# TLC-Densitometric Analysis of Selected 5-Nitroimidazoles

**Alina Pyka-Pająk**

Department of Analytical Chemistry, Faculty of Pharmaceutical Sciences in Sosnowiec, Medical University of Silesia in Katowice, Jagiellońska 4, 41-200 Sosnowiec, Poland
Correspondence: apyka@sum.edu.pl (A.P.-P.); Tel.: +48-32-364-15-30

**Table S1.** Full description the chromatographic peaks of examined metronidazole, secnidazole, ornidazole, and tinidazole and their degradation products formed during pharmaceutical active ingredient heating on silica gel at 120$^{O}$C for 24 h.

| **Compound**[1)] | **$R_F$ Value** | **Band area [%]** | **Maximal Absorption Wavelength [nm]** |
|---|---|---|---|
| M | 0.36 | 87.54 | 308 |
| PM1 | 0.04 | 7.03 | 400 |
| PM2 | 0.29 | 5.43 | 200 |
| S | 0.41 | 89.02 | 309 |
| PS1 | 0.03 | 4.22 | 400 |
| PS2 | 0.50 | 6.76 | 400 |
| O | 0.44 | 26.49 | 310 |
| PO1 | 0.04 | 8.33 | 400 |
| PO2 | 0.19 | 40.25 | 400 |
| PO3 | 0.30 | 9.68 | 400 |
| PO4 | 0.48 | 15.25 | 200 |
| T | 0.55 | 43.19 | 309 |
| PT1 | 0.36 | 56.81 | 282 |

a) M – metronidazole, PM1, PM2 – degradation products of metronidazole,; S – secnidazole, PS1, PS2 - degradation products of secnidazole; T-tinidazole, PT1- degradation product of tinidazole identified as 2-methyl-5-nitroimidazole; O-ornidazole, PO1, PO2, PO3, PO4 – degradation products of ornidazole

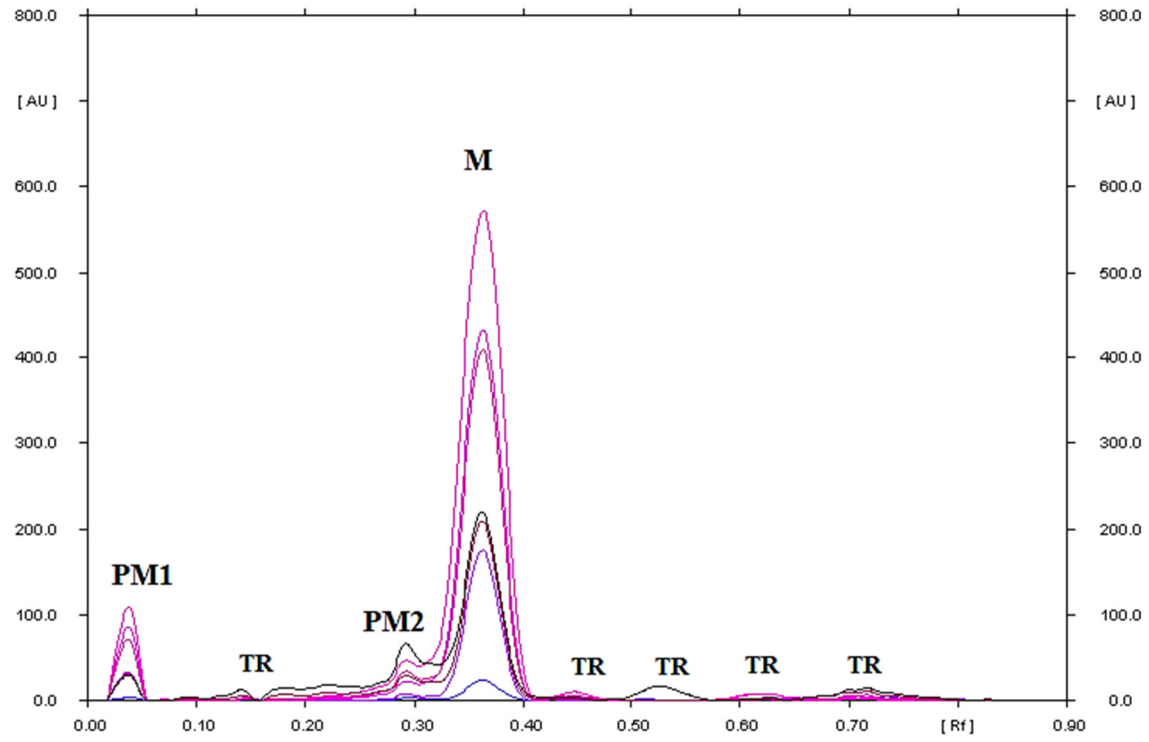


**Figure S1.** TLC-UV densitograms recorded in the multi wavelength from 200 to 380 nm (at wavelength intervals of 30 nm at each step) of metronidazole heated on silica gel at 120°C for 24 h which was next separating using chloroform-methanol (9:1, v/v) as mobile phase; where: M-metronidazole, PM1, PM2 – degradation products of metronidazole, TR – traces.

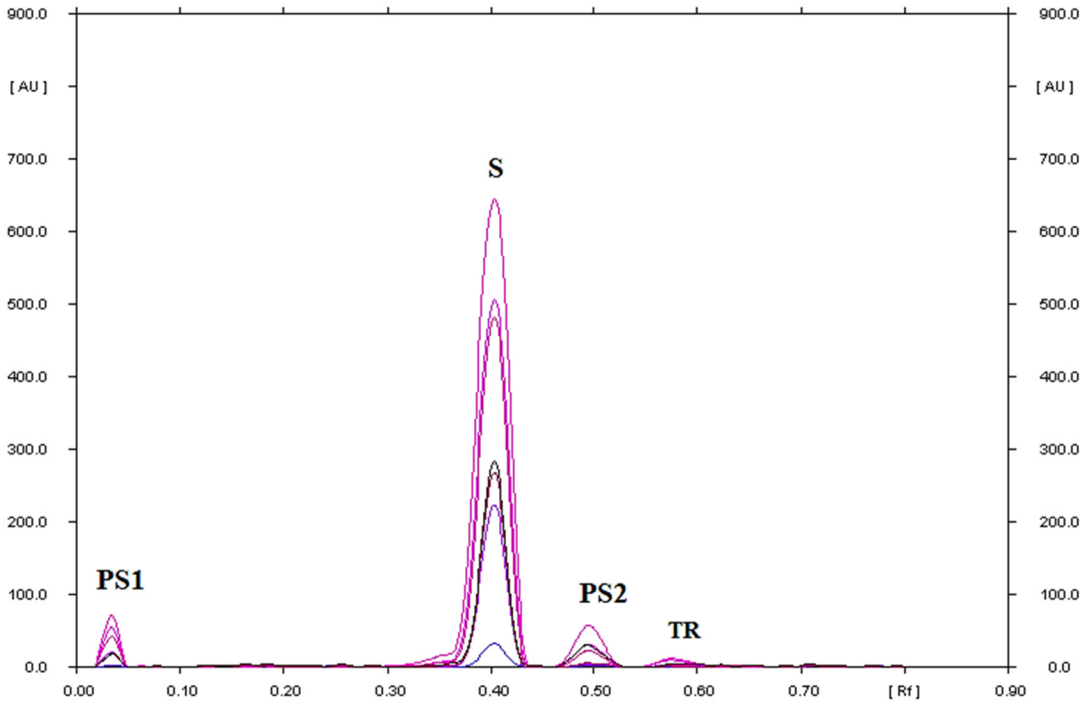


**Figure S2**. TLC-UV densitograms recorded in the multi wavelength from 200 to 380 nm (at wavelength intervals of 30 nm at each step) of secnidazole heated on silica gel at 120°C for 24 h which was next separating using chloroform-methanol (9:1, v/v) as mobile phase; where: S-secnidazole, PS1, PS2 – degradation products of secnidazole, ***TR – trace***.

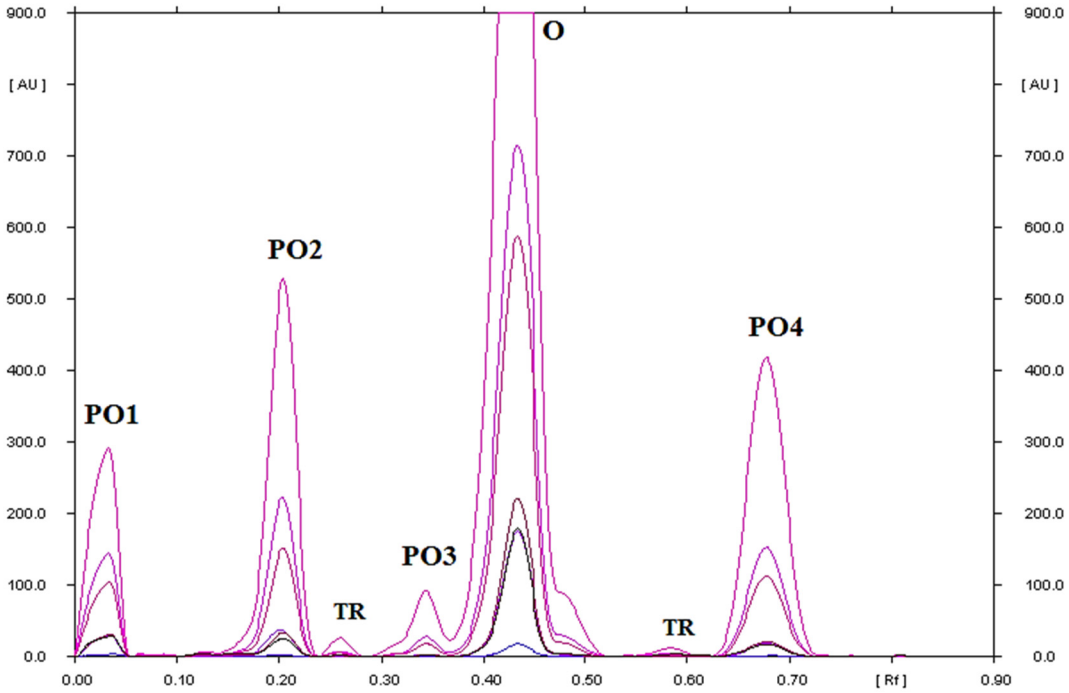


**Figure S3.** TLC-UV densitograms recorded in the multi wavelength from 200 to 380 nm (at wavelength intervals of 30 nm at each step) of ornidazole heated on silica gel at 80$^{o}$C for 24 h which was next separating using chloroform-methanol (9:1, v/v) as mobile phase; where: O-ornidazole, PO1, PO2, PO3, PO4 – degradation products of ornidazole, TR – traces.

**Table S2.** Full description the chromatographic peaks of examined metronidazole and its degradation products formed during metronidazole heating in solutions at 40°C for 1000 h.

| Metronidazole solution[1)] | Compound[2)] | $R_F$ Value | Band area [%] | Maximal Absorption Wavelength [nm] |
|---|---|---|---|---|
| I | M | 0.39 | 100.00 | 312 |
| II | M | 0.36 | 100.00 | 312 |
| III | M | 0.36 | 100.00 | 312 |
| IV | PM1 | 0.05 | 2.17 | 200 |
| | PM2 | 0.23 | 3.42 | 323 |
| | PM3 | 0.27 | 3.10 | 308 |
| | PM4 | 0.31 | 3.02 | 200 |
| | PM5 | 0.45 | 14.39 | 304 |
| | M | 0.36 | 73.90 | 312 |
| V | M | 0.36 | 100.00 | 313 |
| VI | M | 0.37 | 100.00 | 312 |
| VII | M | 0.36 | 100.00 | 313 |

where: [1)]Metronidazole solution: I – in water at pH=2.62; II – in water at pH=5.56; III- in water at pH=8.21; IV- in hydrogen peroxide (3%); V – in physiological salt (0.9% NaCl); VI- in methanol, VII – standard solution; [2)] M-metronidazole, PM1, PM2, PM3, PM4, PM5-degradation products of metronidazole

**Table S3.** Full description the chromatographic peaks of examined secnidazole and its degradation products formed during secnidazole heating in solutions at 40°C for 1000 h.

| Secnidazole solution[1)] | Compound[2)] | $R_F$ Value | Band area [%] | Maximal Absorption Wavelength [nm] |
|---|---|---|---|---|
| I | S | 0.49 | 96.26 | 312 |
| | PS1 | 0.70 | 3.74 | 200 |
| II | S | 0.45 | 100 | 313 |
| III | S | 0.46 | 100 | 312 |
| IV | S | 0.49 | 73.25 | 313 |
| | PS1 | 0.03 | 3.98 | 200 |
| | PS2 | 0.37 | 3.57 | 308 |
| | PS3 | 0.43 | 1.84 | 200 |
| | PS4 | 0.56 | 17.36 | 304 |
| V | S | 0.45 | 100 | 312 |
| VI | S | 0.48 | 100 | 313 |
| VII | S | 0.47 | 100 | 304 |

Where: [1)]Secnidazole solution: I – in water at pH=2.62; II – in water at pH=5.56; III- in water at pH=8.21; IV- in hydrogen peroxide (3%); V – in physiological salt (0.9% NaCl); VI- in methanol, VII – standard solution; [2)] S-metronidazole, PS1, PS2, PS3, PS4-degradation products of secnidazole; PS2 was identified as 2-methyl-5-nitroimidazole

**Table S4.** Full description the chromatographic peaks of examined ornidazole and its degradation products formed during ornidazole heating in solutions at 40°C for 1000 h.

| Ornidazole solution[1)] | Compound[2)] | $R_F$ Value | Band area [%] | Maximal Absorption Wavelength [nm] |
|---|---|---|---|---|
| I | O | 0.51 | 94.87 | 312 |
| | PO1 | 0.68 | 5.13 | 200 |
| II | O | 0.49 | 77.00 | 312 |
| | PO1 | 0.25 | 17.95 | 312 |
| | PO2 | 0.63 | 2.65 | 200 |
| | PO3 | 0.69 | 2.40 | 200 |
| III | O | 0.49 | 80.07 | 313 |
| | PO1 | 0.27 | 17.08 | 312 |
| | PO2 | 0.61 | 2.85 | 200 |
| IV | O | 0.47 | 88.40 | 312 |
| | PO1 | 0.32 | 3.71 | 320 |
| | PO2 | 0.35 | 4.32 | 305 |
| | PO3 | 0.61 | 11.77 | 200 |
| V | O | 0.47 | 83.76 | 312 |
| | PO1 | 0.23 | 11.77 | 315 |
| | PO2 | 0.60 | 2.48 | 200 |
| | PO3 | 0.67 | 1.99 | 312 |
| VI | O | 0.47 | 88.29 | 312 |
| | PO1 | 0.60 | 2.23 | 324 |
| | PO2 | 0.67 | 9.48 | 312 |
| VII | O | 0.47 | 100.00 | 313 |

where: [1)]ornidazole solution: I – in water at pH=2.62; II – in water at pH=5.56; III- in water at pH=8.21; IV- in hydrogen peroxide (3%); V – in physiological salt (0.9% NaCl); VI- in methanol, VII – standard solution; [2)] O-ornidazole, PO1, PO2, PO3-degradation products of ornidazole

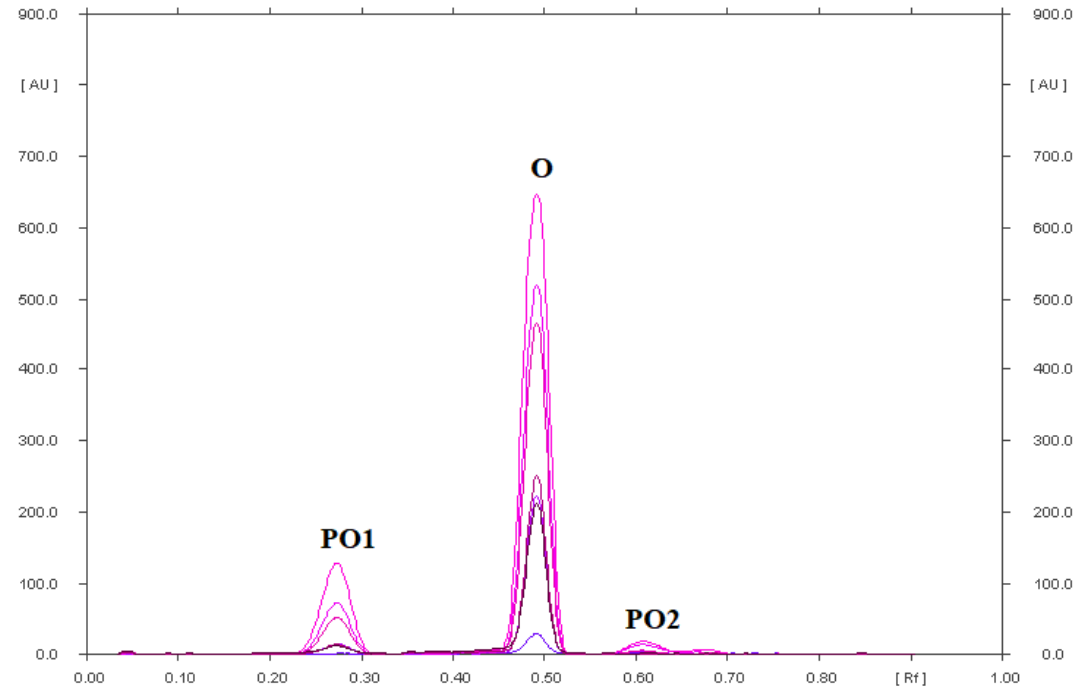


**Figure S4.** TLC-UV densitograms recorded in the multi wavelength from 200 to 380 nm (at wavelength intervals of 30 nm at each step) of ornidazole in water at pH=8.21 solution, which after heating at 40°C for 1000 h was separated on silica gel using a mobile phase chloroform–methanol (9:1, v/v/); where: O-ornidazole, PO1, PO2 - degradation products of ornidazole.

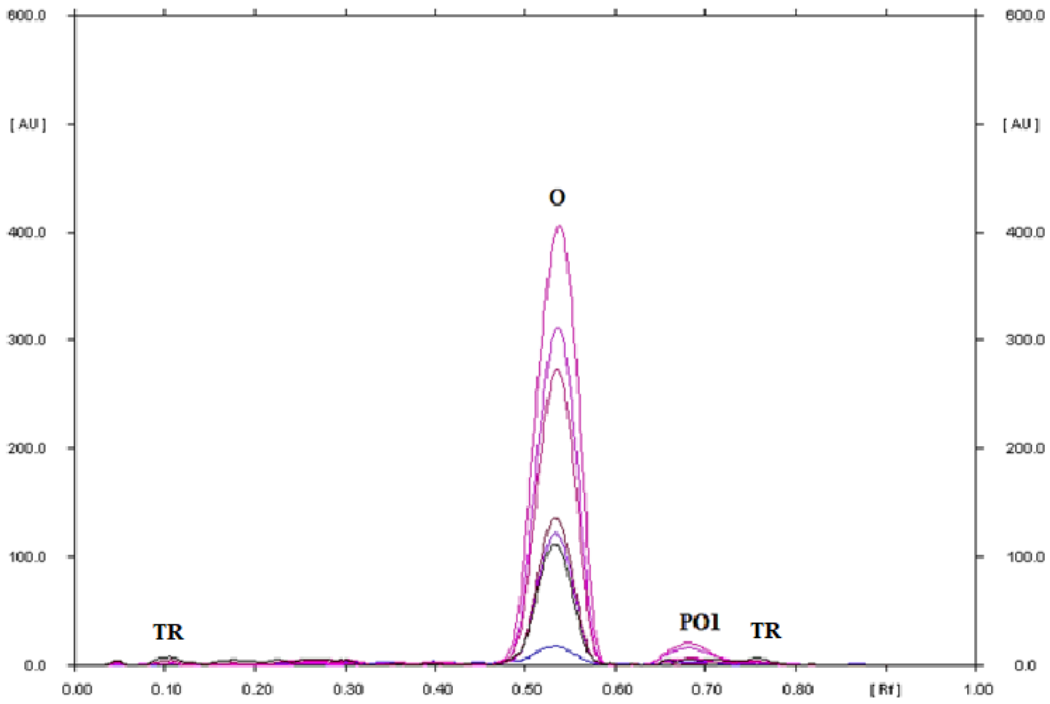


**Figure S5.** TLC-UV densitograms recorded in the multi wavelength from 200 to 380 nm (at wavelength intervals of 30 nm at each step) of ornidazole in water at pH=2.62 solution, which after heating at 40°C for 1000 h was separated on silica gel using a mobile phase chloroform–methanol (9:1, v/v/); where: O-ornidazole, PO1 - degradation product of ornidazole, TR – traces.

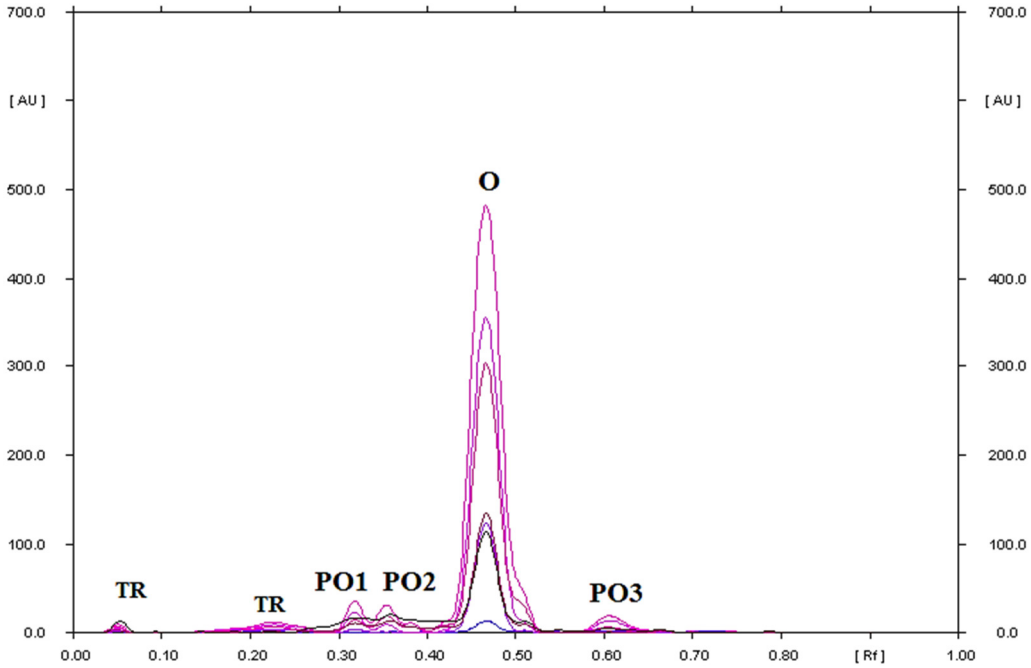


**Figure S6.** TLC-UV densitograms recorded in the multi wavelength from 200 to 380 nm (at wavelength intervals of 30 nm at each step) of ornidazole in hydrogen peroxide (3%) solution, which after heating at 40°C for 1000 h was separated on silica gel using a mobile phase chloroform–methanol (9:1, v/v/); where: O-ornidazole, PO1, PO2, PO3-degradation products of ornidazole, TR – traces.

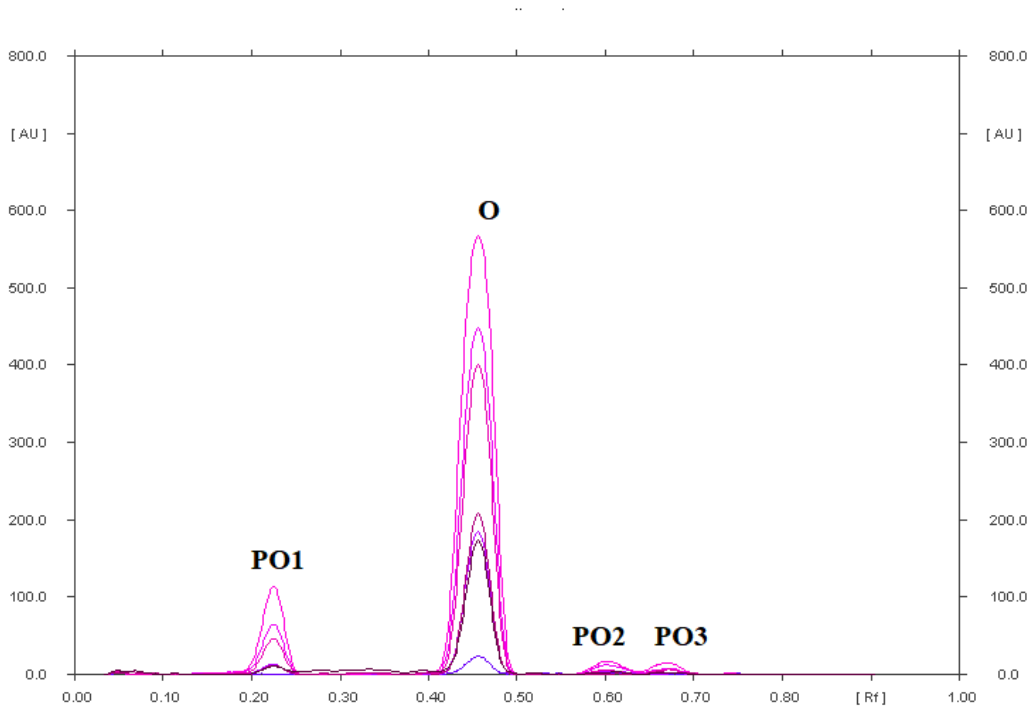


**Figure S7.** TLC-UV densitograms recorded in the multi wavelength from 200 to 380 nm (at wavelength intervals of 30 nm at each step) of ornidazole in physiological salt (0.9% NaCl) solution, which after heating at 40°C for 1000 h was separated on silica gel using a mobile phase chloroform–methanol (9:1, v/v/); where: O-ornidazole, PO1, PO2, PO3-degradation products of ornidazole.

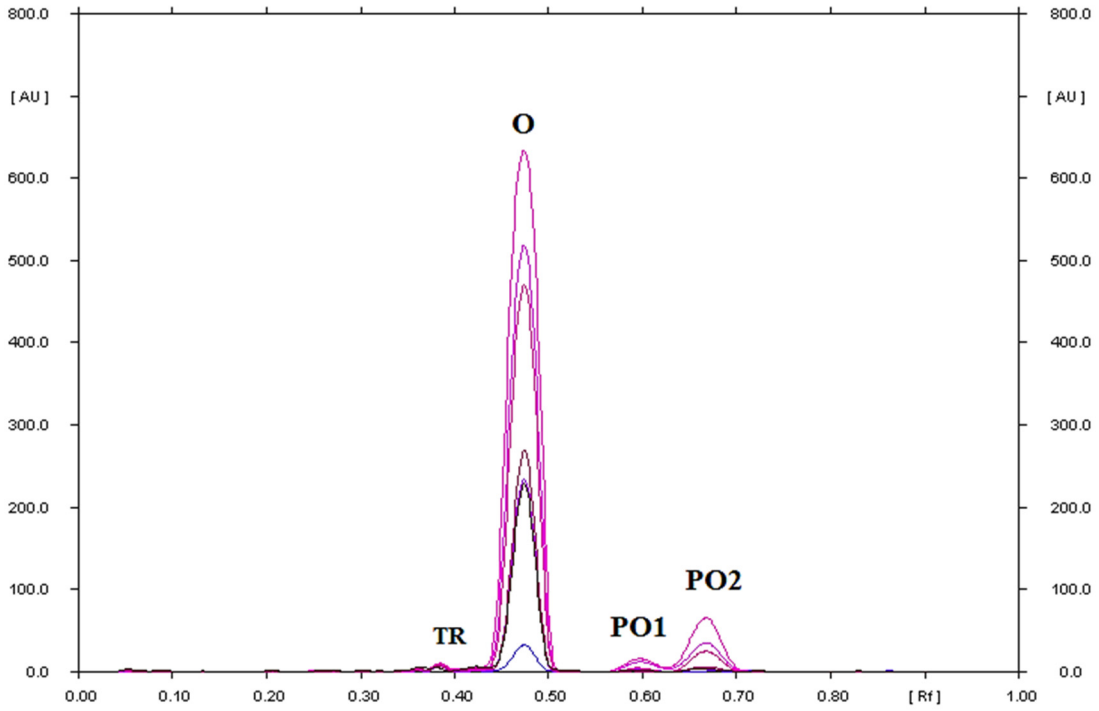


**Figure S8.** TLC-UV densitograms recorded in the multi wavelength from 200 to 380 nm (at wavelength intervals of 30 nm at each step) of ornidazole in methanolic solution, which after heating at 40°C for 1000 h was separated on silica gel using a mobile phase chloroform–methanol (9:1, v/v/); where: O-ornidazole, PO1, PO2 -degradation products of ornidazole, TR – trace.

**Table S5.** Full description the chromatographic peaks of examined tinidazole and its degradation products formed during tinidazole heating in solutions at 40°C for 1000 h.

| Tinidazole solution[1)] | Compound[2)] | $R_F$ Value | Band area [%] | Maximal Absorption Wavelength [nm] |
|---|---|---|---|---|
| I | T | 0. 57 | 100.00 | 312 |
| II | T | 0.53 | 56.73 | 311 |
| | P1 | 0.22 | 1.38 | 200 |
| | P2 | 0.31 | 0.32 | 200 |
| | P3 | 0.34 | 36.76 | 308 |
| | P4 | 0.41 | 4.81 | 200 |
| III | T | 0.53 | 64.47 | 311 |
| | P1 | 0.36 | 35.53 | 307 |
| IV | T | 0.55 | 77.59 | 311 |
| | P1 | 0.40 | 3.85 | 315 |
| | P2 | 0.44 | 5.55 | 307 |
| | P3 | 0.51 | 13.01 | 299 |
| V | T | 0.53 | 61.22 | 311 |
| | P1 | 0.35 | 38.78 | 307 |
| VI | T | 0.54 | 70.73 | 312 |
| | P1 | 0.38 | 29.27 | 308 |
| VII | T | 0.55 | 100.00 | 311 |

Where: [1)]Tinidazole solution: I – in water at pH=2.62; II – in water at pH=5.56; III- in water at pH=8.21; IV- in hydrogen peroxide (3%); V – in physiological salt (0.9% NaCl); VI- in methanol, VII – standard solution; [2)] O-ornidazole, PT1, PT2, PT3, PT4-degradation products of tinidazole; degradation product with $R_F$ equal about 0.34-0.38 was identified as 2-methyl-5-nitroimidazole

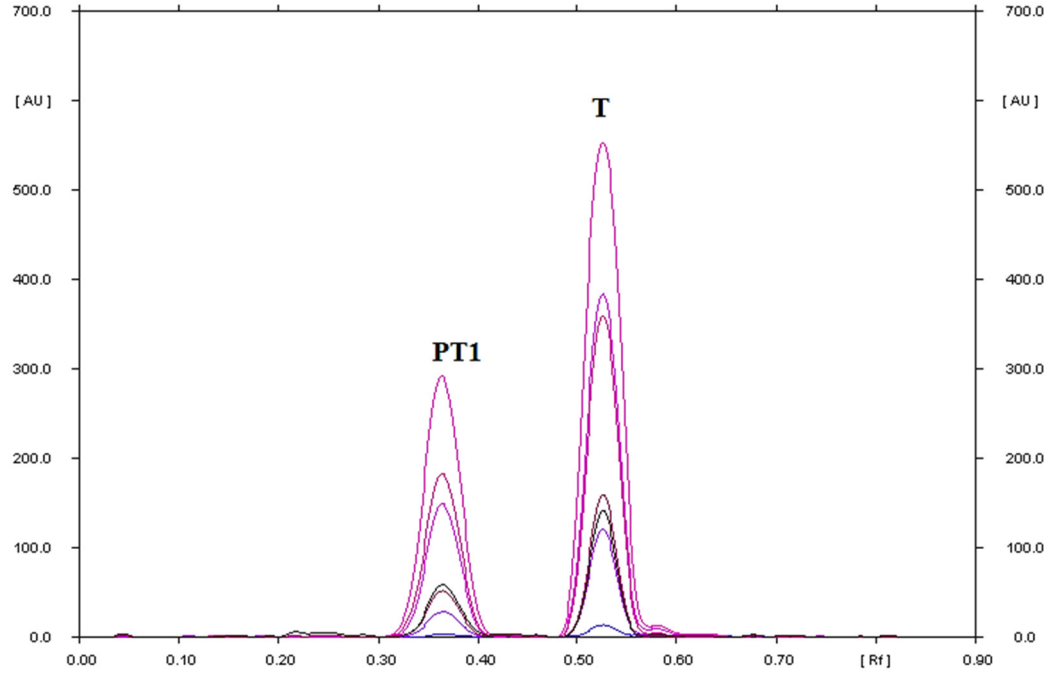


**Figure S9.** TLC-UV densitograms recorded in the multi wavelength from 200 to 380 nm (at wavelength intervals of 30 nm at each step) of tinidazole in water at pH=8.21 solution, which after heating at 40°C for 1000 h was separated on silica gel using a mobile phase chloroform–methanol (9:1, v/v/); where: T-tinidazole, PT1 - degradation product of tinidazole identified as 2-methyl-5-nitroimidazole.

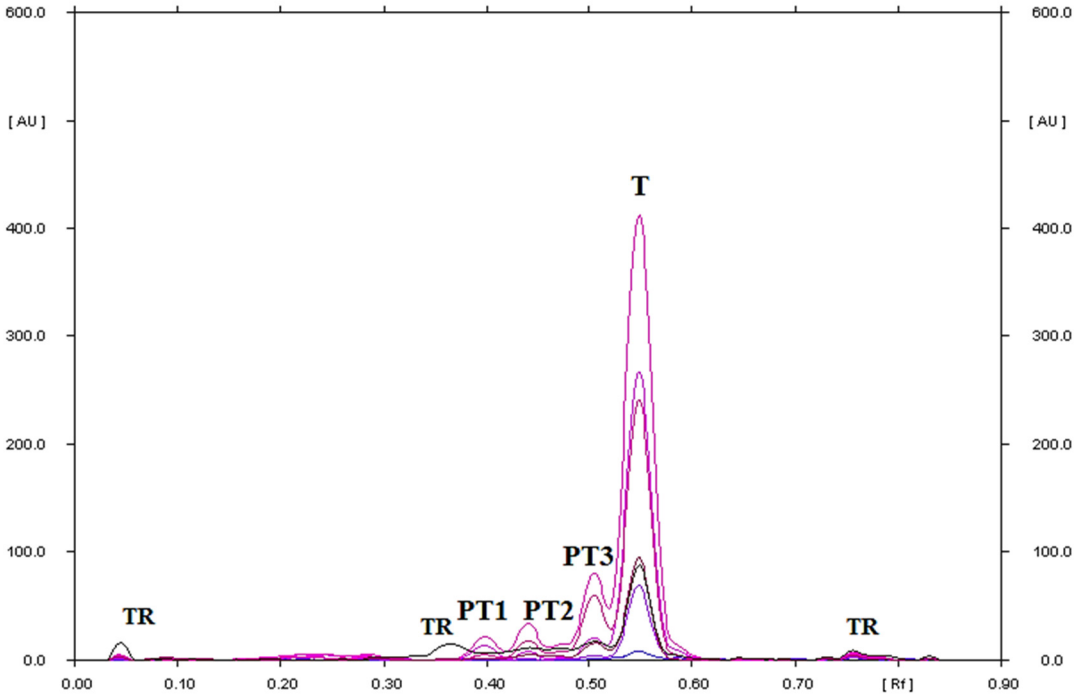


**Figure S10.** TLC-UV densitograms recorded in the multi wavelength from 200 to 380 nm (at wavelength intervals of 30 nm at each step) of tinidazole in hydrogen peroxide (3%) solution, which after heating at 40°C for 1000 h was separated on silica gel using a mobile phase chloroform–methanol (9:1, v/v/); where: T-tinidazole, PT1, PT2, PT3 - degradation products of tinidazole, TR – traces.

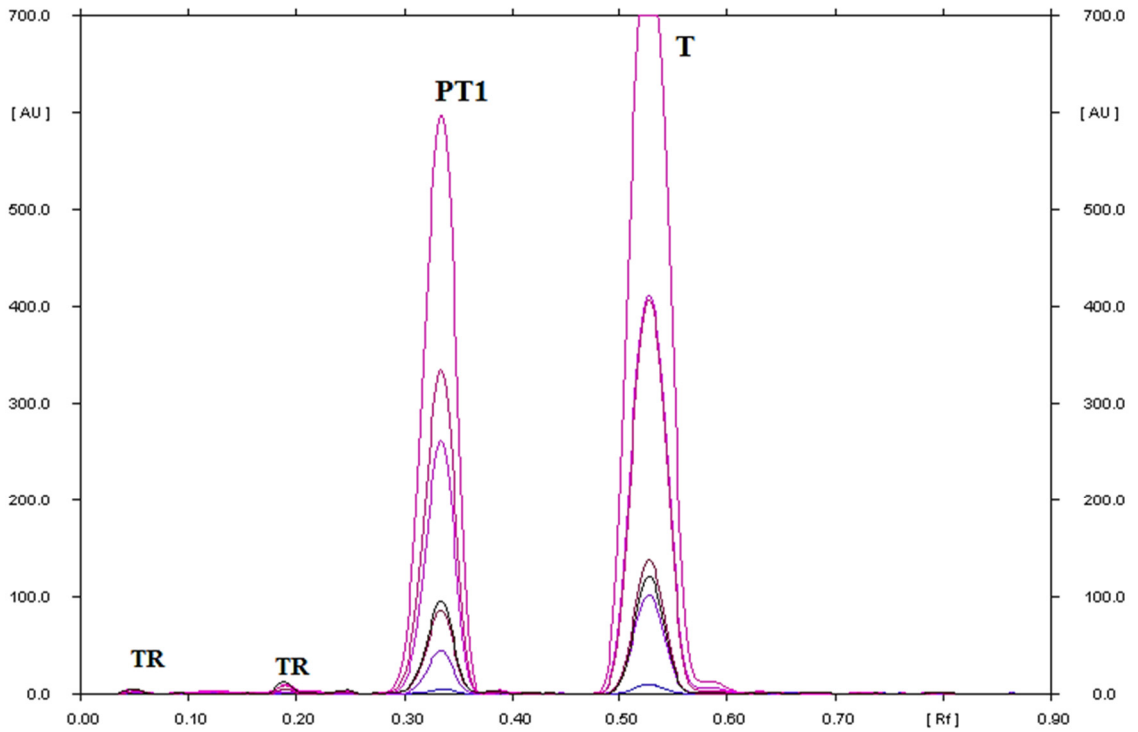


**Figure S11.** TLC-UV densitograms recorded in the multi wavelength from 200 to 380 nm (at wavelength intervals of 30 nm at each step) of tinidazole in physiological salt (0.9% NaCl) solution, which after heating at 40°C for 1000 h was separated on silica gel using a mobile phase chloroform–methanol (9:1, v/v/); where: T-tinidazole, PT1 - degradation product of tinidazole identified as 2-methyl-5-nitroimidazole, TR – traces.

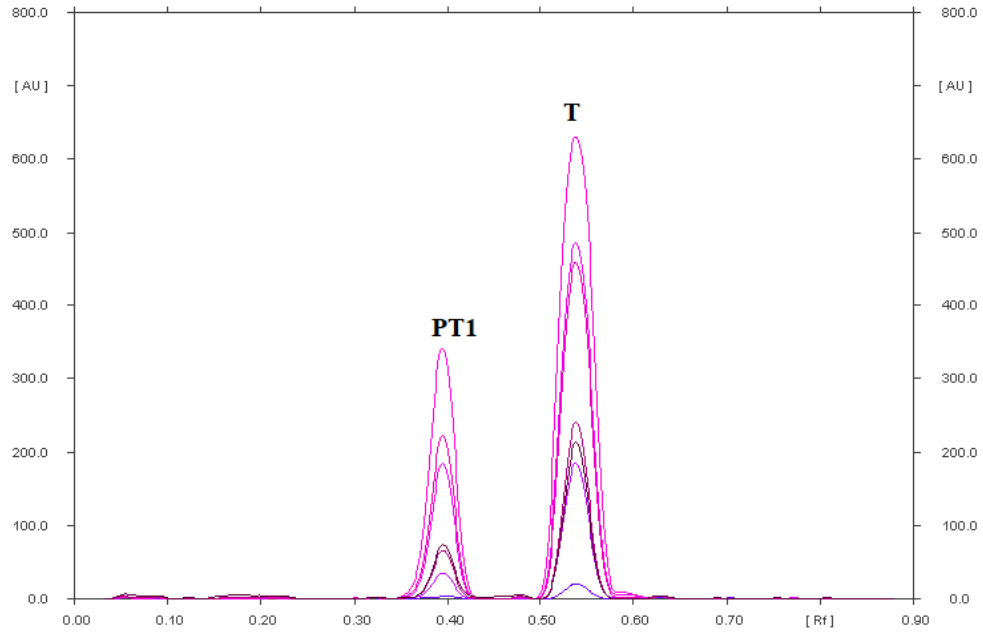


**Figure S12.** TLC-UV densitograms recorded in the multi wavelength from 200 to 380 nm (at wavelength intervals of 30 nm at each step) of tinidazole in methanolic solution, which after heating at 40°C for 1000 h was separated on silica gel using a mobile phase chloroform–methanol (9:1, v/v/); where: T-tinidazole, PT1 - degradation product of tinidazole identified as 2-methyl-5-nitroimidazole.

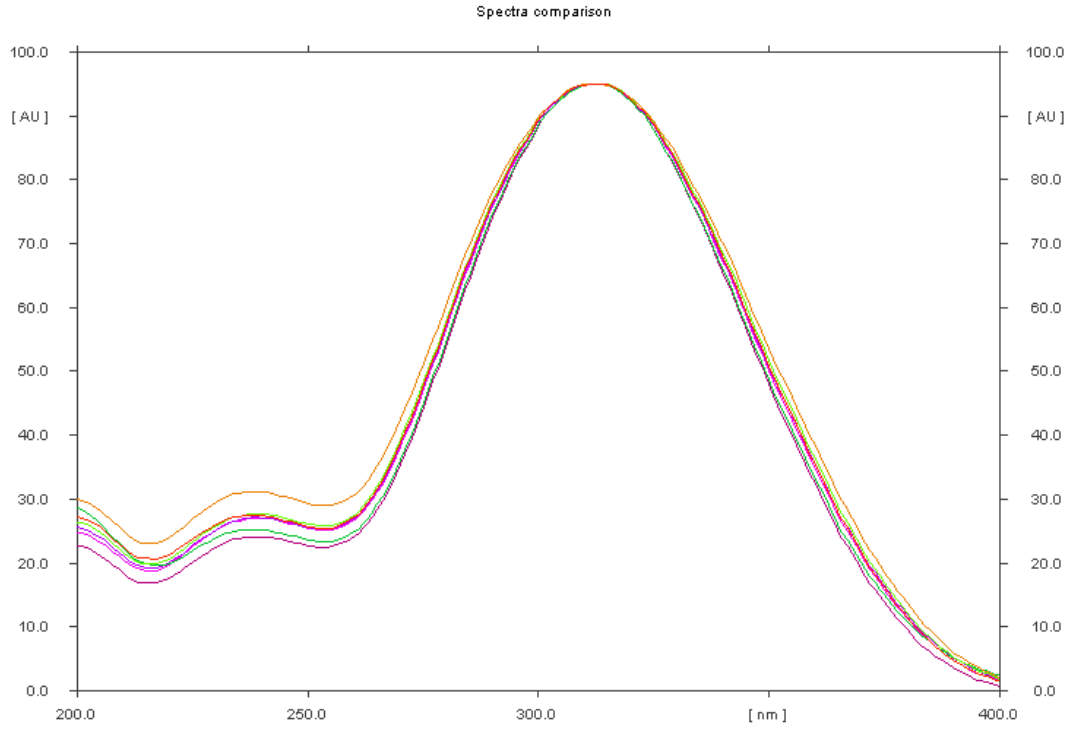


**Figure S13.** Spectra comparison of standard metronidazole and metronidazole after heating in different solutions.

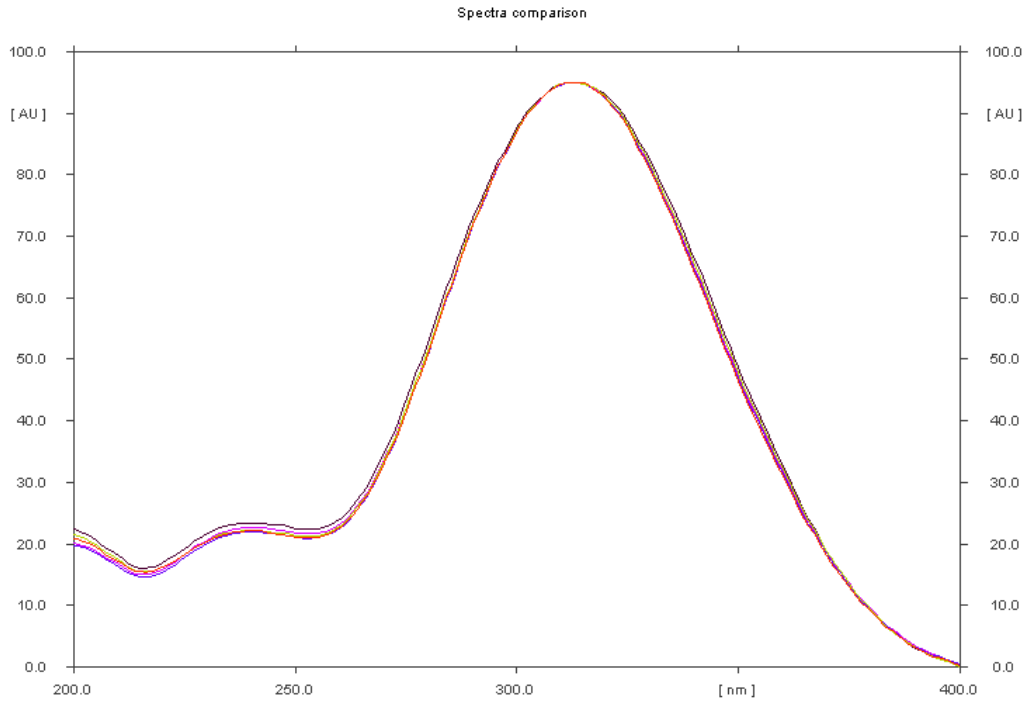


**Figure S14.** Spectra comparison of standard secnidazole and secnidazole after heating in different solutions.

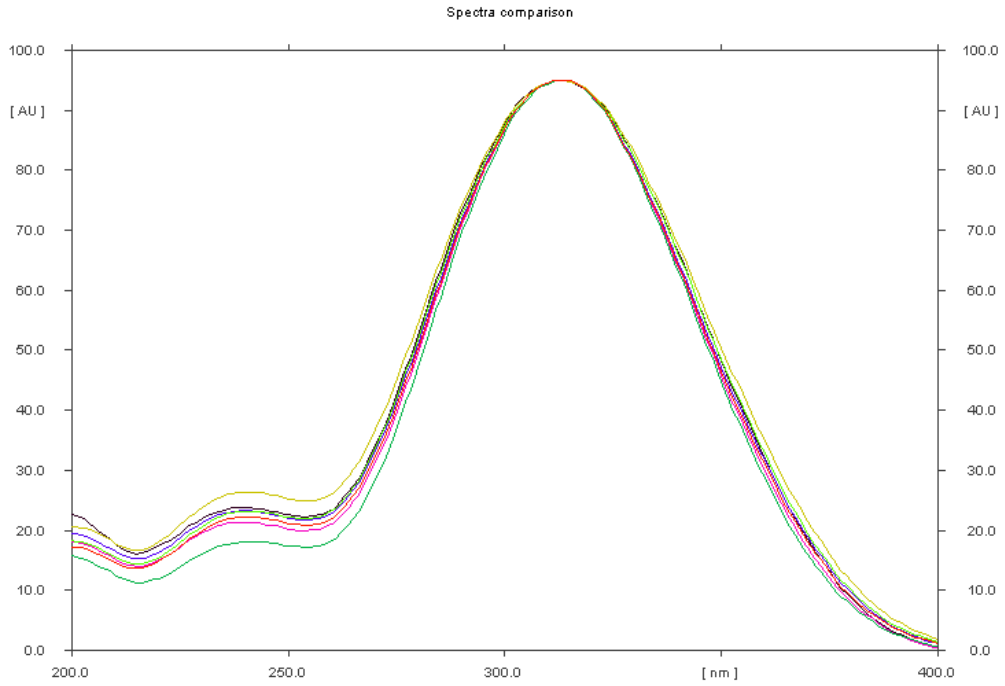


**Figure S15.** Spectra comparison of standard ornidazole and ornidazole after heating in different solutions.

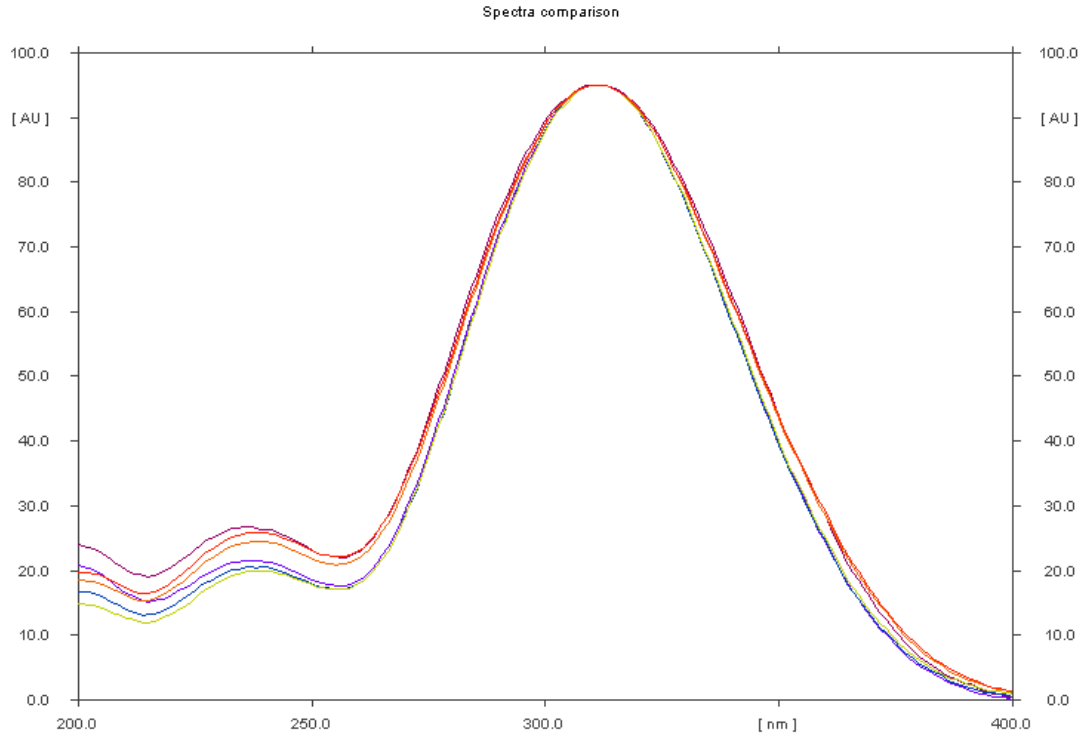


**Figure S16.** Spectra comparison of standard tinidazole and tinidazole after heating in different solutions.