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Analysis of Iranian rosemary essential oil: Application of gas chromatography–mass spectrometry combined with chemometrics

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ABSTRACT

This paper focuses on characterization of the components of Iranian rosemary essential oil using gas chromatography–mass spectrometry (GC–MS). Multivariate curve resolution (MCR) approach was used to overcome the problem of background, baseline offset and overlapping/embedded peaks in GC–MS. The analysis of GC–MS data revealed that sixty eight components exist in the rosemary essential oil. However, with the help of MCR this number was extended to ninety nine components with concentrations higher than 0.01%, which accounts for 98.23% of the total relative content of the rosemary essential oil. The most important constituents of the Iranian rosemary are 1,8-cineole (23.47%), α-pinene (21.74%), berbonone (7.57%), camphor (7.21%) and eucalyptol (4.49%).

1. Introduction

Rosemary (*Rosmarinus officinalis*) is a woody, perennial herb with fragrant evergreen needle-like leaves. It is native to the mediterranean region and is a member of the mint family Lamiaceae. Rosemary is extremely high in iron, calcium, and vitamin B_6. It has a very old reputation for improving memory, and has been used as a symbol for remembrance in Europe. Carnosic acid, found in rosemary, shields the brain from the free radicals. It grows in most regions in Iran. Also it can be found in northern African countries such as Morocco, Tunisia, in southern Europe countries especially in Spain, France, Italy, the area of former Yugoslavia and also it can be found in America. It is useful for remedy of anxiety, bloat, migraine, hypertension, headache, anorexia as an edible compound and it can also be used as a local analgesia in the treatment of muscular pains, rheumatic diseases. In addition, it is suitable for cosmetic–sanitary industry because of its odor and taste. Oil of rosemary is one of the effective oils, its effect on the nervous system is very positive, and also it is very good for prevention of hair loss because of vasodilatation and improved circulation. Eating rosemary makes secretion and repulse gall more facilitated and it is useable in jaundice and hepatic diseases, also in general weakness, excessive fatigue, lethargy for recovery period. Because of remedy properties, rosemary uses to treat Parkinson's [1], Alzheimer [2], also it has antidiabetogenic [3], antifungal [4], antimicrobial [5], anti-inflammatory [6], antiplatelet [7] and antioxidant [3,5] effects. In general, many researchers focus on the analyzing the rosemary essential oil. Numerous researches have been reported on Pharmacia characteristics of rosemary essential oil [8–12]. Also genetic diversity and chemical variation levels of rosemary essential oil have been studied in relation to bioclimatic and geographic location [13]. Some other types of research are reported on the case of using different methods for the extraction of the rosemary components [14–18]. Also, as a sophisticated technique, comprehensive two-dimensional GC and time of flight mass spectrometry (GC × GC–TOFMS) was used [19]. Gas chromatography–mass spectrometry (GC–MS) is one of the most promising techniques for the determination of the components of essential oils [20–24]. Second order instruments involving separation are ideally suited for the analysis of complex samples and are frequently used as powerful tools for chemical analysis. For GC–MS technique, much more components are qualitatively and quantitatively analyzed, but their identifications are performed only through the direct similarity searches in the MS databases attached to the GC–MS instruments. Even under the best experimental conditions, the probability of peak overlap in chromatographic separations can become quite severe, especially for highly complex samples. This is due to the existence of the background, baseline offset, and some overlapping/embedded peaks. These problems can result in a wrong similarity match in the MS library and therefore, true determination of the components cannot be achieved. In these cases, resolution and afterwards quantification of the target compounds becomes a goal. Moreover, even using sophisticated chromatographic technologies, there is a question about the maximum information that

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can be obtained from the instrument and whether the use of appropriate tools for data analysis could improve the interpretation of the results. Fortunately, with the development of chemometric resolution techniques, the extraction of required information about the components in a complex mixture has become possible. Many associated methods, such as evolving factor analysis (EFA) [25,26] fixed size moving window-evolving factor analysis method (FSMW-EFA) [27], window factor analysis (WFA) [28,29], heuristic evolving latent projection (HELP) [30,31], sub-window factor analysis (SFA) [32,33], orthogonal projection regression (OPR) [34] and multivariate curve resolution-alternating least squares (MCR-ALS) [35–39] have been developed to provide more information from the chemical analysis, both in chromatographic separations and in spectral identification [40–43]. Multivariate curve resolution (MCR) methods have been used for the analysis of unresolved peaks in chromatographic separations coupled to multichannel detection such as high performance liquid chromatography–diode array detector (HPLC–DAD), liquid chromatography–mass spectrometry (LC–MS), and GC–MS.

The methods of HELP, OPR and MCR-ALS are the powerful techniques for the mathematical resolution of chromatographic and spectral profiles of pure components of many mixtures and application of these methods are reported in many cases. Hence, the combination of hyphenated instruments and relevant chemometric methods opens a new way for a quick and accurate analysis of the real complex samples. To the best of our knowledge, the components of Iranian rosemary essential oil are not identified yet. Therefore, the main aim of the present work was identification and determination of the components of rosemary essential oil cultivated in Iran. In this paper, the volatile components of Iranian rosemary complex mixture are determined by using GC–MS under appropriate conditions combined with the chemometric techniques. In the present work, the background has been corrected by using Liang et al. method [30,31]. The procedure for noise correction was taken from Savitzky–Golay filter [45] and the number of components for each peak cluster was determined by calculating the morphological scores [44]. Also, the peak purity assessment of the two-dimensional data was controlled by FSMW-EFA [27] and EFA techniques [25,26]. In addition, simple-to-use interactive self-modeling mixture analysis (SIMPLISMA) [46,47] and orthogonal projection approach (OPA) [48–50] were used for the selection of pure variables. HELP [30,31], OPR [34] and MCR-ALS [35–39] methods were used as resolving techniques for the resolution of chromatographic and spectral profiles of the essential oil.

2. Methodology

The detailed theories behind the HELP, OPR and MCR-ALS techniques are given elsewhere [30,31,34–39] and also in Supplementary material section. However, a brief description of the methods is presented in this work to make the article more consistent and understandable.

The method of HELP resolves the overlapping peaks on the base of having selective and zero regions. In GC–MS data, there are selective regions in elution of components in chromatographic direction or concentration profile.

In contrary, the method of OPR resolves species by using projection methodology and do not require selective region. Projection matrix is constructed from $(n-1)$ orthonormal loading vectors and the information of the analyte n will be obtained from the other coexisting $(n-1)$ analytes.

The method of MCR-ALS calculates the concentration and pure spectral profiles in an iterative way. This algorithm starts with initial estimates obtained by using the techniques of EFA [25,26], SIMPLISMA [46,47] or OPA [48–50] and proper constraints (e.g. non-negativity, unimodality, normalization and selectivity) can be applied during the ALS optimization until the concentration and pure spectra optimally fit in the experimental data matrix.

3. Experimental

3.1. Reagents and materials

The plant *R. officinalis* was collected in May 2006 from the north of Tehran, Iran. Chemicals such as n-pentane and sodium sulfate with purity higher than 99% were purchased from Merck (Darmstadt, Germany). Standards of normal alkanes (C-6 to C-18) were purchased from Ultra Scientific (North Kingstown, RI, USA).

3.2. Instrumentation and analysis

GC–MS analyses were performed on an Agilent Technologies (USA) 6890 GC system coupled with a post column splitting 5973 network mass selective detector with a quadrupole analyzer and resolution of 0.1 amu. This system was equipped with a HP5-MS capillary fused silica column (60 m × 0.25 mm i.d.), 0.25 μm film thickness, methyl 5% phenyl polysiloxane (Agilent Technologies, USA). The temperature program initiated at 40 °C, held for 1 min then raised at 3 °C min^{-1} to 250 °C, held for 20 min. Carrier gas, helium (99.999%); with a flow rate of 1 mL min^{-1}; injector temperature, 250 °C; split ratio, 1:50. Mass spectra were taken at 70 eV. Mass range was from 20 to 500 amu. An enhanced ChemStation G1701 DA version D.00.01.27 was used for the data collection and processing. The injections of sample into GC–MS were carried out using a 10-μL micro-syringe model ITO MS-E10 (Japan) with the needle tip of angled cut.

3.3. Isolation of essential oil by hydrodistillation

The plant *R. officinalis* were dried under shade at room temperature for 48 h. Then, 50.00 g of aerial parts were separated, ground and fully submerged in water in a 1000 mL round bottom flask. The mixture was hydro-distilled in a full glass Clevenger-type apparatus. The distillation was prolonged for 3.5 h. When the system cooled down, the essential oil were decanted from the water and dried with anhydrous sodium sulfate. Then, the oil was weighted and stored in a dark glass bottle at 4 °C. The extraction yield was 2.05%.

3.4. Identification

The essential oil components were identified by comparison of their retention indices (RIs) and mass spectral fragmentation patterns with those of standards stored on the NIST v1.7 computer library. The Kovats RIs of the constituents were obtained using gas chromatograms by logarithmic interpolation between bracketing n-alkanes [51,24,52].

3.5. Data analysis

The analyses were performed with the use of an Agilent Technology (HP) gas chromatograph–mass spectrometer. An Enhanced ChemStation G1701DA version D.00.01.27 was used for the data collection and conversion to ASCII format. Data analyses were performed on a Pentium-based HP-Compaq personal computer. Except for the MCR-ALS that downloaded from the website [53], all programs for the chemometric resolution methods were coded in MATLAB 6.5 for Windows by the authors. The library searches and spectral matching of the resolved pure components were conducted on the NIST MS database.

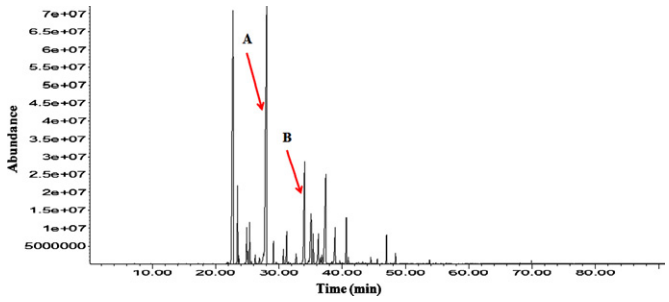

Fig. 1. The total ion chromatogram (TIC) of the rosemary essential oil.

4. Results and discussion

4.1. Qualitative analysis of the Iranian rosemary essential oil

The total ion (current) chromatogram (TIC) of the rosemary essential oil is shown in Fig. 1. This figure demonstrates the complexity of such a mixture by showing several overlapped peaks. The similarity indices obtained from direct searching using MS database are very low for many chromatographic peaks. Also, at different scan points of a single peak one can obtain different compounds using library searching. If the overlapped peaks could not be resolved, the traditional searching based on MS database would fail. It was found that 74 peaks were partially separated from each other in the TIC. Accordingly, the TIC was divided into 74 sub-

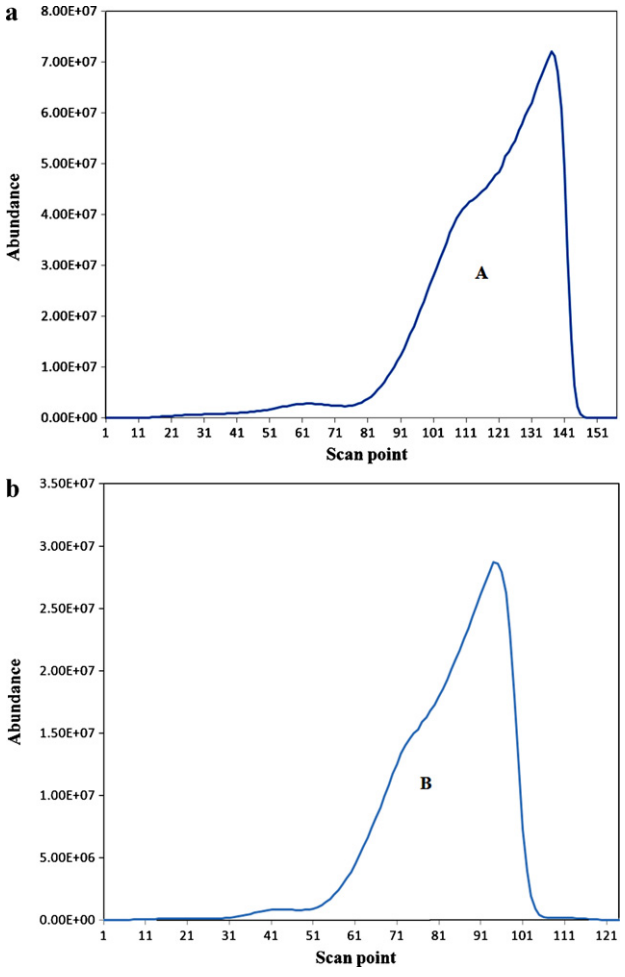

Fig. 2. The local TICs, (a) A and (b) B.

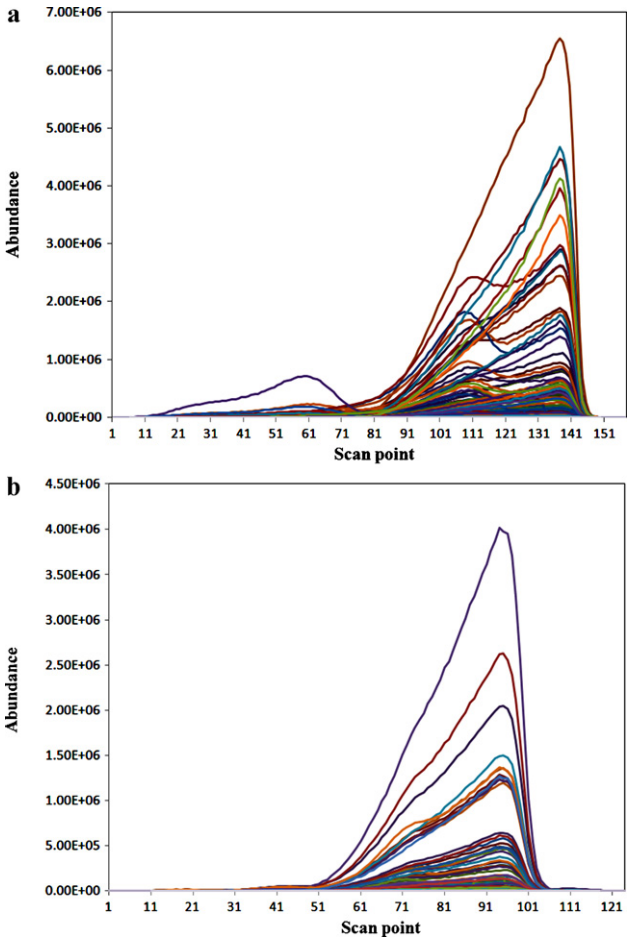

Fig. 3. The peak clusters, (a) A and (b) B.

matrices by using zero component regions along with the elution sequence of the essential oil. Here, matrices were exported by "Tools/Export 3-D Data" that was provided in Agilent MSD ChemStation and saved in a file with the data stored in ASCII format that are compatible with the MATLAB software. Each matrix makes a peak cluster, so there are 74 peak clusters. Among these peaks clusters, there were 35 single component peaks, which were easily identified and quantified by direct library searches. However, 39 peaks were remained from which 26 peak clusters were two-component, 6 peak clusters were three-component, 3 peak clusters were four-component, 3 peak clusters were five-component, and 1 peak cluster was six-component. Essentially, overlapping components must be resolved into pure chromatographic profiles and mass spectra for the quantitative and qualitative analyses. In order to illustrate the resolution procedure, as an example, two peak clusters were selected and labeled by A and B in Fig. 1 and their local TICs were shown in Fig. 2a and b, respectively. The exported matrices in MATLAB were shown in Fig. 3a and 3b, respectively, A (157×251), within range 27.306–28.336 min and B (124×251), within range 33.668–34.486 min.

First, to avoid the effect of background and noise in measured data, it is necessary to remove them. The background correction in this work was performed by using the method of Liang et al. [30,31]. In this method, the local rank analysis of zero component regions can provide sufficient information for uni-variate linear regression with respect to the retention time and then correcting the baseline. The procedure for noise correction applied in this work was taken from Savitzky–Golay filter [45]. Most methods determine the chemical rank on the basis of PCA or singular value decomposi-

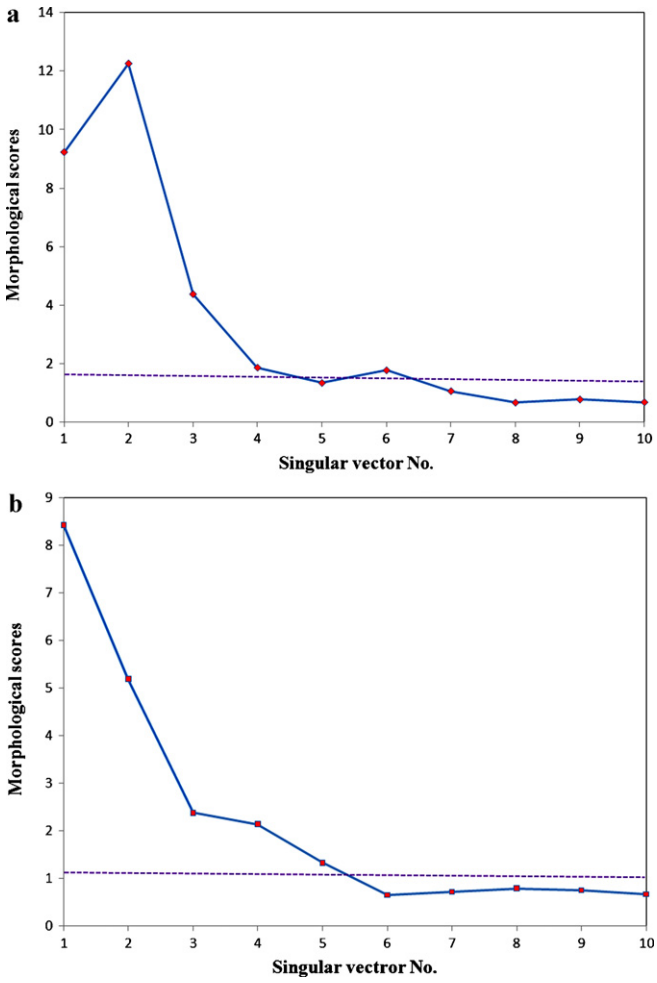

Fig. 4. The morphological score plots of peak clusters, (a) A and (b) B.

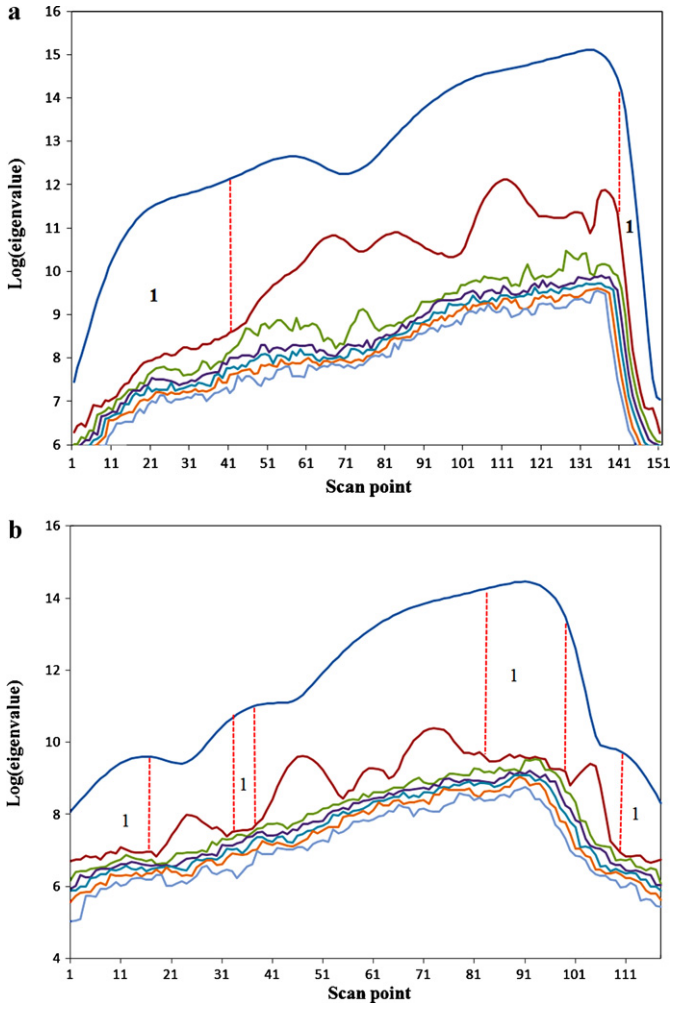

Fig. 5. The plots of FSMW-EFA of peak clusters, (a) A and (b) B.

tion (SVD). However, for complex systems analyzed by hyphenated chromatographic methods (GC–MS, HPLC–DAD, etc.), it is often difficult to arrive at a safe result by using PCA of the full data matrix because of the accumulation of noise. GC–MS data acquired in full scan mode contains many noisy channels. Deleting the noise channel would result in a faster computation. If a channel is due to the analytes, its chromatographic profile is continuous and smooth. On the other hand, channels due to noise consist of random signals. In order to avoid accumulation of noise, key spectra instead of full rank matrix would be analyzed by the morphological score method. In the present contribution, a criterion (the morphological score) is used to discriminate between the noise and the signal channels [44]. Channels whose morphological scores were below than the noise limit were removed. Therefore, prior to starting the chemometric resolution techniques, the noisy mass channels were removed from each peak cluster and then the number of components in each peak cluster was determined by the same procedure (morphological score) [44]. Morphological score plots for the peak clusters A and B are shown in Fig. 4a and b, respectively. It is clear from these plots that there are five components in both peak clusters. This was concluded by counting the number of singular vectors with the morphological scores upper than that of the noise levels. The next step was investigating the peak purity resolution of two-dimensional data which can be controlled by the FSMW-EFA method [27]. Fig. 5a and b shows FSMW-EFA plots for the peak clusters A and B, respectively after the noise correction. In Fig. 5a the selective elution regions of the first and the last components of the peak cluster A, which are marked in the plot by number "1", are shown. Although the HELP method has been used extensively for the resolution of many complex mixtures, but it cannot be applied only for the peak cluster A. This is due to the absence of selective regions for all components and therefore OPR method was also chosen for this purpose. From the number of components identified by the morphological score and the number of components that have selective regions in FSMW-EFA plot, it can be demonstrated that the

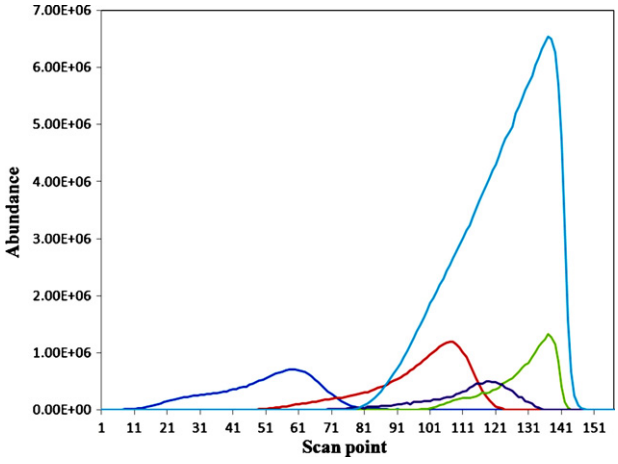

Fig. 6. The resolved concentration profile of the peak cluster A.

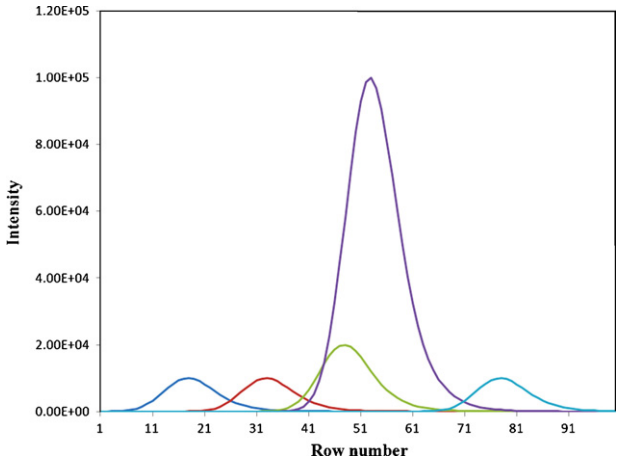

Fig. 7. The stimulated matrix.

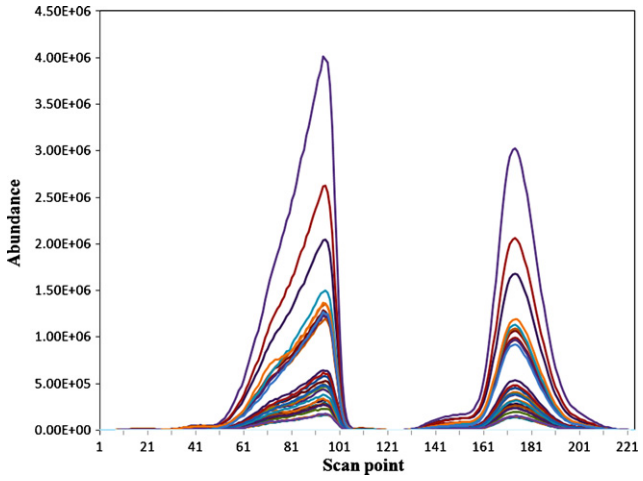

Fig. 8. The pseudo-augmented matrix.

peak cluster A is consisted of two components, which have selective regions and three components without selective regions. First, pure concentration profiles of the two components which have selective regions were extracted by using the HELP technique, and then mass spectra were predicted by a pseudo-inverse method. The pure mass chromatograms of each component can be obtained with multiplying the predicted mass spectra and pure concentration profile. If

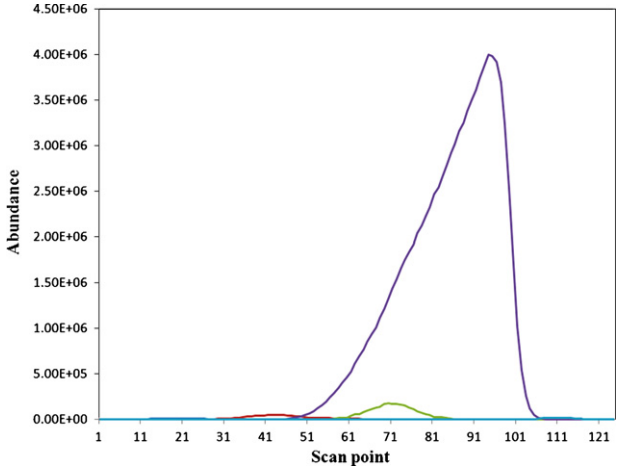

Fig. 9. The resolved concentration profile of the peak cluster B.

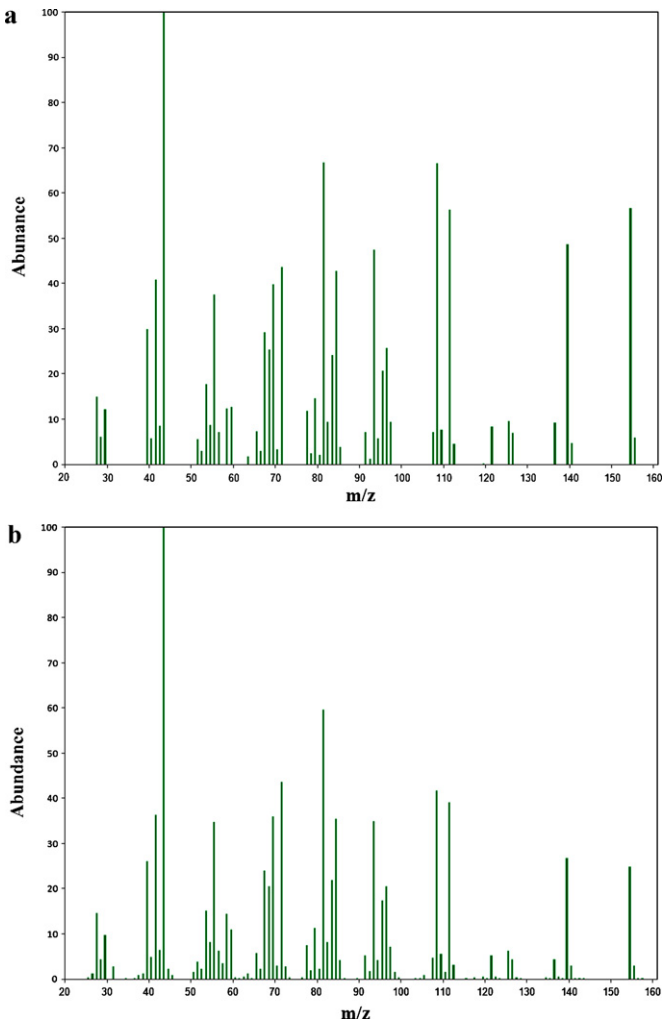

Fig. 10. Resolved and standard mass spectra for one of the components of the peak cluster A, (a) resolved and (b) standard mass spectrum.

the pure mass chromatograms of the two components were subtracted from the real data matrix, the remained matrix will have just three mass chromatograms of components. Since the three remaining components were eluted consequently, so there is no selective region and therefore, the OPR technique was chosen to resolve them. After reaching the pure concentration profiles, the mass spectra were predicted as before. The net resolved chromatographic profile of the peak cluster A is shown in Fig. 6. The peak cluster B also could be resolved by using the non-iterative methods such as HELP alone or its combination with OPR. In Fig. 5b, the selective regions of four components are labeled by number "1" in FSMW-EFA plot. The pure concentration profiles of the four components were resolved by using the HELP technique and then, as before, the mass spectra were predicted. By reducing the mass chromatograms of these components from the real data matrix, the pure mass chromatograms of fifth component can be obtained. However, for showing the comparative power of the iterative and the non-iterative methods and further to examine the results of the HELP and OPR, the MCR-ALS technique was also used. In the MCR-ALS window, some constraints such as non-negativity in concentration and mass spectra and selective concentration matrix were applied. These constraints would help the MCR-ALS method to be more accurate and reliable. In the present work, since we did not have a new run of experiment, therefore we were not being able to perform a thorough augmentation technique. This is due

Table 1
The volatile chemical components of the rosemary essential oil.

No.	Compound	Retention time (min)	Formula	Percentage (%)
1	Ethanol	7.13	C_2H_6O	0.02
2	Mesityle oxide	15.49	$C_6H_{10}O$	0.01
3	1-β-Pinene	21.84	$C_{10}H_{16}$	0.01
4	Tricyclene	22.01	$C_{10}H_{16}$	0.08
5	L-Phellandrene	22.23	$C_{10}H_{16}$	0.08
6	α-Pinene	22.89	$C_{10}H_{16}$	21.74
7	Camphene	23.61	$C_{10}H_{16}$	0.30
8	Unknown	23.78	–	2.94
9	Sabinene	24.75	$C_{10}H_{16}$	0.04
10	β-Pinene	24.91	$C_{10}H_{16}$	0.10
11	2-β-Pinene	25.07	$C_{10}H_{16}$	1.36
12	3-Octanen	25.20	$C_8H_{16}O$	0.52
13	β-Myrcene	25.51	$C_{10}H_{16}$	1.42
14	(Z)-5-Hexenal oxime	25.79	$C_6H_{11}NO$	0.07
15	α-Thunjene	26.40	$C_{10}H_{16}$	0.20
16	α-Terpinene	27.07	$C_{10}H_{16}$	0.29
17	p-Cymene	27.69	$C_{10}H_{14}$	0.75
18	Eucalyptol	28.01	$C_{10}H_{18}O$	4.49
19	Unknown	28.11	–	1.49
20	Unknown	28.17	–	2.43
21	1,8-Cineole	28.20	$C_{10}H_{18}O$	23.47
22	γ-Terpinene	29.30	$C_{10}H_{16}$	0.73
23	Trans-Sabinene hydrate	29.75	$C_{10}H_{18}O$	0.04
24	α-Terpinolene	30.90	$C_{10}H_{16}$	0.41
25	Cyclohexanel-methyl-4(1-methyl ethylidene)	30.93	$C_{10}H_{16}$	0.04
26	(+)-2-Carene	30.99	$C_{10}H_{16}$	0.05
27	L-Linalool	31.33	$C_{10}H_{18}O$	1.52
28	Filifolone	31.70	$C_{10}H_{14}O$	0.07
29	Exo-Fenchol	32.41	$C_{10}H_{18}O$	0.02
30	2,6-Dimethyl-1,6 heptadine-3ol-acetate	32.74	$C_{11}H_{18}O_2$	0.02
31	Chrysanthenone	32.90	$C_{10}H_{14}O$	0.42
32	Unknown	33.74	–	0.01
33	(−) Alcanfor	33.90	$C_{10}H_{16}O$	0.10
34	Cis-dihydro carvone	34.09	$C_{10}H_{16}O$	0.18
35	Camphor	34.24	$C_{10}H_{16}O$	7.21
36	Spiro[bicyclo[2.2.1]]heptan	34.34	$C_{14}H_{20}O_3$	0.02
37	3-Pinanone	34.82	$C_{10}H_{16}O$	0.11
38	Pinocarvone	34.91	$C_{10}H_{14}O$	0.10
39	Methyl bornyl ether	35.16	$C_{11}H_{20}O$	0.06
40	Endo-Borneol	35.20	$C_{10}H_{18}O$	0.01
41	Iso-Borneol	35.27	$C_{10}H_{18}O$	0.39
42	Borneol	35.29	$C_{10}H_{18}O$	3.38
43	1,4-Terpineol	35.61	$C_{10}H_{18}O$	0.90
44	4-Terpineol	35.65	$C_{10}H_{18}O$	1.08
45	β-Fenchyl alcohol	36.09	$C_{10}H_{18}O$	0.11
46	α-Terpineol	36.43	$C_{10}H_{18}O$	1.78
47	Myrtenol	36.79	$C_{10}H_{16}O$	0.42
48	Homo myrtenol	37.01	$C_{11}H_{18}O$	0.49
49	Berbonone	37.57	$C_{10}H_{14}O$	7.57
50	Unknown	37.59	–	2.11
51	Unknown	37.61	–	0.30
52	Z-Citral	38.46	$C_{10}H_{16}O$	0.05
53	Cis-myrtanol	38.58	$C_{10}H_{18}O$	0.11
54	Neirol	38.83	$C_{10}H_{18}O$	0.05
55	Trans-Goraniol	39.08	$C_{10}H_{18}O$	1.96
56	2,5-Bornanediol	39.70	$C_{10}H_{18}O_2$	0.01
57	3,6-Octadienic acid, 3,7-dimethyl-ethyl-ester	39.83	$C_{10}H_{18}O_2$	0.08
58	Iso-piperitenone	40.22	$C_{10}H_{14}O$	0.03
59	Thymol	40.38	$C_{10}H_{14}O$	0.01
60	Endo-bornyl acetate	40.65	$C_{12}H_{20}O_2$	0.03
61	α-Fenchyl acetate	40.86	$C_{12}H_{20}O_2$	1.74
62	2-Methyl-1-thiaindan	41.17	$C_9H_{10}S$	0.20
63	Diazene, actyl phenyl	41.33	$C_8H_8N_2O$	0.01
64	Myrtenyl acetate	42.49	$C_{12}H_{18}$	0.03
65	Sabinol	42.53	$C_{10}H_{16}O$	0.05
66	3-Cyclohexane-1-carboxylic acid-3,7-dimethyl ethyl ester	42.73	$C_{10}H_{16}O_2$	0.08
67	Bornylene	42.82	$C_{10}H_{16}$	0.08
68	Cyclohexane-2ethyl-1,1 dimethyl-3-methylene	43.34	$C_{11}H_{18}$	0.10
69	Piperitenone	43.37	$C_{10}H_{14}O$	0.07
70	Iso-Eugenol	43.93	$C_{10}H_{12}O_2$	0.02
71	1-epi-acetoxy-2-(1-methyl ethenyl)-5-methyl-Cyclohexane	44.34	$C_{12}H_{20}O_2$	0.01
72	Lavanduyl acetate	44.65	$C_{12}H_{20}O_2$	0.04
73	Neryl acetate	44.76	$C_{12}H_{20}O_2$	0.20
74	Methyl eugenol	45.80	$C_{11}H_{14}O_2$	0.14
75	Trans-caryophyllene	47.24	$C_{15}H_{24}$	0.93
76	Geranyl acetone	47.85	$C_{13}H_{22}O$	0.02

Table 1 (*Continued*)

No.	Compound	Retention time (min)	Formula	Percentage (%)
77	α-Humulene	48.67	$C_{15}H_{24}$	0.32
78	3-Buten-1ol-3-methyl, benzoate	49.60	$C_{12}H_{14}O_2$	0.01
79	β-Bisabolene	49.95	$C_{15}H_{24}$	0.01
80	Valencene	50.18	$C_{15}H_{24}$	0.01
81	(−) Caryophyllene oxide	53.94	$C_{15}H_{24}O$	0.15
82	Junipene	54.10	$C_{15}H_{24}$	0.02
83	Ketone, 1-[4-(4-methyl-penthyl)-3-cyclohexane-1-yl]	54.61	$C_{14}H_{22}O$	0.01
84	Humulene oxide	55.07	$C_{15}H_{24}O$	0.02
85	Unknown	55.84	–	0.01
86	Tetracyclo[6.3.2.OE_2, 5.OE_1, 8] tridecan-9-ol, 4,4-dimethyl	56.02	$C_{15}H_{24}O$	0.02
87	Iso-aromadendren oxide	56.72	$C_{15}H_{24}O$	0.02
88	Widdrol	56.98	$C_{15}H_{26}O$	0.01
89	Iso-longifolol	57.18	$C_{15}H_{26}O$	0.02
90	Benzyl benzoate	60.49	$C_{14}H_{12}O_2$	0.01
91	Unknown	65.78	–	0.04
92	Allopteoxylin methyl ether	66.14	$C_{16}H_{16}O_4$	0.05
93	Unknown	68.43	–	0.20
94	4-Oxo-beta-iso damascol	70.25	$C_{13}H_{20}O_2$	0.18
95	1-cyclohexene-1-propanol-2,6,6-trimethyl	70.51	$C_{12}H_{20}O$	0.03
96	Dehydrobietane	70.63	$C_{20}H_{30}$	0.02
97	Phenethyl iodide	70.79	$C_{10}H_{17}N_3$	0.01
98	2,2,6,8-Tetramethyl-7-oxa-tricyclo [6.1.0.0].6. nonane	71.06	$C_{12}H_{20}O$	0.01
99	Cyclohexanepropand,2,2-dimethyl-6-mthylene	71.42	$C_{12}H_{22}O$	0.01
100	Trace 0.01%<			0.09

to the fact that preparation of standards for natural compounds with complex matrix is very time consuming and expensive. So, we have used a method named pseudo-augmentation method in which a simulated matrix was augmented instead of a real data. In this technique, making a pseudo-augmented matrix is a critical step, which can be produced by using the data acquired from the real data matrix. For pseudo-augmentation, it is necessary to have a matrix with the same dimension in row or column. Here, pseudo-augmentation was performed in column-wise. By having the FSMW-EFA plot of the real data matrix, the partial pattern of the components is known. So, with the use of a polynomial modified Gaussian (PMG) model [54], a matrix (100×5) was simulated, which was constructed similar to the real data matrix in positions and intensities of peaks by using the FSMW-EFA plot, the real data matrix and the result of other resolution techniques. In this matrix, 100 stands for the time direction and 5 is representing the number of components. Now, a matrix (5×281), as a mass spectra matrix, which can be multiplied with the matrix of (100×5) is necessary. So, after multiplying these two matrices with each other the matrix of (100×281) will result. The mass spectra, which were predicted as a result of using the non-iterative methods in previous step, are a good source for the matrix (5×281). Finally, the [(124 + 100) × 281] matrix, which is pseudo-augmented matrix, was used in the MCR-ALS window. Figs. 7–9 show the simulated and pseudo-augmented matrices and the resolved concentration profile for the peak cluster B, respectively. Resolved and standard mass spectra for one of the components of the peak cluster A, as an example, are shown in Fig. 10a and b, respectively. After resolving each peak cluster to its pure chromatographic profile and mass spectrum, a total of 138 components were identified, from which 39 components had a lower concentration than 0.01% and only 90 components, from the 99 remaining components, were identified with the NIST-MS library search. The number of identified components of the rosemary essential oil in this work was high. Numerous reports on analyzing the rosemary by using different techniques such as GC, GC–MS and GC × GC–MS are presented [4,8–19] with the aim of improving the number of components, yet the number of components of our study is more than that of all previous reports. The interpretation of GC chromatograms of essential oils requires the information of retention times of each component. Therefore, the availability of all reference compounds is essential. In GC–MS, the interpretation is more accurate than GC, since comparison is based on the similarity matches in the reliability of the MS library. However, the presence of the background and overlapped/embedded peaks decreases the MS similarity. Hence, the use of curve resolution techniques may improve the identification of components obtained from the GC–MS data. The components identified by the proposed procedure are shown in Table 1. For the sake of comparison, five main components of the rosemary reported by previous works with their percentages are listed in Table 2.

4.2. Quantitative analysis of chemical components of rosemary essential oil

From the pure chromatographic profile and mass spectrum of each component, the total two-way response of each component can be obtained by using the outer product of its concentration and spectrum vectors. The total relative amount of each component is then proportional to the overall volume of its two-way response. The advantage of this quantitative method, which is called overall volume integration (OVI) [55], over general peak-area integration, is that all mass spectral points are taken into consideration. However, the results obtained in the present work are not absolute quantitative concentrations (no standards for all compounds are available) but the percentages obtained after internal normalization of all resolved peak areas. The results show that 99 components with concentration higher than 0.01% exist in the Iranian rosemary essential oil. These components account for 98.23% of the

Table 2
Comparison of five main components of different rosemary essential oils in different reports.

1	2	3	4	5	Reference
1,8-Cineole 23.47%	α-Pinene 21.74%	Berbonone 7.57%	Camphor 7.21%	Eucalyptol 4.49%	Present work
1,8-Cineole 44.42%	α-Pinene 12.57%	Borneol 8.52%	β-Pinene 5.18%	Camphene 4.43%	[12]
α-Pinene 24.03%	Verbenone 14.51%	Camphor 12.98%	Limonene 8.57%	Camphene 7.42%	[17]
α-Pinene 44.05%	Camphor 7.82%	Verbenone 6.37%	Camphene 6.14%	Limonene 5.48%	[16]

total relative content. The final relative quantitative values are presented in Table 1. The most important constituents of the Iranian rosemary are 1,8-cineole (23.47%), α-pinene (21.74%), berbonone (7.57%), camphor (7.21%) and eucalyptol (4.49%).

5. Conclusion

In GC–MS technique, the identification is performed through direct similarity searches in MS database attached to the GC–MS instrument. However, for complex samples such as the rosemary essential oil, the probability of overlapped peaks can result in a wrong similarity match in the MS library. Therefore, resolution and afterwards quantification of target compounds becomes a goal. By the use of chemometric resolution techniques a thorough analysis of the rosemary essential oil becomes possible. After resolving the peak clusters to their pure chromatographic and mass spectra using the MCR methods, a total of 138 components were identified from which 39 components had a concentration lower than 0.01%. From the remaining 99 components, only 90 components were recognized with the NIST-MS library search. No previous work using various techniques such as GC, GC–MS and GC × GC–MS were able to report the acquired information.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.chroma.2011.02.048.

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