

Table S1: The sources and state of the eighteen royal jelly samples used in the study:

Sample #	Code	Source*	State
1	RJ1	Seif Pharmaceutical Company	lyophilized
2	RJ2	Seif Pharmaceutical Company	lyophilized
3	RJ3	Shana Apiary stores	lyophilized
4	RJ4	Shana Apiary stores	lyophilized
5	RJ5	Shana Apiary stores	Fresh
6	RJ6	Shana Apiary stores	Fresh
7	RJ7	Imtenan Apiary stores	lyophilized (imported)
8	RJ8	Imtenan Apiary stores	lyophilized (imported)
9	RJ9	Imtenan Apiary stores	Fresh (imported)
10	RJ10	Imtenan Apiary stores	Fresh (imported)
11	RJ11	Imtenan Apiary stores	Fresh (local)
12	RJ12	Imtenan Apiary stores	Fresh (local)
13	RJ13	Royal jelly 1000 capsules [®]	lyophilized
14	RJ14	Royal jelly 1000 capsules [®]	lyophilized
15	RJ15	El-Dakhakhny Apiary stores	Fresh
16	RJ16	El-Dakhakhny Apiary stores	Fresh
17	RJ17	El-Dakhakhny Apiary stores	lyophilized
18	RJ18	El-Dakhakhny Apiary stores	lyophilized

*samples from two different batches were obtained from each source

Table S2: Rf values and peak areas of the bands corresponding to the five quantified royal jelly fatty acids:

	(a) 8-hydroxyoctanoic acid	(b) 3,10-dihydroxydecanoic acid	(c) 10-hydroxy-2-decenoic acid (10-HDA)	(d) decanedioic acid	(e) 10-hydroxydecanoic acid
	Rf= 0.41	Rf= 0.50	Rf= 0.58	Rf=0.67	Rf= 0.73
	Peak area \pm SD*	Peak area \pm SD*	Peak area \pm SD*	Peak area \pm SD*	Peak area \pm SD*
STD MIX-5	20930 \pm 0.025	31219 \pm 0.154	92752 \pm 0.025	1396.991 \pm 0.251	24290 \pm 0.231
RJ1	26648.52747 \pm 0.064	46848.30503 \pm 0.254	131090.4509 \pm 0.214	4550.595271 \pm 0.251	59513.89782 \pm 0.014
RJ2	11065.27473 \pm 0.134	21108.11231 \pm 0.369	91108.39089 \pm 0.325	5123.277767 \pm 0.036	20390.31301 \pm 0.514
RJ3	8927.164835 \pm 0.052	8987.136349 \pm 0.452	57711.84406 \pm 0.412	4254.94468 \pm 0.145	9126.318156 \pm 0.241
RJ4	12319.51648 \pm 0.112	12841.81678 \pm 0.025	69021.78299 \pm 0.652	4259.552116 \pm 0.025	10933.01832 \pm 0.365
RJ5	1895.384615 \pm 0.0321	14037.06205 \pm 0.369	43695.16854 \pm 0.521	2443.857745 \pm 0.522	3870.2669 \pm 0.415
RJ6	10180.92308 \pm 0.054	38096.06237 \pm 0.145	81014.99983 \pm 0.011	3181.938304 \pm 0.215	21725.01186 \pm 0.021
RJ7	3563.468058 \pm 0.065	15041.18572 \pm 0.254	63939.8046 \pm 0.023	2013.288247 \pm 0.113	8604.798519 \pm 0.054
RJ8	5442.948174 \pm 0.147	20925.03232 \pm 0.354	79816.02001 \pm 0.321	2356.713585 \pm 0.214	14872.32356 \pm 0.085
RJ9	1218.218001 \pm 0.254	3999.930552 \pm 0.587	25749.26175 \pm 0.541	1592.914901 \pm 0.415	1047.447431 \pm 0.412
RJ10	1811.910673 \pm 0.087	6912.572414 \pm 0.652	33365.54351 \pm 0.652	2927.320698 \pm 0.051	1722.389666 \pm 0.325
RJ11	1665.291544 \pm 0.036	10696.76767 \pm 0.025	47111.65355 \pm 0.152	1965.286406 \pm 0.651	8256.602684 \pm 0.014
RJ12	1665.291544 \pm 0.154	22458.00173 \pm 0.361	72232.3988 \pm 0.163	1973.24316 \pm 0.214	24809.847 \pm 0.026
RJ13	13285.60856 \pm 0.325	60938.37968 \pm 0.452	14260.52078 \pm 0.251	20872.41193 \pm 0.321	33061.69125 \pm 0.258
RJ14	15433.65828 \pm 0.214	65839.95909 \pm 0.025	41537.78315 \pm 0.351	19072.80922 \pm 0.025	45473.25343 \pm 0.369
RJ15	1940.324949 \pm 0.584	18378.89735 \pm 0.036	54424.9644 \pm 0.423	4769.561871 \pm 0.036	4538.489907 \pm 0.214
RJ16	4781.180688 \pm 0.047	24703.96024 \pm 0.541	64429.24315 \pm 0.142	4405.803843 \pm 0.218	6082.466376 \pm 0.352
RJ17	8616.77479 \pm 0.036	8641.362976 \pm 0.361	55849.38772 \pm 0.025	2506.579997 \pm 0.425	5793.248882 \pm 0.456
RJ18	50243.64961 \pm 0.025	41735.53088 \pm 0.036	123573.1651 \pm 0.542	3707.496536 \pm 0.231	44202.60338 \pm 0.365

*SD is standard deviation of two determinations.

Table S3: Parametric and non-parametric evaluation of the repeatability (intra-day precision) and intermediate precision (inter-day precision) for the determination of royal jelly fatty acids by the developed HPTLC-image analysis method:

	Intra-day precision										Inter-day precision									
	Recovery% ^a																			
	8-hydroxyoctanoic acid		3,10-dihydroxydecanoic acid		10-hydroxy-2-decenoic acid		decanedioic acid		10-hydroxydecanoic acid		8-hydroxyoctanoic acid		3,10-dihydroxydecanoic acid		10-hydroxy-2-decenoic acid		decanedioic acid		10-hydroxydecanoic acid	
Nominal fatty acid concentration ($\mu\text{g spot}^{-1}$)	P	NP	P	NP	P	NP	P	NP	P	NP	P	NP	P	NP	P	NP	P	NP	P	NP
10	97.45	99.45	96.96	99.55	96.45	99.45	98.15	99.85	97.48	99.54	98.47	99.78	96.48	99.78	96.45	99.45	98.45	99.65	97.45	99.78
20	96.15	99.15	97.58	99.13	97.48	99.87	97.45	99.78	97.46	99.71	97.45	99.45	97.45	100.13	96.87	99.65	97.12	100.11	98.22	99.98
30	96.78	99.78	98.45	99.45	98.45	99.31	97.65	99.66	98.45	99.45	96.45	99.34	97.47	100.10	97.45	99.32	96.98	100.05	97.99	100.00
Mean% ^b	96.79	99.46	97.66	99.38	97.46	99.54	97.75	99.76	97.80	99.57	97.46	99.52	97.13	100.00	96.92	99.47	97.52	99.92	97.89	99.92
SD ^c	0.650	0.315	0.749	0.220	1.00	0.291	0.361	0.096	0.566	0.132	1.010	0.229	0.566	0.194	0.502	0.166	0.811	0.240	0.395	0.122
RSD(%) ^d	0.672	0.317	0.766	0.221	1.026	0.293	0.369	0.096	0.579	0.133	1.036	0.230	0.583	0.194	0.518	0.167	0.832	0.240	0.404	0.122
F-test ^e	-	4.49	-	12.05	-	12.29	-	14.67	-	19.04	-	20.29	-	9.020	-	9.611	-	11.98	-	10.99

a the mean recovery of three determinations at each concentration level of the fatty acid.

b the mean of all recoveries at different concentration levels of the fatty acid.

c standard deviation of the mean of recoveries at different concentration levels of the fatty acid.

d percentage relative standard deviation.

e F-test for comparison between two sample variances, F critical=4.433 ($n_1=n_2=9$, $P=0.05$)

