Electronic Supplementary Material (ESI) for Analyst.

Identifying the mode of action of drugs using live-cell FTIR spectroscopy

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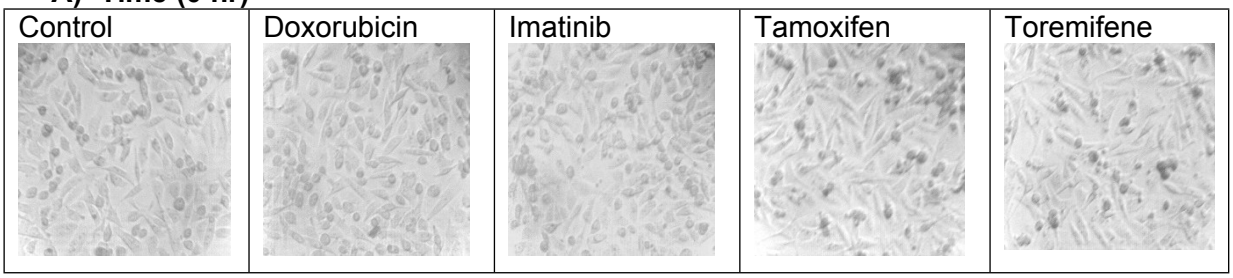
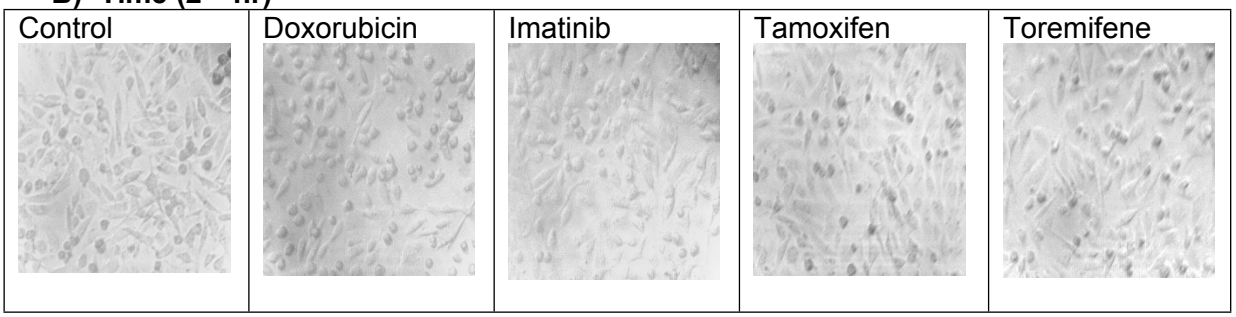
Supplementary:

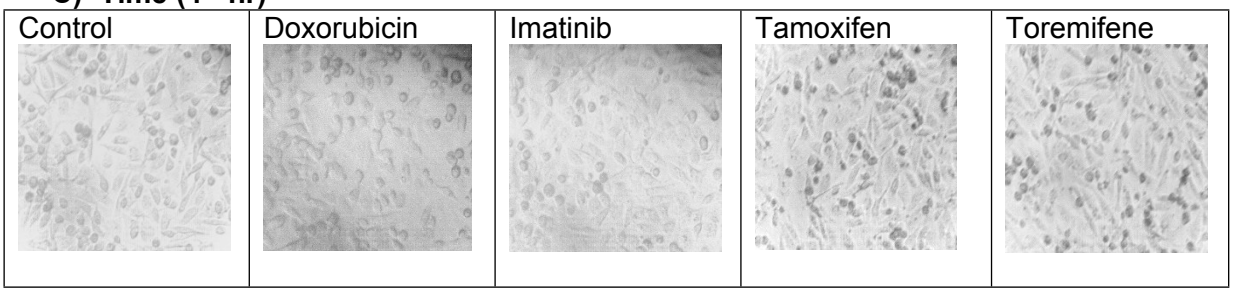
A) Time (0 hr)



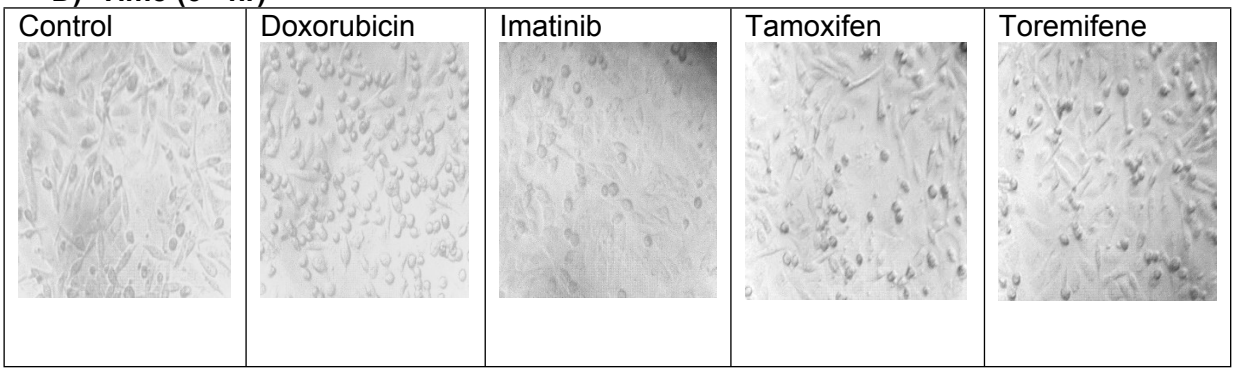
B) Time (2nd hr)



C) Time (4th hr)



D) Time (6th hr)



E) Time (24th hr)

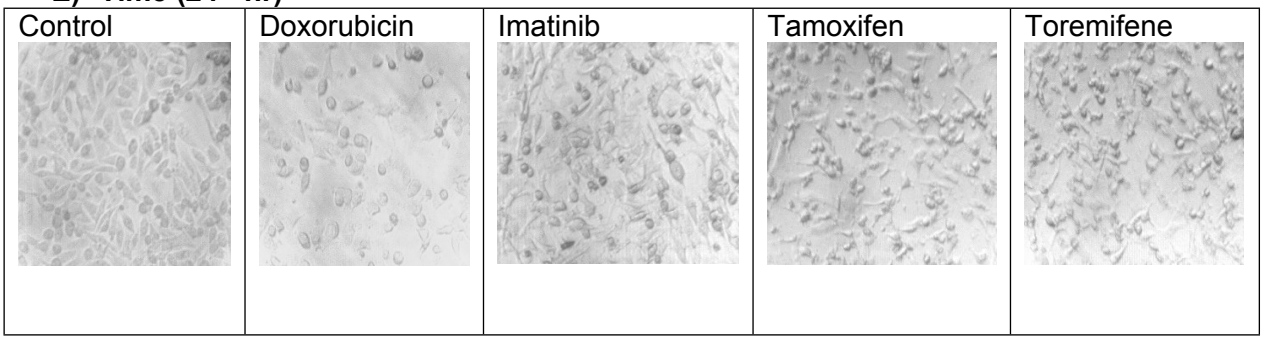


Figure 1: Visible images of live MDA-MB-231 cells attached to ZnS ATR crystal before the exposure to 0.1% DMSO (Control) and IC50 of tamoxifen, toremifene, imatinib and doxorubicin (time 0, A). Visible images in (B), (C), (D) and (E) show cells after the addition of 0.1% DMSO and drugs in the 2nd, 4th, 6th, and 24th hr, respectively.

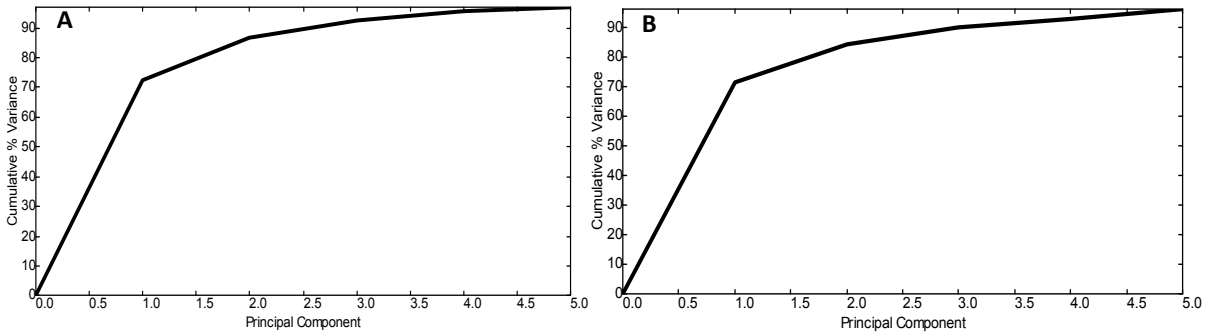
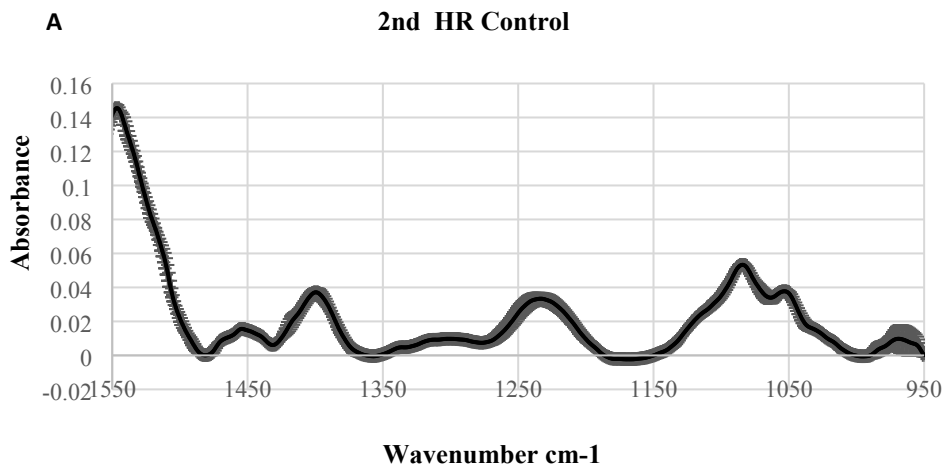
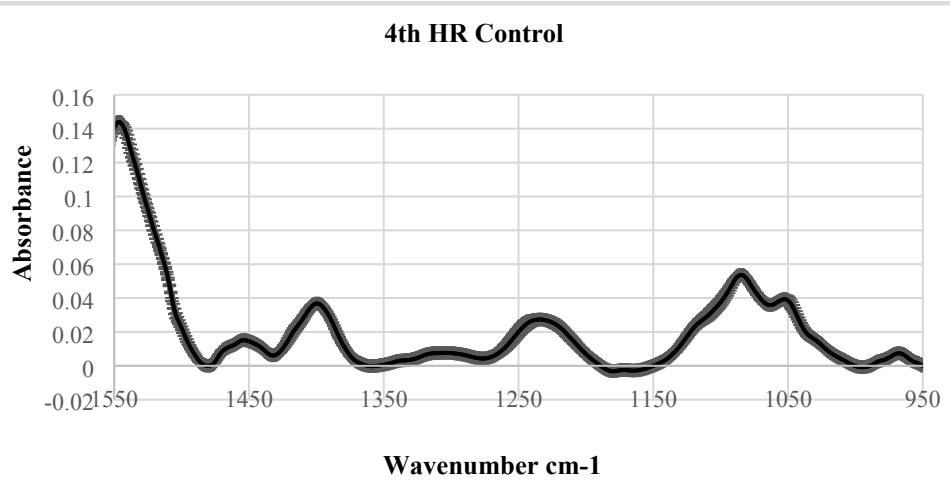
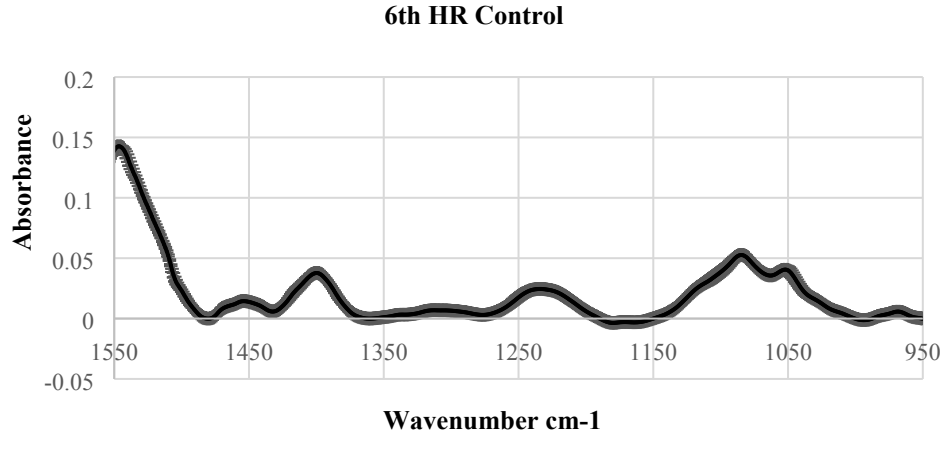


Figure 2: Percentage variance explained as function of the number of principal components of vector normalised difference spectra of live MDA-MB-231 cells for the 6th hr exposure to IC50 **(A)** and 50% IC50 **(B)**.

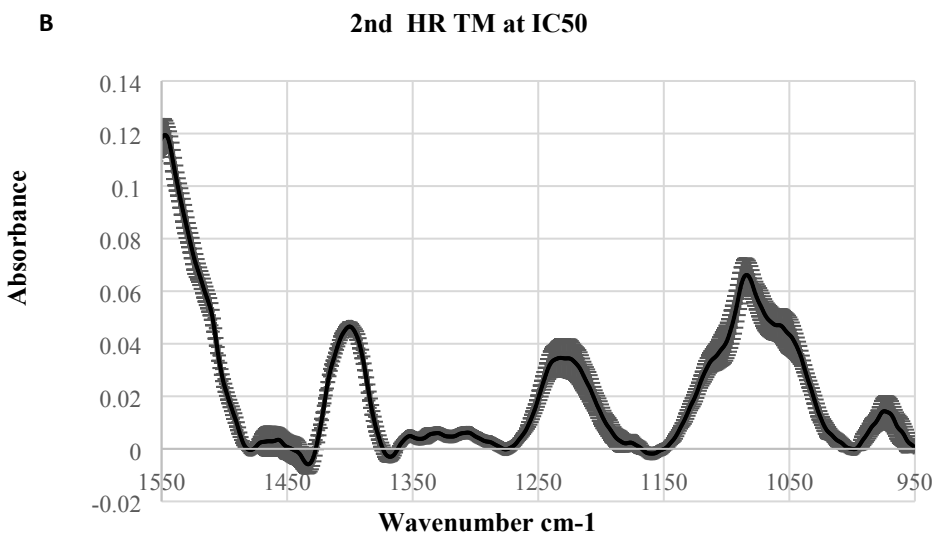
A

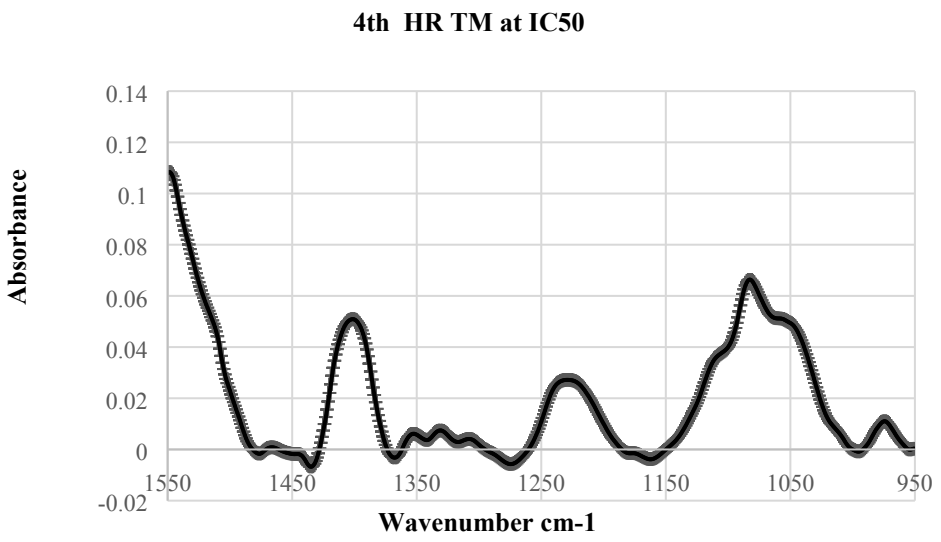

2nd HR Control
Absorbance
0.16
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
-0.02
1550
1450
1350
1250
1150
1050
950
Wavenumber cm-1

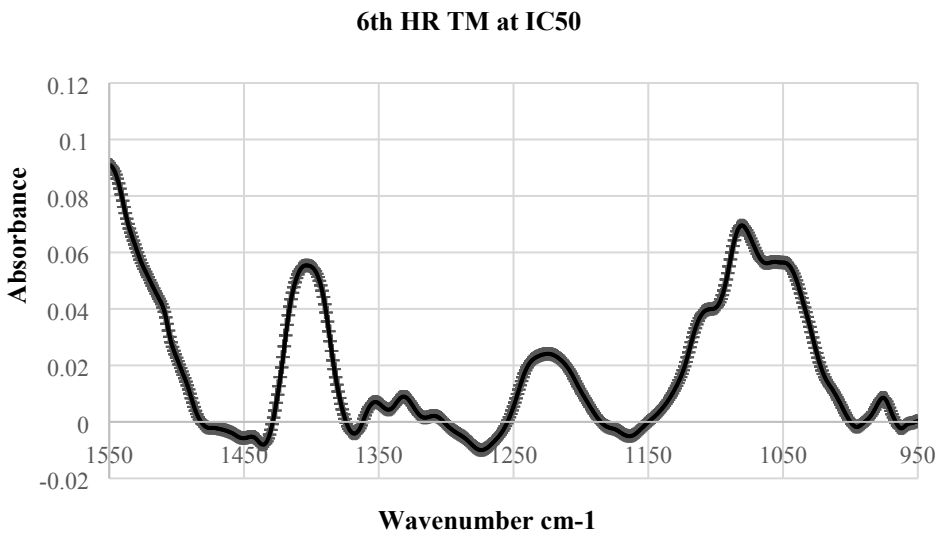

4th HR Control
Absorbance
0.16
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
-0.02
1550
1450
1350
1250
1150
1050
950
Wavenumber cm-1


6th HR Control
Absorbance
0.2
0.15
0.1
0.05
0
-0.05
1550
1450
1350
1250
1150
1050
950
Wavenumber cm-1

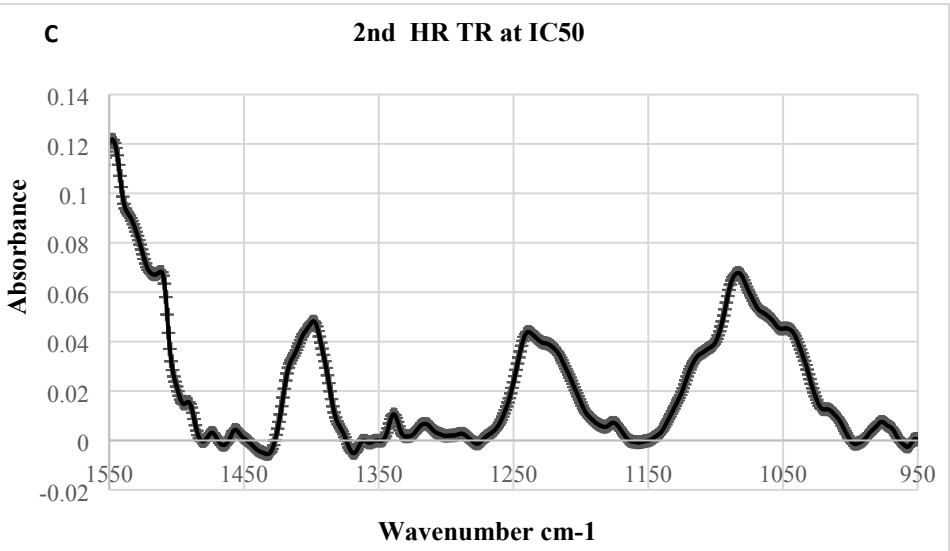
B

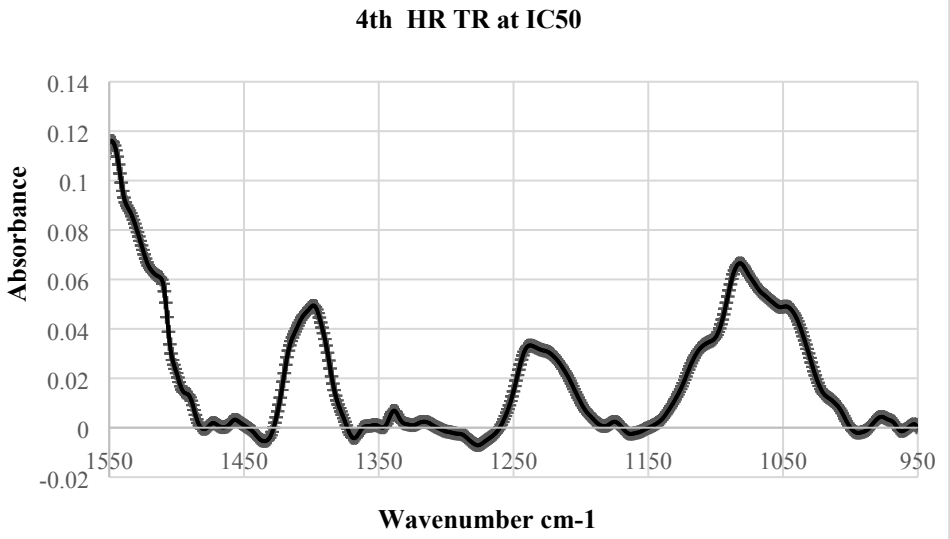

2nd HR TM at IC50
Absorbance
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
-0.02
1550
1450
1350
1250
1150
1050
950
Wavenumber cm-1

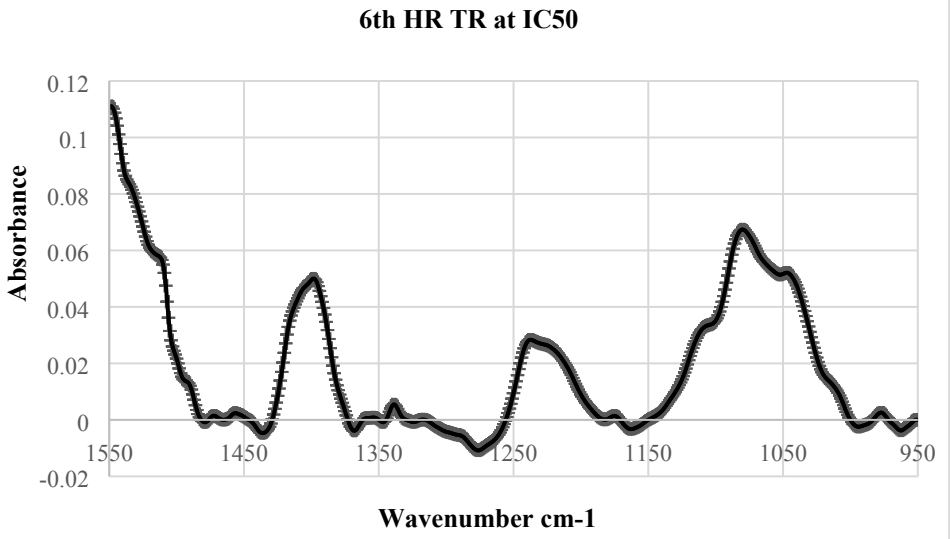

4th HR TM at IC50
Absorbance
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
-0.02
1550
1450
1350
1250
1150
1050
950
Wavenumber cm-1


6th HR TM at IC50
Absorbance
0.12
0.1
0.08
0.06
0.04
0.02
0
-0.02
1550
1450
1350
1250
1150
1050
950
Wavenumber cm-1

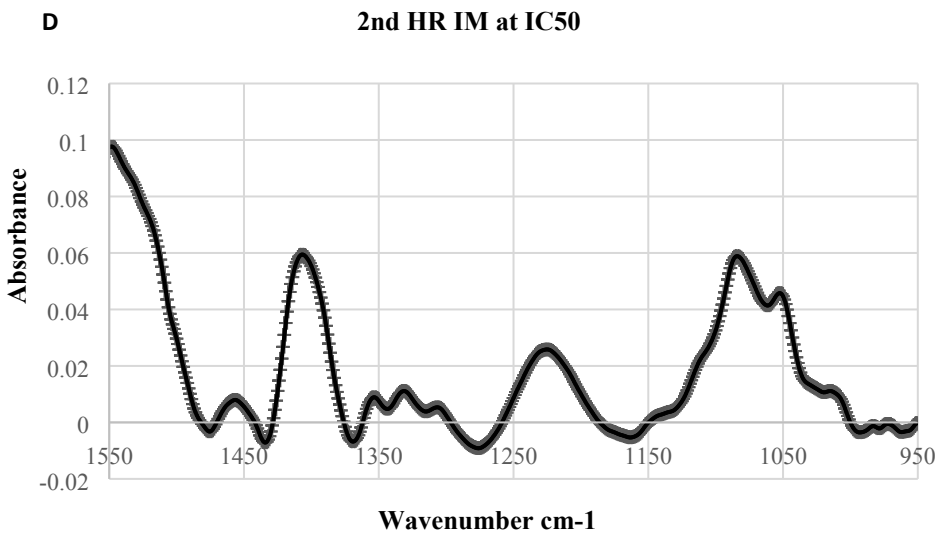
C

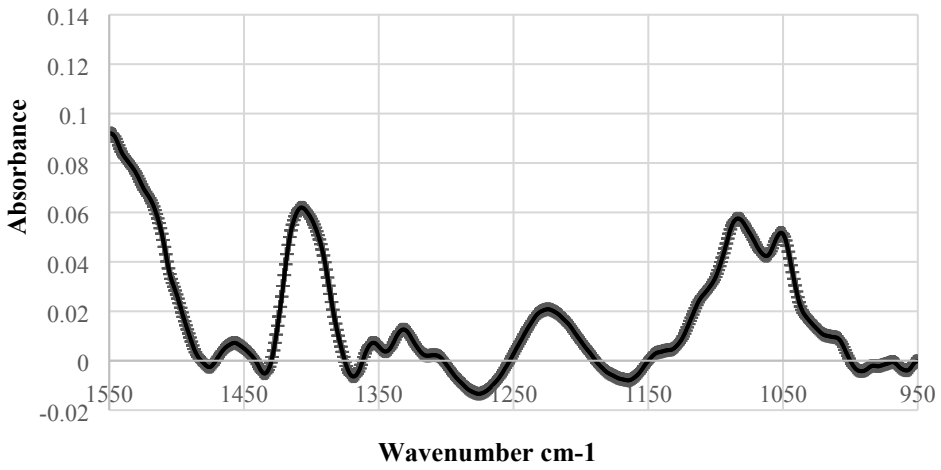

2nd HR TR at IC50
Absorbance
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
-0.02
1550
1450
1350
1250
1150
1050
950
Wavenumber cm-1

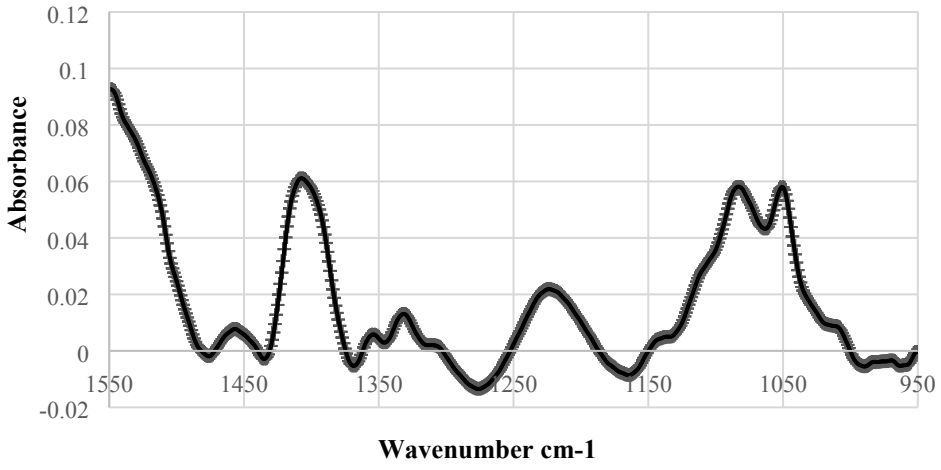

4th HR TR at IC50
Absorbance
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
-0.02
1550
1450
1350
1250
1150
1050
950
Wavenumber cm-1


6th HR TR at IC50
Absorbance
0.12
0.1
0.08
0.06
0.04
0.02
0
-0.02
1550
1450
1350
1250
1150
1050
950
Wavenumber cm-1

D


2nd HR IM at IC50
Absorbance
0.12
0.1
0.08
0.06
0.04
0.02
0
-0.02
1550
1450
1350
1250
1150
1050
950
Wavenumber cm-1


4th HR IM at IC50
Absorbance
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
-0.02
1550
1450
1350
1250
1150
1050
950
Wavenumber cm-1


6th HR IM at IC50
Absorbance
0.12
0.1
0.08
0.06
0.04
0.02
0
-0.02
1550
1450
1350
1250
1150
1050
950
Wavenumber cm-1

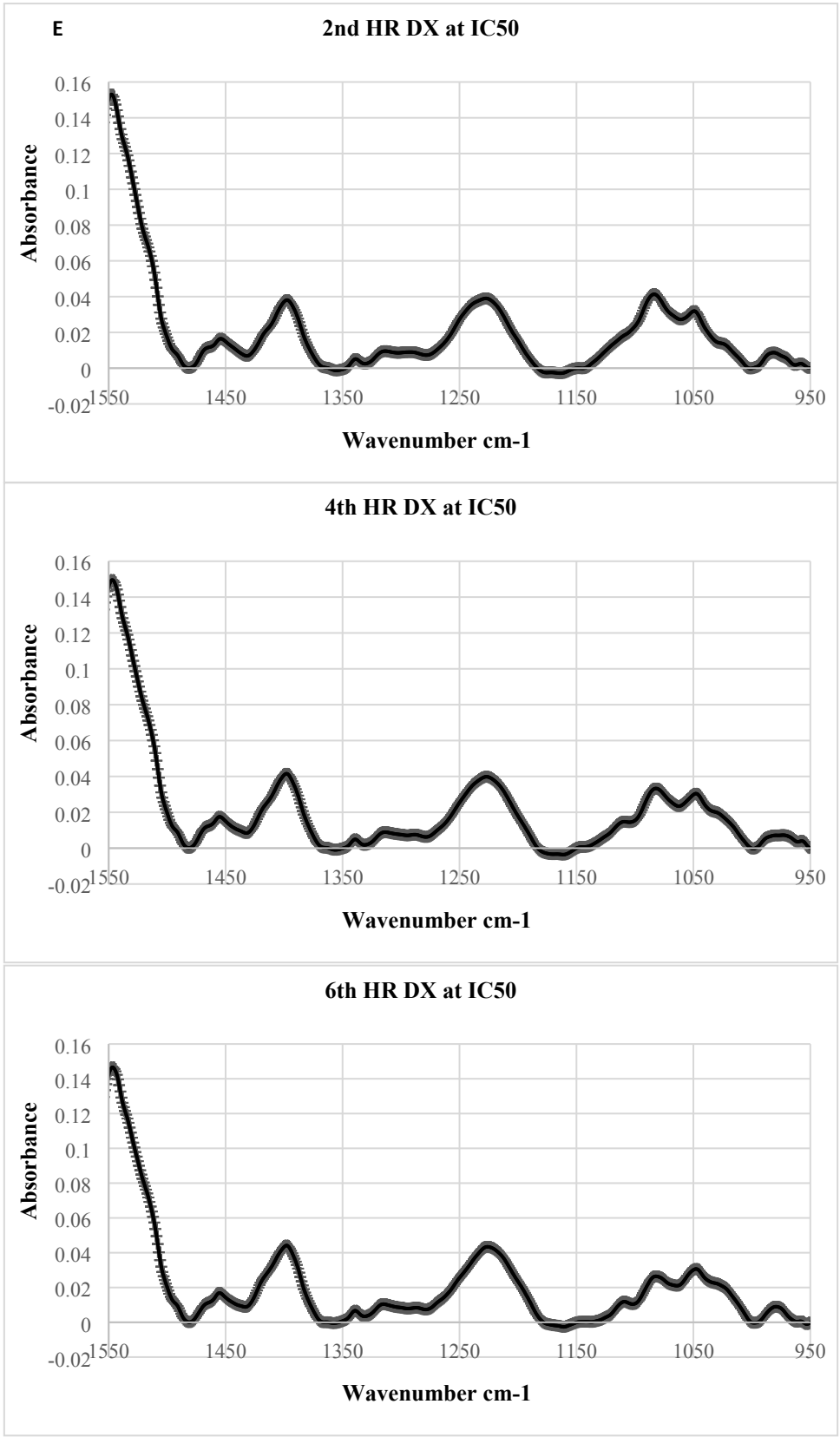
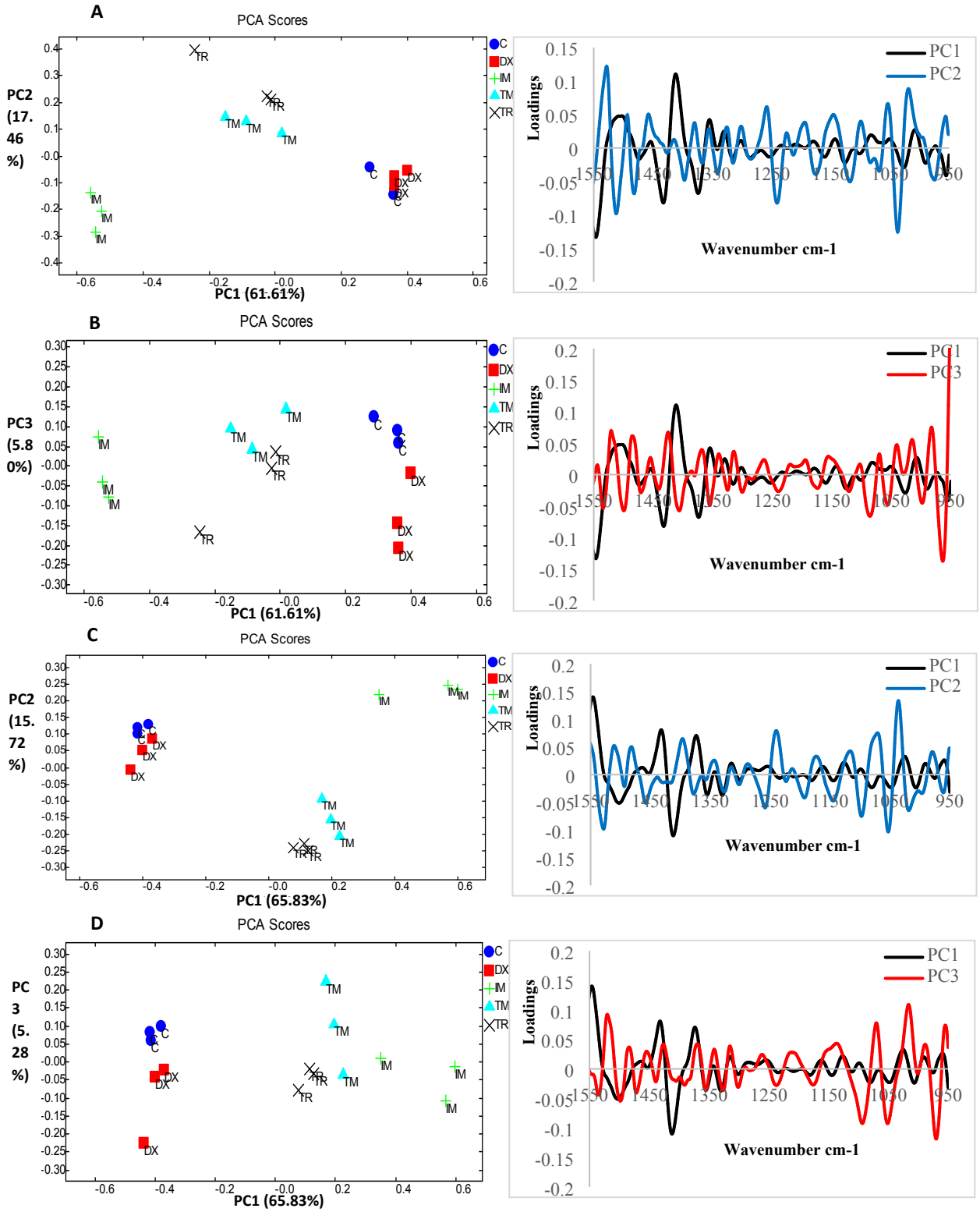


Figure 3: FTIR vector normalised difference spectra of live MDA-MB-231 cells after exposure to 0.1% DMSO (drug vehicle; control(**A**)) and IC50 of tamoxifen (**B**), toremifene (**C**), imatinib (**D**) and doxorubicin (**E**) for 2, 4 and 6 hours. The average spectra of three repeated measurements are presented in (**Black line**) with error bars shown in (**Grey**).


A
PCA Scores
PC2 (17.46 %)
PC1 (61.61%)
C
DX
IM
TM
TR
Loadings
PC1
PC2
Wavenumber cm-1
B
PCA Scores
PC3 (5.80%)
PC1 (61.61%)
PC1
PC3
Wavenumber cm-1
C
PCA Scores
PC2 (15.72 %)
PC1 (65.83%)
PC1
PC2
Wavenumber cm-1
D
PCA Scores
PC 3 (5.28 %)
PC1 (65.83%)
PC1
PC3
Wavenumber cm-1

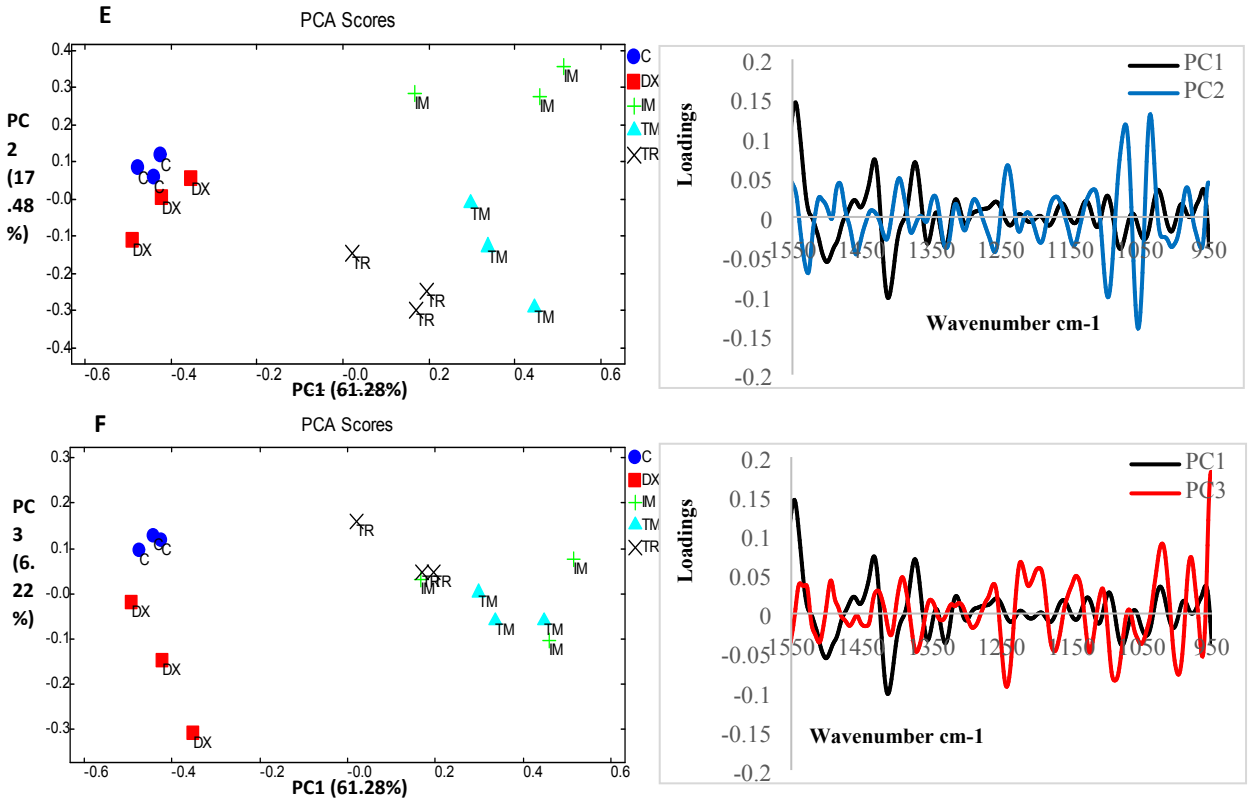
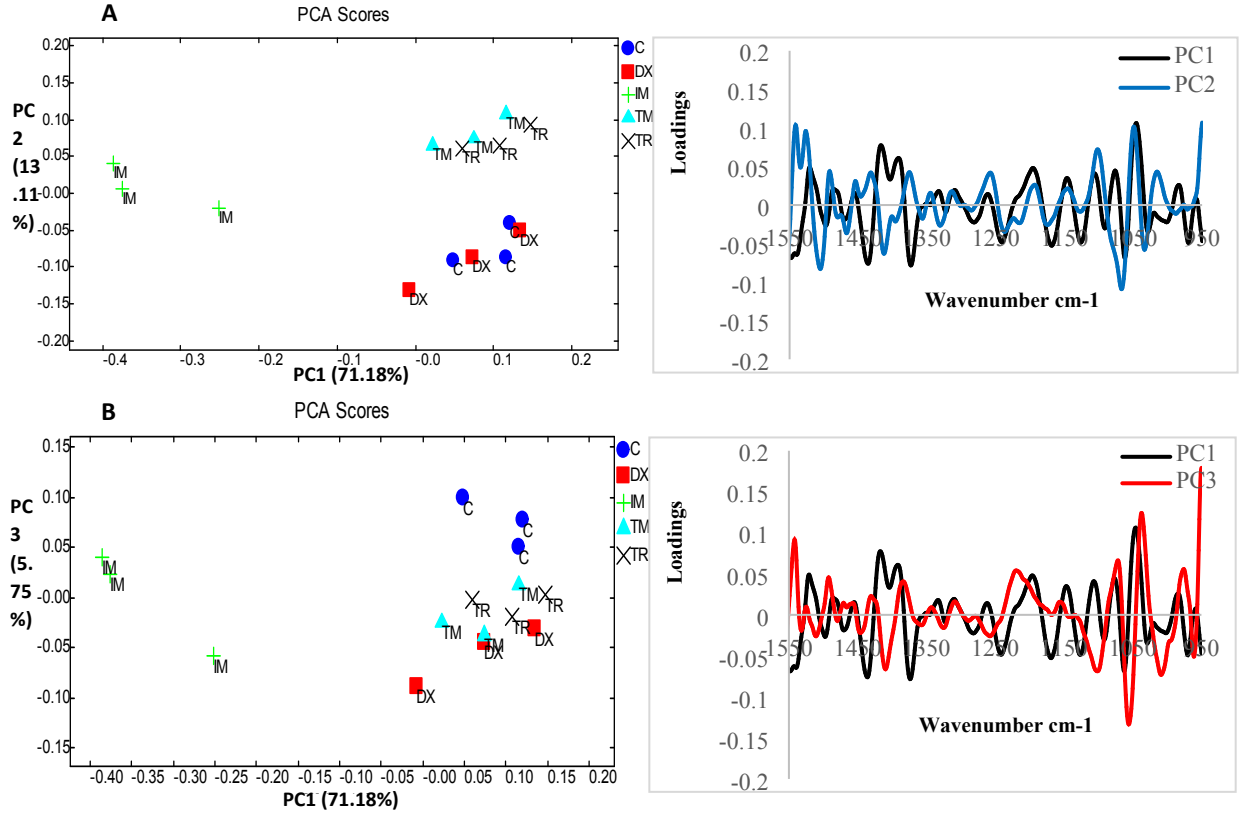


Figure 4: PCA score and its corresponding loading of FTIR vector normalised 2nd derivative difference spectra of live MDA-MB-231 cells after exposure to 0.1% DMSO (control) and IC50 of tamoxifen (TM), toremifene (TR), imatinib (IM) and doxorubicin (DX) in the 2nd hr (A and B), 4th hr (C and D) and 6th hr (E and F).



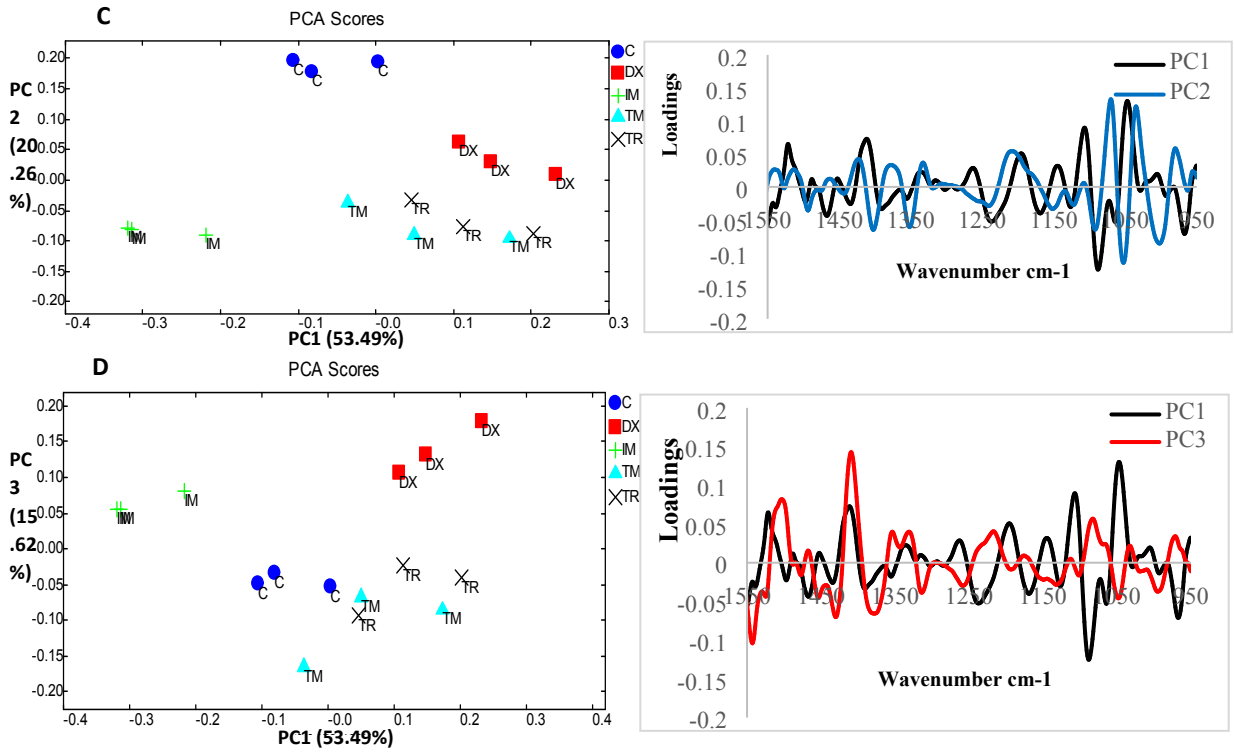


Figure 5: PCA score and its corresponding loading of FTIR vector normalised 2nd derivative difference spectra of live MDA-MB-231 cells after exposure to 0.1% DMSO (control) and 50% IC50 of tamoxifen (TM), toremifene (TR), imatinib (IM) and doxorubicin (DX) in the 6th hr (A and B), 24th hr (C and D).

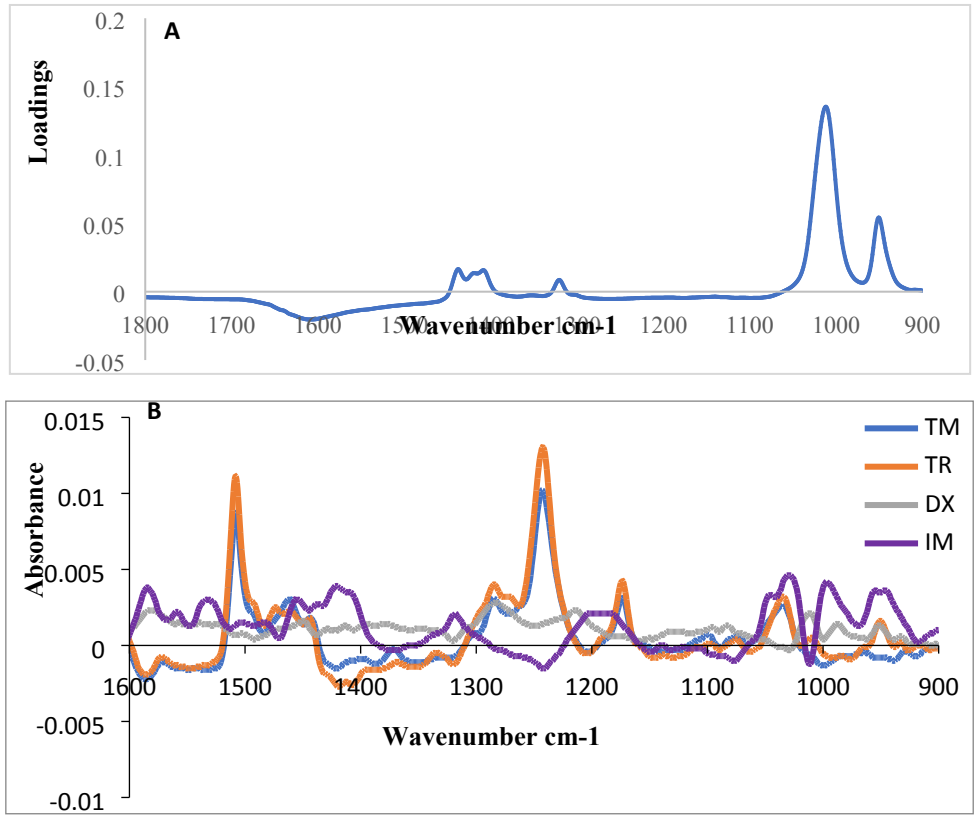
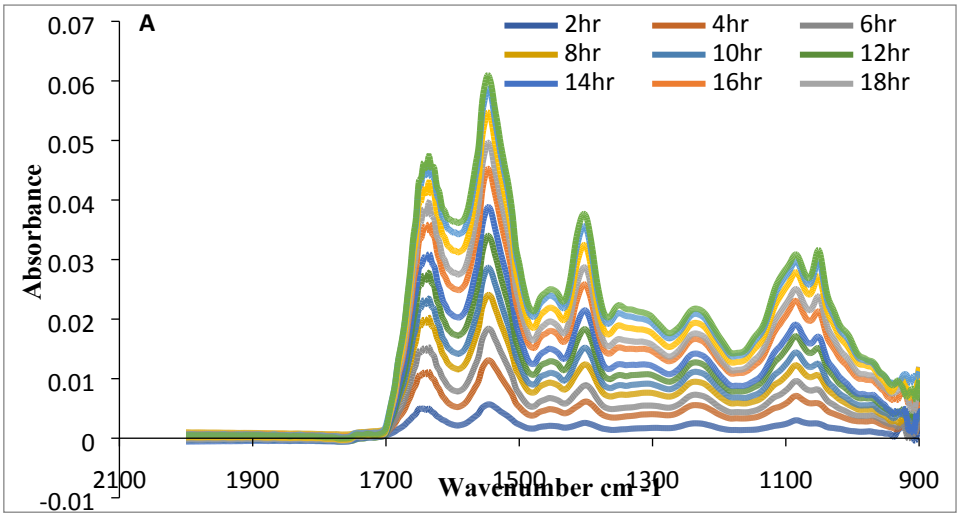
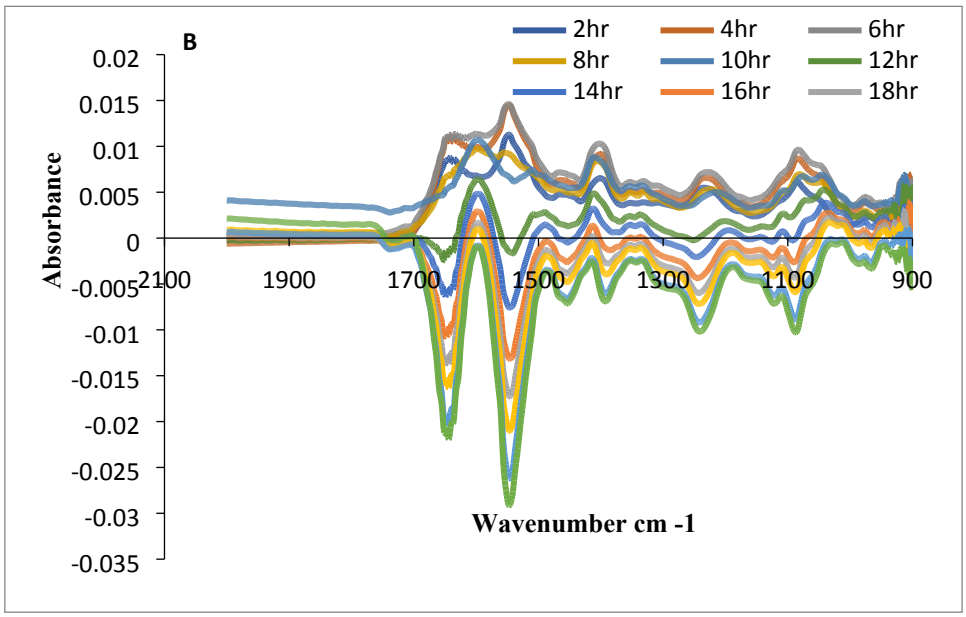
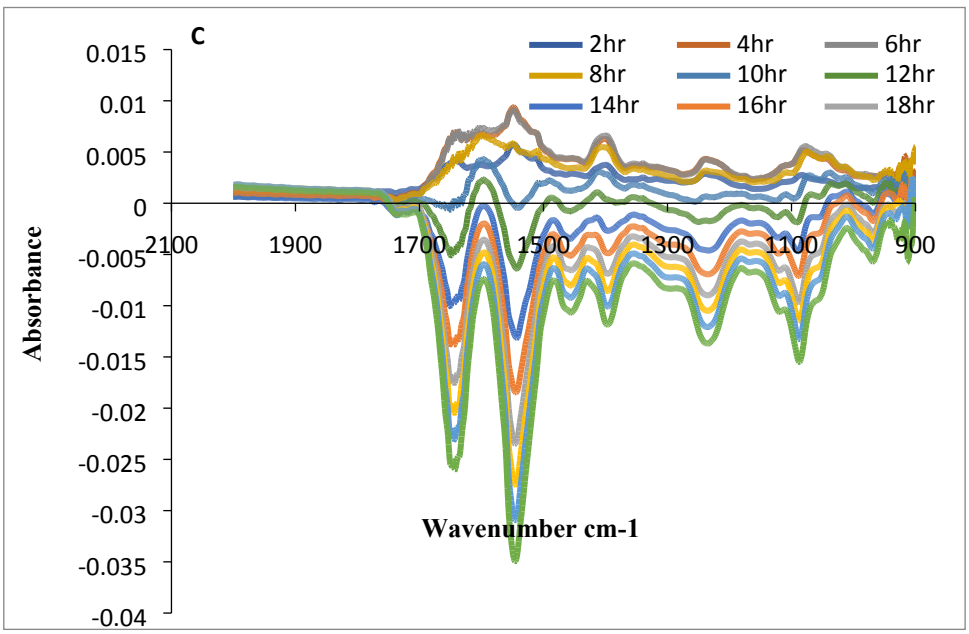


Figure 6: FTIR-ATR Spectra of 10% DMSO and (A) and 10 mM of tamoxifen (TM), toremifene (TR), doxorubicin (DX) and imatinib (IM) shown in (B).


A
2hr
4hr
6hr
8hr
10hr
12hr
14hr
16hr
18hr
Absorbance
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
-0.01
2100
1900
1700
1500
1300
1100
900
Wavenumber cm -1


B
2hr
4hr
6hr
8hr
10hr
12hr
14hr
16hr
18hr
Absorbance
0.02
0.015
0.01
0.005
0
-0.005
-0.01
-0.015
-0.02
-0.025
-0.03
-0.035
2100
1900
1700
1500
1300
1100
900
Wavenumber cm -1


C
2hr
4hr
6hr
8hr
10hr
12hr
14hr
16hr
18hr
Absorbance
0.015
0.01
0.005
0
-0.005
-0.01
-0.015
-0.02
-0.025
-0.03
-0.035
-0.04
2100
1900
1700
1500
1300
1100
900
Wavenumber cm-1

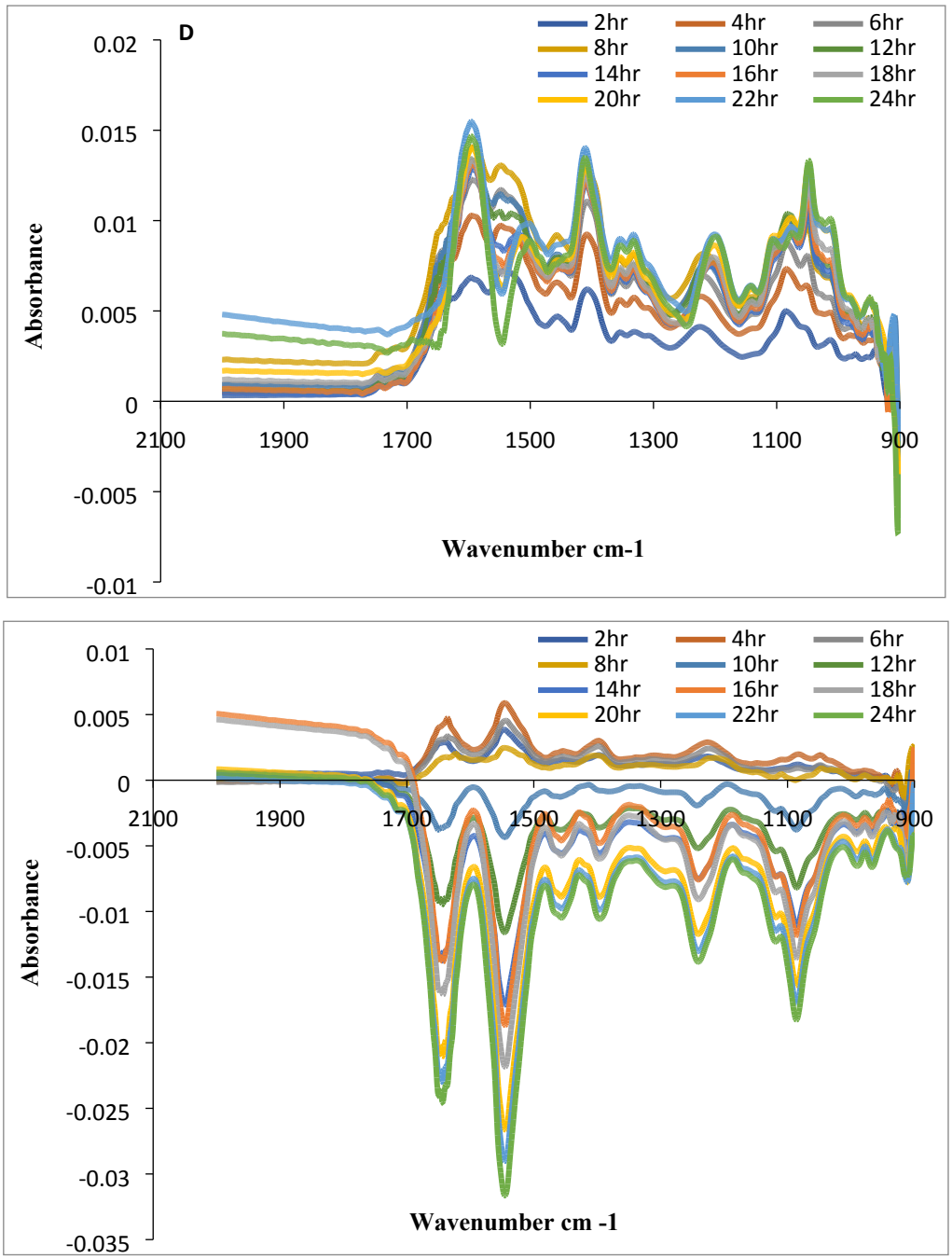


Figure 7: ATR-FTIR non-baselined difference spectra of live MDA-MB-231 cells after exposure to 0.1% DMSO (drug vehicle; control(**A**)) and IC50 of tamoxifen (**B**), toremifene (**C**), imatinib (**D**) and doxorubicin (**E**) for 24 hours.

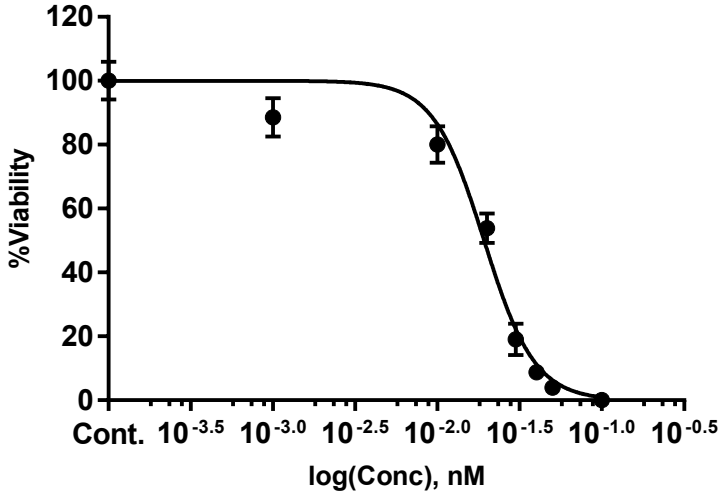


Figure 8: Cell Viability Percentage of MDA-MB-231 treated with tamoxifen for 24 hrs.