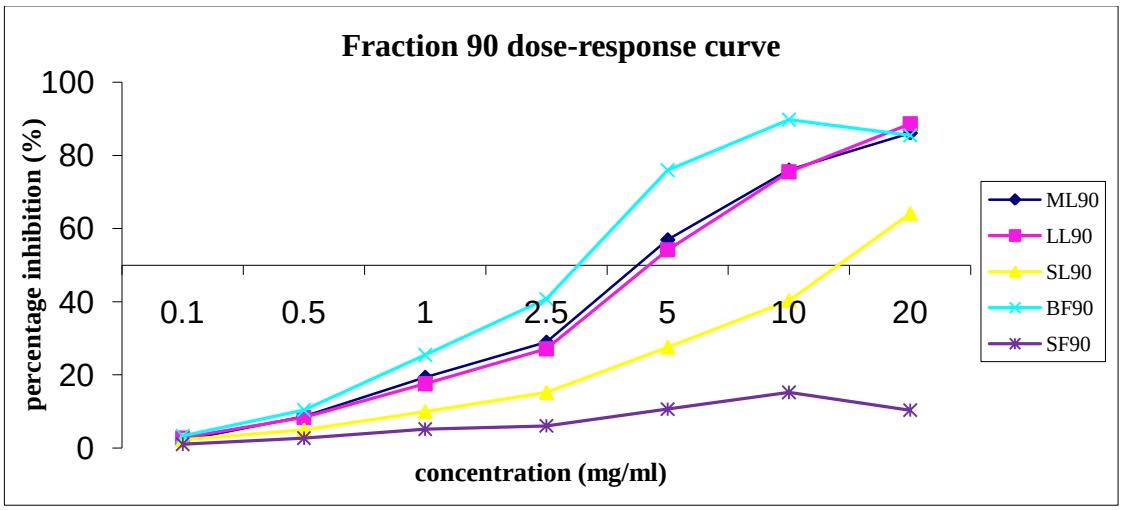
C2: Fraction 30 curve



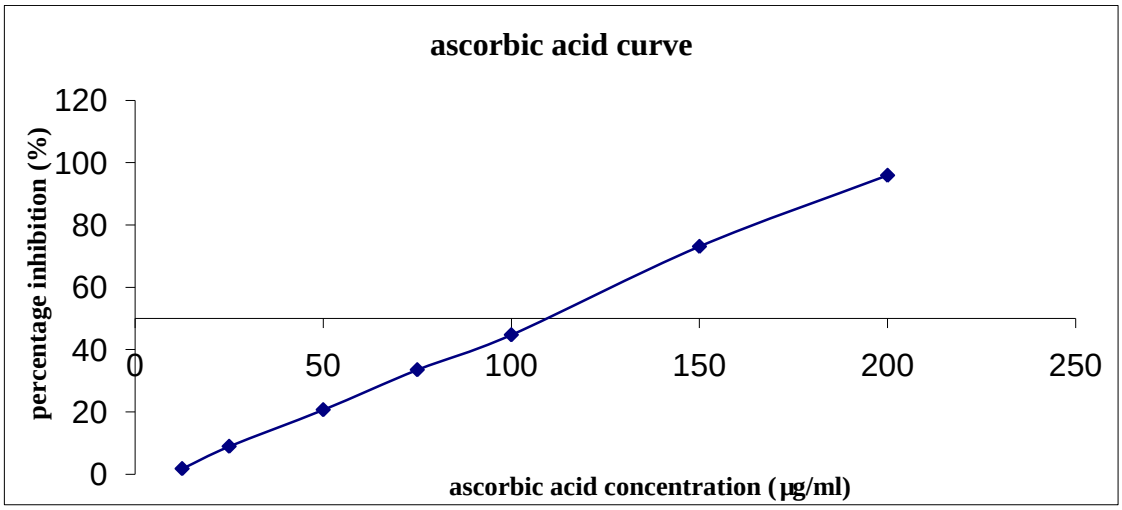
C3: Fraction 60 curve



C4: Fraction 90 curve

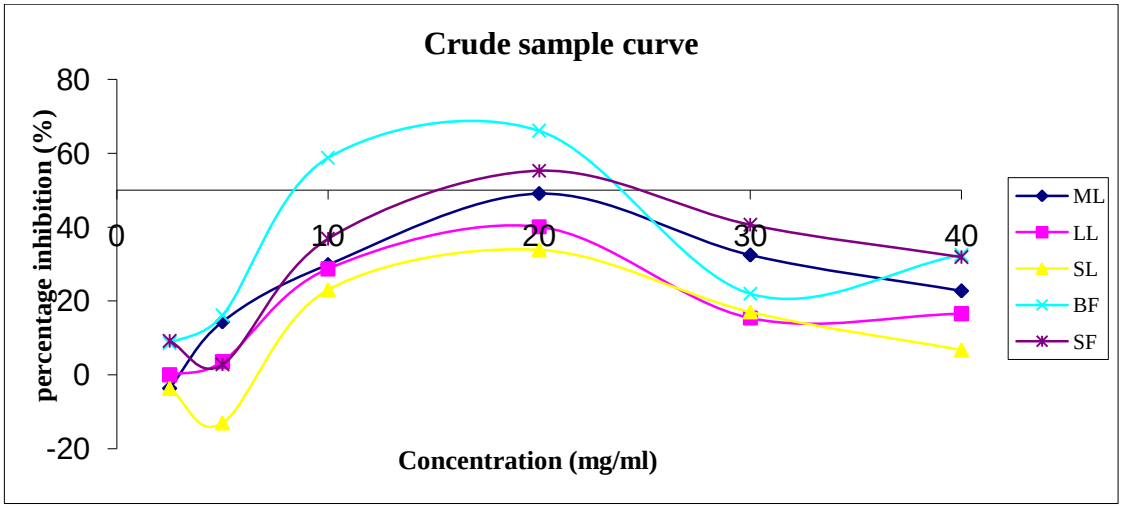


C5: Ascorbic acid curve

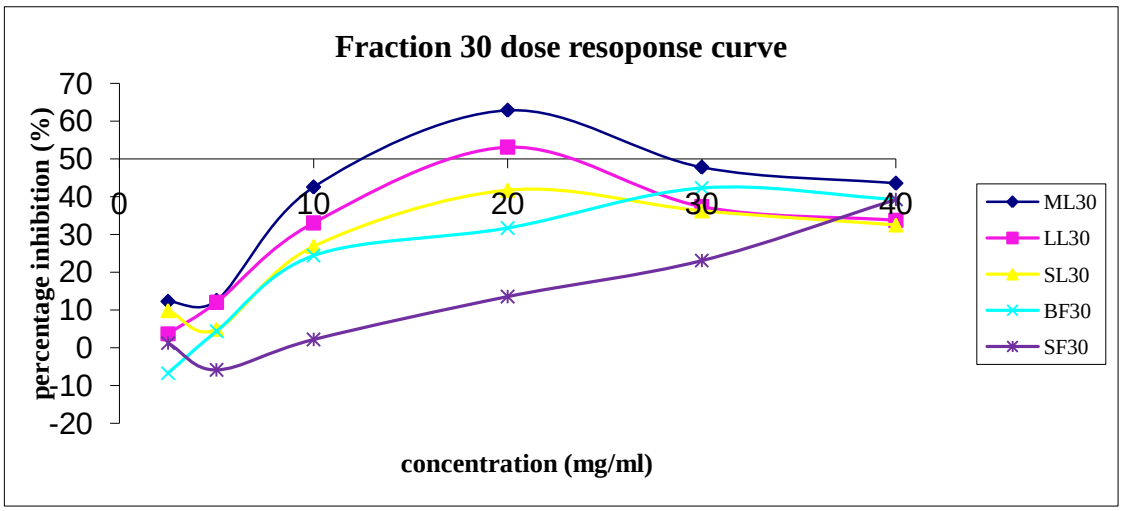


Appendix D: Dose-response curve of samples and ascorbic acid in lipid peroxidation assays

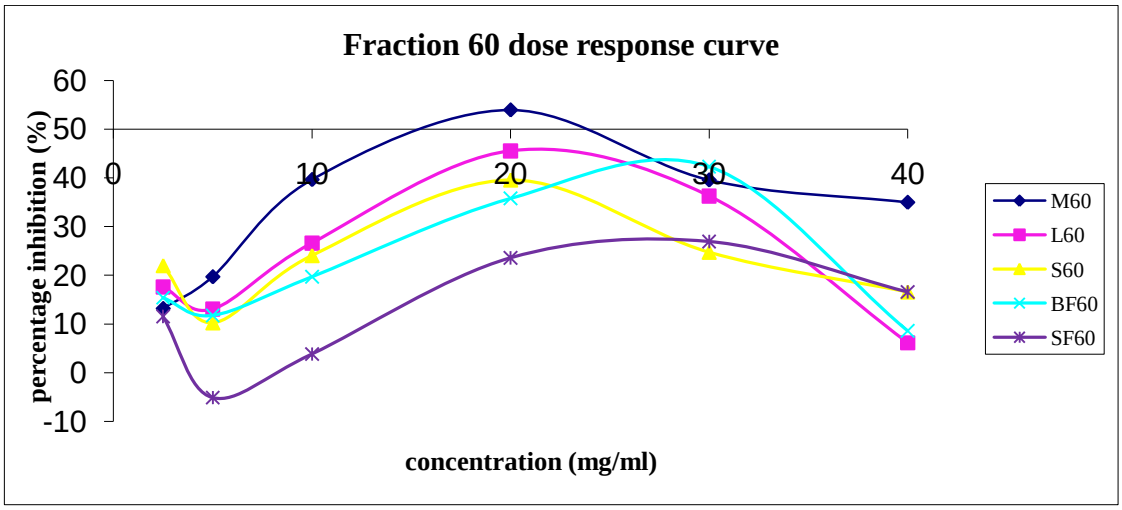
D1: Crude sample curve



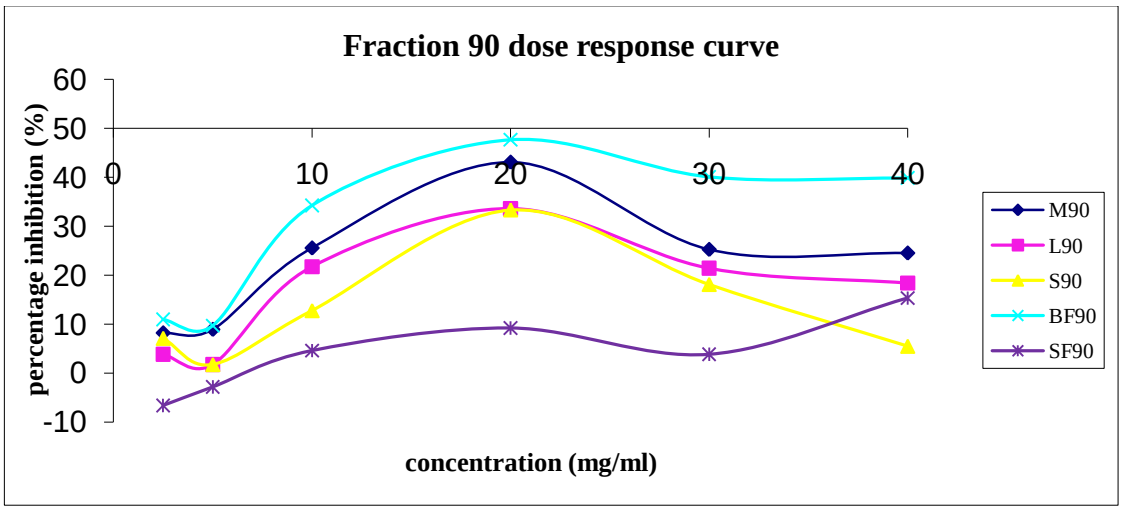
D2: Fraction 30 curve



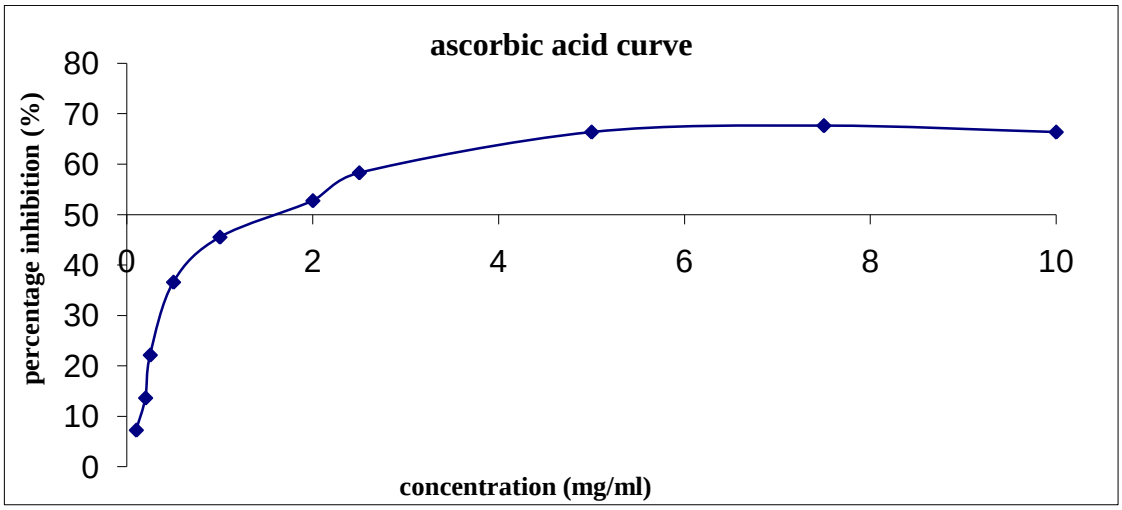
D3: Fraction 60 curve



D4: Fraction 90 curve

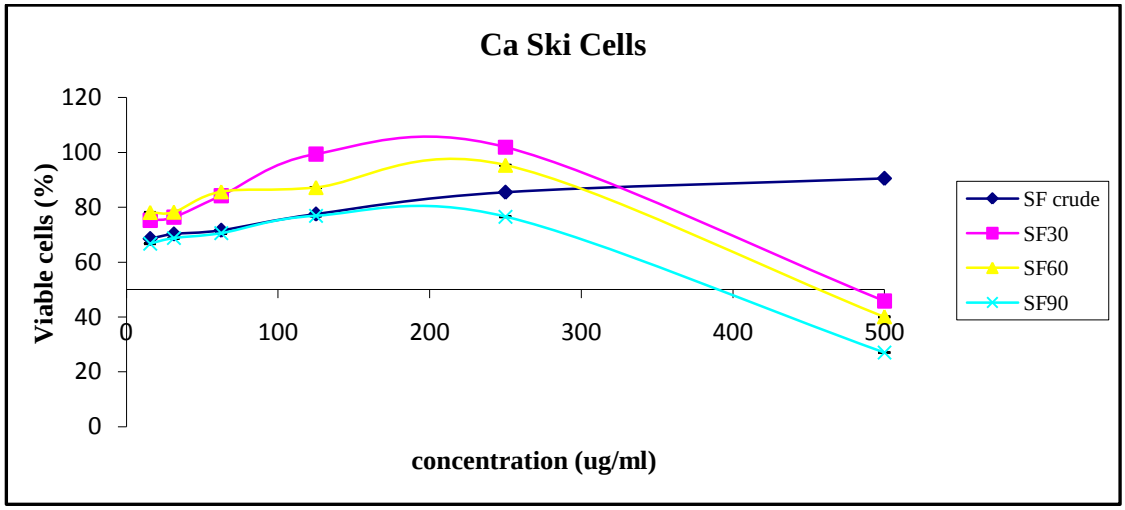


D5: Ascorbic acid curve

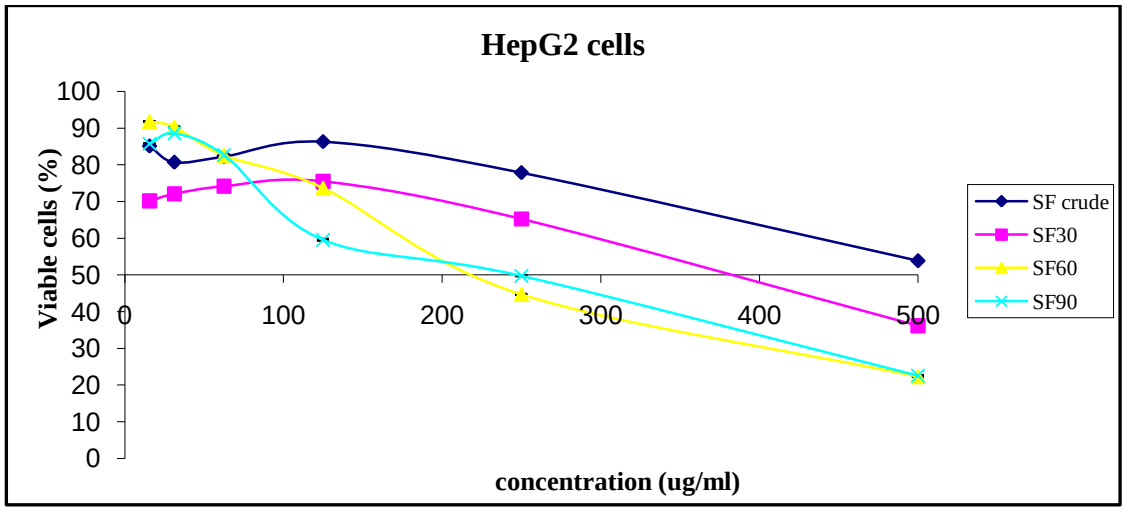


Appendix E: Viable cells curve upon different treatment

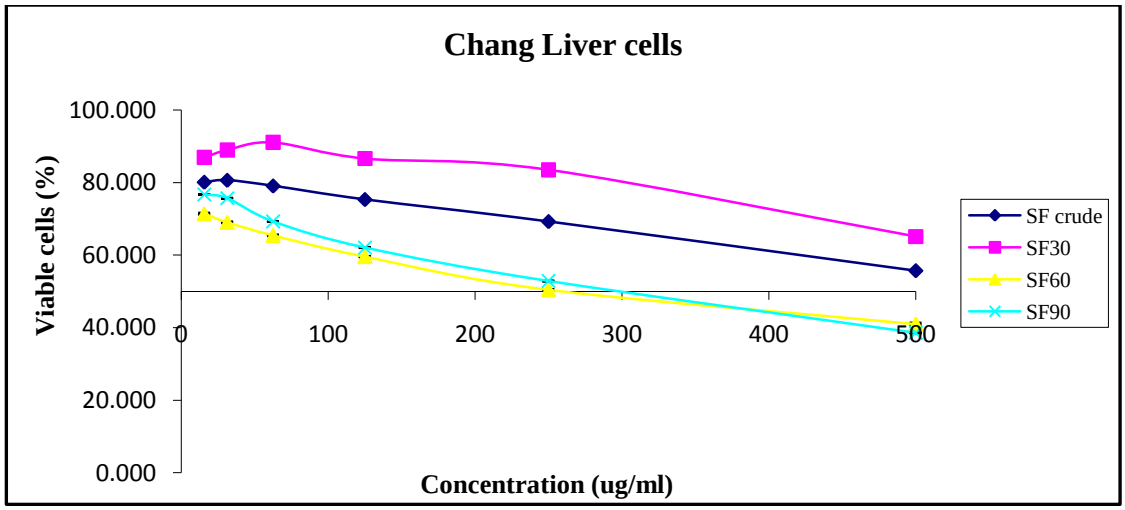
E1: Treatment of SF crude and fractions towards Ca Ski cells



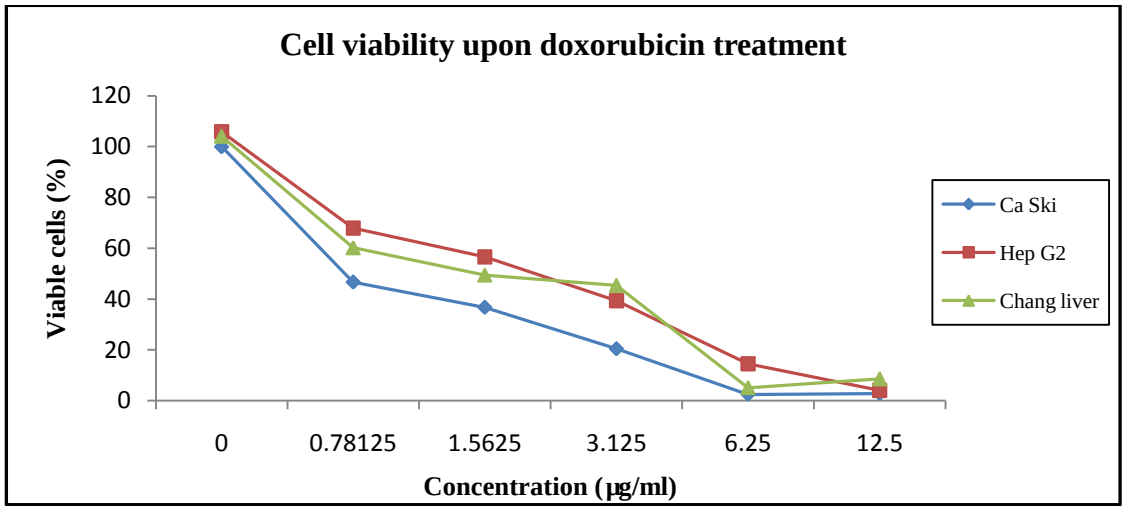
E2: Treatment of SF crude and fractions towards Hep G2 cells



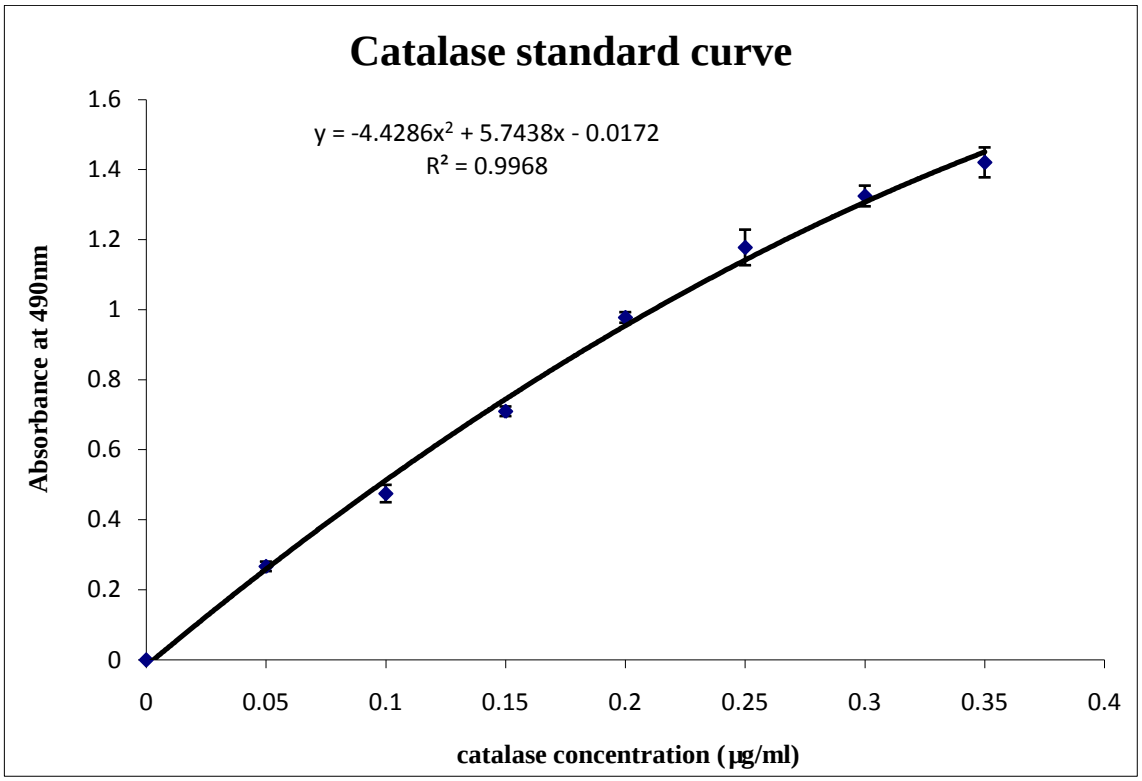
E3: Treatment of SF crude and fractions towards Chang Liver cells



E4: Treatment of doxorubicin towards different cell lines



Appendix F: Catalase standard curve

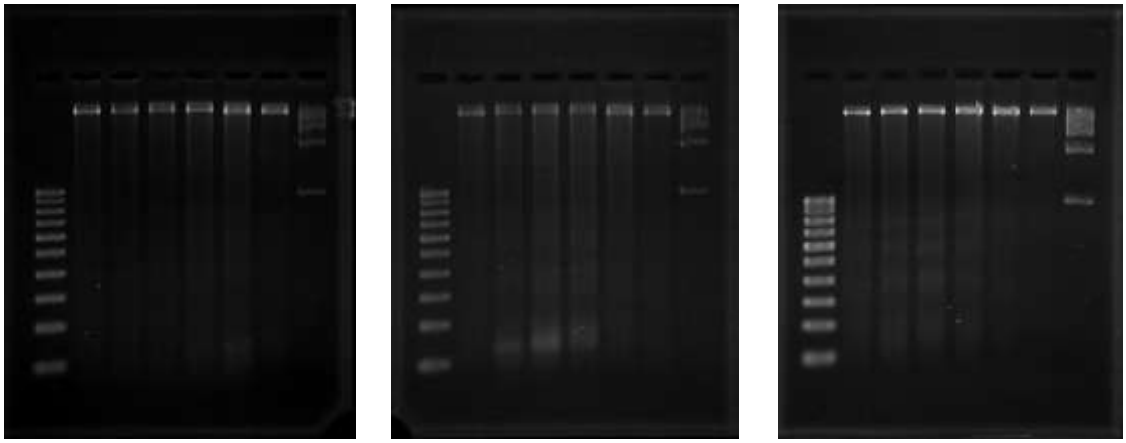


The catalase concentration of cell lysate upon treatment were estimated and calculated based on the equation $y = -4.4286x^2 + 5.7438x - 0.0172$.

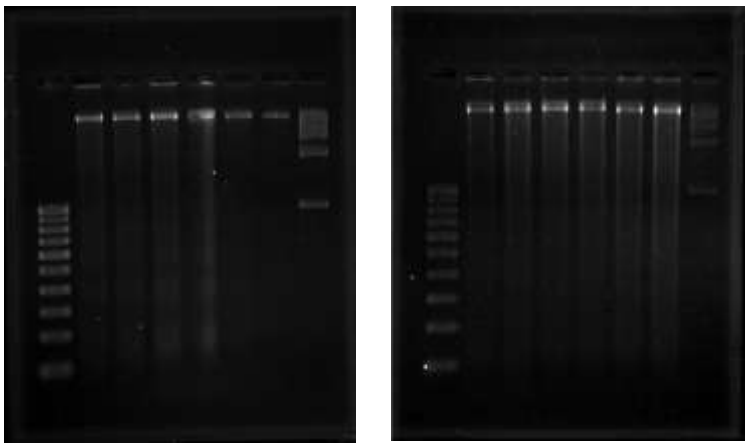
Appendix G: DNA fragmentation results

G1: Ca Ski cells upon different treatments

(a) SF (b) SF30 (c) SF60



(d) SF90 (e) Doxorubicin

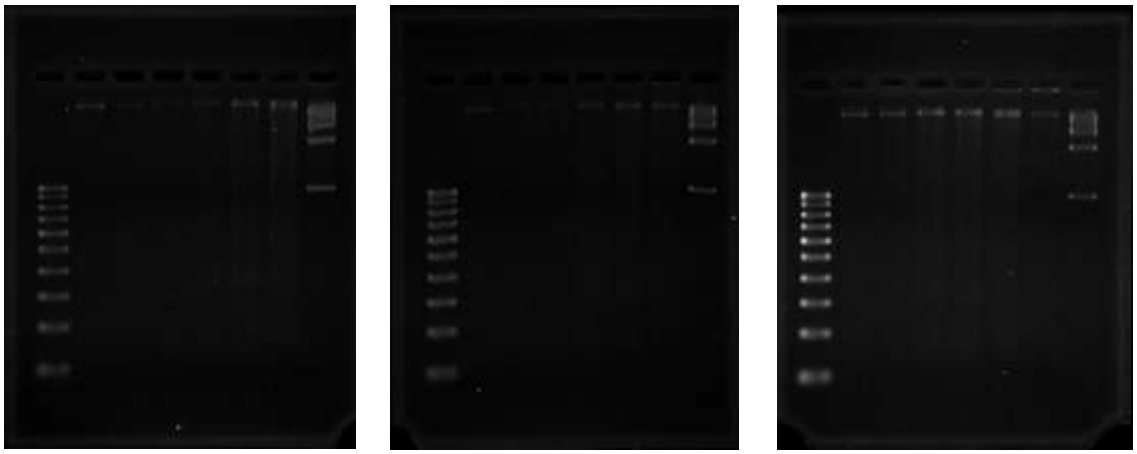


SF samples: left to right
Lane 1: 100bp marker, Lane 2: 0.125mg/ml, Lane 3: 0.25mg/ml, Lane 4: 0.5mg/ml, Lane 5: 0.75mg/ml, Lane 6: 1mg/ml, Lane 7: control, Lane 8: 1kb marker.

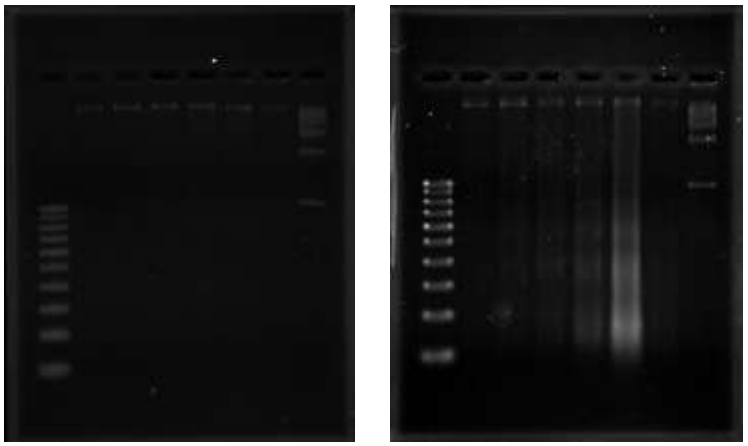
Doxorubicin: left to right
Lane 1: 100bp marker, Lane 2: 0.125 µg/ml, Lane 3: 0.25 µg/ml, Lane 4: 0.5 µg/ml, Lane 5: 1 µg/ml, Lane 6: 2 µg/ml, Lane 7: control, Lane 8: 1kb marker.

G2: Hep G2 cells upon different treatments

(a) SF (b) SF30 (c) SF60



(d) SF90 (e) Doxorubicin

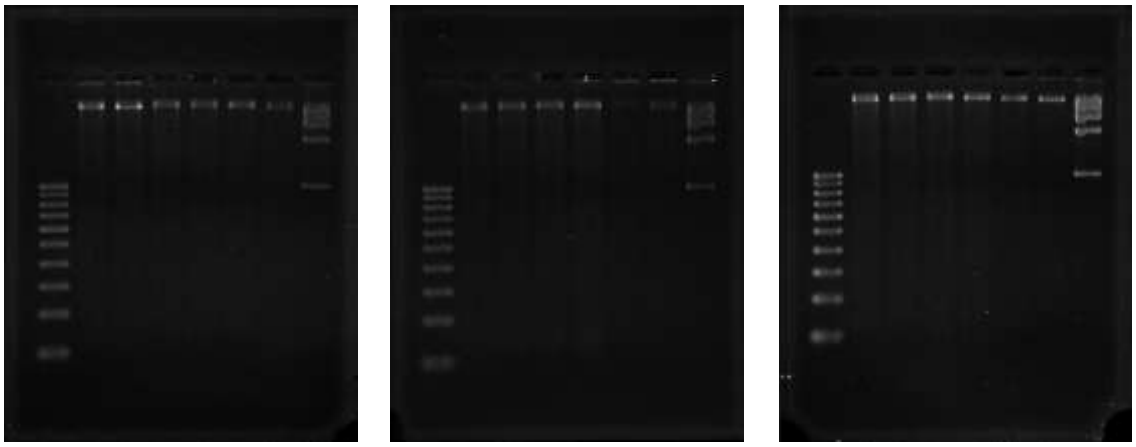


SF samples: left to right
Lane 1: 100bp marker, Lane 2: 0.125mg/ml, Lane 3: 0.25mg/ml, Lane 4: 0.5mg/ml, Lane 5: 0.75mg/ml, Lane 6: 1mg/ml, Lane 7: control, Lane 8: 1kb marker.

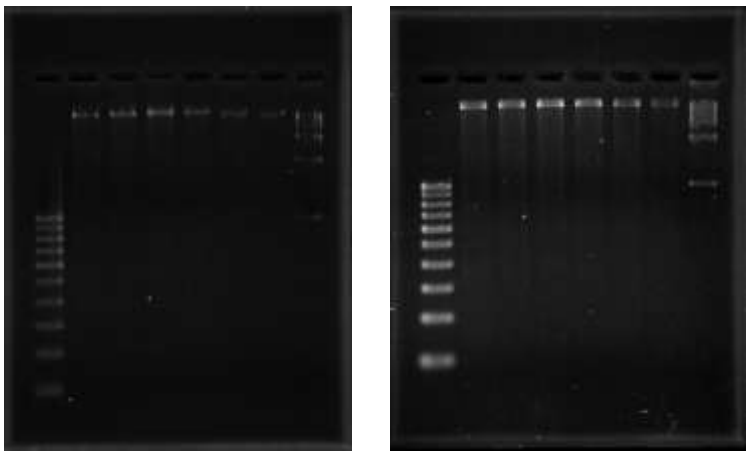
Doxorubicin: left to right
Lane 1: 100bp marker, Lane 2: 0.125 µg/ml, Lane 3: 0.25 µg/ml, Lane 4: 0.5 µg/ml, Lane 5: 1 µg/ml, Lane 6: 2 µg/ml, Lane 7: control, Lane 8: 1kb marker.

G3: Chang Liver cells upon different treatments

(a) SF (b) SF30 (c) SF60



(d) SF90 (e) Doxorubicin

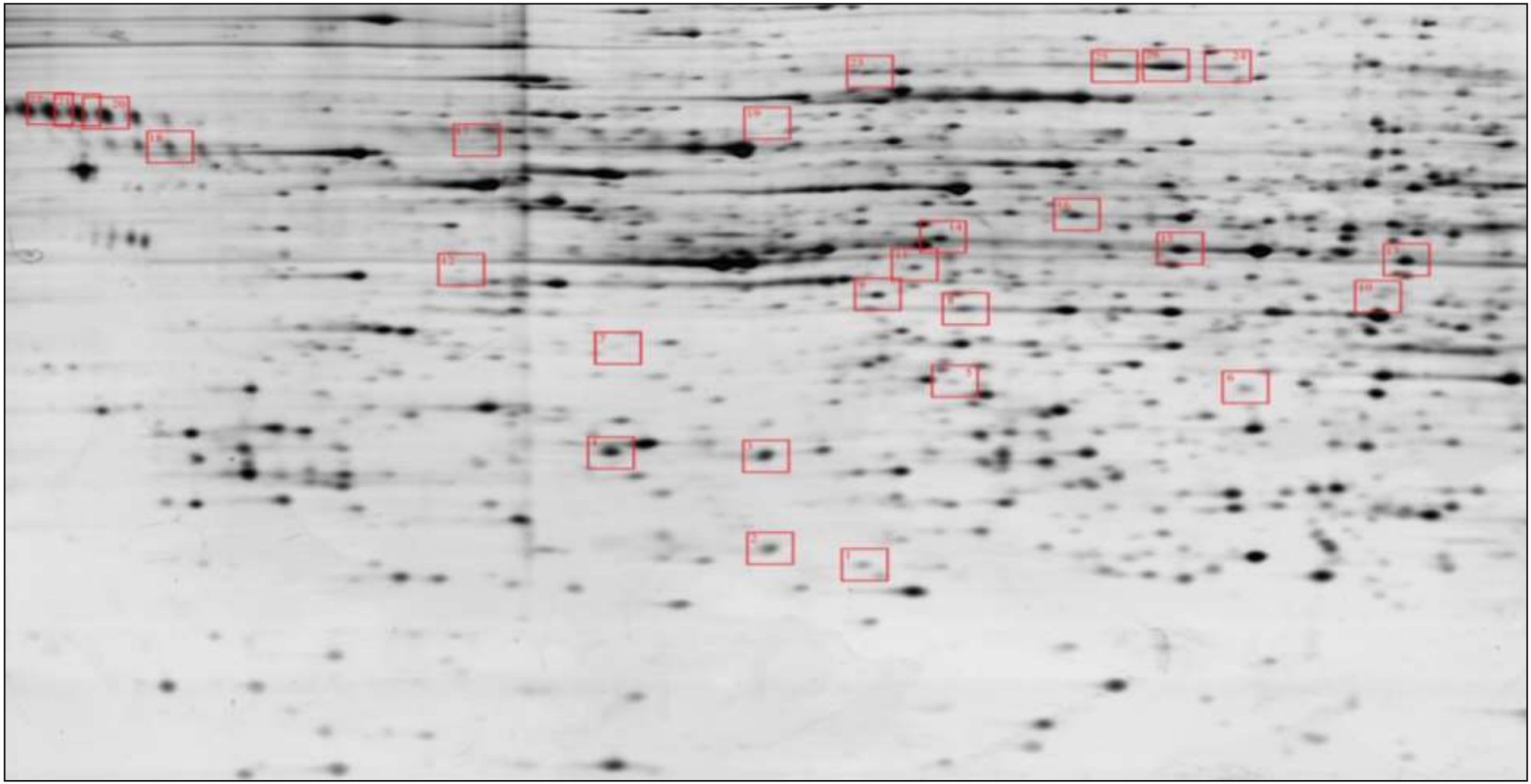


SF samples: left to right
Lane 1: 100bp marker, Lane 2: 0.125mg/ml, Lane 3: 0.25mg/ml, Lane 4: 0.5mg/ml, Lane 5: 0.75mg/ml, Lane 6: 1mg/ml, Lane 7: control, Lane 8: 1kb marker.

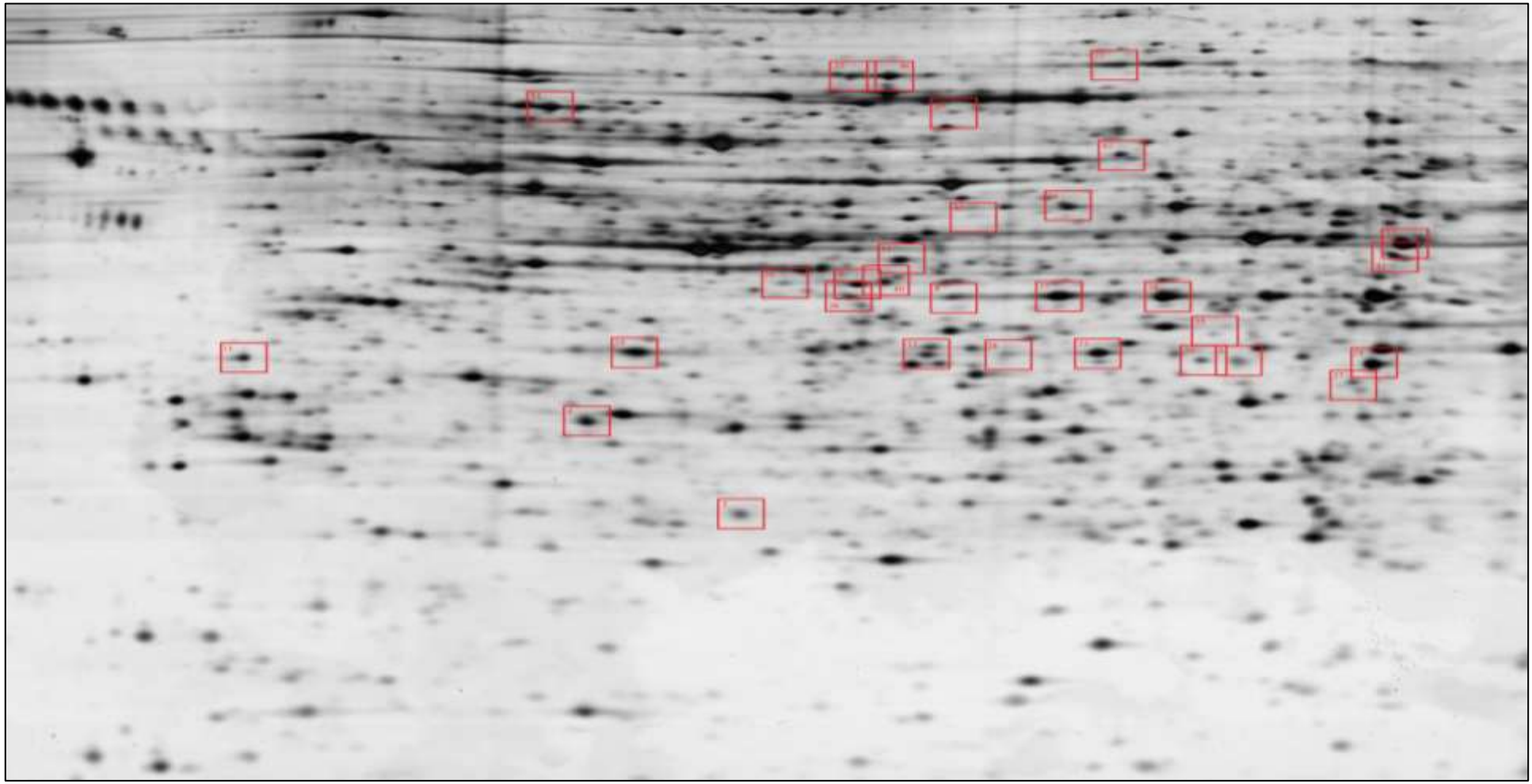
Doxorubicin: left to right
Lane 1: 100bp marker, Lane 2: 0.125 µg/ml, Lane 3: 0.25 µg/ml, Lane 4: 0.5 µg/ml, Lane 5: 1 µg/ml, Lane 6: 2 µg/ml, Lane 7: control, Lane 8: 1kb marker.

Appendix H

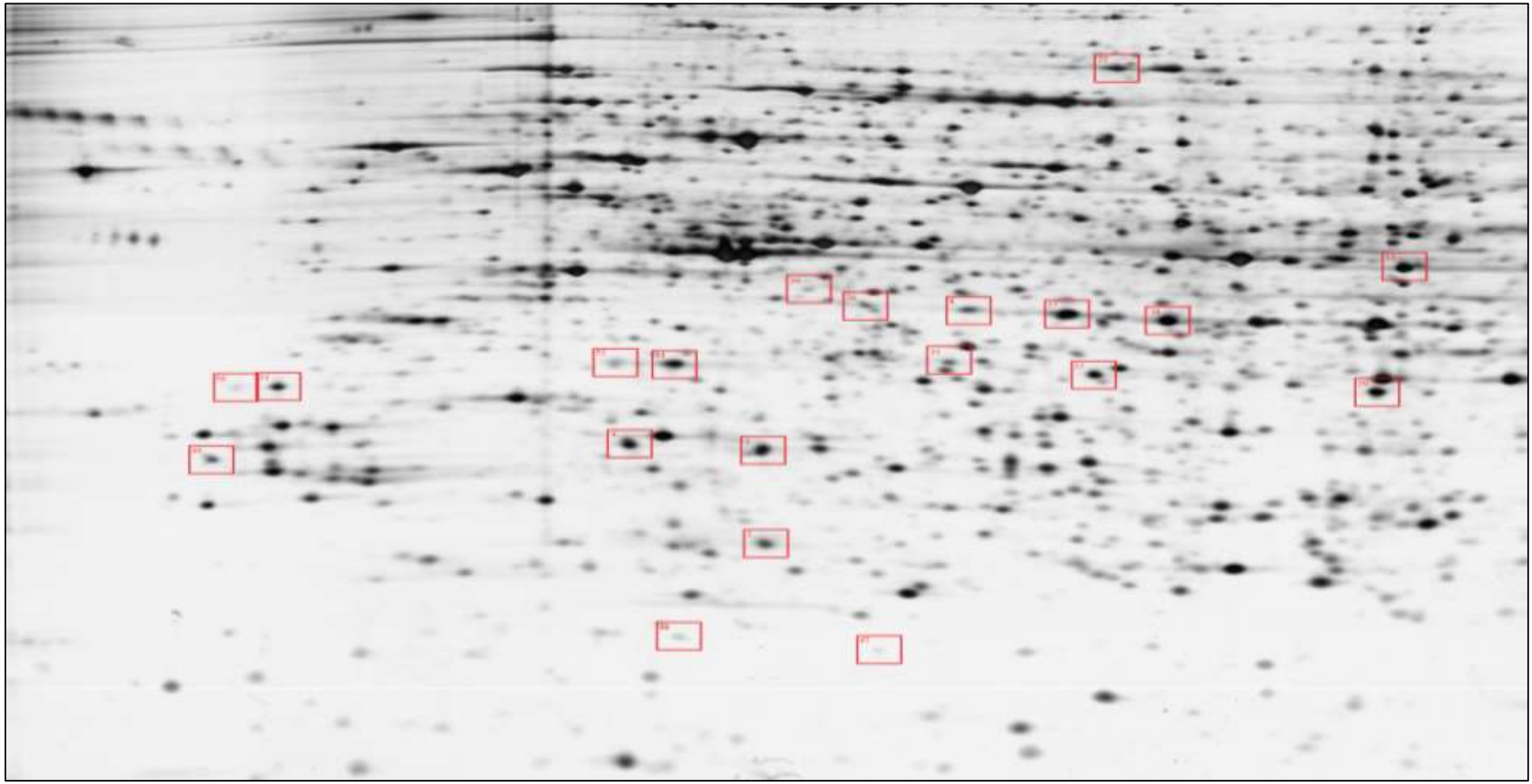
H1: Hep G2 cells treated with SF30



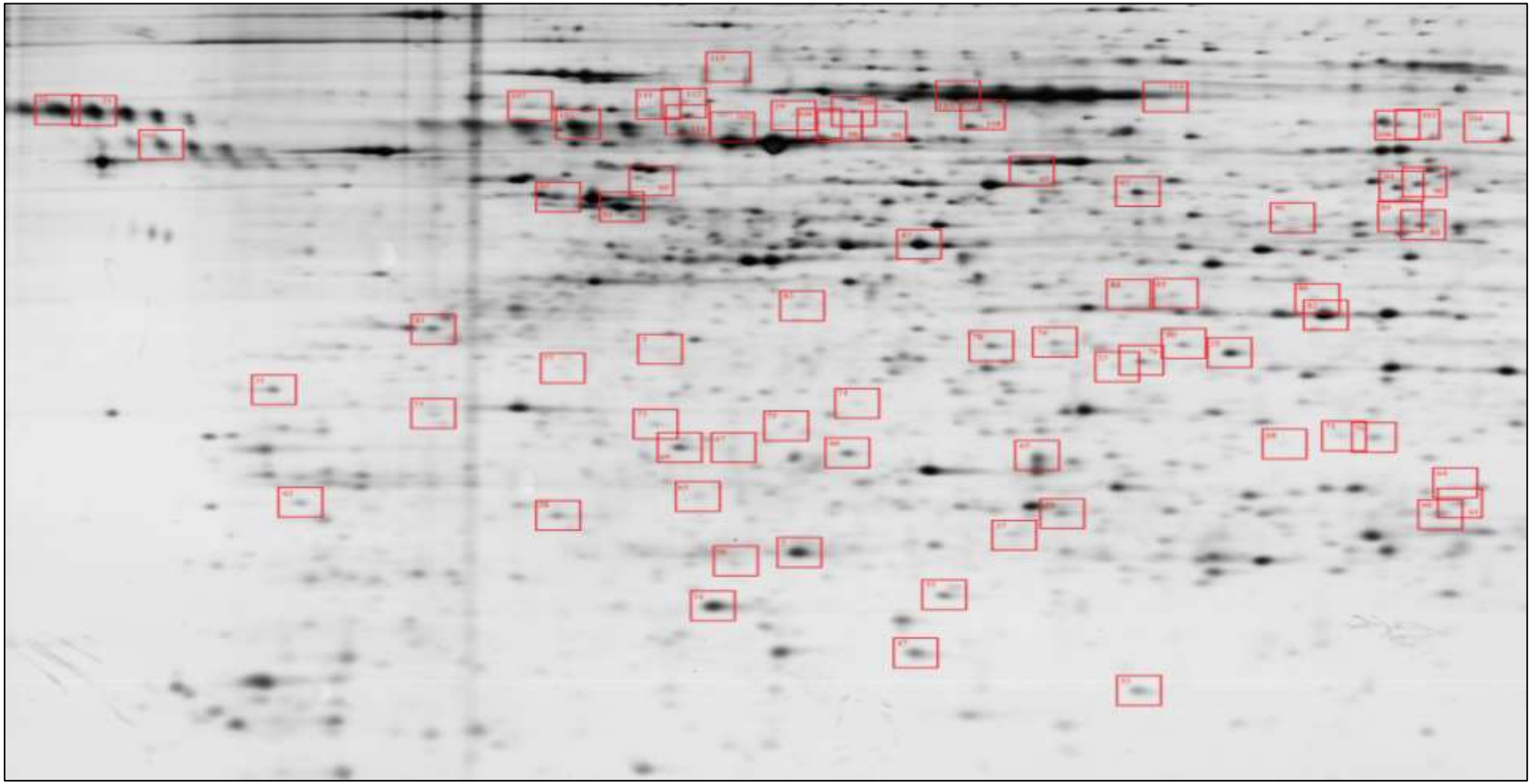
H2: Hep G2 cells treated with SF60



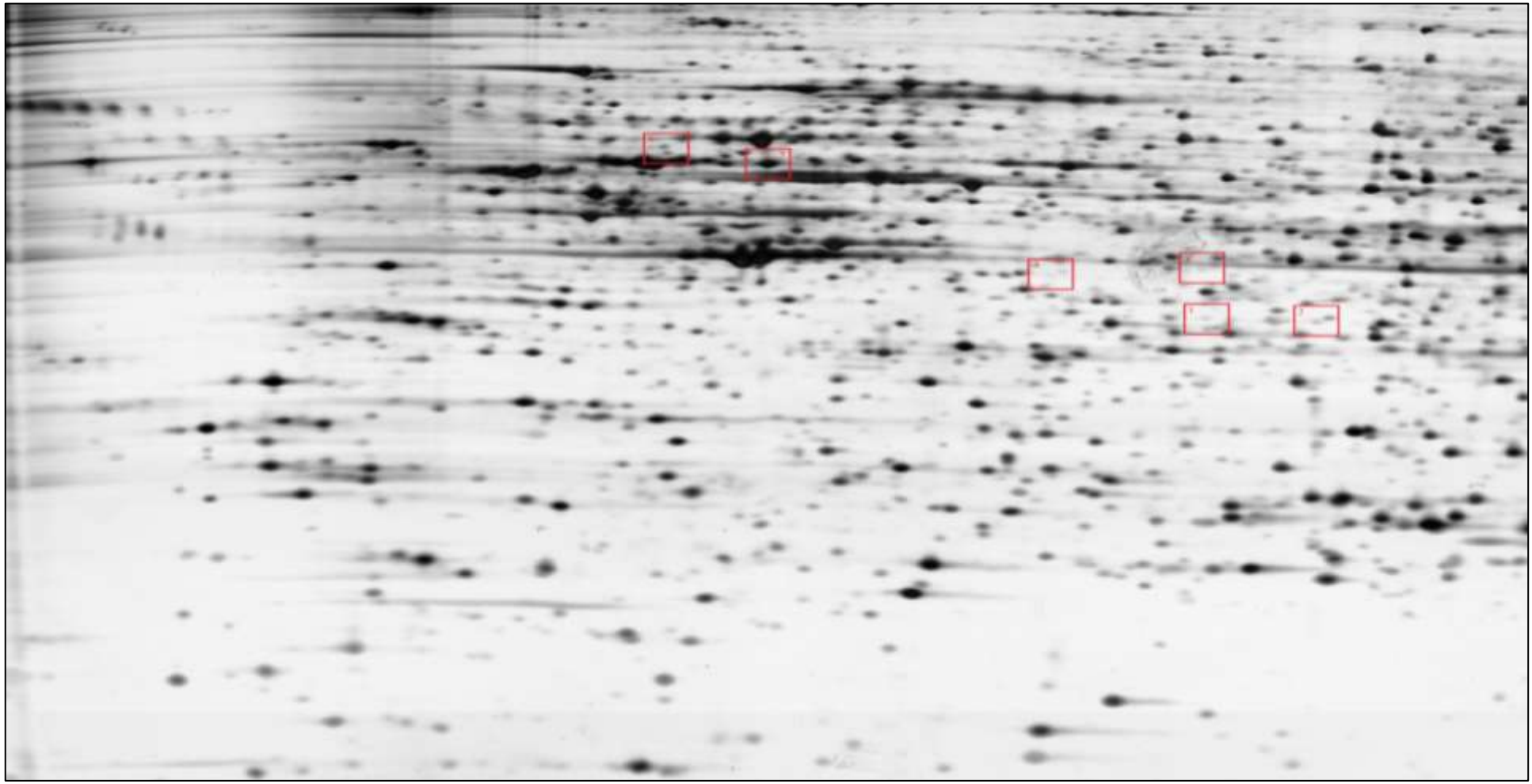
H3: Hep G2 cells treated with SF90



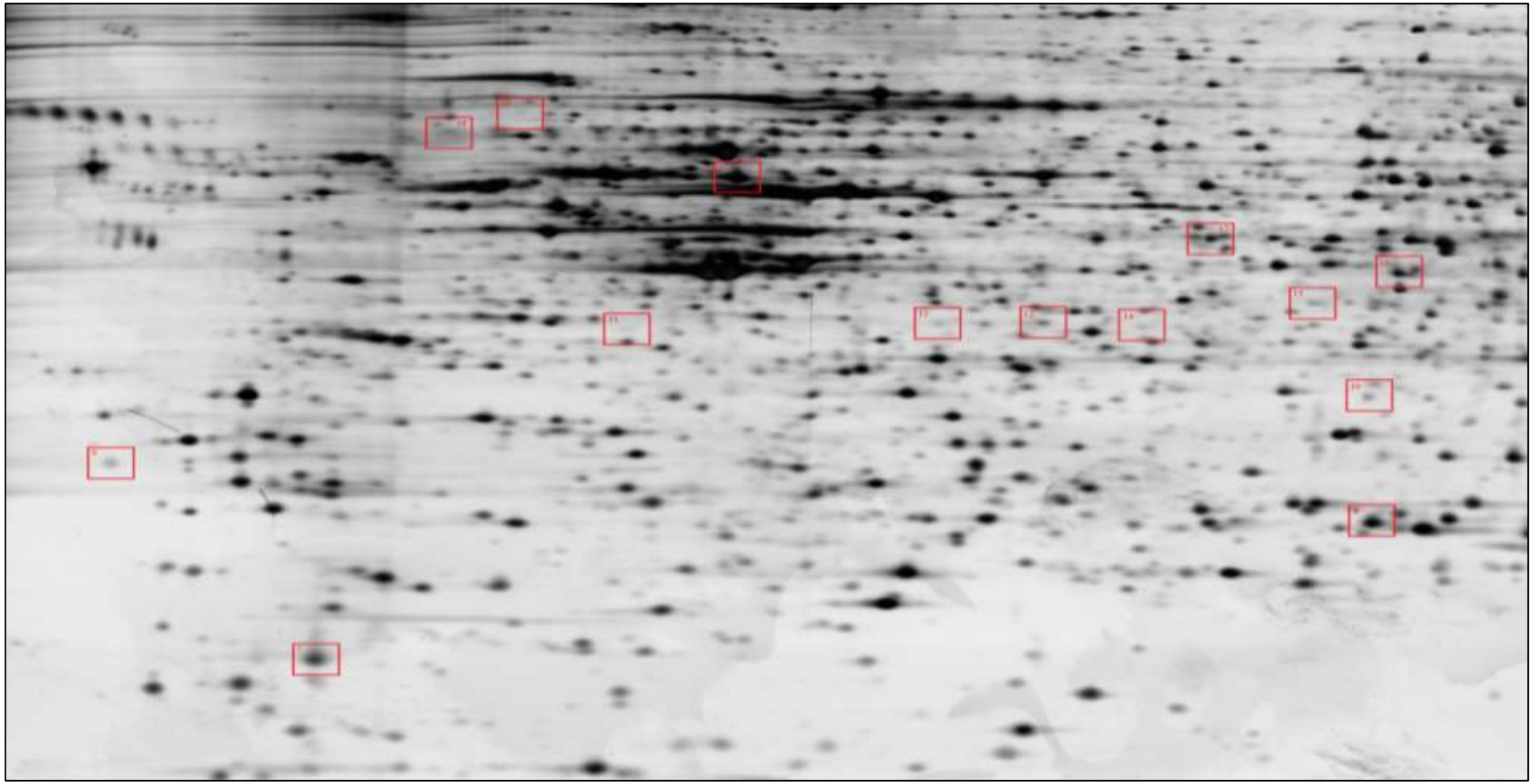
H4: Hep G2 cells treated with doxorubicin



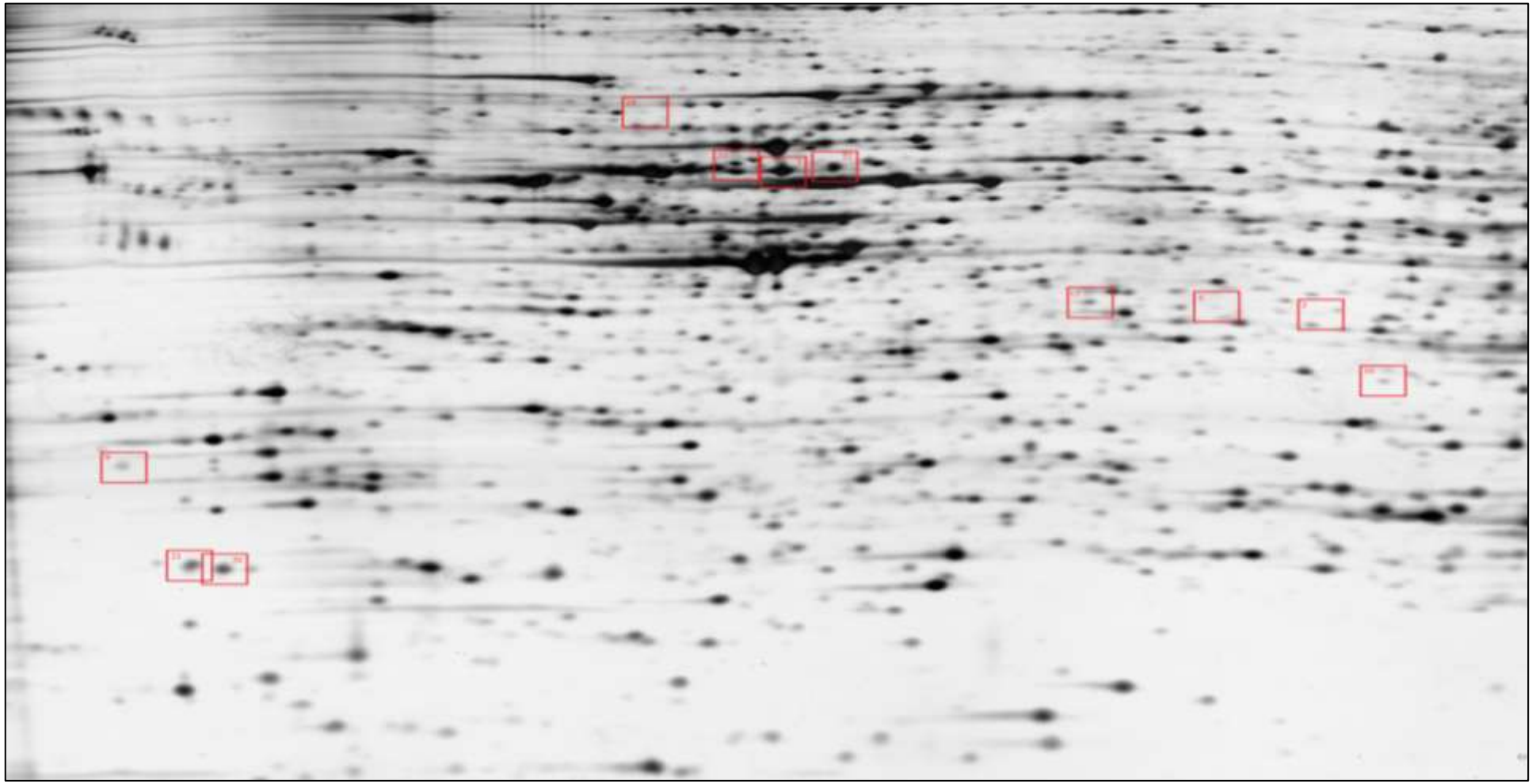
H5: Ca Ski cells treated with SF30



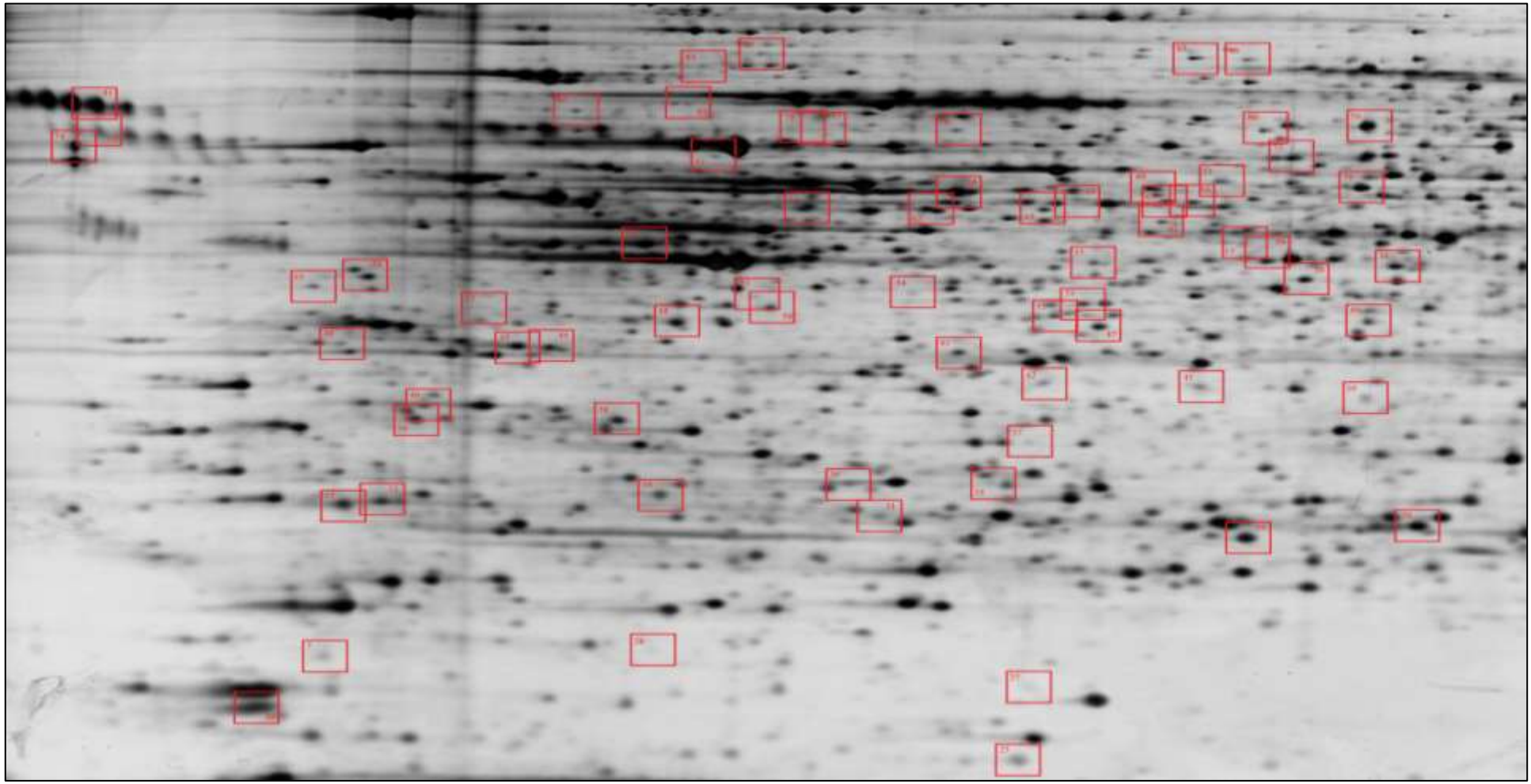
H6: Ca Ski cells treated with SF60



H7: Ca Ski cells treated with SF90

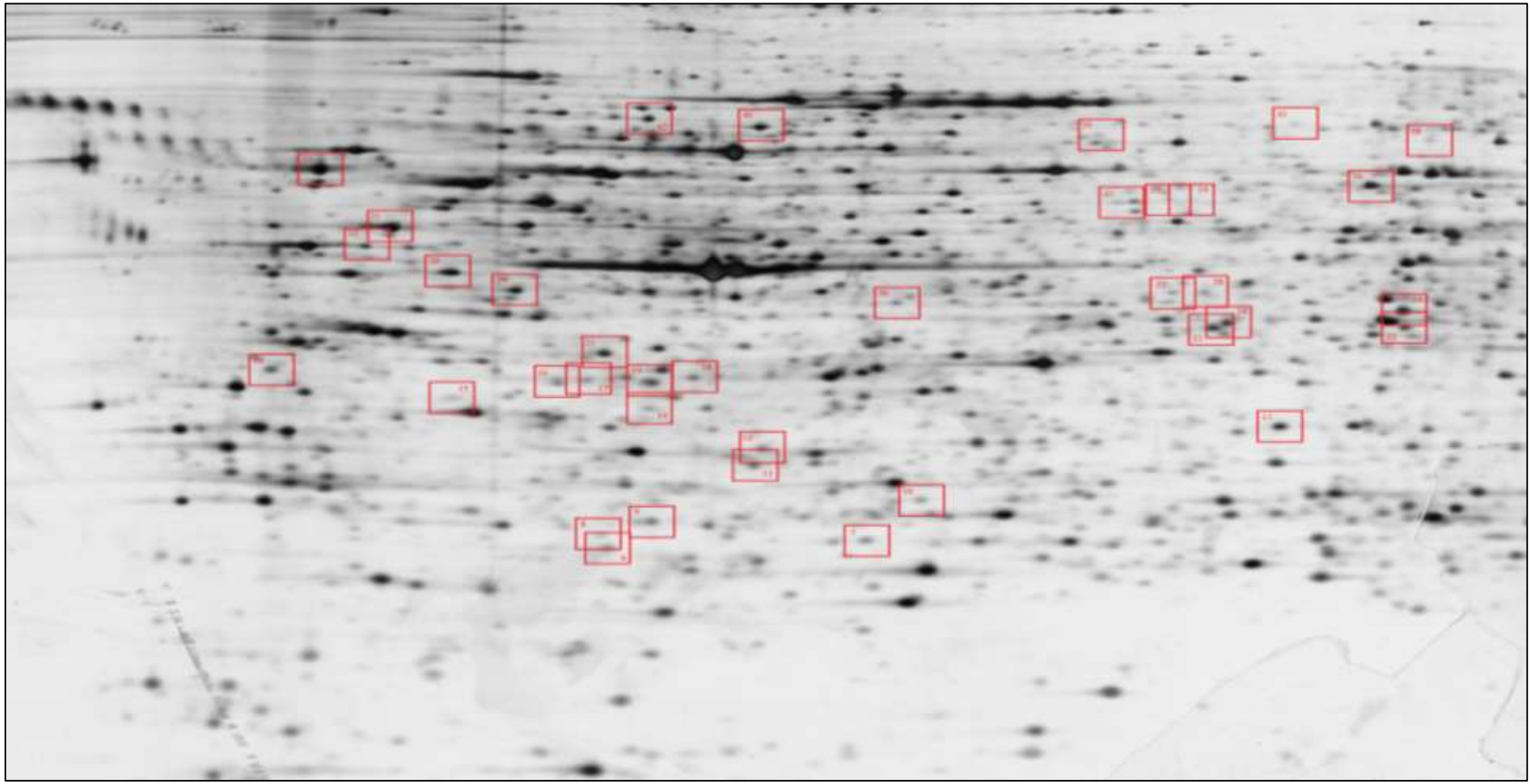


H8: Ca Ski cells treated with doxorubicin

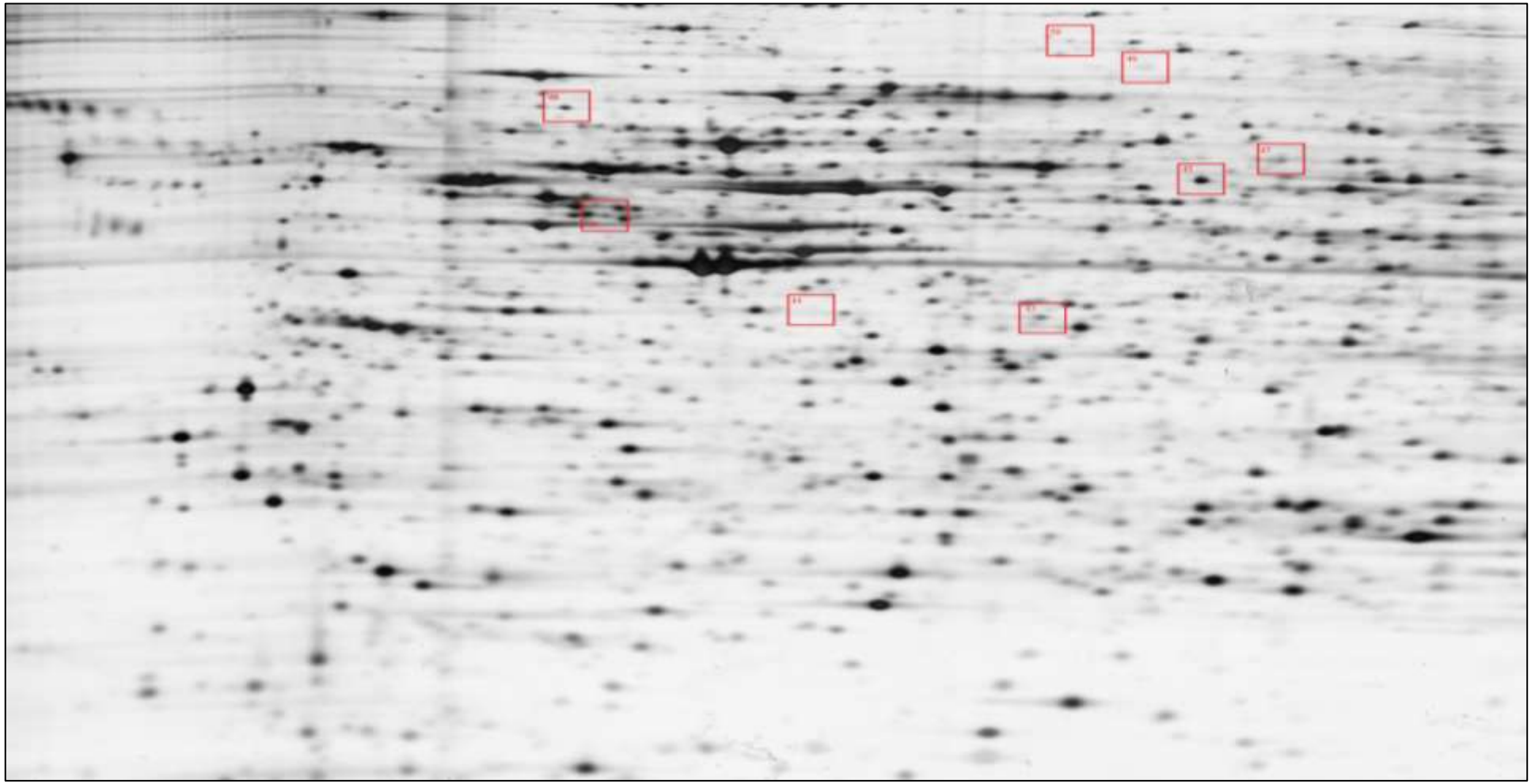


H9: Chang Liver cells treated with SF30

H10: Chang Liver cells treated with SF60



H11: Chang Liver cells treated with SF90



H12: Chang Liver cells treated with doxorubicin