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Short communication

# Quantitative analysis of lovastatin in capsule of Chinese medicine Monascus by capillary zone electrophoresis with UV–vis detector

Min Li [a,b], Liu-Yin Fan [a], Wei Zhang [a], Ji Sun [a], Cheng-Xi Cao [a,*]

[a] *Laboratory of Analytical Biochemistry and Bioseparation, School of Life Science and Biotechnology, Shanghai Jiao Tong University, 800 Dongchuan Road, Minhang, 200240 Shanghai, PR China*

[b] *Laboratory of Analytical Chemistry, Department of Chemistry and Life Science, Chuzhou University, ChuZhou 23912, PR China*

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**Abstract**

A capillary zone electrophoresis (CZE) method was developed for the quantitative analysis of lovastatin (Lvt) in capsule of Monascus-Chinese medicine. Lvt in the capsule was separated using an electrolyte system consisting of 16% ethanol (v/v) in 60 mM Gly-sodium hydroxide buffer, pH 10.5, 16 kV applied voltage, 238 nm detection wavelength with a capillary of 51 cm × 75 μm i.d (43 cm to detector). Under the optimized conditions, the linear response of Lvt concentration ranges from 4.0 to 240 μg/mL with high correlation coefficient ($r = 0.9998$, $n = 9$), the limits of detection (LOD) and quantification (LOQ) for Lvt are 0.73 and 2.42 μg/mL, the precision values (expressed as R.S.D.) of intra-day and inter-day are 1.40–2.12% and 1.47–3.88%, respectively. The recoveries of the analyte at three concentration levels are 90.28–100.71%. The developed method can be well used for the quantification of Lvt in the drug in commercial formulations.


*Keywords:* Capillary zone electrophoresis; Chinese medicine; Lovastatin; Quantitation analysis; Monascus

## 1. Introduction

Lovastatin (Lvt) is a cholesterol-lowering drug isolated from a Monascus and a strain of *Aspergillus terreus* [1,2], which competitively inhibits the biosynthesis of mevalonic acid by HMG-CoA reductase. In recent years, methods for the determination of lovastatin in pharmaceutical formulations and biological matrices have been reported, including high-performance liquid chromatography (HPLC) [3–5] and gas chromatography [6]. However, there are still some problems, such as complexity of procedure, expensive consume of chromatographic column and organic reagents with chromatography grade and pollution of organic reagents, etc. These disadvantages can be overcome by high performance capillary electrophoresis (HPCE).

HPCE has become a useful and powerful separation technique. The most significant features that make CE a successful tool both in basic research and in clinical laboratories are: low costs of analysis, small sample volume, automation, high selectivity. Many methods of CE had been used to analyze drug [7–9]. Srinivasu et al. had reported micellar electrokinetic chromatographic (MEKC) method for the determination of lovastatin and simvastatin in pharmaceutical formulations [7]. However, so far no capillary zone electrophoresis (CZE) method was reported for the separation and quantitative determination of lovastatin in a capsule of Monascus-Chinese medicine.

Lvt is an active pharmaceutical ingredient in the Monascus capsule. Its role is to reduce serum total cholesterol, triglyceride, and low-density lipoprotein cholesterol, increase high-density lipoprotein cholesterol, treat for hyperlipidemia and cardio-cerebro-vascular, diseases caused by hyperlipidemia and atherosclerosis. The determination of Lvt in the capsule of Monascus has great significance for the control of clinical therapy. So, a simple, rapid, and sensitive analytical methods for separation and determination of Lvt in the capsule of Monascus is of great interest. HPLC has been applied for the quantitative analysis of Lvt in the capsule [10]. But the procedure was complex and a large amount of organic solvent was required.

To overcome these problems, in this work, a new, simple but useful CZE method was developed for the separation and determination of Lvt in the capsule of Monascus. Lvt is the

* Corresponding author. Tel.: +86 21 34204207; fax: +86 21 34205820.
*E-mail address:* cxcao@sjtu.edu.cn (C.-X. Cao).

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only ingredient of the API in the Monascus capsule. The label claim of Lvt is no less than 2.5 mg/capsule (0.3 g). The matrix of the capsule is Monascus. Prior to determination, the sample was extracted by ethanol. The parameters affecting the separation and sensitivity of Lvt were optimized. The separation was performed by adding propriety ethanol to the electrolyte buffer. Under the optimized conditions, we conducted a series of investigations on reliability, including specificity, selectivity, linearity, LOD, LOQ, recovery, accuracy, precision, etc. The purpose of this paper was to report a rapid, inexpensive, accurate and sensitive method for quantitative determination of Lvt in the Monascus capsule.

## 2. Materials and methods

### 2.1. Apparatus

An ACS 2000 HPCE apparatus (Beijing Cailu Scientific Inc., Beijing, China) was used. The system was fitted with a power supply (up to voltage 30 kV) with a current limit of 300 μA, a HW-2000 chromatography workstation and an UV–vis detector that could perform wavelength scanning from 190 to 740 nm. A fused-silica capillary was used (Factory of Yongnian Optical Fiber, Hebei, China). The capillary was 51 cm × 75 μm i.d. (43 cm effective length). An Ultra-pure Water System (SG Ultra Clear system, Wasseraufbereitung und Regenerierstation GmbH, Germany) was used to produce ultra pure water with specific conductivity down to 0.055 μS/cm for the analysis of HPCE. HS-1200 ultrasonic apparatus (Shanghai Scientific Institute of Biotechnology, Shanghai, China) was used to extract Lvt from its mixture of the Monascus capsule.

### 2.2. Chemicals

Standard of Lvt was supplied by the Zhejiang Hisun Pharmaceutical Co. Ltd. (Taizhou, China). capsule of Chinese medicine Monascus was purchased from the local drugstore. Glycine (biological grade reagent) was purchased from the Shanghai Shisheng Cell and Bio-technology Company (Shanghai, China). Sodium hydroxide, ethanol (analysis reagent grade, AR) were purchased from the Shanghai Chemistry Reagent Company (Shanghai, China). Phenobarbital was purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China).

### 2.3. Preparation of standard solution

The stock solutions of Lvt (4.0 mg/mL) and internal standard (phenobarbital, 5.0 mg/mL) were prepared by dissolving 40.0 mg Lvt and 5.0 mg phenobarbital in 10,000 μL and 1000 μL ethanol, respectively. The Lvt standard solutions were prepared in accordance with the following procedure. A series of different volume (10, 25, 50, 100, 150, 200, 250, 350, 450 and 600 μL) Lvt stock solutions and 50 μL phenobarbital stock solution were added into blank samples, and then diluted to 1000 μL with ethanol, respectively. The mixtures were then conducted in accordance with the procedure in Section 2.5 shown below. The final concentrations of dilution of Lvt were 4, 10, 20, 40, 60, 80, 100, 140, 180 and 240 μg/mL.

### 2.4. Preparation of buffer

A series of Gly-NaOH buffers with different pH values and concentrations were prepared. The pH of the buffers ranged from 9.2 to 11.0 and the concentrations changed from 10 to 100 mM. Finally background electrolyte was prepared by mixing 84% of 60 mM, pH 10.5 Gly-NaOH buffer and 16% of ethanol. All solutions were filtered through 0.45 μm syringe filter prior to their uses so as to remove solid particles.

### 2.5. Extraction procedure

Lvt can be well dissolved in ethanol, methanol, acetone and ethyl acetate [11]. Ethanol is in common use and innoxious. Phenobarbital (internal standard) can be dissolved in ethanol too. So, ethanol was used for extracting lovastatin in capsule. A 50.0 mg solid content in the capsule and 50 μL phenobarbital standard solution (5.0 mg/mL) were added into a 2 mL-tube, then adding 950 μL ethanol into the tube. The tube was vibrated for 10 min with ultrasonic. After that, the mixture in the tube was centrifuged at 5000 rpm for 2 min, and Lvt was extracted from the solid. The liquor on superstratum was transferred to another tube. The residue was extracted by 1000 μL ethanol again. The two organic phases were combined together and dried by water bath at 80 °C. The residue after dryness in the tube was dissolved in 1000 μL ethanol. A 100 μL of the above solution was diluted to 1000 μL with the buffer for CE analysis. All solutions were stored at 4 °C in a refrigerator until use.

### 2.6. Analytical procedures

HPCE was conducted as following procedure. Before use, the new capillary was conditioned by rinsing with 1 M NaOH for 20 min, ultra-pure water for 10 min, 1 M HCl for 20 min, ultra-pure water for 10 min, and running buffer for 20 min, in order. Between injections the capillary was rinsed with the running buffer for 3 min. Detection wavelength was 238 nm. A 60 mM Gly-NaOH buffer, pH 10.5 containing 16% (v/v) ethanol was used as background electrolyte. Pressure injection of sample was chosen. The pressure was set at 14 mbar and the injection time was controlled at 5 s. The ultimate work voltage was 16 kV. Temperature control of capillary was carried out with 22° C air-cooling. Detection data were collected and processed with the HW-2000 Chromatography Workstation Software.

## 3. Results and discussions

### 3.1. Optimization of conditions

There are some factors that have obvious effect on the separation, peak shape, and electromigrating time of analyte, sensitivity, noise and resolution as well as column efficiency of CE. The

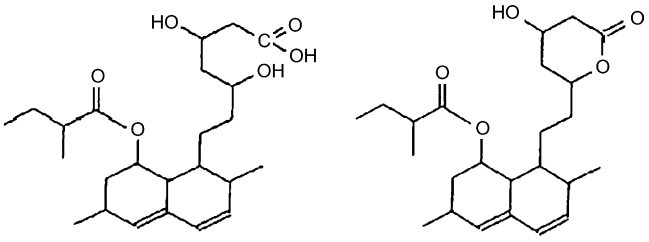


Fig. 1. Structural formulae of acid (left) and lactone (right) of lovastatin.

factors concern pH value and concentration of buffer, organic modifier in buffer and voltage etc. The followings are the results of optimization and investigations.

### 3.1.1. Influence of buffer pH

The first step in the method development process was the selection of optimum pH value. The basic chemical structure of Lvt contains a naphthalene ring system, a β-hydroxy-lactone and methyl butyric acid [12]. Lvt can be dissolved in the alkaline solution, and when pH value is higher than 7.7 [3] the lactone Lvt can be converted to its acidic form. The structures of the lactone and acidic Lvt are shown in Fig. 1. Recent studies in our laboratory showed that $pK_a$ values of acidic Lvt was 9.2 [13]. However, the velocity of conversion was slow and the conversion was not completely at low pH. In this study, high pH was selected in order to make the lactone Lvt can be rapidly and completely converted to its acidic form, so buffer solutions ranging from pH 9.2 to pH 11.0 were investigated in this study. The optimization was initiated with a buffer concentration of 60 mM in the pH range of 9.2–11.0, a voltage of 16 kV and a temperature of 22 °C. Experimental results reveal that the separation of Lvt from other substances was very poor in the studied pH range. Considering $pK_a$ value of acidic Lvt, we chose the pH 10.5 as the optimized pH value at which the lactone Lvt can be converted to its acidic form completely.

### 3.1.2. Influence of buffer concentration

The effect of buffer concentration on the separation was studied by varying it from 10 to 100 mM. The migration time of Lvt decreased with the increase of buffer concentration. Lvt and impurity could not be well separated in the entire studied concentration range without the presence of organic modifier. So, we added organic modifier to the buffer, the effect of buffer concentration on the separation was re-studied in the range of 30–80 mM with 16% ethanol (v/v) in the buffer (see the text below). The results indicated that buffer concentration has influence on migration time, resolution, peak shape, sensitivity, especially electric current. The peak height of Lvt decreased with the increase of buffer concentration. The migration time of Lvt and internal standard, resolution of Lvt and impurity increased with the increase of buffer concentration as shown in Fig. 2. However, the high concentration of buffer led to higher current. So 60 mM buffer was selected for the further experiments as a compromise between resolution, peak shape and analysis time.

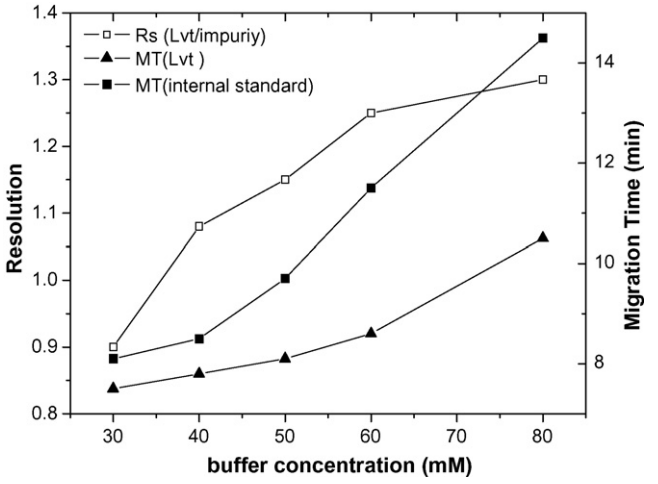


Fig. 2. Effect of buffer concentration on resolution between Lvt and impurity, analysis time. Conditions: 30–80 mM pH 10.5 Gly-NaOH buffer containing 16% (v/v) ethanol, 16 kV. A 51 cm total length (43 cm effective length) and 75 μm i.d. capillary, 238 nm wavelength, 14 mbar 5 s pressure sample injection, 22° C air-cooling.

### 3.1.3. Influence of organic modifier

The organic modifier alters the retention mechanism by changing the electrolyte viscosity and the zeta potential. So, the introduction of organic modifier in the running buffer has played a key role on the separation. In order to improve the separation, ethanol was used as the organic modifier. The effect of ethanol concentration on separation was investigated systematically with 60 mM Gly-NaOH at pH 10.5. Ethanol in the concentration range of 8–24% (v/v) were studied in CZE mode. The results indicated that the analysis time was prolonged and the resolution of Lvt and impurity was improved with the increase of ethanol concentration as indicated in Fig. 3. Finally, 16% (v/v) ethanol was selected as a compromise between resolution and analysis time.

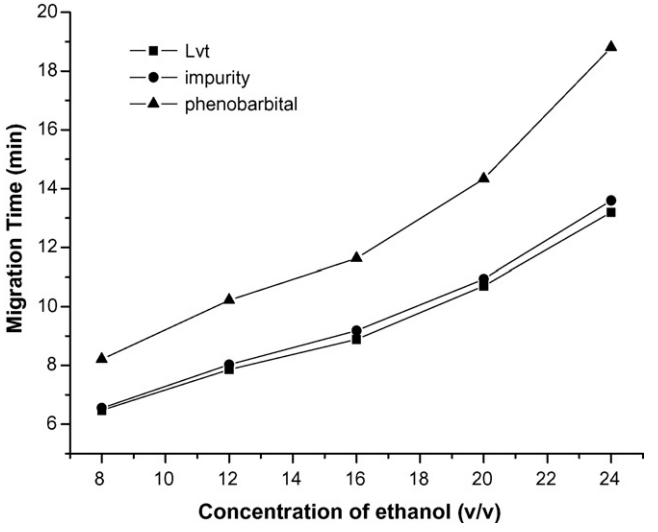


Fig. 3. Influence of ethanol concentration on migration time of Lvt, impurity and internal standard. The conditions are the same as those in Fig. 2, except for the concentration of ethanol.

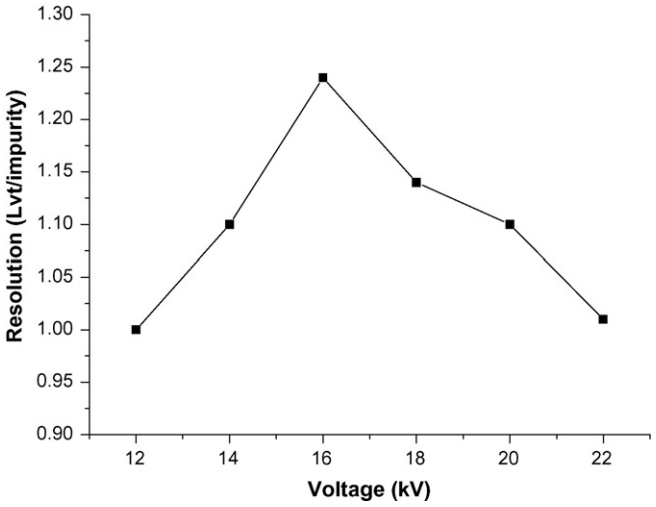


Fig. 4. Effect of applied voltage on resolution of Lvt and impurity. The conditions are the same as those in Fig. 2, except for the voltage.

### 3.1.4. Influence of applied voltage

Applied voltage has very important influence on migration time, current strength and resolution. The experiments showed that the migration time of Lvt and internal standard phenobarbital decreased with the increase of the applied voltage. The resolution increased with the increase of applied voltage up to 16 kV and then decreased with the increase of applied voltage. At higher applied voltage, the analysis time was short but the resolution was poor due to much high Joule's heating within the capillary. A lower voltage produced longer migration time and poorer resolution. Considering fast determination of Lvt and maintain of good resolution, 16 kV was chosen as the applied voltage (see Fig. 4).

### 3.1.5. Optimized conditions

Finally, we achieved the following optimized conditions: 60 mM pH 10.5 Gly-NaOH buffer with 16% (v/v) ethanol, 51 cm total length (43 cm effective) and 75 μm i.d. capillary, 238 nm wavelength, 16 kV voltage, 14 mbar 5 s pressure sample injection and 22 °C air-cooling.

## 3.2. Method validation

Validity of a newly developed analytical technique needs to be strictly demonstrated before its application to actual determination of drug. In this work, various validation criteria of the developed method of CZE, such as specificity, precision, linearity of response, accuracy, detection limit (LOD), quantification limit (LOQ), etc. were assessed as described below.

### 3.2.1. Specificity of method

Specificity of a newly developed analytic method should be firstly demonstrated due to its key importance and priority. In the demonstration of this paper, numerous samples of Lvt extracted from the Monascus with ethanol were analyzed repeatedly under different conditions. The purpose of the analysis was to make sure whether or not the peak of target analyte Lvt in the electrophoregrams was exclusive under the given experimental conditions optimized above. Fig. 5 shows the demonstration of exclusive peak of target analyte. Panel (a) is the electropherogram of extract from blank sample, only ethanol peak can be seen. Panel (b) unveils Lvt extracted from the capsule without internal standard in buffer. Panel (c) is the peaks of ethanol, Lvt and Phenobarbital internal standard extracted from capsule spiked with phenobarbital at 25 μg/mL. Panel (d) proves the peaks Lvt standard and internal standard extracted from sample blank spiked with Lvt at 40 μg/mL and phenobarbital at 25 μg/mL. Panel (e) displays the electrophoregram Lvt and internal standard extract from the capsule spiked with Lvt at 40 μg/mL and phenobarbital at 25 μg/mL. The target peak in panel (e) was higher than that in panel (d). Furthermore, we checked the peak purity by UV diode-array. The results indicated that no interference was noted for Lvt and internal standard. All of the experiments discussed above evidently indicate the specificity of the developed CZE method for the determination of targeted analyte.

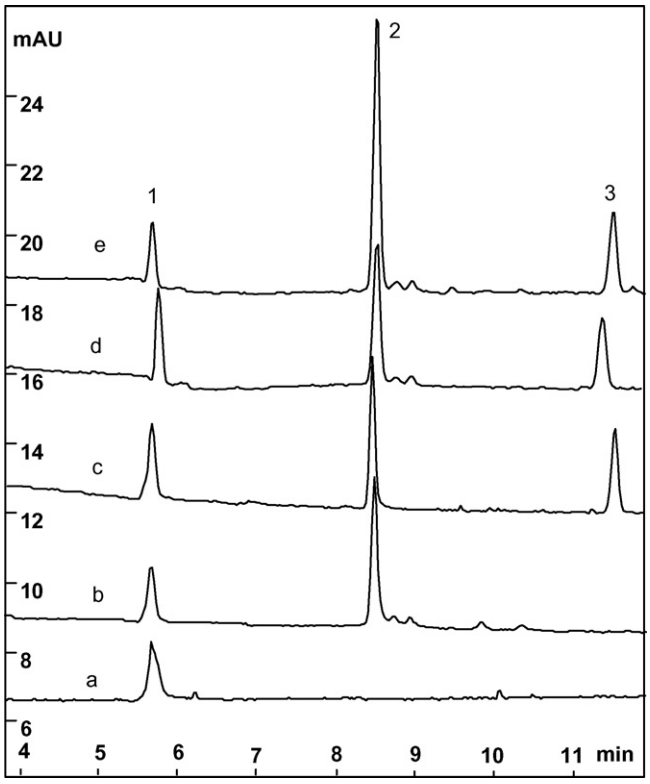


Fig. 5. Electrophoregrams of CZE of: (a) blank sample, (b) Lvt extracted from capsule without phenobarbital internal standard, (c) Lvt and internal standard extracted from capsule, (d) Lvt standard and internal standard extracted from blank sample and (e) Lvt and inernal standard extracted from spiked sample. Peak 1, ethanol; peak 2, Lvt; peak 3, internal standard. The conditions are the same as those in Fig. 2.

### 3.2.2. Linearity

The linearity of detector response versus concentration of analyte was determined by constructing a calibration curve from a set of standard solutions of Lvt extracted from sample blank with nine different concentrations on the optimized conditions.

Table 1
The intra-day variations of peak area ratios between Lvt and phenobarbital ($r = 3$; $n = 6$)

| Sample concentration (μg/mL) | Peak area ratio | S.D. | R.S.D. (%) |
|---|---|---|---|
| 4 | 0.15 | 0.003 | 1.93 |
| 40 | 1.38 | 0.03 | 2.12 |
| 240 | 8.34 | 0.12 | 1.40 |

Table 2
The inter-day variations of peak area ratios between Lvt and phenobarbital ($r = 3$; $n = 5$)

| Sample concentration (μg/mL) | Peak area ratio | S.D. | R.S.D. (%) |
|---|---|---|---|
| 4 | 0.15 | 0.004 | 2.68 |
| 40 | 1.39 | 0.02 | 1.47 |
| 240 | 8.25 | 0.32 | 3.88 |

The linear range was from 4 to 240 μg/mL. The linear equation was $y = 0.01724 + 0.03458x$, where $y$ was the peak area ratio of the analytes and internal standard, $x$ was the concentration of the analyte Lvt. The correlation coefficient for Lvt standards was 0.9998 ($n = 9$), a very high value.

### 3.2.3. Precision

Precision of a new method was evaluated by measuring intra-day and inter-day relative standard deviations (R.S.D.) of peak areas ratio between analyte and internal standard extracted from the sample blank. The intra-day values of R.S.D. were calculated based on six replicate runs of three different concentrations of 4, 40 and 240 μg/mL in a day. The inter-day values of R.S.D. were evaluated using five date sets from the same three standard solutions obtained on 5 days. The two precision results were given in Tables 1 and 2, respectively. The values of R.S.D. of the intra-day runs ranged from 1.40 to 2.12%. The inter-day precision or repeatability, expressed as the R.S.D. was 1.47–3.88%. The results in Tables 1 and 2 implied that the operating conditions selected above could provide a good stable electrophoretic system with quite good repeatability.

### 3.2.4. Recovery

The recovery experiments were performed on standard with three different concentrations. A sample blank without Lvt was spiked with standard solution of Lvt and internal standard of Phenobarbital. The finally concentrations of Lvt were 4, 40, 240 μg/mL, and the concentration of phenobarbital was 25 μg/mL. The samples used for the recovery runs were prepared as described above (see Section 2.5). The recovery was calculated by the ratio value of Lvt and phenobarbital which extracted from the sample blank to the ratio value of them resolved in the run buffer directly. The recovery was 100.71 ± 1.73% at 4 μg/mL, 98.03 ± 1.14% at 40 μg/mL, and 90.28 ± 1.88% at 240 μg/mL (see Table 3), these values were all within the acceptable range. The average Lvt concentration in the capsule was 9.05 ± 0.10 mg/g (i.e. 2.72 ± 0.03 mg/capsule ($n = 5$)). The detected content of Lvt was 2.83 mg/capsule using the LC-method described by reference [10]. Furthermore, the label claim of Lvt was no less than 2.5 mg/capsule. Comparison of the present developed method with LC-method [10] and the label claim indicated that the present developed method can be used for the determination of Lvt in Monascus.

Table 3
The recovery of Lvt under different concentrations ($r = 3$; $n = 3$)

| Added Lvt (μg/mL) | Peak areas ratio | | Recovery (%) |
|---|---|---|---|
| | Extraction from blank sample | Resolving in buffer | |
| 4 | 0.1497 | 0.1486 | 100.71 ± 1.73 |
| 40 | 1.3829 | 1.4107 | 98.03 ± 1.14 |
| 240 | 8.3388 | 9.2364 | 90.28 ± 1.88 |

### 3.2.5. Limits of detection and quantification

The limit of detection (LOD) is a standard which reflects the sensitivity of the method and equipment. The limit of quantification (LOQ) reflects the reliability of the method when used for the determination of low concentration drug. The LOD and LOQ were calculated by setting the signal-to-noise ratio of 3:1 and 10:1, respectively. The LOD and LOQ of the developed method were 0.73 and 2.42 μg/mL, respectively, under 5 s, 14 mbar pressure sample injection.

## 4. Conclusions

The experiment results demonstrate that the proposed CZE method is suitable for the determination of Lvt extracted from capsule of Chinese medicine Monascus. In addition, the method is reliable, simple, secure and low cost. This work also shows that CE is a powerful technique to determine lovastatin in the complex extract of the drug and fermentation. With simple online sample stacking [14,15], the sensitivity of determination can reach at the degree of ng/mL [16]. Recently our laboratory is developing work to enhance the detection sensitivity of Lvt by the simple stacking method. It will be useful for the determination of trace Lvt in biologic fluid (such as human urine, plasma, etc.).

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