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A fast quantification of Salbutamol using automated SPE and Liquid chromatography mass spectrometry

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Introduction

Salbutamol is one of the most common adverse analytical findings at the Doping Control Laboratory of Madrid. In the year 2005, salbutamol was present in 16% of the 6700 total samples analysed. This drug is frequently administered to people that suffer from asthma, but very high doses could have anabolic effects. According to The List of Prohibited Substances (WADA), salbutamol has to be considered as a threshold substance, and a urinary concentration above 1000 ng/ml must be reported as a doping violation as an anabolic agent[1]. On the other hand, WADA in the Technical Document TD2003MRPL indicates that a concentration of salbutamol higher than 100 ng/ml should be reported as a doping violation as a stimulant agent. Therefore there are two threshold concentrations at which accurate and precise salbutamol quantification is mandatory.

The goal of this study has been to develop a quick and robust method for the quantification of salbutamol.

Experimentation

The procedure is based on a solid phase extraction followed by a LC-MS/MS analysis.

Certified reference standards

Salbutamol from British Pharmacopoeia (London, UK), Atenolol from United States Pharmacopeia (USA), β-Glucuronidase E. Coli K 12 from Roche Diagnostics (Manheim, Germany)

Instrumentation and consumables

Samples were extracted with an automatic sample preparative station (Gilson Aspec XL4) and analysed with a LC-MS/MS system, API3000 (Applied Biosystems), equipped with a

TurboIonspray (TIS). OASIS® HLB 1cc extraction cartridges (30mg sorbing mass) from Waters (Ireland) were used.

Table 1 summarises the main LC-MS/MS parameters.

Table 1: Experimental conditions for the LC/MS/MS system.

HPLC CONDITIONS							
Column:	XTerra (Waters) C18 3.5 µm 2.1 x 150mm						
Flow rate:	250 µl/min						
Volume inj:	5 µl						
Solvents:	A: 10mM $NH_4CH_3CO_2$ solution, B: Acetonitrile						
Gradient:	0-1min 35% B; 2-5min 90% B; 5.5-10min 35% B						
MS CONDITIONS							
Source Parameters							
Spray voltage: (+) 4.5 Kv. *TurbolonSpray Temp:* 500°C. *Nebulizer Gas:* 12 *Curtain Gas:* 12							
Method acquisition							
Compound	***Transition***	***Dwell T.***	**DP(Declustering Potencil)**	**FP (Focusing Potencial)**	**CE (Collision Energy)**	**CXP (Collision Cell Exit Potencial)**	**Ret. time**
Salbutamol	240.3/103.1	150	(+)6.0	(+)250.0	(+)51.0	(+)6.0	1.74
	240.3/222.0	150			(+)15.0	(+)12.0	
	240.3/165.8	150			(+)19.0	(+)10.0	
(ISTD)	267.3/74.15	150	(+)46.0	(+)320.0	(+)35.0	(+)4.0	1.67

Extraction procedure

To each 2.5ml aliquot of sample 100µl of pH 7 phosphate buffer, 25µl of E.Coli β-glucuronidase[2] and 25µl of internal standard, atenolol 10µg/mL, were added. Then, each tube was heated for 1 hour at 55°C, and once the hydrolysis was finished the aliquots were centrifuged for 5 minutes at 2500 rpm. The cartridges were conditioned with 500µl MeOH and 500µl water both at a 6 ml/min flow. Salbutamol was loaded by passing 2ml of the urine at a 1 ml/min flow. Next, cartridges were washed with 1 ml of water at a 6 ml/min flow. Finally, a double elution was carried out by passing through 100µl of the mixture methanol/acetonitrile (30:70) at a 2mL/min flow.

130µL of buffer acetate ammonium 10mM were added to each tube which was then directly analysed by LC-MS/MS.

Results and discussion

A total number of 15 calibration curves were prepared. Each curve includes six calibration levels: 50ng/mL, 400ng/mL, 800ng/mL, 1200ng/mL, 1600ng/mL and 2000ng/mL[3]. Figure 1 shows the reconstructed ion chromatograms corresponding to the transition 240.3/103.1 for salbutamol and the ISTD from each calibration sample.

Figure 2 shows the 15 calibration curves obtained during the experimentation process. Only a 3.8 % coefficient of variation was obtained for the slope of the curves[4]. Figure 3

presents the variation of the slope, the average value of the slope and the range of acceptance for the slope. A good characterised normal distribution was obtained for the slope values.

Due to the stability of the calibration curve, an average curve was calculated and used to estimate the salbutamol concentration in the samples for more than two months. Therefore whenever a sample needs to be quantified, only the sample and a quality control have to be prepared. In order to validate the calibration curve, two quality control samples at threshold concentration level (100 ng/ml and 1000 ng/ml) were analysed[3]. A total number of 25 Quality Control (QC) samples for each level were analysed during this time period. Figures 4 and 5 show the variability obtained.

The data present a normal distribution with a mean equal to 982 and a relative standard deviation of 4% for the QC at 1000ng/mL and a mean equal to 98 and a relative standard deviation of 3% for the QC at 100ng/ml, at the 90% or higher confidence level.

One case (#10) present in figure 5 was excluded in order to calculate the previous results[4].

Conclusion

The great stability of the calibration curves obtained with the quantification method developed makes it possible, from the validation data, to calculate an average equation curve (average slope and intercept), and to use it for long time periods.

The method makes possible a high throughput of samples for quantifying salbutamol in a wide range (50-2000ng/mL), with high accuracy (better than 5%) and precision (RSD under 5%)

References

(1) Discrimination of prohibited oral use of salbutamol from Authorised inhaled asthma treatment. R. Berges, J. Segura, R. Ventura. Clinical Chemistry 46:9 1365-1375 (2000)

(2) Preliminary results of excretion studies with salbutamol. Cologne Workshop in Doping Analysis 6, 1998.

(3) Preparation of Calibration Curves. September 2003. LGC/VAM/2003/032.

(4) Statistical Techniques for Data Analysis. John Keenan Taylor. Lewis Publishers, Inc.

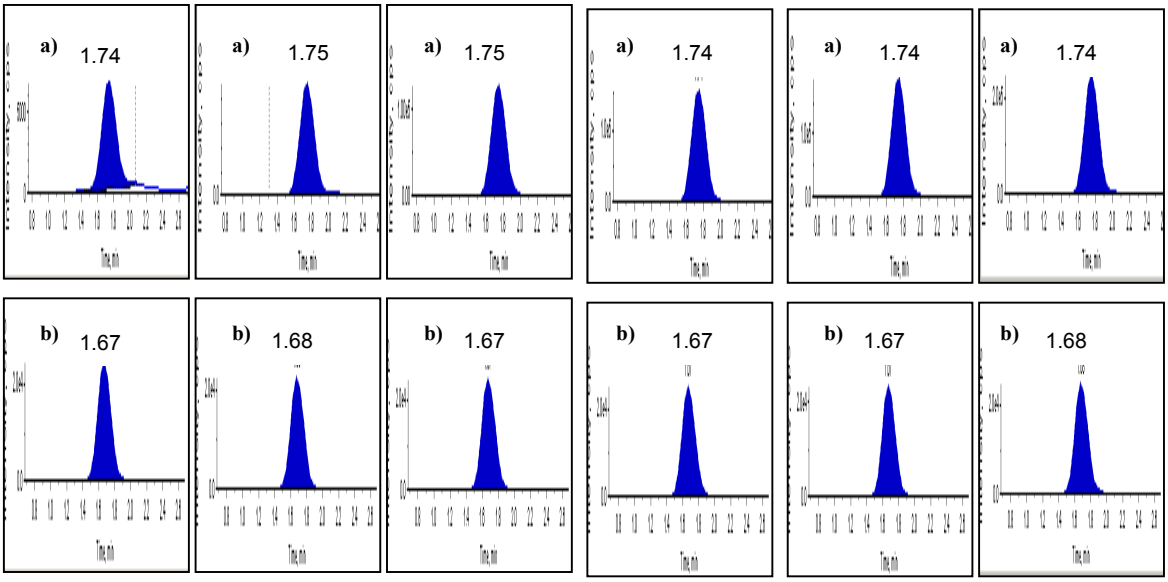

Figure 1: Chromatograms of the transition 240.3/103.1 for salbutamol (a) and ISTD (b) in a calibration curve with the concentrations: 50ng/mL, 400ng/mL, 800ng/mL, 1200ng/mL, 1600ng/mL, 2000ng/mL

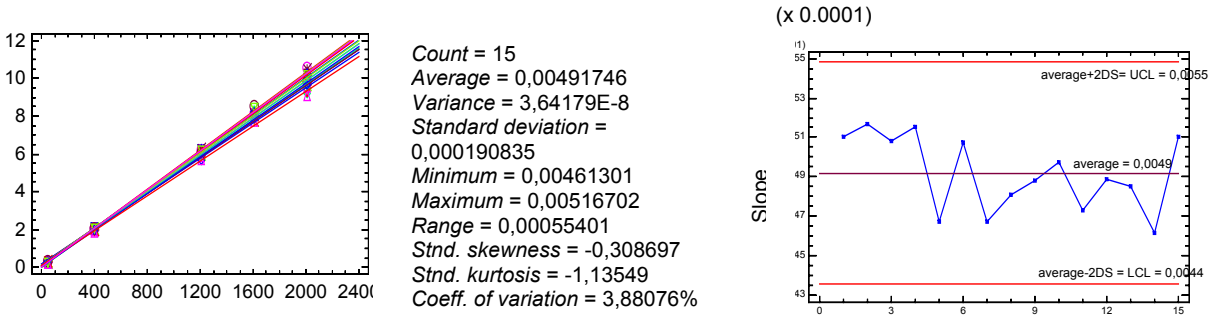

Figure 2 y 3: 15 Calibration curves and the variability of their slopes obtained during the validation process.

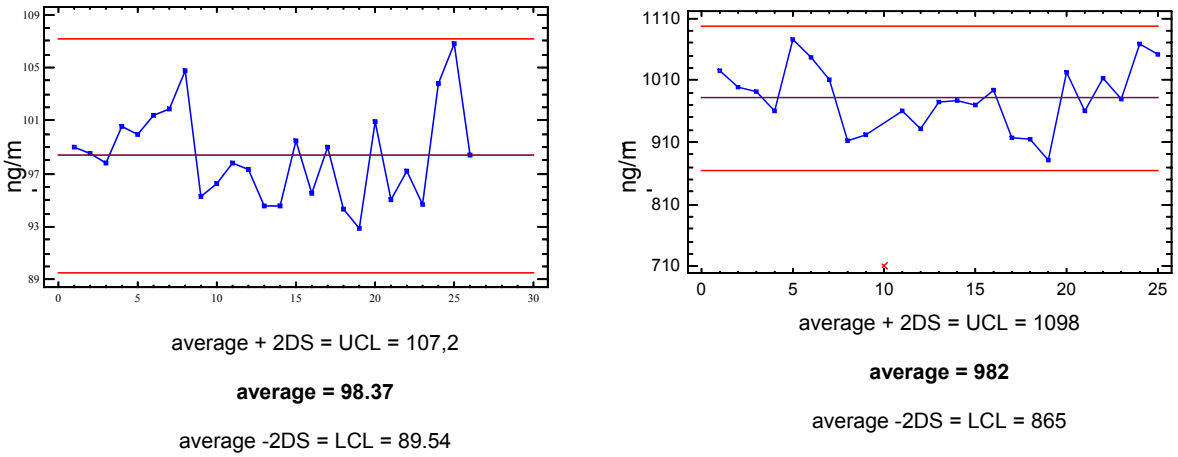

Figure 4 y 5: Quantification of 25 QC samples with a salbutamol concentration of 100ng/mL (left) and quantification of 25 QC samples with a salbutamol concentration of 1000ng/mL.(right)