Reprint from

RECENT ADVANCES IN DOPING ANALYSIS (4)

W. Schänzer
H. Geyer
A. Gotzmann
U. Mareck-Engelke
(Editors)

Sport und Buch Strauß, Köln, 1997

S. ZHU, S. YANG:
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In: W. Schänzer, H. Geyer, A. Gotzmann, U. Mareck-Engelke (eds.) Recent advances in doping analysis (4). Sport und Buch Strauß, Köln, (1997) 371-375

Zhu Shaotang and Yang Shumin

Determination of Benzthiazide in Human Urine by HPLC-ES/MS

Doping Control Centre of China, National Institute of Sport Medicine,
Anding Road 1, Beijing 100029

Abstract

Benzthiazide is a kind of diuretics which was banned by International Olympic Committee. Its detection method with HPLC-ES/MS was reported in this paper.
The standard of the drug and its positive urine sample was analyzed and the conditions of separation and ionization for benzthiazide was optimized.

Keywords: electrospray; diuretic; benzthiazide;

Introduction

Benzthiazide is a diuretic which may be used by athletes to reduce body weight and accelerate the elimination of some banned drugs so as to escape from doping test. In routine analysis of benzthiazide HPLC procedure was used as screening of the suspected sample but, for the confirmation a effectual method has not yet been established, mainly because no successful derivatization method for GC/MS analysis could be established. In our study on the confirmation of benzthiazide by GC-MS, methylation, silylanization and ion-pair extraction derivatization used to be performed but no reliable result was gotten. Only in thermospray-GC/MS analysis parent benzthiazide could be detected in positive urine but the sensitivity is not high-enough to give out a very affirmative detection (1)(2).
Atmosphere Ionization Electrospray interface coupled with 5989B mass spectrometer is a new product of Hewlett-Packard which is mainly applicated in the biochemistry and/or macromolecule studies. The application of the couple in the labile and small molecule drugs is still underdeveloped. In our practice of exploiting this technique a method using on-line HPLC-ES/MS for benzthiazide detection was developed.

Instrument and instrumentation

HP1050 HPLC (Hewlett-Packard GmbH, Waldbronn D-7515, FRG)
HP 5989 mass spectrometer/HP59987A electrospray interface (Hewlett-Packard Company,

Palo Alto 1601 USA)
tune maker: a mixture of valine, trivaline and hexa-tyrosine (Hewlett-Packard Company, Palo Alto 1601, USA)
target ion: m/z118.08, m/z 508.20 and m/z 997.39
CID (collision-induced dissociation) setting: CapEx=120V
electron-multiplier voltage: 1800V
drying nitrogen flow: 8000ml/min
drying gas temperature: 350 °C
nebulizing nitrogen pressure: 550 kPa (80 psi)
HPLC column: 100x2.0mm Hypersil ODS (Hewlett-Packard Company)
mobile phase: A: pure water B: acetonitrile
gradient: 0min: B=40% 20min: B=85%
flow rate: 500μl/min

Reagent and solvent

Benzthiazide standard was purchased from Sigma Company (P.O. Box 14508, St. Louis, MO 63178 USA).
Acetonitrile, methanol and other solvents, chromatographic grade, were purchased from Beijing Chemicals Company (Beijing 100089).

Drug administration and urine collection

5.0mg (single dose) benzthiazide was orally taken by a volunteer. The urine was collected till 20h.

Chemical treatment of urine sample

5ml of the urine was added by 0.5g of solid buffer ($NaHCO_3$:K_2CO_3=3:2, w/w, pH9.0). After vortexed for 15 sec the urine was extracted with ethyl acetate. The organic phase was dried by slow stream of nitrogen and redissolved in 300μl of methanol. 10μl of the solution was subjected to LC-MS analysis.

Result

Benzthiazide standard
2μl of benzthiazide standard solution (100ng/μl) was injected through autosampler of HPLC. Scan mode was adopted to record the spectrum (Fig.1). In the spectrum m/z 432

(M+1) is the molecular ion peak and very small peak m/z 91 (troplyium) could be seen.

Positive urine of benzthiazide

After the chemical treatment as described above the urine sample was subjected to LC-MS analysis. As shown in Fig.2, in the TIC of urine sample a peak could be detected as the parent benzthiazide and its mass spectrum seems to be totally same as that of the benzthiazide standard.

Discussion

Since benzthiazide was banned by IOC the confirmation method for doping test has not yet been successfully developed mainly because of its lower volatility and the inefficiency of derivatization. By our experiment on-line HPLC- ES/MS was demonstrated to be a feasible method to detect the misuse of this drug. The confirmation made by electrospray/mass spectrometer can provide a relatively reliable result but, since only the molecular ion and a very small fragment m/z 91 could be acquired and no GC/MS analysis could participate in the confirmation, it is still remaining questionable that this method can be practically used in doping test.

In regard to the metabolism and /or elimination of benzthiazide, since only a parent form of benzthiazide was found in our experiment and quite limited work on its metabolic fate could be reviewed (3), we failed to make sure whether the elimination of benzthiazide is only in the original form or it would be extensively metabolized.

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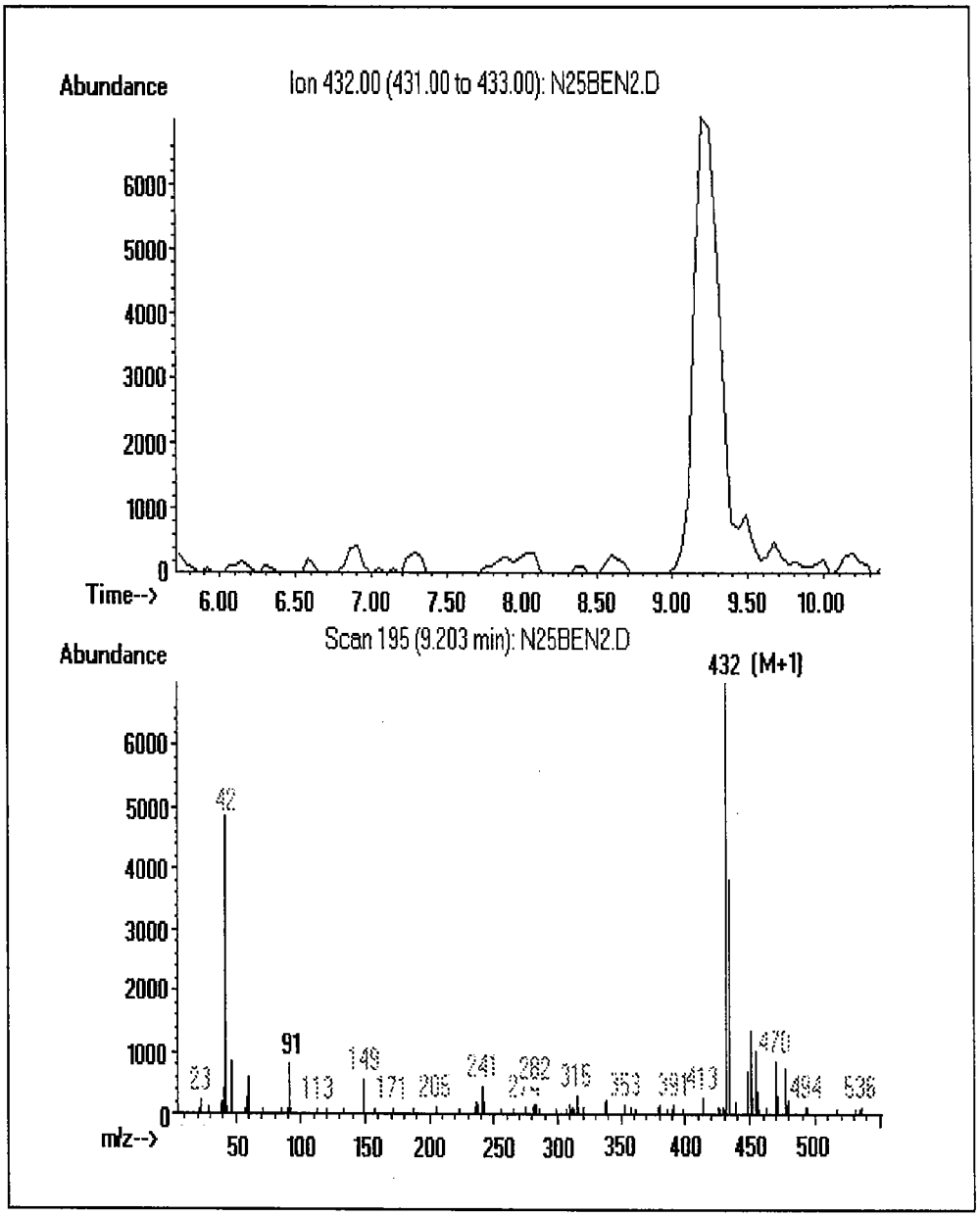

Fig. 1 TIC and mass spectrum of benzthiazide standard

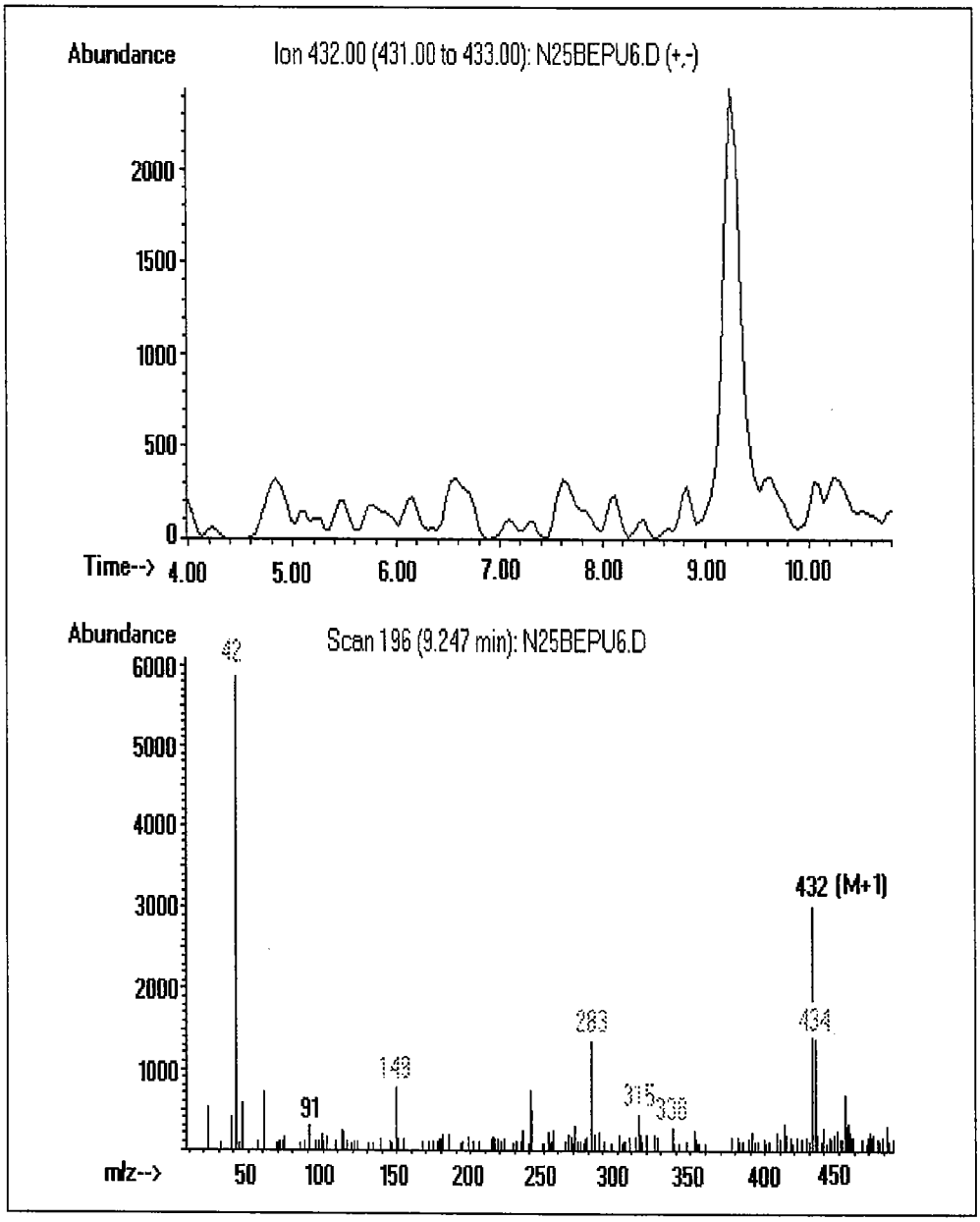

Fig. 2 TIC and mass spectrum of benzthiazide in positive urine (7h)