Table 1: System-suitability data. Mean ± SD, (*n* = 5)

Property	Compounds	Value	RSD (%)	Required limits
Resolution (R)	Diosmin	22.46 ± 0.15	0.65	R > 1.5
	Linarin	20.71 ± 0.07	0.33	
	Pulegone	2.96 ± 0.01	0.44	
Retention time (RT)	Diosmin	3.66 ± 0.008	0.22	RSD £ 1%
	Linarin	7.98 ± 0.010	0.13	
	Pulegone	14.23 ± 0.007	0.05	
Theoretical plates (N)	Diosmin	5439 ± 87.6	1.61	N > 2000
	Linarin	21429 ± 55.2	1.26	
	Pulegone	28813 ± 321	1.11	
Tailing factor (T)	Diosmin	0.95 ± 0.005	0.58	1.05 > T > 0.95
	Linarin	0.99 ± 0.004	0.45	
	Pulegone	1.04 ± 0.004	0.43	

Table 2: Statistical results of linear regression equation analysis in the determination of the three investigated compounds

Compounds	Regression equation	r^2	Linear range (mg/mL)	LOD (µg/mL)	LOQ (µg/mL)
Diosmin	Y = 890.4115X - 0.3198	0.999	0.0122-0.1708	0.313	0.607
Linarin	Y = 1196.5880X - 1.4305	0.999	0.0276-0.3864	0.177	0.504
Pulegone	Y = 3948.4880X - 1.4843	0.999	0.0063-0.0879	0.068	0.171

and LOD values for the three chemical components are also listed in Table 2.

Stability and precision

Intra-day and inter-day precision and accuracy were evaluated by analyzing quality-control samples. The intra-day variation was examined by analyzing five individual sample solutions from the same crude sample of *Z. clinopodioides* Lam on the same day. Inter-day precision and accuracy were determined by once daily trials for three consecutive days. Variations were expressed as relative standard deviation (RSD). The values of the intra-and inter-day variations were less than 2.0%. The instrumental precision was evaluated by five replicate injections of the ban fang ditch sample solution, and RSD value was below 0.89%.

Reproducibility

The reproducibility of extraction was also investigated for the three components by comparing six samples from six independent extractions. Six 0.2000 g samples of *Z. clinopodioides* Lam power from Ban fang ditch were accurately weighed, prepared, and analyzed by RRLC. The RSD values of the six replicates were less than 2.0% for all compounds, demonstrating the high reproducibility of the sample preparation procedure.

Recovery

Recovery tests were performed to further investigate the reproducibility and efficiency of the extraction and analysis method. Recoveries of the three compounds were determined by the method of standard addition. Three concentrations of the compound standard solutions were used to spike *Z. clinopodioides* Lam samples containing known amounts of each compound (namely 50% of the compound). The mixture was extracted and analyzed as described. The mean recoveries of the three compounds were 104.1% for diosmin, 102.3% for linarin, and 97.4% for pulegone, with RSD values of 1.6, 1.2, and 2.1%, respectively.

The obtained results indicate that the developed analytical method was reproducible with high accuracy. It is therefore satisfactory for quantitative analysis.

Application to the analysis of Ziziphora clinopodioides Lam samples

The developed analytical method was successfully applied for the simultaneous determination of the three components in ten different samples of *Z. clinopodioides* Lam. All three compounds were detected from every sample. Each sample was determined in triplicate and the peaks in the chromatograms were identified by comparing the retention times and UV spectra with the authentic standards. The three compounds were quantified in the ten samples [Table 3].

DISCUSSION

We describe a new method of RRLC separation, using 1.8 µm particle size of stationary phase instead of the usual 5 µm columns, is proven to be efficient, precise, accurate, sensitive and time saving, and enabled determination of diosmin, linarin, and pulegone from *Z. clinopodioides* Lam using reversed-phase chromatography with gradient elution and a DAD detector. Simultaneous detection could allow

Table 3: Contents of the three compounds in ten different origin samples of *Ziziphora clinopodioides Lam*. (*n* = 3)

Sources	Diosmin contents (mg/g)	Linarin contents (mg/g)	Polegone contents (mg/g)
Tex	3.1186 ± 0.1156	9.1380 ± 0.1804	0.6102 ± 0.0012
Ban fang ditch	7.0749 ± 0.0501	15.7627 ± 0.1195	3.6721 ± 0.0088
Altay	7.6636 ± 0.1997	7.9919 ± 0.0599	4.0976 ± 0.0063
Tuoli	5.4379 ± 0.0831	24.2941 ± 0.0556	0.5174 ± 0.0047
Xiata Road	4.3156 ± 0.0568	14.1554 ± 0.0545	0.6751 ± 0.0008
Zhaosu Highway	5.7606 ± 0.1123	13.8769 ± 0.0104	1.8499 ± 0.0461
Guozigou	6.1576 ± 0.0338	18.8340 ± 0.0713	2.6950 ± 0.0118
Wulabo	4.9090 ± 0.0499	11.8823 ± 0.0405	0.3493 ± 0.0021
Fukang	7.3029 ± 0.1353	6.1507 ± 0.0564	0.8760 ± 0.0108
Jimsar	4.2613 ± 0.0852	4.7203 ± 0.0284	0.3941 ± 0.0025

for a large number of herbal samples to be analyzed in a relatively short period of time. Further studies are ongoing in our laboratory to further characterize activate compounds of *Z. clinopodioides* Lam. It is clear that there was a significant variation in the contents of the three compounds between the ten samples obtained from different regions. Similar variations have been found for other components and may be attributed to the different growing conditions at the sampling sites. These variations in the bioactive components influence the medicinal quality, so it was necessary to develop an effective qualitative and quantitative method to evaluate the overall quality of *Z. clinopodioides* Lam. The simultaneous analysis of several chemical components is a promising tool for the quality control of medicinal herbs.

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