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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.<sup>1</sup> Exhibiting strong effects on  
26 the central nervous system, some of these opiates are used as analgesic, antitussive and  
27 antispasmodic medicines.<sup>2-4</sup> 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.<sup>1</sup> 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.<sup>5-9</sup> 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).<sup>10-11</sup> 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  $\mu$ 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  $\mu$ L of the supernatant  
54 were recovered. Next 800  $\mu$ 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  $\mu$ 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).

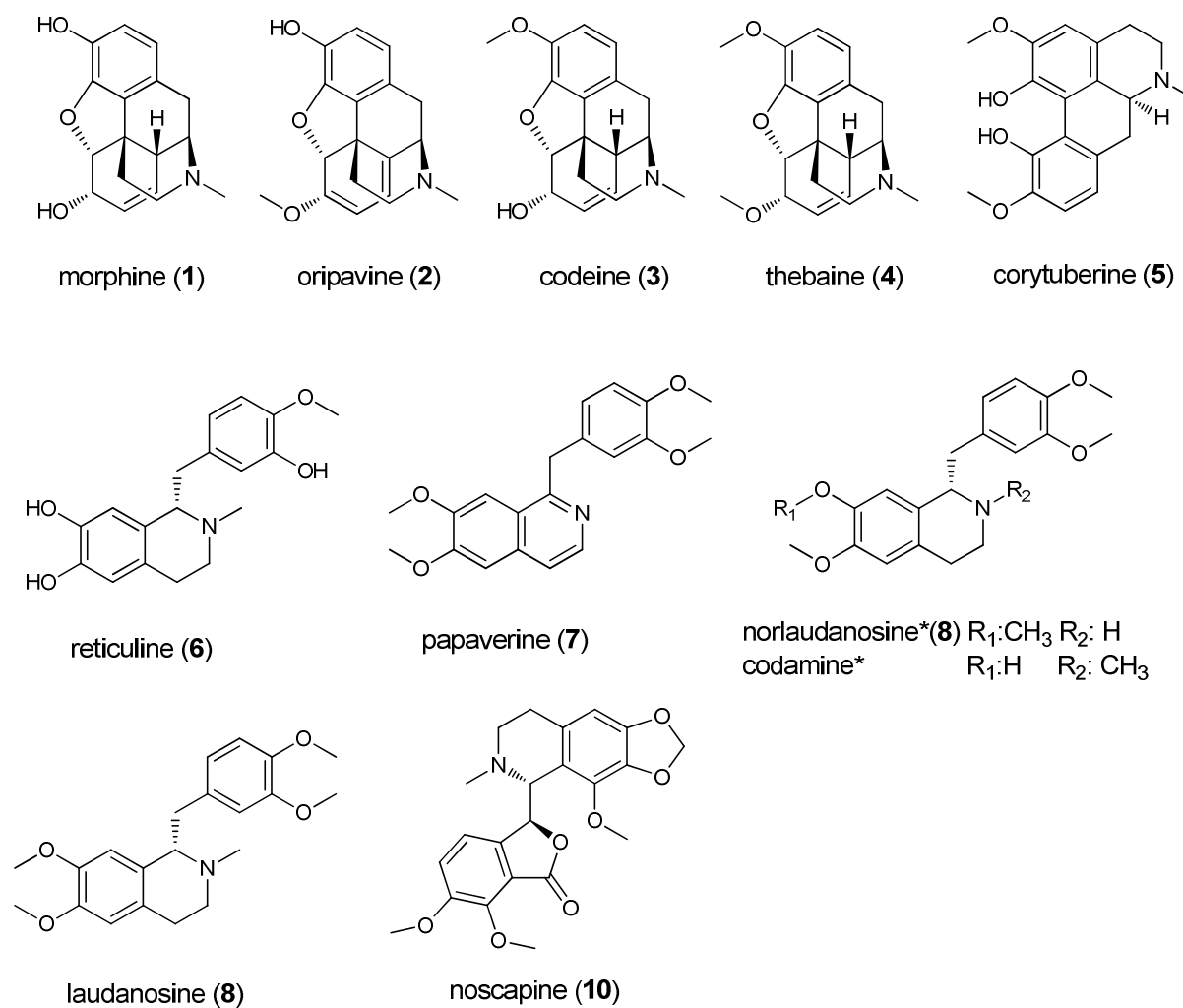
73 High-resolution (HR) experiments were performed on a Spiral-TOF<sup>TM</sup> MALDI TOF/TOF  
74 mass spectrometer (Jeol LTd., Tokyo, Japan) equipped with a pulsed laser operating at  
75 349 nm. Experiments were performed in the positive spiral mode within mass range of  
76 100-1000 Da. Acceleration 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.<sup>12</sup>

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, N<sub>2</sub>, 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.<sup>7</sup> Experiments were conducted on a Gemini C<sub>18</sub> column  
88 (3µm, 110 Å, 150 x 4.6 mm, Phenomenex, Le Pecq, France) protected by a pre-column  
89 security guard cartridge C<sub>18</sub> (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)<sup>12</sup>. 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.<sup>13</sup> 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).<sup>1, 14-15</sup> 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*,<sup>14</sup> 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.



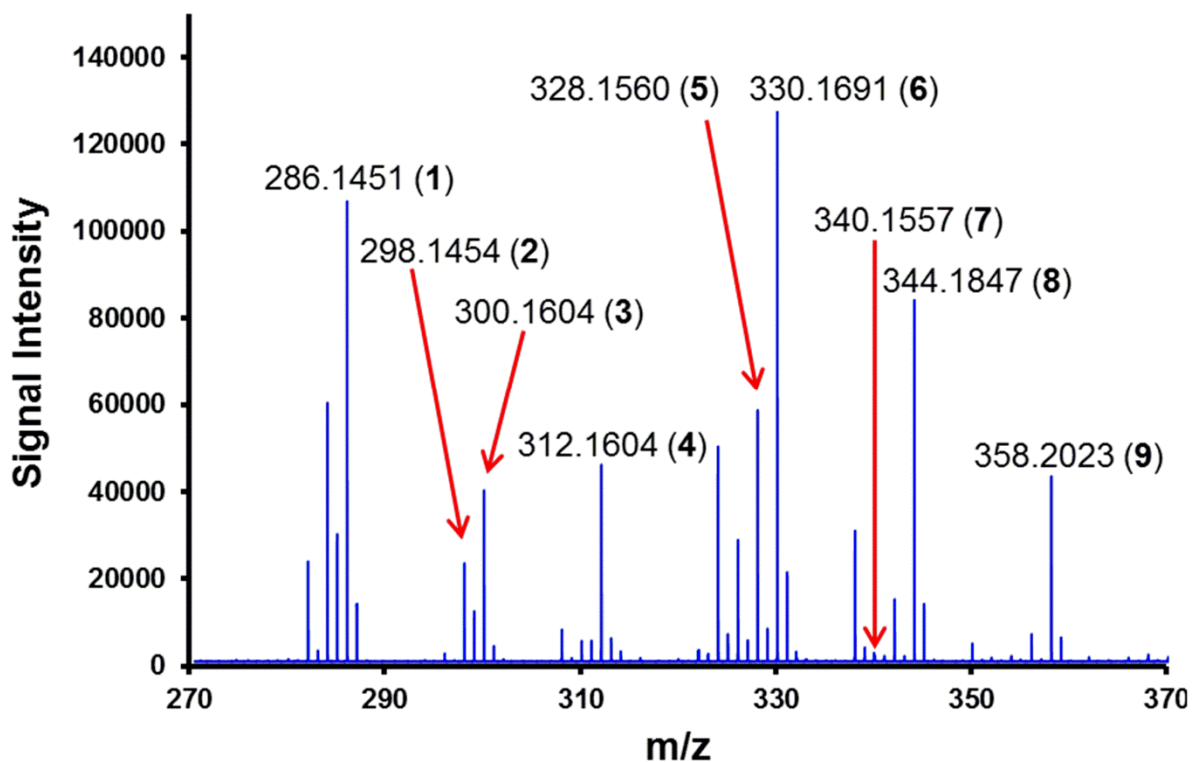
117

118 **Figure 1.** Structures of opiates that have been confirmed by their molecular formulae. Both119 norlaudanosiine and codaminiine ( $C_{20}H_{25}NO_4$ ) are reported constituents of *P. somniferum*,<sup>14</sup> 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] <sup>+</sup>	[M+H] <sup>+</sup>	(Da)	(ppm)	sample	1	2	3	4	5
<b>morphine (1)</b>	C <sub>17</sub> H <sub>19</sub> NO <sub>3</sub>	286.1443	286.1437	-0.0006	-2.1	1	✓	✓	✓	✓	✓
<b>oripavine (2)</b>	C <sub>18</sub> H <sub>19</sub> NO <sub>3</sub>	298.1443	298.1435	-0.0008	-2.7	1	✓	✓	✓	✓	✓
<b>codeine (3)</b>	C <sub>18</sub> H <sub>21</sub> NO <sub>3</sub>	300.1600	300.1596	-0.0004	-1.3	1	✓	✓	✓	✓	✓
<b>thebaine (4)</b>	C <sub>19</sub> H <sub>21</sub> NO <sub>3</sub>	312.1600	312.1590	-0.0010	-3.2	1	✓	✓	✓	✓	✓
<b>corytuberine (5)</b>	C <sub>19</sub> H <sub>21</sub> NO <sub>4</sub>	328.1549	328.1536	-0.0013	-4.0	1	✓	✓	✓	✓	✓
<b>reticuline (6)</b>	C <sub>19</sub> H <sub>23</sub> NO <sub>4</sub>	330.1705	330.1691	0.0014	-4.2	3	✓	✓	✓	✓	✓
<b>papaverine (7)</b>	C <sub>20</sub> H <sub>21</sub> NO <sub>4</sub>	340.1548	340.1550	0.0002	0.6	1	✓	✓	✓	✓	✓
<b>norlaudanosine*(8) codamine*</b>	C <sub>20</sub> H <sub>25</sub> NO <sub>4</sub>	344.1862	344.1847	-0.0015	-4.4	3	✓	✓	✓	✓	✓
<b>laudanosine (9)</b>	C <sub>21</sub> H <sub>27</sub> NO <sub>4</sub>	358.2018	358.2001	-0.0017	-4.7	3	✓	n.d.	✓	✓	✓
<b>noscipine (10)</b>	C <sub>22</sub> H <sub>23</sub> NO <sub>7</sub>	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*.<sup>14</sup>

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

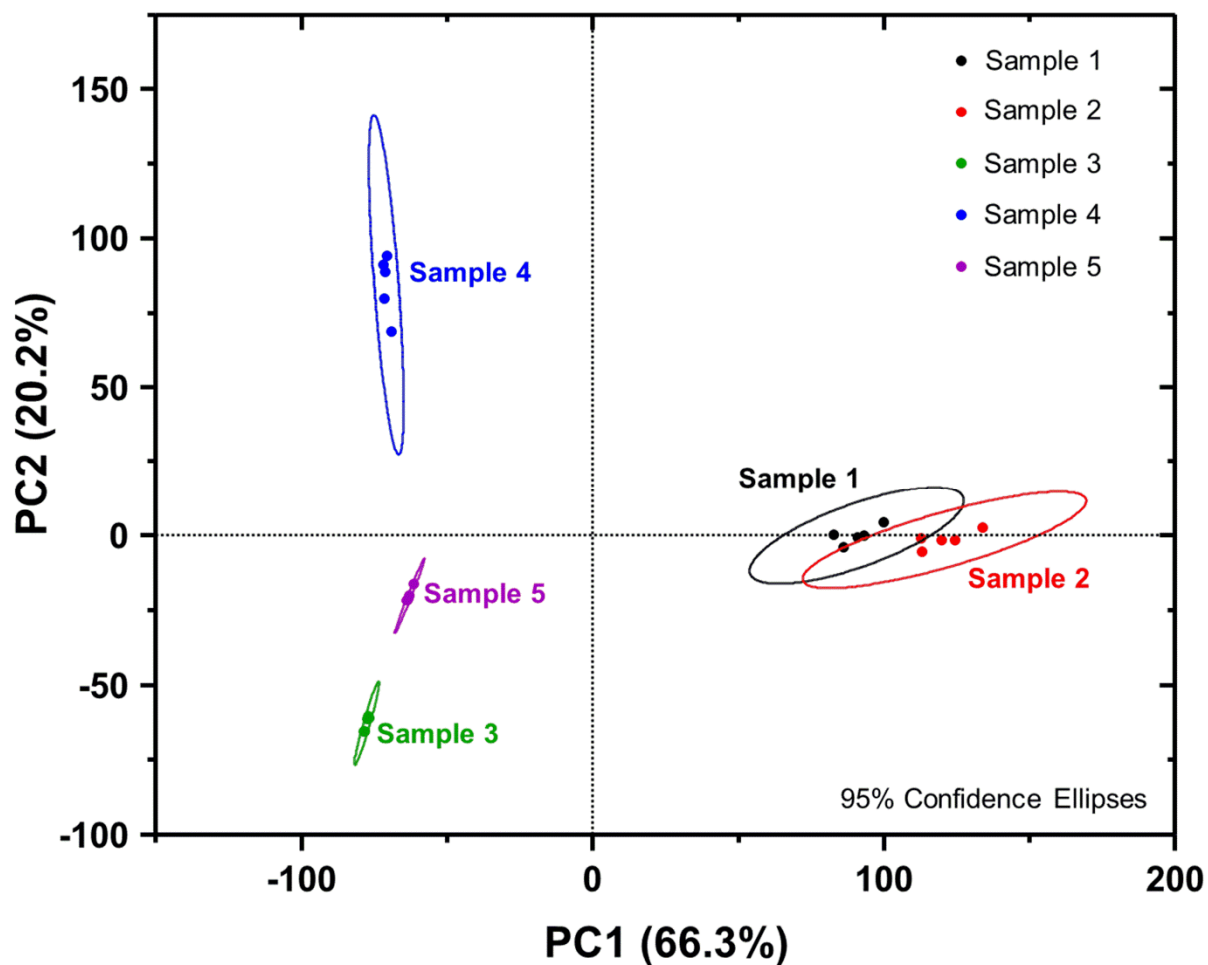
135 Except for noscapine (10) (sample 3 and 4) and laudanosine (9) (sample 2), HR-LDI-MS detected any of the compounds observed by LC-UV-  
136 MS. On the other hand, papaverine (7) was exclusively detected by LDI-MS in sample 4 and 5. A comprehensive summary of all LC-UV-MS  
137 chromatograms is given 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.<sup>5-9</sup> 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,<sup>16-18</sup> 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.<sup>13</sup>

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.<sup>13</sup> 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,<sup>19-21</sup> as well as from a mono-component  
 183 solution<sup>22</sup> and TLC surfaces.<sup>23</sup> 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.<sup>24</sup> 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



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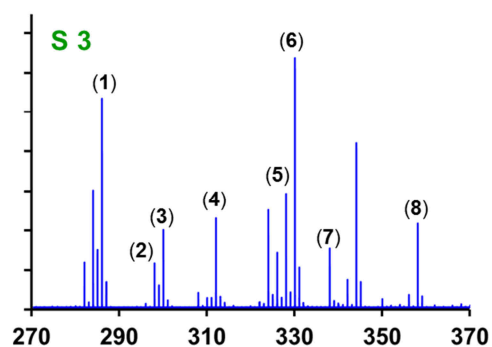
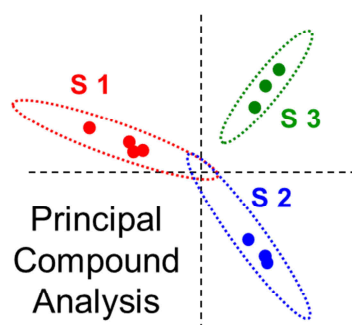
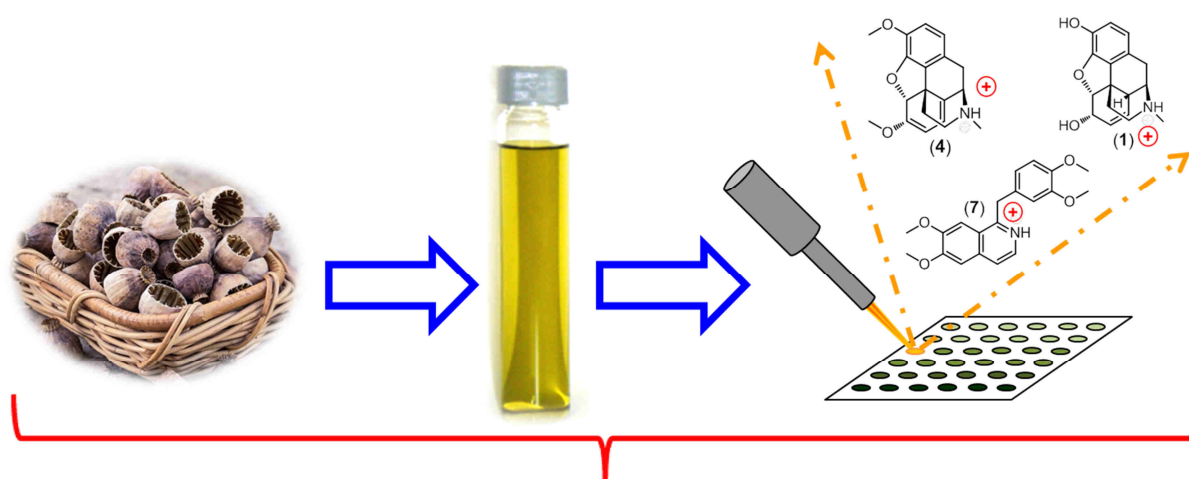
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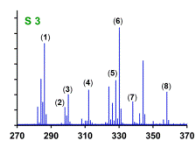
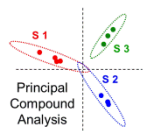
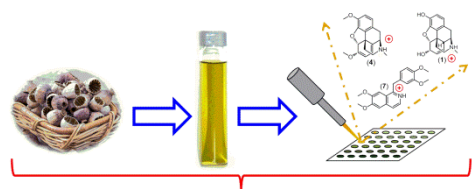
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301 **GRAPHIC FOR TABLE OF CONTENTS:**302 **Original.**

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



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