

Supplementary Materials

Matrix-Assisted DOSY for Analysis of Indole Alkaloid Mixtures

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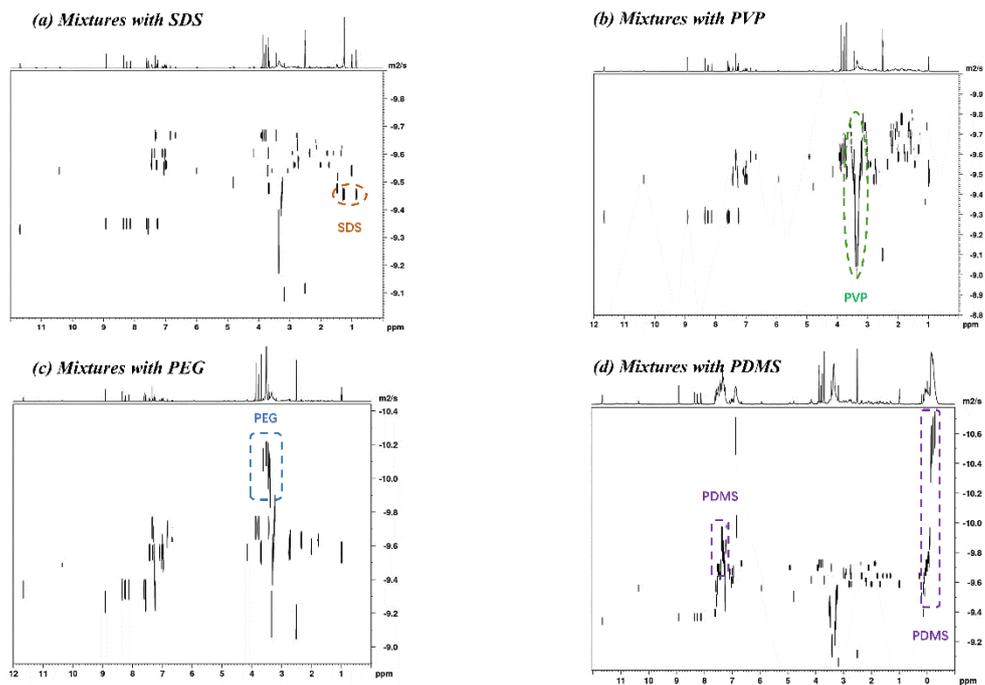


Fig. S1. Experimental results for an indole alkaloid mixture of reserpine, catharanthine, β -carboline and yohimbine in DMSO-*d*₆. 2D DOSY spectral of (a) mixtures with SDS, (b) mixtures with PVP, (c) mixtures with PEG, (d) mixtures with PDMS were obtained. Signals from different matrices were marked by circles of different colors. Diffusion coefficient (*D*) was reported in m²s⁻¹×10⁻¹⁰.

Figure S2. ^1H NMR spectra at 600 MHz of (a) indole alkaloid mixtures (each component was 2 mg) and (b) the same mixtures as in (a), but in the presence of SDS. Signals from SDS micelles were marked by red dot.

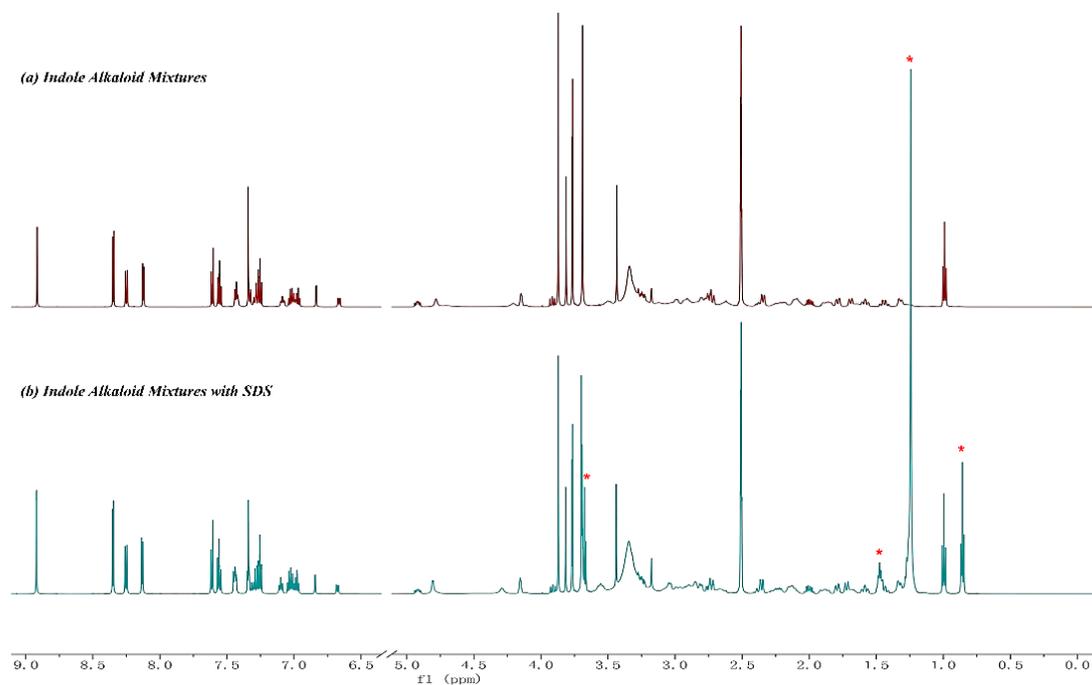


Table S1. Influence of different matrices on the diffusion coefficient and resolution.

	reserpine	yohimbine	catharanthine	β -carboline	ΔD_1	ΔD_2	ΔD_3
None	1.76	2.11	2.41	3.99	0.35	0.30	1.58
SDS	3.08	3.64	4.04	6.80	0.56	0.40	2.76
PEG	1.58	2.02	2.31	3.77	0.44	0.29	1.46
PVP	2.19	2.55	2.81	4.38	0.36	0.27	1.57
PDMS	1.63	2.09	2.22	3.67	0.46	0.13	1.45

$\Delta D_1 = D_{\text{yohimbine}} - D_{\text{reserpine}}$, $\Delta D_2 = D_{\text{catharanthine}} - D_{\text{yohimbine}}$, $\Delta D_3 = D_{\beta\text{-carboline}} - D_{\text{catharanthine}}$
Diffusion coefficient (D) was reported in $\text{m}^2\text{s}^{-1} \times 10^{-10}$.

Table S2. Influence of the number of dummy scans (DS) on diffusion coefficient and resolution.

DS	reserpine	yohimbine	catharanthine	β-carboline	ΔD_1	ΔD_2	ΔD_3
0	1.53	1.70	1.80	2.75	0.17	0.10	0.95
4	1.56	1.72	1.84	2.74	0.16	0.12	0.90
8	3.82	4.31	4.61	6.85	0.49	0.30	2.24
16	1.49	1.69	1.80	2.68	0.20	0.11	0.88

$\Delta D_1 = D_{\text{yohimbine}} - D_{\text{reserpine}}$, $\Delta D_2 = D_{\text{catharanthine}} - D_{\text{yohimbine}}$, $\Delta D_3 = D_{\beta\text{-carboline}} - D_{\text{catharanthine}}$
Diffusion coefficient (D) was reported in $\text{m}^2\text{s}^{-1} \times 10^{-10}$.

TableS3.Influence of the number of scans (NS) on diffusion coefficient and resolution.

NS	reserpine	yohimbine	catharanthine	β -carboline	ΔD_1	ΔD_2	ΔD_3
8	1.56	1.72	1.84	2.74	0.16	0.12	0.90
16	1.51	1.71	1.82	2.73	0.20	0.13	0.91
32	1.51	1.71	1.82	2.72	0.20	0.11	0.90

$\Delta D_1 = D_{\text{yohimbine}} - D_{\text{reserpine}}$, $\Delta D_2 = D_{\text{catharanthine}} - D_{\text{yohimbine}}$, $\Delta D_3 = D_{\beta\text{-carboline}} - D_{\text{catharanthine}}$
Diffusion coefficient (D) was reported in $\text{m}^2\text{s}^{-1} \times 10^{-10}$.

TableS4.Influence of the values of the FID data points (TD) on diffusion coefficient and resolution.

TD	reserpine	yohimbine	catharanthine	β -carboline	ΔD_1	ΔD_2	ΔD_3
16	1.56	1.72	1.84	2.74	0.16	0.12	0.90
32	1.76	1.93	2.06	2.94	0.17	0.13	0.88
64	1.69	1.87	2.00	2.88	0.18	0.13	0.88

$\Delta D_1 = D_{\text{yohimbine}} - D_{\text{reserpine}}$, $\Delta D_2 = D_{\text{catharanthine}} - D_{\text{yohimbine}}$, $\Delta D_3 = D_{\beta\text{-carboline}} - D_{\text{catharanthine}}$
Diffusion coefficient (D) was reported in $\text{m}^2\text{s}^{-1} \times 10^{-10}$.

Table S5. Influence of experimental temperature (T) on diffusion coefficient and resolution.

T (K)	reserpine	yohimbine	catharanthine	β -carboline	ΔD_1	ΔD_2	ΔD_3
293 K	2.90	3.17	3.35	4.80	0.27	0.18	1.45
298 K	3.49	3.78	3.98	5.68	0.29	0.20	1.70
303 K	3.64	3.94	4.14	5.83	0.30	0.20	1.69

$\Delta D_1 = D_{\text{yohimbine}} - D_{\text{reserpine}}$, $\Delta D_2 = D_{\text{catharanthine}} - D_{\text{yohimbine}}$, $\Delta D_3 = D_{\beta\text{-carboline}} - D_{\text{catharanthine}}$
Diffusion coefficient (D) was reported in $\text{m}^2\text{s}^{-1} \times 10^{-10}$.

Table S6. Influence of SDS concentration (C) on diffusion coefficient and resolution.

C	reserpine	yohimbine	catharanthine	β -carboline	ΔD_1	ΔD_2	ΔD_3
2mg	1.98	2.30	2.55	4.16	0.32	0.25	1.61
4mg	3.58	4.02	4.37	6.75	0.44	0.35	2.38
6mg	3.08	3.64	4.04	6.80	0.56	0.40	2.76
8mg	3.63	4.00	4.30	6.53	0.37	0.30	1.23
10mg	2.89	3.26	3.51	5.40	0.37	0.25	1.89

$\Delta D_1 = D_{\text{yohimbine}} - D_{\text{reserpine}}$, $\Delta D_2 = D_{\text{catharanthine}} - D_{\text{yohimbine}}$, $\Delta D_3 = D_{\beta\text{-carboline}} - D_{\text{catharanthine}}$
Diffusion coefficient (D) was reported in $\text{m}^2\text{s}^{-1} \times 10^{-10}$.

Table S7. Influence of the sample stability on diffusion coefficient and resolution.

Time(h)	reserpine	yohimbine	catharanthine	β-carboline
1h	5.22	2.93	2.70	2.21
2h	5.21	2.94	2.58	2.21
4h	5.21	2.93	2.60	2.19
8h	5.22	2.99	2.63	2.21

$\Delta D_1 = D_{\text{yohimbine}} - D_{\text{reserpine}}$, $\Delta D_2 = D_{\text{catharanthine}} - D_{\text{yohimbine}}$, $\Delta D_3 = D_{\beta\text{-carboline}} - D_{\text{catharanthine}}$
Diffusion coefficient (D) was reported in $\text{m}^2\text{s}^{-1} \times 10^{-10}$.

The literature indicates that several types of databases can be used. In the previous work of this research group, the ^1H chemical shift data of natural products was collected from the literature and has been used to establish a large “experimental database”. More than 200 indole alkaloids representing *Apocynaceae* were included in this experimental database. These alkaloids were selected because of the diversity of their structures. For some compounds whose ^1H spectra were not recorded in the literature, we used the NMRP module in the MestReNova software to make predictions in order to enrich the database.

As a routine verification test, the MestReNova software was used to search ^1H spectrum extracted from the DOSY spectrum of the *Alstonia mairei* mixtures and ranked by accuracy. In order to make the results more reliable, we used the search result of the proton spectrum extracted from the model mixture with SDS as a control. The option with the highest score was considered to be the compound we separated. Compared with the score of the experiment without SDS, it is obvious that the addition of a matrix can improve the resolution of DOSY spectra. The analyte signals were mixed together in the DOSY spectrum due to the similar structures of analytes. In practical sample, it can be seen that, the signal overlap was serious without SDS, and we can only roughly extract four ^1H spectra from the spectra. After adding a matrix, the strong overlapping areas can be well separated, we got nine ^1H spectra. Therefore, the matrix can be added to make the search results more accurate. And the results are as follows:

Figure S3. (a) DOSY spectrum of mixtures (each component was 2 mg) dissolved in 600 μ L of DMSO- d_6 . (b) DOSY spectrum of the same mixtures as in (a), but in the presence of 2 mg SDS.

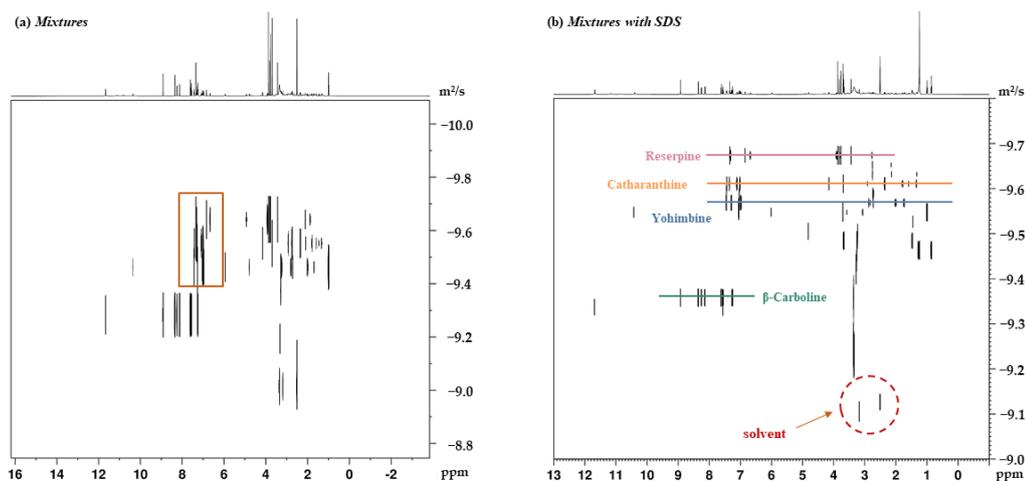


Table S8. Searching ^1H spectrum extracted from the DOSY spectrum of the model mixture in the database, and the following are the options with the highest scores and their corresponding compounds.

(a) Scores obtained from model mixtures without SDS and the corresponding compounds.

Spectra	Compound	Score (%)
C1	Reserpine	858
C2	Yohimbine	756
C3	Catharanthine	616
C4	β -Carboline	787

(b) Scores obtained from model mixtures with the addition of SDS and the corresponding compounds.

Spectra	Compound	Score (%)
C1	Reserpine	923
C2	Yohimbine	953
C3	Catharanthine	989
C4	β -Carboline	998

Figure S4. DOSY spectra of a mixture of the real total alkaloid extract (20 mg) before (a, upper) and after (b, lower) the addition of SDS (10 mg) in DMSO-d6 (600 μ L). Experimental temperature was 298 K.

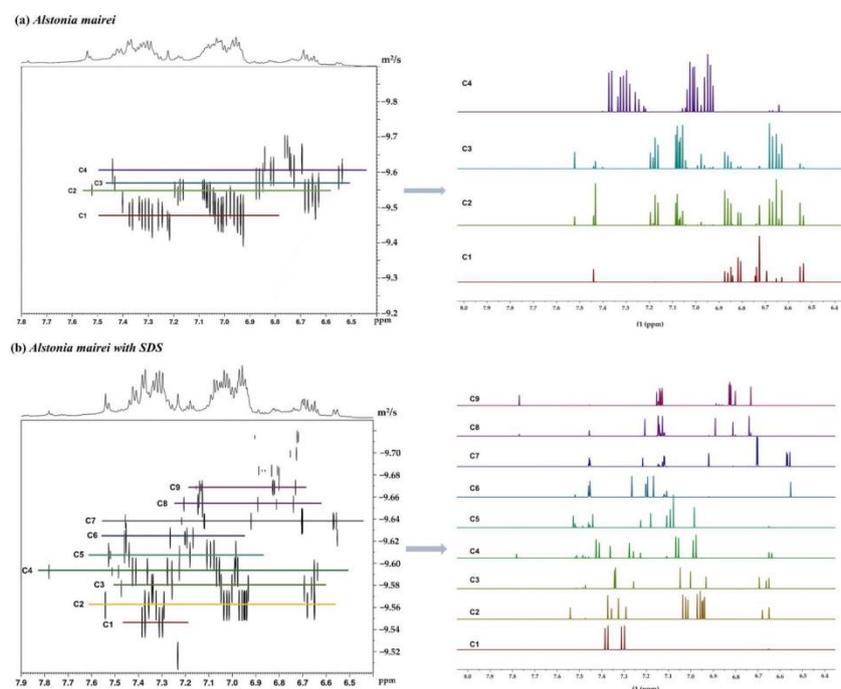


Table S9. Searching ^1H spectrum extracted from the DOSY spectrum of the actual sample mixture in the database, and the following are the options with the highest scores and their corresponding compounds.

(a) Search scores of four ^1H spectra extracted from total alkaloid extract of *Alstonia Mairei* without SDS in the database and the corresponding compounds.

Spectra	Compound	Score (%)
C1	Syringaresino	724
C2	Echitoveniline	538
C3	10-Hydroxy-dihydroperaksine	673
C4	N1-(hydroxymethyl)isositsirikine	848

(b) Search scores of nine ^1H spectra extracted from total alkaloid extract of *Alstonia Mairei* with SDS in the database and the corresponding compounds.

Spectra	Compound	Score (%)
C1	Tueiaoine	833
C2	N1-(hydroxymethyl)isositsirikine	898
C3	Rauvotetraphylline B	894
C4	10-Hydroxy-dihydroperaksine	929
C5	10-methoxypanarine	901
C6	Echitoveniline	868
C7	Syringaresino	870
C8	17-acetylsandwicine	848
C9	Cyclodicaprylactam	808