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1 **Matrix free laser desorption ionization mass spectrometry as an efficient tool for the**
2 **rapid detection of opiates from crude extracts of *Papaver somniferum***

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7 **ABSTRACT**

8 Having a long history of traditional medicinal applications, *Papaver somniferum* is also
9 known as a source of various pharmacologically highly active opiates. Consequently, their
10 detection from plant extracts is an important analytical task and generally addressed by
11 methods of GC and LC-MS. However, opiates do also show structural similarities to matrix
12 molecules used in matrix assisted laser desorption ionization and may therefore ionize upon
13 simple laser irradiation. Following this analytical approach, the present work thoroughly
14 evaluated the direct detection of opiates by matrix free laser desorption ionization (LDI) from
15 crude extracts of *P. somniferum*. The method facilitated the identification of ten reported
16 opiates by their molecular formulae without any chromatographic pre-purification. Moreover
17 a principal compound analysis based on LDI-MS data permitted the correct grouping of all
18 extracts according to their inherent chemistry. Overall these results highlight the promising
19 potential of LDI-MS for the swift detection of opiates from complex mixtures.

20 **KEYWORDS:** opiates detection, *Papaver somniferum*, LDI-MS, LC-MS, PCA

21

22 INTRODUCTION:

23 *Papaver somniferum* L. (Papaveraceae), commonly known as opium poppy, is a globally
24 cultivated medicinal plant and important source of many pharmacologically active alkaloids
25 such as codeine, thebaine, noscapine papaverine and morphine.¹ Exhibiting strong effects on
26 the central nervous system, some of these opiates are used as analgesic, antitussive and
27 antispasmodic medicines.²⁻⁴ Contrary to capsules, seeds of *P. somniferum* do not contain any
28 latex or notable amounts of alkaloids and are a popular food commodity in Central and
29 Eastern Europe. However, opiate contamination of seeds caused by insect damage and bad
30 harvesting practices is a major concern recently highlighted by European food safety
31 authorities.¹ Consequently, the rapid detection and chemical identification of opiates from
32 complex plant matrices is an important issue for food safety and generally addressed by
33 methods of HPLC-DAD, LC-MS or GC-MS.⁵⁻⁹ Any of these methods represent powerful
34 analytical tools however, very little is known about opium alkaloids in the context of laser
35 desorption ionization (LDI). Some of the very few reports describe isoquinolines like 3-
36 aminoquinoline and 1-hydroxyisochinolin as working matrices in matrix assisted laser
37 desorption ionization (MALDI).¹⁰⁻¹¹ As many opiates actually share close structural
38 similarities with these compounds it is reasonable to assume that they should ionize upon
39 simple laser irradiation and without matrix support. With this in mind, the present study
40 thoroughly evaluated the potential of LDI-MS for the direct detection of morphinan and
41 benzyloisoquinoline alkaloids from crude methanolic capsule extracts of *P. somniferum* (I).
42 Moreover results were systematically compared with concurrently performed LC-MS
43 analyses (II). Eventually, a principal component analysis (PCA) verifying whether sample
44 material could be statistically differentiated based on LDI-MS data was conducted (III).

45

46 MATERIAL AND METHODS:

47 Plant Material and Extraction.

48 Voucher samples from five different batches of pulverized capsules of *P. somniferum* were
49 collected at a confidential breeding farm and provided by a private institute that wants to
50 remain anonymous. Reference specimens of each sample are kept at the institute of SONAS.
51 Approximately forty milligrams of powdered plant material were mixed with 1550 μ L
52 analytical grade MeOH (Carlo Erba Reagents, Val de Reuil, France) and sonicated for 20 min.
53 The suspension was then centrifuged for 10 min at 14800 rpm and 800 μ L of the supernatant
54 were recovered. Next 800 μ L of fresh MeOH were added to the plant material and the
55 extraction was repeated. Combined supernatants were dried under nitrogen yielding 4-6 mg
56 dried extract per sample. Detailed information indicating exact weights of sample and plant
57 material is given in Table S1 (Supplementary Information).

58 **Laser Desorption Ionization Mass Spectrometry.** Low-resolution (LR), LDI-MS
59 experiments were carried out on a Bruker Biflex III time of flight (TOF) mass spectrometer
60 (Bruker Daltonik, Bremen, Germany), equipped with a 337 nm pulsed nitrogen laser (model
61 VSL-337i, Laser Sciences Inc., Boston, MA, USA). Samples were analyzed in the reflectron
62 positive mode, within a mass range of 40-2000 Da. Acceleration voltage was set to 19 kV,
63 pulse ion extraction was 200 ns, and laser frequency was 5 Hz. Laser energy was individually
64 adapted to sample requirements and in the range of 80-85%. Stock solutions of extracts poppy
65 were prepared in MeOH at 20 mg/ml before being diluted to 10 mg/mL with a solution
66 comprising MeOH and formic acid (Acros organics, Geel, Belgium) (99:1, v/v). Finally, 0.5
67 μ L were deposited in quintuplicates on a MALDI plate (ground steel, MTP 384 Bruker
68 Daltonics, Bremen, Germany). Data were acquired in manual and automatic mode. The latter
69 facilitated an unbiased operator independent data acquisition, which was essential for
70 consecutively performed PCA. In automatic mode, 300 single spectra from 30 random

71 locations (10 laser shots each) were acquired and summed for each sample deposition spot.
72 Spectra were processed by FlexAnalysis 2.0 (Bruker Daltonik, Bremen, Germany).

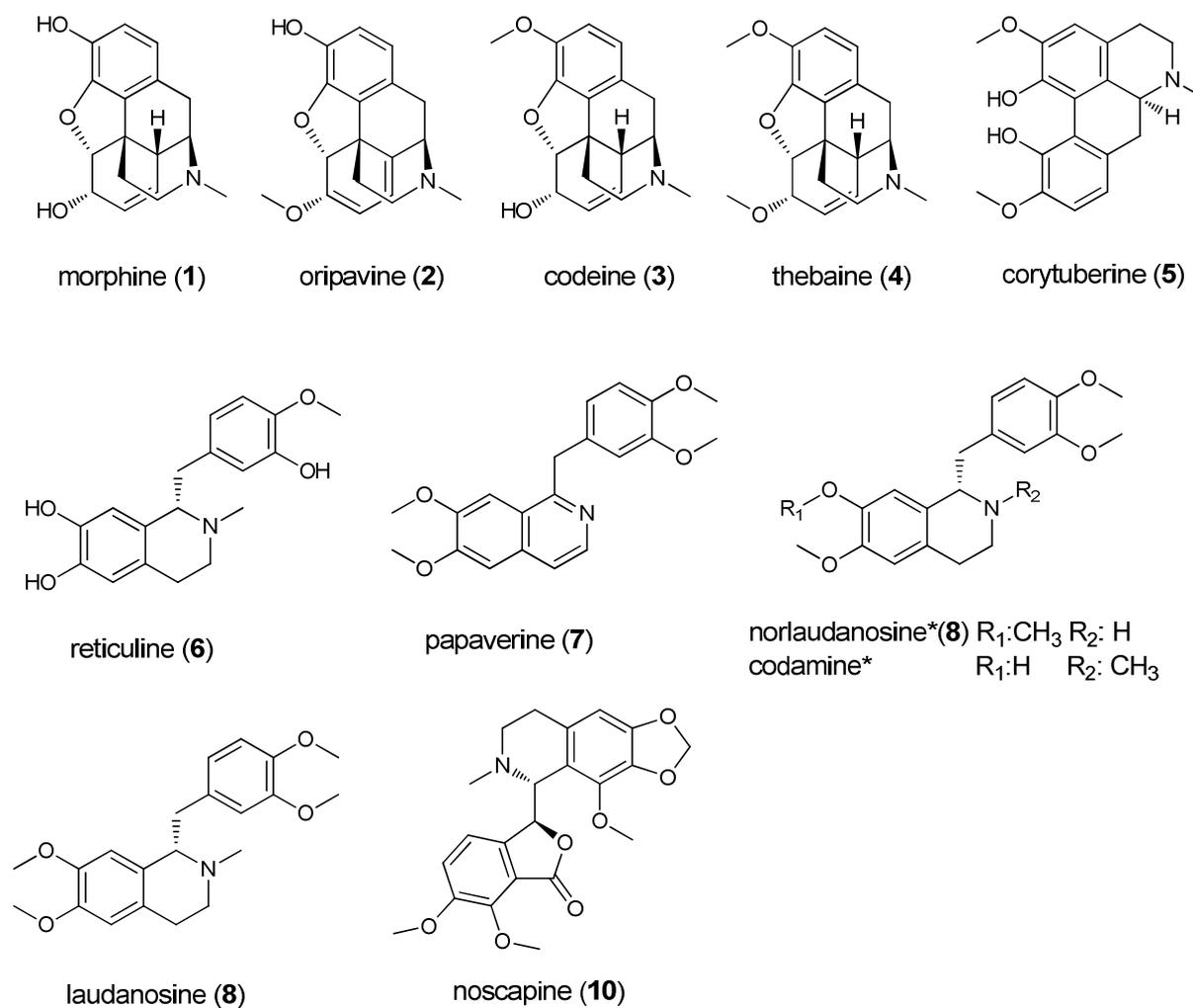
73 High-resolution (HR) experiments were performed on a Spiral-TOFTM MALDI TOF/TOF
74 mass spectrometer (Jeol LTd., Tokyo, Japan) equipped with a pulsed laser operating at
75 349 nm. Experiments were performed in the positive spiral mode within mass range of
76 100-1000 Da. Acceleration voltage was set to 20 kV, laser frequency was 250 Hz and laser
77 power was 59%. Signals exhibiting a signal to noise ratio (S/N) of less than ten were
78 considered not detected. Exported spectra were processed by mMass 5.5.0 a freeware by
79 Martin Strohm.¹²

80 **HPLC-ESI-MS.** Experiments were performed on a Trap Esquire 3000+ instrument (Bruker,
81 Bremen, Germany) equipped with ESI/APCI Ion Trap as well as a UV-VIS wavelength
82 detector. Instrument parameters and experimental conditions were as follows: Collision gas,
83 He; collision energy amplitude, 1.0 V; nebulizer and drying gas, N₂, 7 L/min; pressure of
84 nebulizer gas, 20 psi; dry temperature, 300 °C; flow rate, 0.5 mL/min; solvent split ratio 1:9;
85 scan range, m/z 20–1000. In addition absorption chromatograms were acquired at 230 and
86 280 nm. Chromatographic separation was performed according to a previously published
87 protocol with some modifications.⁷ Experiments were conducted on a Gemini C₁₈ column
88 (3µm, 110 Å, 150 x 4.6 mm, Phenomenex, Le Pecq, France) protected by a pre-column
89 security guard cartridge C₁₈ (4 x 3 mm i.d.) from the same company. The mobile phase
90 contained a solution of ammonium bicarbonate (Servilab, Le Mans, France) at 20 mM
91 adjusted to pH 9.0 by ammonium hydroxide (Acros organics, Geel, Belgium) (solvent A), as
92 well as LC-MS grade MeOH (Honeywell, Seelze, Germany) comprising 5% of solvent A
93 (solvent B). Gradient conditions were as follows: 40% B (0-1 min), 40-100% B (1-30 min),
94 100% B (30-42 min). Signals exhibiting a S/N<10 in MS, as well as less than 0.03 mAU in
95 UV were considered not detected.

96 **Data Processing and Principal Component Analysis.** Data from LR LDI-MS experiments
97 were processed by FlexAnalysis 2.0 (Bruker Daltonik, Bremen, Germany) and mMass 5.5.0
98 (Martin Strohalm, Czech Republic)¹². Principal component analysis (PCA) was performed on
99 Origin Pro (OriginLab Cooperation, Northampton, MA, USA), using the Principal
100 Component Analysis for Spectroscopy software extension. Before conducting PCA, all LDI-
101 MS spectra were normalized to the base peak. A covariance analysis was performed and the
102 number of extracted components was two.

103 **RESULTS AND DISCUSSION:**

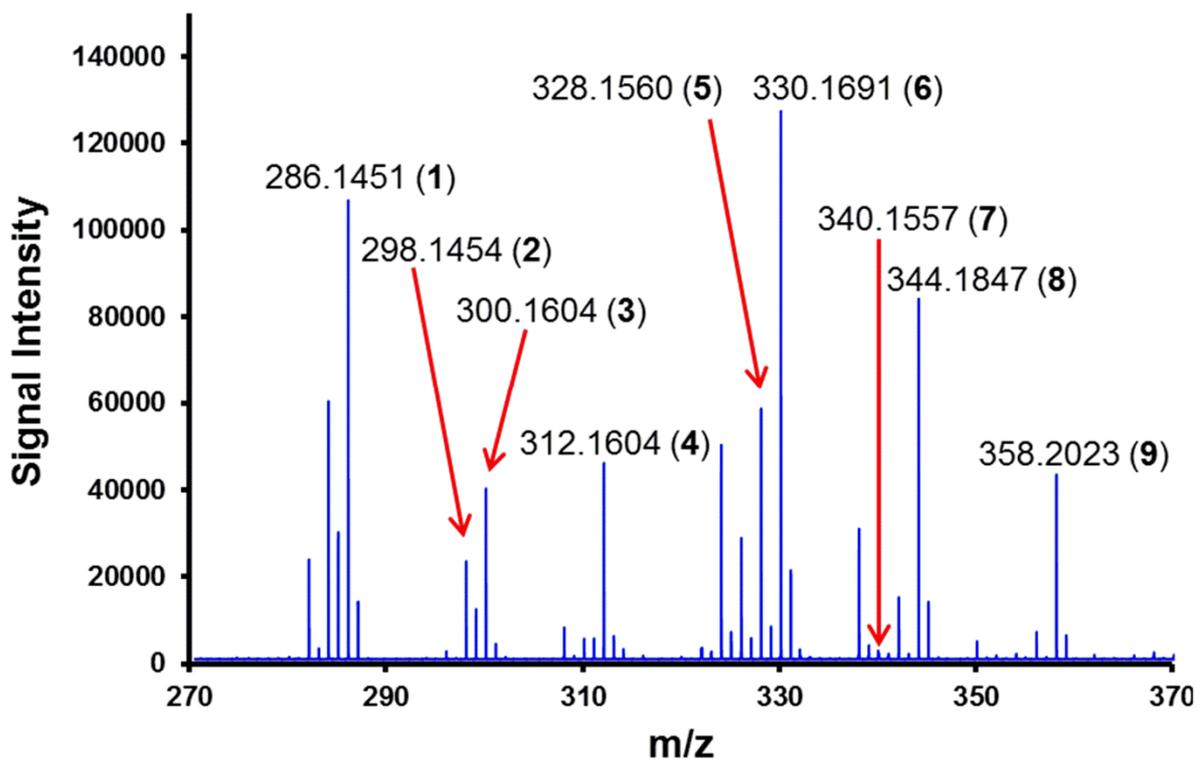
104 Preceding the current work, the concept of direct LDI-MS was successfully applied for the
105 analysis of complex mixtures of phenolic compounds.¹³ Following the same methodological
106 approach, poppy extracts were first analyzed by LR LDI-MS (Biflex III, Bruker). These
107 experiments suggested the presence of ten reported opiates, namely morphine (**1**), oripavine
108 (**2**), codeine (**3**), thebaine (**4**), corytuberine (**5**), reticuline (**6**) papaverine (**7**), norlaudanosine
109 (**8**) and/or its constitutional isomer codamine, as well as laudanosine (**9**) and noscapine (**10**)
110 (Figure 1 and 2).^{1, 14-15} Their molecular formulae were then confirmed by HR-LDI-MS
111 (Table 1). It should be noted that norlaudanosine and codamine are both reported constituents
112 of *P. somniferum*,¹⁴ but, as constitutional isomers cannot be distinguished by their quasi-
113 molecular ion signals. Overall results from LDI-MS revealed a considerable chemical
114 diversity within the analyzed samples, suggesting that the method may provide a valuable tool
115 for discriminative analyses of complex mixtures of alkaloids. This aspect will be discussed in
116 detail later on in the manuscript.



117

118 **Figure 1.** Structures of opiates that have been confirmed by their molecular formulae. Both
 119 norlaudanosine and codamine ($C_{20}H_{25}NO_4$) are reported constituents of *P. somniferum*,¹⁴ but as
 120 constitutional isomers cannot be distinguished by their quasi-molecular ions.

121



122

123 **Figure 2.** High resolution LDI-MS spectrum obtained from the crude methanolic extract of
124 sample 3. The following alkaloids were detected and confirmed by their molecular formulae:
125 Morphine (1), oripavine (2), codeine (3), thebaine (4), corytuberine (5) reticuline (6),
126 papaverine (7), codamine and/or norlaudanosine* (8) and laudanosine (9). Any compound
127 was detected by their quasi-molecular ions $[M+H]^+$. A comprehensive summary of all
128 confirmed alkaloids in all samples is provided in Table 1. Further HR spectra of all extracts
129 are provided in the Supporting Information section (Figure S5 A-E).

130

131 **Table 1.** High resolution LDI-MS performed on samples 1-5

name	molecular	calculated	observed mass	mass error	mass error	data from	Opiates detected in sample				
	formula	mass [M+H] ⁺	[M+H] ⁺	(Da)	(ppm)	sample	1	2	3	4	5
morphine (1)	C ₁₇ H ₁₉ NO ₃	286.1443	286.1437	-0.0006	-2.1	1	✓	✓	✓	✓	✓
oripavine (2)	C ₁₈ H ₁₉ NO ₃	298.1443	298.1435	-0.0008	-2.7	1	✓	✓	✓	✓	✓
codeine (3)	C ₁₈ H ₂₁ NO ₃	300.1600	300.1596	-0.0004	-1.3	1	✓	✓	✓	✓	✓
thebaine (4)	C ₁₉ H ₂₁ NO ₃	312.1600	312.1590	-0.0010	-3.2	1	✓	✓	✓	✓	✓
corytuberine (5)	C ₁₉ H ₂₁ NO ₄	328.1549	328.1536	-0.0013	-4.0	1	✓	✓	✓	✓	✓
reticuline (6)	C ₁₉ H ₂₃ NO ₄	330.1705	330.1691	0.0014	-4.2	3	✓	✓	✓	✓	✓
papaverine (7)	C ₂₀ H ₂₁ NO ₄	340.1548	340.1550	0.0002	0.6	1	✓	✓	✓	✓	✓
norlaudanosine*(8) codamine*	C ₂₀ H ₂₅ NO ₄	344.1862	344.1847	-0.0015	-4.4	3	✓	✓	✓	✓	✓
laudanosine (9)	C ₂₁ H ₂₇ NO ₄	358.2018	358.2001	-0.0017	-4.7	3	✓	n.d.	✓	✓	✓
noscipine (10)	C ₂₂ H ₂₃ NO ₇	414.1552	414.1536	-0.0016	-3.9	1	✓	✓	n.d.	n.d.	n.d.

132 ✓ detected, n.d. not detected (S/N<10). *Constitutional isomers reported for *P. somniferum*.¹⁴

133

134 **Table 2.** Comparison of results from LC-UV (230 nm), LC-MS and HR-LDI-MS experiments

compound	sample 1			sample 2			sample 3			sample 4			sample 5		
	LC-UV	LC-MS	LDI-MS												
morphine (1)	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
oripavine (2)	✓	✓	✓	✓	✓	✓	n.d.	✓	✓	✓	✓	✓	✓	✓	✓
codeine (3)	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
thebaine (4)	co	✓	✓												
corytuberine (5)	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	n.d.	✓	✓
reticuline (6)	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
papaverine (7)	co	✓	✓	co	✓	✓	co	✓	✓	co	n.d.	✓	co	n.d.	✓
norlaudanosine*(8) codamine*	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
laudanosine (9)	✓	✓	✓	n.d.	✓	n.d.	✓	✓	✓	✓	✓	✓	✓	✓	✓
noscapine (10)	✓	✓	✓	✓	✓	✓	✓	✓	n.d.	✓	✓	n.d.	n.d.	n.d.	n.d.

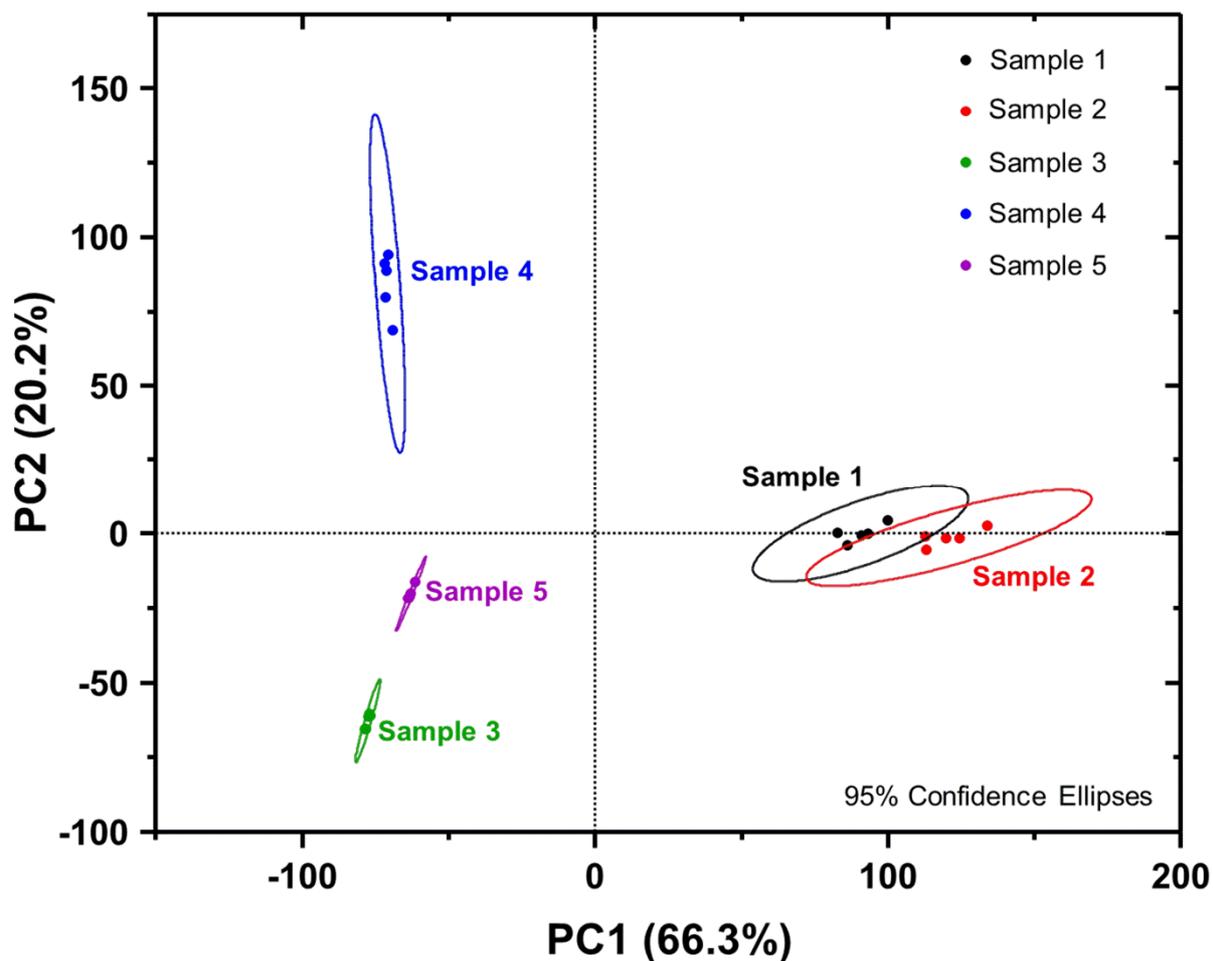
135 Except for noscapine (10) (sample 3 and 4) and laudanosine (9) (sample 2), HR-LDI-MS detected any of the compounds observed by LC-UV-
136 MS. On the other hand, papaverine (7) was exclusively detected by LDI-MS in sample 4 and 5. A comprehensive summary of all LC-UV-MS
137 chromatograms is given in the Supporting Information.

138 With regards to common laboratory practices, the analysis of opiates is mostly performed by
139 methods such as GC-MS, HPLC-UV and LC-UV-MS.⁵⁻⁹ One major advantage of these
140 hyphenated methods is, that complex mixtures undergo chromatographic separation before
141 entering UV or MS detectors. Consequently constituents are individually ionized, or, in case
142 of co-elution, as mixtures of very few compounds. With respect to LDI, the situation is
143 completely different, as all compounds concurrently compete in the ionization process. This
144 may induce so called “analyte suppression effects” (ASE). Initially described for the MALDI
145 process,¹⁶⁻¹⁸ ASE may probably occur in matrix free LDI too, as both methods share similar
146 ionization mechanisms. In order to address this issue and to compare LDI-MS to classical
147 analytical approaches, all samples were systematically analyzed by LC-UV-MS and results
148 are summarized in Table 2. Except for the minor alkaloid noscapine (sample 3, 4) and trace
149 amounts of laudanosine (**9**) in sample 2, LDI-MS detected any compound observed by LC-
150 MS. On the other hand, solely LDI-MS permitted the detection of papaverine in samples 4
151 and 5. Compared to LC-UV, both LC-MS and LDI-MS exhibited higher sensitivity and were
152 not impacted by the co-elution of papaverine and thebaine observed under given
153 chromatographic conditions. A comprehensive summary of all LC-UV-MS experiments is
154 provided in the Supporting Information (file: LC-UV-MS data).

155 With regards to sample processing, LDI-MS did not require any sample pre-conditioning or
156 method optimization steps and yielded instant results. While the automated acquisition of a
157 cumulative LDI spectrum was accomplished within fifty seconds, forty-two minutes were
158 required for an LC-UV-MS experiment. Moreover LDI-MS permitted the use of any solvent
159 for sample solubilization and yielded distinct fingerprint spectra with highly specific relative
160 signals intensity ratios for each sample. (Figure S3 and S4) These findings confirm results
161 previously described for phenolic compounds, which are discussed in detail in the original
162 publication.¹³

163 Concluding the experimental approach, a PCA based on LDI-MS data was performed. Results
164 from this experiment are summarized in Figure 3 and show the correct grouping of all
165 samples according to their chemical composition. As outlined in the Material and Methods
166 section, samples were deposited in quintuplicates, so each data point represent one cumulative
167 spectrum of 300 laser shots randomly acquired from one sample deposition spot. All data
168 points of all samples were located within the 95% ellipse of confidence, confirming the
169 robustness of the method. This is once more in line with previous observations made for
170 phenolic compounds.¹³ Data points of sample 1 and 2 are located rather closely in the PCA
171 with some overlay of their ellipses of confidence, suggesting a similar chemical composition.
172 This also confirms the visual impression observed when looking at single spectra of these
173 samples (Figure S1 and S2 Supporting information).

174



175

176 **Figure 3.** Principal component analysis of poppy samples 1-5 based on their LR LDI-MS
 177 spectra. Samples were analyzed in quintuplicates and each data point represent one
 178 cumulative spectrum of 300 laser shots that were randomly acquired from ten different
 179 locations of one sample spot.

180 For the time being very little is known about the ionization of opiates by laser desorption.
 181 Some reports describe the MALDI detection of morphine from bodily fluids, protein
 182 conjugates and pharmaceutical preparations,¹⁹⁻²¹ as well as from a mono-component
 183 solution²² and TLC surfaces.²³ Like LDI, MALDI yields instant results and may even provide
 184 increased sensitivity. On the other hand MALDI is inevitably linked to the formation of
 185 matrix ions in the low mass region, which may easily superpose analyte signals and prevent
 186 correct spectra interpretation. In addition, redundant matrix ions do severely impair statistical

187 separation by PCA. Bypassing the problem of matrix interference, surface assisted LDI
188 (SALDI) is commonly used for detecting small molecules. The method replaces the matrix by
189 nanotextured materials such as silicon or various specifically structured metal surfaces in
190 order to assist the ionization process. With respect to opiates, one report describes the use of
191 carbon nano-coatings for inducing LDI of papaverine.²⁴ However present results have shown
192 that opiates do sufficiently ionize by simple laser irradiation and without the support of any
193 nano-textured material or surface.

194 In summary the present work has discussed the inherent LDI properties of morphinan and
195 benzyloquinoline alkaloids as well as their simultaneous detection from crude extracts of
196 *P. somniferum*. Permitting the detection of ten opiates by their molecular formulae, results
197 from LDI-MS were comparable with those obtained by LC-MS. Moreover, all poppy samples
198 exhibited highly specific “fingerprint” spectra allowing their unambiguous identification as
199 well as their discrimination by PCA. Facilitating an automated and rapid qualitative analysis
200 of large sample batches, LDI-MS may provide an interesting analytical tool for industrial
201 application.

202

203 ABBREVIATIONS:

204	APCI	Atmospheric Pressure Chemical Ionization
205	ASE	Analyte Suppression Effects
206	ESI	Electro Spray Ionization
207	HPLC	High Performance Liquid Chromatography
208	LC-MS	Liquid Chromatography coupled with Mass Spectrometry
209	LDI-MS	Laser Desorption Ionization coupled with Mass Spectrometry
210	MALDI	Matrix Assisted Laser Desorption Ionisation
211	PCA	Principle Component Analysis
212	TOF	Time of Flight
213	UV	Ultra Violet (used for absorption chromatograms in combination with HPLC)

214 ACKNOWLEDGEMENT:**215 SUPPORTING INFORMATION:**

216 Low (Figures S1-S3) and high resolution (Figure S5 A-E) LDI-MS spectra, as well as a
217 detailed summary of extraction yields (Table S1) are provided for samples 1-5. Further a
218 detailed analysis of the repeatability of LDI-MS experiments is given (Figure S3, S4). A
219 complete summary of all LC-UV-MS experiments conducted on samples 1-5 is provided as
220 well. Finally, original, high resolution LDI-MS spectrum files of samples 1-5 were exported.
221 These files can be opened and analyzed with mMass (freeware).

222

223 **REFERENCES**

- 224 1. Knutsen, H.K.; Alexander, J; Barregård, L.; Bignami, M.; Brüschweiler, B.;
225 Ceccatelli, S.; Cottrill, B.; Dinovi, M.; Edler, L.; Grasl-Kraupp, B.; Hogstrand, C.;
226 Hoogenboom, L.R.; Nebbia, C.S.; Oswald, I.P.; Petersen, A.; Rose, M.; Roudot, A.C.;
227 Schwerdtle, T.; Vollmer, G.; Wallace, H.; Benford, D.; Calò, G.; Dahan, A.; Dusemund, B.;
228 Mulder, P.; Németh-Zámboriné, E.; Arcella, D.; Baert, K.; Cascio, C.; Levorato, S.; Schutte,
229 M.; Vleminckx, C. Update of the Scientific Opinion on opium alkaloids in poppy seeds.
230 *EFSA J* **2018**, *16*, e05243.
- 231 2. Weintraub, M.; Moscucci, M. Taste preference for cough syrups: a comparative study
232 of three codeine-containing medications. *Clin Ther* **1986**, *8*, 348-353.
- 233 3. Matoba, M. Use of morphine and adjuvant drugs according to the condition. *Eur J*
234 *Pain* **2001**, *5 Suppl A*, 59-62.
- 235 4. Kimoto, Y.; Kessler, R. Constantinou, C.E. Relaxation mechanisms of antispasmodics
236 papaverine and thiphenamil on the human corpus cavernosum. *J Urol* **1990**, *144*, 1497-1499.
- 237 5. Acevska, J.; Stefkov, G.; Petkovska, R.; Kulevanova, S.; Dimitrovska, A.
238 Chemometric approach for development, optimization, and validation of different
239 chromatographic methods for separation of opium alkaloids. *Anal Bioanal Chem* **2012**, *403*,
240 1117-1129.
- 241 6. Yoshimatsu, K.; Kiuchi, F.; Shimomura, K.; Makino, Y. A rapid and reliable solid-
242 phase extraction method for high-performance liquid chromatographic analysis of opium
243 alkaloids from papaver plants. *Chem Pharm Bull* **2005**, *53*, 1446-1450.
- 244 7. Sproll, C.; Perz, R.C.; Lachenmeier, D.W. Optimized LC/MS/MS analysis of
245 morphine and codeine in poppy seed and evaluation of their fate during food processing as a
246 basis for risk analysis. *J Agric Food Chem* **2006**, *54*, 5292-5298.

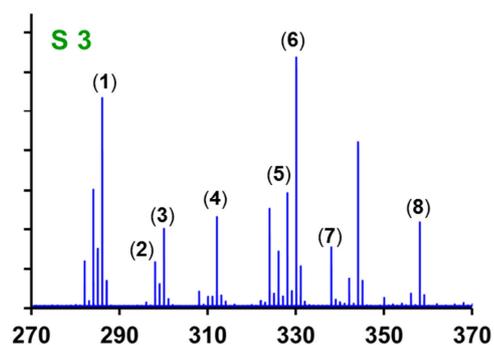
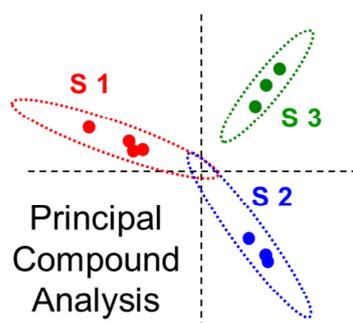
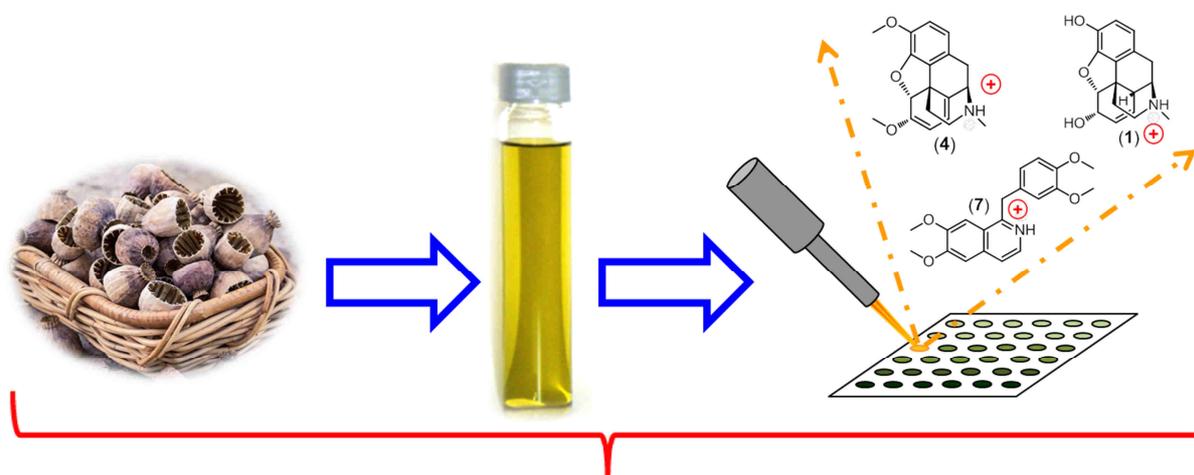
- 247 8. López, P.; Pereboom-de Fauw, D.P.K.H.; Mulder, P.P.J.; Spanjer, M.; de Stoppelaar,
248 J.; Mol, H.G.J.; de Nijs, M. Straightforward analytical method to determine opium alkaloids
249 in poppy seeds and bakery products. *Food Chem* **2018**, *242*, 443-450.
- 250 9. Liu, C.; Hua, Z.; Bai, Y. Classification of Opium by UPLC-Q-TOF Analysis of
251 Principal and Minor Alkaloids. *J Forensic Sci* **2016**, *61*, 1615-1621.
- 252 10. Rohmer, M.; Meyer, B.; Mank, M.; Stahl, B.; Bahr, U.; Karas, M. 3-Aminoquinoline
253 acting as matrix and derivatizing agent for MALDI MS analysis of oligosaccharides. *Anal*
254 *Chem* **2010**, *82*, 3719-3726.
- 255 11. Mohr, M.D.; Börnsen, K.O.; Widmer, H.M. Matrix-assisted laser
256 desorption/ionization mass spectrometry: Improved matrix for oligosaccharides. *Rapid*
257 *Commun Mass Spectrom* **1995**, *9*, 809-814.
- 258 12. Strohalm, M.; Kavan, D.; Novák, P.; Volný M.; Havlíček, V. mMass 3: a cross-
259 platform software environment for precise analysis of mass spectrometric data. *Anal Chem*
260 **2010**, *82*, 4648-4651.
- 261 13. Schinkovitz, A.; Boisard, S.; Freuze, I.; Osuga, J.; Mehlmer, N.; Brück, T.; Richomme
262 P. Matrix-free laser desorption ionization mass spectrometry as a functional tool for the
263 analysis and differentiation of complex phenolic mixtures in propolis: a new approach to
264 quality control. *Anal Bioanal Chem* **2018**, *410*, 6187–6195.
- 265 14. Dang, T.T.; Facchini, P.J. Characterization of three O-methyltransferases involved in
266 noscapine biosynthesis in opium poppy. *Plant Physiol* **2012**, *159*, 618-31.
- 267 15. Cuimei, L.; Zhendong, H.; Yanping, B. Classification of Opium by UPLC-Q-TOF
268 Analysis of Principal and Minor Alkaloids. *J Forensic Sci* **2016**, *61*, 1615-1621.
- 269 16. Lou, X.; van Dongen, J.L.J.; Milroy, L.G.; Meijer, E.W. Generation of gas-phase ions
270 from charged clusters: an important ionization step causing suppression of matrix and analyte

- 271 ions in matrix-assisted laser desorption/ionization mass spectrometry. *Rapid Commun Mass*
272 *Spectrom* **2016**, *24*, 2628-2634.
- 273 17. Knochenmuss, R. Ion formation mechanisms in UV-MALDI. *Analyst* **2006**, *131*, 996-
274 986.
- 275 18. McCombie, G.; Knochenmuss, R. Small-Molecule MALDI using the Matrix
276 Suppression Effect to reduce or eliminate matrix background interferences. *Anal Chem* **2004**,
277 *76*, 4990-4997.
- 278 19. Shoyama, Y.; Fukada, T.; Tanaka, T.; Kusai, A.; Nojima, K. Direct determination of
279 opium alkaloid-bovine serum albumin conjugate by matrix-assisted laser
280 desorption/ionization mass spectrometry. *Biol Pharm Bull* **1993**, *16*, 1051-1053.
- 281 20. Ho, Y.C.; Tseng, M.C.; Lu, Y.W.; Lin, C.C.; Chen, Y.J.; Fuh, M.R. Nanoparticle-
282 assisted MALDI-TOF MS combined with seed-layer surface preparation for quantification of
283 small molecules. *Anal Chim Acta* **2011**, *697* (1-2), 1-7.
- 284 21. Brondz, I.; Brondz, A. Supercritical Fluid Chromatography-Mass Spectrometry (SFC-
285 MS) and MALDI-TOF-MS of Heterocyclic Compounds with Trivalent and Pentavalent
286 Nitrogen in Cough Relief Medical Forms Tuxi and Cosylan. *Am J Anal Chem* **2012**, *3*, 870-
287 876.
- 288 22. Schinkovitz, A.; Kenfack, G.T.; Seraphin, D.; Levillain, E.; Dias, M.; Richomme, P.
289 Selective detection of alkaloids in MALDI-TOF: the introduction of a novel matrix molecule.
290 *Anal Bioanal Chem* **2012**, *403*, 1697-1705.
- 291 23. Kuwayama, K.; Tsujikawa, K.; Miyaguchi, H.; Kanamori, T.; Iwata, Y.T.; Inoue, H.
292 Rapid, simple, and highly sensitive analysis of drugs in biological samples using thin-layer
293 chromatography coupled with matrix-assisted laser desorption/ionization mass spectrometry.
294 *Anal Bioanal Chem* **2012**, *402*, 1257-1267.

295 24. Kosyakov, D.S.; Sorokina, E.A.; Ul'yanovskii, N.V.; Varakin, E.A.; Chukhchin, D.G.;
296 Gorbova, N.S. Carbon nanocoatings: A new approach to recording mass spectra of low-
297 molecular compounds using surface-assisted laser desorption/ionization mass spectrometry. *J*
298 *Anal Chem* **2016**, *71* (13), 1221-1227.

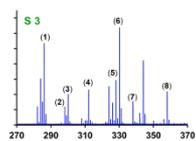
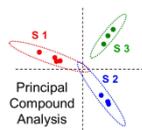
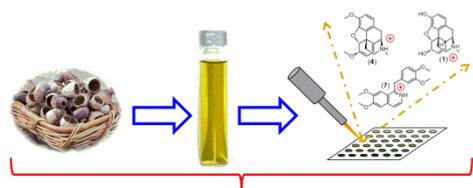
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301 **GRAPHIC FOR TABLE OF CONTENTS:**302 **Original.**

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304 **Resized according to the journal's requirements.**



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