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## GC–MS vs. GC–MS–MS analysis of pentacyclic terpanes in crude oils from Libya and Serbia – A comparison of two methods

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**Abstract:** The values of parameters calculated from distribution and abundance of the selected pentacyclic terpanes in crude oils from Libya and Serbia, which were originally derived from gas chromatography-mass spectrometry (GC–MS) were compared with results of quantification based on gas chromatography–mass spectrometry-mass spectrometry (GC–MS–MS). The parameters analyzed are the most often used terpane source and maturity parameters, which were applied to a large sample set of 70 oils, originating from five oil fields. The aim of the paper was to investigate to which extent the measurements of the selected parameters by these two instrumental techniques agree and to determine the influence of differences between parameter values on geochemical interpretation. For that purpose two methods, concordance correlation coefficient and mean-difference plot were used. The obtained results indicate that calculation of  $C_{27}18\alpha(H)-22,29,30$ -trisorneohopane/ $C_{27}18\alpha(H)-22,29,30$ -trisorneohopane +  $C_{27}17\alpha(H)-22,29,30$ -trisorhopane),  $C_{29}18\alpha(H)-30$ -norneohopane/ $C_{29}17\alpha(H)21\beta(H)-30$ -norhopane and  $C_{29}17\alpha(H)21\beta(H)-30$ -norhopane/ $C_{30}17\alpha(H)21\beta(H)$ -hopane ratios either by GC–MS or GC–MS–MS do not significantly influence interpretation. On the other hand, the determination of  $C_{30}17\beta(H)21\alpha(H)$ -moretane/ $C_{30}17\alpha(H)21\beta(H)$ -hopane ratio, gammacerane index and oleanane index by GC–MS vs. GC–MS–MS could notably affect interpretation. These differences can be explained by the co-elution and the peak overlapping in GC–MS but also by better separation, higher precision and better selectivity of the GC–MS–MS. Deviation of the almost all studied para-

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meters from the line of equality was similar for the oils from the same oil field but some differences were observed for the oils from different oil fields. Therefore, when GC–MS–MS results are to be used in organic geochemical interpretations, a regional calibration of GC–MS vs. GC–MS–MS relationship for each petroleum system is highly recommended.

*Keywords:* oils; terpanes; GC–MS; GC–MS–MS; concordance correlation coefficient; mean-difference plot.

## INTRODUCTION

Crude oil is a complex mixture of a large number of chemical compounds, commonly dominated by hydrocarbons.<sup>1</sup> Due to the complexity of crude oil composition, the development of organic geochemistry, from its beginnings until the present day, followed the development of the instrumental techniques for the analysis of organic compounds. From the organic geochemical point of view, the most important compounds in crude oils are sterane and terpane biomarkers, as well as bicyclic and tricyclic aromatic compounds.

Sterane and terpane biomarkers are in geological samples (crude oils and extracts of source rocks and coals) usually present at ppm to ppb level. Due to the complex composition of these samples and to the low abundance of biomarker compounds in them, gas chromatographic–mass spectrometric (GC–MS) analysis was, for many years, the method of choice for identification and quantification of biomarkers in crude oils. Most of the organic geochemical parameters, based on the abundance and distribution of sterane and terpane biomarkers were originally defined on the basis of the results of GC–MS analysis.<sup>2</sup> However, in the GC–MS analyses interferences often occur, due to co-elution and peak overlapping, and quite often an additional selectivity is needed to resolve complex biomarker mixtures.

With the development of tandem or quadrupole mass spectrometers coupled with GC (GC–MS–MS), many of the shortcomings encountered in analytical separations with GC–MS were overcome. The major advantage of a GC–MS–MS system over GC–MS is a possibility to resolve individual biomarkers or biomarker groups in complex mixtures and to precisely quantify all resolved isomers.<sup>3</sup> Nowadays, GC–MS–MS and other hyphenated techniques are routinely used in many organic geochemical laboratories.

Although the knowledge on the discrepancies between GC–MS and GC–MS–MS results in quantification of sterane and terpane parameters is not a novelty for the organic geochemical scientific community, a detailed comparison of the results of the analysis of the same samples using these two techniques, to the best of our knowledge, has not been published so far.

In this study a comparison of the values of parameters calculated from distribution and abundance of the selected pentacyclic terpanes in crude oils from Libya and Serbia, derived from gas chromatography–mass spectrometry (GC–

-MS) and gas chromatography-mass spectrometry-mass spectrometry (GC-MS-MS) the quantification of results was performed, using a large sample set of 70 oils. The parameters analyzed are the most often used terpene source and maturity parameters:  $C_{27}18\alpha(H)-22,29,30$ -trisorneohopane/ $(C_{27}18\alpha(H)-22,29,30$ -trisorneohopane +  $C_{27}17\alpha(H)-22,29,30$ -trisnorhopane (Ts/(Ts+Tm)),  $C_{29}18\alpha(H)-30$ -norneohopane/ $C_{29}17\alpha(H)21\beta(H)-30$ -norhopane ( $C_{29}Ts/C_{29}H$ ),  $C_{29}17\alpha(H)21\beta(H)-30$ -norhopane/ $C_{30}17\alpha(H)21\beta(H)$ -hopane ( $C_{29}H/C_{30}H$ ),  $C_{30}17\beta(H)21\alpha(H)$ -moretane/ $C_{30}17\alpha(H)21\beta(H)$ -hopane ( $C_{30}M/C_{30}H$ ), gamma-cerane index ( $GI = 100G/C_{30}H$ ) and oleanane index ( $OI = 100O/C_{30}H$ ). The aim of this paper was to investigate to which extent the measurements of the selected parameters by these two instrumental techniques agree and to understand the nature of their differences.

## EXPERIMENTAL

### *Samples*

Following the conclusion of some authors that a sample size of at least 50 should be used in the regression analysis,<sup>4</sup> a large data set of 70 crude oil samples was chosen for our present research. The crude oils were carefully selected to comprise samples different in source, maturity and biodegradation level. In order to avoid erroneous conclusions that might be drawn from the analyses of the oils from one oil field, the samples from four oil fields from the Pannonian Basin (Serbia) were selected. Furthermore, in order to make the conclusions even more general, 14 crude oil samples from different parts of the Sirte Basin (Libya) were included in this research as well. All investigated oils are of Cretaceous or Tertiary age.<sup>5-7</sup>

The Serbian crude oils investigated originate from the southeastern part of the Pannonian Basin, from the oil fields Elemir, Rusanda, Zrenjanin and Velebit. All these oils were generated from clay-rich source rocks containing mixtures of terrestrial and marine organic matter. However, they are different in their maturity level. The Zrenjanin oils have low level of thermal maturity, corresponding to calculated vitrinite reflectance,  $R_c$ , of 0.60–0.70 %.<sup>6</sup> The Elemir and Rusanda crude oils were generated from the source rocks at medium maturity level, equivalent to vitrinite reflectance between 0.70 and 0.80 %.<sup>8,9</sup> The source rocks of the Velebit oils are estimated to be of high maturity, consistent with a vitrinite reflectance of > 0.80 %  $R_c$ .<sup>7</sup> Another distinctive feature of the Velebit oils is that they are biodegraded. In a biodegradation-based classification,<sup>2</sup> these oils were found to be altered to an index value of 3 to 4.<sup>7</sup>

The crude oils from the Sirte Basin are in the literature described as mature to post mature oils. Most of them are of marine origin, although a mixed marine-terrestrial source is proposed for some oils as well. The Sirte Shale is considered the main source for most of the oils in this area.<sup>10,11</sup> The Libyan crude oils investigated in this research originate from different parts of the Sirte Basin, from the oil fields: Amal, En Naga, Intisar, Messla, Nafoora, Samah, Sarir C, Waha and Zelten.

### *Analytical methods*

The crude oils were fractionated into saturated hydrocarbons, aromatic hydrocarbons and polar compounds by column chromatography.<sup>12,13</sup> The terpene biomarkers were analyzed in the fractions of saturated hydrocarbons by gas chromatography-mass spectrometry (GC-MS) and gas chromatography-mass spectrometry-mass spectrometry (GC-MS-MS).

The GC–MS analysis was conducted using an Agilent 7890A gas chromatograph fitted with a HP5-MS capillary column (30 mm×0.25 mm, film thickness 0.25 μm, temperature range: 80 °C for 0 min; then 2 °C min<sup>-1</sup> to 300 °C and held for 20 min), with the exception of Velebit oils for which column was heated in the same temperature range, but with a heating rate of 3 °C min<sup>-1</sup>. Helium was used as the carrier gas (flow rate 1.5 cm<sup>3</sup> min<sup>-1</sup>). The GC was coupled to a Hewlett-Packard 5975C mass selective detector operated at 70 eV in the 45–550 scan range. The analysis of the target compounds was performed from *m/z* 191 ion chromatograms. The individual compounds were identified by the comparison of mass spectra and retention times with literature data.

The GC–MS-MS analysis was performed using an Agilent 6890N gas chromatograph connected to a Waters (Micromass) Quattro Micro GC tandem quadrupole mass spectrometer. A Phenomenex ZB-5 column (30 m×0.25 mm i.d., film thickness 0.10 μm) was used. The temperature programme was: 30 °C/min from 70 to 100 °C and 4 °C/min from 100 to 308 °C (hold 8 min). GC–MS-MS analysis was carried out using separate runs with relevant parent–daughter transitions for C<sub>27</sub>–C<sub>35</sub> hopanes (M<sup>+</sup> → *m/z* 191).

Characteristic terpane parameters were calculated from GC–MS and GC–MS-MS chromatogram peak areas (softwares: Agilent ChemStation and Waters MassLynx V4.0). The ratios are listed in Table S-I of the Supplementary Material to this paper.

#### Statistical methods

In this study, we applied two approaches which are considered the best in the method comparison studies:<sup>14</sup> concordance correlation coefficient and mean-difference plot. All statistical analyses were conducted using MedCalc v.12.5 statistic software package.

#### Concordance correlation coefficient

The concordance correlation coefficient (CCC),  $\rho_c$  was introduced and developed by Lin<sup>15,16</sup> as a measure of agreement between paired continuous measurements obtained by two analysts or by two measurement methods. This coefficient can be calculated as a product of two components:

$$\rho_c = \rho C_b \quad (1)$$

The term  $\rho$  is the Pearson product-moment correlation coefficient. Being a measure of the closeness (or distance) of the results to the best-fit line, Pearson  $\rho$  is interpreted as a measure of precision. The term  $C_b$  is a bias correction factor, interpreted as a measure of accuracy. It determines how far the best-fit line deviates from the line of perfect concordance (*i.e.*, the line at 45° on a square scatter plot). The bias can have values:  $0 < C_b \leq 1$ . When  $C_b = 1$ , the best-fit line coincides with the 45° line. The lower the value of  $C_b$ , the greater the deviation is from the 45° line.

Similar to other correlation coefficients,  $\rho_c$  can have values between -1 and +1.  $\rho_c = +1$  means perfect concordance;  $\rho_c = -1$  means perfect negative concordance and  $\rho_c = 0$  means no correlation (no linear relationship exists between two continuous variables). Regarding the values between 0 and  $\pm 1$ , some authors suggested different magnitude guidelines to express the strength of the agreement.<sup>17,18</sup> However, it should be emphasized that the extent of the agreement depends on the samples to be analyzed, the methods to be used and the features to be compared. Because of that there are no universal scales in the method comparison studies, but they are rather unpredictable and might be quite different from one case to another.

Considering the nature of the samples and the values of the parameters we compare, in our present research we will use the following descriptive scale to interpret the values of the

concordance correlation coefficient and the strength of agreement:  $\rho_c < 0.65$  as poor agreement; 0.65–0.80 as moderate; 0.80–0.95 as substantial, and 0.95–1 as almost perfect agreement.

#### *Mean-difference plot*

The mean-difference plot is a graphical method to evaluate the accordance between two different instruments or two measurements techniques. This approach is based on the construction and the analysis of the plot, which is in the literature known as Tukey mean-difference plot,<sup>19,20</sup> or the Bland–Altman plot.<sup>21,22</sup> The plot is constructed as a difference of two paired measurements against their average. Usually, at least three horizontal lines are drawn within this plot: the line at the mean difference and the lines for the 95 % confidence interval for the mean (the mean difference  $\pm 1.96$  times the standard deviation of the differences). The former two lines are called “limits of agreement”, and they “tell us” how far apart the measurements by two methods (or two instruments) are more likely to be for most individuals.

The next step is a visual and the statistical analysis of the results in order to identify outliers and/or systematic bias between the paired measurements.

If a one-sample *t*-test, shows that the mean value of the difference does not differ significantly from 0, this indicates a good agreement between the two methods and that they may be used interchangeably. If a one-sample *t*-test, reveals that the mean value of the difference differs significantly from 0, this indicates the presence of fixed or proportional bias, meaning that the methods do not agree equally through the range of measurements.

The visual analysis of the results is probably the most important part of the method comparison studies. The purpose of this analysis is to estimate the degree of accordance between two methods, taking into the account the analytical significance of the difference between the results for the analyzed set of parameters, the precision of the analytical methods and the instruments used, as well as the nature of the samples.

## RESULTS AND DISCUSSION

### *The analysis of concordance correlation coefficients*

As a first step in the method comparison study, the concordance correlation analysis was conducted for the six organic geochemical parameters determined by GC–MS and GC–MS-MS instrumental techniques. As mentioned before, the parameters analyzed were:  $Ts/(Ts+Tm)$ ,  $C_{29}Ts/C_{29}H$ ,  $C_{29}H/C_{30}H$ ,  $C_{30}M/C_{30}H$ , gammacerane and oleanane index (Tables I and S-I). The correlation analysis graphs are shown in Fig. 1.

The concordance correlation analysis of the  $Ts/(Ts+Tm)$  parameter (Fig. 1a and Table S-I) showed high similarity between the values of this parameter determined by GC–MS and GC–MS-MS techniques. This observation is additionally confirmed by high Pearson correlation coefficient ( $\rho = 0.97$ , Table I), indicating the high precision in measurement of this parameter by these two instrumental techniques. High concordance correlation coefficient  $\rho_c = 0.82$  (Table I) with narrow 95 % confidence interval, ranging from 0.76 to 0.87 (Table I) indicate that similar values of the  $Ts/(Ts+Tm)$  parameter determined by GC–MS and GC–MS-MS can be expected for wide range of the values.

However, the visual inspection of the similarity graph (Fig. 1a) revealed a slight deviation from the line of equality ( $y = x$  line) with the increase of the

values of the  $Ts/(Ts+Tm)$  parameter. This deviation is also reflected in accuracy in the determination of this parameter, as shown by bias correction factor  $C_b$  (accur-

TABLE I. Concordance correlation analysis;  $Ts/(Ts+Tm) = C_{27}18\alpha(H)-22,29,30$ -trisorneohopane/ $(C_{27}18\alpha(H)-22,29,30$ -trisorneohopane +  $C_{27}17\alpha(H)-22,29,30$ -trisorhopane;  $C_{29}Ts/C_{29}H = C_{29}18\alpha(H)-30$ -norneohopane/ $C_{29}17\alpha(H)21\beta(H)-30$ -norhopane;  $C_{29}H/C_{30}H = C_{29}17\alpha(H)21\beta(H)-30$ -norhopane/ $C_{30}17\alpha(H)21\beta(H)$ -hopane;  $C_{30}M/C_{30}H = C_{30}17\beta(H)21\alpha(H)$ -moretane/ $C_{30}17\alpha(H)21\beta(H)$ -hopane; *GI*: gammacerane index,; *OI*: oleanane. Values of all parameters for individual samples are given in Table S-I of the Supplementary material to this paper

Parameter	Sample size	Concordance correlation coefficient (CCC)	CCC 95 % confidence interval	Pearson correlation coefficient, $\rho$ (precision)	Bias correction factor, $C_b$ (accuracy)
$Ts/(Ts+Tm)$	68	0.82	0.76-0.87	0.97	0.85
$C_{29}Ts/C_{29}H$	68	0.53	0.35-0.67	0.59	0.90
$C_{29}Ts/C_{29}H^a$	44	0.94	0.90-0.96	0.98	0.96
$C_{29}H/C_{30}H$	68	0.27	0.05-0.47	0.29	0.94
$C_{30}M/C_{30}H$	68	0.10	0.06-0.14	0.68	0.15
<i>GI</i>	68	0.29	0.19-0.37	0.73	0.39
<i>OI</i>	55	0.33	0.22-0.43	0.79	0.41

<sup>a</sup>Velebit oils were excluded from the analysis

acy < precision, Table I). According to these results it might be presumed that when the values of the  $Ts/(Ts+Tm)$  parameter are high, the values of this parameter determined by GC–MS–MS might be higher than those determined by GC–MS. Considering the fact that the  $Ts/(Ts+Tm)$  parameter increases with increase in oil maturity,<sup>2</sup> it can also be concluded that in more mature crude oils a higher difference in  $Ts/(Ts+Tm)$  parameter, determined by GC–MS and GC–MS–MS, can be expected than in less mature oils.

Furthermore, it can also be noticed that the deviation of this parameter from the line of equality is similar for the oils from the same oil field but it is different for the oils from different oil fields. In the case of  $Ts/(Ts+Tm)$  parameter, the discrepancies between the values determined by GC–MS and GC–MS–MS instrumental techniques, for the oils originating from different oil fields, are not surprising. Actually, it is well known that  $Ts$  and  $Tm$  in GC–MS  $m/z = 191$  chromatograms co-elute with some tri- and tetracyclic terpanes.<sup>2</sup> Furthermore, the relative content of tri-, tetra- and pentacyclic terpanes in crude oils is influenced by the source rocks organic contents, but also by lithology and oxicity of the depositional environment.<sup>2</sup> As a consequence, the difference between the values of the  $Ts/(Ts+Tm)$  parameter determined by GC–MS and GC–MS–MS instrumental techniques is different for crude oils from different oil fields. Because of that, it should be emphasized that, when GC–MS–MS results are to be used in organic geochemical interpretations, a regional calibration of GC–MS vs. GC–MS–MS relationship for each crude oil system investigated is highly recommended.

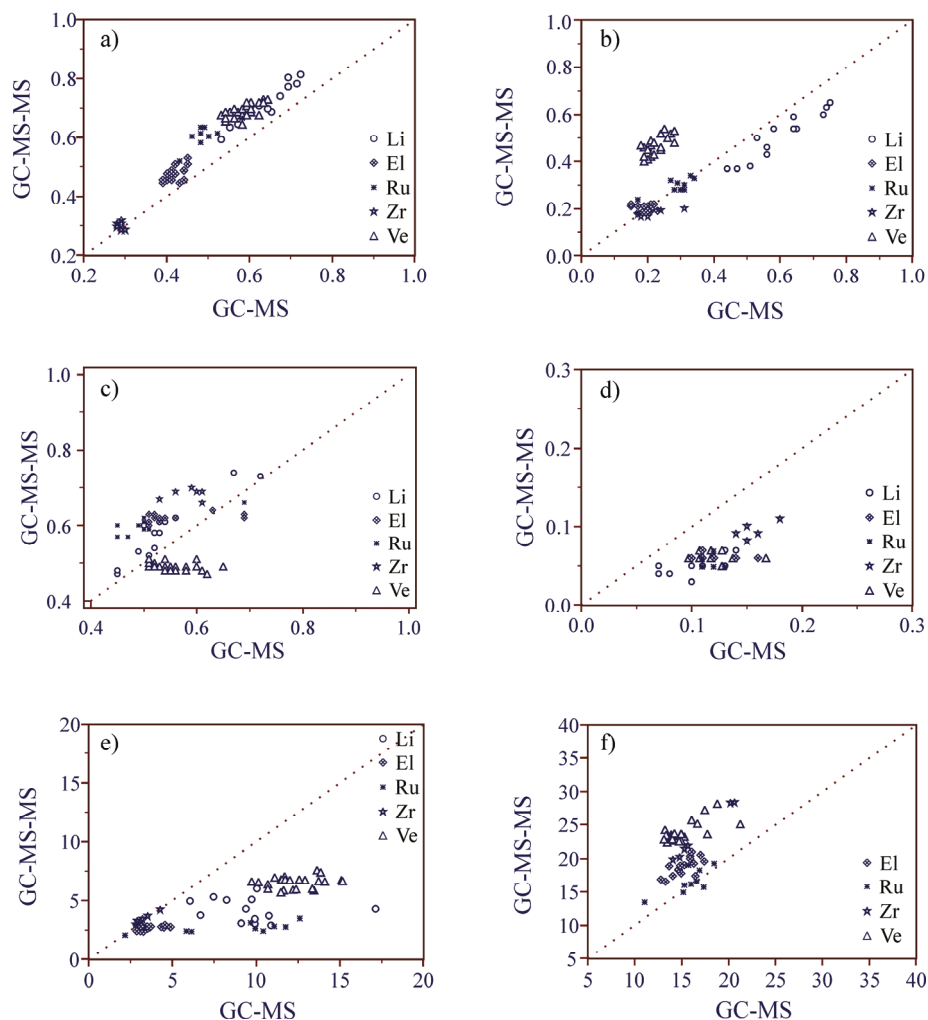


Fig. 1. Concordance correlation analysis scatter diagrams for parameters:  $Ts/(Ts+Tm)$  (a);  $C_{29}Ts/C_{29}H$  (b);  $C_{29}H/C_{30}H$  (c);  $C_{30}M/C_{30}H$  (d);  $GI$  (e) and  $OI$  (f). Li – Libya oils; El – Elemir oils; Ru – Rusanda oils; Zr – Zrenjanin oils; Ve – Velebit oils.

Additionally, it should be stated that all samples investigated in this study were not analyzed at the same time. The samples from the oil fields Elemir, Rusanda and Zrenjanin were analyzed as one set of samples; the Velebit crude oils were analyzed as the second set of samples, and the crude oils from Libya were analyzed as the third set of samples. Accordingly the differences in the agreement of the values of the  $Ts/(Ts+Tm)$  parameter between these three groups of samples can also be interpreted as a consequence of the variability in the working conditions of the different parts of the instruments over time.



It is noticeable that the values of the  $Ts/(Ts+Tm)$  parameter for the Velebit oils follow, more or less, the same trend as for all other samples (Fig. 1a). As stated earlier, the Velebit oils were shown to be biodegraded to the biodegradation level 3, and some samples even to the level 4. Accordingly, it can be concluded that  $Ts$  and  $Tm$  are not affected by biodegradation in oils biodegraded to the level 4, or if they are somewhat degraded, they are removed to the same extent, leaving their ratio unaltered. This conclusion is in accordance with the published literature data.<sup>2</sup>

The concordance correlation analysis of the  $C_{29}Ts/C_{29}H$  parameter (Fig. 1b; Table S-I) indicated a low correlation coefficient ( $\rho = 0.53$ ; Table I) and a wide 95 % confidence interval for  $CCC$  (0.35–0.67; Table I). All these results point to the significant difference between the values of this parameter determined by GC–MS and GC–MS–MS instrumental techniques. However, the visual examination of the similarity graph (Fig. 1b) revealed a different correlation trend for the  $C_{29}Ts/C_{29}H$  ratio in the Velebit oils and other samples.

In the case of the Velebit crude oils, the  $C_{29}Ts/C_{29}H$  parameter, determined by two instrumental techniques, showed significant deviation from the line of equality ( $y = x$  line) with systematic shift between the GC–MS and GC–MS–MS values (Fig. 1b). These results indicate that the GC–MS–MS analysis may result in  $C_{29}Ts/C_{29}H$  parameter values which are twice as high than those determined by GC–MS technique. The obtained result can be attributed to the fact that among the investigated samples, only Velebit oils were analysed by GC–MS with heating rate of  $3\text{ }^{\circ}\text{C min}^{-1}$ , whereas for other oils heating rate of  $2\text{ }^{\circ}\text{C min}^{-1}$  was used. Since  $C_{29}Ts$  and  $C_{29}H$  have very similar retention times and eluted much closer than corresponding  $C_{27}$  homologues ( $Ts$  and  $Tm$ ) it can be supposed that under heating rates higher than  $2\text{ }^{\circ}\text{C min}^{-1}$  separation of  $C_{29}Ts$  and  $C_{29}H$  in routine GC–MS is not efficient, particularly in mature samples, such as Velebit oils, which are usually rich in  $C_{29}Ts$ , resulting in erratic values of  $C_{29}Ts/C_{29}H$  ratio.

Taking into account the different pattern of the Velebit oils, the concordance correlation analysis of the  $C_{29}Ts/C_{29}H$  parameter was repeated using the results for other oils from the analyzed data set (oils: Libya, Elemir, Rusanda and Zrenjanin; Tables I and S-I). This analysis revealed a high similarity between the values of this parameter determined by two instrumental techniques. High  $CCC$  coefficient (0.94; Table I) reflects both, high precision and accuracy in measurement of this parameter by these two techniques. A narrow 95 % confidence interval for  $CCC$ , ranging from 0.90 to 0.96 indicate that similar values of the  $C_{29}Ts/C_{29}H$  ratio determined by GC–MS and GC–MS–MS can be expected in wide range of values. However, visual inspection of the similarity graph revealed a slight deviation from the line of equality ( $y = x$  line) for the Libya crude oil samples. These results indicate that a difference in the strength of the agreement between them can be expected for crude oils originating from different oil fields



and, again point to the importance of regional calibration of GC-MS and GC-MS-MS results in organic geochemical studies. Difference in the strength of the agreement for  $C_{29}Ts/C_{29}H$  ratio in different basins could be attributed to the fact that certain oleanane/taraxastane derivatives such as  $18\alpha(H)$ -28-noroleanane and  $19\alpha(H)$ -24,28-bisnortaraxastane may co-elute with  $C_{29}H$  under routine GC-MS equipped with 25 m column.<sup>23</sup> This influence can be particularly expected in deltaic oils of late Cretaceous or younger age, rich in oleanane/taraxastane. On the other hand, a rearranged oleanane (MW 412, base peak 191) coelutes with  $C_{29}H$ . In some oils this compound can be even more abundant than  $C_{29}H$ , making the quantification of  $C_{29}H$  from  $m/z$  191 chromatograms impossible.<sup>24</sup> Nevertheless, from the results of this study, a general conclusion can be drawn that the calculation of  $C_{29}Ts/C_{29}H$  ratio either by GC-MS or GC-MS-MS does not significantly influence the geochemical interpretation, if routine GC-MS is performed with column heating rate at  $2\text{ }^{\circ}\text{C min}^{-1}$ .

In the case of all other parameters investigated ( $C_{29}H/C_{30}H$ ,  $C_{30}M/C_{30}H$ ,  $GI$ , and  $OI$ ; Table S-I) the concordance correlation analysis of the results calculated from two instrumental techniques revealed poor agreement with the concordance correlation coefficients lower than 0.33 (Table I) and significant deviation from the line of equality (Fig. 1c-f).

#### *The analysis of the mean-difference plots*

Further investigation comprised the construction of difference plot for each parameter (the plot of the difference between the values of the parameter calculated from GC-MS and GC-MS-MS results for each sample against their mean) and their visual and statistical analysis. The difference plots for the geochemical parameters analysed are shown in Fig. 2.

The statistical analysis comprised a one-sample  $t$ -test of the difference for the paired measurements of the organic geochemical parameters analyzed. The purpose of this test was to determine if the difference between the values obtained from GC-MS and GC-MS-MS is statistically considered not to be zero. The results are shown in Table II.

The  $t$ -test of the difference of the  $Ts/(Ts+Tm)$  parameter (Tables II and S-I) showed that the mean value of the difference between GC-MS-MS and GC-MS results is statistically significantly different from 0. These results indicate that these two methods do not agree equally through the investigated range of measurements. The visual analysis of the difference plot (Fig. 2a) revealed that the GC-MS-MS gives higher values for the  $Ts/(Ts+Tm)$  parameter (Fig. 2a), and that the difference from the GC-MS results for this parameter is in the range from 0.01 to 0.14, with the arithmetic mean at 0.07 (Table II). Although the statistical analysis indicated significant difference in calculation of  $Ts/(Ts+Tm)$  parameter based on GC-MS and GC-MS-MS analyses, from the organic geochemical point

of view these differences cannot be considered significant.  $Ts/(Ts+Tm)$  ratio depends on both, depositional environment and maturity and therefore is usually used in geochemical research only to confirm the other biomarker ratios. Accordingly, it can be concluded that the differences observed in this research would not probably affect geochemical interpretation.

TABLE II. One-sample *t*-test of the difference; if the calculated *P*-value is lower than 0.05 the conclusion is that, statistically, the sample mean is significantly different from zero; for the abbreviations of parameters, see the legend of Table I. Values of all parameters for individual samples are given in Table S-I of the Supplementary material to this paper

Parameter	Sample size	Arithmetic mean	Lower limit	Upper limit	Significance level ( <i>P</i> )
$Ts/(Ts+Tm)$	68	0.07	0.01	0.14	< 0.0001
$C_{29}Ts/C_{29}H^a$	44	-0.03	-0.13	0.08	0.0018
$C_{29}H/C_{30}H$	68	0.02	-0.14	0.18	0.0493
$C_{30}M/C_{30}H$	68	-0.06	-0.09	-0.02	< 0.0001
GI	68	-4.10	-10.10	1.90	< 0.0001
OI	55	5.60	-0.70	12.00	< 0.0001

<sup>a</sup>Velebit oils were excluded from the analysis

In the difference plot, it is also visible that the difference between GC-MS-MS and GC-MS results is similar for the oils from the same oil field but it is dissimilar for the oils from different oil fields. These results are in agreement with the previous concordance correlation analysis and confirm the importance of the analysis of the agreement between GC-MS-MS and GC-MS results in organic geochemical interpretations. Moreover, the influence of variability of the instrument's working parameters over time might also be the reason for some of these differences between the samples which were not analysed at the same time.

Considering the fact that the concordance correlation analysis of the  $C_{29}Ts/C_{29}H$  revealed a different pattern for the Velebit oils (most probably affected by the higher heating rate during GC-MS analysis), these samples were excluded from the further analysis of this parameter. The *t*-test of the difference of the  $C_{29}Ts/C_{29}H$  ratio (Tables II and S-I) showed that the mean value of the difference between GC-MS-MS and GC-MS results is statistically significantly different from 0. These results imply that these two methods do not agree well in the investigated range of measurements.

The visual inspection of the difference plot (Fig. 2b) indicated a similar pattern for the oils originating from the same oil basin. On one hand, the results for the Serbian oils are centred around the zero line, demonstrating low difference in the values of this parameter obtained from different instrumental methods and good agreement between them. On the other hand, the results for the oils from the Libyan Sirte basin indicate a systematic shift between the GC-MS-MS and GC-MS values. The values of the  $C_{29}Ts/C_{29}H$  ratio in the Sirte oils determined

by GC-MS-MS are lower and the difference from the GC-MS results for this parameter is in the range from 0.08 to -0.13, with the arithmetic mean at -0.03 (Table II). Even though the statistical analysis suggested a significant difference in calculation of  $C_{29}Ts/C_{29}H$  parameter based on GC-MS and GC-MS-MS analyses, these differences cannot be considered significant in geochemical investigations. Since  $C_{29}Ts/C_{29}H$  ratio as an analogue to the  $Ts/(Ts+Tm)$  ratio depends on both, depositional environment and maturity, it is therefore usually used in geochemical research to confirm other biomarker ratios. Because of that it can be concluded that the observed difference would not significantly influence geochemical interpretation.

The *t*-test of the difference of the  $C_{29}H/C_{30}H$  parameter (Tables II and S-I) revealed that the mean value of the difference between GC-MS-MS and GC-MS results is statistically significantly different from 0, suggesting a possible disagreement between these two methods for the investigated set of measurements. The observed differences could be attributed to already discussed co-elution of certain compounds with  $C_{29}H$ , as well as to contribution of 30-nor-17 $\alpha$ (H)-hopane,<sup>2</sup> and some other non-identified compounds to  $C_{30}H$  peak.

The visual analysis of the difference plot (Fig. 2c) showed that the difference between the GC-MS-MS and GC-MS results is scattered around the zero line within the  $\pm 0.16$  range (Table II). In organic geochemical research the  $C_{29}H/C_{30}H$  ratio is used for the distinction of anoxic carbonate or marl sourced oils having the value of this parameter  $\geq 1$  from other samples. Accordingly, it can be concluded that the observed differences between the GC-MS-MS and GC-MS results in most cases would not significantly influence the geochemical interpretation.

In the difference plot (Fig. 2c) a similar pattern for the oils from the same oil field is noticeable, indicating that this parameter is influenced by both, origin and maturity of crude oils. However, a considerably different pattern for the oils from the different oil fields is noticeable as well. According to these results it can be concluded that the agreement between the GC-MS-MS and GC-MS results in analysis of the  $C_{29}H/C_{30}H$  parameter can vary from one oil field to another in both, the sign and magnitude.

The *t*-test of the difference of the  $C_{30}M/C_{30}H$  parameter (Tables II and S-I) showed that the mean value of the difference between GC-MS-MS and GC-MS results is statistically significantly different from 0, implying a possible disagreement between these two methods for the investigated set of measurements. The difference between GC-MS-MS and GC-MS results for this parameter is in the range from -0.02 to -0.09, and the arithmetic mean is at -0.06 (Table II). In geochemical interpretations the  $C_{30}M/C_{30}H$  ratio is used as maturity and age indicator. Its values usually range from 0.05 to 0.20 in oils, having boundary value for interpretation of 0.10. Considering all these facts, it can be concluded that the differences we observed between the GC-MS-MS and GC-MS results in

the analysis of the  $C_{30}M/C_{30}H$  parameter should be considered important in geochemical studies.

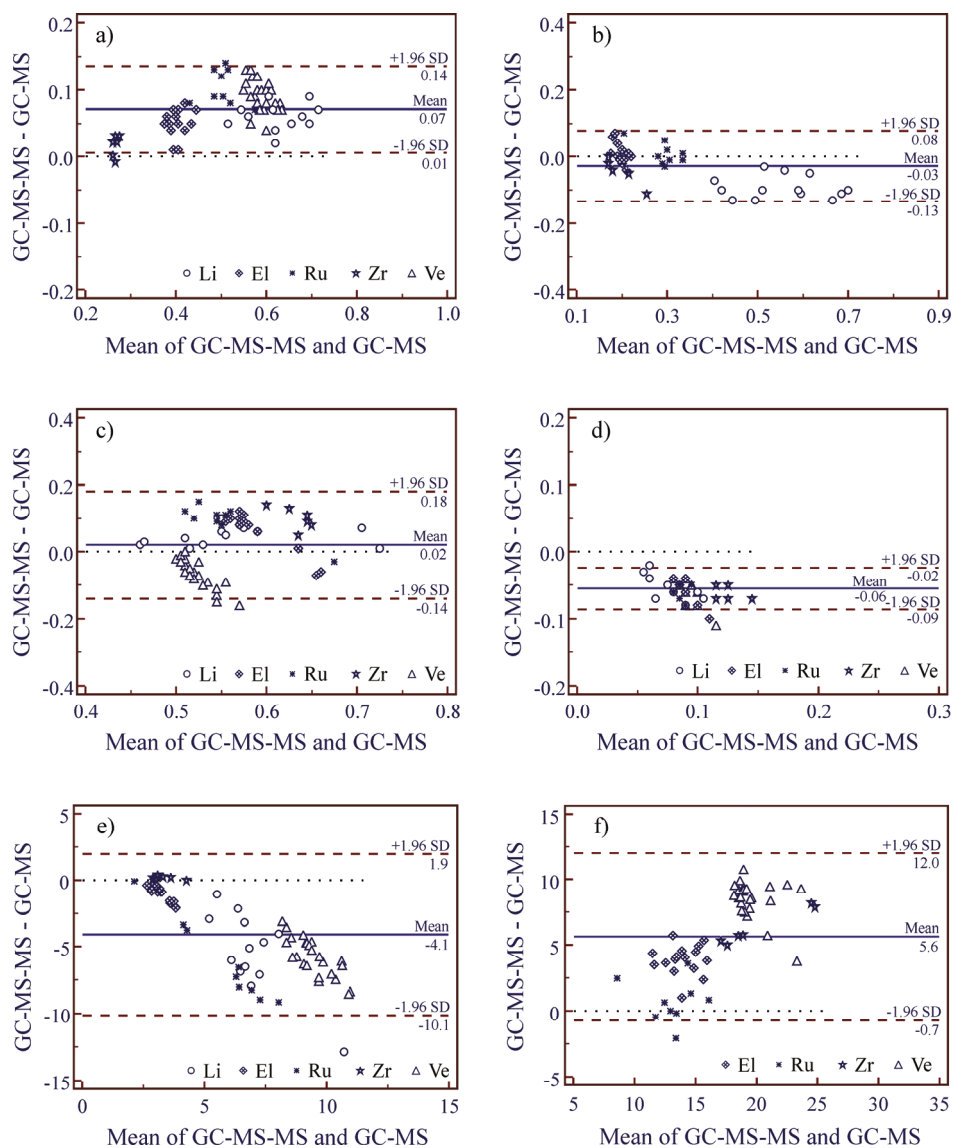


Fig. 2. Mean-difference plots for parameters: Ts/(Ts+Tm) (a);  $C_{29}Ts/C_{29}H$  (b);  $C_{29}H/C_{30}H$  (c);  $C_{30}M/C_{30}H$  (d); GI (e) and OI (f). Li – Libya oils; El – Elemir oils; Ru – Rusanda oils; Zr – Zrenjanin oils; Ve – Velebit oils.

The examination of the difference plot (Fig. 2d) revealed that the values of the  $C_{30}M/C_{30}H$  parameter determined by GC-MS-MS are lower than those det-

etermined by GC-MS. This analysis (Fig. 2d) also revealed a very similar pattern for all oils investigated indicating a systematic difference between the two analytical methods, which is in this case very similar for all oils investigated regardless of their origin.

Possible influence of co-elution in GC-MS traces on C<sub>30</sub>H peak has already been discussed. However, C<sub>30</sub>M peak can also be affected by the co-elution of C<sub>30</sub>17 $\alpha$ (H)21 $\alpha$ (H)-hopane or C<sub>31</sub>17 $\alpha$ (H)22(S)-diahopane during GC-MS analyses, particularly under conditions of poor column performance. Finally, the differences are also related to different relative responses of compounds. C<sub>30</sub>M has a smaller molecular ion than C<sub>30</sub>H in its mass spectrum leading to a lower response in GC-MS-MS.

The *t*-test of the difference of the *GI* (Tables II, S-I) indicated that the mean value of the difference between GC-MS-MS and GC-MS results is statistically significantly different from 0. These results suggest that these two methods do not agree through the investigated range of measurements. Visual inspection of the difference plot (Fig. 2e) showed that the lower values measured by GC-MS-MS, comparing to GC-MS should always be expected. Furthermore, from this figure is obvious that a proportional difference exists between these results, with an increase in the differences between GC-MS and GC-MS-MS results corresponding to larger values. All these results demonstrate the significant disagreement between the GC-MS-MS and GC-MS results in *GI* analysis. The reason for this disagreement can be explained by the fragmentation pattern of gammacerane during GC-MS analysis. Due to its high symmetry, gammacerane molecule always gives two identical fragments in *m/z* 191 mass chromatograms. Because of that, the gammacerane peak always suggests much larger values and the correction of the results is usually needed for proper interpretation.<sup>25</sup> Furthermore, some authors found that gammacerane co-elutes with a C<sub>31</sub> methylhopane, which can be another source of unrealistically high gammacerane index values measured from GC-MS.<sup>26</sup> Other authors found that under conditions of poor column performance, gammacerane can nearly co-elute with the 22R epimer of C<sub>31</sub>17 $\alpha$ (H)21 $\beta$ (H)-homohopane, which can additionally result in unrealistically high values of gammacerane ratio.<sup>2</sup> However, it should be emphasized that when gas chromatographic columns different in selectivity are used, gammacerane can have different relative retention times. As a result, the resolution of gammacerane peak from other neighbouring compounds can be different, and accordingly, the number of coeluting compounds can differ as well.

Other probable causes of erroneous gammacerane quantification are coelution of a hexacyclic C<sub>31</sub> hopane (MW 424),<sup>27</sup> coelution of one of the C<sub>31</sub> neohopanes (C<sub>31</sub>Ts) and coeluting tricyclic terpanes.

Finally, it can be concluded that the difference between GC-MS-MS and GC-MS results in analysis of *GI* is both, statistically and geochemically significant.

ant. Due to the all aforementioned reasons the GC–MS analysis always gives higher results requiring careful geochemical interpretation.

The *t*-test of the difference of the *OI* (Tables II, S-I) indicated that the mean value of the difference between GC–MS-MS and GC–MS results is statistically significantly different from 0. These results also indicated a possible disagreement between these two methods for the investigated set of measurements. The visual analysis of the difference plot (Fig. 2f) showed that the GC–MS-MS method always gives higher values of this parameter comparing to the GC–MS results. Furthermore, a proportional difference between these results can be noticed, with an increase in the differences between GC–MS and GC–MS-MS results corresponding to larger values. All these results point to the significant disagreement between these two methods. These differences can be explained by co-elution and peak overlapping in GC–MS analyzes but also by better separation, higher precision and better selectivity of the GC–MS-MS system used.

Numerous terrigenous triterpanes may coelute with both oleanane and hopane. Additionally, similarly to some other geochemical parameters, the differences between GC–MS and GC–MS-MS results are also related to the dissimilar relative responses of compounds. The molecular ion of oleanane is larger than that of C<sub>30</sub>H in its mass spectrum leading to a higher response in GC–MS-MS.

The visual analysis of the difference plot (Fig. 2f) also revealed that the difference magnitude between GC–MS-MS and GC–MS results in *OI* determination depends on the oil field studied. For the Rusanda oil samples the difference is close to 0 indicating a good agreement between the two methods and that they may be used interchangeably for the analysis of these crude oils.

However, for the oils from the other oil fields the difference between GC–MS-MS and GC–MS results is higher and they are increasing in the following order Elemir < Zrenjanin < Velebit. For these three oil fields the difference between two analytical methods is not only statistically but also geochemically significant, and can significantly affect geochemical interpretation. Considering the fact that oleanane in crude oils is an indicator for both source input and geologic age,<sup>2,28</sup> these results are not surprising. Nevertheless these results also stress the importance of the regional calibration between GC–MS-MS and GC–MS results for each oil field and each parameter to be analysed.

#### CONCLUSION

In this study a comparison of the values of parameters calculated from distributions and abundances of selected pentacyclic terpanes in crude oils, derived from gas chromatography-mass spectrometry (GC–MS) and gas chromatography–mass spectrometry-mass spectrometry (GC–MS-MS) quantification results was performed. The parameters analyzed are the most often used terpane source and maturity parameters, which were applied to a large sample set of 70 oils.

Two statistical methods were used: concordance correlation coefficient and mean-difference plot.

The analyses of the  $T_s/(T_s+T_m)$ ,  $C_{29}Ts/C_{29}H$  and  $C_{29}H/C_{30}H$  parameters indicated a good agreement between the results obtained by the GC-MS-MS and GC-MS methods and that they may be used interchangeably in the determination of these parameters. However, it was also noticeable that the agreement between the GC-MS-MS and GC-MS results in analysis of these three parameters can vary from one oil field to another in the sign and/or magnitude. Although these differences were found to be statistically significantly different, they are not geochemically significantly different because in most cases they would not affect geochemical interpretations.

The analyses of the  $C_{30}M/C_{30}H$  parameter,  $GI$  and  $OI$  showed that the difference between the GC-MS-MS and GC-MS results is both statistically and geochemically significant. The difference between two analytical methods in determination of these three parameters can significantly affect the geochemical interpretation. Because of that  $C_{30}M/C_{30}H$  parameter,  $GI$  and  $OI$  require careful analysis and interpretation depending on the analytical methods used.

The results obtained in this study point to the importance of comparison between GC-MS-MS and GC-MS results in organic-geochemical studies. Considering the fact that most of the organic geochemical parameters were originally defined on the basis of the results of GC-MS analysis, whenever GC-MS-MS results are to be used in organic geochemical interpretations, a regional calibration of GC-MS vs. GC-MS-MS relationship for each petroleum system is highly recommended.

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#### SUPPLEMENTARY MATERIAL

Values of terpane parameters in investigated oils are available electronically at the pages of the journal website: <http://www.shd.org.rs/JSCS/>, or from the corresponding author on request.



## ИЗВОД

## GC–MS НАСПРАМ GC–MS–MS АНАЛИЗЕ ПЕНТАЦИКЛИЧНИХ ТЕРПАНА У СИРОВИМ НАФТАМА ИЗ ЛИБИЈЕ И СРБИЈЕ – ПОРЕЂЕЊЕ ДВЕ МЕТОДЕ

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Вредности параметара израчунатих на основу расподела и обилности одабраних пентацикличних терпана у сировим нафтами из Либије и Србије, који су оригинално добијени из резултата гасно хроматографско–масено спектрометријске (GC–MS) анализе упоређене су са квантификационим резултатима који су засновани на гасно хроматографско–масено спектрометријско–масено спектрометријској (GC–MS–MS) анализи. Анализирани параметри су најчешће коришћени терпански изворни и матурациони параметри, који су примењени на велику групу узорака од 70 сирових нафти, које потичу из пет нафтних поља. Циљ овог рада био је да испита у којој мери се мерења одабраних параметара овим два техникама слажу и да одреди утицај разлика између вредности ових параметара на геохемијску интерпретацију. У том циљу, коришћене су две статистичке методе: коефицијент слагања корелација и дијаграм средња вредност–разлика. Добијени резултати показују да израчунавање  $C_{27}18\alpha(H)-22,29,30$ -триснорнеохопан +  $C_{27}17\alpha(H)-22,29,30$ -триснорхопан,  $C_{29}18\alpha(H)-30$ -норнеохопан/ $C_{29}17\alpha(H)21\beta(H)-30$ -норхопан и  $C_{29}17\alpha(H)21\beta(H)-30$ -норхопан/ $C_{30}17\alpha(H)21\beta(H)$ -хопан односа било GC–MS или GC–MS–MS техником не утиче значајно на интерпретацију. С друге стране, одређивање  $C_{30}17\beta(H)21\alpha(H)$ -моретан/ $C_{30}17\alpha(H)21\beta(H)$ -хопан односа, гамацеранског индекса и олеананског индекса GC–MS или GC–MS–MS техником може значајно утицати на интерпретацију. Ове разлике се могу објаснити коелуирањем и преклапањем пикова током GC–MS анализе али такође и бољим раздвајањем, већом прецизношћу и бољом селективношћу GC–MS–MS технике. Одступање скоро свих параметара од линије једнакости је слично за нафте из истог нафтног поља, али су уочене разлике при анализи нафти из различитих нафтних поља. Стога, када се планира примена GC–MS–MS резултата у органско геохемијским интерпретацијама, препоручује се и регионална калибрација односа између GC–MS и GC–MS–MS резултата за сваки нафтни систем.

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