A Non-aqueous Capillary Electrophoresis for Determination of Eugenol in Cloves and Dental Preparations

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Abstract

A total non-aqueous capillary electrophoresis method was developed and applied for the first time for the quantification of eugenol in cloves and dental preparations. The optimized conditions included a buffer consisting of 150 mM sodium acetate and 300 μL 1 M acetic acid methanol solution (30 mM), an applied voltage of 25 kV, and a temperature of 25 °C and an applied wavelength of 214 nm. The developed method of determining the eugenol was characterized by the following parameters: a detection time within 1.97 min, good linearity ($R^2 = 0.9989–0.9999$), detection limit at the level from 0.19 to 0.35 µg mL⁻¹, very good extraction yield of 99.6–100.6% from both methanol standard solutions, clove buds’ matrix, and dental preparations. Limit of quantitation at the level from 0.81 to 0.98 µg mL⁻¹. The method is based on the developed one-step extraction procedure. Moreover, the developed method does not require the use of any eugenol solubility enhancers such as SDS.

Keywords Biological fluids · Cloves · Dental preparations eugenol · Non-aqueous capillary electrophoresis

Introduction

The plant world provides a huge amount of biologically active compounds that are widely used in medicine and the pharmaceutical industry. One of such compounds is eugenol (2-methoxy-4-(2-propenyl)phenol) (EUG) found in cloves derived from Syzygium aromaticum L. plants [1, 2]. It is mainly obtained from cloves in the process of steam distillation or microwave extraction [3, 4]. It is an oily liquid, very sparingly soluble in water and well soluble in methanol [5]. Eugenol has found a wide application in the food industry, where it is mainly used as an antibacterial and antifungal agent to prevent food spoilage [6]. In the cosmetics industry, however, it is used for the production of various types of creams, lotions, and oils with antiseptic action [7]. However, the most important seems to be its antiseptic and bactericidal properties used in medicine, especially in dentistry, where eugenol performs a significant role in killing or inhibiting the multiplication of bacteria and has anti-inflammatory and analgesic properties [8, 9]. Some toxicological studies have shown other, very important and significant actions of eugenol. It turns out that besides the commonly known antiseptic and antibacterial properties, eugenol also shows many important intra-body effects. Some studies indicate a hepatoprotective effect of eugenol [10]. Another work described the eugenol inhibitory effect of amyloid-induced hemolysis [11]. On the other hand, it should also be remembered that not all properties of eugenol may have a positive effect on our body. There are many studies describing the toxic effects of eugenol related to the production of phenoxy radicals [12]. Eugenol in certain concentrations may have a toxic effect on liver cells by reducing the concentration of cytoprotective thiol compounds [13]. It can also reduce the viability and proliferation of certain cells, increase the level of reactive oxygen species, and reduce the adenosine tri-phosphate (ATP) level [14].

Therefore, taking into consideration the above-described applications, properties, and effects of eugenol, research on the development of new techniques enabling the quantification of eugenol seems to be important.

Many of such techniques already exist. The most commonly used methods in eugenol research are HPLC [15–17], GC, MS, and their various configurations [18, 19], EC [20], and enzymatic biosensor [21].
These methods are often characterized by a fairly large number of used reagents and being time-consuming.

Additionally, the MEKC method is based on the determination of eugenol in the aqueous environment of the separation system.

Therefore, the aim of this study was to develop the simplest and most stable CE method, allowing for the study of the total and actual eugenol content in various biological preparations.

The non-aqueous CE method presented in this paper allows for the determination of eugenol without the need to use any means to improve its solubility and eliminates any difficulties related to eugenol solubility. This simple CE method allows for the fast determination of eugenol in three different materials such as plant preparation (cloves) and dental preparations, which is a very important aspect considering that eugenol can occur in many raw materials, preparations, and biological materials.

**Materials and Methods**

**Chemical and Reagents**

Eugenol was purchased from Sigma-Aldrich (St Louis, USA). Methanol, hexane, sodium acetate, and acetic acid were purchased from Sigma-Aldrich (Darmstadt, Germany). β-Glucuronidase for enzymatic cleavage was purchased from Sigma-Aldrich (Poznań, Poland). Human plasma SEROSTANDARD N was purchased from Biomed (Kraków, Poland). Tryptophan as an internal standard (IS) (Fig. 1B) was purchased from POCH (Gliwice, Poland). All reagents were of analytical grade (99.5–99.8%). The cloves were purchased from a herbal store (Warsaw, Poland).

**Preparation of Stock and Working Standard Solutions**

The primary standard stock solution of eugenol with a concentration of 10 mg mL⁻¹ was prepared by dissolving 100 mg of eugenol in 10 mL of methanol and stored in –15 °C. This solution was further diluted with methanol every day to obtain working standard solutions of appropriate concentrations form 0.5 to 300.0 μg mL⁻¹. Each concentration of the analyte was evaluated based on the peak area with respect to a quantitative calibration.

The separation process was carried out in the presence of a buffer consisting of 150 mM sodium acetate and 300 μL 1 M acetic acid methanol solution—NACE separation system. The acetate buffer was prepared by dissolving 1230.4 mg of sodium acetate with 300 μL 1 M acetic acid methanol solution in 100 mL of methanol. The solution of NACE separation system was filtered through a syringe filter Milex-GP Sigma-Aldrich (Poznań, Poland) with a pore diameter of 0.22 μm.

**Extraction Procedure of Eugenol from Clove Bud**

In the extraction method developed in this study, 5 g of clove buds were weighed and ground into a very fine powder in a high-speed electric grinder. Then, 100 mg of the preparation was taken from this, and added 100 μL of methanolic tryptophan solution (IS) in a concentration of 10 μg mL⁻¹ and 5 mL of n-hexane. The choice of tryptophan as the internal standard was dictated by its high extraction efficiency of 99.9%. A certain limitation resulting from the use of tryptophan as an internal standard may be the lack of similarity in chemical structure to the determined eugenol. However, despite this limitation, tryptophan appears to have performed well as an internal standard.

The whole sample was thoroughly and vigorously mixed for 2 min using a Vortex, then heated in a water bath at 50 °C for 10 min, and vigorously mixed again (Vortex) for 5 min. Then, it was centrifuged at 8600×g for 5 min. The organic phase was transferred to a second tube and evaporated under nitrogen at 37 °C. The residue was dissolved in 1 mL of the solution of NACE separation system and introduced into a capillary.

**Extraction Procedure of Eugenol from Eugenol from Dental Preparation (Oil Preparation)**

To develop a method of extracting eugenol from a dental preparation in the form of an oil preparation, the extraction process was based on the initial use of a minimum amount of aqueous solution to remove any possible water-soluble pollutants. A methanolic tryptophan solution as an internal standard (IS) in a concentration of 10 μg mL⁻¹ and 5 mL of n-hexane have been added to this layout and subjected to the sequential extraction steps described above. The organic phase was transferred to another tube and evaporated under nitrogen at 37 °C. Finally, the residue was dissolved in 1 mL of solution of NACE separation system and introduced into a capillary.
Extraction Procedure of Eugenol from Dental Preparation (Mixture of Zinc Oxide and Eugenol)

A dental preparation was prepared in the amount of 2 g consisting of a mixture of zinc oxide and eugenol in a 2:1. From this, 100 mg of the mass was weighed and placed in a dialysis bag, and 2000 µL of methanol was added. After the specified time (2, 4, 6, 8, 12, and 24 h), the entire solution (dialysate) was transferred to a glass test tube. From the tube containing the dialysate, 20 µL of the solution was withdrawn and diluted with background electrolyte (solution of NACE separation system) 5000 times. This operation was repeated many times at specified time intervals (2, 4, 6, 8, 12, and 24 h) and the diluted analyses were subjected to electrophoretic analysis.

Extraction Procedure of Eugenol and Eugenol Glucuronide from Serum

To develop the serum extraction method, three standardized serum solutions (SEROSTANDARD N) were used in the amount of 1 mL, to which were added 5, 50, and 300 µg of eugenol and 100 µL of methanol solution of eugenol in a concentration of 10 µg mL⁻¹. The mixture was placed in a narrow 10 mL tube closed with a Teflon stopper and mixed (Vortex) for 15 min. Then, 5 mL of n-hexane was added to the tube and mixed (Vortex) for 3 min to avoid foaming of the mixture. Then heated whole in a water bath at 50 °C for 10 min and vigorously mixed again (Vortex) for 5 min. This procedure was repeated twice. Then, it was centrifuged at 8600×g for 5 min. The organic phase was transferred to a second tube and evaporated under nitrogen at 37 °C. The residue was dissolved in 1 mL of solution of NACE separation system and introduced into a capillary as described earlier. The above-described procedure was also used to extract eugenol glucuronide from serum.

Instrumentation

A Beckman–Coulter P/ACE MDQ Capillary Electrophoresis system equipped with a UV/visible detector was used. For the analysis of the investigated compound, an eCAP fused-silica capillary (40 cm total length, 30 cm effective length, 75 µm id, 375 µm od) was used. A Karat software version 32 was used for the control of all parameters of the CE.

CE Conditions

Electrophoretic analysis was carried out using an eCAP fused-silica capillary described in the instrumentation section Extraction Procedure of Eugenol and Eugenol Glucuronide from Serum. All the samples containing eugenol were determined using a buffer consisting of 150 mM sodium acetate and 30 mM of acetic acid in methanol. The detector was set at 214 nm. The applied voltage was 25 kV. The capillary temperature was set at 25 °C.

New capillary was rinsed for 10 min with 1 M NaOH under a pressure of 20 psi and electroconditioned with the 1 M NaOH by applying a separation voltage (10 kV) for 5 min. Then, the capillary was flushed with the solution of NACE separation system (15 min, 20 psi). Samples were hydrodynamically injected using a pressure of 2 psi for 5 s. Between runs, the capillary was rinsed with BGE for 2 min. The working BGE was refreshed after every analysis to ensure optimum separation repeatability. The vials containing the buffer were emptied at the end of the day and refilled with the running buffer at the beginning of the next working day, prior to the analysis.

Method Validation

The developed method was validated for linearity, specificity, precision, accuracy, extraction recoveries, and limits of detection (LODs) and quantification (LOQs), according to the International Council on Harmonization (ICH) guidelines [22] and guideline on bioanalytical method validation [23].

Specificity of the method was defined as its ability to differentiate and quantify an analyte of interest and an internal standard from the endogenous components in the matrix, or other components in the sample.

Linearity of the method was evaluated based on the measurements of areas under the peaks for eugenol standard solutions at concentrations of 1.0 µg mL⁻¹, 10.0 µg mL⁻¹, 50.0 µg mL⁻¹, 100.0 µg mL⁻¹, 200.0 µg mL⁻¹, and 300.0 µg mL⁻¹, containing an IS at a concentration of 10 µg mL⁻¹. The peak area values obtained for each sample were determined from the ratio of the analyte peak area/IS versus the analyte concentrations with respect to the six-point calibration curves.

Precision of the method was carried out by analyzing the eugenol standard solutions at two different concentration levels (5.0 µg mL⁻¹ and 300 µg mL⁻¹) containing IS at a constant concentration of 10 µg mL⁻¹.

To evaluate the repeatability and intermediate precision, each measurement was carried out six times within the same day and on six different days. Results for the precision and repeatability were expressed as RSD%.

Accuracy and extraction recoveries were evaluated for standard solutions, clove buds, dental preparation, and serum. To the clove buds, the known amount of eugenol was added and subjected to an extraction process and diluted 1:100, 1:1000, and 1:10,000. Each analysis included the addition of an internal standard (IS). Measurements were made for low (5.0 µg mL⁻¹), medium (50.0 µg mL⁻¹), and
high (300.0 µg mL⁻¹) concentration of eugenol for each extraction system. Each sample was measured six times. Based on the obtained recovery values, the accuracy of the method was calculated.

LOD was determined at the lowest eugenol concentrations, with an S/N (signal-to-noise) ratio of at least 3 for this compound.

LOQ has been determined by quantitative measurement of the lowest eugenol concentration with a stated and acceptable accuracy and precision (CV% < 20).

Results and Discussion

To determine the best conditions for eugenol determination, the following parameters were tested with the developed CE method: analytical wavelength ($\lambda$), buffer consisting [sodium acetate (mM) and acetic acid methanol solution (mM)] ($C$), voltage (kV), and temperature ($T$). Present in the buffer, the sodium acetate did not cause disturbances in the background image, nor did it affect contamination with the analysis components. Each of the parameters was tested in the order of their increasing values and presented in the form of the dependence of the detection level and migration rate on the value of five measurement points (Fig. 2). The measurement points presented the following parameter values:

A. $\lambda = 300$ nm, $C = 25$ mM sodium acetate and 30 mM acetic acid, kV = 10, $T = 10$ °C
B. $\lambda = 280$ nm, $C = 50$ mM sodium acetate and 30 mM acetic acid, kV = 15, $T = 15$ °C
C. $\lambda = 254$ nm, $C = 100$ mM sodium acetate and 30 mM acetic acid, kV = 20, $T = 20$ °C
D. $\lambda = 214$ nm, $C = 150$ mM sodium acetate and 30 mM acetic acid, kV = 25, $T = 25$ °C
E. $\lambda = 200$ nm, $C = 200$ mM sodium acetate and 30 mM acetic acid, kV = 30, $T = 30$ °C.

For choosing the detection wavelength, the wavelength scanning was performed at five different wavelengths. The results of the conducted studies showed that the detection level of the substance under test decreased with the increase of the wavelength. The best wavelength with the highest detection level was $\lambda = 214$ nm. The detection sensitivity was taken according to the peak area.

As the buffer concentration increased, the migration time decreased and the detection level increased. After exceeding the concentration of 150 mM sodium acetate, the migration time increased and the detection level deteriorated. Among the five tested buffer concentrations, the shortest migration time and the highest level of detection was obtained at 150 mM of sodium acetate with 300 µL 1 M acetic acid methanol solution (30 mM).

The current voltage was tested in the range from 10 to 30 kV. The successively increasing voltage accelerated the migration of the investigated compound and thus shortened the migration time. The use of a voltage greater than 25 kV caused disturbances and the lack of stability of the current intensity. Therefore, the best voltage was 25 kV.

The study of the influence of temperature changes on the level of detection and migration rate of the determined compound showed that the best temperature is 25 °C. At lower temperatures, the rate of migration was slower, whereas increasing the temperature to 30 °C resulted in the instability of the current flow.

Specificity

The specificity of the method was determined by comparing the retention times and eugenol ratios in standard solution and in the presence of clove buds’ matrix, dental preparation, and serum, and by adding a solvent standard to the matrix and testing the influence of foreign substances from the extracted material on the possibility of interference with the tested compound. The developed method showed no interference of foreign/accompanying substances with the tested compound (Figs. 3A, B 4A, B, 5A, B), which means that the method is specific. Read from the electropherogram (Fig. 3B), the value of eugenol concentration (198 µg mL⁻¹) after the extraction process from a given amount of cloves is its percentage content in the dry substance and is 19.8%.

Linearity

The developed method was linear in the range from 0.5 to 300 µg mL⁻¹ with the correlation coefficient
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Accuracy

The accuracy of the method was performed as described in section CE Conditions and the results obtained are showed in Table 2. The accuracy of the method was established at the level of 95.8–100.6%.

Precision

Precision of the method (described in section CE Conditions) was tested based on standard solutions containing 5.0 and 300.0 μg mL\(^{-1}\) of eugenol, and a constant concentration (10 μg mL\(^{-1}\)) of the IS. The results obtained for peak areas were at the level of values 0.9–1.1 ( %RSD) for the concentration of 5.0 μg mL\(^{-1}\) and 0.1 ( %RSD) for the eugenol concentration of 300.0 μg mL\(^{-1}\) and results obtained for migration times were at the level of values 1.1–2.2 ( %RSD) for the concentration of 5.0 μg mL\(^{-1}\) and 0.6–1.1 ( %RSD) for the eugenol concentration of 300.0 μg mL\(^{-1}\) (Table 3).

Limit of Detection (LOD) and Quantification (LOQ)

The LOD and LOQ methods were performed as described in section Extraction Procedure of Eugenol and...
Table 1  Regression equation, limits of detection, and quantification for compound (eugenol) \((n=6)\)

| Compound       | Linearity range (μg mL\(^{-1}\)) | \(R^2\) (mean) | (%RSD) | LOD (μg mL\(^{-1}\)) | LOQ (μg mL\(^{-1}\)) | Regression equation (mean) | (± SD) | Slope | Intercept |
|----------------|----------------------------------|----------------|--------|----------------------|----------------------|---------------------------|--------|-------|-----------|
| Eugenol\(_{CLOVES}\) | 1–300                           | 0.9999         | 1.58   | 0.28                 | 0.90                 | \(y = 1.004x - 0.708\)    | ± 0.13 | ± 2.25|
| Eugenol\(_{DentalPrep}\) | 0.5–300                         | 0.9999         | 1.14   | 0.19                 | 0.81                 | \(y = 1.014x - 0.028\)    | ± 0.10 | ± 1.92|
| Eugenol\(_{SERUM}\)   | 2–300                           | 0.9989         | 1.92   | 0.35                 | 0.98                 | \(y = 1.025x - 0.046\)    | ± 0.19 | ± 2.96|

LOD and LOQ were determined at S/N (of signal-to-noise ratio) of 3 and 10, respectively. Eugenol\(_{CLOVES}\)—eugenol from cloves. Eugenol\(_{DentalPrep}\)—eugenol from dental preparation. Eugenol\(_{SERUM}\)—eugenol from serum.

Table 2  Recovery data of compound (eugenol) from clove buds, dental preparation, and serum

| Compound       | From standard solutions after extraction process \((n=6)\) calculated in relation to IS | From clove buds samples after extraction process \((n=6)\) calculated in relation to IS | Added amount (μg mL\(^{-1}\)) | Observed amount (Mean ± SD) | %Recovery | % RSD |
|----------------|---------------------------------------------|---------------------------------------------|-------------------------------|-------------------------------|-----------|-------|
| Eugenol        |                                             |                                             | 5.0                           | 4.98 ± 0.17                   | 99.6      | 3.41  |
|                |                                             |                                             | 50.0                          | 49.95 ± 0.87                  | 99.9      | 1.74  |
|                |                                             |                                             | 300.0                         | 300.10 ± 1.07                 | 100.0     | 0.36  |

| Compound       | From dental preparation samples after extraction process \((n=6)\) calculated in relation to IS | From serum samples after extraction process \((n=6)\) calculated in relation to IS | Added amount (μg mL\(^{-1}\)) | Observed amount (Mean ± SD) | %Recovery | % RSD |
|----------------|---------------------------------------------|---------------------------------------------|-------------------------------|-------------------------------|-----------|-------|
| Eugenol        |                                             |                                             | 5.0                           | 5.00 ± 0.07                   | 100.0     | 1.40  |
|                |                                             |                                             | 50.0                          | 50.01 ± 0.09                  | 100.0     | 0.18  |
|                |                                             |                                             | 300.0                         | 300.01 ± 0.05                 | 100.0     | 0.02  |

Table 3  Intra-day and inter-day precision of compound (eugenol)

| Compound       | Concentration (μg mL\(^{-1}\)) | Intra-day precision \((n=6, \text{mean})\) (± SD) | Day 1 | Day 2 | Day 3 |
|----------------|-------------------------------|-----------------------------------------------|-------|-------|-------|
|                |                               | \(t_m^a\) | \(P^b\) | \(t_m^a\) | \(P^b\) | \(t_m^a\) | \(P^b\) | \(t_m^a\) | \(P^b\) |
| Eugenol        | 5.0                           | 1.81 ± 0.02 | 1116 ± 11.04 | 1.80 ± 0.02 | 1110 ± 11.50 | 1.81 ± 0.02 | 1118 ± 10.60 |
| RSD (%)        | 1.1                           | 1.0       | 1.1       | 1.0       | 1.1       | 1.1       | 0.9       |
|                | 300.0                         | 1.80 ± 0.01 | 66,958 ± 64.56 | 1.81 ± 0.01 | 66,962 ± 65.54 | 1.81 ± 0.01 | 66,965 ± 62.72 |
| RSD (%)        | 0.6                           | 0.1       | 0.6       | 0.1       | 0.6       | 0.1       | 0.1       |

Inter-day precision \((n=18, \text{mean})\) (± SD)

| Compound       | Concentration (μg mL\(^{-1}\)) | Inter-day precision \((n=18, \text{mean})\) (± SD) |
|----------------|-------------------------------|--------------------------------------------------|
|                |                               | \(t_m^a\) | \(P^b\) | \(t_m^a\) | \(P^b\) |
| Eugenol        | 5.0                           | 1.82 ± 0.04 | 1116 ± 12.50 |
| RSD (%)        | 2.2                           | 1.1       | 1.1       |
|                | 300.0                         | 1.81 ± 0.02 | 66,967 ± 63.86 |
| RSD (%)        | 1.1                           | 0.1       | 0.1       |

\(^a\) (± SD) and %RSD of migration time
\(^b\) (± SD) and %RSD of peak area
EugenolGlucuronide from Serum and the results obtained are shown in Table 1.

The LOD for eugenol was established at the level of 0.19–0.35 µg mL⁻¹, and the LOQ for eugenol was established at the level of 0.81–0.98 µg mL⁻¹ (Table 1).

As mentioned earlier, eugenol was also measured by many other methods. It should be emphasized that each of them was developed only for the determination of eugenol in one research material.

This work also presents the possibility of determining the released fraction of eugenol from a dental preparation (Fig. 6) used as a paste filling a cavity in a tooth. The mean results of measurements of the areas under the peaks for the fractions of free eugenol released in specific time periods (Table 4) show the possibility of testing the release of free eugenol from a dental preparation (a mixture of zinc oxide and eugenol). Of course, testing the amount of eugenol released is limited by the limit of quantification of a given method. However, with the assumed 24-h time period and the sum of peak areas obtained in this way, it can be concluded that 42.28% of eugenol from the dental preparation was released.

An attempt was also made to demonstrate that the NACE method used in this study enables the investigation of eugenol in serum, however, after its release from its connections with glucuronic acid (Fig. 7). For this purpose, the serum was subjected to electrophoretic analysis with a defined amount (30.0 µg mL⁻¹) of eugenol glucuronide (Fig. 7A).

![Fig. 6](image_url)  
**Fig. 6** A Representative electropherograms of a standard solution containing 10.0 µg mL⁻¹ of I.S. (migration time: 0.95 min) and 250.0 µg mL⁻¹ of eugenol (E) (migration time: 1.97 min). B Level of eugenol after 2 h of extraction. C Level of eugenol after 4 h of extraction. D Level of eugenol after 6 h extraction. E Level of eugenol after 8 h of extraction. F Level of eugenol after 12 h extraction. G Level of eugenol after 24 h extraction from the dental preparation.

### Table 4 Mean values of the areas under the peaks for the free fraction of eugenol released in specific time periods from the dental preparation (n = 6)

| Time (h) | Area under the peak (µg mL⁻¹) | Concentration (µg mL⁻¹) |
|---------|------------------------------|------------------------|
| 0       | 10,236,558—for the amount added to the formulation | 250.0                   |
| 2       | 1,602,243                    | 39.1                   |
| 4       | 1,112,669                    | 27.2                   |
| 6       | 890,135                      | 21.7                   |
| 8       | 623,094                      | 15.5                   |
| 12      | 89,013                       | 2.2                    |
| 24      | 0                            | 0.0                    |
The used amount of eugenol glucuronide is much higher than expected of this compound in serum, because its use in the amount actually present in the serum would not allow its determination. Eugenol glucuronide was then completely hydrolyzed with beta-glucuronidase and re-analyzed by electrophoresis (Fig. 7B). Unfortunately, this method, due to too low-serum eugenol concentrations, is not available for the quantification limit of the NACE method, compared to other methods [15–21] for the determination of eugenol in the serum at a detectable level. For this purpose, it would be necessary to develop an additional method of concentration of the sample allowing the determination of the eugenol fraction in the serum.

There are many types of methods described in the literature, which are characterized by better parameters [15, 17–21] compared to the method presented in this paper. The exception may be the UHPLC method [16] where NACE method can be considered competitive in relation to the ultra-high-performance liquid chromatography. However, an important advantage of the method presented here is the analysis time, which seems to be the shortest compared to the above-mentioned methods.

Taking into account the analysis times (24 min [15] and 11 min [16]), the NACE method, for which the analysis time is 1.81 min, is an incomparably faster method. Comparing the total analysis times depending on the type of material from which eugenol was extracted, the analysis times ranged from 30 to more than 80 min [17–19]. In the case of the NACE method described here, the total analysis time ranged from 14 to 71 min, which is also a significant advantage. The only method that can be compared is the mentioned MEKC method [24], which allows the determination of eugenol and derivatives present in cloves. However, in this case, the NON-AQUEOUS CE method presented in this paper is better in terms of all compared parameters (Table 5).

Summing up, it should be stated that the optimized non-aqueous CE method may be competitive to some HPLC methods considered as reference methods. However, due to its versatility, simplicity, and very short analysis time, the non-aqueous CE method may be the best solution for the determination of eugenol in various preparations and biological materials characterized by high eugenol contents achievable for the quantification limit for the NACE method presented here.

### Concluding Remarks

The optimized NON-AQUEOUS CE method is the first CE method for the determination of eugenol in a completely anhydrous environment. As it is known, the solubility of eugenol in water is much lower than in organic solvents. The complete elimination of water from the extraction process and the creation of a separation system based on an anhydrous buffer allowed to obtain very good determination parameters characterized by a very fast analysis time, high sensitivity of the method, and a low limit of detection and quantification. As demonstrated, it is possible to determine eugenol in various biological preparations. However, the use of the NACE method for the determination of eugenol in the patient’s serum is not possible at the moment due to too low amounts of eugenol, not available for the LOQ of the presented method. However, the NON-AQUEOUS CE method is a much faster, simpler, more stable method that does not require the use of additional reagents to modify the dissolution process. This method may be an attractive alternative to other methods.

| Table 5 | Comparison of the CE method in the aquatic environment using SDS [24] with the non-aqueous CE method |
|---------|--------------------------------------------------------------------------------------------------|
| CE in the aquatic environment using SDS [24] | Non-aqueous CE method |
| Migration time (min) | 4.67 | 1.97 |
| LOQ (μg mL⁻¹) | 3 | 0.90 |
| LOD (μg mL⁻¹) | 1 | 0.28 |
| Linearity range (μg mL⁻¹) | 25–400 | 1–300 |
| \(R^2\) | 0.998 | 0.9999 |
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Declarations

Conflict of Interest The authors declare that there are no conflicts of interest.

Ethical Approval This article does not contain any studies with human participants or animals performed by any of the authors.

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