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Separation of lactic acid and by-products obtained by catalytic conversion of glycerol using high-performance liquid chromatography

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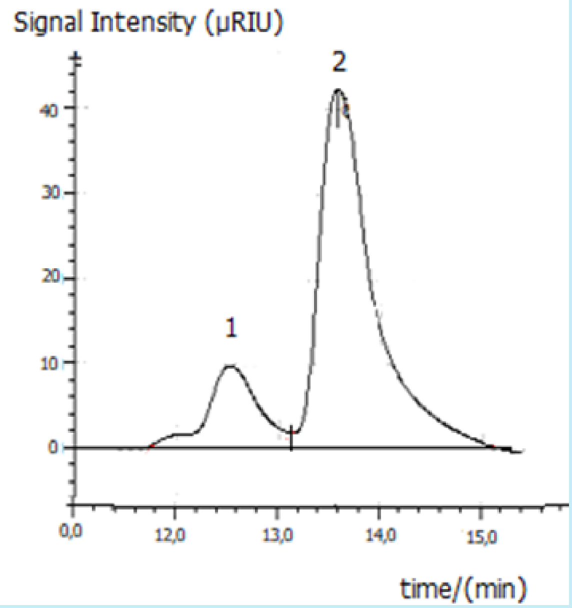
ABSTRACT: Lactic acid is an attractive raw material in synthesizing many products. A new method for quantifying glycerol, lactic acid, and the by-products (pyruvaldehyde) obtained in this reaction was developed using high-performance liquid chromatography (HPLC) with a refractive index detector (HPLC-RI) in a column (300 × 7.7 mm, 8 µm) using H_2SO_4 0.001 M + 10% ACN (organic modifier) as mobile phase (0.6 mL min^{-1}). This method indicated outstanding linearity for glycerol and lactic acid concentration from 0.6 to 6.6 g L^{-1} (coefficient of determination (R^2) = 0.9912 and 0.9961, respectively) and accuracy between 98.33 and 100.00%. From this, it was possible to conclude that the method is applicable and concise for separating the primordial products in this reaction.



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1. Introduction

Biodiesel has significant potential as an alternative diesel due to its biodegradability, renewable energy source, better lubricity, combustion efficiency, and low toxicity compared to other fuels (Tan *et al.*, 2015). Glycerol is produced as a by-product, representing around 10% of the total volume of biodiesel produced (Bilck *et al.*, 2015; Chi *et al.*, 2007).

Studies show that the conversion of glycerol to other chemicals with high added value, such as lactic acid, makes the glycerol industry more profitable (Arcanjo *et al.*, 2017; Manfro and Souza, 2014), making biodiesel production a more suitable option to replace fossil fuels (Huang *et al.*, 2012).

Glycerol can be dehydrogenated to form dihydroxyacetone (Santos *et al.*, 2018). This intermediate went through dehydration to pyruvaldehyde on solid acid catalysts and rearranged via Cannizzaro reaction to lactic acid. Therefore, from the literature, pyruvaldehyde is a crucial intermediate on glycerol valorization in lactic acid (Lari *et al.*, 2016; Santos *et al.*, 2018).

Lactic acid is a raw material to benefit from synthesizing several products, applying in the textile industry and cosmetic industry (Drumright *et al.*, 2000). In the food industry, its main applications are as a pH regulator and food additive (Bruno *et al.*, 2020; Drumright *et al.*, 2000). It has been used as a precursor of solvents in green chemistry, such as ethyl lactate, and used in the synthesis of polylactic acid (PLA), applied in the production of biodegradable packaging (Bruno *et al.*, 2020; Drumright *et al.*, 2000).

Lactic acid is commonly sold as an 88% solution, varying its price according to its application in the market. In general, lactic acid price follows the cost of starch and sugar raw materials used for fermentation (Biddy *et al.*, 2016). With a market growth of 16.2% per year, it is expected that this demand will reach 960.1 kt in 2025, which should represent U$ 9.8 billion in the global market. As with lactic acid, PLA demand is also expected to grow, reaching $ 6.5 billion worldwide in 2025. One reason for this would be the Asia-Pacific PLA market growth rate of 22.4% per year due to various beverages and foods and the cosmetics manufacturing units (Oliveira *et al.*, 2018).

The hydrothermal conversion of glycerol to lactic acid has been widely studied recently as an alternative to the fermentation route, with the advantage of using a low-cost raw material. Furthermore, for environmental reasons, there is an increase in demand for lactic acid, as it is the raw material for producing a biodegradable polymer (PLA) (Bruno *et al.*, 2018; Shen *et al.*, 2009).

Some methods described in the literature involve the determination of different organic acids by high-performance liquid chromatography (HPLC). However, these methods cannot efficiently separate glycerol and lactic acid, observing the overlap of peaks. In this context, the present work describes a methodology for separating the lactic acid produced by hydrothermal conversion of glycerol using HPLC.

2. Materials and methods

2.1 Standard samples of glycerol and lactic acid

Glycerol and lactic acid HPLC grade were obtained from Sigma-Aldrich (St. Louis, U.S.A.) and Proquímios (Rio de Janeiro, Brazil). Two calibration curves were obtained using the following concentrations 0.6, 1.8, 3.0, 4.2, 5.4 and 6.6 g L^{-1} for the composition of the curve points. Standards were diluted in Milli-Q water to 10.0% vol. (Millipore, Bedford, USA) for method analysis. Diluted solutions acted as standards for linearity assessment.

2.2 Design of Experiments

A central composite design (CCD) was proposed to study the influence of experimental variables on glycerolysis reaction. The effect of two variable parameters, time and temperature, requiring nine experiments using face-centered stair points was investigated. The low and high values of the variables were chosen based on preliminary investigations. For this purpose, sodium hydroxide and glycerol concentrations, catalyst content, time, and temperature were used as independent variables, and lactic acid concentration was used as the dependent variable, as presented in Tab. 1. The experimental design and optimization were conducted with Statistica 12.0 software.

Table 1. Experimental conditions of central composite design.

Experiment	[NaOH] (g L^{-1})	[glycerol] (g L^{-1})	Catalyst (%)	t (h)	T (°C)
1	0.6	0.6	5	3	220
2	0.6	0.6	5	3	250
3	0.6	0.6	5	3	280
4	0.6	0.6	5	4.5	220
5	0.6	0.6	5	4.5	250
6	0.6	0.6	5	4.5	280
7	0.6	0.6	5	6	220
8	0.6	0.6	5	6	250
9	0.6	0.6	5	6	280

2.3 Reaction Procedure

A 100 mL 4590 series reactor (Parr Instrument Company, Moline, U.S.A.) was used for hydrothermal conversion of glycerol. The operating conditions were modulated by a 4848 reactor controller (Parr Instrument Company), and the reagents were fed into the reactor in the desired mass ratio of glycerol: NaOH (1:1) and desired temperatures (220, 250 and 280 °C), with the use of 5% of a copper catalyst supported on silica. Then, the reactor contents were stirred at 1000 rpm at a vacuum pressure of 800 psi. The total reaction time was 3, 4.5 and 6 h.

2.4 High-performance liquid chromatography method

Analyses were performed employing the Nexera series UHPLC system (Shimadzu, Japan) equipped with an LC-40 XR binary pump, a RID-20A detector, and a SIL-40XR autosampler. A Hi-Plex H column (Agilent Technologies, Santa Clara, U.S.A.), 300 mm long by 7.7 mm internal diameter and 8 µm particle size was used to separate the products. The mobile phase used was H_2SO_4 0.001 mol L^{-1} + 10% ACN (organic modifier), using ultrasound previously to remove bubbles. The conditions used for this method were the temperature of detector and oven at 60 °C, 20 min of running time, and flow rate of 0.6 mL min^{-1}. The flow rate of 0.6 mL min^{-1}, column oven and detector temperature at 60 °C and 20 min for the total execution time. Samples were diluted to 10% vol in ultrapure water (Milli-Q) as a diluent and injected in triplicate. The results were analyzed using the LabSolutions software (Shimadzu).

2.5 Method validation

The developed method was validated according to ICH (2005) guidelines in terms of the following analytical parameters: linearity, limit of detection (LOD), limit of quantification (LOQ), precision, and accuracy (Dias *et al.*, 2020).

2.6 Linearity

Linearity was given by injection in triplicate of the standards of each point used to build the calibration curve (Novaes *et al.*, 2018). Two distinct curves (glycerol and lactic acid) were drawn in the interval of 0.6–6.6 g L^{-1} (0.6; 1.8; 3.0; 4.2; 5.4 and 6.6 g L^{-1}). Homogeneity was assessed by the Cochran test (Shen *et al.*, 2009), through Eq. 1.

$$C = \frac{s_{max}^2}{\sum_k^{i=1} s_i^2} \tag{1}$$

where s_{max}^2 is the highest value variance and $\sum_k^{i=1} s_i^2$ is the total of all samples variances.

2.7 Limit of detection and limit of quantification

The LOD and LOQ were calculated using Eqs. 2 and 3, respectively.

$$LOD = \frac{3.3\sigma_{LC}}{S} \tag{2}$$

$$LOQ = \frac{10\sigma_{LC}}{S} \tag{3}$$

where σ_{LC} is the standard deviation of the minor number concentration (LC) tested and S is the slope of the curve obtained.

2.8 Precision

Precision was calculated by repeatability, using three different concentrations (lowest, average, and highest) in triplicate (Rocha and Bacelar Júnior, 2018). Concentrations were 0.6, 3.0 and 6.6 g L^{-1} for both curves, and the result was expressed as relative standard deviation (RSD, in %), as in Eq. 4.

$$RSD\ (\%) = \frac{\sigma}{\bar{C}} \times 100 \tag{4}$$

where σ represents the deviation from the lowest standard concentration and $\bar{C}$ is the mean concentration.

2.9 Accuracy

This parameter was represented by the recovery (R, in %) by calculating different concentrations (lowest, average, and highest) in triplicate for the different curves. Concentrations used were 0.6, 3.0 and 6.6 g L^{-1} for both curves. Accuracy was calculated with Eq. 5.

$$R\ (\%) = \frac{C_m}{C_e} \times 100 \tag{5}$$

where C_m and C_e are the obtained and theoretical concentrations, respectively.

3. Results and discussion

3.1 Reactions

Table 2 shows the results of conversion and selectivity for lactic acid under the reaction conditions of the experimental design.

Table 2. Selectivity and conversion to lactic acid under the reaction conditions of the experimental design.

Reaction	Conversion (%)	Selectivity for lactic acid (%)
1	33.90	2.42
2	31.40	9.90
3	38.04	35.73
4	32.26	1.80
5	34.60	8.29
6	42.82	34.63
7	29.96	2.04
8	33.81	13.22
9	53.72	58.84

According to Tab. 2, it can be observed that the method proposed by HPLC was efficient in separating the two compounds, glycerol and lactic acid, allowing the quantification of each one of these compounds and enabling the calculation of the conversion and the selectivity for lactic acid. It is essential to mention that pyruvaldehyde was obtained as a by-product, in smaller amounts, in all reactions, which is expected for catalysis reactions in the primary medium due to the greater predisposition of formation of this intermediate by the Cannizzaro reaction (Lari *et al.*, 2016; Santos *et al.*, 2018).

It can also be noticed that, according to Fig. 1, when comparing the temperatures, increasing this parameter favors the formation of secondary compounds, such as pyruvaldehyde. When the reaction time increases, the appearance of lactic acid is preferred, consequently increasing its selectivity. Therefore, temperature increase combined with the increase in reaction time is favorable to the rise of the conversion and the rise in the selectivity of the lactic acid, which corroborates the results expected in the literature (Evans *et al.*, 2020).

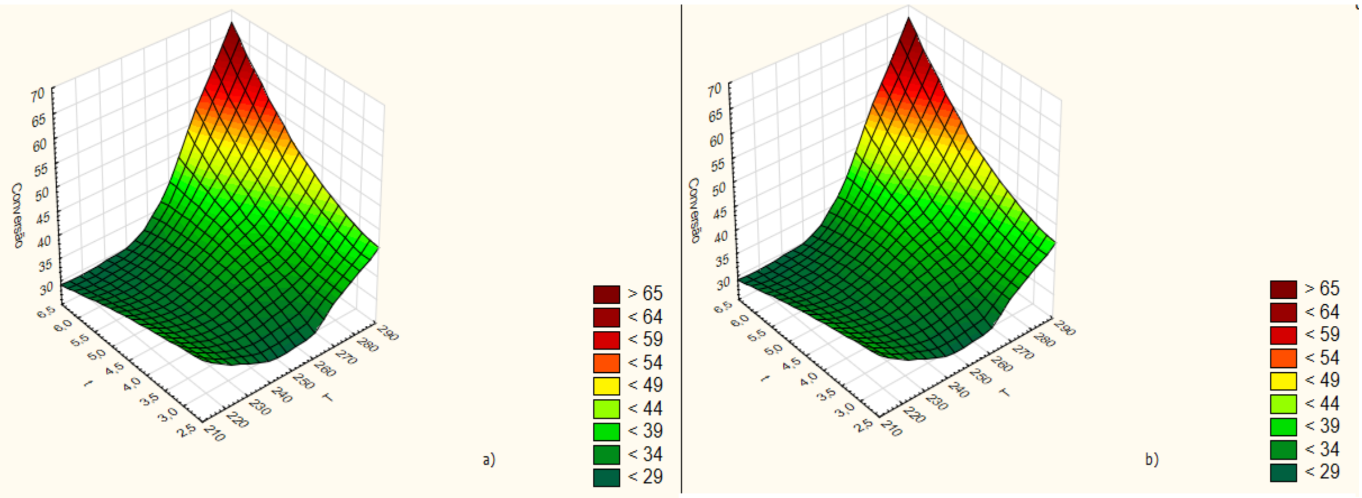

Figure 1. (a) Response surface Selectivity for lactic acid vs. T and t; **(b)** Response surface conversion vs. T and t.

3.2 Analysis by HPLC

All standards and reaction products were examined by HPLC and showed similar chromatographic appearance, which can be seen in Fig. 2. The overlapping peaks indicate coelution of interference that shares the same transition, with consequences such as inadequate quantification of the compound of interest or even the absence of identification of the combination of interest (Welch *et al.*, 2009). Diluted sulfuric acid as a mobile phase showed this coelution of desired compounds, generating an incorrect quantification.

Beltrán-Pietro *et al.* (2013) validated a chromatographic method using an ionic column at a temperature of 70 °C with a *refractive index detector* (RID) detector connected to a UV detector, with a phase composed of 3 mmol L^{-1} of sulfuric acid (H_2SO_4, pH 2.00). with a flow rate of 0.5 mL min^{-1} in an analysis time of 30 min. With this method, it is possible to identify compounds such as glyceraldehyde, oxalic acid, dihydroxyacetone, among others; however, it presents coelution of other compounds such as glycerol and lactic acid.

Although some methods in the literature report the separation of glycerol and lactic acid in the chromatographic profile, it is observed that, in fact, there is a co-elution of these compounds, not allowing an efficient quantification of these products, requiring the development of a new method.

As an alternative, using the mobile phase with the organic modifier was essential for separating glycerol and lactic acid compounds, allowing a better chromatographic profile to visualize both peaks. The choice of acetonitrile as an organic modifier was due to its low viscosity (0.4 cP at 25 °C), which generates less pressure and allows a better retention time response (Leite, 2008; Sadek, 1996). Some tests were performed with 5, 10 and 20% vol of acetonitrile to decide the best proportion of acetonitrile in the mobile phase. The best chromatographic separation occurred with the 10% vol of acetonitrile.

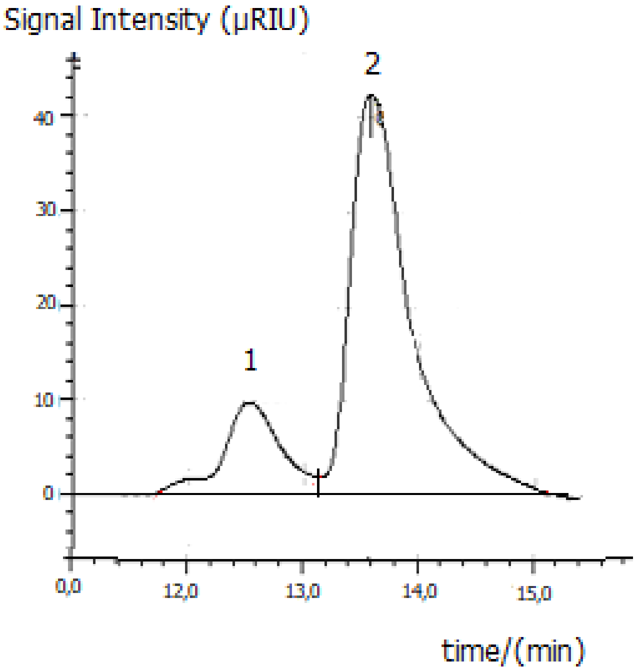

Figure 2. High-performance liquid chromatography chromatogram of the sample obtained through the reaction, using chromatographic conditions. Conditions: dilution of 10% vol, the flow rate at 0.6 mL min^{-1}, and injection volume of 20.0 µL. 1: lactic acid; 2: glycerol.

3.3. Method validation

3.3.1 Linearity

Six standard samples of glycerol and lactic acid were analyzed in triplicate by HPLC for each curve constructed, as seen in Fig. 3. This parameter was evaluated in the interval of 0.6–6.6 g L^{-1}. The determination coefficient (R^2) was above the acceptance rule values (0.990) (Leite, 2008; Ribani *et al.*, 2004; Sadek, 1996), indicating excellent linearity.

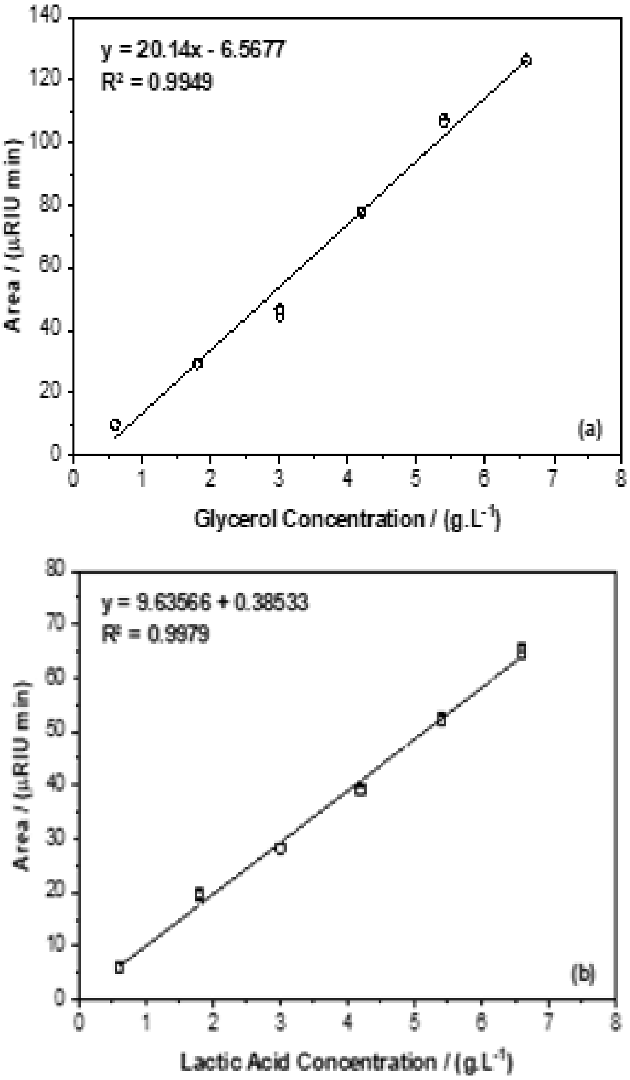

Figure 3. Fitted curves in HPLC analysis for **(a)** glycerol and **(b)** lactic acid. Range: 0.6 – 6.6 gL^{-1}.

The Cochran test was used with a 95% confidence level using the number of samples (n = 6) in triplicate. The calculated values of C were 0.661 for the glycerol curve and 0.130 for the lactic acid curve, being lower values when compared to the tabulated (0.616), indicating homoscedastic variances (Novaes *et al.*, 2018).

3.3.2 Limit of detection and limit of quantitation

For the glycerol analytical curve, LOD and LOQ values were 0.076 and 0.385 g L^{-1}, respectively, while for lactic acid, the LOD and LOQ values were 0.221

and 1.166 g L^{-1}. The results showed the possibility of differentiation and quantification of the different samples of each curve. The responsible standards require values below 0.5 g L^{-1}, and the present method is applicable (Cassini *et al.*, 2013; Souza and Junqueira, 2005), except only for lactic acid that showed a value above that specified by the standard.

3.3.3 Precision

Through Tab. 3, it is possible to conclude that the values are accurate about repeatability since they are lower than the 5% determined by the standards (Novaes *et al.*, 2018; Ribani *et al.*, 2004; Sadek, 1996).

Table 3. Validation parameters for the HPLC method.

Parameter	*Glycerol*	*Lactic Acid*
Linearity range (n = 6) (g.L^{-1})	0.6–6.6	0.6–6.6
Regression equation	y = 20.14x – 6.5677	y = 9.63566x + 0.38533
Determination coefficient (R^2)	0.9949	0.9979
LOD (g L^{-1})	0.076	0.385
LOQ (g L^{-1})	0.231	1.166
Repeatability (RSD, %)		
Lower	1.500	0.751
Middle	0.683	0.568
Higher	0.158	0.889
Recovery (%)		
Lower	100.00 ± 0.01	100.00 ± 0.01
Middle	98.33 ± 0.08	100.00 ± 0.01
Higher	100.00 ± 0.01	100.00 ± 0.05

3.3.4 Accuracy

The values obtained for this parameter are between 98.33 and 100%. Regarding the glycerol curve, the mean recovery was 99.44 ± 0.03%, while for lactic acid, it was 100.00 ± 0.02%, indicating an amicable agreement between experimental and theoretical data. Furthermore, these results are within what is expected by regulatory standards (70–120%) (Goutal *et al.*, 2016; Lanças, 2004).

4. Conclusions

The methodology described in this article allowed the separation of lactic acid and glycerol by HPLC in a quick and straightforward run of 20 min. In addition, it was possible to quantify each one, allowing the calculation of conversion and selectivity for lactic acid for the reactions mentioned in the experimental design.

Authors' contribution

Conceptualization: Cruz, A. F. A.
Data curation: Cruz, A. F. A.
Formal Analysis: Cruz, A. F. A.; Dias, G. P.; Bittencourt, F. R.
Funding acquisition: Not applicable
Investigation: Cruz, A. F. A.
Methodology: Cruz, A. F. A.; Dias, G. P.; Bittencourt, F. R.
Project administration: Aranda, D. A. G.
Resources: Aranda, D. A. G.
Software: Cruz, A. F. A.; Dias, G. P.; Bittencourt, F. R.
Supervision: Aranda, D. A. G.
Validation: Cruz, A. F. A.
Visualization: Cruz, A. F. A.; Aranda, D. A. G.
Writing – original draft: Cruz, A. F. A.; Aranda, D. A. G.
Writing – review & editing: Cruz, A. F. A.; Aranda, D. A. G.

Data availability statement

All data sets were generated or analyzed in the current study.

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