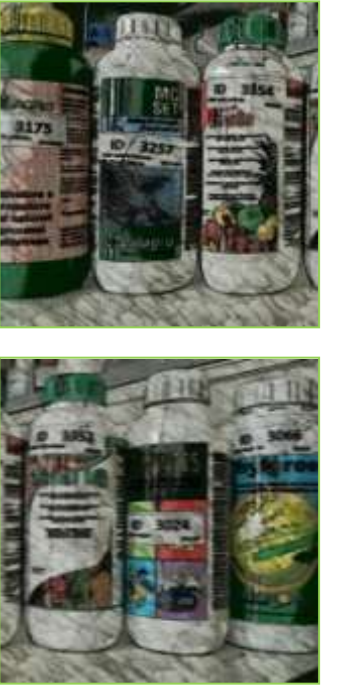




HPLC/MS-MS detection of alkaloids from *Sophora* spp. in bio-pesticide products for agriculture

Leonardo Sabatino, Michele Scarangella, Francesco Lazzaro, Monica Scordino, Giovanna Picariello, Claudia Leotta, Pasqualino Traulo, Giacomo Gagliano.



Ministero delle Politiche Agricole Alimentari e Forestali, Dipartimento dell'Ispettorato Centrale della Tutela della Qualità e Repressione Frodi dei Prodotti Agroalimentari (ICQRF) - Laboratorio di Catania, Via A. Volta, 19 - Catania
e-mail: l.sabatino@politicheagricole.it

INTRODUCTION

Plants of genus *Sophora* are commonly used in traditional Chinese medicine. Among them, such as the roots of *S. flavescens*, the roots of *S. tonkinensis* and the seeds of *S. alopecuroides* are widely used for the treatment of some skin, sore throat and inflammation. *S. flavescens* extracts contain tetracyclo-quinolizidine matrine-like alkaloids, and the main constituent are matrine and oxymatrine. Many Chinese and Korean scientific papers report the use of *Sophora* extract as "botanical-insecticide". The use of plants extract alkaloids are becoming an important issue due to the continuous demand of alternatives for synthetic pesticide to be used as plant protection products.

The registration of bio-pesticide in Europe is fraught by a complex regulatory framework. High costs, slow approval system, big data set with toxicological and eco-toxicological studies are required in order to authorize product on the market. As a consequence the EU-countries present a significant difficulty for bio-pesticide entering on the market. Alkaloids matrine and oxymatrine present in *Sophora* extracts are not authorized bio-pesticide.

The present method reports a suitable analytical method able to establish the occurrence of matrine and oxymatrine in market corroborants and fertilizers. The reverse phase separation coupled with MS-MS detection allowed the secure identification of the alkaloids. The precision and the accuracy of the method developed make it adequate to cope its quantification in fertilizers and corroborant plant extracts of the market. No time-consuming extraction procedure and toxic derivatization reagents were used, making the analysis appropriate for routine application. Due to the complexity of the matrix, quantification of the alkaloids is required by standard additions method. This screening should be carried out as routine analysis to assess quality of European fertilizers and corroborant plant extracts and could be integrated with the detection of unpermitted pesticides, preserving consumers and importers from commercial illicit and from potential toxicological consequences.

MATERIALS AND METHODS

Chemicals. Matrine (purity $\geq 97\%$), Oxymatrine (purity $\geq 98\%$) was obtained by Sigma-Aldrich (Milan, Italy). HPLC-grade methanol was supplied by Romil (Milan, Italy), analytical grade formic acid was by VWR International (Milan, Italy). Distilled water was purified at 18.2 M Ω with a Millipore MilliQ ULTRA (Milan, Italy) purification system.

Alkaloids free matrix. A liquid extract from algae and a liquid hydrolyzed animal epithelium were used as representative matrine and oxymatrine free matrixes for validation studies.

Standard solutions preparation and storage. Working standard solutions of alkaloids were prepared each time by diluting stock solutions (1'000 mg/L) in methanol/water (4:1 v/v).

HPLC/MS-MS conditions for alkaloids quantification. The analysis was performed with a liquid chromatograph consisting of a Finnigan Surveyor MS-pump, a Finnigan Surveyor autosampler and a Finnigan LCQ DECA XP MAX detector (Thermo Scientific, Milan, Italy). The analytical column was an Aquasil-C18, 150 x 2.1 mm (Thermo), 3 μ m, the column temperature 30 °C and the injection volume 10 μ L. The mobile phase for HPLC/MS-MS analysis was methanol (A) and formic acid 0.3% in water (B), at a flow rate of 200 μ L/min. The gradient program is reported in **Table 1**. Mass spectral analyses were performed using a LCQ ion-trap mass detector operating in the positive ion mode using an ion spray LC/MS interface. The MS-MS spectra were obtained using an applied collision energy of 35% of instrument maximum and the scan range was 50-300 m/z, sheath gas 18 units, auxiliary gas 16 units, capillary temperature 220°C, spray voltage 3.50 KV, positive mode.

Alkaloids quantification. 0.5 g of sample were added of 50 mL of methanol/water (4:1 v/v), sonicated for 10 minutes and centrifuged at room temperature at 3000 rpm with an ALC 4236 centrifuge (Italy). After filtration with 0.45 μ m PTFE filter (VWR, Italy), 100 μ l were added to 1 ml of methanol/water (1:1 v/v) and analyzed by HPLC/MS-MS.

HPLC/MS-MS identification of compounds, Figure 1. Studied alkaloids were characterized in terms of retention times (Rt)(\pm standard deviation, n=20) and MS-MS data as follows:

Matrine: (Rt), 6.4 \pm 0.1 min; MS, 249 [M+H]⁺ (100); MS-MS focused on [M+H]⁺, 249 [M+H]⁺ (100), 231 [M-H₂O+H]⁺ (10), 150 [M-C₅H₉NO+H]⁺ (30), 148 [M-C₆H₁₅N+H]⁺ (100).

Oxymatrine: (Rt), 7.6 \pm 0.1 min; MS, 265 [M+H]⁺ (100); MS-MS focused on [M+H]⁺, 265 [M+H]⁺ (10), 247 [M-H₂O+H]⁺ (100), 220 [M-CH₃NO+H]⁺ (20), 205 [M-C₂H₆NO+H]⁺ (60), 148 [M-C₅H₉NO-H₂O+H]⁺ (40).

RESULTS

A reversed phase high performance liquid chromatographic method (HPLC/MS-MS) has been developed and validated for detection of alkaloids *matrine* and *oxymatrine* in fertilizer with labeled enhancer plant defense activities. The analytical method was statistically validated (**Table 2**). The results show a strong matrix effect, requiring quantification by standard addition method. The regression lines showed $r^2 > 0.994$. Recoveries ranging from 97 to 104% were obtained for the fortification level of 0.01% w/w and the relative standard deviations ranged from 3 to 4% (n = 10). The limits of detection were below 0.0001% w/w, while the limits of quantification did not exceed 0.0004 % w/w. The method is currently applied in ICQRF Laboratory of Catania on fertilized and corroborant plant extract. In the frame of MIPAAF institutional quality control activity, 7 fertilizers and 3 corroborant plant extracts, contained one or both the alkaloids showing a mean content ranging from 0.02 to 2.4% w/w of these unpermitted active substances.

Table 1. HPLC Gradient:

A = Methanol; B = Water formic acid 0.3 %

	Time, minutes	A, %	B, %
1	0.00	15	85
2	9.00	50	50
3	24.50	95	5
4	28.00	40	60
5	30.00	15	85
6	32.00	15	85

Table 2. Method validation parameters

Parameter	Matrine	Oxymatrine
System precision, (% RSD) ^a	0.8	1.1
Linearity range (mg/L) ^b	1 - 100	1 - 100
Correlation coefficient, (r ²) ^b	0.994	0.995
LOD (% w/w)	0.0001	0.0001
LOQ (% w/w)	0.0004	0.0004
Precision, (% RSD) ^a	2.9	3.8
Accuracy, (%) ^a	97	104

^a n=10. ^b referred to standard addition method.

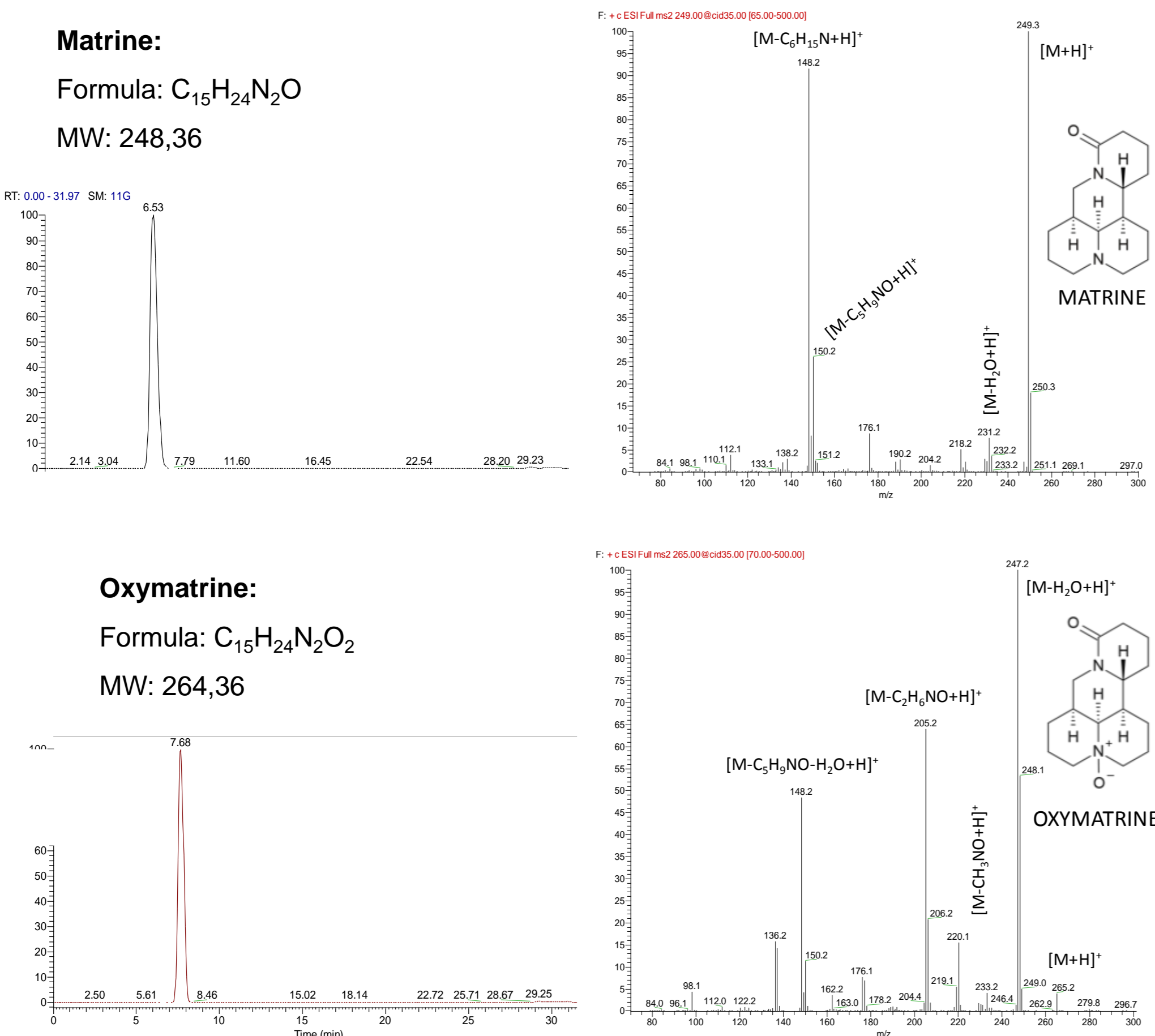


Figure 1. HPLC/MS-MS chromatogram of matrine and oxymatrine in spiked matrix.

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