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Direct analysis of quaternary alkaloids by *in situ* reactive desorption corona beam ionization MS

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Abstract:

The direct detection of quaternary alkaloids by atmospheric pressure chemical ionization (APCI)-base ambient MS is difficult because of their poor volatility. In this paper, a reactive protocol was developed for the *in situ* determination of quaternary alkaloids with desorption corona beam ionization (DCBI) mass spectrometry (MS). The model compounds of 8 quaternary alkaloids including sanguinarine, chelerythrine, cyclanoline, nitidine, coptisine, jatrorrhizine, berberine, palmatine and 2 tertiary alkaloids including protopine and allocryptopine were investigated in different state such as on Polytetrafluoroethylene (PTFE) plate, in raw herbal materials, and in silica gel. After various reactive reagents were studied, the mixture of saturated aqueous NaOH solution and CH₃OH solvent (3:7, v/v) was selected as the optimized reactive reagent for the reactive DCBI-MS detection. All of the target molecules can be detected with high sensitivity. On a PTFE plate the limit of detection were 0.0795, 0.1060, 0.4860, 0.9665, 0.8879, 0.3987, 0.5557, 0.4591, 0.0889, and 0.1929 mg/L for sanguinarine, chelerythrine, cyclanoline, nitidine, coptisine, jatrorrhizine, berberine, palmatine, and protopine, allocryptopine respectively. The reactive protocol was also applied to the direct detection of raw herbal material and thin layer chromatography successfully.

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3 **Keywords:** reactive DCBI-MS; thin-layer chromatography; quaternary alkaloids; raw
4 herbal materials
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7 **1. Introduction**

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9 Ambient ionization mass spectrometry (MS) allows the direct analysis of
10 ordinary objects in the open atmosphere in the laboratory or their natural environment.
11 According to the discharge mechanism, the ambient ionization techniques can be
12 classified into electrospray ionization (ESI)-related, including desorption electrospray
13 ionization (DESI), and atmospheric pressure chemical ionization (APCI)-related, such
14 as direct analysis in real time (DART). The new MS technologies have been applied
15 in different areas¹⁻³.
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23 After DESI and DART were reported in 2004⁴ and 2005⁵ respectively, there are
24 more than 30 ambient ionization methods have been developed. One of the
25 APCI-related plasma based ionization sources, termed desorption corona beam
26 ionization (DCBI)⁶⁻⁸ for direct analysis, was proposed by our group in 2009. In DCBI
27 source, plasma produced by heated helium in electric field. The visible heated helium
28 beam form in a metal ring electrode of DCBI, can quickly desorb and ionize
29 compounds that are in the gas, liquid and solid phase. There are three advantages of
30 DCBI: 1) it does not need solvent to perform the experiment; 2) visible and
31 temperature controlled beam support the desorption area determination; 3) rapid
32 analysis of diverse phase sample. As a typical example of APCI related ambient
33 ionization technique, the fundamental mechanisms of DCBI have been identified as a
34 combination of thermal desorption and momentum ionization with a heated gas
35 stream. Lots of compounds including pesticides, veterinary additives, explosive
36 materials and Over-The-Counter (OTC) drugs have been detected by DCBI-MS
37 successfully. Coupling with a poly-dimethylsiloxane (PDMS) substrate for detection
38 of trace amount of pesticides had broadened the application of DCBI for water
39 sample⁹. Thereafter, it has been applied to the direct analysis in different area such as
40 raw plant materials, ionic liquid matrix, Thin-layer chromatography (TLC) plate
41 etc¹⁰⁻¹⁴.
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3 The alkaloids are part of group of secondary metabolites. They are found broadly
4 in nature with diverse structures, and have important biological activities. The
5 alkaloid contents are always the quality control indexes of plant medicines. Ambient
6 MS technologies have been also introduced to rapidly determine the alkaloids.
7 Hitherto, all of the methods for quaternary alkaloids analysis besides the quaternary
8 ammonium bases were based on ESI-related technologies¹⁵⁻²⁵. The most used source
9 is DESI which is based on droplet-pick-up mechanism. There is no evidence of
10 quaternary alkaloids analysis by APCI-related ambient MS technique due to poor
11 volatility of quaternary alkaloids and thermo desorption mechanism of APCI-related
12 technique.

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14 In this paper, we report a reactive strategy for the *in situ* determination of the
15 quaternary alkaloids by DCBI-MS. After dropping the reactive reagent, quaternary
16 alkaloids can be efficiently detected from the raw herbal materials and silica matrix.
17 This strategy provides the possibility for APCI-related ambient MS technique to
18 analyze low volatile species directly. Hence, it offers one more option ionization
19 potential contrast to DESI and produces relatively simple mass spectra.

2. Experimental section

2.1 Reagents and instruments

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21 Methanol (HPLC grade) was purchased from Chemical Research Institute of
22 Fine Chemical Industry (Changsha, China). Triethylamine, ammonia, m-xylene,
23 chloroform (analytical grade) were purchased from Shanghai Sinopharm Chemical
24 Reagent Co., Ltd. (Shanghai, China). Sodium hydroxide, ethanol (analytical grade),
25 formic acid and acetic acid (HPLC grade) were purchased from Tianjin Hengxing
26 Chemical Preparation Co., Ltd. (Tianjin, China). Standards of protopine, sanguinarine,
27 chelerythrine, allocryptopine, cyclanoline, and nitidine were prepared in our
28 laboratory by preparative HPLC and identified with MS, H¹-NMR and C¹³-NMR.
29 Coptisine, jatrorrhizine, berberine and palmatine were purchased from the National
30 Institute for the Control of Pharmaceutical and Biological Products (Beijing, China).
31 The purity of all these 10 alkaloids standards was not less than 98%. The chemical
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3 structures of them are shown in Figure 1. The fresh *Macleaya cordata* were collected
4 from suburb of Changsha (located in the northeast Hunan Province, China). The 6
5 herbs (*Coptis chinensis* Franch, *Phellodendron amurense* Rupr, *Zanthoxylum nitidum*,
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7 *Fibraurea recisa* Pierre, *Radix Stephaniae Tetrandrae*) were purchased in local
8 pharmacy.
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12 Commercial silica-coated TLC plate (0.20-0.25mm silica gel with fluorescent
13 indicator) was purchased from Branch of Qingdao Haiyang Chemical Co., Ltd.,
14 Qingdao, China. Quantitative capillary was purchased from Huaxi medical university
15 instrument factory, Chengdu, China. DCBI source and its controller were provided by
16 Shimadzu Research Laboratory (Shanghai) Co., Ltd. Mass spectrometric analysis was
17 carried out on a Finnigan LCQ Advantage MAX ion trap mass spectrometer (Thermo,
18 San Jose, CA, USA).
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20 21 22 **2.2 DCBI conditions.**

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25 In this study, the desorption corona beam ionization source was in positive mode
26 (DCBI⁺) for all samples. The flow rate of helium gas was 0.6 L/min. Discharge
27 voltage of DCBI was set to +3.5 kV. The heating temperature was investigated and
28 kept at 220 °C for the further experiments.
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32 LCQ mass spectrometer worked in full scan mode without further specification.
33 The capillary temperature of LCQ was set at 260 °C. The capillary voltage was 0 V,
34 and the tube lens voltage was 10 V.
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36 37 38 **2.3 Preparation of standard solutions and sample solutions.**

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41 Stock solution of all the alkaloids was prepared with methanol. The
42 concentration of each standard was 1.00 mg/mL.
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46 Different parts of *Mecleagua cordata* (wild) R. Br. were dried and grinded into
47 powder (20 mesh). 1.0 g powder was extracted with 50 mL chloroform-ethanol (1:1,
48 v/v). After overnight soaking and 30 min ultrasonic bath, the extracted solution was
49 concentrated to 2 mL in a vacuum rotational evaporator.
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51 52 53 **2.4 DCBI-MS**

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56 Each aliquot of 2 µL sample solution was loaded on a substrate by a
57 microsyringe. After natural drying in air, the condensed sample on the substrate was
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3 moved into the visible corona beam for detection. The spatial location of target
4 sample spot was 5 mm axially from the capillary inlet and 2 mm below. The same
5 method was applied for both raw herbal analysis and the TLC plate detection. The
6 quantitative determination for each sample was carried out 5 times repeatedly.
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10 11 12 13 14 **3. Results and discussion**

15 16 **3.1 Reactive reagent**

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18 Quaternary alkaloids are present in salt form in nature mostly. The volatility is
19 very low due to their high level of polarity. Since the thermo-desorption mechanism is
20 postulated in DCBI, it is hard to desorb the quaternary alkaloids with heating gas.
21 Preliminary findings proved that eight quaternary alkaloids have no MS response
22 without reaction. According to the acid/base equilibrium, alkaloids tend to be free
23 base in alkaline environment. Free quaternary alkaloid is not so polar and prone to
24 evaporation. Naturally, quaternary alkaloids derived with the alkaline reagent were
25 detected by the DCBI-MS.
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33 To optimize the reactive DCBI-MS conditions, 8 quaternary alkaloids including
34 coptisine, cyclanoline, jatrorrhizine, nitidine, berberine, chelerythrine, sanguinarine,
35 and palmatine were selected as model analytes. 6 types of alkaline reactive reagent, i.e,
36 A. water/methanol (1:1, v/v); B. triethylamine/methanol (1:10, v/v); C.
37 triethylamine/methanol/water (1:8:1, v/v/v); D. ammonia water/methanol (1:10, v/v);
38 E. ammonia water/methanol/water (1:8:1, v/v/v); F. saturated aqueous NaOH
39 solution/methanol (1:9, v/v) were prepared for reactive DCBI-MS investigation. The
40 pH of reactive reagents A to F were tested by pH test strips, and were 6.7, 8.0, 8.9, 7.8,
41 8.6, 13.7 respectively. Each reagent mixture (20 μ L) was added on the dry sample
42 spots on the PTFE substrate. DCBI-MS detection was then carried out 10 seconds
43 later. The MS responses (peak areas of extracted ion chromatogram) of the alkaloids
44 are shown in Figure 2. From the results, it can be found that the MS responses under
45 the reagent A-E are very low, almost same as response that without any reactive
46 reagent. Otherwise when using reagent F (saturated aqueous NaOH solution/methanol,
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1:9, v/v), the DCBI-MS responses were increased significantly.

The observed molecular ion of coptisine (MW: 320.3) is m/z 320, cyclanoline (MW: 342.4) is m/z 342, jatrorrhizine (MW: 338.4) is m/z 338, nitidine (MW: 348.4) m/z 348, berberine (MW: 336.4) is m/z 336, chelerythrine (MW: 348.4) is m/z 348, sanguinarine (MW: 332.1) is m/z 332 and palmatine (MW: 352.4) is m/z 352 respectively. They can all be detected with high sensitivity. The MS behaviors of them are similar with that in ESI source.

This demonstrated that quaternary alkaloids can react with strong base solution and then generate their equilibrium mixture, i.e., $R_4N^+X^- + OH^- \rightarrow R_4N^+OH^- + X^-$. $R_4N^+OH^-$ in the equilibrium mixture have higher volatility than $R_4N^+X^-$. Consequently, $R_4N^+OH^-$ can be desorbed by heating helium and generate M^+ in corona beam. The procedure can be described as $R_4N^+OH^- + H^+ \rightarrow R_4N^+H_2O \rightarrow R_4N^+ + H_2O$. Therefore, quaternary alkaloid was detected by DCBI-MS. As for triethylamine and ammonia water, due to their relative weak alkaline compared with NaOH, they cannot transform $R_4N^+X^-$ to $R_4N^+OH^-$ sufficiently. Hence, the DCBI-MS detection was not realized under this condition.

To study the possibility of DCBI-MS detection for thin layer chromatography, the detection of alkaloids on a commercial silica-coated TLC plate was conducted with reactive DCBI-MS. The results showed that the responses of target analyte on TLC plate had not significant difference with that on PTFE plate when using reactive reagent F.

To optimize NaOH reactive reagent, effect on DCBI-MS response of volume ratio (1:9, 2:8, 3:7, 4:6 and 5:5, respectively) of saturated aqueous NaOH solution with methanol were investigated. The results are shown in Figure 3. It can be found that the 8 quaternary alkaloids responses increased with the increasing of the NaOH content. When the ratio was 3:7, the MS responses of the alkaloids reached the maximum. Therefore, we chose the volume ratio of 3:7 for the following experiments. With the optimal reactive reagent (saturated aqueous NaOH solution : methanol =3:7), the limit of detection of sanguinarine, chelerythrine, cyclanoline, nitidine, coptisine, jatrorrhizine, berberine, palmatine, and protopine, allocryptopine on a PTFE plate

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3 were 0.0795, 0.1060, 0.4860, 0.9665, 0.8879, 0.3987, 0.5557, 0.4591, 0.0889, and
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5 0.1929 mg/L, respectively.
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7 **3.2 Effect of desorption temperature**

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9 Because of the thermo-desorption mechanism¹¹, the temperature of DCBI source
10 is important. Under the optimal reactive condition, we investigated a series of
11 desorption temperatures (120 °C, 180 °C, 220 °C, 240 °C) for 8 quaternary alkaloids.
12 The MS response of target compounds under different temperatures is shown in
13 Figure 4. Under the temperature of 120 °C, all compounds are barely detected. Their
14 signals increased significantly as the temperature rises. The signal increasing rates of
15 different targets are diverse. It indicated that the volatility difference is still significant
16 for the investigated quaternary alkaloids. In addition, despite of the good intensity for
17 all compounds at 240 °C, the signal to noise ratio (S/N) of them is poor. Therefore, the
18 optimal temperature choice was 220 °C for the further experiments.
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28 **3.3 In situ detection of raw herbal materials**

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30 6 types of raw herbal materials were investigated to verify the proposed reactive
31 DCBI-MS method. Dropping the saturated aqueous NaOH solution/methanol (3:7, v/v)
32 reagent on the surface of herbal materials, 8 quaternary alkaloids were detected in
33 them. The relative quantitative (peak height in extracted ion chromatogram) results
34 are listed in Table 1. It should be noticed that there is a pair of isomer, chelerythrine
35 and nitidine. Without chromatography separation it is practically impossible to
36 distinguish them. In our preview LC experiments, we did not find the nitidine in
37 *Macleaya cordata*. Also we did not find the chelerythrine in *Zanthoxylum nitidum*.
38 However, there are some reports²⁶ that have detected nitidine and chelerythrine in the
39 *Zanthoxylum nitidum* simultaneously. To avoid mistake, the ion of m/z 348 that are
40 detected in *Zanthoxylum nitidum* is not assigned to certain nitidine but to total
41 alkaloids that including nitidine and chelerythrine. The peak height of each alkaloid is
42 at least one order of magnitude higher than in non-reactive detection. Without reactive
43 reagent, direct analysis of these 8 alkaloids in herbal material was difficult. For
44 example, the MS spectrums of *Zanthoxylum nitidum* with and without reactive
45 reagent were compared in Figure 5. There is no obvious peak of m/z 348 in untreated
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sample spectrum (figure 5A). After adding reagent, m/z 348 (Probably molecular ion of nitidine) appeared prominently (figure 5B). The results demonstrated that reactive DCBI-MS method can be effectively applied to in situ detection of quaternary alkaloids for raw herbal materials.

3.4 Reactive DCBI-MS detection for the TLC separation

Recently, ambient MS combined with TLC have been applied in the different fields¹⁵⁻¹⁷. This combination was conducted to provide a more versatile and specific information on TLC. We used proposed reactive DCBI-MS method to detect 4 alkaloids (including 2 quaternary alkaloids, chelerythrine and sanguinarine and 2 tertiary alkaloids allocryptopine, and protopine) after TLC separation.

The TLC plate was dried naturally after separation, and irradiated under UV light to locate the alkaloid position through dark spot. After adding a reactive reagent, the dark spot was exposed to visible corona beam in DCBI source. A typical result of 4 alkaloids standard mixture is shown in Figure 6. The peak assignment of m/z 370.36 is allocryptopine (MW: 369.4), m/z 354.50 is protopine (MW: 353.4), m/z 348.35 is chelerythrine (MW: 348.4) and m/z 332.37 is sanguinarine (MW: 332.1) respectively. A series of mixture of standards were carried out with TLC-reactive-DCBI to get the regression equation by the means of external standard method. The quantitative results including limit of detection, regression equation, relative standard deviation are listed in Table 2. The determination of 4 alkaloids in different parts of fresh *Mecleagua cordata* was achieved with same TLC-reactive-DCBI method. The results are listed in Table 3.

4. Conclusion

A reactive DCBI-MS method was proposed for the direct detection of quaternary alkaloids. The method was successfully used in situ analysis of raw herbal materials. It was also applied to the TLC detection successfully. The optimal reactive reagent was the mixture of saturated aqueous NaOH solution and methanol (3:7, v/v). The optimized DCBI source temperature was 220 °C. The results of six herbs showed that

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3 the analysis of quaternary alkaloids can be completed within 1 minute. The proposed
4 reactive strategy can be applied in other APCI-relative ambient MS techniques.
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8 9 **Acknowledgment**

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3 Figure captions:

4 Figure 1 Structures of 10 alkaloids.
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7 Figure 2 Peak area of 8 alkaloids in extracted ion chromatogram with different reactive reagents.

8 A. water/methanol (1:1, v/v), B. triethylamine/methanol (1:10, v/v), C.
9 triethylamine/methanol/water (1:8:1, v/v/v), D. ammonia water/methanol (1:10, v/v), E. (ammonia
10 water/methanol/water (1:8:1, v/v/v), F. saturated aqueous NaOH solution/methanol (1:9, v/v)
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13 Figure 3 Eight quaternary alkaloids responses (peak areas) in reagents of saturated aqueous NaOH
14 solution and methanol mixture that with different volume ratio.
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17 Figure 4 Eight quaternary alkaloids responses (peak areas) at different source temperature
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19 Figure 5 The MS spectra of alkaloid (m/z 348, probably nitidine) in *Zanthoxylum nitidum* by
20 reactive DCBI-MS.
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22 A. without reactive reagent; B. with saturated NaOH solution/methanol (3:7, v/v), C. extraction
23 ion chromatogram of m/z 348
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26 Figure 6 TLC chromatogram of 4 standard alkaloids (sanguinarine, chelerythrine, protopine,
27 allocryptopine) mixture sample and their *in situ* spectra with reactive DCBI-MS method
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Figure 1

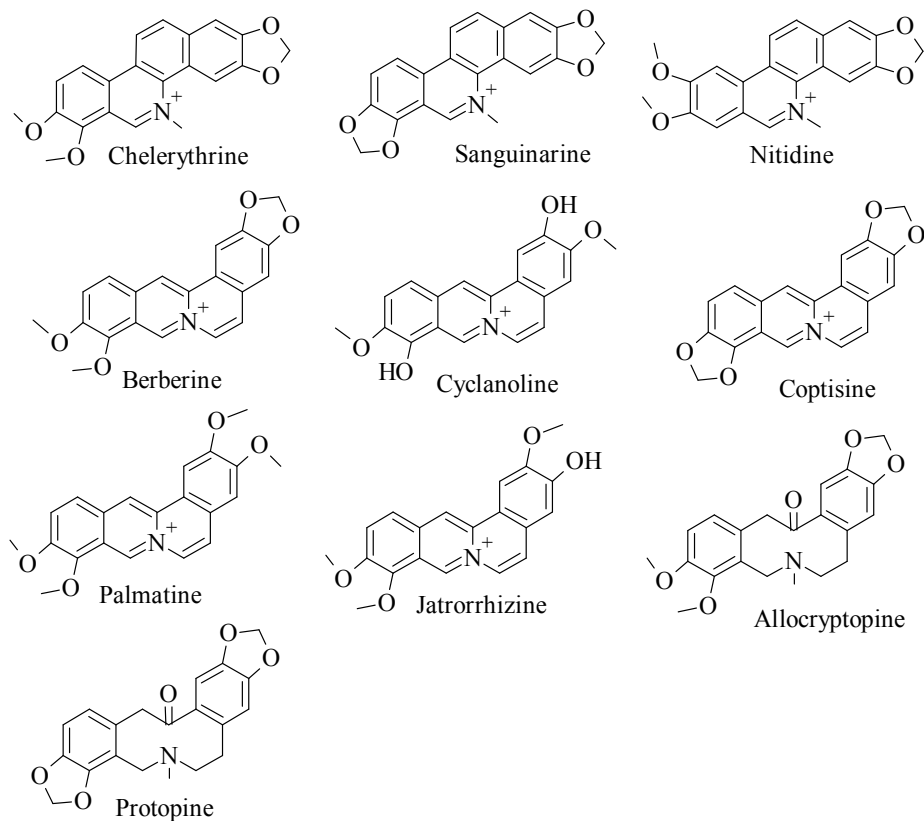


Figure 2

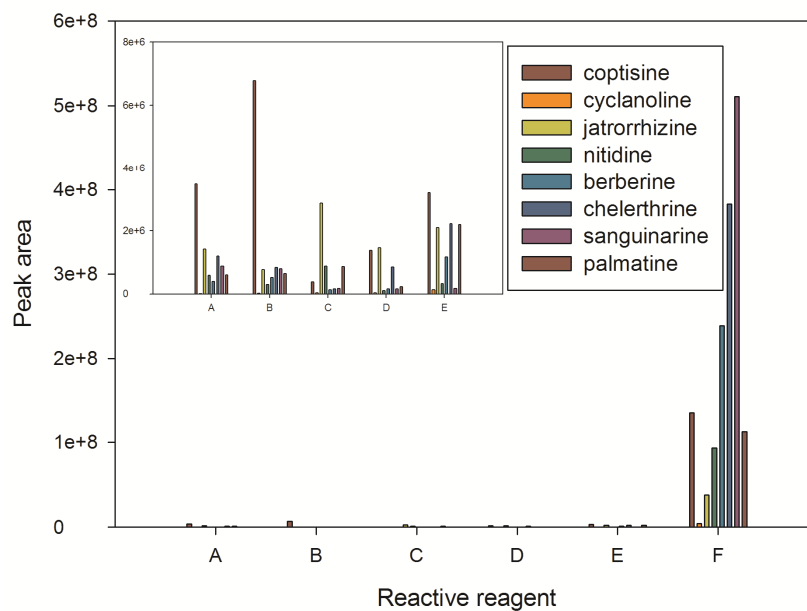


Figure 3

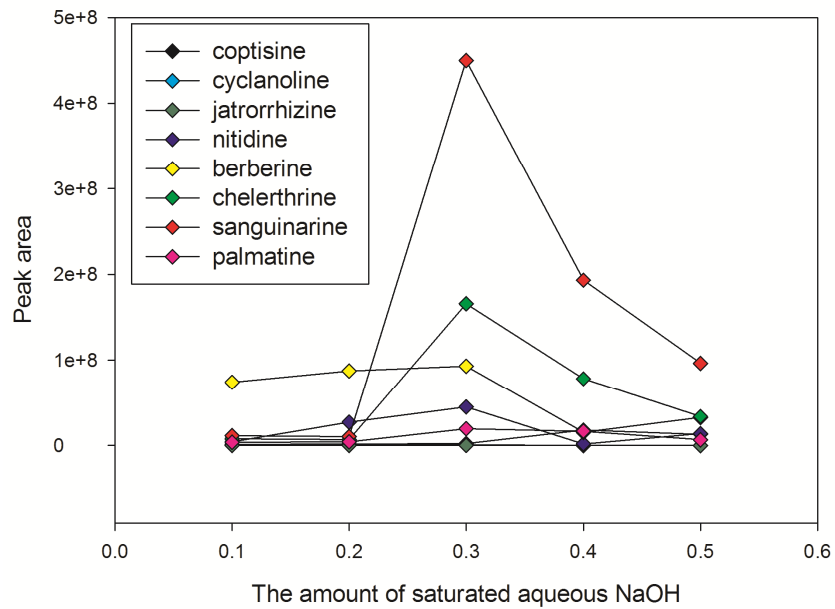
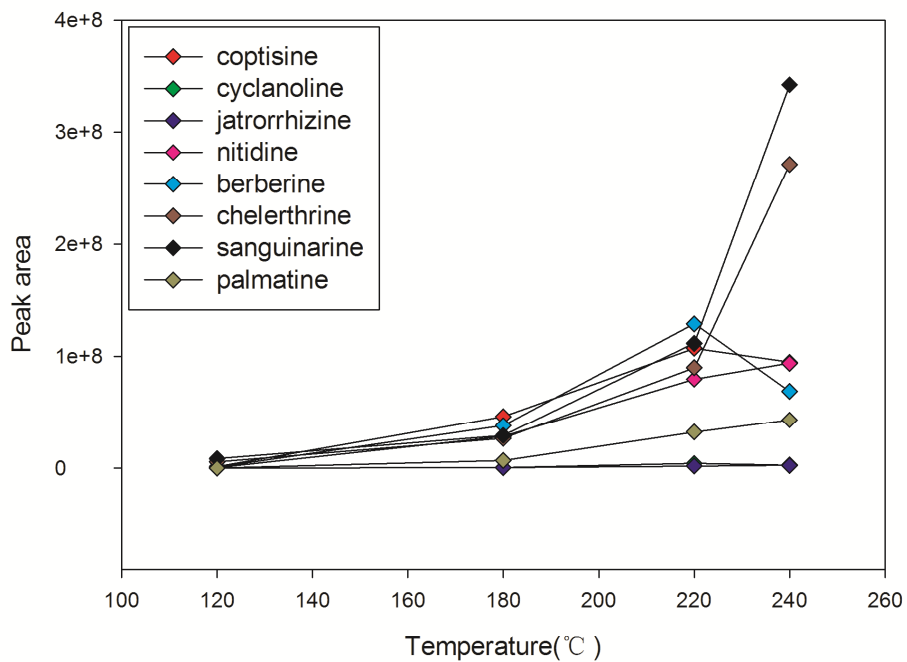


Figure 4

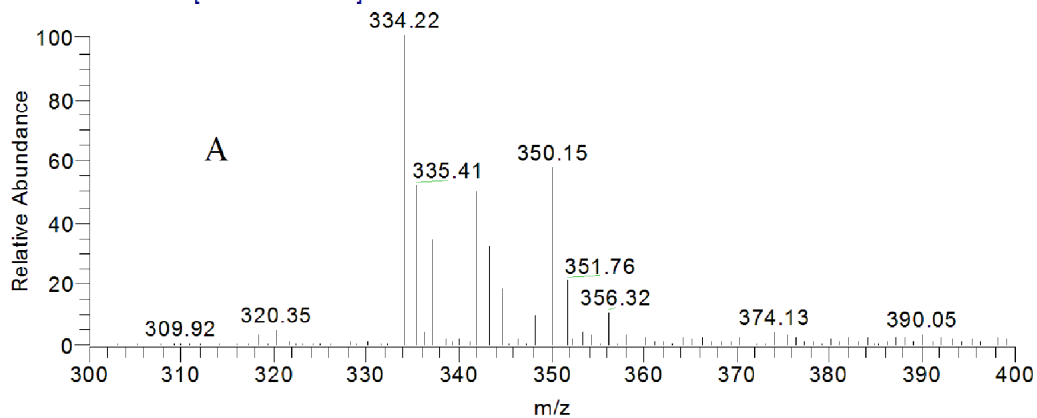


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Figure 5

liangmianzhen #225-239 RT: 1.88-1.98 AV: 15 SB: 32 2.36-2.68 NL: 5.11E6

T: + c ESI Full ms [300.00-400.00]



liangmianzhen #382-393 RT: 3.36-3.44 AV: 12 SB: 32 2.36-2.68 NL: 1.49E7

T: + c ESI Full ms [300.00-400.00]

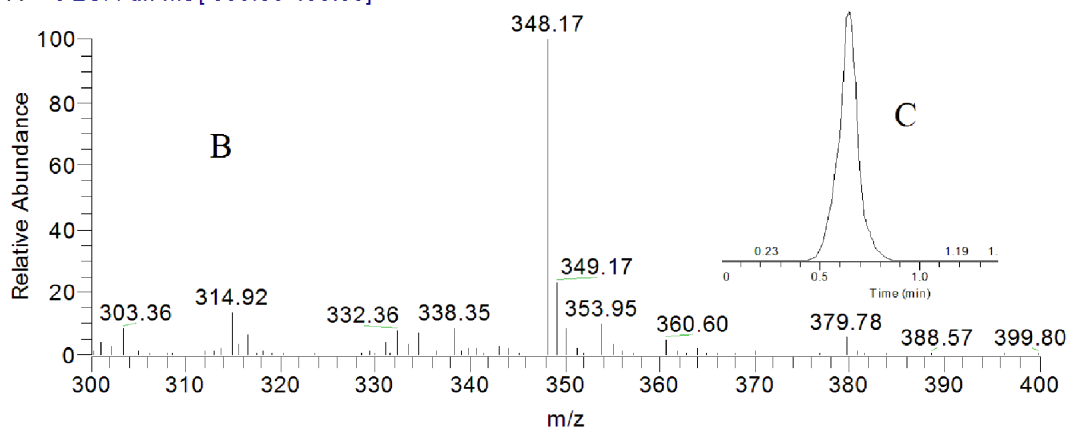


Figure 6

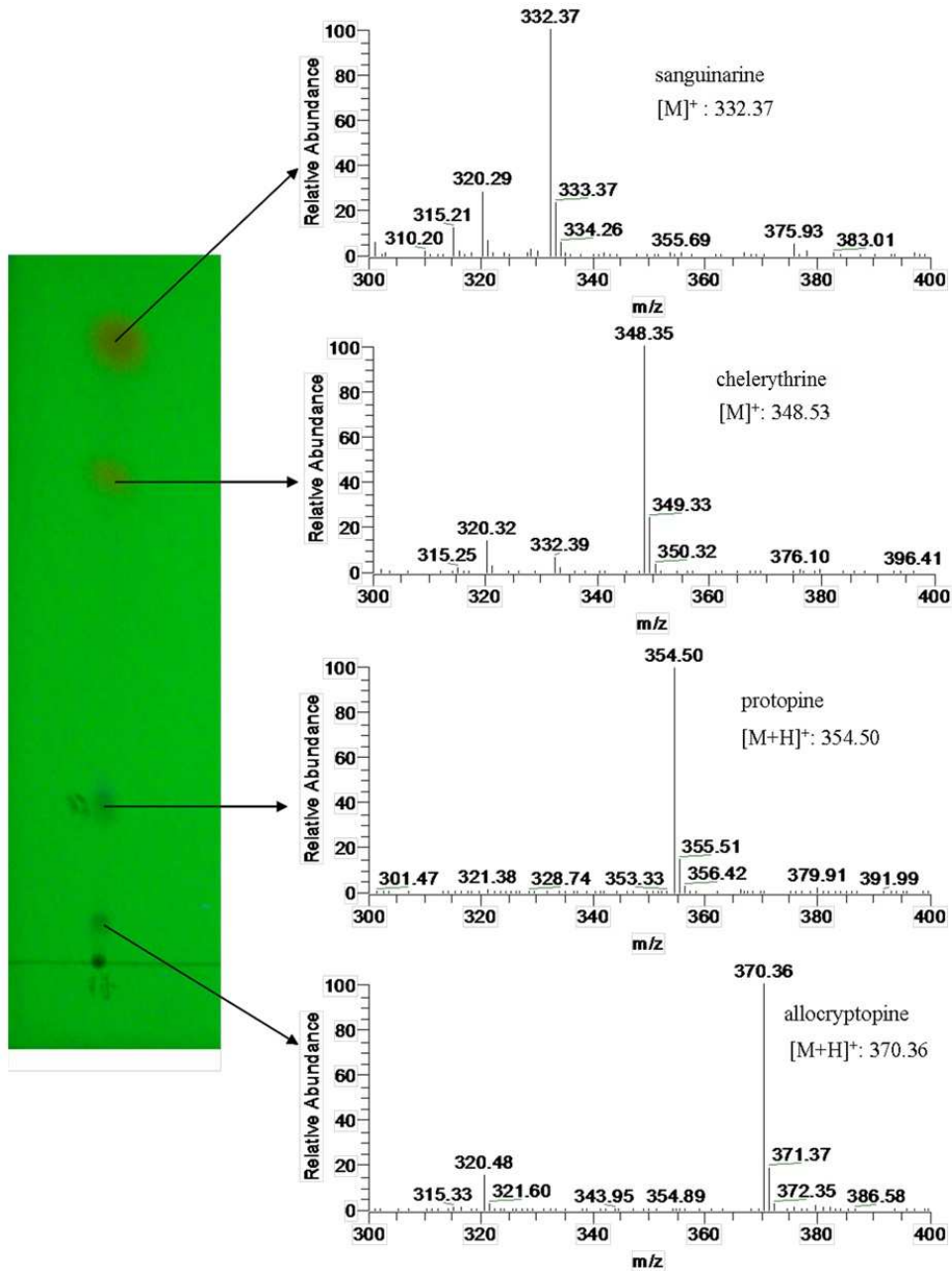


Table 1. Peak height in MS spectra of alkaloids in 6 kinds of herbs by direct DCBI detection with and without the reactive reagent saturated aqueous NaOH solution/methanol (3:7, v/v)

Herbs	alkaloids	Height (without)	Height (with)
<i>Coptis Chinesis Franch</i>	Coptisine	268592	2694316
	Berberine	1090183	17999090
<i>Phellodendron amurense Rupr</i>	Jatrorrhizine	1052487	11686123
<i>Zanthoxylum nitidum</i>	Ion* of m/z 348	878829	17370694
<i>Macleaya cordata.</i>	Chelerythrine	4096912	23342921
	Sanguinarine	1038478	15653795
<i>Fibraureapierre</i>	Palmatine	944694	8839026
<i>Radix Stephaniae Tetrandrae</i>	Cyclanoline	422561	5365210

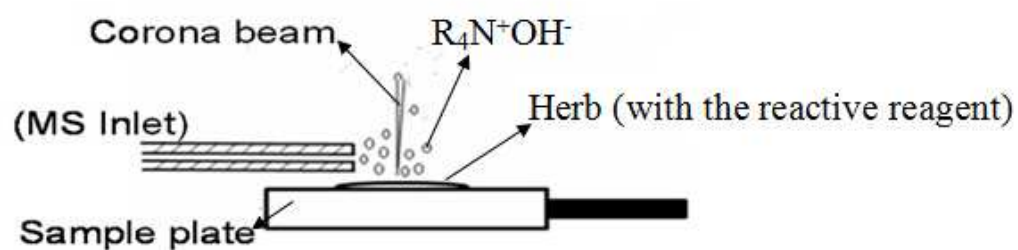
*Probably nitidine although chelerythrine cannot be excluded.

Table 2. Linearity of 4 alkaloids by reactive TLC-DCBI-MS

Alkaloids	Regression equation	Correlation coefficients	LOD (mg/L)	Concentration (mg/L)	RSD (% , n=5)
Allocriptopine	Y=18.63X-59.46	0.9969	0.5	1	7.22
				25	33.67
				100	10.63
Protopine	Y=1.12X+1.30	0.9931	0.25	1	24.86
				25	22.33
				100	30.89
Chelerthrine	Y=0.07X-1.50	0.9963	2	5	17.58
				25	5.25
				100	28.58
Sanguinarine	Y=0.42X-9.32	0.9926	1	5	14.51
				25	22.49
				100	8.73

Table 3. Concentration (mg/g, n=3) of 4 alkaloids in different part of the *Macleaya cordata*.

Sample	Allocriptopine	Protopine	Chelerythrine	Sanguinarine
Skin of the roots	5.594	4.95	0.84	0.6196
Woodiness of the roots	0.891	0.6953	0.1117	0.1312
Stems	0.7072	1.529	0.1537	0.3748
Fruits	6.758	13.39	4.83	15.95
Leaves	6.05	14.52	2.743	3.973



This developed reactive DCBI-MS is simple, rapid and sensitive for rapid qualification and semi-quantification of quaternary alkaloids in herbs.