Optimization of the conditions of guaiacol and glyoxylic acid condensation to vanillylmandelic acid as an intermediate product in vanillin synthesis

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Abstract. The study is devoted to optimization of synthesis of vanillylmandelic acid as an intermediate product in vanillin production and analytical determination of all components of the reaction mixture. It was noted that variation of synthesis temperature, reaction time and rate of initial reagents affects on the yield of vanillylmandelic acid. Guaiacol disubstituted by two glyoxylic acid molecules is the main impurity in vanillylmandelic acid synthesis what was identified by high resolution HPLC-MS. It is determined that it is optimal to carry out the condensation of guaiacol and glyoxylic acid at the temperature of the reaction mixture no higher than 30 °С and a mole ratio glyoxylic acid : guaiacol not less than 1: 1.5.

1. Introduction
Vanillylmandelic acid (VMA) is the end-product of the metabolism of catecholamines (adrenaline, noradrenaline), as well as an intermediate product in the synthesis of vanillin by the Guaiacol method [1]. The Guaiacol method is more environmentally friendly than the process of vanillin production from lignin-containing raw materials and it allows obtaining a high quality product with low cost as compared with natural vanillin and vanillin extracted from natural raw materials.

Composition of the products of guaiacol and glyoxylic acid (GA) condensation affects significantly on the yield of the final product (vanillin) in the Guaiacol method, since this reaction can be accompanied by the formation of a number of by-products (figure 1).

This is due to the fact that hydrogen in the ortho-position of guaiacol can also be substituted by GA to form 2-hydroxy-3-methoxymandelic (3) and 4-hydroxy-5-methoxy-1,3-benzenediglyoxylic acid (4). Gas chromatography investigation showed [2] that the substitution preferably occurs at the para-position with respect to the hydroxyl group, therefore the amount of impurity compounds 3 and 4 is low. However, since these impurities cannot be selectively extracted from an aqueous solution of VMA (2), which is the product of the first stage of the Guaiacol method, their presence affects the purity of vanillin obtained in the second stage of the synthesis.
Another significant problem is the propensity of GA to disproportionate on the glycolic and oxalic acids in an alkaline medium. The effect of this side reaction can be minimized by conducting it with a pH control of the medium, the value of which shouldn’t be more than 11 [3].

To optimize the conditions of the first stage of the Guaiacol method of vanillin production, it is necessary to control not only guaiacol and VMA content in the reaction mixture, but also by-products and closely related impurities that can affect the quality of vanillin synthesized from VMA at the second stage. The method of quantitative determination should be specific for VMA, guaiacol and compounds related to VMA.

![Diagram of vanillylmandelic acid synthesis and by-products formation]

Figure 1. Scheme of vanillylmandelic acid synthesis and by-products formation.

In addition to the quantitative analysis of the reaction mixture, it is necessary to confirm the authenticity and chromatographic purity of the synthesized VMA, identify by-products of the reaction and impurities that can affect the quality of the target vanillin. The most suitable selective method solving this problem is HPLC method.

Thus, the goal of the research is optimization of the conditions of VMA synthesis by condensation of guaiacol and GA and development of the methods of analytic determination of both main products and by-products of the reaction.
2. Experimental part

2.1. Materials and methods

Guaiacol (Sigma Aldrich) and sodium hydroxide, hydrochloric acid, ethylacetate, benzene (Vekton) were purchased from the commercial vendors. Gradient-grade acetonitrile was supplied by PanReac AppliChem. The ultrapure water used in all experiments was purified with the Milli-Q Simplycy ultrapure water purification system. For identification and quantitative analysis used Guaiacol (98.0 %, Acros Organics), Vanillin (99.0 %, Sigma-Aldrich), Vanillic acid (97.0 % Sigma-Aldrich) and 4-Hydroxy-3-methoxymandelic acid (≥98 %, Sigma). Glyoxylic acid aqueous solution (50 wt.%) was obtained according to our developed method [4].

Vanillinmandelic acid, Guaiacol, Vanillin and its impurities were separated by HPLC method using columns Zorbax Eclipse Plus Phenyl-Hexyl 150 × 4.6 mm, 3.5 μm particle size (Agilent, USA). The mobile phase consisted of aqueous phosphate 50 mM buffer solution with pH = 2.5 (A) and acetonitrile (B) in gradient mode: 0 min. – 5 % of B, 14 min – 70 % of B. Summary time – 20 min; temperature of column 45°C; flow rate 1.5 ml/min, the detection wavelength of UV detector was 272 nm. Samples were dissolved in mixture of phosphate 200 mM buffer solution with pH = 2.5 and acetonitrile (4:1).

The experiments were carried out using an Agilent 1260 Infinity Liquid Chromatography System (Agilent, USA) equipped with a Poroshell EC-C18 reverse-phase 120 column 2.1 × 100 mm with 3.5 μm particle size (Agilent, USA). The column was maintained at 35°C; the injected sample volume was 1 μL. Solvent A – water; solvent B – acetonitrile. Gradient conditions were: 0-3 min 1 % B, 3–8 min linear gradient from 1 to 10% B, 8–9 min return to 1 % B, from 9 to 13 min column equilibration with 1 % B. The flow rate was 300 μL/min. The MS experiments were performed on an Agilent 6550 iFunnel Q-TOF LC-MS system (Agilent, USA), equipped with an electrospray ionization source. An electrospray interface was operated in positive ion mode. The conditions for the acquisition parameters were: gas temperature 200°C, drying gas 14 ml/min, nebulizer pressure 35 psig, sheath gas temperature 350 °C and sheath gas flow 11 ml/min, capillary voltage 3.5 kV. The scan range was 100–500 with 2 Hz sampling rate.

The NMR analysis was carried out using an NMR-spectrometer Bruker AVANCE 400 III HD (Bruker, USA). One-dimensional spectra were recorded on nuclei of atoms H (frequency was 400.17 MHz) to confirm the structure. Dimethylsulfoxide DMSO D-6 (with 99.9 % atoms D) used as the solvent.

2.2. Condensation of guaiacol and GA

An aqueous solution of NaOH (30 wt.%) was added to the mixture of guaiacol and water up to pH = 11. Then, the mixture of 7.4 g (0.05 mol) of glyoxylic acid aqueous solution (50 wt.%) and 6.6 g (0.05 mol) of NaOH aqueous solution (30 wt.%) in 50 mL of water was added dropwise during 1 hour. The reaction was kept at a constant temperature in the range of 8 to 40 °C. The progress of the reaction was controlled by HPLC. After the end of the condensation concentrated hydrochloric acid was added to the dark-brown solution up to pH = 4-5. Reaction mixture was extracted with benzene (2x100 mL). The aqueous layer was then extracted with ethyl acetate (3x100mL). The ethyl acetate extracts were combined, dried with sodium sulphate and concentrated using the rotary evaporator. The solid residue was recrystallized with ethyl acetate to give almost colorless crystals of VMA with melting point 133-134 °C.

3. Results and Discussion

3.1. Identification and quantitative analysis of components of the reaction mixture

Reversed-phase chromatography is optimal for the chromatographic separation of guaiacol, VMA and their related compounds in various solvents (ethylacetate, benzene and toluene) [5]. Spherical silica gel modified by phenylhexylsilane has been chosen as a stationary phase because of its high efficiency
and optimum selectivity in the determination of VMA and its relative substances. This phase has an orthogonal selectivity in the distribution mode due to additional adsorption effects [5].

Mobile phase is a mixture of aqueous phosphate 50 mM buffer solution with pH = 2.5 and acetonitrile (95:5 vol.%). The mobile phase is selected in such a way as to ensure the retention of the most polar components of the reaction mixture such as VMA and its derivatives. An increase in the proportion of acetonitrile leads to an increase in the eluting power of the mobile phase and significantly reduces the retention of the determinate components [6]. To normalize the retention of guaiacol and related components, a gradient elution mode with a linear profile was used.

In addition to guaiacol and VMA, peaks of vanillin and of several impurities formed during the synthesis are present at the chromatogram of the reaction mixture (figure 2).

![Figure 2. Typical chromatogram of reaction mass; retention of compounds: 1 (t_r = 7.3), 2 (t_r = 5.0), 4 (t_r = 2.6), 5 (t_r = 6.8) and its unknown impurity (t_r = 5.8).](image)

Identification of guaiacol, VMA and vanillin was carried out by comparison of their retention times with retention times of standard samples. The main impurity (t_r = 2.6) is the product of disubstitution of guaiacol with glyoxalic acid (C_11H_12O_8) which was confirmed by high resolution HPLC-MS (figure 3). Mass spectrum contains molecular ion (M–H)^– = 271.0459 and ion of sodium adduct (M–2H+Na)^– = 293.0278 corresponding to compound 4.

![Figure 3. Mass-spectra of 4 (t_r = 2.6).](image)

VMA as the main product of the synthesis was isolated by extraction with ethyl acetate from the acidified reaction mixture, purified by recrystallization from ethyl acetate and identified by IR, NMR ^1H spectroscopy and HPLC methods. IR spectrum: ν, cm^{-1}: 3336 (OH), 2971 (CH_3), 2932 (CH_3), 1744 (C=O), 1713 (C=O). NMR ^1H spectrum: δ, ppm (DMSO-d6): s. 8.96, d. 6.96, dd. 6.78, d.
6, 72, s. 5, 68, s. 4, 89, s. 3, 75. Chromatographic purity of VMA was determined by HPLC method at the same conditions.

The quantitative determination of guaiacol and VMA in the reaction mixtures was carried out according to the calibration curves (table 1). Determination of the product of disubstitution of guaiacol and other components was carried out in terms of the VMA along a calibration curve.

**Table 1.** Some parameters of calibration curve of guaiacol and VMA determination.

| Component | Range of calibration | \( C = a \times S + b \) | \( a \) | \( b \) | \( R^2 \) |
|-----------|----------------------|--------------------------|------|------|-------|
| Guaiacol  | 0.05–1.2 %           | 0.002906                 | 0.0  | 0.0  | 0.9998|
| VMA       | 0.05–1.2 %           | 0.000450                 | 0.0  | 0.0  | 0.9999|

3.2. **Optimization of GA and guaiacol condensation**

Based on the results of pilot experiments that were conducted to develop methods for analytical control of components, it was found that a significant amount of only one impurity is formed in the reaction mixture of the condensation products. By HPLC-MS, this impurity was identified as the product of the addition of two molecules of GA to one guaiacol molecule (4, figure 4). The main parameters affecting the yield of the VMA and the content of 4 in the final mixture are synthesis temperature, reagent ratio and duration of the reaction. To optimize the conditions of the condensation stage, the influence of synthesis temperature in the range from 8 °C to 40 °C and the molar ratio of the initial reagents (Guaiacol: GA = 1: 0.75-1.5) were investigated.

3.2.1. **Influence of reaction temperature**

The reaction temperatures were 8 °C, 20 °C, 30 °C, 40 °C and reaction times were 96 h, 72 h, 15 h and 7 h, respectively. The molar ratio of GA: guaiacol was 1:1. The maximum yield of the desired product was obtained during the synthesis in the range of 20-25 °C (figure 4). There was no significant effect of the process temperature on the content of 4 in the reaction mixture (figure 5, line 1).

![Figure 4](image1.png)  
**Figure 4.** Influence of reaction temperature on the yield of VMA.

![Figure 5](image2.png)  
**Figure 5.** Influence of reaction temperature on the content of 4.

An increase of the reaction mixture holding time at temperatures of 30 and 40 °C up to 72 h does not lead to increase of VMA yield. It is connected with expenditure of VMA on the side reaction of disubstituted impurities formation, which is confirmed by the growth of its concentration in the reaction mixture (figure 5, line 2).
3.2.2. Influence of reaction time
The results of the study of the influence of the reaction mixture holding time for the system with the molar ratio of GA: guaiacol = 1: 1 at the temperature of the process of 30 °C are shown in figure 6. It is shown that during the reaction with the chosen parameters, the concentration of the target substance (VMA) ceases after 15 h. Further aging of the mixture does not lead to an increase in the yield of VMA, while a gradual increase in the amount of disubstituted impurity is observed, which make worse the quality of the target product.

![Figure 6](image6.png)

**Figure 6.** The dependence of VMA, guaiacol and impurity concentrations on the reaction time at 30 °C

The maximum content of VMA in the mixture is reached after 7 hours when carrying out the same synthesis at 40 °C, (figure 7). Further aging of the mixture not only leads to an increase in the impurity content, but also to a gradual decrease in the concentration of the product, which is associated with an increase in the rate of its expenditure on the side reaction of formation of 4.

![Figure 7](image7.png)

**Figure 7.** The dependence of VMA, guaiacol and impurity concentrations on the reaction time at 40 °C

3.2.3. Influence of the ratio of the initial components
Experiments were carried out with a molar ratio of GA: Guaiacol = 1: (0.75 - 1.5) at 20 °C. The results were shown in figures 8 and 9. The maximum yield of VMA (82 %) was achieved at a ratio of 1: 1.5. Carrying out the reaction with an excess of guaiacol allows not only to significantly increasing the yield of VMA, but also contributes to a decrease in the content of 4.
Thus, it is preferable to carry out the condensation stage in the presence of an excess of guaiacol not less than 1.5 times.

4. Conclusion
In the present work, a method for the qualitative and quantitative analysis of guaiacol, VMA, 4-hydroxy-5-methoxy-1,3-benzenediglyoxalic acids and vanillin was developed in a co-presence in the reaction mixture. The influence of the ratio of the initial reagents, temperature and time of synthesis on the yield and purity of VMA was studied. It is determined that it is optimal to carry out the condensation stage at the temperature of the reaction mixture no higher than 30 °C and a mole ratio GA : guaiacol not less than 1: 1.5.

Acknowledgements
The study was carried out with the financial support of the Federal Target Program "Research and Development in Priority Areas for the Development of the Scientific and Technological Complex of Russia for 2014-2020" (Agreement No. 14.575.21.0170, unique identifier of the work (draft) RFMEFI57517X0170).

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