ISSN- 2350-0530(O), ISSN- 2394-3629(P)
DOI: 10.5281/zenodo.1133862

INTERNATIONAL JOURNAL OF RESEARCH – GRANTHAALAYAH
A knowledge Repository

Science

CHARACTERIZATION OF NON-STEROIDAL ANTI-INFLAMMATORY DRUGS IN TABLETS BY DIRECT ANALYSIS IN REAL TIME/TIME OF FLIGHT/MASS SPECTROMETRY (DART/TOF/MS) WITHOUT SAMPLE PREPARATION

Nawaf Masfer Saad Al-Otaibi [*1]

[*1] King Saud University, Faculty of Science, Department of Chemistry, P.O. Box 2455, Riyadh, 11451, Saudi Arabia

Abstract

This powerful mass spectrometric technique was used to investigate four profen drugs which are widely used as non-steroidal anti-inflammatory drugs for treating pain, fever and inflammation: Ibuprofen (M = 206), Flurbiprofen (M = 244), Naproxen (M = 230) and Ketoprofen (M = 254). The DART ion source was operated in positive ion mode with helium as the ionizing medium heated to 250°C. A solution containing a mixture of poly (ethylene glycol) standards PEG 600 and PEG 200 was used for mass calibration. A standard solution of each pure standard was manually introduced by using a small glass rod. The high resolution mass spectra of Ibuprofen, Flurbiprofen, Naproxen and Ketoprofen showed the presence of an intense protonated molecular ion $[M + H]^+$ at 207.13860, 245.09776, 231.10632 and 255.09966 Da, respectively. Moreover, a non-protonated molecular ion M^+ was also observed except for Ketoprofen. On the other hand, an intense adduct ion $[M + H2O]^+$ was also present in the four mass spectra. Since these profens are 2-arylpropionic acid derivatives they have a known tendency to form dimers; indeed, an intense protonated ion corresponding to this dimer $[2M + H]^+$ was present in the spectrum of Ibuprofen, Flurbiprofen, Naproxen and Ketoprofen at 413.27102, 489.19944, 461.19266 and 509.19662 Da, respectively. The presence of profens as active ingredients was then investigated in various pharmaceutical tablets collected from the local market. A small piece of each solid tablet was submitted to the DART ion source without any sample preparation. The obtained mass spectrum showed the characteristic peaks corresponding to each profen as active principle, as well as the other ingredients of the tablet.

Keywords: Ibuprofen; Flurbiprofen; Naproxen; Ketoprofen; DART.

Cite This Article: Nawaf Masfer Saad Al-Otaibi. (2017). "CHARACTERIZATION OF NON-STEROIDAL ANTI-INFLAMMATORY DRUGS IN TABLETS BY DIRECT ANALYSIS IN REAL TIME/TIME OF FLIGHT/MASS SPECTROMETRY (DART/TOF/MS) WITHOUT SAMPLE PREPARATION." *International Journal of Research - Granthaalayah*, 5(12), 191-199. https://doi.org/10.5281/zenodo.1133862.

ISSN- 2350-0530(O), ISSN- 2394-3629(P)
DOI: 10.5281/zenodo.1133862

1. Introduction

Direct analysis in real time (DART) is an ambient ionization technique which was recently designed for mass spectrometry and showed many advantages such as high sample throughput and ease of operation [1]. DART-MS analysis can be carried out at atmospheric pressure; thus it allows for direct investigation of various liquid or solid samples without any pretreatment such as extraction, purification or separation. The DART ion source proved to be very convenient for the analysis of small molecules [2]. In positive ion mode, metastable helium atoms generate protonated gaseous water clusters; then, by proton exchange, these clusters form $[M + H]^+$ ions, which are generally the predominant species [3].

Profens or 2-arylpropionic acids (2-APAs) are a group of non-steroidal anti-inflammatory drugs (NSAIDs) having anti-inflammatory and analgesic activities due to their ability to inhibit cyclooxygenase enzymes that promote inflammation [4,5]. These profens form a group of analgesic, antipyretic and anti-inflammatory agents that are used with great frequency in both humans and animals since they do not induce sedation, respiratory depression or addiction [6]. This set includes Ibuprofen, Ketoprofen, Naproxen and Flurbiprofen, among others. In particular, ibuprofen is among the most widely used pharmaceuticals in the world [7].

Hložek *et al.* developed and validated a gas chromatographic method with flame ionization detection (GC–FID) for the measurement of Ibuprofen, Naproxen and Ketoprofen for clinical toxicology purposes. Plasma sample was treated and prepared for quantitative analysis. The method was successfully applied to quantify the selected compounds in serum of patients from emergency units [8].

Yilmaz and Alkan described a gas chromatography–mass spectrometry (GC–MS) method for the determination of Flurbiprofen in pharmaceutical preparations. For GC–MS, electron ionization mode (EI = 70 eV) and selected ion monitoring (SIM) mode were used for quantitative analysis (m/z 180 for Flurbiprofen). The mean recovery of Flurbiprofen was 99.4% for pharmaceutical preparations. The limits of detection and quantification of Flurbiprofen were 0.05 and 0.15 μg/mL, respectively. Also, the method was applied for the quality control of five commercial Flurbiprofen dosage forms to quantify the drug and to check the formulation content uniformity [9].

Nochea *et al.* described a new analytical method for the determination of trace levels of five non-steroidal anti-inflammatory drugs (NSAIDs: clofibric acid, Ibuprofen, Naproxen, diclofenac and Ketoprofen) in water samples. The detection limits of the method in water varied from 0.042 μg/L for Ibuprofen to 1.2 μg/L for Ketoprofen. The relative standard deviations (RSD) values were found to be relatively low (<10% for all compounds). The results obtained show the presence of ibuprofen and naproxen in the influent waste water sample [10].

Hashim and Khan developed a highly sensitive and reliable method for the enantioselective analysis of Ibuprofen, Ketoprofen and Naproxen in waste water and environmental water samples. These three pharmaceuticals are chiral molecules and the variable presence of their individual (R)- and (S)-enantiomers is of increasing interest for environmental analysis. Separation and detection of the individual diastereomers was undertaken by gas

ISSN- 2350-0530(O), ISSN- 2394-3629(P)
DOI: 10.5281/zenodo.1133862

chromatography–tandem mass spectrometry (GC–MS/MS). Method detection limits were shown to be within the range of 0.2–3.3 ng L^{-1} for individual enantiomers in ultrapure water, drinking water, surface water and a synthetic waste water [11].

DART time-of-flight mass spectrometer was used to investigate four profen drugs which are widely used as non-steroidal anti-inflammatory drugs for treating pain, fever and inflammation: Ibuprofen (M = 206), Flurbiprofen (M = 244), Naproxen (M = 230) and Ketoprofen (M = 254).

The time-of-flight mass spectrometer equipped with a direct analysis in real time (DART-TOF-MS) proved to be capable of analyzing drugs in pills and tablets with no sample preparation. A small piece of the solid sample can simply be placed in front of the DART ion source and the active ingredients as well as the excipients can be detected and characterized within seconds. The high throughput of this technique coupled with the high mass accuracy and accurate isotopic patterns make it especially suitable for the rapid identification of unknown species in solid and liquid materials [12].

The aim of the study is the rapid identification of Ibuprofen, Flurbiprofen, Naproxen and Ketoprofen without any columns.

2. Materials and Methods

The DART ion source was operated in positive ion mode with helium as the ionizing medium heated to 250°C. A solution containing a mixture of poly (ethylene glycol) standards PEG 600 and PEG 200 was used for mass calibration. A standard solution of each pure standard (Ibuprofen, Flurbiprofen, Naproxen and Ketoprofen, all are standard references from Sigma-Aldrich, purity 99.9%) was manually introduced by using a small glass rod. The commercial tablets were purchased from the local market, grinded and about 2 mg of each pharmaceutical was submitted to the DART ion source without any extraction, purification or sample preparation.

2.1. DART-TOF-MS Experimental Conditions

- Instrument : Accu TOF-DART mass spectrometer from JEOL (Tokyo, Japan) equipped with a DART ion source and a high resolution time of flight analyzer (TOF).
- Solid samples were directly evaporated with hot helium at the DART outlet. Liquid samples were injected with a small glass rod (melting point tube)
- Vacuum (Pirani gauge): $1.8\times10^{+2}$ Pa
- Vacuum (TOF analyzer): 1.3×10^{-5} Pa
- Nebulizing and ionization gas: helium at flow: 2.5 L/min
- Helium flow temperature: 350 °C
- Ionization mode: positive (by protonation)
- Discharge needle voltage : 3000 V
- Grid electrode voltage : 350 V
- Mass range: m/z 50 to 1000 mass units (Da)
- Acquisition time of a spectrum: 0.2 s

ISSN- 2350-0530(O), ISSN- 2394-3629(P)
DOI: 10.5281/zenodo.1133862

- Mass calibration based on a mixture of polyethylene glycol (PEG 600 + PEG 1500) injected with each sample as internal mass reference

2.2. Submission of A Liquid Sample At the DART Ionization Source

2.2.1. Submission of Liquid Samples

The liquid samples were directly injected under atmospheric pressure using a small glass rod placed between the outlet of the DART ion source and the inlet orifice of the TOF analyzer.

2.2.2. Submission of Solid Samples

The solid samples were prepared as follows: one tablet of each commercial pharmaceutic was finely grinded and homogenized. The powder was submitted to the hot helium flow at the DART outlet at 350°C. Before recording any spectrum, a solution of polyethylene glycol in methanol was first introduced as reference mixture for mass calibration to allow an accurate determination of all masses.

3. Results and Discussion

3.1. Mass Spectra of the Profens Standard Solutions

The four investigated profens were first injected as standard solutions with a concentration of 1000 ppm. The obtained high resolution mass spectra with the proposed formula corresponding to their main peaks are shown below:

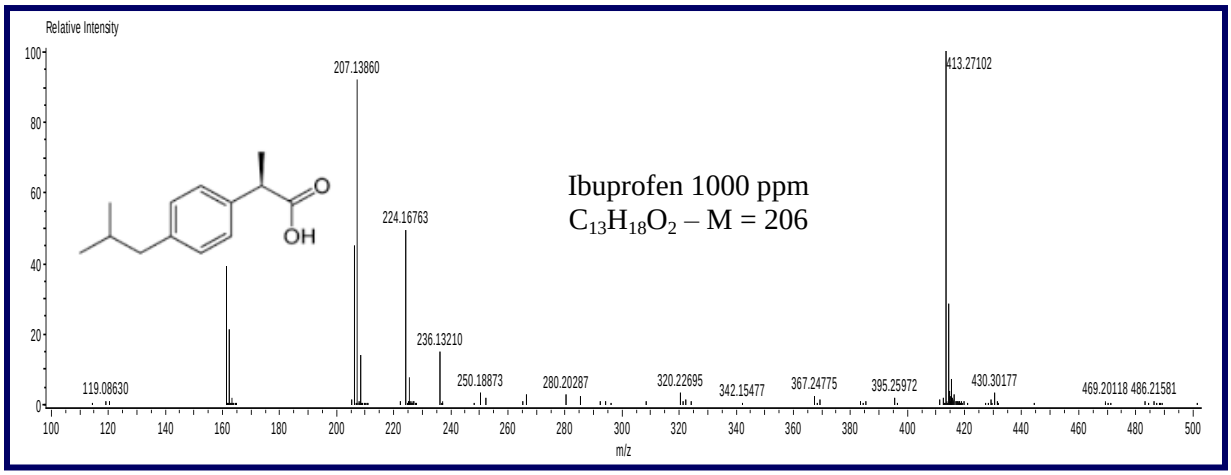

Figure 1: The mass spectrum of Ibuprofen standard solution.

Figure 1 represents the most important peaks resulting from the molecular ions and the fragments of Ibuprofen molecule. At *m/z* = 161, we notice that formate group was eliminated from Ibuprofen molecule giving the ion $[M\text{-}HCO_2]^+$. Also, we noticed the formation of the molecular ion $[M]^+$ and the protonic ion $[M + H]^+$ at *m/z* = 207, as shown in Table 1. Since Ibuprofen molecule is a carboxylic acid, so it forms hydrogen bonds giving the protonated dimer $[2M + H]^+$ at *m/z* = 413.

ISSN- 2350-0530(O), ISSN- 2394-3629(P)
DOI: 10.5281/zenodo.1133862

Table 1: The mass fragments in Ibuprofen standard solution

No.	Experimental mass	Calculated mass	Mass diff. (mu)	Formula	Unsaturation degree	Proposed structure
1	161.13241	161.13303	-0.61	$C_{12}H_{17}$	4.5	$[M - HCO_2]^+$
2	206.13129	206.13068	0.62	$C_{13}H_{18}O_2$	5.0	$[M]^{+\cdot}$
3	207.13860	207.13850	0.10	$C_{13}H_{19}O_2$	4.5	$[M + H]^+$
4	224.16649	224.16505	1.43	$C_{13}H_{22}NO_2$	3.5	$[M + H_2O]^+$
5	413.27102	413.26918	1.84	$C_{26}H_{37}O_4$	8.5	Dimer $[2M + H]^+$

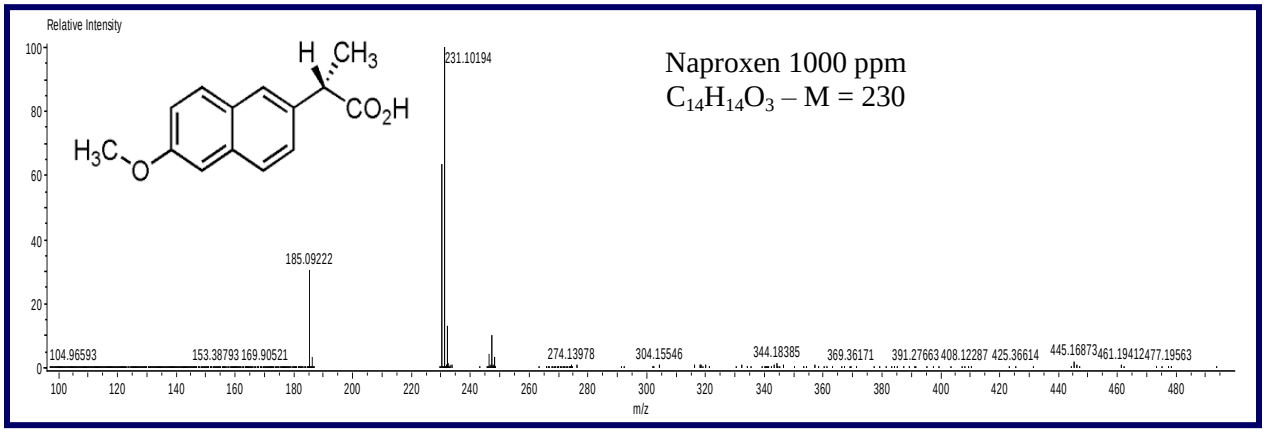

Figure 2: The mass spectrum of Naproxen standard solution

Figure 2 represents the most important peaks resulting from the molecular ions and the fragments of Naproxen molecule. At *m/z* = 230, we noticed the formation of the protonic ion $[M+H]^+$ at *m/z* = 231, as shown in Table 2.

Table 2: The mass fragments in Naproxen standard solution

No.	Experimental mass	Calculated mass	Mass diff. (mu)	Formula	Unsaturation degree	Proposed structure
1	230.09551	230.09429	1.22	$C_{14}H_{14}O_3$	8.0	Naproxen
2	231.10194	231.10212	-0.18	$C_{14}H_{15}O_3$	7.5	$[M + H]^+$

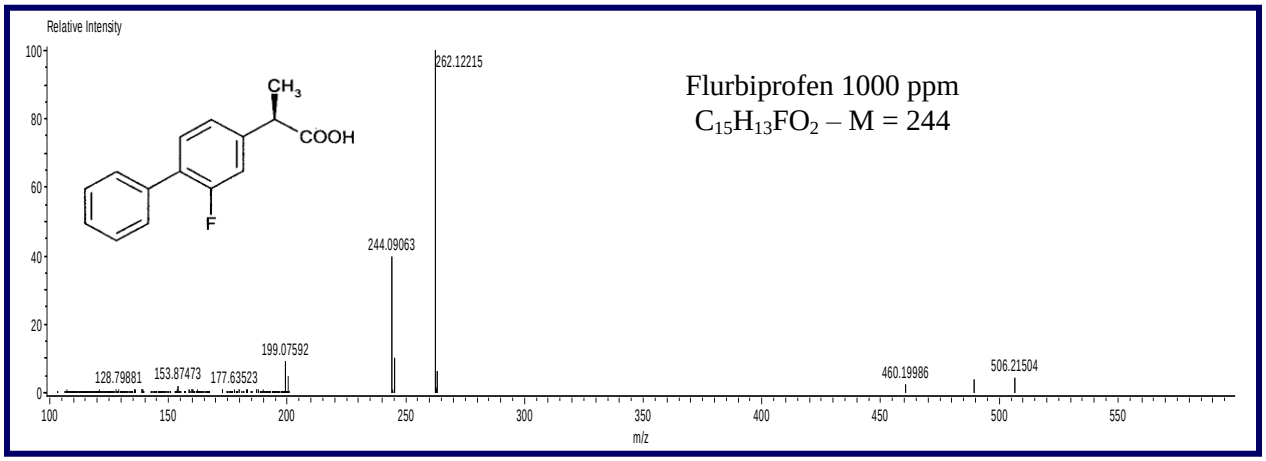

Figure 3: The mass spectrum of Flurbiprofen standard solution

ISSN- 2350-0530(O), ISSN- 2394-3629(P)
DOI: 10.5281/zenodo.1133862

Figure 3 represents the most important peaks resulting from the molecular ions and the fragments of Flurbiprofen molecule. At *m/z* = 199, we notice that formate group was eliminated from Flurbiprofen molecule giving the ion $[M–HCO_2]^+$. Also, we noticed the formation of the molecular ion $[M]^+$ at *m/z* = 244 and 245, respectively, because of the presence of (C-13). Also, we observed the attachment of ammonium group with Flurbiprofen molecule $[M + NH_4]^+$ at *m/z* = 262, as shown in Table 3. Since Flurbiprofen molecule is a carboxylic acid, so it forms hydrogen bonds giving the protonated dimer $[2M + H]^+$.

Table 3: The mass fragments in Flurbiprofen standard solution

No.	Experimental mass	Calculated mass	Mass diff. (mu)	Formula	Unsaturation degree	Proposed structure
1	199.07592	199.09230	-16.38	$C_{14}H_{12}F$	8.5	$[M – HCO_2]^+$
2	244.09063	244.08996	0.68	$C_{15}H_{13}FO_2$	9.0	$[M]^+$
3	245.09385	245.09331	0.54	$C_{14}{}^{13}CH_{13}FO_2$	9.0	$[M]^+$
4	262.12215	262.12433	-2.18	$C_{15}H_{17}FNO_2$	16.5	$[M + NH2]^+$
5	489.19211	489.18774	4.37	$C_{30}H_{27}F_2O_4$	16.5	Dimer $[2M + H]^+$

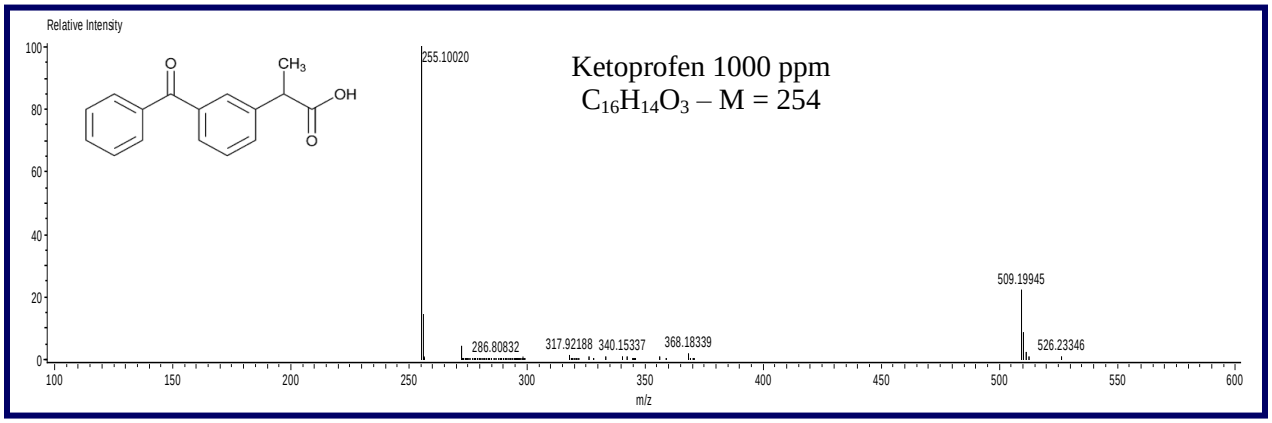

Figure 4: The mass spectrum of Ketoprofen standard solution

Figure 4 represents the most important peaks resulting from the molecular ions and the fragments of Ketoprofen molecule. At *m/z* = 255, we noticed the formation of the protonated molecular ion $[M + H]^+$. Also, we observed the formation of the protonated dimer $[2M + H]^+$ at *m/z* = 509, as shown in Table 4, where we noticed that there is no great difference between experimental mass and calculated mass, which indicates that TOF is preferred because of its high separation.

Table 4: The mass fragments in Ketoprofen standard solution

No.	Experimental mass	Calculated mass	Mass diff. (mu)	Formula	Unsaturation degree	Proposed structure
1	255.10020	255.10212	-1.92	$C_{16}H_{15}O_3$	9.5	$[M+H]^+$
2	509.19945	509.19641	3.04	$C_{32}H_{29}O_6$	18.5	Dimer $[2M + H]^+$

3.2. Mass Spectra of the Pharmaceutical Preparations

Several pharmaceutical products obtained from the local market (Riyadh, Saudi Arabia) were analyzed by DART-TOF-MS. The tablets were grinded and the powder was submitted to the

ISSN- 2350-0530(O), ISSN- 2394-3629(P)
DOI: 10.5281/zenodo.1133862

helium flow of the DART ionization source at 350°C. The high resolution mass spectra of the whole tablets without any extraction, purification or separation are shown below:

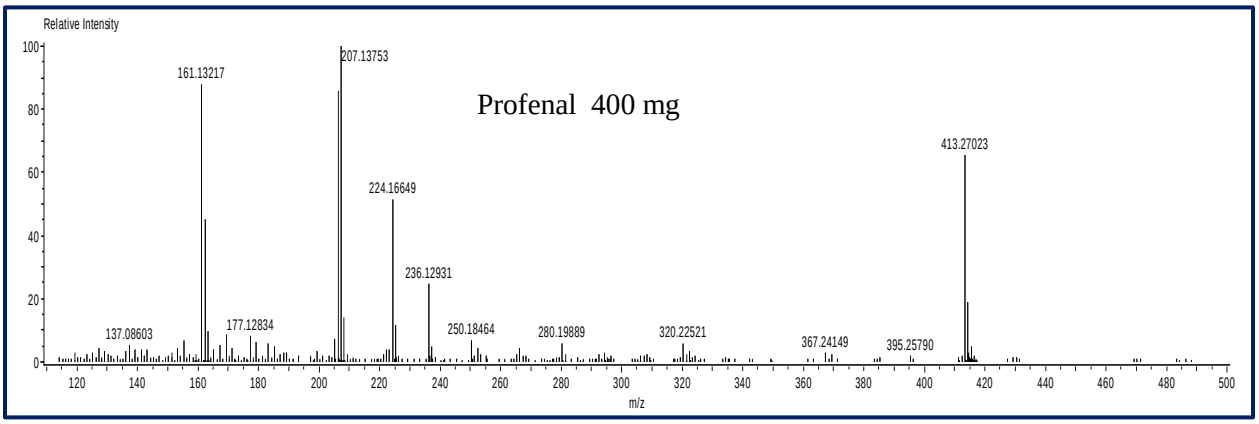

Figure 5: The mass spectrum of Profenal drug (400 mg) containing Ibuprofen

Figure 5 represents the most important peaks resulting from the molecular ions and the fragments of Profenal drug (400 mg) containing Ibuprofen molecule. We noticed the formation of the following ions respectively: $[M - HCO_2]^+$, $[M - CO_2]^+$, $[M]^+$, $[M + H]^+$, $[M + NH_4]^+$ and $[2M]^+$, as shown in Table 5, where we noticed that there is no great difference between experimental mass and calculated mass, which indicates that Profenal drug is free from any another impurities because most of the masses are congruent with the standard solution of Ibuprofen.

Table 5: The mass fragments in Profenal drug (400 mg)

No.	Experimental mass	Calculated mass	Mass diff. (mu)	Formula	Unsaturation degree	Proposed structure
1	161.13241	161.13303	-0.86	$C_{12}H_{17}$	4.5	$[M - HCO_2]^+$
2	162.14127	162.14085	0.42	$C_{12}H_{18}$	4.0	$[M - CO_2]^+$
3	206.13010	206.13068	-0.57	$C_{13}H_{18}O_2$	5.0	$[M]^+$
4	207.13753	207.13850	-0.98	$C_{13}H_{19}O_2$	4.5	$[M + H]^+$
5	224.16649	224.16505	1.43	$C_{13}H_{22}NO_2$	3.5	$[M + NH_4]^+$
6	413.27023	413.26918	1.04	$C_{26}H_{37}O_4$	8.5	Dimer

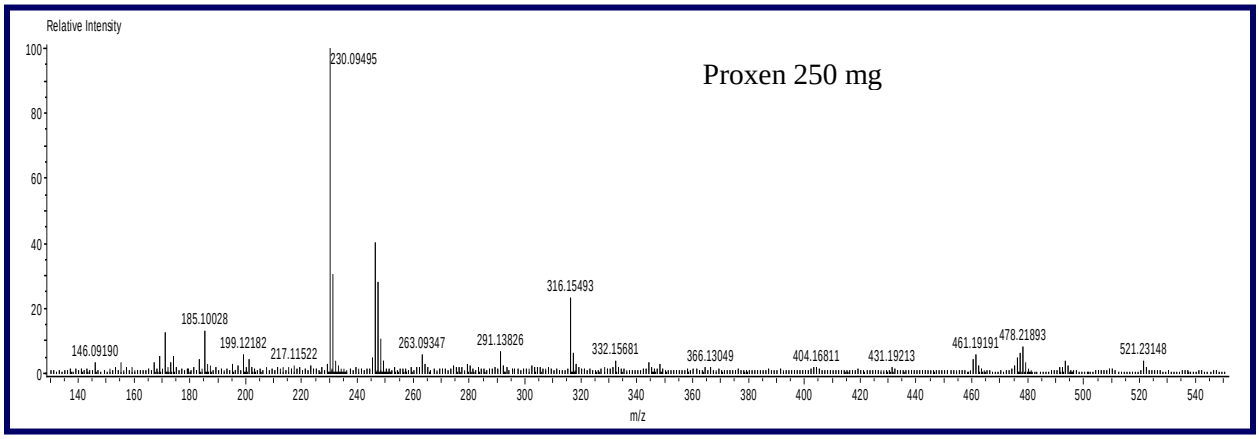

Figure 6: The mass spectrum of Proxen drug (250 mg) containing Naproxen

ISSN- 2350-0530(O), ISSN- 2394-3629(P)
DOI: 10.5281/zenodo.1133862

Figure 6 represents the most important peaks resulting from the molecular ions and the fragments of Proxen drug (250 mg) containing Naproxen molecule. As shown in Table 6, we noticed the formation of the following ions respectively: $[M + H]^+$, $[M + O]^+$ and the protonated dimer $[Dimer + H^+]^+$. Fast analysis appears that Proxen drug contains codeine oxide in its pharmaceutical composition as an additive substance at *m/z* = 316, which indicates that Proxen drug is free from any another impurities.

Table 6: The mass fragments in Proxen drug (250 mg)

No.	Experimental mass	Calculated mass	Mass diff. (mu)	Formula	Unsaturation degree	Proposed structure
1	230.09495	230.09429	0.66	$C_{14}H_{14}O_3$	8.0	Naproxen M
2	231.10018	231.10212	-1.94	$C_{14}H_{15}O_3$	7.5	$[M+H]^+$
3	246.08858	246.08921	-0.62	$C_{14}H_{14}O_4$	8.0	$[M+O]^+$
4	316.15493	316.15488	0.05	$C_{18}H_{22}NO_4$	8.5	Codeine oxide
5	460.19023	460.18859	1.64	$C_{28}H_{28}O_6$	15.0	Dimer
6	461.19191	461.19641	-4.50	$C_{28}H_{29}O_6$	14.5	Dimer + H^+

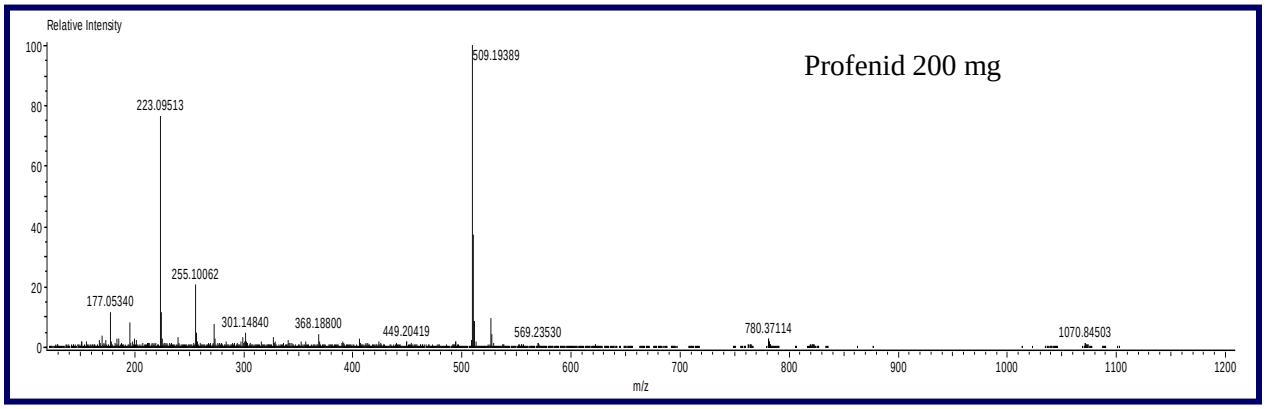

Figure 7: The mass spectrum of Profenid drug (250 mg) containing Ketoprofen

Figure 7 represents the most important peaks resulting from the molecular ions and the fragments of Profenid drug (250 mg) containing Ketoprofen molecule. As shown in Table 7, we noticed the formation of the protonated dimer of Ketoprofen $[M + H]^+$ at *m/z* = 255, and the molecular ion of Ketoprofen hydride $[M + H_2O]^+$ at *m/z* = 272. Also, we observed the molecular ion of the protonated dimer and the molecular ion of hydride dimer. We noticed here that Profenid drug is free from any another impurities and did not contains the active material like other drugs. Therefore, DART/MOF/MS gives us a rapid and accurate idea about the contents of these drugs without any chemical treatment or any chromatographic columns.

Table 7: The mass fragments in Profenid drug (250 mg).

No.	Experimental mass	Calculated mass	Mass diff. (mu)	Formula	Unsaturation degree	Proposed structure
1	255.10062	255.10212	-1.50	$C_{16}H_{15}O_3$	9.5	Ketoprofen $[M+H]^+$
2	272.12712	272.12599	1.13	$C_{13}H_{20}O_6$	4.0	$[M + H_2O]^+$
3	509.19389	509.19641	-2.52	$C_{32}H_{29}O_6$	18.5	Dimer $[2M+H]^+$
4	526.22270	526.22028	2.42	$C_{29}H_{34}O_9$	13.0	Dimer $[2M+H_2O]^+$

ISSN- 2350-0530(O), ISSN- 2394-3629(P)
DOI: 10.5281/zenodo.1133862

4. Conclusion

DART-TOF-MS was successfully applied for rapid characterization of some widely used profens. This technique proved to be very convenient for identification of the active components in liquid or solid samples without need to any extraction, purification or chromatographic separation. The other constituents of the samples are also observed in the spectrum, such as impurities or degradation products, with determination of their formula. This promising technique could be also applied for quantitative determination of specific compounds but it needs addition of a suitable internal standard.

References

[1] Robert B. Cody; James A. Laramee and H. Dupont Durst. Versatile New Ion Source for the Analysis of Materials in Open Air Under Ambient Conditions. Anal. Chem., 2005, 77, 2297-2302.
[2] Jürgen H. Gross. Direct analysis in real time – A critical review on DART-MS. Anal. Bioanal. Chem., 406: 63–80, 2014.
[3] Robert B. Cody. Observation of Molecular Ions and Analysis of Nonpolar Compounds with the Direct Analysis in Real Time Ion Source, Anal. Chem., 81 (2009) 1101-1107.
[4] Inger L. Meek; Mart A.F.J. Van de Laar; Harald E. Vonkeman. Non-Steroidal Anti-Inflammatory Drugs: An Overview of Cardiovascular Risks. Pharmaceuticals, 2010, 3, 2146-2162.
[5] John Robert Vane. The mechanism of action of anti-inflammatory drugs. Int. J. Clin. Prac. Suppl., 135 (2003) 2.
[6] Zakharov S., Navratil T. and Pelclova D. Suicide attempts by deliberate self-poisoning in children and adolescents. Psychiatry Res., 210 (2013) 302–7.
[7] Yen Sun, Humiko Takaba, Hideaki Kido, Mihoko N Nakashima and Kenichiro Nakashima, Simultaneous Determination of Aryl propionic Acid Nonsteroidal Anti-Inflammatory Drugs in Pharmaceutical Formulations and Human Plasma by HPLC with UV Detection, Journal of Pharmaceutical and Biomedical Analysis, 30(5) (2003) 1611-1619. doi:10.1016/S0731-7085(02)00549-6
[8] Tomáš Hložek; Miroslava Bursová and Radomír Čabala. Fast ibuprofen, ketoprofen and naproxen simultaneous determination in human serum for clinical toxicology by GC–FID. Clinical Biochemistry, 47 (2014) 109–111.
[9] Bilal Yilmaz, and Emrah Alkan. Determination of flurbiprofen in pharmaceutical preparations by GC–MS. Arabian Journal of Chemistry, xxx, (2015) xxx-xxx.
[10] Gloria Grueiro Noche; María Esther Fernández Laespada; José Luis Pérez Pavón; Bernardo Moreno Cordero and Soledad Muniategui Lorenzo. In situ aqueous derivatization and determination of non-steroidal anti-inflammatory drugs by salting-out-assisted liquid–liquid extraction and gas chromatography–mass spectrometry. Journal of Chromatography A, 1218 (2011) 6240–6247.
[11] Nor H Hashim and Stuart J. Khan. Enantioselective analysis of ibuprofen, ketoprofen and naproxen in wastewater and environmental water samples. Journal of Chromatography A, 1218 (2011) 4746– 4754.
[12] Robert B. Cody, James A Laramee, and H. Dupont Durst. Versatile New Ion Source for the Analysis of Materials in Open Air under Ambient Conditions. Anal. Chem., 77(8) (2005) 2297–2302.

*Corresponding author.
E-mail address: 434108458@ student.ksu.edu.sa