Analysis of Clenbuterol in Urine

Abstract

As a β2 sympathomimetic doping, clenbuterol can be detected from urine if it goes into human body, however, the traditional sample preparation is complicated and time-consuming. In this paper, LabTech Automated Solid Phase Extraction (SPE) instrument and LabTech Concentration System were applied to extract and concentrate clenbuterol from urine sample. The whole automatic sample preparation process includes solid phase extraction, quantitative concentration and solvent exchange, which is simple, of high efficiency and of good result reproducibility. The prepared clenbuterol sample was accurately and fast analyzed by High Performance Liquid Chromatography (HPLC). The results showed that the calibration curve had a linear coefficient (R^2) of 0.9998, recovery rate of 89.53%, and RSD of 1.04%.

Keywords：Clenbuterol, Urine, Solid Phase Extraction (SPE), Quantitative Concentration, HPLC

Clenbuterol is one type of β- dopings, which can promote protein synthesis, and accelerate the transformation and decomposition of the fat in the metabolic process. After clenbuterol goes into the human body, the body is gradually poisoned with the accumulated clenbuterol. The clenbuterol in human body can be excreted through the urine, so the test of clenbuterol in urine can detect whether there are clenbuterol in body.

In this experiment, LabTech Automated Solid Phase Extraction (SPE) instrument - Quantitative Concentration system were applied for sample pretreatment. After urine samples were collected and preliminary purified, automatic pretreatment system completed the sample preparation through solid phase extraction - eluent concentration - the solvent exchange process. The treated sample can be directly used for the analysis by liquid chromatography. The whole process greatly simplified the experimental work, and the results by liquid chromatography analysis are reliable and accurate, and of high reproducibility and recovery.

1 Instrument and Reagents

1.1 Instrument

LabTech PrepElite-VS automated SPE and concentration instrument
LabTech LC600 Gradient HPLC system

1.2 Reagents

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Ethyl acetate
1.0 mol/L sodium chloride solution
0.1mol/L potassium hydrogen phthalate buffer solution（pH=4.0）
Methanol （Chromatographically pure）
4% ammonia methanol
Deionized water

2 Experimental methods

2.1 Sample collection and preparation

5.00ml of urine sample was collected, and adjusted to pH9.5 with 2.5 mol / L sodium hydroxide solution, followed by adding 1mL 1mol/L of sodium chloride. After complete mixing, 10mL of ethyl acetate was added and the mixture was shaken for 10 min, and then the sample was centrifuged for 15 min. The supernatant was collected. 10 ml of ethyl acetate was used to repeatedly extract the sample. After centrifugation, the supernatant was collected and merged with previous supernatant. The total supernatant was dried by nitrogen gas. 5mL 0.1mol/L potassium hydrogen phthalate buffer solution (pH = 4.0) was added for further experiment.

2.2 Sample purification

SPE column: ProElut PXC 90 mg/6 mL

a. Conditioning：Orderly adding 4.5mL methanol, 4.5 mL water and 4.5 mL 30 mmol/L HCl, to condition SPE column.
b. Sample injection: injecting the pretreated sample into SPE column, wasting the effluent.
c. Rinsing: Orderly rinsing the column with 4.5 mL water and 4.5 mL methanol, nitrogen gas purge to dry for 10min.
d. Elution: 7.5 mL 4% ammonia methanol to elute.
e. Online concentration: At 50 ºC, concentrating online with mobile phase (methanol: H_2O = 35:65）to exchange solvent, and automatically make the volume to 1mL.

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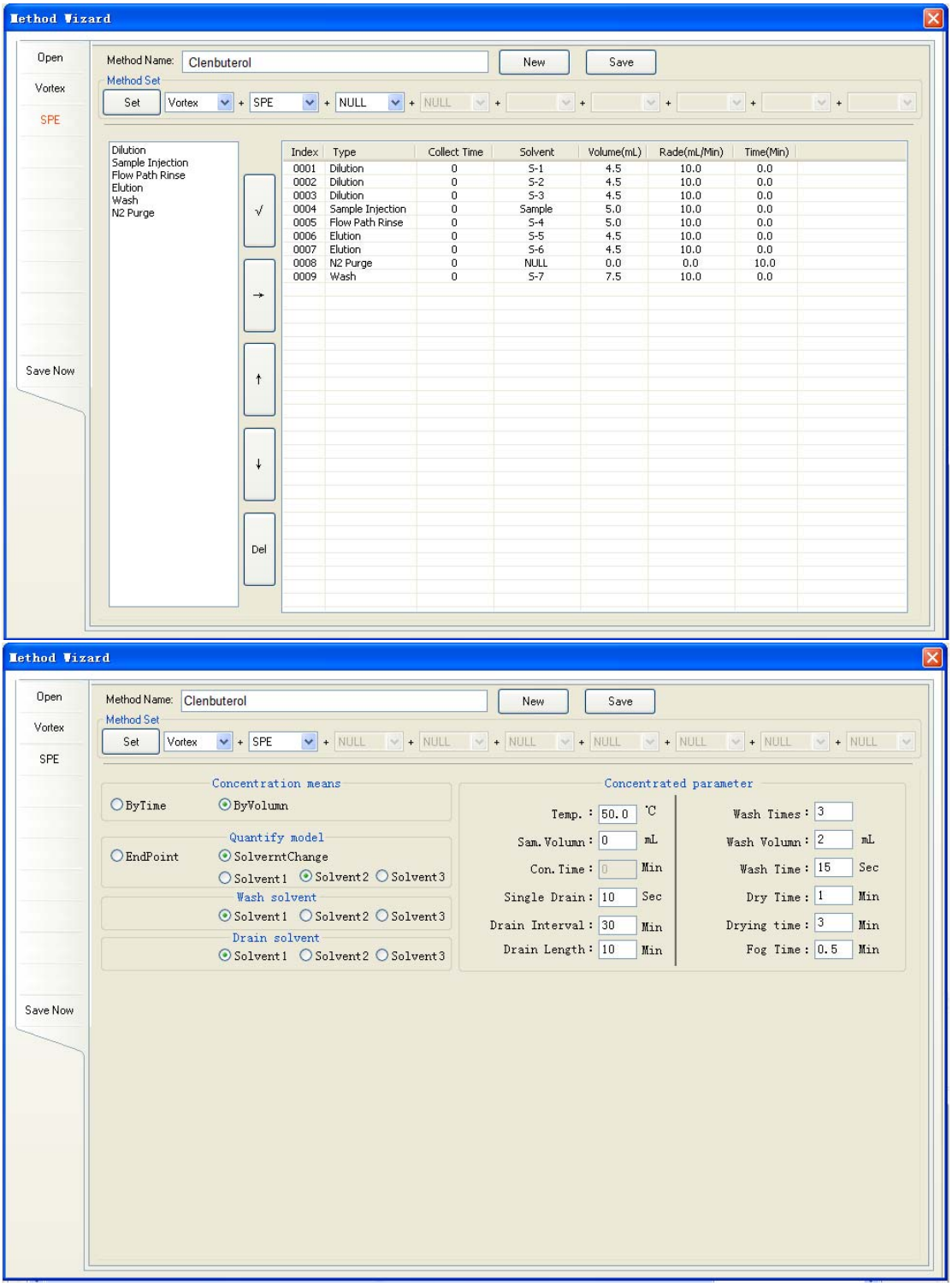

Figure 1 Method setting interface for PrepElite-VS

2.3 Sample analysis

2.3.1 HPLC operation parameters

HPLC column: C18 column, 250mm×4.6mm, 5μm

Mobile phase: Methanol: H2O=35:65

Flow rate: 1mL/min
Injection volume: 20μL
UV detector: 244nm

2.3.2 Calibration and sample analysis
Standard clenbuterol solution: Accurately weight standard clenbuterol chemical, and solve it with methanol to make 25μg/mL standard stock solution. Use the mobile phase (methanol: H_2O = 35:65) to dilute the stock solution to serial standard solutions: 0.025μg/mL, 0.05μg/mL, 0.25μg/mL, 1.0μg/mL, and 2.5μg/mL.

The treated sample from urine was filtered with 0.45μm syringe filter.

50μL standard solution and sample was injected into HPLC for quantitative analysis.

3 Results and Discussion

3.1 LC chromatograms of standard clenbuterol

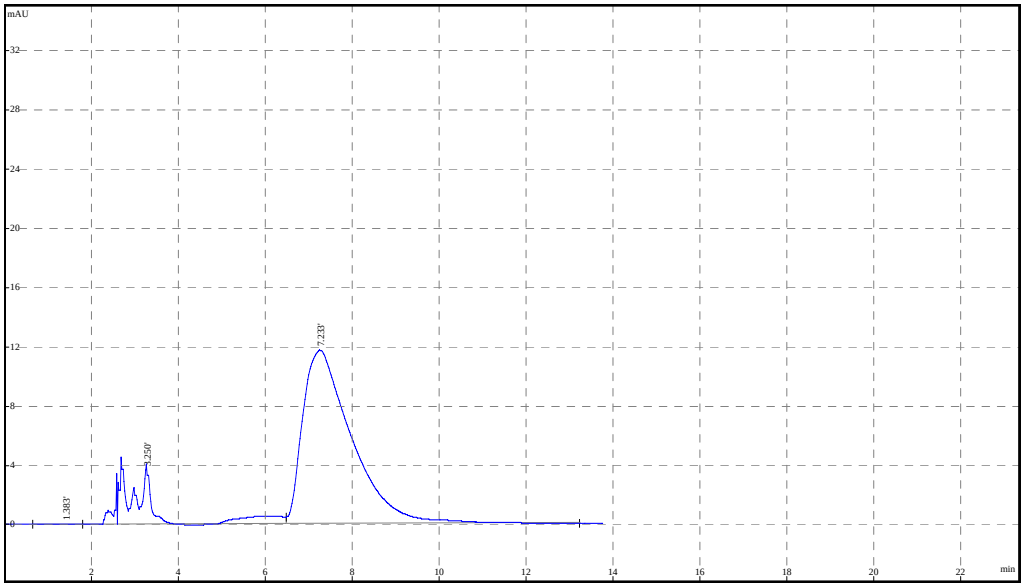

Figure 2 Chromatogram of standard clenbuterol (1.0μg/mL)

3.2 Calibration curve of clenbuterol

Concentration of clenbuterol standard solutions: 0.025μg/mL, 0.05μg/mL, 0.25μg/mL, 1.0μg/mL, 2.5μg/mL，linear equation: Y=361552X-4061.8, correlation coefficient: R^2=0.9998.

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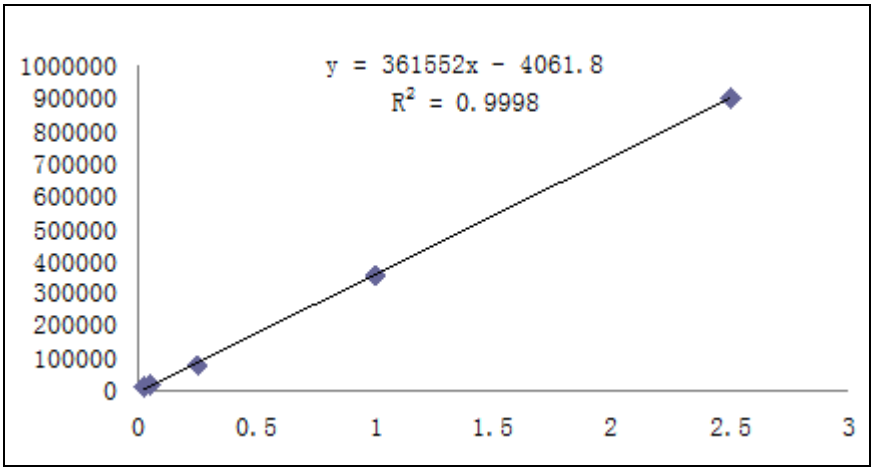

Figure 3 Calibration curve of standard clenbuterol

3.3 Recovery of spiked internal standard

Results of recovery are presented in Table 1.

Table 1 Recovery of spiked internal standard

#	Concentration of clenbuterol (μg/mL)	Recovery (%)	RSD（%）
1	0.9056	90.56	1.04
2	0.8975	89.75	
3	0.8897	88.97	
4	0.8823	88.23	
5	0.9012	90.12	
Average	0.8953	89.53	

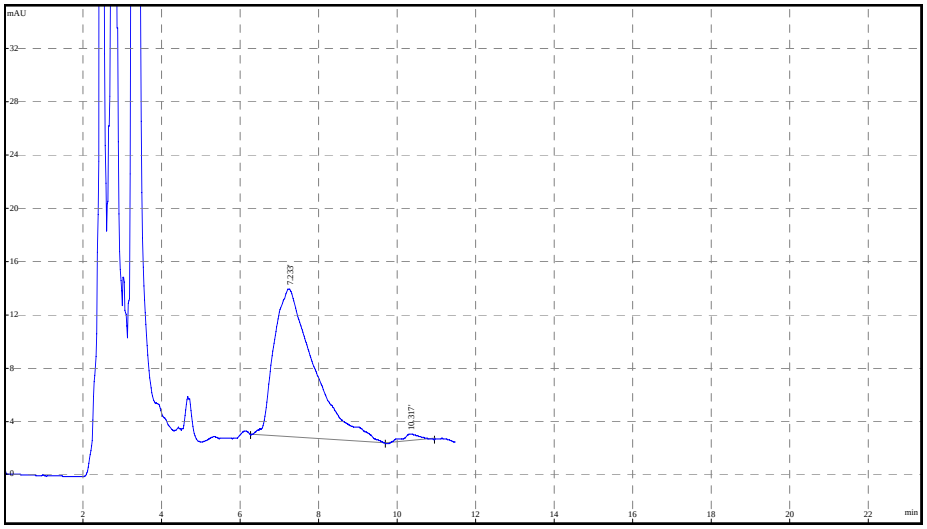

Figure 4 Chromatogram of sample with internal standard

3.4 Sample without internal standard

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The result without spiking internal standard is showed in Figure 5. After calculation, there is no clenbuterol that can be detected without spiked internal standard.

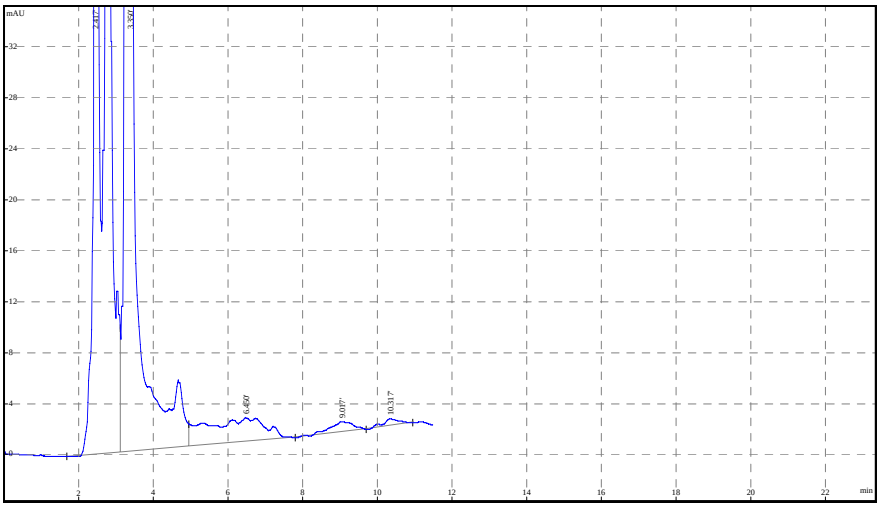

Figure 5 Chromatogram of sample without spiked internal standard

3.5 Comparison of three samples

Clenbuterol standard (1.0μg/mL), sample without internal standard, and sample with spiked internal standard are compared, and chromatograms are shown in Figure 6.

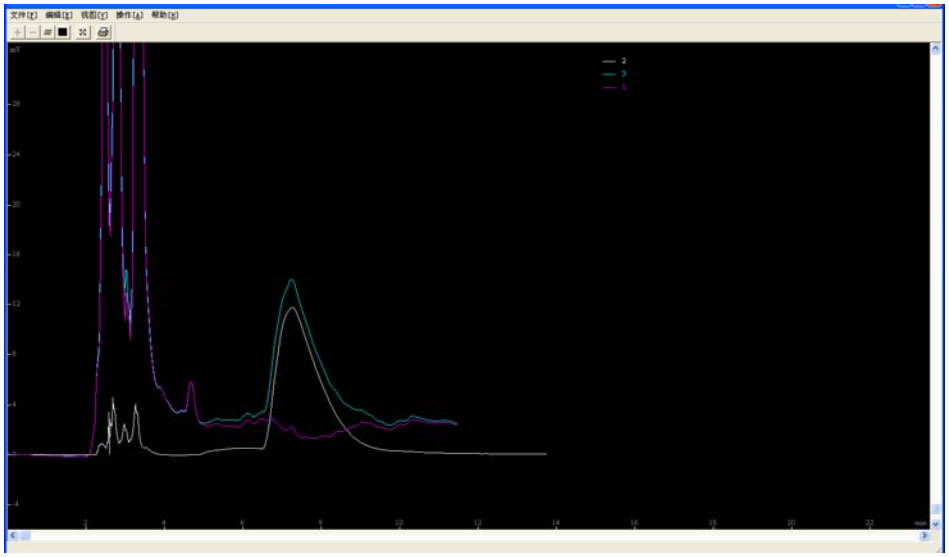

Figure 6 Comparison of chromatograms of three samples

4 Conclusion

In this study, LabTech automated SPE instrument and Quantitative Concentration system were applied for urine sample pre-treatment. The high degree of automation solved the problems by traditional pre-treatment, such as complicated SPE steps,

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solvent evaporation, and uncontrolled flow rate through column. The collected eluent from SPE was concentrated online, exchanged solvent and controlled to the constant volume, which greatly simplified the tedious sample pre-treatment work and protected the operator from exposing in a variety of organic solvents, while the recovery rate was stable, reliable, recovery rate as high as 89.53%.

HPLC was applied to do clenbuterol qualitative and quantitative analysis. The test was fast and easy, and the calibration curve was linear (coefficient R^2 = 0.9998). The exact concentration can be measured for samples with or without spiked standards. The recovery rate can be accurately calculated. The test had advantages of simple operation and high stability, and RSD was 1.04%. No clenbuterol was detected in collected urine samples.

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