

Retraction

Retracted: Quantitative Detection of Amino Acids and Carnitine in Human Blood and Quality Control of Peptide Drugs

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This article has been retracted by Hindawi following an investigation undertaken by the publisher [1]. This investigation has uncovered evidence of one or more of the following indicators of systematic manipulation of the publication process:

- (1) Discrepancies in scope
- (2) Discrepancies in the description of the research reported
- (3) Discrepancies between the availability of data and the research described
- (4) Inappropriate citations
- (5) Incoherent, meaningless and/or irrelevant content included in the article
- (6) Peer-review manipulation

The presence of these indicators undermines our confidence in the integrity of the article's content and we cannot, therefore, vouch for its reliability. Please note that this notice is intended solely to alert readers that the content of this article is unreliable. We have not investigated whether authors were aware of or involved in the systematic manipulation of the publication process.

In addition, our investigation has also shown that one or more of the following human-subject reporting requirements has not been met in this article: ethical approval by an Institutional Review Board (IRB) committee or equivalent, patient/participant consent to participate, and/or agreement to publish patient/participant details (where relevant).

Wiley and Hindawi regrets that the usual quality checks did not identify these issues before publication and have since put additional measures in place to safeguard research integrity.

We wish to credit our own Research Integrity and Research Publishing teams and anonymous and named external researchers and research integrity experts for contributing to this investigation.

The corresponding author, as the representative of all authors, has been given the opportunity to register their agreement or disagreement to this retraction. We have kept a record of any response received.

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- [1] Y. Ma, Y. Zhu, Z. Lu et al., "Quantitative Detection of Amino Acids and Carnitine in Human Blood and Quality Control of Peptide Drugs," *Journal of Chemistry*, vol. 2022, Article ID 1652592, 6 pages, 2022.

Research Article

Quantitative Detection of Amino Acids and Carnitine in Human Blood and Quality Control of Peptide Drugs

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In order to deal with the biochemical metabolic disorder and the lack of end metabolites caused by hereditary metabolic diseases, a quantitative detection method of amino acids and carnitine in human blood and the quality control method of polypeptide drugs were proposed. First, a method for the detection of 10 amino acids and 32 acylcarnitine metabolites in human blood was established and analyzed by liquid chromatography tandem mass spectrometry. Through the experimental research on typical peptide drugs, combined with the classical synthesis process and the PLS-DA model of human body in different regions, the relevant detection methods for the quality control of peptide drugs were established, which provided a reference for the formulation of peptide drug quality standards. The experimental results show that the quantitative detection method proposed in this paper can effectively detect the content of most amino acids and acylcarnitine. The classical detection method achieved a loss of 16.74% smaller mass-to-charge ratio, and based on this, the quantitative standard for polypeptide drug delivery was determined, which verifies the correctness and superiority of the detection method in this paper.

1. Introduction

In recent years, the quantitative detection of amino acids and carnitine in human blood has become a hot research topic worldwide. In the related background, inherited metabolic diseases (IMD), also known as inborn errors of metabolism, refer to the function of certain enzymes, transport proteins, membranes, or receptors that maintain normal metabolism in the body due to gene mutation. A type of disease that causes changes in the body's biochemical metabolism, resulting in accumulation of intermediate or bypass metabolites or lack of terminal metabolites, and a series of clinical symptoms [1]. At present, more than 500 hereditary metabolic diseases have been found, most of which are autosomal recessive inheritance, mainly including amino acids, organic acids, fatty acids, lysosomes, mitochondria, peroxidase, purines, metals, urea cycle, carbohydrate compounds and porphyrin, bilirubin, red blood cells, and other abnormal substance metabolic diseases [2].

The quality control of peptide drugs associated with it has also attracted widespread attention from scholars. Taking the typical peptide drug exenatide as an example, exenatide is a polypeptide diabetes treatment drug containing 39 amino acids. Glucagon-like peptide-1 (GLP-1) analogs have a good effect on patients with type 2 diabetes who cannot control blood sugar with oral metformin or sulfonylureas and can promote pancreatic β -cell proliferation and insulin secretion, inhibit glucagon release and slow gastric emptying, and inhibit food intake. It is the first new drug for the treatment of type 2 diabetes approved worldwide in recent years. The technical means of quality control of peptide drugs include chromatography, mass spectrometry, nuclear magnetic resonance, electrophoresis, and other methods, of which it is worth mentioning the full-column imaging capillary isoelectric focusing electrophoresis method. Capillary isoelectric focusing-whole column imaging detection (CIEF-WCID) is a new type of electrophoresis system that combines capillary isoelectric focusing and whole-column imaging technology [3, 4]. Figure 1 is a

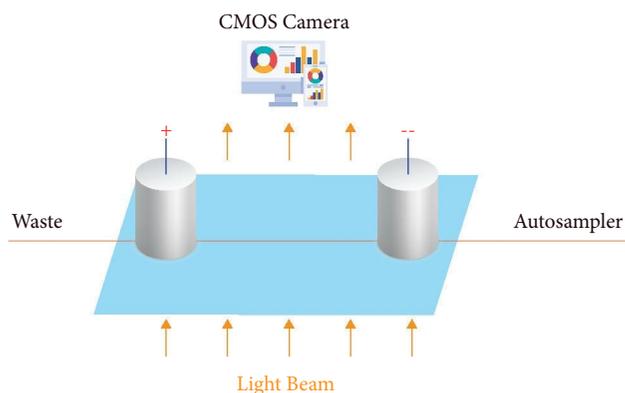


FIGURE 1: Schematic diagram of full-column imaging capillary isoelectric focusing electrophoresis apparatus.

schematic diagram of a full-column imaging capillary isoelectric focusing electrophoresis apparatus, in which one end of the capillary column is connected to an automatic sampler, and the other end is connected to a waste liquid bottle. When high-voltage direct current is applied to both ends of the cathode and the anolyte, the carrier ampholyte forms a pH gradient in the capillary column, and the sample moves to the anode or cathode according to the charged property in the 50 mm-long separation channel. When the sample moves to its isoelectric point at the same pH value, the net charge of the sample is zero, that is, isoelectric focusing separation is completed. During the entire focusing process, complementary metal oxide semiconductor (CMOS) is used as an imaging detection technology to perform dynamic imaging detection throughout the focusing process of the entire focusing separation channel.

2. Literature Review

Ashrafuzzaman et al. pointed out that one of the main characteristics of IMD is age dependence, the earlier symptoms appear, the more severe the disease, and the higher the mortality rate. In the neonatal period, abnormal symptoms of the nervous system first appear in IMD, which are mainly manifested as difficulty in sucking and feeding, abnormal breathing, slow heart rate, hiccups, and even coma. Digestive symptoms such as refusal to eat, vomiting, and diarrhea also occur shortly after eating [5, 6]. Zhang et al. found after investigation that some patients will not develop disease until months or even years after birth. The cause of the disease may be infection or eating a lot of protein-containing food, common hypoglycemia, hyperlactatemia, metabolic symptoms such as sexual acidosis, cyclic ataxia, and coma are often misdiagnosed because they are similar to the symptoms of hypoxia, severe dehydration, infection, diabetes, and endocrine disorders. In the early stage, some diseases can be controlled, and patients can recover partially or completely through treatment methods such as drug therapy, diet control, hormone replacement therapy, gene therapy, enzyme replacement therapy, and stem cell transplantation [7]. Prof. Gao et al. successfully established a diagnostic method for phenylketonuria by the

bacterial inhibition method, started the screening of hereditary metabolic diseases, and applied enzymatic analysis to screen galactosemia [8]. Zhang et al. carried out screening for metabolic diseases such as phenylketonuria, maple syrup, and homocystinemia [9]. The results of Song et al. showed that, in China, the screening of genetic metabolic diseases took the lead in screening newborns for phenylketonuria, congenital hypothyroidism, and galactosemia [10]. Li et al. discovered the first case of isovaleric acidemia using gas chromatography-tandem mass spectrometry. Since then, gas chromatography-tandem mass spectrometry has been widely used in the screening of genetic metabolic diseases, especially organic aciduria. However, this method has the problems of long detection time and high analysis cost [11]. Liu et al. and other researchers found that electrospray tandem mass spectrometry can rapidly detect acylcarnitines in blood samples and introduced tandem mass spectrometry technology to the screening of inherited metabolic diseases for the first time. This method requires a small amount of samples, and the analysis time is only 2-3 minutes to screen for a variety of diseases. Studies have shown that the detection rate of tandem mass spectrometry is twice that of traditional screening methods [12].

Bf et al. were among the first scholars to enable tandem mass spectrometry for newborn screening for inborn errors of metabolism. They screened 944,078 infants over an 8-year period, of whom 219 had disorders of amino acid or acylcarnitine metabolism, an overall incidence rate of 1 in 4300 [13]. Louge et al. pointed out that the application of tandem mass spectrometry for the screening of inherited metabolic diseases has been gradually carried out in many economically developed regions of China. Among 748 high-risk children with clinically suspected genetic metabolic diseases in a certain province, amino acid and acylcarnitine were detected, and a total of 95 cases were abnormal, with a detection rate of 12.7%, including 54 cases of amino acid metabolism diseases and 19 cases of organic acidemia and 22 cases of fatty acid oxidation deficiency disease. Researchers in another province screened 116,000 newborns, and the results showed that the incidence of amino acid and acylcarnitine-based metabolic diseases in this region was about 1/5,800. With the gradual development of this method in China, more and more regions have successively used tandem mass spectrometry to screen various genetic and metabolic diseases [14]. Deng et al believe that the screening of inherited metabolic diseases by tandem mass spectrometry was first applied to detect abnormal metabolic diseases related to amino acids and acylcarnitines. For example, amino acid diseases such as phenylketonuria, maple syrup, citrullinemia, and argininemia; propionic acidemia, glutaric acidemia, 2-methylcrotonyl-CoA carboxylase deficiency, and other organic acid diseases; and medium and long-chain acyl-CoA dehydrogenase deficiency, trifunctional protein deficiency, and other fatty acid metabolism diseases. To enhance detection sensitivity, pre- or postcolumn derivatization is usually employed. Routine mass spectrometry screening for some diseases produces false-positive or false-negative results, and researchers are working to improve detection methods for these diseases. L-isoleucine is a

specific marker for diagnosing maple syrup, but because it has the same molecular weight as leucine and isoleucine, it is directly injected without chromatographic separation and mass spectrometry cannot distinguish these substances [15]. Wang et al. successfully separated leucine, isoleucine, and alloisoleucine by chromatographic separation and quantified it from valine and hydroxyproline. The results show that alloisoleucine can only be detected in patients with maple syrup diabetes mellitus. Some researchers have also explored the use of nonderivatized methods to quantify amino acids and acylcarnitines, using high-performance liquid chromatography electrospray tandem mass spectrometry for simple and rapid quantification of 20 amino acids. To obtain good chromatographic separation, perfluoroheptanoic acid was added to the mobile phase as an ion-pairing reagent [16].

On this basis, a method for the determination and control of amino acids and carnitine in human blood was established. First, the quantitative detection method of 42 amino acids and acylcarnitine in human blood was established. 37 genetic metabolic diseases of amino acid, organic acid, and fatty acid were screened by liquid chromatography and tandem mass spectrometry at 2 min simultaneously, expanding the type of disease screening. Screening of genetic metabolic diseases in 205 samples. Then, the data of 205 cases were analyzed and found a different amino acid and acylcarnitine content in different age groups, providing a guiding role in further improving the screening of genetic metabolic diseases.

3. Research Method

In this paper, a method for quantitative detection of 42 endogenous metabolites of amino acids and acylcarnitines in human blood was established mainly by liquid chromatography tandem mass spectrometry, which can simultaneously diagnose 37 kinds of amino acid, organic acid, and fatty acid metabolic diseases. The multivariate statistical analysis of the results was carried out by means of target metabolomics to study the influence of age and geographical factors on the content of amino acids and acylcarnitines, so as to control the delivery quality of peptide drugs and find possible differential metabolites [17].

3.1. Sample Collection. For newborns, blood collection should be carried out at 72 hours after birth and after 6 times of breastfeeding, and 1–1.5 hours after breastfeeding is the best time for blood collection. Sterilize the baby's heel with 75% alcohol, pierce the triangular needle or vertebral needle obliquely into the inner 0.5–0.8 cm, 2.5–3.0 mm deep from the outer edge of the heel, 1–2 cm away from the heel, discard the first drop of blood, when the blood drips naturally, touch the blood drop with 903 filter paper, so that the blood can be sucked in and penetrate the filter paper by itself, and three blood spots are collected at a time [18]. For adults, sample blood from the fingertip. After the blood samples were collected, they were naturally dried at room temperature for at least 5 hours, sealed in plastic bags, and stored in a refrigerator at 4°C.

3.2. Configuration of Working Fluid. The internal standard of amino acid and acylcarnitine isotope was dissolved in 1 mL of methanol extractant, and after shaking for 2 h, it was used as a stock solution and stored in a –20°C refrigerator. When using, the stock solution was diluted 200 times with methanol extractant as the working solution, and the working solution should be prepared on the same day [19].

3.3. Sample Preparation Method. The dry filter paper blood slices of the quality control and samples were punched into circular slices with a diameter of 3 mm with a hole punch and placed in a flat-bottom 96-well plate, and 90 µL of methanol working solution containing amino acids and acylcarnitine internal standard was added to each well to cover the viscosity. The membrane was incubated at 700 r/min at a speed of 700 r/min and shaken for 30 min at a temperature of 30°C to extract amino acids and acylcarnitines from the blood slices. Add 50 µL of 3.0 mol/L n-butanol hydrochloride derivatization reagent to each well of a 96-well plate with a pointed bottom, cover with a heat-sealing film, heat-seal at 175°C for 8 s, and then incubate at 60°C for 30 min to make amino acids and acylcarnitines after being fully derivatized [20]. The heat-sealing film was lifted off, dried with nitrogen at 50°C, 75 µL of acetonitrile reconstituted solution was added, covered with aluminum foil, the speed was 600 r/min, and the sample was reconstituted at 27°C for 10 min, and then the sample was injected with an autosampler.

3.4. Liquid Phase Method. 90% acetonitrile was used as the mobile phase, the injection volume was 10 µL, and the flow rate was set to 0–0.16 min 0.15 mL/min, 0.16–1.20 min 0.01 mL/min, and 1.20–1.40 min 0.6 mL/min [21].

3.5. Mass Spectrometry. Electrospray positive ion detection mode was used, Ala, Leu, Met, Phe, Tyr, Val, and corresponding isotope internal standards were scanned by neutral loss; the mass-to-charge ratio of neutral loss group was 102.1 Da, cone voltage: 22 V, collision energy: 13 V. C4, C4-OH, C5, C5-OH, C5: 1, C6, C8, C8: 1, C10, C10: 1, C10: 2, C12, C12: 1, C14, C14-OH, C14: 1, C14:2, C16, C16-OH, C18, C18-OH, and C18:2; and the corresponding isotope internal standard was scanned by parent ion, product ion was 85 Da, cone voltage: 30 V, collision energy: 27 V. Gly, Orn, Arg, Cit, C0, C2, and the corresponding isotope internal standards were scanned by multiple reaction monitoring. The mass spectrometry parameters are shown in Table 1 [22].

3.6. Data Processing and Statistical Analysis. MassLynx software was used for data processing, and the contents of amino acids and acylcarnitine metabolites were calculated. The calculation formula is

$$C = \frac{m_1}{m_2} + \text{IS} + \text{RRF} + X, \quad (1)$$

TABLE 1: Multiple reaction monitoring scan mode mass spectrometry parameters.

	Parent (m/z)	Daughter (m/z)	Dwell (s)	Cone (V)	Collision (V)
Gly	132.1	76	0.05	20	8
Orn	189.2	70.1	0.05	21	16
Arg	231.2	70.1	0.05	24	20
Cit	232.2	113.1	0.05	22	19
C0	218.3	103	0.05	26	17
C2	260.3	85	0.05	28	21

where m_1 is the peak area of the detected component, m_2 is the peak area of the internal standard, IS is the concentration of the internal standard, RRF is the relative response factor, and X is a custom factor. Using SIMCA-P+ (version 12.0, Umetrics, Umea, Sweden) statistical analysis software, multivariate statistical analysis was carried out on the content of amino acids and acylcarnitine metabolites. The pattern recognition method included principal component analysis (PCA) and partial least squares-discriminate analysis (PLS-DA). The differential variables were screened by the VIP value (variable importance on projection) of the PLS-DA model variables, and the variables with $VIP > 1$ were selected as differential metabolites, which were further screened by combining the T test. When $P < 0.05$, there was a significant difference [23].

4. Result Analysis

When using tandem mass spectrometry to detect amino acids and acylcarnitines in blood slices, the samples are not separated by a chromatographic column, but a lower flow rate is selected to allow the samples to enter the mass spectrometer directly and slowly, using electrospray ionization. Select the appropriate liquid phase conditions to obtain the ideal mass spectral response [24]. Figure 2 shows the total ion current chromatogram of metabolites in normal human body, and the total running time of each sample does not exceed 2 min.

Most α -amino acids react with the derivatizing reagent *n*-butanol to generate the corresponding butyl esterification products, and the neutral fragments with a molecular weight of 102.1 Da are lost under the action of collision-induced cleavage. Therefore, the neutral loss scanning method is adopted. The scanning range is the same. The butyl esterification product formed by the reaction of acylcarnitine with *n*-butanol undergoes 1,4 rearrangement and α -cleavage under the action of collision-induced cleavage to lose 1-butene, fatty acid, and trimethylamine, resulting in a molecular weight of 85 Da. The fragment ion is suitable for the method of parent ion scanning, and Q3 only allows ions with a molecular weight of 85 Da to pass and detect. A small number of amino acids and acylcarnitines are more sensitive in the scanning mode of multiple reaction monitoring [25]. Therefore, in the experiment, we used a combination of neutral loss scanning, parent ion scanning, and multiple reaction monitoring scanning to detect amino acids and acylcarnitines. The experimental results showed that the detection method proposed in this paper achieved a 16.74%

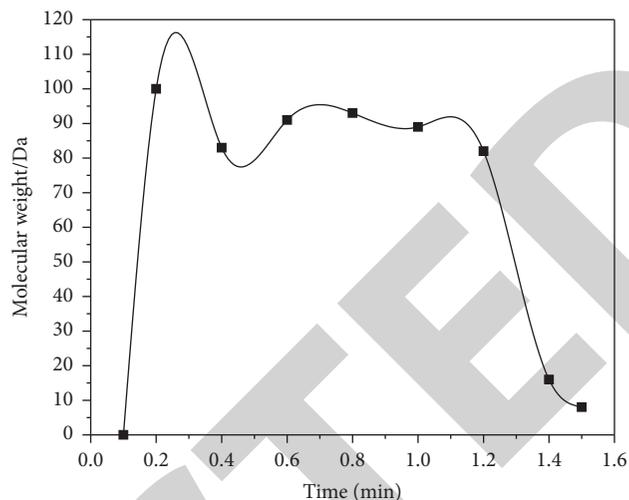


FIGURE 2: Total ion chromatogram of metabolites in normal humans.

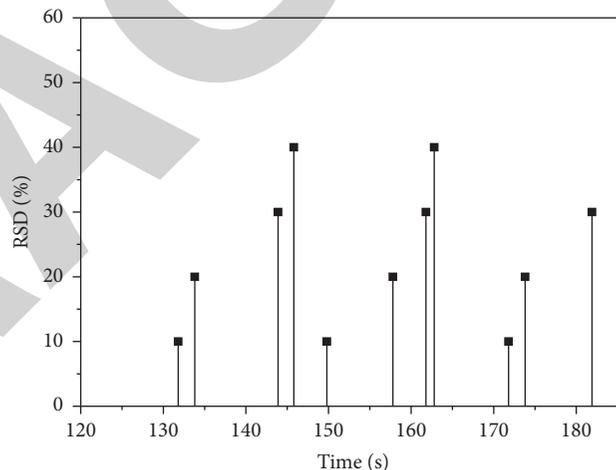


FIGURE 3: α amino acid neutral loss scan mass spectrum.

of the missing mass-to-charge ratio. Figure 3 is a mass spectrogram of metabolites in a normal human body.

In this experiment, the intraday and interday precision of the method was also investigated, and the RSDs of amino acids and acylcarnitines in the low- and high-concentration quality controls were calculated, respectively. The experimental results are shown in Table 2. The intraday and interday RSDs of the low-concentration quality control were 2.55%–20.58% and 7.42%–23.68%, respectively, and the intraday and interday RSDs of the high-concentration quality control were 1.31%–17.15% and 7.35%–22.01%, respectively (%). This method can fully meet the experimental needs in which the slightly higher RSD may be due to the different sampling locations of the quality control blood slices each time or because of the longer time interval between repeated measurements during the day.

According to different regions, people aged 22–35 can be divided into high-altitude group and nonaltitude group, and a PLS-DA model was established to determine the delivery

TABLE 2: Within-day ($n = 3$) and between-day precision ($n = 18$) of metabolites in quality control.

Metabolites	Intraday RSD (%)		Daytime RSD (%)	
	Low concentration quality control	High concentration quality control	Low concentration quality control	High concentration quality control
Gly	4.15	4.88	7.42	7.35
Cit	5.26	1.31	8.31	7.82
Ala	6.52	17.15	14.50	17.19
Val	10.98	14.24	14.09	22.01
Leu	5.58	3.88	11.80	8.48
Met	7.38	4.90	9.23	10.20
Phe	3.76	4.06	9.49	8.47
Tyr	8.97	7.72	10.83	10.57
C0	19.53	14.64	19.74	19.14
C2	11.70	6.66	13.22	12.62

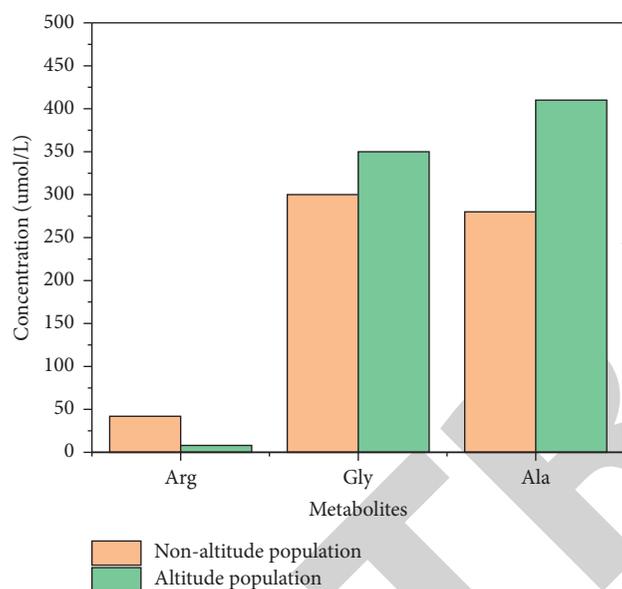


FIGURE 4: Reference amount of human peptide drug quality delivery in different regions.

quality of peptide drugs. The model $R2X = 0.758$, $R2Y = 0.893$, and $Q2 = 0.717$. There is a separation trend between the endogenous metabolite PLS-DA group in the 20–35-year-old high-altitude population and the non-altitude population, indicating that there are differences in the metabolite content in the blood of the two populations. Ala, Arg, Gly, Leu, Tyr, Orn, Val, and Phe are the main differential metabolites. Among them, Ala, Gly, Orn, Leu, and Val are higher in the plateau population, while Arg, Tyr, and Phe are in the nonaltitude population. In order to further verify the significant difference of the differential metabolites, combined with T test, the differential metabolites Arg, Ala, and Gly were found ($P < 0.05$). Figure 4 shows the reference amount of peptide drug quality delivery in different geographical populations based on the experimental results. The average Arg value detected in 9 high-altitude samples was $9.12 \mu\text{mol/L}$, and the average Arg value in 10 nonaltitude samples was $46.09 \mu\text{mol/L}$; in the corresponding two groups, the mean values of data Ala were

$432.26 \mu\text{mol/L}$ and $291.74 \mu\text{mol/L}$ and Gly contents were $365.39 \mu\text{mol/L}$ and $306.18 \mu\text{mol/L}$, respectively. The obvious decrease of Arg content in the population in the plateau area may be due to the increase in the utilization of Arg. Arg can not only be used for protein synthesis but also a substrate for NO synthase and Arg enzymes. The enhanced utilization of Arg leads to an increase in NO synthesis and a significant expansion of NO. Vascular effect and some studies have also shown that the exhaled NO level in the plateau population is higher than that in the nonaltitude population. The Arg enzyme converts Arg to Orn, which is consistent with the increase in Orn. The different contents of amino acids in different regions may be related to plateau hypoxia and dietary habits, which may provide some guidance for the evaluation of nutritional health status of plateau population.

5. Conclusion

This paper is mainly divided into two parts, the first part is the quantitative detection of amino acids and carnitine in human blood, and the second part is the quality control research of polypeptide drugs. First, a quantitative detection method for 42 amino acids and acylcarnitines in human blood was established, and 37 amino acids, organic acids, and fatty acids were screened simultaneously by liquid chromatography tandem mass spectrometry in 2 minutes, and the disease screening was expanded. Type: cthe screening of 205 samples of hereditary metabolic diseases was completed. Then, the data of 205 samples were analyzed and counted, and it was found that the contents of amino acids and acylcarnitines in the blood of different age groups were different, which provided guidance for further improving the screening of inherited metabolic diseases. The contents of some amino acids in different regions are also different, which will provide a basis for the quality of peptide drug delivery required by different regions. The method of electrofocused electrophoresis is rapid, accurate, and reproducible. By optimizing the experimental conditions, the detection method of related substances of exena skin is established, proving the feasibility of the method and checking the related substances of exena skin samples, to provide a new technical means for the determination of electric points and the inspection of related substances.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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