Current Science
CS 4 (4), 615– 624 (2024)
Received 26 May 2024 | **Revised** 28June 2024 | **Accepted** 25 July 2024 | **Online Available** 20 **August** 2024
https://doi.org/10.52845/CS/2024-4-4-4

Current Science

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OPEN ACCESS

ISSN(O)2795-8639

ORIGINAL ARTICLE

Qualitative and Quantitative Analysis of Beta-2 Agonists in Urine Samples by Ultra High Performance Liquid Chromatography-Tandem Mass Spectrometry

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Abstract:

Objective: Formoterol and salbutamol, β2-agonists, are listed in the Prohibited List of the World Anti-Doping Code International Standard, requiring both qualitative and quantitative analysis. To accurately determine the content of formoterol and salbutamol in urine, we developed an ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) method.

Methods: The samples were enzymatically hydrolyzed and extracted with organic solvents for liquid-liquid extraction, followed by purification and concentration. Qualitative and quantitative analyses were performed using tandem mass spectrometry with an electrospray ionization (ESI) source in multiple reaction monitoring mode. Results: The method demonstrated relative recovery rates between 90%-105%, matrix effects within 80-120%, and intra-day and inter-day precision below 15%.

Conclusion: This method is efficient, accurate, stable, and highly sensitive, fully meeting the detection requirements for the stimulants formoterol and salbutamol.

Keywords: β2-Agonists; UPLC-MS/MS; Formoterol; Salbutamol

Introduction

The term "doping" originated from "Dope," which initially referred to a concoction of complex ingredients. Athletes later consumed these concoctions to enhance their performance, leading "Dope" to acquire the meaning of "performance-enhancing drugs," and eventually, it became synonymous with "doping". The use of such substances is associated with considerable health risks, including but not limited to cardiovascular diseases, diabetes, cancer, and mental health issues [1]. Despite efforts by sports organizations and legislative bodies to implement various mechanisms to prevent the use of substances that improve performance and alter appearance, such as anabolic-androgenic steroids known for their ability to increase muscle mass and strength as well as enhance male characteristics, a significant portion of athletes are still driven by potential benefits to pursue victory at all costs [2,3].

β2-agonists constitute a significant category of doping agents, known for their bronchodilatory, anti-allergic, and anti-edema effects [4]. Clinically, they are mainly used to treat respiratory difficulties caused by obstructive pulmonary diseases, such as bronchial asthma, acute and chronic bronchitis, asthmatic bronchitis, and emphysema [5]. These drugs bind to β2-adrenergic receptors on airway target cell membranes, activating excitatory G proteins, which in turn activate adenylate cyclase. This enzyme catalyzes the conversion of ATP to cAMP within cells, increasing intracellular cAMP levels, thereby activating cAMP-dependent protein kinase (PKA). Through mechanisms such as the reduction of intracellular free calcium concentrations, inactivation of myosin light chain

kinase (MLCK), and opening of potassium channels, these actions ultimately lead to smooth muscle relaxation [6]. Additionally, β-agonist stimulation can also inhibit the release of inflammatory mediators from mast cells and neutrophils, enhance ciliary motion in the airways, promote airway secretion, reduce vascular permeability, and alleviate submucosal edema in the airways, all of which contribute to relieving or eliminating asthma symptoms [7]. In sports, athletes might use these prohibited drugs to improve personal respiratory efficiency and increase oxygen carrying capacity [8].

Previous studies have developed various conventional methods for the detection of β2-agonists, including immunoassay techniques [9] like enzyme-linked immunosorbent assay (ELISA), capillary electrophoresis [10], and gas chromatography-mass spectrometry (GC/MS) analysis based on derivatization [11,12]. However, these methods have been less used due to their poor sensitivity and cumbersome pretreatment processes. The advancement of mass spectrometry instruments has popularized liquid chromatography-tandem mass spectrometry (LC-MS/MS) technology, which has found applications in biology, chemistry, environment, materials, and other fields. LC-MS/MS, with its simple pretreatment and rapid, direct sampling advantages, has been increasingly applied in the detection of doping agents [13–15]. There have been numerous studies on the quantitative analysis of β2-agonists using LC-MS/MS, mainly involving β-glucuronidase enzyme hydrolysis and direct injection methods [16].

For formoterol, in 2014, Lee et al. developed a dilution direct injection method after enzymatic hydrolysis to detect formoterol in human urine, with a detection limit of 2 ng/mL [17]. Sardela et al. investigated the stability of formoterol under different pH conditions, finding good stability at physiological pH values of 5.2 and 7.0, while degradation was highest at pH 1.0 and 9.5 [18]. Therefore, it is recommended to keep the sample pH close to neutral during pretreatment. In 2013, Monica and colleagues developed a method to detect formoterol and its O-desmethyl metabolite, discovering that the metabolite could only be detected after inhaling high doses of formoterol [19].

Regarding salbutamol, in 2011, Kang Mi Lee and others developed an enzymatic method to detect salbutamol in urine, with a detection limit of 8 ng/mL [20]. In 2015, Ting Zhou et al. developed a method to determine the enantiomers of salbutamol in human plasma and urine, conducting the first study on the chiral transformation mechanism of R-salbutamol [21]. That same year, Ammari and team developed an LC-MS/MS detection method for the long-acting β2-agonist indacaterol using liquid-liquid extraction [22].

Given that formoterol and salbutamol are two β2-agonists explicitly required for qualitative and quantitative determination in the Prohibited List of the World Anti-Doping Code International Standard, we have established a more sensitive method that can simultaneously analyze and quantify these two substances. This method requires a small sample volume (2 μL), minimizes matrix interference, and has detection and quantification limits superior to those reported in current literature [17,18,23,24], offering a new detection method for the qualitative and quantitative analysis of these two β2-agonists in various sports competitions.

1. Instrument

The ultra-high performance liquid chromatography system (Agilent 1290 Infinity II, USA) is equipped with a vacuum degasser, column oven, and the 1290 Infinity II quaternary pump, among others. The triple quadrupole-linear ion trap mass spectrometer (QTRAP 6500+, Sciex, USA) features the Analyst® 1.7 data acquisition and processing workstation, SCIEX OS® 1.4 quantitative software, and an ESI electrospray ionization source. Other essential equipment includes the Eppendorf high-speed refrigerated centrifuge (Centrifuge 5804 R, Germany), Milli-Q ultrapure water system (Merck Millipore, USA), Thermo Fisher -80°C freezer (Forma 907, USA), Vortex-GENIE-2 vortex mixer (Scientific Industries), SB-5200DTD ultrasonic cleaner (Ningbo Xinzhi Biotechnology Co., Ltd.), a low-temperature constant temperature circulating bath (XT5201-D31-R40HG), and a nitrogen blower (Dri-Block DB-3D).

2. Materials and reagents

The standard substances formoterol (1ST1306A), formoterol-D6 (1ST1396-100M), salbutamol

(1ST1310X), and salbutamol-D3 (1ST1353) were all acquired from Tianjin Alta Scientific Co., Ltd. Methanol (MeOH) and acetonitrile (ACN) were purchased from MERCK (Darmstadt, Germany). LC-MS grade formic acid (FA) was obtained from SIGMA (Deisenhofen, Germany), and LC-MS grade ammonium formate (AF) from ROE SCIENTIFIC INC (Newark, USA). Water (H2O) was produced using a Milli-Q ultrapure water system (Merck Millipore, USA). Sodium dihydrogen phosphate and disodium hydrogen phosphate (analytical grade, AR) were purchased from Sinopharm Chemical Reagent Co., Ltd. β-Glucuronidase (G7396-2MU) was acquired from Sigma-Aldrich.

3. Experimental method

3.1 Preparation of standard solution

To prepare the stock solutions, formoterol, formoterol-D6, salbutamol, and salbutamol-D3 are precisely weighed and dissolved in methanol to make the respective stock solutions. Then, 1 mL of formoterol and 1 mL of salbutamol stock solutions are accurately pipetted into the same 25 mL volumetric flask. Methanol is added to dilute the mixture to the mark, creating a mixed stock solution with mass concentrations of 100 μg/mL for formoterol and 4 μg/mL for salbutamol. This mixed stock solution can be further diluted in a stepwise fashion to obtain a series of mixed working solutions for use.

3.2 Chromatographic conditions

Chromatographic column: Waters BEH C18 column (1.7 μm, 2.1 mm × 100 mm); Column temperature: 35°C; Flow rate: 0.3 mL/min; Mobile phase: Mobile phase A (water with 10 mM ammonium formate and 0.1% formic acid) and Mobile phase B (acetonitrile), with a gradient elution program as follows: 0-1 min, 5% B; from 5% B to 60% B over 1-6 min; 90% B from 6.5 to 7.5 min; from 90% B back to 5% B over 7.5-7.6 min; hold at 5% B from 7.6 to 10 min; Injection volume: 2 μL.

3.3 Mass spectrum conditions

Using electrospray ionization in positive mode (ESI+), and multiple reaction monitoring (MRM) mode, the ion source temperature is set at 550°C with an ion spray voltage of 5500 V. The nebulizer gas is set at 55 psi, auxiliary heating gas at 55 psi, and curtain gas at 35 psi. The specific mass spectrometry parameters for the compounds can be found in Table 1. Under these chromatographic and mass spectrometric conditions, the total ion chromatogram (TIC) of the LC-MS/MS is shown in Figure 1. Furthermore, the secondary fragment mass spectra of formoterol, salbutamol, and their respective internal standards, as illustrated in Figures 2 and 3, can serve as references for qualitative analysis.

Table 1 MS/MS parameters for compounds

Compound	Precursor ion(m/z)	Product ion (m/z)	CE/eV	DP/eV
formoterol	345.1	149.1*	26	60
	345.1	121.1	38	60
formoterol-D6	351.3	155.2*	27	60
	351.3	123.3	38	60
salbutamol	240.0	148.1*	24	40
	240.0	166.1	18	40
salbutamol-D3	243.1	151.2*	25	40
	243.1	169.2	18	40

note： *quantitative ion

3.4 Sample pretreatment

Take 1 mL of urine, add 10 μL of internal standard (formoterol-D6 and salbutamol-D3 at mass concentrations of 100 μg/mL and 4 μg/mL, respectively), add 500 μL of PBS (phosphate-buffered saline) and 50 μL of β-glucuronidase. Vortex to mix thoroughly, incubate in a 55°C water bath for 1 hour, then cool to room temperature. Add 3 mL of ethyl acetate, vortex vigorously for 2 minutes twice, and let stand for 10 minutes. Centrifuge (5000 rpm, 5 minutes, 4°C) to separate the layers, then freeze the upper

ethyl acetate layer at -30°C in a low-temperature constant temperature circulating bath for ten minutes. Dry the upper layer under nitrogen, redissolve in 1 mL of the initial mobile phase, and after centrifugation, dilute 20 times with a solvent in the same ratio as the initial mobile phase. Centrifuge again and inject the supernatant for analysis. The method development for this study utilized urine samples from a total of 6 different individuals, with sample collection starting on April 2, 2024.

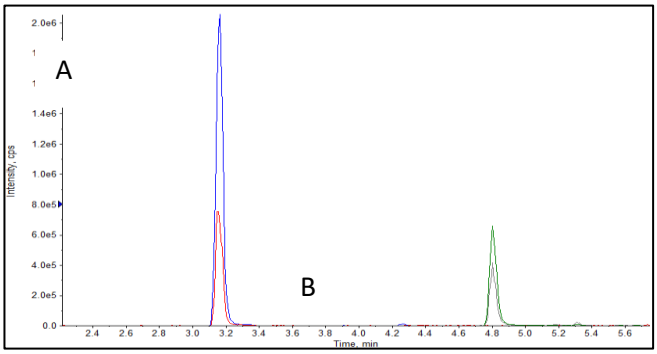

Figure 1 MRM chromatograms of salbutamol (A) and formoterol (B)

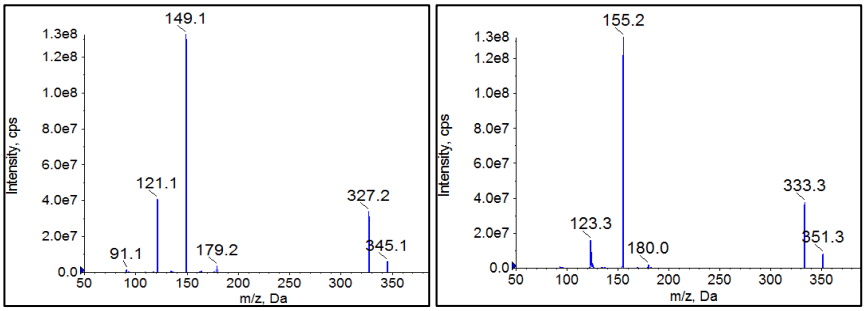

Figure 2 MS2 spectraof formoterol (left) and formoterol-D6 (right)

4. Methodological verification

4.1 Selectivity

The selectivity, also known as specificity, of a method evaluates the interference of target analytes in blank samples under certain pretreatment and detection conditions. By comparing the detection situations between blank urine samples and spiked urine samples, including the appearance of target chromatographic peaks, retention times, and ion ratios, the interference on the analytes can be assessed. Figures 4 and 5 show that in 20 actual blank samples, no chromatographic peaks for the analytes and internal standards were observed at the expected retention times, indicating no interference from endogenous substances in the blank urine. Conversely, the corresponding analyte peaks were detected in the spiked urine samples, indicating that the pretreatment does not cause false-negative results. This demonstrates that the method's selectivity meets the detection requirements.

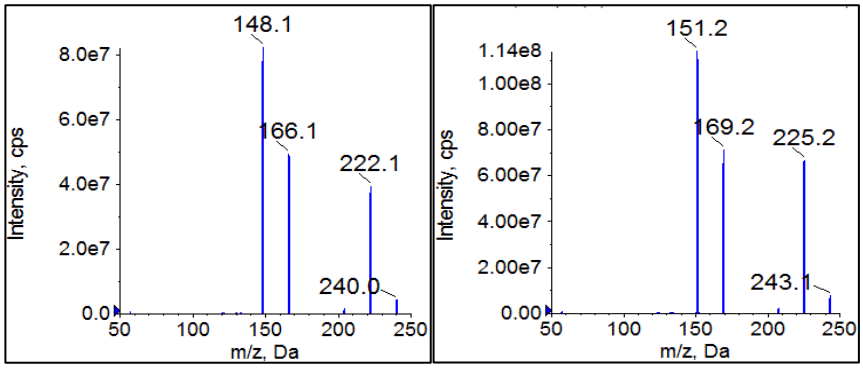

Figure 3 MS2 spectra of salbutamol (left) and salbutamol-D3 (right)

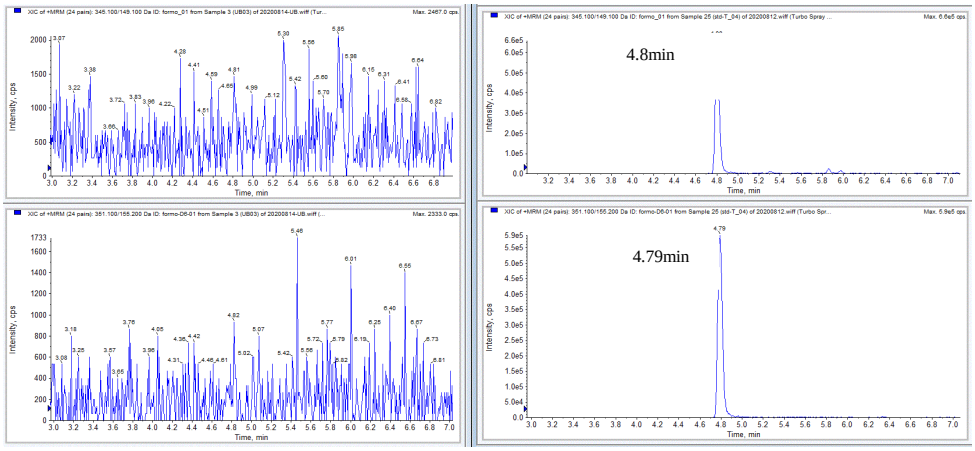

Figure 4 Chromatogramsof formoterol(up) and formoterol-D6 (down) in blank urine samples（n=20）and labeled urine samples

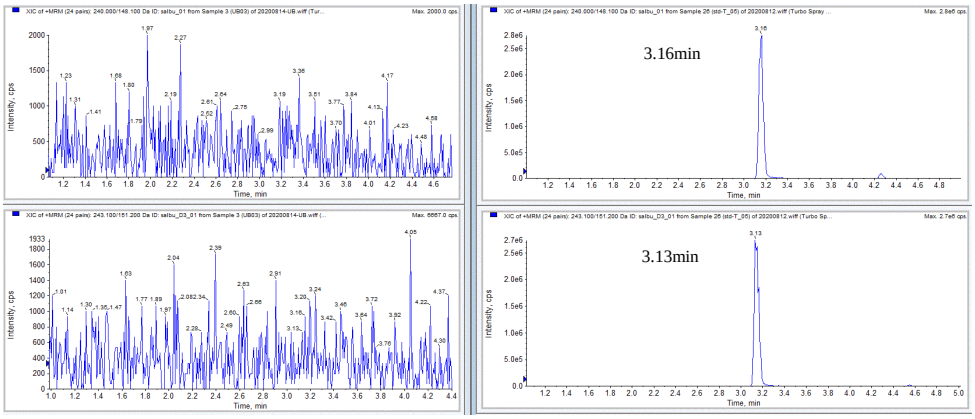

Figure 5 Chromatogramsof salbutamol (up) and salbutamol-D3 (down) in blank urine samples（n=20）and labeled urine samples

4.2 Linearity and LOD, LOQ

A series of mixed working solutions were prepared in varying amounts and added to urine to create six different concentration gradients of spiked samples (formoterol concentrations of 5, 10, 20, 40, 60, 80 ng/mL; salbutamol concentrations of 0.125, 0.25, 0.5, 1, 1.5, 2 μg/mL). Following the pretreatment conditions described in section "3.4," and the detection conditions outlined in sections "3.2" and "3.3," the standard curves were plotted with the ratio of the target substance to the internal standard's quantification ion chromatographic peak area as the ordinate (Y) and the mass concentration as the abscissa (X) (μg/mL or ng/mL), obtaining the regression equation and correlation coefficient. The experimentally determined correlation coefficient squared values were all greater than 0.999, fully meeting routine detection requirements.

The detection limit (LOD) and quantification limit (LOQ) were determined by preparing dilutions corresponding to signal-to-noise ratios (S/N) of 3 and 10, respectively. The LOD for formoterol was found to be 0.4 ng/mL and the LOQ was 0.8 ng/mL; for salbutamol, the LOD was 1 ng/mL and the LOQ was 2.5 ng/mL. Peak area acquisition and data analysis were performed using SCIEX OS software, with relevant results presented in Table 2.

Table 2 Linear range and limit of detection (LOD), limit of quantitation (LOQ) for compounds

Compound	Linearity	r^2	Linear Range	LOD (ng/ml)	LOQ (ng/ml)
Formoterol	y=0.02581x-0.00733	0.9993	5-80 (ng/mL)	0.4	0.8
Salbutamol	y=1.01609x-0.02179	0.9992	0.125-2 (μg/mL)	1	2.5

4.3 Precision and accuracy

Spiked urine samples were prepared at low (10 ng/mL), medium (40 ng/mL), and high (60 ng/mL) concentrations for formoterol, and low (0.25 μg/mL), medium (1 μg/mL), and high (1.5 μg/mL) concentrations for salbutamol to serve as Quality Control (QC) samples. Six replicates of each concentration were prepared for both pretreatment and analysis, with intra-day and inter-day precision calculated. Intra-day precision, also known as repeatability, and inter-day precision, showed that the relative standard deviation (RSD) for both formoterol and salbutamol at different mass concentrations was less than 10%. The accuracy for different concentration QCs ranged from 90.18% to 102.46%, as shown in Table 3, meeting the requirements for methodological validation.

Table 3 Intra-day and inter-day precision and accuracy of compounds

Compound	Conc.(ng /mL)	intra-day precision		inter-day precision	
		Accuracy (Mean)	RSD (%)	Accuracy (Mean)	RSD (%)
Formoterol	10	94.03	7.83	90.18	8.13
	40	92.93	4.18	93.58	5.36
	60	94.48	3.43	96.14	6.44
Salbutamol	0.25	94.85	4.57	97.81	6.45
	1	100.29	5.24	102.46	6.02
	1.5	102.37	6.34	101.42	6.70

Table 4 Recovery and matrix effect of compounds

Compound	Conc.(ng/mL)	Recovery (mean±SD,%)	Matrix effect (mean±SD,%)
Formoterol	10	98.35±6.92	95.61±5.45
	40	96.24±2.72	96.25±4.85
	60	98.60±4.10	99.98±6.55
Salbutamol	0.25	97.70±7.27	97.66±6.07
	1	96.25±11.09	94.84±10.80
	1.5	92.59±9.44	106.35±4.45

Table 5 Relative deviation of quantitative concentration of compound

Compound	Relative deviation (mean , %)	SD%
Formoterol	5.06	3.11
Salbutamol	5.73	3.37

Table 6 Maximum Tolerance Windows for Relative Abundances to ensure appropriate confidence in identification

Ion Ratio (%)	Ion ratio acceptance (%)	Ion Ratio* (%)	Acceptable window* (%)
50-100	±10（Absolute）	60	50-70
25-50	±20（Relative）	40	32-48
1-25	±5（Absolute）	10	5-15

Note: * For example

Table 7 Maximum acceptable range of ion ratio for formoterol and salbutamol

Compound	Product ion	Ion Ratio(Mean, %)
Formoterol	149.1*	100%

Salbutamol	121.2	57.23
	148.1*	100%
	166.1	41.04

Note： * Quantitative ion

4.4 Recovery and matrix effects

Blank urine samples were taken and spiked at low (10 ng/mL), medium (40 ng/mL), and high (60 ng/mL) concentrations for formoterol, and low (0.25 μg/mL), medium (1 μg/mL), and high (1.5 μg/mL) concentrations for salbutamol. For each group, six replicate urine samples were prepared and pretreated according to the conditions described in section "2.4," followed by sample analysis to measure the response of the target analytes relative to the internal standard.

Additionally, three sets of blank urine samples were processed, and then spiked with corresponding concentrations of formoterol, salbutamol, and internal standards post-treatment to measure the response of the target analytes relative to the internal standard. The relative recovery rates were calculated using the response value ratios obtained from these two batches for each concentration. The final determined recovery rates were all above 90%, as shown in Table 4, meeting the methodological validation requirements.

Lastly, three sets of blank water samples were processed and then spiked with corresponding concentrations of formoterol and salbutamol, as well as internal standards post-treatment, to measure the response of the target analytes relative to the internal standard. The data from these last two batches were used to calculate the matrix effect, which was found to be between 90%-110%, as shown in Table 4. This also meets the requirements for methodological validation.

4.5 Bias

Deviation refers to the difference between the measured value and the true value or the mean of measured values. The World Anti-Doping Agency (WADA) technical document requires the calculation of quantitative deviation for doping substances near their threshold concentrations when testing substances with a threshold. The relative deviation between the concentrations determined by standard curve fitting and the actual concentrations for formoterol (40 ng/mL) and salbutamol (1 μg/mL) in ten samples at threshold concentrations was calculated after processing. The relative deviations were all less than 10%, and the standard deviation (SD) values of the ten samples were less than 15%, as shown in Table 5, meeting the requirements for methodological validation.

4.6 Carryover

To assess the carryover of formoterol and salbutamol in the analytical system, blank urine samples were injected immediately after the highest concentration point of the calibration curve. In this detection method, when checking for formoterol and salbutamol in blank urine samples immediately after the highest concentration point, no residual chromatographic peaks were observed at their respective chromatographic retention times. Therefore, when applying this detection method for quantification, there will be no false positives caused by residual interference, meeting the methodological validation requirements.

4.7 Ion Ratio

Under the same liquid chromatography-mass spectrometry (LC-MS) conditions, the fragmentation patterns of a given substance should remain consistent, which allows the ratio of the response values between the qualitative and quantitative ions, known as the ion ratio, to be used as a means of verification. Variations in the ion ratio can indicate whether a substance is being interfered with, leading to false positives and thus enhancing the qualitative accuracy for the detection of formoterol and salbutamol. The international requirements for ion ratios are detailed in Table 6.

In this detection method, the ion ratios for formoterol and salbutamol standards were studied, and the acceptable maximum ranges were established. These ion ratio standards were set to improve the qualitative accuracy for these two β2-agonists, as shown in Table 7. Establishing these ion ratio standards is crucial for confirming the presence of formoterol and salbutamol in samples,

thus ensuring the reliability of the detection method and meeting the qualitative requirements.

Discussion

Urine samples are widely used in doping control due to their rapid, non-invasive collection, and easy transportation. Testing for doping agents in athletes using urine samples is the most common and broadly applied method. Compared to GC-MS (Gas Chromatography-Mass Spectrometry), LC-MS (Liquid Chromatography-Mass Spectrometry) offers simpler sample pre-treatment without the need for derivatization, higher sensitivity, and a broader range of detectable substances. Formoterol and salbutamol, as two β2-agonists that the World Anti-Doping Agency (WADA) explicitly requires to be quantified, are substances of significant interest for monitoring.

In optimizing the pre-treatment process for this study, the main focus was on examining the water bath process and the choice of extraction reagent. Due to the unavailability of glucuronide metabolites of the analytes for testing, the study relied on literature that suggested a 55°C water bath for one hour as suitable conditions for the enzymatic hydrolysis process. Enzymatic hydrolysis efficiency shows a certain correlation within a specific temperature range and hydrolysis duration. In our experiments, it was observed that if the hydrolysis temperature exceeded 60°C or the duration surpassed two hours, the response values of the non-conjugated analytes would decrease, indicating that excessive temperature or prolonged time might lead to degradation of the non-conjugated analytes.

Regarding the choice of extraction solvent, the study primarily optimized and compared the extraction efficiency of tert-butyl methyl ether and ethyl acetate under the same conditions. The results indicated that ethyl acetate yielded higher response values for the analytes, suggesting better extraction efficiency with ethyl acetate.

The liquid chromatography-mass spectrometry (LC-MS) method developed in this study for the detection of formoterol and salbutamol exhibits high sensitivity and strong selectivity, with a minimum detection limit reaching 1 ng/mL and below. The method satisfies routine testing requirements through evaluations of analyte linearity and correlation coefficients, minimum detection limits and quantification limits, and residual analysis. Both inter-day and intra-day precisions are less than 15%, the relative recovery rates are above 80%, and the matrix effects are within the range of 80%-120%, aligning with the general requirements for method validation. This established method provides technical support and a reserve for the detection of β2-agonists formoterol and salbutamol in athletes.

Conclusion

This study developed and validated a method for simultaneously detecting formoterol and salbutamol in urine samples using LC-MS/MS, combined with enzymatic hydrolysis and liquid-liquid extraction as the pre-treatment approach. This method is proven to be effective and feasible for the detection and determination of the β2-agonists formoterol and salbutamol in athletes during sporting events.

Funding Declaration

This study was supported by the Youth Research Fund Project of Shanghai University of Sport (2023STD021).

Competing Interest declaration

No potential conflict of interest was reported by the authors.

References

1. Bird S R, Goebel C, Burke L M, Greaves R F. Doping in sport and exercise: anabolic, ergogenic, health and clinical issues[J]. Annals of clinical biochemistry, 2016, 53(Pt 2): 196-221.
2. Vorona E, Nieschlag E. Adverse effects of doping with anabolic androgenic steroids in competi tive athletics, recreational sports and bodybuilding[J]. Minerva endocrinologica, 20 18, 43(4): 476-488.
3. Birzniece V. Doping in sport: effects, harm and misconceptions[J]. Intern Med J, 2015, 45 (3): 239-48.
4. Billington C K, Penn R B, Hall I P. β2 Agonists[J]. Handbook of experimental pharmacology, 2017, 237: 23-40.
5. Johnson M. Molecular mechanisms of beta(2)-adrenergic receptor function, response , and regulation[J]. The Journal of allergy and clinical immunology, 2006, 117(1): 18-24; quiz 25.
6. Monaco T J, Hanania N A. Emerging inhaled

long-acting beta-2 adrenoceptor agonists for the trea tment of COPD[J]. Expert opinion on emerging drugs, 2017, 22(3): 285-299.
7. Norris S L, Yen P Y, Dana T L, Care B R, Burda B U: Drug Class Reviews, Drug Class Review: Beta(2)-Agonists: Final Report, Portland (OR): Oregon Health & Science University
8. Hostrup M, Jacobson G A, Jessen S, Lemminger A K. Anabolic and lipolytic actions of beta(2) -agonists in humans and antidoping challenges[J]. Drug Test Anal, 20 20, 12(5): 597-609.
9. Wada. Technical Document For The Decision Limits For The Confirmatory Quantification Of Threshold Substances: World Anti-Doping Agency., 2019.
10. Neubert H, Olah T, Lee A.2018 White Paper on Recent Issues in Bioanalysis: focus on immunogenicity assays by hybrid LBA/LCMS and regulatory feedback (Part 2 - PK, PD & ADA assays by hybrid LBA/LCMS & regulatory agencies' inputs on bioanalysis, biomarkers and immunogenicity)[J]. Bioanalysis, 2018, 10(23): 1897-1917.
11. Ventura R, González G, Smeyers M T, De La Torre R, Segura J. Screening procedure for beta-adrenergic drugs in sports drug testing by immunological methods[J]. J Anal Toxicol, 19 98, 22(2): 127-34.
12. Chen Z, Cai Y, Zhang L, Zhang L. Capillary electrochromatography of three β2-agonists in human urine using a lauryl methacrylate-based monolithic column[J]. J Sep Sci, 2012, 35(9): 1138-45.
13. Ahrens B D, Kucherova Y, Butch A W. Detection of Stimulants and Narcotics by Liquid Chromatography-Tandem Mass Spectrometry and Gas Chromatography-Mass Spectrometry for Sports Doping Control[J]. Methods in molecular biology (Clifton, N.J.), 2016, 1383: 247-63.
14. Polet M, Van Gansbeke W, Van Eenoo P. Development and validation of an open screening method for doping substances in urine by gas chromatography quadrupole time-of-flight mass spectrometry[J]. Anal Chim Acta, 2018, 1042: 52-59.
15. Cuervo D, Loli C, Fernández-Álvarez M, Muñoz G, Carreras D. Determination of doping peptides via solid-phase microelution and accurate-mass quadrupole time-of-flight LC-MS[J]. J Chromatogr B Analyt Technol Biomed Life Sci, 2017, 1065-1066: 134-144.
16. Elmongy H, Masquelier M, Ericsson M. Development and validation of a UHPLC-HRMS method for the simultaneous determination of the endogenous anabolic androgenic steroids in human serum[J]. J Chromatogr A, 2020, 1613: 460686.
17. Fragkaki A G, Angelis Y S, Kiousi P, Georgakopoulos C G, Lyris E. Comparison of sulfo-conjugated and gluco-conjugated urinary metabolites for detection of methenolone misuse in doping control by LC-HRMS, GC-MS and GC-HRMS[J]. J Mass Spectrom, 2015, 50(5): 740-8.
18. Handelsman D J, Bermon S. Detection of testosterone doping in female athletes[J]. Drug Test Anal, 2019, 11(10): 1566-1571.
19. Khamis M M, Adamko D J, El-Aneed A. Mass spectrometric based approaches in urine metabolomics and biomarker discovery[J]. Mass Spectrom Rev, 2017, 36(2): 115-134.
20. Mazzarino M, De La Torre X, Fiacco I, Pompei C, Calabrese F, Botrè F. A simplified procedure for the analysis of formoterol in human urine by liquid chromatography-electrospray tandem mass spectrometry: application to the characterization of the metabolic profile and stability of formoterol in urine[J]. J Chromatogr B Analyt Technol Biomed Life Sci, 2013, 931: 75-83.
21. Deventer K, Pozo O J, Delbeke F T, Van Eenoo P. Quantitative detection of inhaled formoterol in human urine and relevance to doping control analysis[J]. Drug Test Anal, 2012, 4(6): 449-54.
22. Lee K M, Kim H J, Son J, Park J H, Kwon O S, Lee J. Simple quantitation of formoterol and 11-nor-Δ(9)-tetrahydrocannabinol-9-carboxylic acid in human urine by liquid chromatography-tandem mass spectrometry in doping control[J]. J Chromatogr B Analyt Technol Biomed Life Sci, 2014, 967: 8-12.
23. Sardela V F, Deventer K, Pereira H M, De Aquino Neto F R, Van Eenoo P. Development and validation of a ultra high performance liquid chromatography-tandem mass spectrometric method for the direct detection of formoterol in human urine[J]. J Pharm Biomed Anal, 2012, 70: 471-5.

24. Lee K M, Kim H J, Jeong E S, Yoo H H, Kwon O S, Jin C, Kim D H, Lee J. Simple and accurate quantitative analysis of seven prohibited threshold substances in human urine by liquid chromatography/tandem mass spectrometry in doping control[J]. Rapid Commun Mass Spectrom, 2011,25(16):2261-7.
25. Zhou T, Zeng J, Liu S, Zhao T, Wu J, Lai W, He M, Xu B, Qu S, Xu L, Tan W. Study on the determination and chiral inversion of R-salbutamol in human plasma and urine by liquid chromatography-tandem mass spectrometry[J]. J Chromatogr B Analyt Technol Biomed Life Sci, 2015, 1002: 218-27.
26. Ammari W G, Al-Qadhi Z, Khalil M, Tayyem R, Qammaz S, Oriquat G, Basheti I A, Chrystyn H. Indacaterol determination in human urine: validation of a liquid-liquid extraction and liquid chromatography-tandem mass spectrometry analytical method[J]. J Aerosol Med Pulm Drug Deliv, 2015, 28(3): 202-10.
27. Suo D C, Zhao G L, Wang P L, Su X O. Simultaneous determination of β-agonists and psychiatric drugs in feeds by LC-MS-MS[J]. J Chromatogr Sci, 2014, 52(7): 604-8.
28. Wang L, Pu R C, Wang X X, Luo C Y, Zhang L C, Zhang X S. Multiresidue Determination of β2-Agonists Including Phenylethanolamine A in Animal-Derived Food by Ultra-High Performance Liquid Chromatography/Tandem Mass Spectrometry[J]. J Chromatogr Sci, 2015, 53(6): 925-31.
29. Tsikas D, Zoerner A A. Analysis of eicosanoids by LC-MS/MS and GC-MS/MS: a historical retrospect and a discussion[J]. J Chromatogr B Analyt Technol Biomed Life. Sci, 2014, 964: 79-88.