Matrix-Free Laser Desorption Ionization Mass Spectrometry as an Efficient Tool for the Rapid Detection of Opiates in Crude Extracts of Papaver somniferum

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

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ABSTRACT

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

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

INTRODUCTION:

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

MATERIAL AND METHODS:

Plant Material and Extraction.

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

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

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

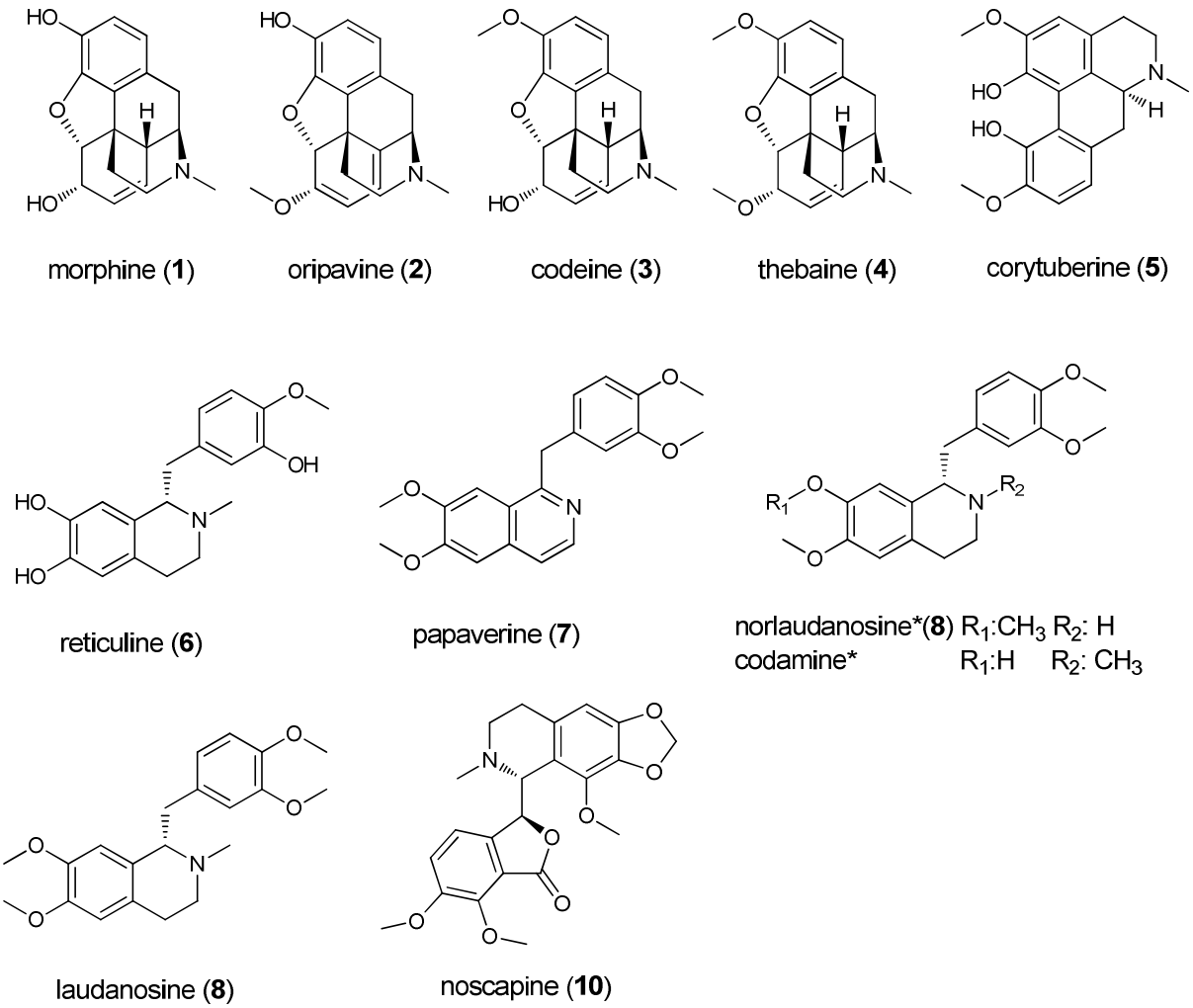
High-resolution (HR) experiments were performed on a Spiral-TOF™ MALDI TOF/TOF mass spectrometer (Jeol LTd., Tokyo, Japan) equipped with a pulsed laser operating at 349 nm. Experiments were performed in the positive spiral mode within mass range of 100⁻1000 Da. Acceleration voltage was set to 20 kV,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

HPLC-ESI-MS. Experiments were performed on a Trap Esquire 3000+ instrument (Bruker, Bremen, Germany) equipped with ESI/APCI Ion Trap as well as a UV-VIS wavelength detector. Instrument parameters and experimental conditions were as follows: Collision gas, He; collision energy amplitude, 1.0 V; nebulizer and drying gas, N_2, 7 L/min; pressure of nebulizer gas, 20 psi; dry temperature, 300 °C; flow rate, 0.5 mL/min; solvent split ratio 1:9; scan range, m/z 20−1000. In addition absorption chromatograms were acquired at 230 and 280 nm. Chromatographic separation was performed according to a previously published protocol with some modifications.[7] Experiments were conducted on a Gemini C_{18} column (3µm, 110 A, 150 x 4.6 mm, Phenomenex, Le Pecq, France) protected by a pre-column security guard cartridge C_{18} (4 × 3 mm i.d.) from the same company. The mobile phase contained a solution of ammonium bicarbonate (Servilab, Le Mans, France) at 20 mM adjusted to pH 9.0 by ammonium hydroxide (Acros organics, Geel, Belgium) (solvent A), as well as LC-MS grade MeOH (Honeywell, Seelze, Germany) comprising 5% of solvent A (solvent B). Gradient conditions were as follows: 40% B (0-1 min), 40-100% B (1-30 min), 100% B (30-42 min). Signals exhibiting a S/N<10 in MS, as well as less than 0.03 mAU in UV were considered not detected.

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

RESULTS AND DISCUSSION:

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



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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 consituens of *P. somniferum*,[14] but as

120 constitutional isomers cannot be disdiguished by their quasi-molecular ions.

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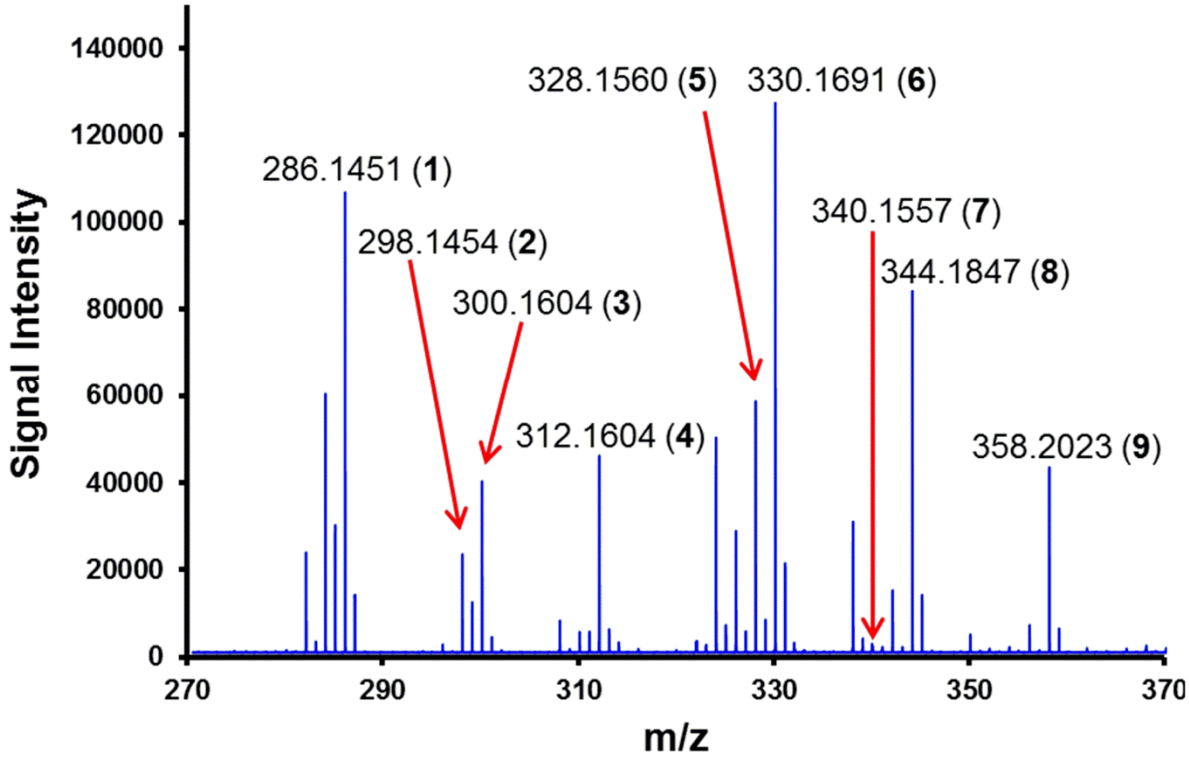


Figure 2. High resolution LDI-MS spectrum obtained from the crude methanolic extract of sample 3. The following alkaloids were detected and confirmed by their molecular formulae: Morphine (**1**), oripavine (**2**), codeine (**3**), thebaine (**4**), corytuberine (**5**) reticuline (**6**), papaverine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

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

name	molecular formula	calculated mass $[M+H]^+$	observed mass $[M+H]^+$	mass error (Da)	mass error (ppm)	data from sample	Opiates detected in 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.	✓	✓	✓
noscapine (10)	$C_{22}H_{23}NO_7$	414.1552	414.1536	-0.0016	-3.9	1	✓	✓	n.d.	n.d	n.d.

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

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

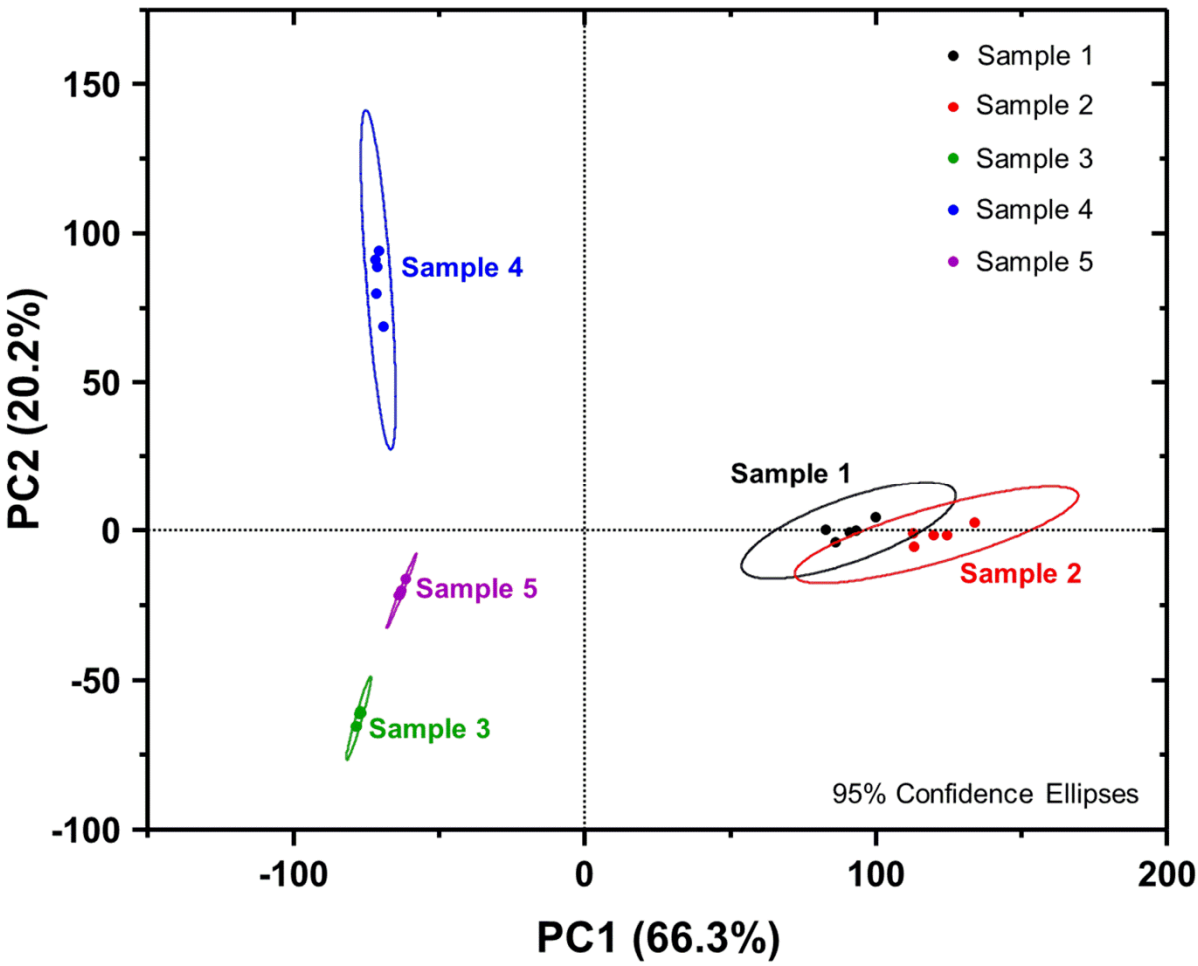
compound	sample 1			sample 2			sample 3			sample 4			sample 5		
	LC-UV	**LC-MS**	**LDI-MS**	**LC-UV**	**LC-MS**	**LDI-MS**	**LC-UV**	**LC-MS**	**LDI-MS**	**LC-UV**	**LC-MS**	**LDI-MS**	**LC-UV**	**LC-MS**	**LDI-MS**
morphine (1)	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
oripavine (2)	✓	✓	✓	✓	✓	✓	n.d.	✓	✓	✓	✓	✓	✓	✓	✓
codeine (3)	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
thebaine (4)	co	✓	✓	co	✓	✓	co	✓	✓	co	✓	✓	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.

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-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 chromatograms is given in the Supporting Information.

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

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

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



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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 solution [22] 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

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

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

ABBREVIATIONS:

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

ACKNOWLEDGEMENT:

SUPPORTING INFORMATION:

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

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GRAPHIC FOR TABLE OF CONTENTS:

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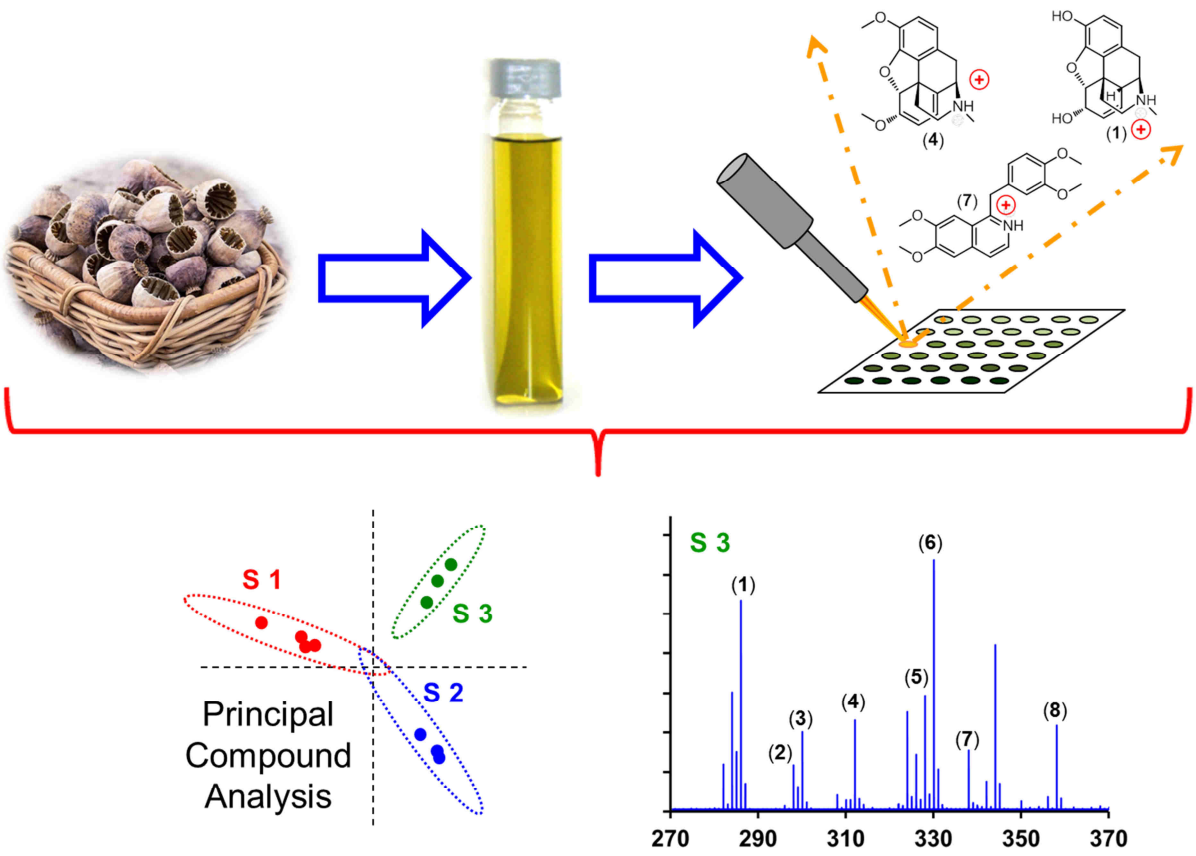

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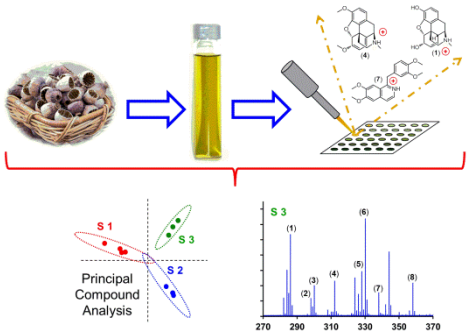
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