



HAL
open science

Imaging Matrix-Assisted Laser Desorption/Ionization Fourier Transform Ion Cyclotron Resonance Mass Spectrometry of oxaliplatin derivatives in human tissue sections

Justine Ferey, Marion Larroque, Isabelle Schmitz-Afonso, Johann Le Maître,
Olivia Sgarbura, Sébastien Carrere, François Quenet, Brice Bouyssière,
Christine Enjalbal, Sandra Mounicou, et al.

► To cite this version:

Justine Ferey, Marion Larroque, Isabelle Schmitz-Afonso, Johann Le Maître, Olivia Sgarbura, et al..
Imaging Matrix-Assisted Laser Desorption/Ionization Fourier Transform Ion Cyclotron Resonance
Mass Spectrometry of oxaliplatin derivatives in human tissue sections. *Talanta*, 2022, 237, pp.122915.
10.1016/j.talanta.2021.122915 . hal-03400641

HAL Id: hal-03400641

<https://univ-pau.hal.science/hal-03400641v1>

Submitted on 24 Nov 2021

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution - NonCommercial 4.0 International License

1 **Imaging Matrix-Assisted Laser Desorption/Ionization Fourier Transform**
2 **Ion Cyclotron Resonance Mass Spectrometry of oxaliplatin derivatives in**
3 **human tissue sections**

4
5 Justine Ferey^{1,2,3}, Marion Larroque⁴, Isabelle Schmitz-Afonso^{1*}, Johann Le
6 Maître¹, Olivia Sgarbura⁵, Sébastien Carrere⁵, François Quenet⁵, Brice
7 Bouyssiere⁶, Christine Enjalbal⁷, Sandra Mounicou⁶, Carlos Afonso¹

8
9 ¹*Normandie Univ, COBRA, UMR 6014 and FR 3038, Université de Rouen, INSA de Rouen,*
10 *CNRS, IRCOF, 1 rue Tesnières, 76821 Mont-Saint-Aignan, Cedex, France.*

11 ²*UMR1331 Toxalim (Research Centre in Food Toxicology), Toulouse University, INRAE,*
12 *ENVT, INP-Purpan, UPS, 31027 Toulouse, France*

13 ³*Metatoul-AXIOM platform, National Infrastructure for Metabolomics and Fluxomics:*
14 *MetaboHUB, Toxalim, INRAE, 31027 Toulouse, France*

15 ⁴*Unité de Recherche Translationnelle, Institut du Cancer de Montpellier (ICM), 208 rue des*
16 *apothicaires, 34298 Montpellier, France.*

17 ⁵*Service Chirurgie, Institut du Cancer de Montpellier (ICM), 208 rue des apothicaires, 34298*
18 *Montpellier, France.*

19 ⁶*Universite de Pau et des Pays de l'Adour, E2S UPPA, CNRS, IPREM, Institut des Sciences*
20 *Analytiques et de Physico-chimie pour l'Environnement et les Matériaux, UMR5254,*
21 *Hélioparc, 64053 Pau, France*

22 ⁷*Univ Montpellier, IBMM, CNRS, ENSCM, 34095 Montpellier, France*

23 *Corresponding author at: Normandie Univ, COBRA, UMR 6014 and FR 3038, Université de
24 Rouen, INSA de Rouen, CNRS, IRCOF, 1 rue Tesnières, 76821 Mont-Saint-Aignan, Cedex,
25 France. E-mail address : isabelle.schmitz-afonso@univ-rouen.fr
26

27

28

29

30

31

32 **Abstract**

33 Mass Spectrometry Imaging is an effective technology that allows to determine the *in-situ*
34 distribution of endogen and/or exogen small molecules. It is a rapidly emerging approach for
35 visualizing drugs and their metabolites within biological tissues. Matrix-Assisted Laser
36 Desorption Ionization (MALDI) Mass Spectrometry Imaging (MSI) coupled to high resolving
37 power analyzer (e.g. TOF) was already investigated for metallodrug localization and
38 metabolization studies, but was proved to suffer from a lack of sensitivity and resolution,
39 leading to poor coverage and assignment. To counter these technological limitations, the use of
40 ultra-high resolving power analyzer such as Fourier Transform Ion Cyclotron Resonance
41 (FTICR) could be revealed as a technique of choice. The high field FTICR MS provides ultra-
42 high resolving power and mass accuracy that allows exhaustive molecule coverage and non-
43 ambiguous molecular formula assignments. Platinum derivatives, such as oxaliplatin, are
44 widely used as therapeutic agents for cancer treatment. The assessment of their intake,
45 distribution and metabolism within the organs is important to know the risks associated with
46 their use. In this study, MALDI FTICR MSI analyses were performed to better understand the
47 penetration and metabolization of platinum derivatives in ovaries of women treated by
48 Hyperthermic Intraperitoneal Chemotherapy (HIPEC) for peritoneal metastasis of colorectal or
49 appendicular origin. Twelve ovary sections, from six ovary samples in six women donors,
50 before and after treatment, were analyzed with 120 μm spatial resolution. For the first time, the
51 high resolving power (220,000 at m/z 457) and sub-ppm accuracy (< 1 ppm) of the FTICR
52 combined with an Isotopic Fine Structure study enabled to distinguish two Pt-isobaric species
53 derived from oxaliplatin in biological tissues. One of these, which is unknown, was specifically
54 localized at the contour of the ovary.

55

56 **Keywords** : Mass Spectrometry Imaging; MALDI-FTICR ; ovary ; platinum metabolites ; HIPEC ;
57 Isotopic Fine Structure

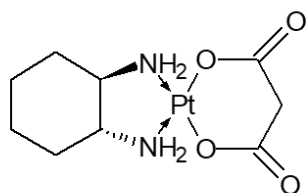
59 **1. Introduction**

60 Mass Spectrometry Imaging (MSI) has entered the field of tissue-based research. It is an
61 effective technology that allows to determine the *in-situ* distribution of endogen and/or exogen
62 small molecules. Introduced by Caprioli et al [1], MSI using Matrix-Assisted Laser
63 Desorption/Ionization Time-Of-Flight (MALDI-TOF) technology has become an appropriate
64 alternative to radioactive labeling, to visualize simultaneously initial drug distribution and their
65 metabolism in specific tissues or whole animal body [2]. This became possible by spatially
66 imaging the mass to charge (m/z) ratios of molecules to generate molecular pictures of the tissue
67 [1]. Currently, application of MALDI MSI in drug research and development has attracted
68 intense attention from the pharmaceutical industry [3, 4]. MALDI imaging is complementary
69 to more conventional optical imaging techniques (such as histology, fluorescence, RAMAN
70 microscopy, Xray spectroscopy [5]) and lies in its label-free, unparalleled chemical information
71 and spatially resolved detection. In this way, the MSI technique is raising interest, especially in
72 clinical research, for various kinds of applications like drug biodistribution and metabolization
73 studies. However, it must be noted that the use of Formalin-Fixed Paraffin-Embedded (FFPE)
74 tissues is not recommended for metabolite studies (loss and delocalization of the metabolites
75 during the washing steps), which may reflect a limitation of this technique regarding the large
76 FFPE samples library. This limitation is of less importance for MSI of proteins, more robust to
77 FFPE sample preparation steps. [6, 7]

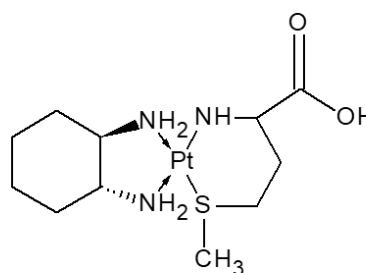
78 In particular, platinum-based drugs play an important role in cancer treatment [8, 9]. Given the
79 clinical use of these drugs, evaluation of their intake, distribution and metabolism within organs
80 and/or tumors is important to study the efficacy, metabolization and the fate of the drug in the
81 organism. Various methodological developments for the imaging of this metal-based anti-

82 cancer compound as Pt-metallodrugs in biological samples were improved in the last years [10].
83 The main analytical tools for MS-based metallodrug imaging related in the literature are SIMS-
84 MSI, LA-ICP-MSI and MALDI-MSI. These three techniques can be compared in term of
85 sensitivity, spatial resolution and type of analytes detected. While SIMS-MSI and MALDI-MSI
86 are useful in providing molecular information on the drug metabolites, they suffer from a lower
87 sensitivity than LA-ICP-MS [10-12]. Indeed, LA-ICP-MS technique is very sensitive (platinum
88 detection around ng/mg of tissue [13]), offers a spatial resolution of 1 μm , does not require
89 chemical derivatization of the sample, allows for FFPE sections analysis but does not allow
90 molecular characterization of the metallodrug. Besides, SIMS-MSI offers the best spatial
91 resolution (100 nm) with a sensitivity up to 0.1 pg/mg of tissue [5] for elemental and molecular
92 fragments while MALDI-MSI provides the visualization of biomolecules leading to molecular
93 information on intact drug species formed, at the μm scale. However, MALDI-TOF MSI [14]
94 shows low sensitivity (picogram level detection) for platinum compound detection due to poor
95 ionization of metallodrugs in comparison to LA-ICP-MSI. As detailed below, MALDI-MSI
96 limitations can be overcome thanks to FTICR instruments instead of TOF analyzers.

97 Nowadays, oxaliplatin is the most administrated anti-cancer drug of all platinum based-
98 treatments and the study of its metabolization, as oxaliplatin-methionine complex metabolite,
99 is of great interest (formulas shown in Figure 1).



Chemical Formula $\text{C}_8\text{H}_{14}\text{N}_2\text{O}_4\text{Pt}$
Exact Mass: 397.0601
Oxaliplatin



Chemical Formula $\text{C}_{11}\text{H}_{23}\text{N}_3\text{O}_2\text{PtS}$
Exact Mass: 456.1159
Oxaliplatin-methionine

101 **Figure 1:** Structural formulas of oxaliplatin and oxaliplatin-methionine.
102

103 Indeed, a MALDI TOF-MSI approach has been previously developed to study the distribution
104 of oxaliplatin in rat kidney, in which platinum-containing compounds were detected as
105 complexes that might be attributed to oxaliplatin-methionine and oxaliplatin-cysteine [15].
106 These findings were complemented by a combined MALDI-TOF and LA ICP-MS imaging in
107 which the presence of platinum drugs was confirmed in human tumors [16] and in ovaries [13]
108 from HIPEC treated patients. However, in many cases, *in vivo* administrated drugs can be more
109 difficult to analyze than endogenous small molecules due to their low abundance (ng/mg of
110 platinum in the ovaries [13]) and due to the ionization competition phenomenon with more
111 abundant and more easily ionizable molecules. In addition, the mass resolution of the TOF MS
112 instruments (typically between 20,000 to 50,000) may not be sufficient to separate all isobaric
113 species in the absence of prior chromatographic separation [17]. Consequently, in some cases,
114 it is not possible to evidence the presence of some species when high abundance isobars are
115 present. Therefore, it is difficult to precisely identify individual signal in MSI spectra and
116 resulting MS/MS experiments are the combination of fragments from several isobaric precursor
117 ions [18].

118 The MALDI-TOF-MSI tool limitations [13] can be overcome with the use of ultra-high
119 resolution mass analyzer such as Fourier Transform Ion Cyclotron Resonance (FTICR) MS
120 [19-21]. High resolution instruments are defined by a resolving power, Full Width at Half
121 Maximum (FWHM), above 10,000 [22] whereas ultra-high resolution analyzers provide a
122 resolving power above 200,000 [17]. Thus, the FTICR MSI at high magnetic field provides
123 high mass accuracy (< 1 ppm) and ultra-high resolving power (> 200,000) enabling the
124 separation of most isobaric species. In addition, the resolution obtained during FTICR MS
125 tissue imaging is very suitable for unambiguous molecular formula attribution taking advantage

126 of the Isotopic Fine Structure (IFS) [17-19, 23-26]. Therefore, this technique is of great interest
127 for exhaustive analysis, rapid and precise annotation of compounds in biological samples [27]
128 and already have proved its merit for MALDI imaging analysis of biological tissues in terms of
129 resolving power, mass accuracy and dynamic range [27].

130 In this way, the objective of this study was to investigate the insight of MALDI-MSI coupled
131 to FTICR analyzer for the visualization and the identification of oxaliplatin and its potential
132 metabolites in human ovary surgically resected from woman treated with HIPEC for a
133 peritoneal carcinomatosis. Briefly, peritoneal carcinomatosis is a common extension of colon
134 cancer whose recurrence is characterized by the multiplication of tumor nodules on the
135 peritoneum. HIPEC is one of the most recent treatments enabling a significantly improved
136 survival of the patients. It consists in a local application of a high concentration of warmed anti-
137 cancer Pt-metallodrugs, such as oxaliplatin, through the peritoneal cavity [28-31]. In treated
138 women, the intra peritoneal location of ovaries raises the possibility for their contamination
139 with the Pt-drug [13]. Thus, the objective of the related clinical work is to optimize oxaliplatin
140 distribution to tumoral nodules issued from peritoneal metastasis during the HIPEC while
141 limiting its diffusion to adjacent organs like ovaries in order to preserve fertility. The eventual
142 contamination of the ovaries has been studied in this work. As a result, the analyses of 12 ovary
143 sections before and after treatment enabled to highlight the presence of a Pt-metabolite on the
144 outskirts of the organ. The ultra-high mass accuracy and resolution of the FTICR analysis
145 combined with the study of IFS allowed to determine with a high level of confidence the
146 molecular formula of this Pt-containing metabolite, derived from oxaliplatin.

147

148 **2. Materials and Methods**

149 *2.1. Reagents and chemicals*

150 Acetonitrile (ACN) and water quality grade LC-MS were purchased from Fisher Scientific
151 (Loughborough, UK). α -Cyano-4-hydroxycinnamic acid (CHCA) matrix, and trifluoroacetic
152 acid (TFA), were purchased from Sigma-Aldrich (S^t Louis, US). Sodium trifluoroacetate
153 (NaTFA, Sigma-Aldrich, S^t Louis, US), at a concentration of 0.1 mg mL⁻¹ (ACN/H₂O 50/50
154 (v/v)) was used as external calibrant before each analysis. Oxaliplatin-methionine was prepared
155 by reacting Eloxatin solution (1.3mM) with the amino acid methionine (1.3mM) in
156 physiological buffer (23mM NaHCO₃, 5mM NaH₂PO₄, pH7.4) at 37°C under 5% CO₂ for 24h.
157 All chemicals and solvents for this reaction were purchased from Sigma Aldrich (St. Louis,
158 MO. USA) [15].

159

160 2.2. Tissue collection

161 For this prospective study, all patients gave written informed consent before the procedure, and
162 the institutional review board approved the study, which was performed in accordance with the
163 ethical standards of the Helsinki Declaration of 1975. Six ovary samples were surgically
164 removed from women donors diagnosed with colorectal peritoneal carcinomatosis before and
165 after HIPEC treatment of the peritoneal tumoral nodule with oxaliplatin drug. Clinical patient's
166 data and HIPEC treatments were previously published [13]. Briefly, the patients in this group
167 presented peritoneal metastasis of colorectal (4 patients) and appendicular (2 patients) origin.
168 The appendicular origin was low grade appendiceal mucinous neoplasm (LAMN) [32]. The
169 two entities were included as the HIPEC protocol is similar, based on the administration of
170 oxaliplatin. The prognostic of the two entities, however, is quite different with a median overall
171 survival (OS) of 41.7 months for the patients with peritoneal metastasis of colorectal origin,
172 while it is of 196 months for patients with LAMN [32, 33]. The difference of prognosis of these
173 patients has no impact on the results of the current study that aims to detect penetration of the
174 ovaries. This latter aspect can be highly important for the female patients that have fertility

175 projects that are compatible with the long survival of some of these patients [34]. For each
176 donor, one ovary was removed before HIPEC treatment and used as a reference sample for
177 MALDI-FTICR MSI. The ones removed after treatment were used to image the drug
178 localization and to identify the drug metabolite. In addition, one peritoneal tumoral nodule
179 section was also sampled for analysis. The samples were frozen in liquid nitrogen and stored at
180 -80°C until analysis.

181 *2.3. Tissue preparation for MALDI FTICR imaging*

182 Fresh frozen $10\ \mu\text{m}$ ovary sections were obtained with a Microm HM 550 cryotome (Thermo
183 Scientific, Germany) using -18°C and -20°C temperatures for the specimen and the chamber
184 respectively. Sections were mounted on conductive Indium tin oxide (ITO)-coated slides
185 (Bruker, Bremen, Germany, $75\times 50\ \text{mm}$) and stored at -80°C . Before analysis, the slides were
186 first taken back at room temperature for 1h in a desiccator. CHCA matrix solution ($5\ \text{mg mL}^{-1}$
187 in ACN/ H_2O 70/30 (v/v), 0.1% TFA) was applied to the tissue sections with an automatic
188 microsyringe HTX TM-Sprayer (HTX Imaging, Chapel Hill, NC, US) with the following
189 parameters: flow rate $100\ \mu\text{L min}^{-1}$, number of passes 12, track spacing 2 mm, nozzle
190 temperature 85°C , nozzle velocity $1200\ \text{mm min}^{-1}$, N_2 pressure 10 psi and N_2 flow rate $2\ \text{L min}^{-1}$.
191 Slides were dried under vacuum before analysis.

192 *2.4. Mass spectrometry instrumentation*

193 MALDI imaging analyses were performed on a FTICR instrument (solariX XR, Bruker,
194 Bremen, Germany) equipped with a 12 Tesla superconducting magnet and a dynamically
195 harmonized ICR cell. This instrument is equipped with both laser desorption ionization source
196 (smartbeam II, 1 kHz Nd:YAG $\times 3$ laser at 355 nm, Bruker) and an electrospray ionization (ESI)
197 source. Mass spectrometric analyses were performed in positive ion mode in the mass range
198 m/z 150-1000. Each MALDI mass spectrum for each position is the result of 250 consecutive

199 laser shots. Spectra were acquired with a 120 μm spatial resolution. The analytical resolution is
200 220,000 at m/z 457.

201 Before each image acquisition, external calibration was performed in positive ion mode by
202 NaTFA infusion *via* ESI source. Internal calibration of the MALDI mass spectra was then
203 performed by assigning known metabolites in the mass range m/z 150-1000. During the image
204 acquisition, an auto-calibration was performed assigning $\text{C}_5\text{H}_{14}\text{NO}_4\text{P}$ (m/z 184.07332, $[\text{M}+\text{H}]^+$,
205 phosphocholine), $\text{C}_{10}\text{H}_7\text{NO}_3$ (m/z 399.03778, $[2\text{M}+\text{K}]^+$, matrix peak), $\text{C}_{24}\text{H}_{50}\text{NO}_7\text{P}$ (m/z
206 496.33977, $[\text{M}+\text{H}]^+$, lipid), $\text{C}_{37}\text{H}_{75}\text{N}_2\text{O}_6\text{P}$ (m/z 675.54355, $[\text{M}+\text{H}]^+$, lipid), $\text{C}_{42}\text{H}_{82}\text{NO}_8\text{P}$ (m/z
207 782.56703, $[\text{M}+\text{Na}]^+$, lipid), $\text{C}_{44}\text{H}_{80}\text{NO}_8\text{P}$ (m/z 820.52531, $[\text{M}+\text{K}]^+$, lipid).

208 Data size was set at 2 million points for a transient length of 0.87 s and mass spectra were
209 acquired with a 97% data file reduction. All images were acquired with the FTMS control and
210 FlexImaging (v 5.0, Bruker, Bremen) softwares. Images were processed with SCLs Lab Pro
211 software (Bruker Daltonics, Bremen, Germany).

212 To unambiguously determine the molecular formula of the compound of interest, the IFS was
213 evaluated by accumulation of 20 scans on the region of interest by zone profiling. Molecular
214 formulas were generated using Bruker Data Analysis, considering the following elements and
215 limits ($\text{C}_{0-20}\text{H}_{0-40}\text{O}_{0-10}\text{N}_{0-10}\text{S}_{0-4}\text{Pt}_{0-1}$) with m/z errors < 1 ppm.

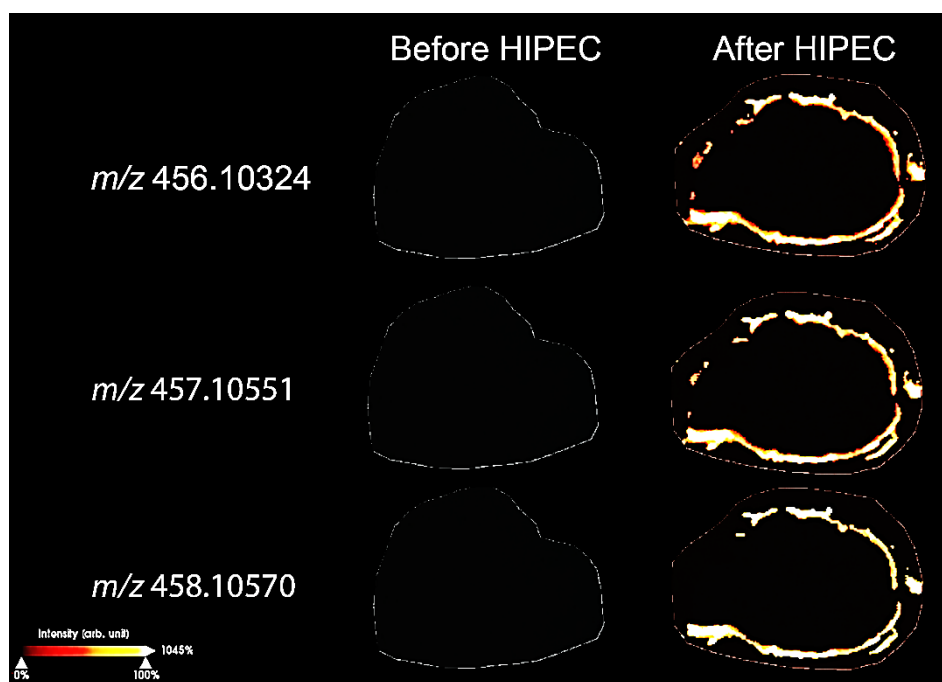
216

217 **3. Results and discussions**

218 *3.1. Pt localization inside ovary tissues*

219 The aim of the study is to localize and identify by MALDI-FTICR MSI analysis the
220 administrated Pt-drug and/or its metabolites after HIPEC treatment of the peritoneal metastasis
221 in adjacent ovarian tissue section. Mass spectrum of a Pt-containing compound has a
222 characteristic isotopic profile, characterized by the m/z values of each isotope. This profile is
223 due to the presence of the three most abundant platinum isotopes namely ^{194}Pt , ^{195}Pt and ^{196}Pt

224 with respective relative abundance of 32.9%, 33.9% and 25.9%. For the 6 patients, one ovary
225 was removed before HIPEC treatment (reference tissue) and the other one after treatment.
226 Overall, 12 ovary sections were analyzed. Due to the high number of detected metabolites (more
227 than 1,200 peaks for an ovary tissue analysis), the isotope profile of Pt-compounds is difficult
228 to detect. To evidence Pt-containing compounds, a non-targeted search of discriminating m/z
229 values was carried out between the sections before and after treatment, with SCiLS software.



230

231 **Figure 2:** MALDI FTICR MSI of ovary tissues before and after HIPEC treatment.
232 Visualization of discriminative values at m/z 456.10324, m/z 457.10551 and m/z 458.10570.
233 Spatial resolution of 120 μm .

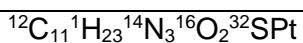
234 **In Figure 2,** MALDI FTICR MS images show a peripheral location of a discriminative
235 compound at three m/z values, m/z 456.10324, m/z 457.10551 and m/z 458.10570, which do not
236 correspond to the original oxaliplatin compound (m/z 397.0653, m/z 398.0675, m/z 399.0679).
237 The same peripheral distribution could indicate that they are from the same molecule.
238 Moreover, the nominal masses of these three m/z values are separated by one mass unit, which
239 could reflect the presence of the platinum isotopic profile.

240 3.2. Determination of molecular formula of peripheral Pt-based compound by FTICR

241 With ultra-high resolution instruments, the molecular formula of a compound can be
 242 determined thanks to a high mass accuracy and to an isotopic pattern study [24]. To get a higher
 243 signal to noise ratio and therefore better define the isotopic structure, 20 spectra were
 244 accumulated in the peripheral regions of the tissue.

245 The three detected masses at m/z 456.10324, m/z 457.10551 and m/z 458.10570 did not
 246 correspond to the oxaliplatin ion. With a mass difference of 40 ppm, this species is an isobar of
 247 the oxaliplatin-methionine complex $C_{11}H_{23}N_3O_2SPt$ (**Figure 1, Table 1**) described in the study
 248 of Bouslimani *et al.* [15]. The resolution, around 20,000, and mass measure precision,
 249 difference of 0.06 Da, would not have been sufficient in this study to differentiate the two
 250 isobars. Thus, to validate the presence of another compound than oxaliplatin-methionine in
 251 these tissue sections, drops of 1 μ L of this complex were deposited at the tissue peripheries. As
 252 shown in **Figure S1**, the FTICR high resolution (220,000 at m/z 457.12309) enabled to
 253 distinguish these two isobaric species. The overall internally calibrated mass spectrum showed
 254 an average error of 0.133 ppm in the mass range m/z 150-1000. From this accurate mass
 255 measurement, the molecular formula of oxaliplatin-methionine complex was confirmed
 256 ($^{12}C_{11}^{1}H_{23}^{14}N_3^{16}O_2^{32}S^{195}Pt$) with a mass accuracy of 0.453 ppm. This statement was reinforced
 257 by the analysis of a section from a peritoneal tumoral nodule itself, resected after HIPEC.
 258 Results, shown in **Figure S2**, highlight the presence of three oxaliplatin-methionine complex
 259 isotopes (**Table 1**) as previously described [15], with respective errors of 0.145 ppm, 0.060 ppm
 260 and 0.388 ppm. As shown in **Figure S3**, the other platinum complex at m/z 457.10551 was also
 261 visualized in these tissues after HIPEC treatment with the presence of the three co-localized
 262 isotopes ^{194}Pt , ^{195}Pt , and ^{196}Pt .

	^{194}Pt (M) [M+H] ⁺	^{195}Pt (M+1) [M+H] ⁺	^{196}Pt (M+2) [M+H] ⁺
Oxaliplatin $^{12}C_8^{1}H_{14}^{14}N_2^{16}O_4Pt$	397.065314	398.067522	399.067585
Oxaliplatin-methionine	456.121055	457.123297	458.123319



263 **Table 1:** Theoretical m/z values of the isotopes M (^{194}Pt), M+1 (^{195}Pt) and M+2 (^{196}Pt) for
 264 oxaliplatin and oxaliplatin-methionine complex.

265
 266 The use of high field FTICR analyzer, providing high mass accuracy and resolution, was
 267 demonstrated to be essential for the unambiguous differentiation of these isobaric compounds,
 268 which reveals the presence of a platinum-derivative at the contours of the ovary.

269 In the ovary tissue sections mass spectra, the molecular formula of the ion of interest was
 270 determined from the monoisotopic peak, calculated by using the most abundant isotope of each
 271 element (m/z 457.10551 for ^{195}Pt). Then, the molecular formula of this compound was precisely
 272 determined by study of the IFS. In major cases, mass spectrometry studies have to consider
 273 atomic isotopes that play an important role in the atomic elucidation of a compound. Generally,
 274 each element has one most abundant isotope (such as ^{12}C) and others with minor abundance
 275 (such as ^{13}C). These low abundance isotopic peaks can be separated from other isobaric
 276 isotopologues when working at sufficiently high resolution, thus facilitating the atomic
 277 composition determination [27]. Primary, the high mass accuracy of the spectrum considerably
 278 reduced the number of possible molecular formulas especially for Pt-containing compound. The
 279 monoisotopic peak detected at m/z 457.10551 had 3 candidate formulas, namely $\text{C}_{10}\text{H}_{19}\text{N}_3\text{O}_5\text{Pt}$,
 280 $\text{C}_{11}\text{H}_{15}\text{N}_7\text{OPt}$ and $\text{C}_{11}\text{H}_{23}\text{N}_3\text{S}_2\text{Pt}$, at respective mass errors of -1.86 , 1.09 and 0.06 ppm (**Table**
 281 **2**). The mass accuracy of each isotopologue (containing ^{194}Pt , ^{195}Pt and ^{196}Pt) and their relative
 282 intensity were compared to the theoretical mass spectra, generated at the same resolving power
 283 as the experimental mass spectrum (220,000). All m/z values and relative intensities of the
 284 clearly resolved isotope peaks are listed in **Table 2**.

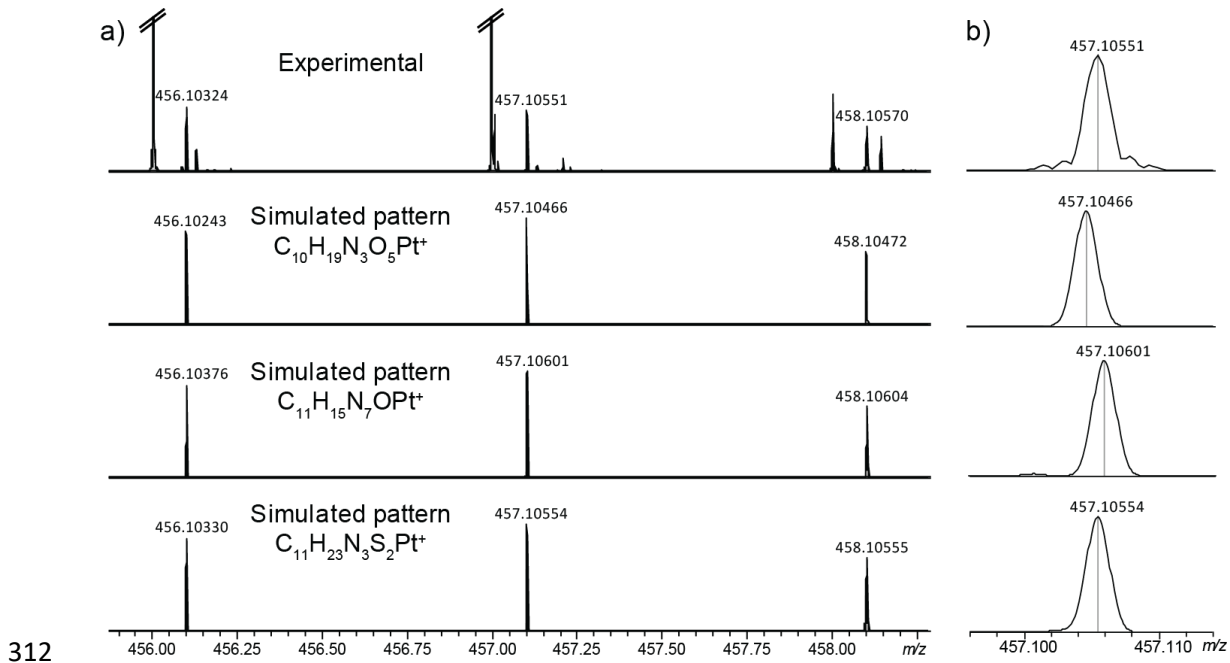
		^{194}Pt (M)	^{195}Pt (M+1)	^{196}Pt (M+2)
Theoretical m/z (relative abundance)	$^{12}\text{C}_{10}^{1}\text{H}_{19}^{14}\text{N}_3^{16}\text{O}_5\text{Pt}$	456.10243 (87%)	457.10466 (100%)	458.10472 (68%)
	$^{12}\text{C}_{11}^{1}\text{H}_{15}^{14}\text{N}_7^{16}\text{OPt}$	456.10376 (87%)	457.10601 (100%)	458.10604 (68%)
	$^{12}\text{C}_{11}^{1}\text{H}_{23}^{14}\text{N}_3^{32}\text{S}_2\text{Pt}$	456.10330 (87%)	457.10554 (100%)	458.10555 (68%)

Experimental m/z (relative abundance)		456.10324 (93%)	457.10551 (100%)	458.10570 (70%)
Errors between theoretical and experimental values (ppm)	$^{12}\text{C}_{10}^{1}\text{H}_{19}^{14}\text{N}_3^{16}\text{O}_5\text{Pt}$	-1.78	-1.86	-2.19
	$^{12}\text{C}_{11}^{1}\text{H}_{15}^{14}\text{N}_7^{16}\text{OPt}$	1.15	1.09	0.736
	$^{12}\text{C}_{11}^{1}\text{H}_{23}^{14}\text{N}_3^{32}\text{S}_2\text{Pt}$	0.125	0.06	-0.319

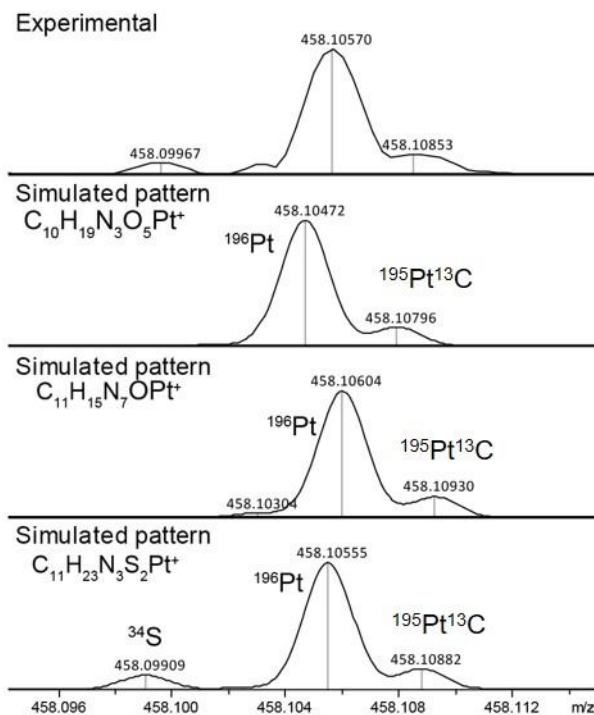
285 **Table 2:** Theoretical and experimental m/z values and relative abundance (%) of the isotopes
286 M (^{194}Pt), M+1 (^{195}Pt) and M+2 (^{196}Pt) for the determined raw formulas $\text{C}_{10}\text{H}_{19}\text{N}_3\text{O}_5\text{Pt}$,
287 $\text{C}_{11}\text{H}_{15}\text{N}_7\text{OPt}$ and $\text{C}_{11}\text{H}_{23}\text{N}_3\text{S}_2\text{Pt}$ of the unknown compound with associated m/z errors.

288 **Figure 3** shows the complete pattern spectra containing the M (^{194}Pt), M+1 (^{195}Pt) and M+2
289 (^{196}Pt) isotopes for the experimental analysis and the simulated patterns with an expanded
290 window of the monoisotopic peak M+1. For the three molecular formulas, the three isotopes
291 are present in the spectra with excellent mass accuracies (**Table 2**). The experimental relative
292 abundances are adequate with the theoretical ones with 93%, 100% and 70% for M, M+1 and
293 M+2 isotope, respectively. The precise identification was therefore performed by studying the
294 IFS of the M+2 isotope showing three resolved isotopologues. As shown in **Figure 4**, for each
295 molecular formula, we observed a peak generated by ^{13}C substitution (m/z 458.10796 for
296 $^{12}\text{C}_9^{13}\text{C}_1^1\text{H}_{19}^{14}\text{N}_3^{16}\text{O}_5^{195}\text{Pt}$, m/z 458.10930 for $^{12}\text{C}_{10}^{13}\text{C}_1^1\text{H}_{15}^{14}\text{N}_7^{16}\text{O}^{195}\text{Pt}$ and m/z 458.10882 for
297 $^{12}\text{C}_{10}^{13}\text{C}_1^1\text{H}_{23}^{14}\text{N}_3^{32}\text{S}_2^{195}\text{Pt}$). The experimental one is observed at m/z 458.10853. For
298 $\text{C}_{11}\text{H}_{23}\text{N}_3\text{S}_2\text{Pt}$, the peak observed at m/z 458.09909 is produced by a ^{34}S substitution. This peak
299 is also encountered in the experimental profile at m/z 458.09967 which proves the presence of
300 sulfur in the compound. The peak caused by a ^{34}S substitution (m/z 458.09909) is resolved from
301 the peaks caused by a ^{196}Pt substitution (m/z 458.10555) and a $^{195}\text{Pt}^{13}\text{C}$ substitution (m/z
302 458.10882). This isotope study also confirmed the presence of platinum. This IFS confirmed
303 the molecular formula $\text{C}_{11}\text{H}_{23}\text{N}_3\text{S}_2\text{Pt}$ that does not fit with the oxaliplatin-methionine compound
304 previously reported to be identified in rat tumoral kidney tissue and in tumoral peritoneum
305 tissue [15]. Consequently, we could confirm the presence of oxaliplatin-methionine in tumoral
306 peritoneum tissue but not in the ovary tissue sections from the treated women, implying that
307 the Pt-drug diffuses to the ovary with the metabolization of another compound. These results

308 were confirmed by measurements on tissue from six patients. Platinum metabolite distribution
 309 in the 6 ovary sections before treatment and 6 ovary sections after treatment is shown in **Figure**
 310 **S4**. MSI based on FTICR mass analyzer showed here its great potential for unambiguous
 311 metabolite identification.



313 **Figure 3:** MALDI FTICR MS spectra of (a) Experimental and theoretical isotopic
 314 distribution patterns of $C_{10}H_{19}N_3O_5Pt$, $C_{11}H_{15}N_7O_7Pt$ and $C_{11}H_{23}N_3S_2Pt$ for M (^{194}Pt), M+1
 315 (^{195}Pt) and M+2 (^{196}Pt) isotopes (b) expanded m/z window of the experimental monoisotopic
 316 peak at m/z 457,10551 and the three theoretical monoisotopic peaks of $C_{10}H_{19}N_3O_5Pt$,
 317 $C_{11}H_{15}N_7O_7Pt$ and $C_{11}H_{23}N_3S_2Pt$.
 318



319

320 **Figure 4:** MALDI FTICR MS spectra of observed and theoretical isotopic fine structure of

321 $C_{10}H_{19}N_3O_5Pt$, $C_{11}H_{15}N_7OPt$ and $C_{11}H_{23}N_3S_2Pt$ for $M+2$ (^{196}Pt).

322 Although the confirmation of a developed structural formula could not be performed by tandem
 323 MS/MS mass spectrometry due to the very low signal intensity of the Pt compound, hypothesis
 324 could be made from the literature.

325 Previously reported platinum and sulfur containing complexes were described, involving the
 326 formation of a complex with a diethyldithiocarbamate (DDTC) molecule [14]. According to the
 327 determined molecular formula, we can hypothesize that the DACH (diaminocyclohexane)-Pt
 328 group has complexed with a DDTC molecule to form the complex (DACH) (DDTC) Pt(II)
 329 **(Figure S5)**. Even if DDTC acts as rescue agent, recommended by the World Health
 330 Organization, to detoxify platinum after chemotherapy treatment, to our knowledge, this
 331 molecule was not administrated during HIPEC [35-37]. In other way, the presence of DDTC
 332 could come from its use as a pesticide. Some studies revealed the accumulation and the effect

333 of this kind of carbamates on the male and female reproductive organs [38, 39]. Nevertheless,
334 its presence as a complex into the ovaries after (during) an HIPEC procedure would need to be
335 confirmed by complementary studies to assign the detected Pt-containing compound. Further
336 investigations have to be carried out to formally identify the structure of this Pt metabolite
337 ($C_{11}H_{23}N_3S_2Pt$ molecular formula) and explain its origin.

338 Therefore, this study showed that oxaliplatin still diffuses to the non-diseased ovarian tissues
339 despite HIPEC optimization. It highlighted the great interest of FTICR-MSI to help in
340 optimizing treatment protocols by understanding drug metabolism and fate in living cells.

341 **4. Conclusion**

342 This study showed that detailed characterization of biological samples by MALDI imaging can
343 require instrumentation with high mass resolving power and mass accuracy. This work shows
344 the potential of MALDI imaging coupled to FTICR analyzer for the non-targeted study of Pt-
345 drug in ovary tissue after HIPEC treatment of the peritoneal metastasis. The high mass accuracy
346 and resolving power allowed to distinguish the presence of two Pt-isobars. Both the oxaliplatin-
347 methionine complex and the new isobaric complex were detected in the tumoral peritoneal
348 tissue whereas the new complex only was localized at the periphery of the ovary. The use of
349 lower resolution mass analyzer such as TOF, would not allow the separation of these isobaric
350 species, leading to wrong assignments. The molecular formula of this unknown compound was
351 then determined thanks to high mass accuracy and high resolution enabling an isotopic fine
352 structure study. To our knowledge, this study showed for the first time the powerful of MALDI
353 FTICR MS for imaging and molecular elucidation of metallodrug species in treated biological
354 tissues. Besides, it showed, for the first time, the presence of an unknown Pt-species in ovary
355 tissues after HIPEC treatment, which open new perspective in the study of Pt-metallodrug
356 metabolization understanding.

357

358 **Acknowledgments**

359 This work has been partially supported by University of Rouen Normandy, INSA Rouen
360 Normandy, the Centre National de la Recherche Scientifique (CNRS), European Regional
361 Development Fund (ERDF), Labex SynOrg (ANR-11-LABX-0029), Carnot Institute I2C, the
362 graduate school for research XL-Chem (ANR-18-EURE-0020 XL CHEM), and by Région
363 Normandie. This work was supported the European Union's Horizon 2020 Research
364 Infrastructures program (Grant Agreement 731077). Access to a CNRS FTICR research
365 infrastructure (FR3624) is gratefully acknowledged.

366

367 **Competing interest**

368 The authors declare no competing financial interest.

369

370 **Appendix A. Supplemental information.**

371 Supplementary data associated with this article can be found in the online version at

372

373 **References**

- 374 [1] R.M. Caprioli, T.B. Farmer, J. Gite, Molecular imaging of biological samples: localization of
375 peptides and proteins using MALDI-TOF MS, *Anal. Chem.* 69 (1997) 4751-4760.
376 <https://doi.org/10.1021/ac970888i>
- 377 [2] S. Khatib-Shahidi, M. Andersson, J.L. Herman, T.A. Gillespie, R.M. Caprioli, Direct Molecular
378 Analysis of Whole-Body Animal Tissue Sections by Imaging MALDI Mass Spectrometry, *Anal. Chem.* 78
379 (2006) 6448-6456. <https://doi.org/10.1021/ac060788p>
- 380 [3] S. Schulz, M. Becker, M.R. Groseclose, S. Schadt, C. Hopf, Advanced MALDI mass spectrometry
381 imaging in pharmaceutical research and drug development, *Curr. Opin. Biotechnol.* 55 (2019) 51-59.
382 <https://doi.org/10.1016/j.copbio.2018.08.003>
- 383 [4] Y. Hsieh, J. Chen, W.A. Korfmacher, Mapping pharmaceuticals in tissues using MALDI imaging
384 mass spectrometry, *J. Pharmacol. Toxicol. Methods* 55(2) (2007) 193-200.
385 <https://doi.org/10.1016/j.vascn.2006.06.004>
- 386 [5] D.J. Hare, E.J. New, M.D. de Jonge, G. McColl, Imaging metals in biology: balancing sensitivity,
387 selectivity and spatial resolution, *Chem. Soc. Rev.* 44(17) (2015) 5941-58.
388 <https://doi.org/10.1039/C5CS00055F>
- 389 [6] A. Ly, A. Buck, B. Balluff, N. Sun, K. Gorzolka, A. Feuchtinger, K. P. Janssen, P. J. K. Kuppen, P. J. K.
390 Kuppen, C. J. H. Van de Velde, G. Weirich, F. Erlmeier, R. Langer, M. Aubele, H. Zitzelsberger, L.
391 McDonnell, M. Aichler, A. Walch, High-mass-resolution MALDI mass spectrometry imaging of
392 metabolites from formalin-fixed paraffin-embedded tissue, *Nat. Protoc.* 11 (2016) 1428-1443.
393 <https://doi.org/10.1038/nprot.2016.081>

394 [7] J. Stauber, R. Lemaire, J. Franck, D. Bonnel, D. Croix, R. Day, M. Wisztorski, I. Fournier, M. Salzet,
395 MALDI Imaging of Formalin-Fixed Paraffin-Embedded Tissues: Application to Model Animals of
396 Parkinson Disease for Biomarker Hunting, *J. Proteome Res.* 7(3) (2008) 969-978.
397 <https://doi.org/10.1021/pr070464x>

398 [8] T. Makovec, Cisplatin and beyond: molecular mechanisms of action and drug resistance
399 development in cancer chemotherapy, *Radiol. Oncol.* 53(2) (2019) 148-158.
400 <https://doi.org/10.2478/raon-2019-0018>

401 [9] S.J. Park, W. Ye, R. Xiao, C. Silvin, M. Padget, J.W. Hodge, C. Van Waes, N.C. Schmitt, Cisplatin and
402 oxaliplatin induce similar immunogenic changes in preclinical models of head and neck cancer, *Oral*
403 *oncol.* 95 (2019) 127-135. <https://doi.org/10.1016/j.oraloncology.2019.06.016>

404 [10] S. Theiner, A. Schoeberl, A. Schweikert, B.K. Keppler, G. Koellensperger, Mass spectrometry
405 techniques for imaging and detection of metallodrugs, *Curr Opin Chem Biol* 61 (2021) 123-134.
406 <https://doi.org/10.1016/j.cbpa.2020.12.005>

407 [11] R.F.S. Lee, S. Theiner, A. Meibom, G. Koellensperger, B.K. Keppler, P.J. Dyson, Application of
408 imaging mass spectrometry approaches to facilitate metal-based anticancer drug research,
409 *Metallomics* 9(4) (2017) 365-381. <https://doi.org/10.1039/c6mt00231e>

410 [12] H.U. Holtkamp, C.G. Hartinger, Advanced metallomics methods in anticancer metallodrug mode
411 of action studies, *TrAC* 104 (2018) 110-117. <https://doi.org/10.1016/j.trac.2017.09.023>

412 [13] M. Larroque, S. Mounicou, O. Sgarbura, C. Arnaudguilhem, L. Rebel, C. Leaha, P.-A. Faye, C.
413 Enjalbal, F. Quénet, B. Bouyssiére, S. Carrere, Study of oxaliplatin penetration into ovaries of patients
414 treated with hyperthermic intraperitoneal chemotherapy (HIPEC) for peritoneal metastases of
415 colorectal and appendiceal origin using mass spectrometry imaging, *Pleura Peritoneum* 6 (2021) 67-
416 74. <https://doi.org/10.1515/pp-2020-0149>

417 [14] X. Liu, A.B. Hummon, Chemical Imaging of Platinum-Based Drugs and their Metabolites, *Sci. Rep.*
418 6 (2016) 38507. <https://doi.org/10.1038/srep38507>

419 [15] A. Bouslimani, N. Bec, M. Glueckmann, C. Hirtz, C. Larroque, Matrix-assisted laser
420 desorption/ionization imaging mass spectrometry of oxaliplatin derivatives in heated intraoperative
421 chemotherapy (HIPEC)-like treated rat kidney, *RCM* 24(4) (2010) 415-21.
422 <https://doi.org/10.1002/rcm.4408>

423 [16] A.B. J. Bianga, N. Bec, F. Quenet, S. Mounicou, B. Bouyssiére, R. Lobinski, J. Szpunar, C. Larroque,
424 Complementary of MALDI and LA ICP mass spectrometry for platinum anticancer imaging in human
425 tumor, *Metallomics* 6 (2014) 1382-1386. <https://doi.org/10.1039/c4mt00131a>

426 [17] M. Aichler, A. Walch, MALDI Imaging mass spectrometry: current frontiers and perspectives in
427 pathology research and practice, *Lab. Invest.* 95(4) (2015) 422-31.
428 <https://doi.org/10.1038/labinvest.2014.156>

429 [18] Y. Fujimura, D. Miura, MALDI Mass Spectrometry Imaging for Visualizing In Situ Metabolism of
430 Endogenous Metabolites and Dietary Phytochemicals, *Metabolites* 4(2) (2014) 319-46.
431 <https://doi.org/10.3390/metabo4020319>

432 [19] D.S. Cornett, S.L. Frappier, R.M. Caprioli, MALDI-FTICR imaging mass spectrometry of drugs and
433 metabolites in tissue, *Anal. Chem* 80(14) (2008) 5648-53. <https://doi.org/10.1021/ac800617s>

434 [20] J. Ferey, F. Marguet, A. Laquerriere, S. Marret, I. Schmitz-Afonso, S. Bekri, C. Afonso, A. Tebani, A
435 new optimization strategy for MALDI FTICR MS tissue analysis for untargeted metabolomics using
436 experimental design and data modeling, *Anal. Bioanal. Chem.* 411(17) (2019) 3891-3903.
437 <https://doi.org/10.1007/s00216-019-01863-6>

438 [21] A. G. Marshall, C. L. Hendrickson, G.S. Jackson, Fourier Transform Ion Cyclotron Resonance Mass
439 Spectrometry: a primer, *Mass Spectrom. Rev.* 17 (1998) 1-35. [https://doi.org/10.1002/\(SICI\)1098-
440 2787\(1998\)17:1<1::AID-MAS1>3.0.CO;2-K](https://doi.org/10.1002/(SICI)1098-2787(1998)17:1<1::AID-MAS1>3.0.CO;2-K)

441 [22] F. Xian, C.L. Hendrickson, A.G. Marshall, High resolution mass spectrometry, *Anal. Chem.* 84(2)
442 (2012) 708-19. <https://doi.org/10.1021/ac203191t>

443 [23] Y.H. Kim, Y. Fujimura, T. Hagihara, M. Sasaki, D. Yukihiro, T. Nagao, D. Miura, S. Yamaguchi, K.
444 Saito, H. Tanaka, H. Wariishi, K. Yamada, H. Tachibana, In situ label-free imaging for visualizing the

445 biotransformation of a bioactive polyphenol, *Sci. Rep.* 3 (2013) 2805.
446 <https://doi.org/10.1038/srep02805>
447 [24] T. Nagao, D. Yukihiro, Y. Fujimura, K. Saito, K. Takahashi, D. Miura, H. Wariishi, Power of isotopic
448 fine structure for unambiguous determination of metabolite elemental compositions: in silico
449 evaluation and metabolomic application, *Anal. Chim. Acta.* 813 (2014) 70-6.
450 <https://doi.org/10.1016/j.aca.2014.01.032>
451 [25] M. Kihara, Y. Matsuo-Tezuka, M. Noguchi-Sasaki, K. Yorozu, M. Kurasawa, Y. Shimonaka, M.
452 Hirata, Visualization of (57)Fe-Labeled Heme Isotopic Fine Structure and Localization of Regions of
453 Erythroblast Maturation in Mouse Spleen by MALDI FTICR-MS Imaging, *J. Am. Soc. Mass. Spectrom.*
454 28(11) (2017) 2469-2475. <https://doi.org/10.1007/s13361-017-1768-y>
455 [26] L. Xu, X. Li, X. Wang, A. Song, F. Han, A feasible strategy based on isotopic fine structures to
456 enhance the reliability of metabolite identification by Fourier transform ion cyclotron resonance
457 mass spectrometry, *Rapid comm in mass spectrom* 34(1) (2020) 1-8.
458 <https://doi.org/10.1002/rcm.8560>
459 [27] S.A. Stopka, L.Z. Samarah, J.B. Shaw, A.V. Liyu, D. Velickovic, B.J. Agtuca, C. Kukolj, D.W.
460 Koppenaal, G. Stacey, L. Pasa-Tolic, C.R. Anderton, A. Vertes, Ambient Metabolic Profiling and
461 Imaging of Biological Samples with Ultrahigh Molecular Resolution Using Laser Ablation Electrospray
462 Ionization 21 Tesla FTICR Mass Spectrometry, *Anal. Chem.* 91(8) (2019) 5028-5035.
463 <https://doi.org/10.1021/acs.analchem.8b05084>
464 [28] D. Elias, B. Raynard, F. Farkhondeh, D. Goéré, D. Rouquie, R. Ciuchendea, M. Pocard, M. Ducreux,
465 Peritoneal carcinomatosis of colorectal origin, *Gastroen Clin Biol* 30(10) (2006) 1200-1204.
466 <https://doi.org/10.1097/O1.sla.0000197702.46394.16>
467 [29] A. Burnett, M. Lecompte, N. Trabulsi, P. Dubé, M. Gervais, B. Trilling, A. Cloutier, L. Sideris,
468 Peritoneal carcinomatosis index predicts survival in colorectal patients undergoing HIPEC using
469 oxaliplatin: a retrospective single-arm cohort study, *World J Surg Oncol* 17(1) (2019).
470 <https://doi.org/10.1186/s12957-019-1618-4>
471 [30] Y.L. Klaver, T. Hendriks, R.M. Lomme, H.J. Rutten, R.P. Bleichrodt, I.H. de Hingh, Intraoperative
472 versus early postoperative intraperitoneal chemotherapy after cytoreduction for colorectal
473 peritoneal carcinomatosis: an experimental study, *Ann. Surg. Oncol.* 19 (2012) S475-82.
474 <https://doi.org/10.1245/s10434-011-1984-9>
475 [31] J. Esquivel, D. Elias, D. Baratti, S. Kusamura, M. Deraco, Consensus statement on the loco
476 regional treatment of colorectal cancer with peritoneal dissemination, *J. Surg. Oncol.* 98(4) (2008)
477 263-7. <https://doi.org/10.1002/jso.21053>
478 [32] T.C. Chua, B.J. Moran, P.H. Sugarbaker, E.A. Levine, O. Glehen, F.N. Gilly, D. Baratti, M. Deraco,
479 D. Elias, A. Sardi, W. Liauw, T.D. Yan, P. Barrios, A. Gomez Portilla, I.H. de Hingh, W.P. Ceelen, J.O.
480 Pelz, P. Piso, S. Gonzalez-Moreno, K. Van Der Speeten, D.L. Morris, Early- and long-term outcome
481 data of patients with pseudomyxoma peritonei from appendiceal origin treated by a strategy of
482 cytoreductive surgery and hyperthermic intraperitoneal chemotherapy, *Journal of clinical oncology :*
483 *official journal of the American Society of Clinical Oncology* 30(20) (2012) 2449-56.
484 <https://doi.org/10.1200/JCO.2011.39.7166>
485 [33] F. Quénet, D. Elias, L. Roca, D. Goéré, L. Ghouti, M. Pocard, O. Facy, C. Arvieux, G. Lorimier, D.
486 Pezet, F. Marchal, V. Loi, P. Meeus, B. Juzyna, H. de Forges, J. Paineau, O. Glehen, P. Mariani, C.
487 Brigand, J.-M. Bereder, S. Msika, G. Portier, P. Rat, Cytoreductive surgery plus hyperthermic
488 intraperitoneal chemotherapy versus cytoreductive surgery alone for colorectal peritoneal
489 metastases (PRODIGE 7): a multicentre, randomised, open-label, phase 3 trial, *Lancet. Oncol* 22(2)
490 (2021) 256-266. [https://doi.org/10.1016/S1470-2045\(20\)30599-4](https://doi.org/10.1016/S1470-2045(20)30599-4)
491 [34] C. Violette, T. Kim, L. Shandley, R. Lee, C. Staley, J. Winer, S. Maithel, H. Hipp, J. Kawass, M.
492 Russej, Fertility after cytoreductive surgery and hyperthermic intraperitoneal chemotherapy: A call to
493 action, *J. Surg. Oncol.* 123 (2021) 1045-1049. <https://doi.org/10.1002/jso.26387>
494 [35] M.W. DeGregorio, D.R. Gandara, W.M. Holleran, E.A. Perez, C.C. King, H.G. Wold, T.J. Montine,
495 R.F. Borch, High-dose cisplatin with diethyldithiocarbamate (DDTC) rescue therapy: preliminary

496 pharmacologic observations, *Cancer Chemother Pharmacol* 23 (1989) 276-278.
497 <https://doi.org/10.1007/BF00292403>
498 [36] J. Reedijk, Why does cisplatin reach guanine-N7 with competing S-Donor ligands available in the
499 Cell?, *Chem. Rev.* 99 (1999) 2499-2510. <https://doi.org/10.1021/cr980422f>
500 [37] D. Bouvet, A. Michalowicz, S. Crauste-Manciet, D. Brossard, K. Provost, EXAFS and IR structural
501 study of platinum-based anticancer drugs' degradation by diethyl dithiocarbamate, *Inorg. Chem.* 45
502 (2006) 3393-3398. <https://doi.org/10.1021/ic051904u>
503 [38] D.T. Vengayil, J. Singh, A.L. Singh, V.K. Das, P.B. Singh, Bioaccumulation of carbamate and
504 pyrethroid insecticides in fishes of the river gomti at jaunpur during breeding season, *J. Ecophysiol.*
505 *Occup. Hlth* 11 (2011) 1-8. <https://doi.org/10.18311/jeoh/2011/2243>
506 [39] S. Soloneski, M. Gonzalez, E. Piaggio, M.A. Reigosa, M.L. Larramendy, Effect of dithiocarbamate
507 pesticide zined and its commercial formulation, azzuro III. Genotoxic evaluation on chinese hamster
508 ovary (CHO) cells, *Mutat. Res.* (2002) 201-212. [https://doi.org/10.1016/s1383-5718\(01\)00337-0](https://doi.org/10.1016/s1383-5718(01)00337-0)
509
510

511 **Figure captions**

512

513 **Figure 1:** Structural formulas of oxaliplatin and oxaliplatin-methionine.

514 **Figure 2:** MALDI FTICR imaging of ovary tissues before and after HIPEC treatment.

515 Visualization of discriminative values at m/z 456.10324, m/z 457.10551 and m/z 458.10570.

516 Spatial resolution of 120 μm .

517 **Figure 3:** MALDI FTICR MS spectra of (a) Experimental and theoretical isotopic distribution

518 patterns of $\text{C}_{10}\text{H}_{19}\text{N}_3\text{O}_5\text{Pt}$, $\text{C}_{11}\text{H}_{15}\text{N}_7\text{OPt}$ and $\text{C}_{11}\text{H}_{23}\text{N}_3\text{S}_2\text{Pt}$ for M (^{194}Pt), M+1 (^{195}Pt) and M+2

519 (^{196}Pt) isotopes (b) expanded m/z window of the experimental monoisotopic peak at m/z

520 457,10551 and the three theoretical monoisotopic peaks of $\text{C}_{10}\text{H}_{19}\text{N}_3\text{O}_5\text{Pt}$, $\text{C}_{11}\text{H}_{15}\text{N}_7\text{OPt}$ and

521 $\text{C}_{11}\text{H}_{23}\text{N}_3\text{S}_2\text{Pt}$.

522 **Figure 4:** MALDI FTICR MS spectra of observed and theoretical isotopic fine structure of

523 $\text{C}_{10}\text{H}_{19}\text{N}_3\text{O}_5\text{Pt}$, $\text{C}_{11}\text{H}_{15}\text{N}_7\text{OPt}$ and $\text{C}_{11}\text{H}_{23}\text{N}_3\text{S}_2\text{Pt}$ for M+2 (^{196}Pt).

524

525 **Table captions**

526

527 **Table 1:** Theoretical m/z values of the isotopes M (^{194}Pt), M+1 (^{195}Pt) and M+2 (^{196}Pt) for
528 oxaliplatin and oxaliplatin-methionine complex.

529 **Table 2:** Theoretical and experimental m/z values and relative abundance (%) of the isotopes
530 M (^{194}Pt), M+1 (^{195}Pt) and M+2 (^{196}Pt) for the determined raw formulas $\text{C}_{10}\text{H}_{19}\text{N}_3\text{O}_5\text{Pt}$,
531 $\text{C}_{11}\text{H}_{15}\text{N}_7\text{OPt}$ and $\text{C}_{11}\text{H}_{23}\text{N}_3\text{S}_2\text{Pt}$ and the unknown compound with associated m/z errors.

532