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# DETERMINATION OF S-LACTIC ACID CONCENTRATION FROM FERMENTATION OF ALOE VERA GEL USINGHIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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**Abstract:** A method of determining the concentration of s-lactic acid using high-performance liquid chromatography (HPLC) was developed. The method used a HIQSIL 100 C18 column (Length: 250 mm, Diameter: 4.6 mm, Particle size: 5 μm) with potassium dihydrogen phosphate as a mobile phase. The separation was performed using a UV detector at 210 nm wavelength. The flow rate was kept at 1mL/min and the injection volume was 20 μL. The results showed that the linearity was in the range 0-180 ppm, and the correlation coefficient was found to be ($R^2$= 0.999). The limit of detection was 1.15 ppm, and the limit of quantification was 3.79 ppm. The mean recoveries were from 95% to 99%, and the repeatability RSD was 2.87%. The method was used to determine the s-lactic acid concentrations from the fermentation of aloe veragel. The method seems to be accurate, precise, simple and economical.

**Keywords:** s- lactic acid, HPLC, repeatability, recoveries

## 1 Introduction

Fermentation has significant advantages over the chemical production of lacticacid. The advantages include the high specificity of product (optically pure isomeric form), the capacity to transform raw materials i.e.whey, molasses, sugar cane biogases, and starch, which are rich in fermentable sugars, reduction of the use of substrates derived from petrochemicals, and low energy consumption [5,7]. Nevertheless, the commercial use of this raw material would require low costs, high production rates, consistent availability, fewer by-products, and easy fermentation with little pre-treatment [8]. Aloe vera could potentially be used as a raw material that can be processed into lactic acid. Aloe verais suitable for fermentation because it is high in fermentable sugars such as mannose and glucose, which facilitate pre-treatment through hydrolysis. Aloe veraalso contains traces of other sugars such as arabinose, galactose and xylose [4]. Importantly, aloe vera is also low in lignin and hemicelluloses [6]. Besides, aloe veracontains many othertypes of sugar, so it is suitable for lactic acid fermentation. As a result, the need for an accurate method to determine lactic acid concentration is essential. This paper deals with the development of a method of determining s-lactic acid concentration from the fermentation of aloe veragel using high-performance liquid chromatography.

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## 2 Material and methods

All reagents were of analytical grade. Distilled and deionized water (Milli-Q Millipore 18.2 MΩ-cm resistivity) was used for all dilutions. s-lactic acid standard was purchased from Merck (Darmstadt, Germany). Working standard solutions were prepared by dilution of a stock and intermediate standards. The working standards were as follows: s-lactic acid 0, 11.25, 22.5, 45, 90, 180 ppm diluted in 10 mM potassium dihydrogen phosphate.

The HPLC model Shimadzu, SIL 20A (Kyoto, Japan) equipped with an LC solution software, quaternary pump and online degasser model LC20AD and injection valve with a loop capacity of 20 μL was used. The detector used was a programmable UV-VIS detector model SPD-20A. The s-lactic acid compound was separated on reverse-phase HIQSIL100 (250 mm× 4.6 mm, i.e. 5 μm), C18 column.

The sample was homogenized. 650 μL of the sample was transferred to a 100 ml volumetric flask and dissolved in a mobile phase. The solution was made up to the mark with mobile phase and filtered through a 0.45-μm-membrane filter. Aliquots of solutions were prepared and injected into the system and the chromatograms were recorded. The peak area of the sample was calculated.

Chromatographic analysis was performed on HIQSIL 100 C18 (250 × 4.6 mm, 5 μm) column with a mobile phase containing10 mM potassium dihydrogen phosphate. The mobile phase was degassed and filtered as above before being pumped into the HPLC system. The isocratic analysis was performed on a SPD-20A beam UV at 210 nm at a flow rate of 1.0 mL/min. The injection volume was 20 μL.

The developed analytical method was validated according to [2, 1, 3] ICH guidelines for the parameters like linearity, limit of detection (LOD), limit of quantification (LOQ), accuracy, and repeatability.

The data were evaluated based on statistical parameters using Microsoft office Excel 2007 and SPSS 18 soft wares.

## 3 Results discussion

### 3.1 Specificity

The specificity study of the method was carried out using a12 replication analysis of the 18 ppm standard s-lactic acid and the sample.The method was evaluated by analyzing the retention time. The chromatograms of the standard and the sample arethe same (Fig. 1 and Fig. 2). The chromatography system was specific for the analyte.

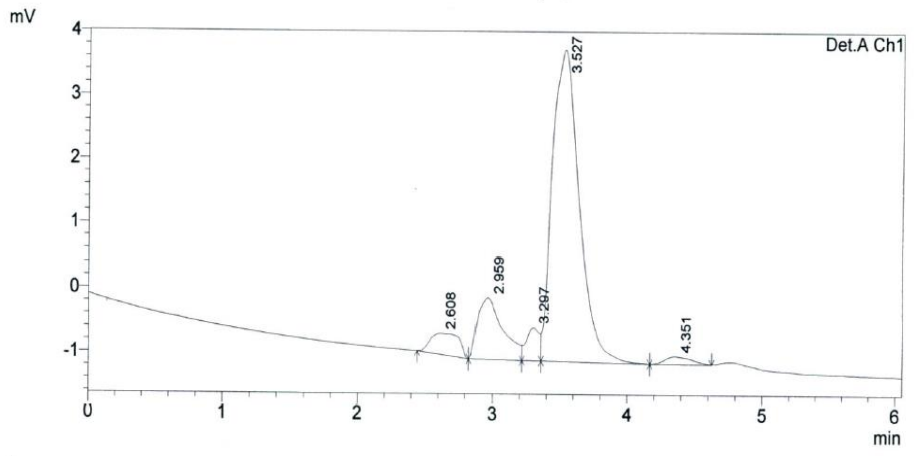


**Fig.1.** Standard chromatogram of s-lactic acid

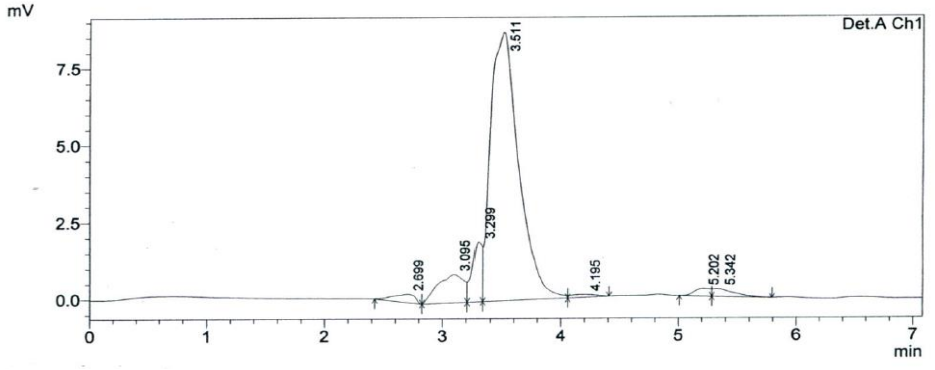


**Fig.2.** Sample chromatogram of s-lactic acid

### 3.2 System suitability

The system suitability parameters such as relative standard deviation (RSD) of analyte, retention time, and the peak areas of the 12 replications were calculated from the chromatogram (Table 1). The results show that RSD of the peak area and retention time of s-lactic acid was within 2%, indicating system suitability.

**Table 1.** Retention time and peak area of the s-lactic acid analyzed by HPLC

| Number | Peak area (mV) | Retention time (min) |
|---|---|---|
| 1 | 28329 | 3.527 |
| 2 | 27523 | 3.511 |
| 3 | 28109 | 3.526 |
| 4 | 27387 | 3.517 |

| | | |
|---|---|---|
| 5 | 27890 | 3.481 |
| 6 | 28013 | 3.513 |
| 7 | 27654 | 3.469 |
| 8 | 27701 | 3.459 |
| 9 | 27802 | 3.509 |
| 10 | 28129 | 3.533 |
| 11 | 28821 | 3.497 |
| 12 | 27653 | 3.578 |
| Average | 27918 | 3.51 |
| SD | 394.69 | 0.03 |
| RSD (%) | 1.41 | 0.88 |

### 3.3 Linearity

By using a calibration curve solution, we have $y = ax + b$, where $y$ is the signal intensity and $x$ is the known concentration of the given analyte in the calibration solution. The linearity of response was assessed by plotting the absorbance values ($y$-axis) of standard solutions of s-lactic acid in comparison with its final concentration ($x$-axis). The concentrations of s-lactic acid were 0, 11.25, 22.5, 45, 90, and 180 ppm, and a linear relationship existed for theregression equation with a high coefficient of determination ($R^2$) (Table 2). According to [2], the analytical response is linear over certain concentration ranges if the $R^2$ value obtained is greater than 0.995. Fig. 3 shows the regression between absorbance values ($y$-axis)and the concentrations of s-lactic acid ($x$-axis).

**Table 2.** Linear regression data of s-lactic acid calibration curve

| | **s- lactic acid** |
|---|---|
| Linear range | 0–180 ppm |
| $R^2$ | 0.999 |
| Slope | 1591±7 |
| Intercept | -256±583 |
| Confidence limit of slope | 1572–1610 |
| Confidence limit of intercept | 1874–132 |

95% confidence limit. The regression equationis$y = ax+b$, where $a$ is the slope, and $b$is the intercept.

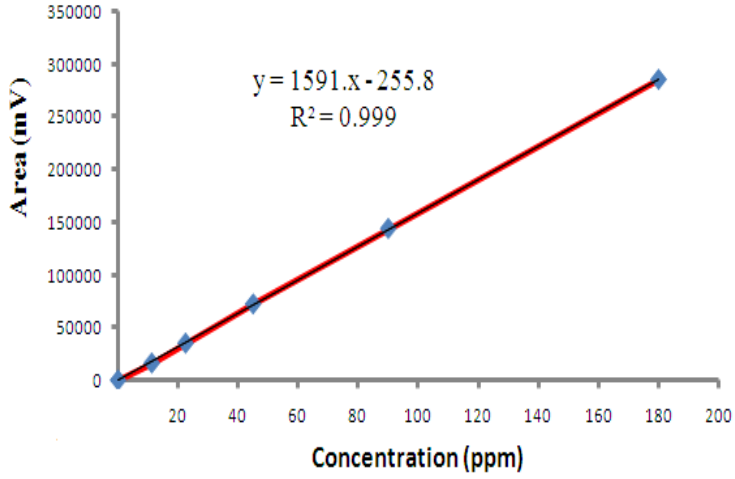


**Fig.3.** Calibration curve

### 3.4 Repeatability

The repeatability of the method was determined by performing the analysis of seven samples treated according to the procedure described previously with the selected HPLC conditions. s-lactic acid content in the sample was calculated using the following formula

$$\text{s-lactic acid content (ppm)} = C_0 * DF \quad (1)$$

where $C_0$ is the concentration calculated from calibration curve, and$DF$ is the dilution factor.

The results presented in Table 3 show that under the selected HPLC conditions, the relative standard deviation of the peak area of s-lactic acid is 2.87%. According to AOAC, the maximum acceptable RSD value is 11%, and the repeatability of this determination is very good.

**Table3**. Repeatability of the HPLC method for s-lactic acid

| Sample | Peak area (mV) | Content (%) |
|---|---|---|
| AL2.1 | 164350 | 1.58 |
| AL2.2 | 159801 | 1.54 |
| AL2.3 | 163210 | 1.57 |
| AL2.4 | 150641 | 1.45 |
| AL2.5 | 162430 | 1.56 |
| AL2.6 | 157097 | 1.51 |

| | | |
|---|---|---|
| AL2.7 | 158543 | 1.53 |
| SD (%) | | 0.044 |
| RSD (%) | | 2.87 |

*Note*: Samples were diluted.

### 3.5 Recovery

For the recovery determination, spiked samples at concentrationsof 22.5, 45, 90, and 180 ppm were prepared and then treated according to the previously described procedure. The results are presented in Table 3. The mean recoveries of s-lactic acid from the samples spiked at 22.5-180 ppm were in the range of 95–99%. The performance characteristics of the method presented in this paper indicate that it may be used for the determination of s-lactic acid from fermentation of aloe vera gel.

**Table 3.** Recovery results of s-lactic acid for spiked samples

| Concentration spiked to sample (ppm) | | Found Concentration (ppm) | Recovery (%) | RSD (%) |
|---|---|---|---|---|
| s-lactic acid | 22.5 | 21.36 ± 0.99 ($n$ = 7) | 95.0±4,5 ($n$ = 7) | 4.7 |
| | 45 | 45.28 ± 0.76 ($n$ = 7) | 99.7±2.6 ($n$ = 7) | 2.6 |
| | 90 | 89.58 ± 1.45 ($n$ = 7) | 99.4 ± 2.0 ($n$ = 7) | 2.0 |
| | 180 | 187.23 ± 3.43 ($n$ = 7) | 99.0 ± 2.0 ($n$ = 7) | 2.0 |

*Note*: Samples were diluted.

### 3.6 Limit of detection and limit of quantification

The limit of detection (LOD) is the lowest concentration of the analyte that can be detected by the proposed method, and limit of quantification (LOQ) is the lowest concentration of analyte that can be detected with acceptable accuracy and precision [2]. The peak areas of seven samples were measured (Table 4). The analysis was conducted under the conditions mentioned above. The standard deviation ($\sigma$), LOD, and LOQ values were calculated using the following formula [2]

$$LOD = \frac{3 \times \sigma}{a} \quad (1)$$

$$LOQ = 3.3 \times LOD \quad (2)$$

where $\sigma$ is the standard deviation of $y$-intercepts of the regression line, and $a$ is the slope of the calibration curve. The limit of detection for s-lactic acid was 1.15 ppm and the limit of quantification of s-lactic acid was 3.79 ppm, which shows that this method is very sensitive.

**Table 4.**Limit of detection (ppm) and limit of quantification (ppm) for the s-lactic acid analyzed using HPLC

| **No** | **1** | **2** | **3** | **4** | **5** | **6** | **7** | $\sigma$ |
|---|---|---|---|---|---|---|---|---|
| Area (mV) | 16352 | 15478 | 16782 | 16532 | 16891 | 15432 | 15780 | 610 |

### 3.7 Application

The method is applied to determine s-lactic acid concentration from fermentation of aloe veragel.The results obtained are reported in Table 5.

**Table 5.** s-lactic acid (%) found in the process of fermentation of aloe vera gel*

| **Sample** | **Peak area (mV)** | **s-lactic acid (%)** |
|---|---|---|
| W1 | 159087 | 1.50 |
| W1 | 143990 | 1.36 |
| W1 | 137598 | 1.30 |
| W1 | 145981 | 1.38 |
| W1 | 135679 | 1.28 |
| W1 | 135421 | 1.28 |
| W3 | 167892 | 1.59 |
| W3 | 164350 | 1.55 |
| W3 | 169803 | 1.60 |
| W3 | 154321 | 1.46 |
| W3 | 176893 | 1.67 |
| W3 | 180461 | 1.70 |
| W4 | 288126 | 2.72 |
| W4 | 284961 | 2.69 |
| W4 | 274992 | 2.60 |

| Sample | Peak area (mV) | s-lactic acid (%) |
|---|---|---|
| W4 | 279043 | 2.63 |
| W4 | 268902 | 2.54 |
| W4 | 298764 | 2.82 |

* Samples were diluted.

In the analysis of s-lactic acid from the fermentation of aloe vera gel, the s-lactic acid concentrations ranged from 1.28 to 2.82 %.

## 4 Conclusions

The method described in this paper was found to be simple, sensitive, accurate, precise, rapid, robust and economical. The method parameters including linearity, sensitivity, precision and accuracy areacceptable. Furthermore, the developed method can be used for determination of s-lactic acid concentration from the process of fermentation of aloe vera gel.

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