Analytical and Bioanalytical Chemistry

Electronic Supplementary Material

New CZE-DAD method for honeybee venom analysis and standardization of the product

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Table S1. The variation coefficients (CV) of related peak areas (A) and migration times (t_M) for different concentrations of apamine, MCDP, phospholipase A_2 and melittin (analysis repeatability and injection repeatability).

Standard	Conc. [μg/g]	Analysis repeatability		Injection repeatability	
		CV [%]		CV [%]	
		A	t_M	A	t_M
MCDP	4.9	9.21	0.64	-	-
	9.6	7.22	1.17	4.57	5.14
	14.4	2.20	1.02	5.69	6.85
	19.2	3.30	0.36	2.10	8.46
	23.2	1.30	1.14	8.44	8.03
	32.0	3.46	1.22	1.87	1.14
Apamine	1.8	3.59	2.07	9.45	0.71
	3.6	8.44	0.34	10.29	0.12
	6.1	3.98	1.13	2.28	0.34
	9.0	6.29	0.53	2.09	0.33
	11.9	5.89	0.31	2.83	0.61
	14.8	1.66	0.61	1.05	0.44
	20.1	8.18	1.32	2.30	1.21
Phospholipase A_2	9.9	12.82	2.74	8.17	1.33
	21.1	10.87	2.67	6.32	1.92
	42.1	5.47	0.94	10.11	1.81
	62.0	3.46	5.16	11.65	7.33
	84.4	5.75	1.63	5.95	5.85
	103.2	1.44	0.48	1.50	6.49
Melittin	27.6	3.87	6.82	2.03	0.76
	49.1	3.14	0.15	2.82	1.11
	104.1	4.15	0.31	2.58	0.46
	154.4	3.02	0.38	3.45	0.44
	202.0	4.94	0.61	2.14	0.29
	256.6	3.41	0.23	2.11	0.67
	306.3	3.75	5.31	2.96	2.23

Table S2. Recoveries [%] of analytical standards.

Standard	Method I*	Method II**	Method III***
MCDP	79.17	87.31	92.16
	72.53	89.43	
	78.37	81.64	
Apamine	91.12	97.35	147.58
	84.65	92.43	
	87.48	87.20	
Phospholipase A_2	111.62	104.96	118.06
	109.48	105.19	
	112.31	111.94	
Melittin	101.16	100.09	108.27
	92.38	98.70	
	106.34	108.18	

* Method I – Bee venom solutions (301.6 μg/g) were mixed with analytical standards in 1:1 ratio. The total contents of standards were approximately 80%, 100% and 120% in relation to dry mass of bee venom.

* Method II – Bee venom solutions (602.1 μg/g) were mixed with analytical standards in 1:1 ratio. The total contents of standards were approximately 125%, 150% and 175% in relation to dry mass of bee venom.

***Method III– The mixture with the known concentrations of the analyzed standards was prepared, and then recovery of them was established. The total contents of standards in mixture obtained were similar to those from natural honeybee venom.

Table S3. The calibration data of the HPCE and HPLC for MCDP, apamine, phospholipase A_2 and melittin.

Standard	HPCE			HPLC		
	R	a	b	R	a	b
MCDP	0,9985	0,0137	– 0,0143	0,9986	0,0305	– 0,0539
Apamine	0,9976	0,0331	– 0,0229	0,9996	0,0466	– 0,0115
Phospholipase A_2	0,9968	0,0443	– 0,3179	0,9996	0,0418	0,1795
Melittin	0,9999	0,0376	– 0,1475	0,9999	0,0551	– 0,3473

Table S4. Factor loadings for all analyzed constituents (apamine, MCDP, phospholipase A_2, melittin) of bee venom (samples number 1 to 19).

	PC1	PC2	PC3	PC4
Apamine	-0.972	0.071	0.084	0.209
MCDP	-0.939	0.319	0.023	-0.126
Phospholipase A_2	-0.944	-0.275	0.159	-0.092
Melittin	-0.957	-0.114	-0.265	0.001
Eigenvalue	3.634	0.195	0.103	0.068
Proportion	0.908	0.049	0.026	0.017
Cumulative	0.908	0.957	0.983	1.000

Table S5. The correlation matrix for all analyzed constituents (apamine, MCDP, phospholipase A_2, melittin) of bee venom (samples number 1 to 19).

	Apamine	MCDP	Phospholipase A_2	Melittin
Apamine	1.000	0.911	0.892	0.900
MCDP	0.911	1.000	0.814	0.857
Phospholipase A_2	0.892	0.814	1.000	0.893
Melittin	0.900	0.857	0.893	1.000