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Simultaneous HPLC-UV determination of lactic acid, glycolic acid, glycolide, lactide and ethyl acetate in monomers for producing biodegradable polymers

M.K. Zamanova *, V.N. Glotova, T.N. Izhenbina, D.S. Krutas, V.T. Novikov

Department of Technology of Organic Substances and Polymer Materials
Tomsk Polytechnic University, Lenin Avenue 30, Tomsk, Russia

Abstract

A simple, rapid high-performance liquid chromatographic (HPLC) method was developed for the simultaneous determination of glycolic acid, lactic acid, glicolide, lactide and ethyacetate in monomers for obtaining biopolymers. The separation was effected on the reversed-phase C18 column 250mm×4.6mm with particle size 5μ using a mobile phase mixture buffer and acetonitrile in a ratio 88:12 v/v and elution was isocratic at a flow-rate of 1.0 mL/min. The determinations were performed with a UV-Vis detector at 200 nm. The volume of the injected sample was 20 μL. Detection limits for acids and its dimers (glycolic acid, lactic acid, glicolide, lactide) and ethylacetate range between 82 and 182 ng/mL. The analytes are separated in 13 min. Recovery studies showed good results for all solutes (99–102%). The method is linear for all compounds over the concentration range tested, and shows good precision and accuracy, making it suitable for quantitation of acids and its dimers (glycolic acid, lactic acid, glicolide, lactide) and ethyl acetate in monomers.

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Keywords: HPLC, lactide, glycolide, biodegradable polymers.

1. Introduction

Poly-lactic acid (PLA) is a biodegradable aliphatic polyester produced industrially both on a large and small scale. It

* Corresponding author. Tel.: +7-962-782-9516; fax: +7(3-822)563-383.
E-mail address: m.k.zamanova@gmail.com

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is used for a wide variety of applications, ranging from biomedical applications to raw material for food packaging, bottles and consumables in general. Due to its excellent mechanical properties, permeability, transparency and environmental compatibility, in fact, PLA is a one of the most interesting polymeric candidate to replace on the market of non-biodegradable petroleum based synthetic polymers[1]. Recently, polymers based on lactic acid have received special attention in the field of medical applications because these polyesters degrade in the human body by hydrolysis of the ester backbone to non-harmful and non-toxic compounds. These compounds are also used in the production of implantable medical devices, in dental applications and, more recently, as scaffolds for autografted new skin, wound covers, anastomose systems and stents[2]. All of these devices can be loaded with a large number of different compounds such as drugs, pharmacological active principles, release modifiers and molecules suitable for Magnetic Resonance Imaging (MRI).

Nomenclature

LA - lactic acid;
GA - glycolic acid;
EA - ethyl acetate;
HPLC - high performance liquid chromatography;
RT - retention time;
RSD - relative standard deviation;
SD - standard deviation;
RE - relative error.

The synthesis of PLA and copolymers can follow three routes: (1) condensation polymerization, (2) azeotropic dehydrative condensation, and (3) ring-opening polymerization from the cyclic dimers. The third method, based on ring opening polymerization (ROP) of cyclic dimers is the only practical technique for producing pure high molecular weight polymers (Mw≥100,000) and the one that has been developed most widely for industrial-scale production. ROP also has the advantage that the chemistry and therefore the properties of the final polymer can be accurately controlled and tuned to requirements.

It is known that the impurities like lactic acid, glycolic acid, solvents in the monomer have a significant bearing on the molecular weight of PLA and copolymers. In addition, varying levels of water-soluble acid impurities are well known to exist in PLGAs, which can influence their solid-state stability, drug encapsulation efficiency, and drug release behavior. And it is important to determine the impurities content before polymerization, after storage and transportation. Impurities have been analysed in various matrices by various techniques. High performance liquid chromatography (HPLC) has been used to analyse short fatty acids[3,4] and lactide content in polymer[5]. Gas chromatography (GC) has been used for LA and GA analysis with the most reported methods[6,7,8,9,10] involving derivatization typically using t-butyldimethylsylil or oxidation to aldehyde. Derivatization is often the lengthy experiment and may not be appropriate for a high throughput laboratory. The potentiometric titration and pre-derivatization HPLC usage for the analysis of LA and GA has been reported, where the pre-derivatization HPLC method was developed for separation and quantification of water-soluble acid impurities and degradation products in PLGA and was compared with potentiometric titration by using tetrabutyl ammonium hydroxide[11]. Various other methods have already been reported for the detection and determination of LA and GA including amperometric lactate biosensors[12,13,14], electrophoresis[15,16], spectrophotometric method[16] and ion-exclusion HPLC.

However, these studies could not achieve simultaneous determination of glycolic acid, lactic acid, glycolide, lactide and ethyl acetate. The main purpose of this study was to develop a rapid, specific, precise and accurate HPLC method for analytical determination of acids and its dimers (GA, LA, glycolide, lactide) and ethyl acetate in monomers used in our project for obtaining PLA, PLGA and other copolymers. Thus, the validation of the analytical method is a necessary procedure and involves experimental studies of analytical parameters, in order to guarantee the analytical results. Some important parameters such as specificity and selectivity, linearity, accuracy and precision must be verified

2. Methods

2.1. *Chemicals*

Lactic acid and glycolic acid were purchased from Purac (USA); acetonitrile was supplied by Cryochrom chemical company (Russia); ethyl acetate (REAHIM, Russia) was used for recrystallisation, glycolide and lactide were obtained in laboratory, initial purity were not less than 99.9 %. Ultrapure water for HPLC eluent was prepared using a Laboratory water purification system ADRONA Crystal-B (Latvia).

2.2. *Instrumentation and chromatographic conditions*

Reversed phase HPLC analysis was done using a YL9100 HPLC system (YoungLin Clarity, South Korea) which consists of YL9101 vacuum degasser, YL9110 quarternary pump, YL9131 column compartment, YL9120 UV/Vis detector with manual sample injector Rheodyne 9725i. The analytical column was a Tracer Excel 120 ODSA, 250 mm×4.6 mm and with particle size 5 μm. Chromatographic separation was carried out at the column oven temperature 40ºC ± 2ºC with a flow rate 1.0 mL/min of isocratic elution using two solvents: A – ($3\cdot10^{-2}$ mol/L H_3PO_4 in water) and B – acetonitrile HPLC grade in ratio 88:12 v/v. The injected sample volume was 20 μL. The quantitation wavelength was set at 200 nm.

For optimization of separation of analytes, aqueous solutions of H_3PO_4 in three different concentrations were tested: 1st – $1.2\cdot10^{-2}$ mol/L, 2nd – $2.1\cdot10^{-2}$ mol/L and 3rd – $3.1\cdot10^{-2}$ mol/L. Influence of temperature of the column oven on the separation of analytes was investigated.

2.3. *Preparation of standard and sample solutions*

Standard stock solution of glycolic acid (3.0 mg/mL), lactic acid (2.5 mg/mL), glicolide (12.8 mg/mL), lactide (12.8 mg/ml) and ethylacetate (2.2 mg/mL) were prepared in HPLC grade of acetonitrile and filtered using membrane disc filter (0.45 μm). The retention time of each compound was determined. Stock solution containing five analytes was prepared and diluted to appropriate different concentrations and calibration curves were established. Samples were prepared in HPLC grade of acetonitrile and filtered using membrane disc filter (0.45 μm).

2.4. *Standard curves and sample analysis*

For quantitative analysis, five different concentrations of a standard stock solution containing five analytes were injected. By using YL Clarity software (version 3.0.4.444), a calibration curve for each compound was obtained by plotting the peak areas versus the concentration of each analyte. Chromatograms of samples obtained were analyzed using the YL Clarity software (version 3.0.4.444) based on comparing retention times of the sample with those of the standards for qualitative analysis and the calibration curve for quantitative analysis. A straight line standard curve was obtained by linear regression of 6 independent sets of experimental data.

3. Results

The method was validated to demonstrate that it is suitable for its intended purpose by the standard procedure to evaluate adequate validation characteristics (system suitability, accuracy, precision, linearity, robustness and stability-indicating capability). The excellent absorption of GA, LA, glycolide, lactide and EA together as detected at 200 nm and this wavelength were chosen for the analysis.

The chromatogram at 200 nm showed a complete resolution of all peaks (Figure 1).

3.1. *Influence of H_3PO_4 concentration of the mobile phase and column oven temperature on separation of analytes*

Separation of the GA, LA, glycolide, lactide and EA on a Tracer Excel 120 ODSA (250mm×4.6mm, 5μm) was

tested with three H_3PO_4 solutions and three different column oven temperature. Mobile phase 3rd with $c(H_3PO_4)$ = $3 \cdot 10^{-2}$ mol/L was the best for HPLC separations of the analytes tested as shown in Figure 2. And it was found that column oven temperature 40ºC is optimal and provides good separation, and no column deterioration was observed even after prolonged use.

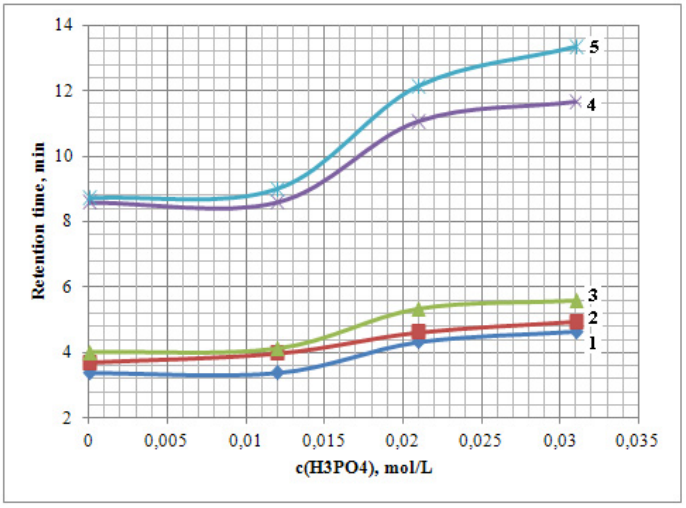

Figure 1. Influence of the H_3PO_4 concentration of the mobile phase on the separation of GA (1), LA (2), glycolide (3), lactide (4) and ethyl acetate (5)

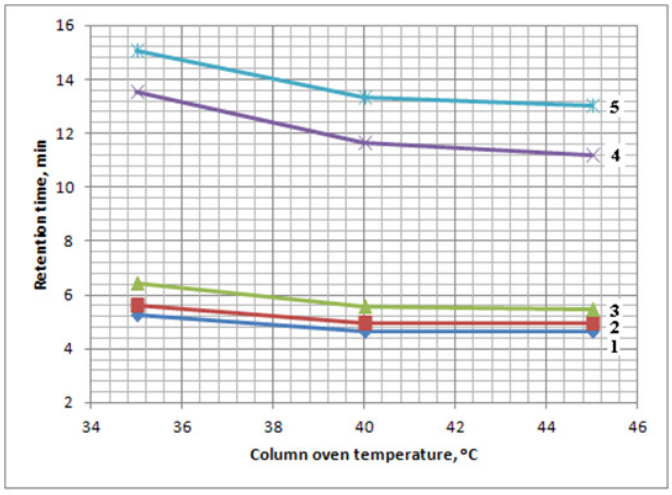

Figure 2. Influence of the column oven temperature on the separation of GA (1), LA (2), glycolide (3), lactide (4) and ethyl acetate (5)

3.2. Specificity and selectivity and system suitability (SST)

Specificity and selectivity were determined by comparing the chromatograms of the standard solution and the blank samples. SST is commonly used to verify resolution, column efficiency, and repeatability of the chromatographic system to ensure its adequacy for a particular analysis. It is performed by performing six injections

of standard solution. The peaks of all analytes were symmetrical and well separated. The percentage relative standard deviation (RSD) of the area and retention time of each compound from six replicate injections were below 1.5%. Low values of RSD for replicated injections indicate that the system is precise. The results of other system suitability parameters such as peak asymmetry and theoretical plates are presented in Table 1. As seen from these data, the acceptable system suitability parameters would be as follows: the relative standard deviation of replicate injections is not more than 5.0 %, the tailing factor is not more than 1.5 and the theoretical plates are not less than 2000 (excluding glycolide).

Table 1. SST parameters of the developed HPLC method

Compounds	RT (min)	RSD (Area), %	RSD (RT), %	Theoretical plates	Asymmetry
Glycolic acid	4.69	0.45	0.32	11180	1.47
Lactic acid	4.99	0.80	0.30	16221	1.23
Glycolide	5.64	0.58	0.41	678	0.81
Lactide	11.78	0.46	0.35	2700	1.00
Ethylacetate	13.51	1.23	0.33	3988	0.85

3.3. Linearity of the method

The linearity was analyzed by calculating the correlation coefficient for the (straight line) analytical curve of GA, LA, glycolide, lactide and EA.

A calibration curve for each component was constructed by linear regression of the observed average peak area versus concentration. The coefficients of the regression curves (the slope and the intercept on the y axis) and the squares of the correlation coefficients (r^2) were calculated by the least squares method. Linearity was evaluated using standard samples over five calibration points with six measurements for each calibration point. The calibration curve at all concentration ranges was better described by a quadratic equation with the correlation coefficient > 0.99. Calibration curves were linear for all analytes investigated. Table 1 presents the equation of the regression line, correlation coefficients (r^2), relative standard deviation (RSD), values of the slope and intercept for each compound., the results are shown in Table 2.

Table 2. Linearity Results

Compounds	Concentration range mg/mL	Response equation	r^2
Glycolic acid	0.600 – 3.000	y = 1204.8x – 40.912	0.997
Lactic acid	0.500 – 2.500	y = 2568.6x – 199.68	0.995
Glicolide	2.533 – 12.672	y = 2147x + 362.07	0.994
Lactide	2.561 – 12.816	y = 3803.3x + 2064.2	0.991
Ethylacetate	0.436 – 2.181	y = 1137.2x – 8.7695	0.995

3.4. Accuracy and precision

Accuracy of the method was determined for both intra-day and inter-day variations using the six times analysis of the samples. Precision was determined by repeatability (intra-day) and intermediate (inter-day). Assay precision of the method was evaluated by repeatability (intra-day) and intermediate (inter-day) by analysis of six replicates of the standard solution for two concentrations in 3 days. The mean of percentage recoveries and the relative standard

deviation were also calculated. The results are shown in Table 3. The RSD values for intra-day precision was ≤0.83% and for intra-day ≤2.66%. The relative error values for intra-day accuracy were ≤3.75% and for inter-day accuracy was ≤3.60%. The results show good reproducibility and precision of the developed method.

Table 3. Precision and accuracy of method for determination of GA, LA, glycolide, lactide and EA

Compounds	Amount taken (mg/ml)	Intra day			Inter-day		
		Amount found ± SD (mg/mL)	Accuracy RE, %	Precision RSD %	Amount found ± SD (mg/mL)	Accuracy, %	Precision RSD %
Glycolic acid	1.49	1.48 ± 0.010	0.79	0.70	1.50 ± 0.016	0.25	1.10
	2.49	2.46 ± 0.011	1.09	0.45	2.50 ± 0.066	0.55	2.66
Lactic acid	1.29	1.26 ± 0.010	2.28	0.77	1.26 ± 0.001	2.30	0.04
	2.15	2.12 ± 0.014	1.58	0.65	2.08 ± 0.043	3.55	2.09
Glycolide	5.63	5.50 ± 0.010	2.45	0.18	5.50 ± 0.005	2.35	0.09
	9.39	9.15 ± 0.047	2.57	0.52	9.23 ± 0.180	1.74	1.94
Lactide	6.06	6.11 ± 0.020	0.73	0.32	6.14 ± 0.035	1.22	0.57
	10.11	9.99 ± 0.046	1.17	0.46	10.05 ± 0.065	0.59	0.65
Ethylacetate	0.93	0.90 ± 0.007	3.75	0.83	0.90 ± 0.008	3.60	0.89
	1.56	1.55 ± 0.002	0.66	0.15	1.54 ± 0.010	0.87	0.66

3.5. Application

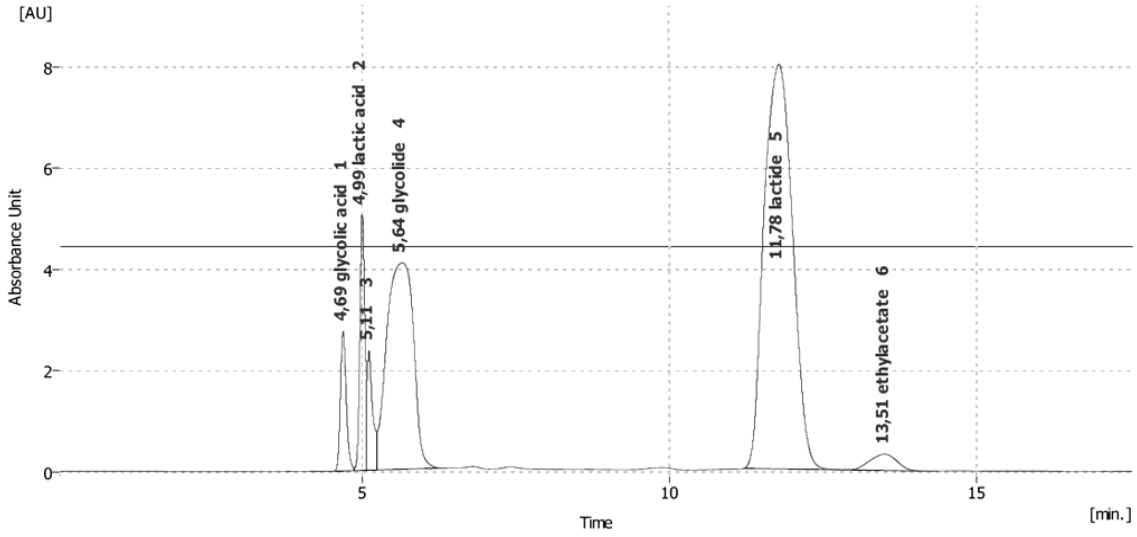

Figure 3. HPLC separation of glycolic acid, lactic acid, glycolide, lactide and ethyl acetate on Tracer Excel 120 ODSA, with $c(H_3PO_4) = 3 \cdot 10^{-2}$ mol/L and column oven temperature 40ºC, 200 nm.

Under the optimized experimental conditions, the HPLC method was then applied to study the contents of

analytes in some real samples, the results are shown in Table 4, where every value represents the mean of triplicate analysis. The representative chromatograms of the standard solution (Figure 3) and the test preparations show identical retention times.

Table 4. Mass concentration glycolic acid, lactic acid, glycolide, lactide and ethyl acetate in different monomers for obtaining bioplastics

Sample type	Content, % mass				
	Glycolic acid	Lactic acid	Glycolide	Lactide	Ethyl acetate
Lactide-crude	n/d	27.00±0.19	n/d	69.80±0.12	n/d
Lactide, 1st recristallization	n/d	2.29±0.02	n/d	97.51±0.21	0.20±0.00
Lactide, 2nd recristallization	n/d	1.53±0.01	n/d	98.36±0.22	0.11±0.00
Lactide, 3rd recristallization	n/d	0.28±0.00	n/d	99.95±0.22	n/d
Lactide, storage time 2 weeks	n/d	2.03±0.01	n/d	97.95±0.25	n/d
Glycolide, storage time 1 week	0.93±0.00	n/d	73.56±0.13	n/d	n/d
Glycolide, storage time 2 week	1.56±0.01	n/d	57.65±0.10	n/d	n/d

4. Conclusions

A new analytical method has been developed and applied in routine for screening and quantitation glycolic acid, lactic acid, glycolide, lactide and ethylacetate in the monomers for receiving biopolymers. This method was found to be satisfactory in terms of linearity of response, system precision, assay accuracy and quantification. Recovery studies showed good results for all solutes (99–102%). The method is linear for all compounds over the concentration range tested.

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