Matrine: Screening and Quantification

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Background Information

Matrine is contained in several plants of the Fabaceae family (legumes) such as Sophora, Goebilia, Vexibia and Euchresta. Due to their content in alkaloids, these plants are considered poisonous to humans and livestock. Still, various species, especially from the genus Sophora are used in traditional Chinese medicine. The quinolizidine alkaloids matrine and oxymatrine are regarded as the main active ingredients of Sophora extracts. Both are considered hepatotoxic, but at the same time are employed in medicine against various illnesses.

In agriculture, matrine and oxymatrine have been shown to exhibit considerable efficacy against various insect pests, pathogenic fungi, bacteria, and nematodes. In Asian countries the use of S. flavescens extracts is common, and matrine is registered as a pesticide active substance in several countries. In China MRLs are established, e.g. for citrus fruits (1 mg/kg); pears and cucumbers (5 mg/kg each).

Within the EU, neither extracts containing matrine and oxymatrine nor the two active components themselves have ever been subjected to any official authorization process and are thus considered illegal.

In 2013/14 Italian authorities initiated broad enforcement actions after being alarmed about imports and illegal distributions of "natural fertilizers" and "plant extracts" from China containing Sophora flavescens. Since 2018 matrine and oxymatrine have been also in the focus of food control labs.

In 2019 matrine (but not oxymatrine) was included in the EU-list of pesticides. As there is no registration in place, the default MRL of 0.01 mg/kg applies. This also reinforces the position that Sophora extracts, containing matrine as an active ingredient, are not allowed to be used in organic agriculture.

Increasing controls and enforcement actions have reduced matrine findings, but there is still need for residue controls.

Aims

This study aimed at developing qualitative and quantitative methods for the control of matrine residues at low levels. For screening the priority was on methods routinely used in our lab (QuPPe+LC-MS/MS; QuEChERS+LC-MS/MS and QuEChERS+GC-Orbitrap-MS).

Extraction

Analytically, matrine poses difficulties due to its high, and pH-dependent, polarity (see logP curve in Fig. I). In theory acceptable QuEChERS recoveries can only be achieved at alkaline conditions and as can be seen in Figure I, this was also confirmed by recovery experiments. The QuEChERS-based method developed for nicotine was proved suitable also for matrine.

The QuPPe method, which does not involve a partitioning step, also provides good recoveries, but isotope labelled matrine is typically needed to adjust for the strong matrix effects. Initially, an ILIS was not available on the market, making it difficult to employ QuPPe for routine quantitative analysis of matrine.

In practice, matrine was thus initially screened using CEN-QuEChERS (combined with LC-MS/MS or GC-Orbitrap, later also with HILIC-LC-MS/MS) and in case of a positive result, quantitative analysis was conducted using alkaline QuEChERS combined with HILIC-LC-MS/MS or an optimized RP-LC-MS/MS. More info under "Measurement".

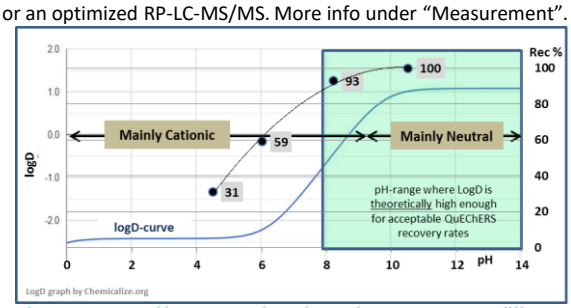

Figure I: computed logP curve of matrine and recovery rates at different partitioning pH values

Measurement

LC-MS/MS analysis: Chromatographic separation was accomplished by HILIC and RP columns. Initial trials showed that increasing eluent pH had a positive impact on intensity and shape of the matrine peak (see Fig. II). Sophoridine, a potentially interfering matrine isomer, fortunately eluted at a different RT in these cases (not shown here).

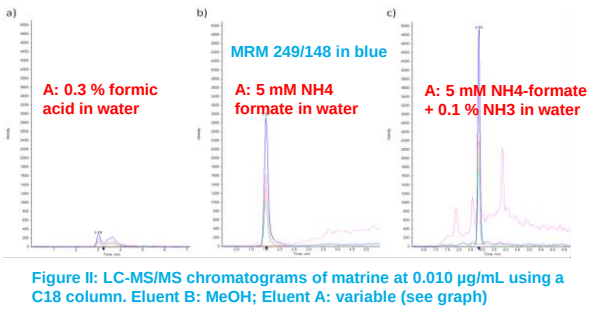

Figure II: LC-MS/MS chromatograms of matrine at 0.010 µg/mL using a C18 column. Eluent B: MeOH; Eluent A: variable (see graph)

GC-Orbitrap analysis: Sensitivity was less favorable compared to LC-MS/MS. Furthermore, matrine and oxymatrine signals were undistinguishable in terms of RT, peak shape and mass spectrum, raising the suspicion that a conversion of matrine to oxymatrine takes place during injection. Sophoridine fortunately eluted at a different RT.

Routine screening: As matrine recoveries using standard MRMs are low and as findings are rare there is great interest for introducing screening this compound. But with the default MRL applying, a sensitive screening approach is needed. Various methods routinely running in our lab were checked:

i. QuPPe 4.2 (HILIC using BEH Amide column)
ii. CEN QuEChERS followed by RP(C_{18})-LC-MS/MS
iii. CEN QuEChERS followed by GC-Orbitrap

Despite the poor recovery using CEN-QuEChERS (~30 %) and the strong matrix effects in QuPPe, screening at 0.01 mg/kg was possible with both approaches, i) and ii). In iii), apart of the specificity issues (not necessarily disadvantageous), the signals were in some cases too weak for deciding to trigger further actions. Type of matrix and addition of APs had a strong influence on signal intensity. Further tests, are planned to check whether GC-MS/MS is more useful for screening.

Quantification: Both, the HILIC method (i) and the optimized C18 method (ii; using eluent c) of Figure II) proved to be very suitable for quantification. After the ILIS became available, quantification is being done by QuPPe 4.2 from the first routine shot, together with the other compounds covered by this approach.

Extraction and Validation

The method was validated in cucumber, grape and lemon. With CEN-QuEChERS absolute recoveries were too low. Best results for matrine were achieved by QuPPe and alkaline QuEChERS (see Poster PO40).

Matrix	Matrine D_3 (as IS)	QuEChERS		Alk. QuEChERS		QuPPe	
		Mean Rec.	RSD %	Mean Rec.	RSD %	Mean Rec.	RSD %
Cucumber	No	35 %	2.2	90 %	1.8	92 %	4.5
	Yes*	94 %	2.4	100 %	2.1	95 %	3.4
Grape	No	24 %	0.7	41 %	3.2	93 %	3.5
	Yes	94 %	2.6	103 %	4.0	97 %	1.2
Lemon	No	39 %	1.3	63 %	9.3	89 %	5.1
	Yes	102 %	1.9	104 %	0.8	98 %	2.8

Table I: Matrine validation data at 0.01 mg/kg using different approaches

* Calc. using matrix matched calibration and nicotine D4 as IS as matrine D3 was not available at that time

Using the latter two approaches the SANTE validation requirements were met at the 0.01 mg/kg level in all cases using ILIS and in most cases using matrix-matched calibration without ILIS.

Summary

Methodologies for screening and quantitative analysis of matrine were developed. For more information see the EURL-SRM Analytical Observation report on matrine (to be published soon).

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