Thermogravimetric analysis of teeth for forensic purposes

Diego Lozano-Peral[1,2] • Ana Arango-Díaz[3] • Stella Martín-de-las-Heras[4] • Leticia Rubio[1]

[1] Department of Forensic Medicine, University of Malaga, 29071 Malaga, Spain

[2] Supercomputing and Bioinnovation Center, University of Malaga, 29590 Malaga, Spain

[3] Servicios Centrales de Apoyo a la Investigación, University of Malaga, 29071 Malaga, Spain

[4] Department of Forensic Medicine and Forensic Dentistry, University of Granada, 18071 Granada, Spain

✉ Stella Martin-de-las-Heras
e-mail: stella@ugr.es
Telephone number: (+34) 958249932

ORCID of the authors:

Leticia Rubio: 0000-0002-8233-624X

Stella Martin-de-las-Heras: 0000-0002-1554-951X

Diego Lozano-Peral: 0000-0001-8072-0355

Abstract

The objectives of this study were to characterize the thermal decomposition of human teeth and to evaluate the decomposition of organic matter, including DNA, at different temperatures. Eight teeth were chemically characterized by thermogravimetric analysis coupled with mass spectroscopy, conducting evolved gas analyses at temperatures up to 1000 ºC and 60-minute isothermal assays at 50, 100, 150, 200, 250, 300, 350, and 400 ºC. Mass losses (total of 25.2 %) were associated with: loss of free water at temperatures between 44 and 210 ºC, combustion of organic matter between 211 and 603 ºC, and decomposition of inorganic matter between 604 and 940 ºC. The first organic fragment detected was sulfur dioxide (linked to protein decomposition), which showed a major peak at around 270 ºC, while volatile DNA residues were recorded between 330 and 347 ºC. Isothermal assay results showed that carbon dioxide molecules (associated with organic matrix decomposition) were already present between 150 and 200 ºC, indicating the start of organic matter degradation and a potential negative effect on DNA integrity at this temperature range. The most severe decomposition of organic matter started between 200 and 250 ºC. These data contribute to knowledge on the decomposition of organic matter, particularly DNA, under thermal conditions encountered in forensic scenarios requiring the genetic identification of fire-damaged humans.

Keywords Teeth • DNA • Identification • Thermogravimetric analysis • Mass spectroscopy

Introduction

The identification of human remains that have endured extreme environmental conditions can pose a major challenge to forensic scientists [1,2]. Fire can be involved in accidents [3], homicides, and suicides [4,5] or used to conceal crimes [6]. DNA typing is sometimes the only forensic technique capable of identifying individuals severely damaged by fire [7]. Samples for this purpose are generally drawn from skeletal elements [8,9], especially from teeth [6,10], which surround and protect DNA with hard dental tissue [11,12]. Knowledge of the decomposition of teeth components at different temperatures is therefore of forensic interest.

Thermogravimetry analysis coupled to mass spectrometry (TG-MS) has been used to characterize the chemical composition and structure of bones [13,14] and teeth [15,16] and for clinical medical and odontological purposes [17,18,19]. It can serve to characterize the decomposition at different temperatures of DNA, among other chemical components. This technique yields data on mass loss and evolved gas of interest for analyzing the organic and inorganic content of samples and determining decomposition phases. It has been used in this way by archaeologists to determine the composition of organic matter in bones [20].

Slightly discrepant results have been published in studies on thermal DNA degradation using varied techniques under different conditions [21,22]. Thermal analysis of the chemical decomposition of enamel and dentin components has been conducted for the purposes of restorative dentistry [15]. However, we have found no previous TG-MS study in complete teeth from crown to root (enamel, dentine, and pulp) in a forensic context.

The objectives of this study were: (1) to characterize the thermal decomposition of human teeth at increasing temperatures using TG-MS; and (2) to determine the thermal decomposition of the organic matrix of human teeth, including DNA among other chemical compounds, by isothermal assay.

Material and methods

Human tooth sampling, decontamination, and pulverization

Eight erupted permanent teeth (7 molars and 1 premolar) were obtained from three females and five males (mean age, 39.4 years; range, 19-72 years) attending oral health clinics of the Andalusian Public Health Service in Cadiz (Spain). Only healthy teeth were included, i.e., free from cavities, endodontics, reconstruction, breakage, or abnormal staining (e.g., from tetracycline). Informed consent was obtained from all participants enrolled in the study, which was approved by the Ethics Committee for Human Research at the University of Malaga (Spain) and complied with the Declaration of Helsinki.

After extraction for valid clinical reasons (periodontal disease, malocclusion, or orthodontic treatment), teeth were immersed in saline solution and delivered to the laboratory for analysis. Blood was washed from the teeth with distilled water, and any attached soft tissue was carefully removed with a scalpel. Teeth were stored at -20 ºC until analysis, when a 6770 freezer mill was used to pulverize samples in liquid nitrogen (SpexSamplePrep, CertiPrep®, Stanmore, London).

Thermogravimetric analysis coupled with Mass Spectroscopy

Pulverized samples were examined in a Mettler-Toledo TGA/DSC1 thermogravimetric analyzer (Schwerzenbach, Switzerland). In brief, 20 mg of each sample was placed in a 70-µl alumina crucible and heated at 10 ºC/min under a stream of oxygen (50 mL/min) to generate an oxidizing environment. Temperature range analyses and isothermal assays were performed. In both experiments, the sample mass used was 20 mg of each pulverized teeth. In the temperature range analyses, 20 mg of two samples with four replicates each was heated from 30 to 1000 ºC. In the isothermal assay, 20 mg of each sample (n=8) was heated for 60 min at 50, 100, 150, 200, 250, 300, 350, or 400 ºC.

The analyzer was calibrated using indium, aluminum, and gold. Baseline curves were constructed under the same experimental conditions to take account of buoyancy effects on the balance. Empty crucibles were always heated under the same conditions as the samples, and the resulting thermograms were subtracted from those obtained for the samples. Thermal decomposition analyses were performed to construct thermogravimetric (TG) curves using STAR^{e} v.13.00 software (Mettler-Toledo System). The derivative of the thermogravimetric curve (DTG) was used to differentiate distinct decomposition phases.

The thermogravimetric analyzer was coupled to a ThermoStar™ GSD 320 mass spectrometer (Pfeiffer Vacuum, Asslar, Germany) for evolved gas analysis during combustion. Mass spectrometry (MS) was used to identify released gases and display their profiles over time, yielding information on the decomposition mechanism. Based on previous thermal studies in human bones, enamel, dentine, and DNA, the following MS mass-to-charge (m/z) ratios were selected for analysis: 15 (methyl), 17 (ammonia; hydroxyl), 18 (water), 30 (nitrous oxide), 44 (carbon dioxide), 64 (sulfur dioxide), 79 (ion phosphate), and 98 (phosphoric acid) [13,15,20,22].

Percentage mass loss data from the isothermal assays were exported to an Excel (Microsoft Corporation, Redmond, WA) spread sheet. Statistical analyses and mass loss graph were performed using R v.3.4.4 and RStudio software v.1.1.442 (http://www.r-project.org) [23]. One-way analysis of variance (ANOVA) was used for comparisons, considering $p<0.05$ to be statistically significant. When F-values were significant, the Tukey honestly significant difference (HSD) *post-hoc* test was applied for multiple comparisons.

Results

Temperature range analysis

Temperature range assay results revealed TG profiles that could be divided into three well-differentiated steps (Fig. 1): Step 1, between 44 and 210 ºC, with peak at 78 ºC (DTG curve) and mass loss of 7.3 %; Step 2, between 211 and 603 ºC, with major peak at 340 ºC, shoulder at 420 ºC, and mass loss of 16%; and Step 3, between 604 ºC and 940 ºC, with peak at 721 ºC and mass loss of 1.9%. Total mass loss in the thermal decomposition process was 25.2% of the original mass.

Results of the evolved gas analysis of volatile products are depicted in Figure 2 along with the DTG curve. Table 1 exhibits the molecular identities of the evolved species detected. In Step 1, the peak at 78 ºC corresponds to water (m/z = 18). In Step 2, a peak was observed at 270 ºC for sulfur dioxide (m/z=64), followed by phosphate groups with peaks at 330 and 338 ºC (m/z=98 and m/z=79, respectively); methyl residues (m/z=15), water and carbon dioxide (m/z=44) were detected at 339, 343, and 346 ºC, respectively; and nitrous oxide groups (m/z=30) were detected at 347 ºC (Table 2). Step 3 only showed changes in weak signals for water and carbon dioxide molecules. Changes in signals for water were detected in all three steps.

Isothermal assays

Figure 3 depicts TG-DTG and MS curves from isothermal assays at 50, 100, 150 and 200 ºC for 60 min. According to TG-DTG curves (Fig. 3, left), masses loss in these assays took place in a single step. Intermediate limits of decomposition phases are indicated in the DTG curves with arrows. In the 200 ºC assay, a very small shoulder can be observed in DTG curve above the intermediate limit (marked by a triangle ▲). Figure 4 depicts TG-DTG curves and MS curves from isothermal assay data at 250, 300, 350 and 400 ºC for 60 min, showing the two well-differentiated patterns of mass loss at these temperatures (Fig. 4, left). The DTG curve at 400 ºC shows a shoulder above the second intermediate limit (indicated by a square ■).

Figure 5 depicts the mean percentage mass loss in the isothermal assays. After 60 min, the mass loss was: 3.05% at 50ºC, 5.32% at 100 ºC, 5.92% at 150 ºC, 6.85% at 200 ºC, 8.95% at 250 ºC (6.53% in a first and 2.43% in a second step); 15.86% at 300 ºC (6.5% in first and 9.36% in second step); 18.99% at 350 ºC (6.72% in first and 12.27% in second step; and 21.00% at 400ºC (6.2% in first and 14.80% in second step). One-way analysis of variance (ANOVA) showed significant differences (sum of squared deviations = 2579.2; 7 degrees of freedom; $p<0.01$). Tukey's HSD test revealed significant differences in all multiple comparisons (*$p< 0.01$) but not in 100-150 ºC and 150-200 ºC pairs.

MS curves (Fig. 3 and 4, right) showed signal changes for the detected molecules (see Table 1). At 50 ºC and 100 ºC, signal changes evidenced water alone (m/z=18 and m/z=17-18, respectively), which was recorded in the isothermal assays at all temperatures; at 150 and 200 ºC, carbon dioxide was also observed (m/z=44); at 250 ºC, a higher signal was recorded for carbon dioxide (m/z=44) and sulfur dioxide was also detected (m/z=64), with both components detected from 150 ºC but with a major peak at 245 ºC; at 300 ºC, carbon (m/z=44) and sulfur dioxide (m/z=64) were also detected, with a peak at 290 ºC; at both 350 and 400 ºC, phosphate groups (m/z=79 and 98) and methyl fragments (m/z=15) were detected. Nitrous oxide molecules (m/z=30) were observed in one sample at 350 ºC but not at 400ºC. All of these components were released after the release of carbon and sulfur dioxide. The peak at 340 ºC showed the highest decomposition of organic matter in both isothermal assays.

Discussion

Teeth are the hardest structures in the human body, and their pulp is well protected by dentin,

enamel, and cementum, explaining their frequent use for obtaining DNA from burnt corpses [24]. Chemical and morphological changes in incinerated teeth depend on the intensity and the duration of the fire but also on the rate of temperature rise. This study is the first to use TG-MS to investigate complete human teeth from crown to root (enamel, dentine and pulp) for forensic purposes, exploring the decomposition pattern of whole teeth when subjected to increasing heat stress. In temperature range assays, loss of teeth mass (total of 25.2%) occurred in three well-differentiated steps, from 44 to 210 ºC, from 211 to 603 ºC, and from 604 ºC to 940 ºC. A similar TG pattern was previously reported for the enamel and dentine of teeth from humans and other mammal species in tests of dental materials [19], which also described three well-differentiated steps but with slightly different temperature ranges (up to 200 ºC, from 200 to 560 ºC and from 560 ºC to 1100 ºC). These small discrepancies with our study may be attributable to their use of a mill rather than freezer-mill to pulverize the teeth. We studied not only enamel and dentine but also pulp in our study of complete teeth, which are of particular relevance in forensic investigations, given that teeth are generally recovered in one piece from crown to root.

In our evolved gas analysis, the peak detected at 78 ºC corresponds to water associated with the loss of free water [13]. However, water molecules are released throughout the studied temperature range due to the loss of humidity of organic/inorganic components or their combustion. Volatile fragments detected during the second mass loss step result from the combustion of different components of organic matter. An interesting study about thermal properties of fossilized mammal bones pointed out that the mass loss of organic combustion occurred at two stages, interpreted as follows: B1 (230-400 ºC)—structural water loss and low-molecular organic compounds combustion (denominated as "low temperature organic content," e.g., non-collagen peptide like albumin); B2 (400-600 ºC)—high-molecular organics combustion (denominated as "high-temperature organic content," mostly collagen) [25]. In our study, the first organic molecule detected, sulfur dioxide (major peak at 270 ºC), is related to protein decomposition [15]. It was followed by phosphate groups (peaks at 330 and 338 ºC) that may be related to organic matter such as DNA. This is because the decomposition of hydroxyapatite, which is the largest inorganic component of teeth and contains phosphate groups [15], is only observed at temperatures of around 1500 ºC [26]. Methyl residues, water, and carbon dioxide were then detected at 339, 343, and 346 ºC, respectively [13–15]. Nitrous oxide groups were observed at 347ºC, showing a signal (m/s=30) that can also be associated with organic fragments such as formaldehyde. Both nitrous oxide and formaldehyde are possible

products of protein and amide pyrolysis [14]. Amides are present in proteins and also in DNA. The shoulder in the DTG curve at 420 ºC indicates a complex degradation step involving multiple chemical processes in this temperature range [14,27]. The third mass loss step corresponds to the decomposition of inorganic matter in teeth. Only water and carbon dioxide were detected, likely attributable to the dehydroxylation and decarboxylation of carbonated apatite [13,14,28]. According to these findings, almost all of the organic matter in complete teeth decomposes between 270 and 400 ºC, with DNA most likely decomposing at temperatures between 330 and 400 ºC.

We conducted isothermal assays to examine organic matter decomposition when teeth undergo a constant temperature for a period of time, as in certain forensic scenarios. Free water alone was released at 50 and 100 ºC, associated with a loss of humidity, while carbon dioxide was also detected by mass spectrometry at both 150 and 200 ºC. The slight shoulder in the DTG curve after the intermediate limit at 200 ºC indicates a decomposition sub-stage compatible with small emissions of carbon dioxide at this temperature range [14,27], and the release of water and carbon dioxide is associated with decomposition of the organic matrix. Hence, organic matter, including DNA among other organic compounds, may start to be degraded after one hour at 150 ºC and especially at 250 ºC, which may compromise its usefulness for reliable forensic human identification. A DNA thermal degradation study [22] previously reported that the sugar-base bonds of DNA largely break between 160 and 200 ºC. Another study [21] found that thermal degradation of DNA began at 130 ºC under dry conditions, being completely degraded at around 190 ºC. Under natural conditions, however, DNA is found in aqueous solution and should be studied in this state in order to obtain outcomes of interest in the forensic setting.

TG-DTG profiles show that mass was lost in a single step at temperatures between 50 and 200 ºC but in two well-differentiated steps between 250 and 400 ºC. This change in decomposition pattern was observed between 200 and 250 ºC, when the greatest decomposition of organic components began. This is consistent with the detection by MS of different volatile organic molecules between 250 and 400 ºC. Stronger carbon dioxide signals were detected at 250 ºC than at 200 ºC alongside sulfur dioxide molecules, with both being linked to protein decomposition (major peaks at 245 ºC). Our observations indicate that the decomposition of organic matter in teeth could occur earlier after a longer exposure to lower temperatures than after a shorter exposure to higher temperatures.

The shoulder observed in 400 ºC DTG curve after the second intermediate peak demonstrates that complex chemical processes occur at this temperature range due to decomposition of organic matrix. Methyl and carbon dioxide residues correspond to decomposition of organic matrix and sulfur dioxide detection was associated to proteins decomposition. The detection of phosphate groups in all samples at around 350 ºC might evidence degradation of DNA [15,26], compromising the reliability of identification based teeth exposed to this temperature.

Our application of TG-MS to study the effect of heat on whole teeth contributes further knowledge on the decomposition of organic matter, including DNA, which is of interest in fire-related forensic scenarios. The data yielded by TG-MS on altered components in fire-damaged teeth could also provide complementary information on fire conditions, including the temperature reached and the duration of exposure [29–31]. One study limitation is that thermal effects were investigated in a controlled laboratory environment for a given exposure time, and no account was taken of accelerant type or fire extinction method, among other influential factors. There was also no examination of the potential protective effects of alveolar bone, maxillary bone, or soft tissue. Further research is needed to study different circumstances in all possible forensic scenarios.

Conclusion

Chemical and morphological changes in incinerated teeth depend on the intensity and duration of the fire and the rate of temperature rise. To our best knowledge, this is the first thermal analysis on whole teeth, including pulp, which is of particular relevance in forensic investigations, given that teeth are generally recovered in one piece from crown to root. TG-MS analysis provided data on the decomposition of organic matter, including DNA, in teeth subjected to different temperatures. Organic matter degradation may occur earlier in teeth after a longer exposure to lower temperatures than after a shorter exposure to higher temperatures. These data are of interest in fire-related forensic situations for which DNA identification is required.

Conflict of interest: The authors declare that they have no conflict of interest.

Ethical approval:

The study and protocols for recruitment were approved by the Human Research Ethics

Committee of the University of Malaga (Approval number: CEUMA 2013-0048-H) in accordance with the "Ethical Principles for Medical Research Involving Human Subjects" adopted in the Declaration of Helsinki by the World Medical Association (64th WMA General Assembly, Fortaleza, Brazil, October 2013), Recommendation No. R (97) 5 of the Committee of Ministers to Member States on the Protection of Medical Data (1997), and Spanish data protection act (Ley Orgánica 15/1999 de Protección de Datos, LOPD).

References

1. Holland MM, Cave C a, Holland C a, Bille TW. Development of a quality, high throughput DNA analysis procedure for skeletal samples to assist with the identification of victims from the World Trade Center attacks. Croat Med J. 2003;44:264–72.

2. Foran DR, Gehring ME, Stallworth SE. The recovery and analysis of mitochondrial DNA from exploded pipe bombs. J Forensic Sci. 2009;54:90–4.

3. Ubelaker DH. The forensic evaluation of burned skeletal remains: A synthesis. Forensic Sci Int. 2009;183:1–5.

4. Makhlouf F, Alvarez JC, de la Grandmaison GL. Suicidal and criminal immolations: An 18-year study and review of the literature. Leg Med. 2011;13:98–102.

5. Margiotta G, Gabbrielli M, Carnevali E, Alberti T, Carlini L, Lancia M, et al. Genetic Identification by Using Short Tandem Repeats Analysis in a Case of Suicide by Self-incineration. Am J Forensic Med Pathol. 2014;35:172–5.

6. Fanton L, Jdeed K, Tilhet-Coartet S, Malicier D. Criminal burning. Forensic Sci Int. 2006;158:87–93.

7. Butler JM. The future of forensic DNA analysis. Philos Trans R Soc Lond B Biol Sci. 2015; https://doi.org/10.1098/rstb.2014.0252

8. Mundorff AZ, Bartelink EJ, Mar-Cash E. DNA preservation in skeletal elements from the world trade center disaster: Recommendations for Mass Fatality Management. J Forensic Sci. 2009;54:739–45.

9. Schwark T, Heinrich A, Preuße-Prange A, Von Wurmb-Schwark N. Reliable genetic identification of burnt human remains. Forensic Sci Int Genet. 2011;5:393–9.

10. Valenzuela A, Martin-de las Heras S, Marques T, Exposito N, Bohoyo JM. The application of dental methods of identification to human burn victims in a mass disaster. Int J Legal Med . 2000;113:236–9.

11. Woodward SR, King MJ, Chiu NM, King MJ, Chiu NM, Kuchar MJ, et al. Amplification of ancient nuclear DNA from teeth and soft tissues . Technical Amplification of Ancient Nuclear DNA From Teeth and Soft Tissues. 1994;244–7.

12. Ricaut FX, Keyser-Tracqui C, Crubézy E, Ludes B. STR-genotyping from human medieval tooth and bone samples. Forensic Sci Int. 2005;151:31–5.

13. Peters F, Schwarz K, Epple M. The structure of bone studied with synchrotron X-ray diffraction, X-ray absorption spectroscopy and thermal analysis. Thermochim Acta. 2000;361:131–8.

14. Onishi A, Thomas P, Stuart B. TG-MS characterisation of pig bone in an inert atmosphere. J Therm Anal Calorim. 2007;88:405–9.

15. Teruel JDD, Alcolea A, Hernández A, Ruiz AJO. Comparison of chemical composition of enamel and dentine in human, bovine, porcine and ovine teeth. Arch Oral Biol . 2015;60:768–75.

16. Vargas-Becerril N, Reyes-Gasga J, García-García R. Evaluation of crystalline indexes obtained through infrared spectroscopyand x-ray diffraction in thermally treated. Mater Sci Eng C Mater Biol Appl. 2019;97:644–649.

17. Chowdhury ND, Ghosh KS. Calorimetric studies of Ag–Sn–Cu dental amalgam alloy powders and their amalgams. J Therm Anal Calorim. 2017;130:623–637.

18 . Buriti JS, Barreto MEV, Santos KO, Fook MVL. Thermal, morphological, spectroscopic and biological study of chitosan, hydroxyapatite and wollastonite biocomposites. J Therm Anal Calorim. 2018;134:1521–1530.

19. Lörinczy D. Thermal analysis in biological and medical applications. J Therm Anal Calorim. 2017;130:1263–1280.

20. Devièse T, Colombini MP, Regert M, Stuart BH, Guerbois JP. TGMS analysis of archaeological bone from burials of the late Roman period. J Therm Anal Calorim. 2010;99:811–3.

21. Karni M, Zidon D, Polak P, Zalevsky Z, Shefi O. Thermal degradation of DNA. DNA Cell Biol. 2013;32:298–301.

22. Alongi J, Di Blasio A, Milnes J, Malucelli G, Bourbigot S, Kandola B, et al. Thermal degradation of DNA, an all-in-one natural intumescent flame retardant. Polym Degrad Stab. 2014;113:1–9.

23. R Core Team. R: A language and environment for statistical computing [Internet]. R Found. Stat. Comput. Viena, Austria. 2013. Available from: https://www.r-project.org/

24. Manjunath BC, Chandrashekar BR, Mahesh M, Vatchala Rani RM. DNA profiling and forensic dentistry - A review of the recent concepts and trends. J Forensic Leg Med. 2011;18:191–7.

25. Votyakov S, Kiseleva D, Shchapova Yu, Sadykova N. Thermal properties of fossilized mammal bone remnants of the Urals. J Therm Anal Calorim. 2010;101:63–70.

26. Liao CJ, Lin FH, Chen KS, Sun JS. Thermal decomposition and reconstitution of hydroxyapatite in air atmosphere. Biomaterials. 1999;20:1807–13.

27. Janković B. Thermal characterization and detailed kinetic analysis of Cassava starch thermo-oxidative degradation. Carbohydr Polym. 2013;95:621–9.

28. Enax J, Prymak O, Raabe D, Epple M. Structure, composition, and mechanical properties of shark teeth. J Struct Biol. 2012;178:290–9.

39. Fereira JL, Fereira ÁE De, Ortega AI. Methods for the analysis of hard dental tissues exposed to high temperatures. Forensic Sci Int. 2008;178:119–24.

30. Muller M, Berytrand MF, Quatrehomme G, Bolla M RJ. Macroscopic and microscopic aspects of incinerated teeth. J Forensic Odontostomatol. 1998;16:1–7.

31. Rubio L, Sioli JM, Suarez J, Gaitan MJ, Martin-de-las-Heras S. Spectrophotometric analysis of color changes in teeth incinerated at increasing temperatures. Forensic Sci Int. 2015;252:193.e1-193.e6.

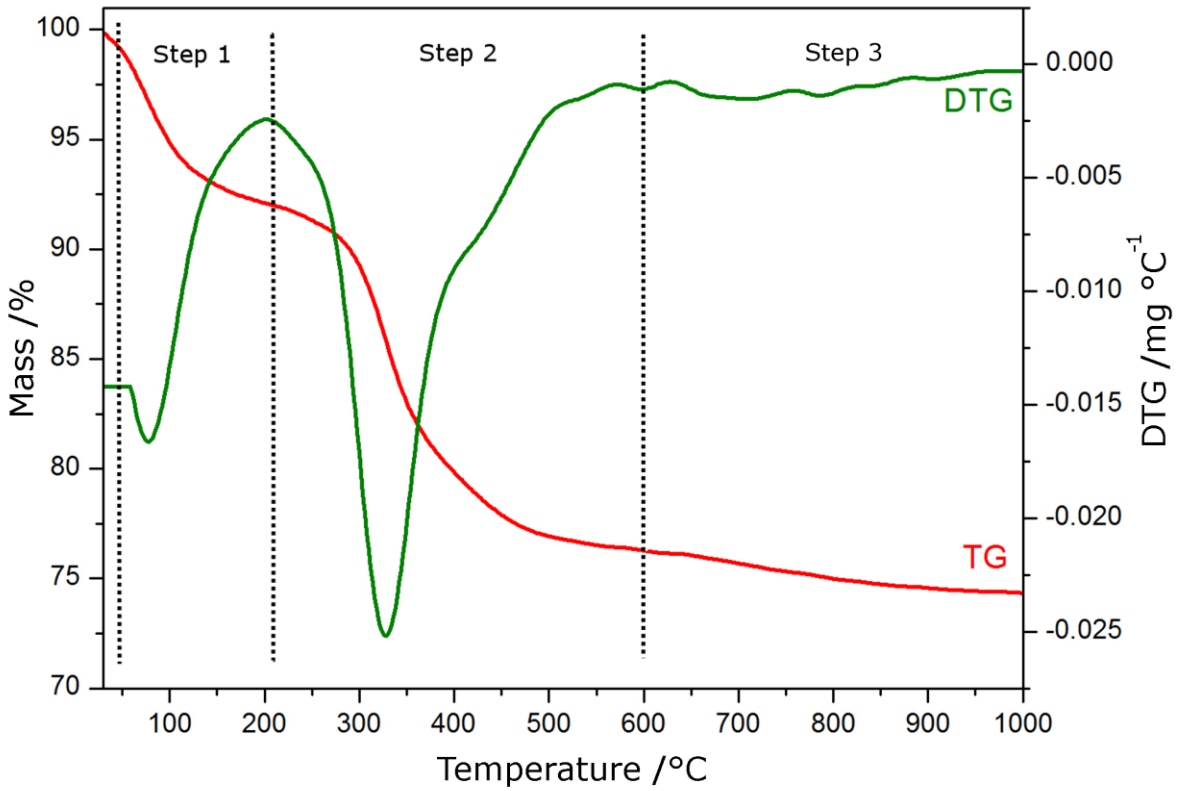

Fig.1 Thermogravimetric curve in red color (TG) and Derivate of Thermogravimetric curve in green (DTG) are depicted for decomposition of human teeth in an oxidizing atmosphere. Percentage of mass (left Y axis) and mg $ºC^{-1}$ (right Y axis) are shown in TG and DTG curves, respectively. Temperature in ºC is shown on X axis

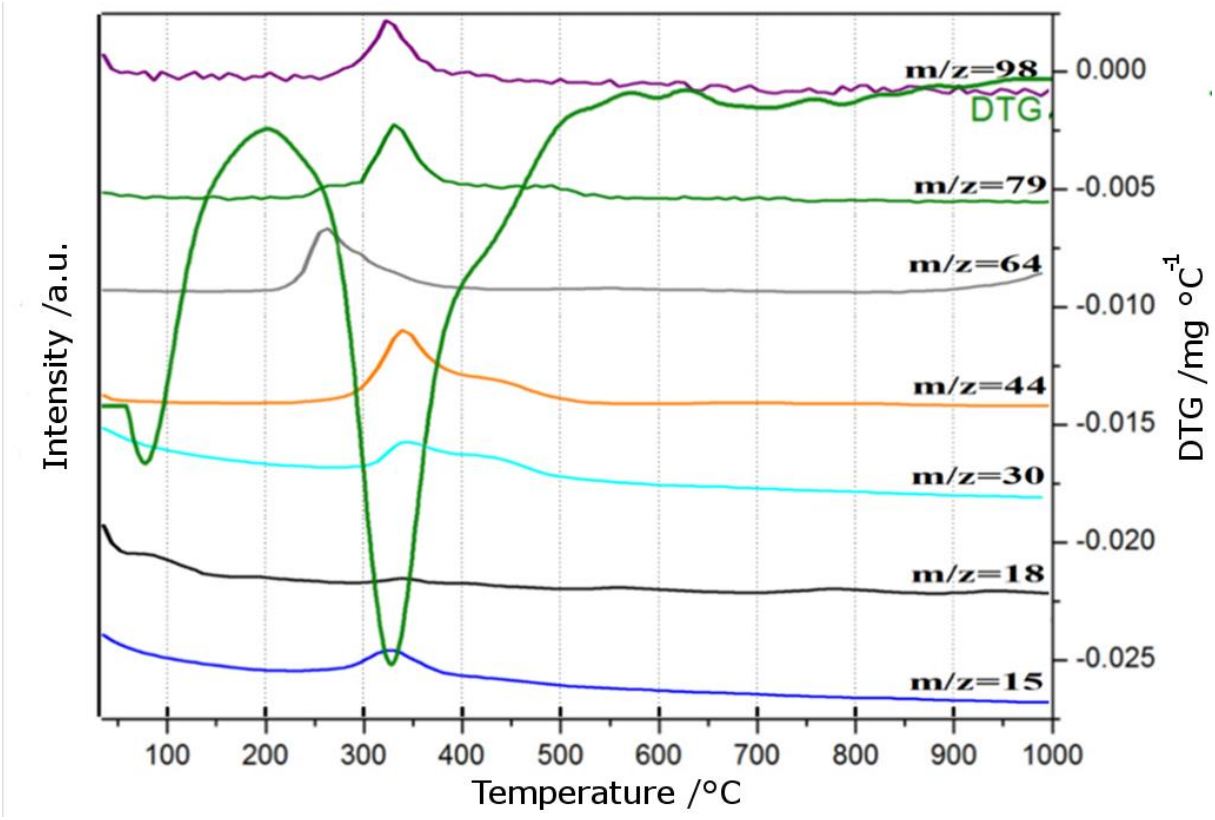

Fig.2 Combined Derivate of Thermogravimetric and Mass spectrometric curves (DTG-MS) for decomposition of human teeth in an oxidizing atmosphere. DTG curve (green) is measured in mg ºC^{-1} on the right Y axis. MS signal intensity is measured in arbitrary units (a.u.) on the left Y axis. Volatile products are represented in different colors. Molecule names of each m/z signal are described in table 2. Temperature in ºC is shown on X axis

Table 1 Molecules and their formulas and mass-to-charge ratios detected in the temperature range analysis and isothermal assays

Mass-to-charge /*m/z*	Formula	Molecule name
15	$-CH_3$	Methyl
17	NH_3; OH^-	Ammonium; Hidroxil
18	H_2O	Water
30	NO, NO_2, (NO_X)	Nitrous oxide
44	CO_2	Carbon dioxide
64	SO_2	Sulfur dioxide
79	PO_3^{-2}	Phosphate ion
98	H_3PO_4	Phosphoric acid

Table 2 Molecules, mass-to-charge ratios and temperature at major peaks detected in the second step, ranged from 211-613 ºC of the temperature range assay with evolved gas analysis

Mass-to-charge /*m/z*	**Molecule name**	**Temperature at major peak /ºC**
64	Sulfur dioxide	270
98	Phosphoric acid	330
79	Phosphate ion	338
15	Methyl	339
18	Water	343
44	Carbon dioxide	346
30	Nitrous oxide	347

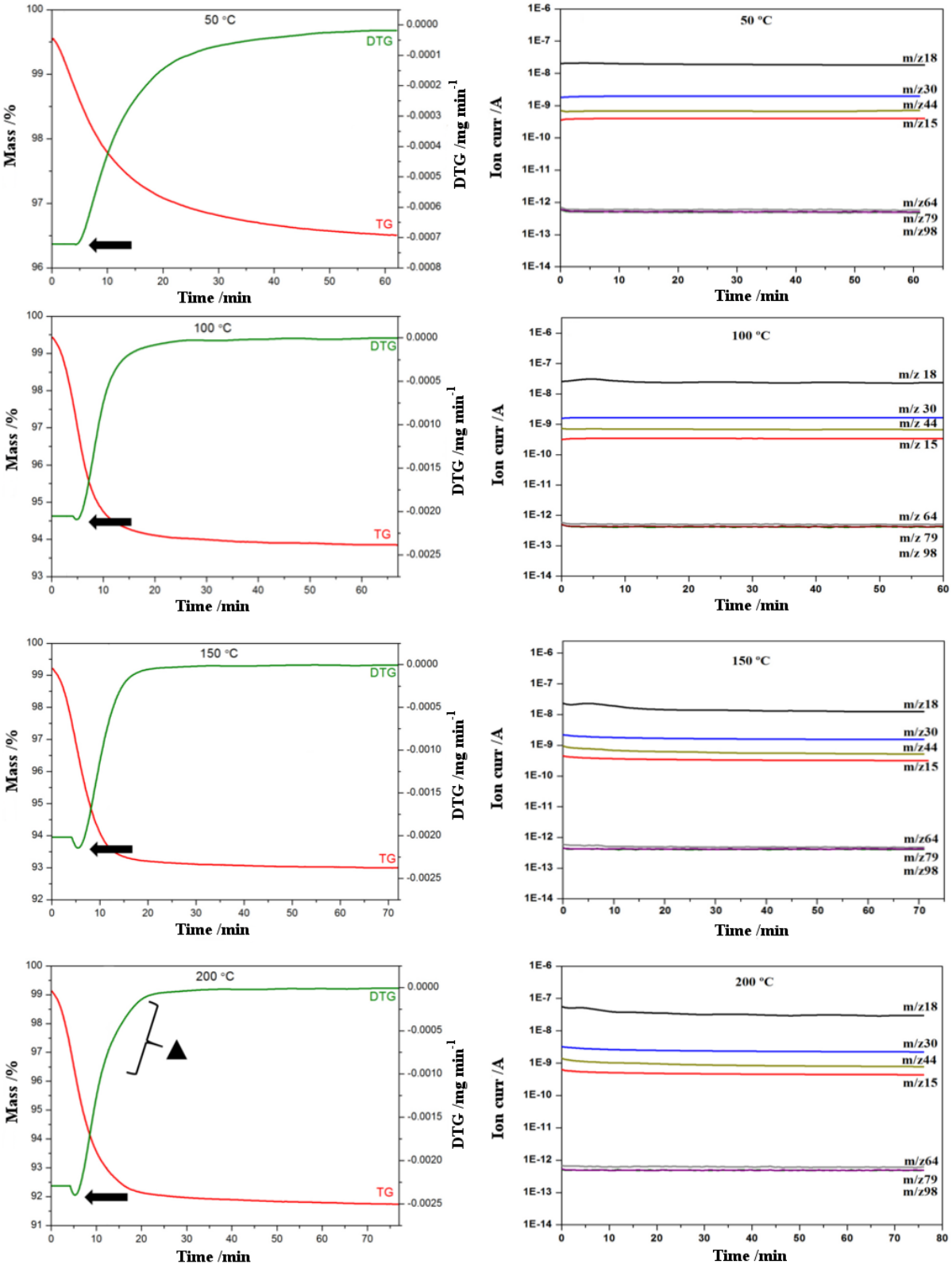

Fig. 3 Left: Thermogravimetric curve in red color (TG) and Derivate of Thermogravimetric curve in green (DTG) are depicted for isothermal assays at 50, 100, 150 and 200 ºC. Mass percentage (left Y axis) and mg min^{-1} (right Y axis) are shown in TG and DTG curves, respectively. Time in minutes (min) is shown on X axis. Intermediate limit of decomposition step is indicated by an arrow. In 200 ºC DTG curve a very slight shoulder after the intermediate limit is indicated by a triangle (▲). **Right:** Mass Spectrometric curves (MS) are represented for isothermal assays at 50, 100, 150 and 200 ºC. Ion Current is shown in Y axis. Volatile products are represented in different colors. Molecule names of each m/z signal are described in table 1. Time in minutes (min) is shown on X axis

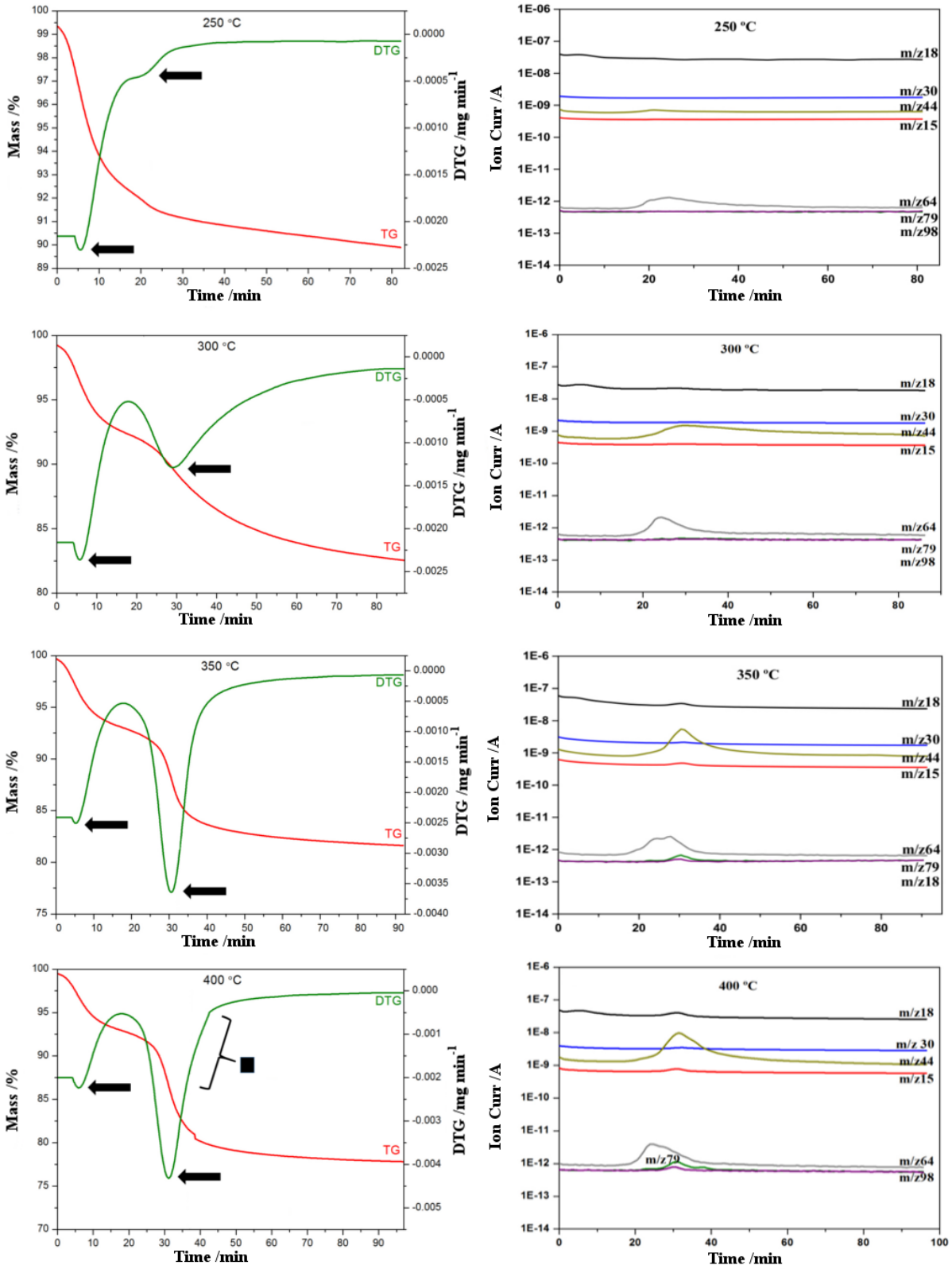

Fig.4 Left: Thermogravimetric curve in red color (TG) and Derivate of Thermogravimetric curve in green (DTG) are depicted for isothermal assays at 250, 300, 350 and 400 ºC. Mass percentage (left Y axis) and mg min^{-1} (right Y axis) are shown in TG and DTG curves, respectively. Time in minutes (min) is shown on X axis. Intermediate limits of decomposition steps are indicated by arrows. In 400 ºC DTG curve, a shoulder after the second intermediate limit is marked by a square (■). **Right:** Mass Spectrometric curves (MS) are represented for isothermal assays at 250, 300, 350 and 400 ºC. Ion Current is shown in Y axis. Volatile products are represented in different colors. Molecule names of each m/z signal are described in table 1. Time in minutes (min) is shown on X axis

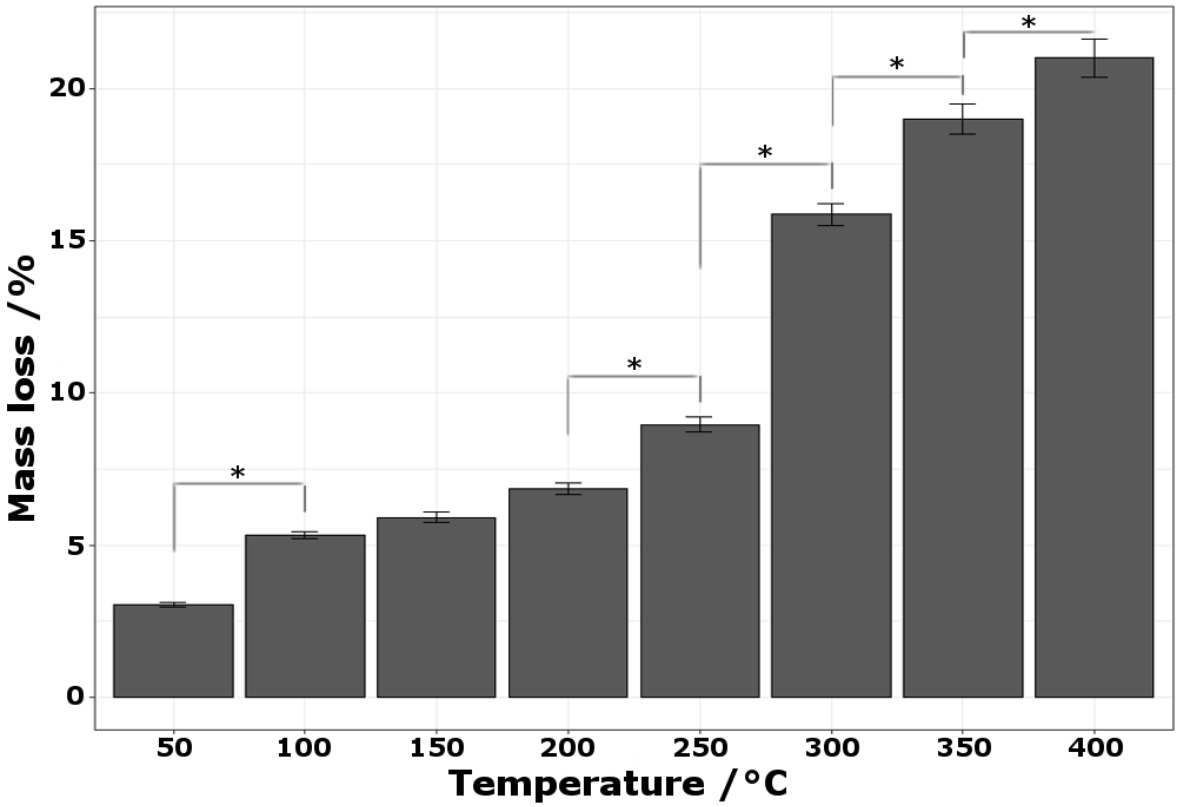

Fig. 5 Mean of mass loss percentages in isothermal assays (50, 100, 150, 200, 250, 300, 350 and 400 ºC). The bars represent the mean of the mass loss (M). Error bars represent standard deviations of the mean (SEM). Significant differences for multiple comparisons were found between all the groups (*p< 0.01), except between 100-150 ºC and 150-200 ºC groups

Figure 1

Click here to access/download;Figure;Fig 1.png

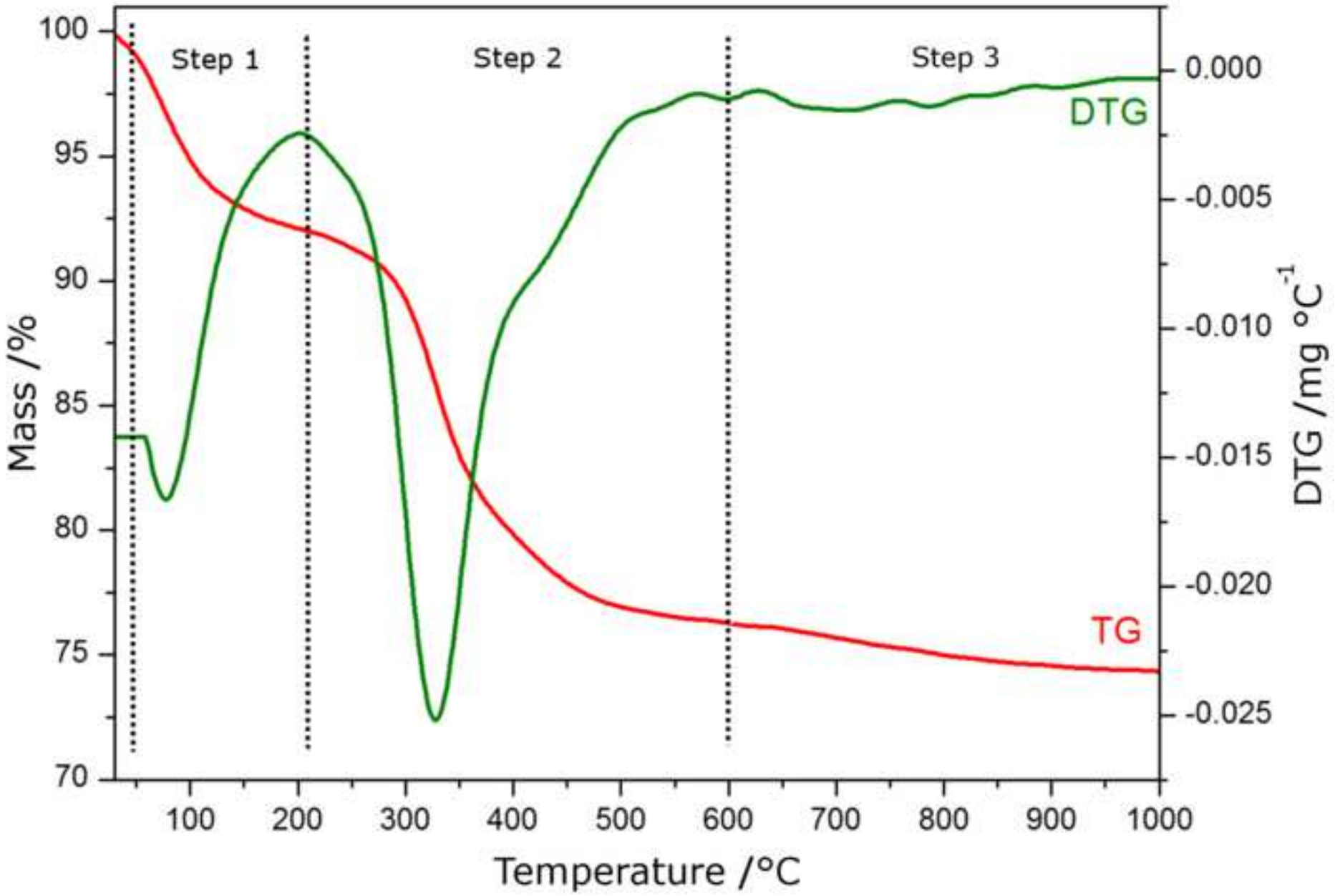

Figure 2

Click here to access/download;Figure;Fig 2.png

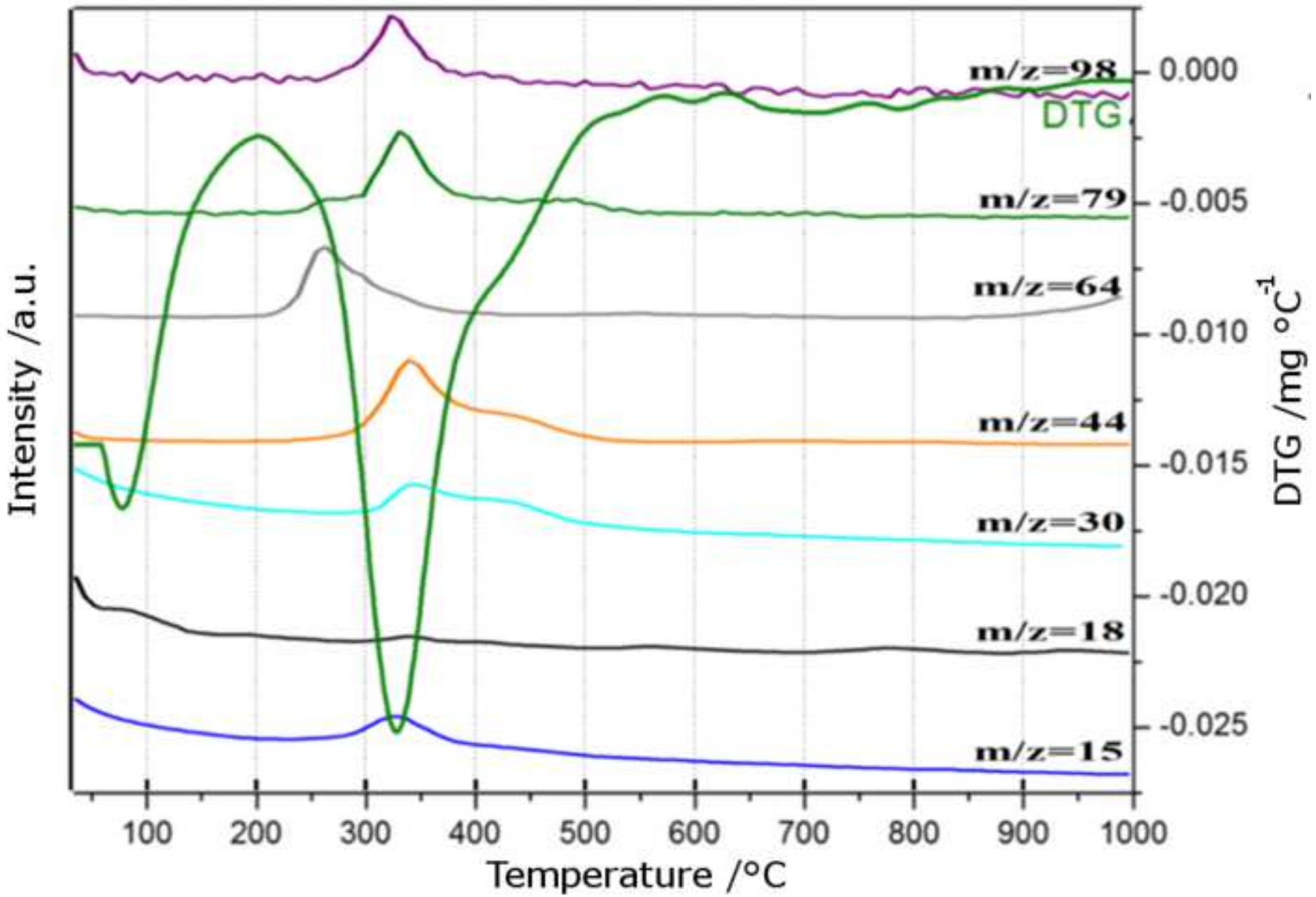

Figure 3

Click here to access/download;Figure;Fig 3.png

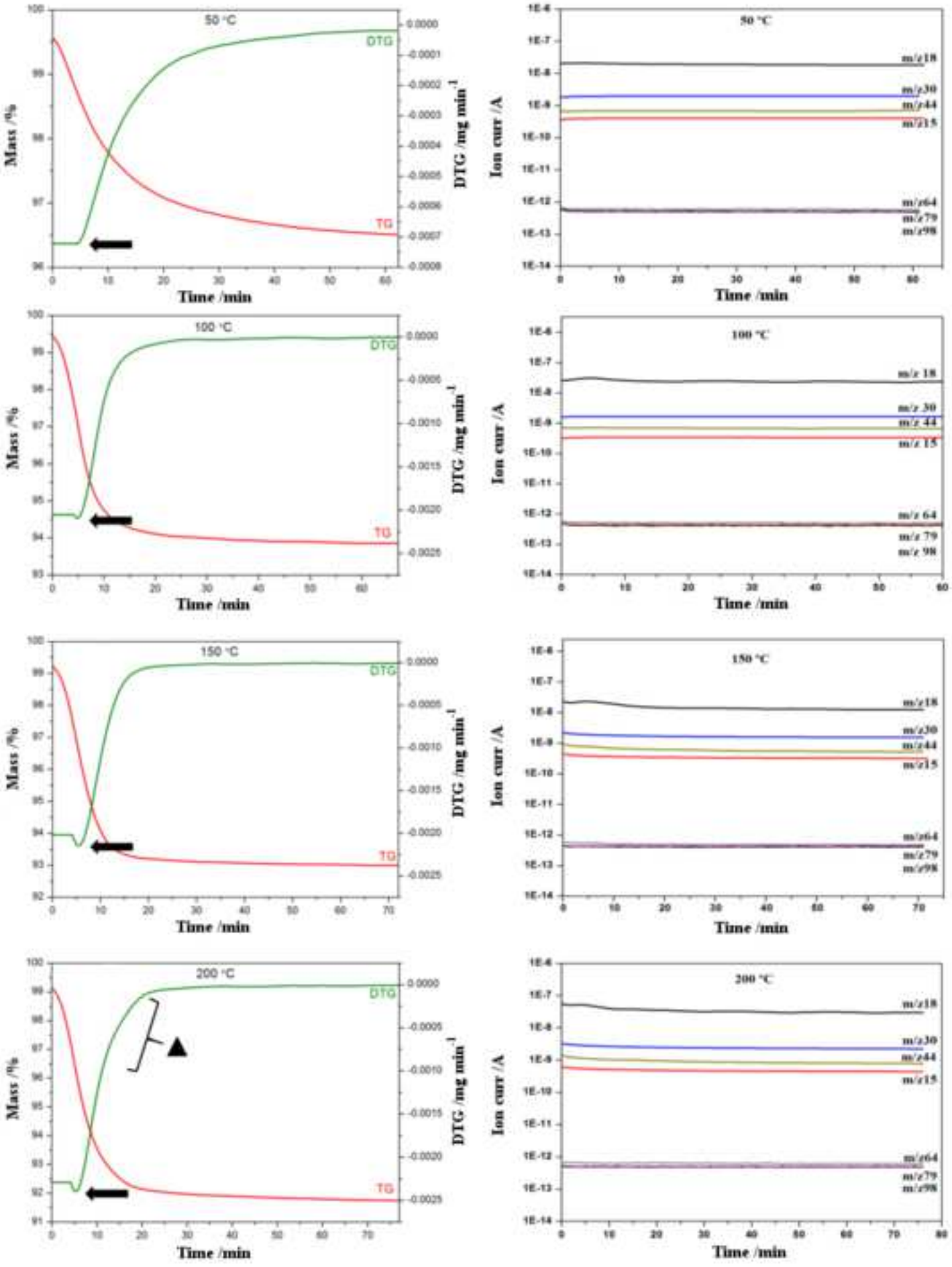

Figure 4

Click here to access/download;Figure;Fig 4.png

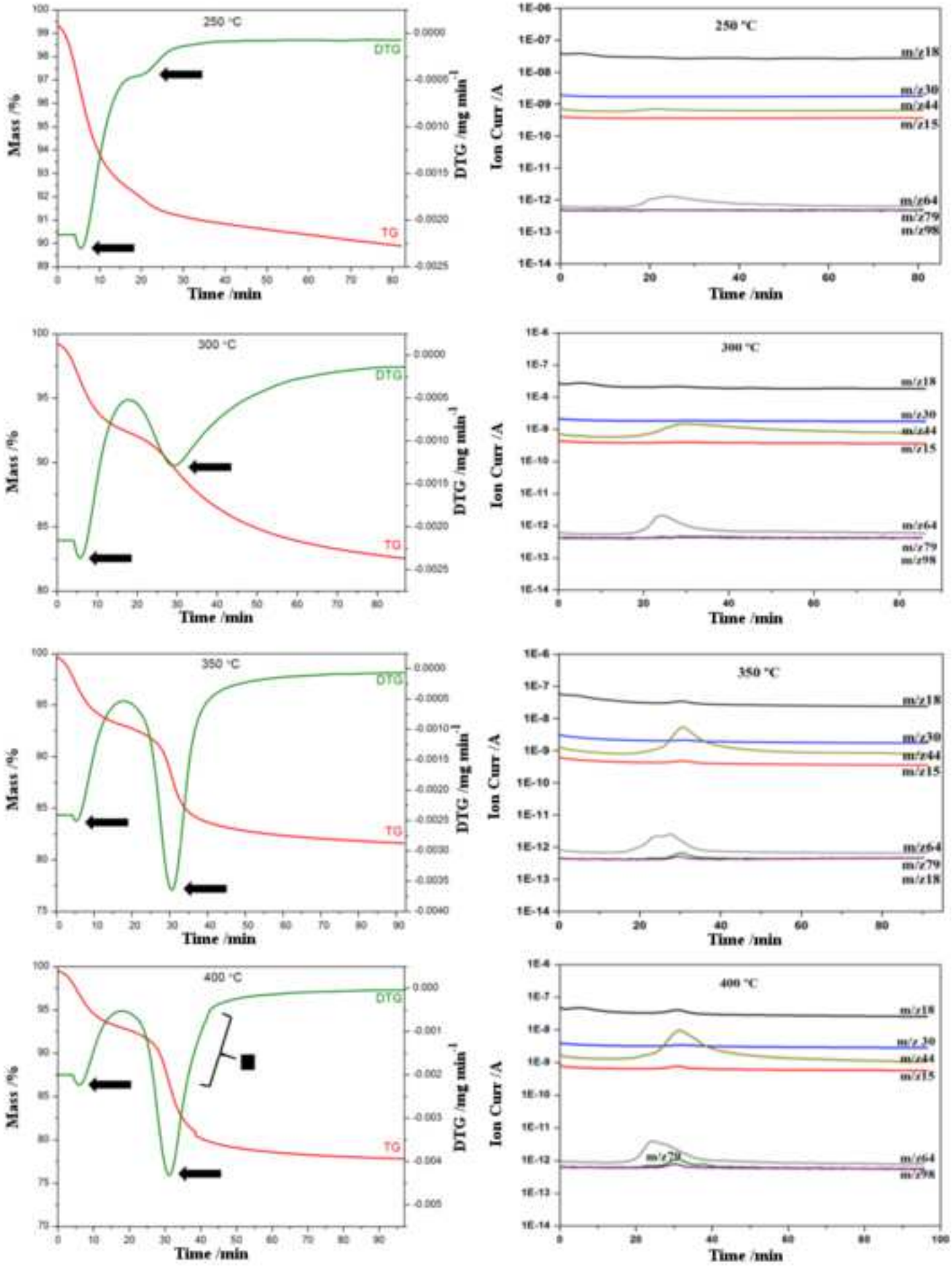

Figure 5

Click here to access/download;Figure;Fig 5.png