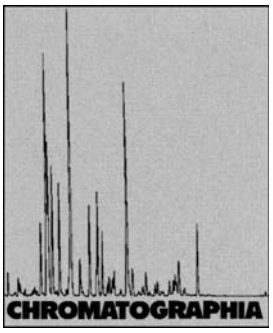
Determination of Atenolol in Human Plasma by Pseudo Reversed Phase Liquid Chromatography-Tandem Mass Spectrometry



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Abstract

Previous HPLC determination of atenolol on reversed-phase packings has often required a mobile phase containing three components: organic modifier, buffer and ion-pairing reagent or organic amine. In addition to the complexity of the eluents employed, alkyl sulphonates and organic amines in the mobile phase can reduce the life of silica-based bonded columns. A new simple, rapid and sensitive method—pseudo reversed-phase liquid chromatography/tandem mass spectrometry has been developed for the analysis of atenolol in human plasma using bare silica as the stationary phase coupled with a simple mobile phase consisted of 5% acetonitrile and 95% formate buffer. The optimization of separation is fast and easy. The assay was validated for the concentration range 1–100 ng mL^{-1} with a detection limit of 1 ng mL^{-1}. Moreover, the silica column was durable with the mainly aqueous eluents. No obvious loss in performance was observed for 30,000 column volumes of eluent.

Keywords

Column liquid chromatography
Pseudo-RPLC
MS-MS
Bare silica as stationary phase
Atenolol

Introduction

Atenolol [4-(2-hydroxy-3-isopropylaminopropoxy)phenylacetamide] is a long-established β-blocker widely used in the treatment of high blood pressure, arrhythmias and angina pectoris [1]. It is also used in sport and in stressful activities as a doping agent [2]. Accordingly, the development of a rapid analysis procedure has a practical interest in diverse areas, including forensic, toxicology and doping control.

There are many methods published for the determination of atenolol incorporating a variety of analytical techniques. In the last decade, gas chromatography with mass spectrometry (GC-MS) [3], high performance liquid chromatography (HPLC) [4–6] or capillary zone electrophoresis [7] have been the techniques generally used for the determination of atenolol. Although GC-MS is sensitive, derivation is needed, which makes sample preparation laborious and time consuming. HPLC, particularly reversed-phase liquid chromatography (RPLC) [8] has been favoured as the best technique for screening analysis of atenolol, with mobile phases generally consisting of acetonitrile or methanol, buffer, an ion-pairing reagent to provide adequate retention and organic amines to reduce peak tailing [9, 10]. However, alkyl sulphonates and organic amines dramatically reduced the life of reversed-phase packings such as C_{18} silica [8]. Additionally, as one of the most polar β-blockers, atenolol is difficult to retain on a C_{18} column, unless a mobile phase with only a small amount of organic solvent is used but highly aqueous mobile phases can cause the collapse of high density C_8 or C_{18} chains [11, 12]. One way out of this dilemma is to use a polar stationary phase. Due to its high hydrophilicity, atenolol is not soluble in mobile phases such as hexane or chloroform used in typical normal-phase liquid chromatography (NPLC) but there is an alternative chromatographic mode where a polar stationary phase is used with an aqueous-organic solvent as mobile phase.

Basic compounds can be separated on plain, unbonded silica with aqueous-rich mobile phases (the so-called pseudo-reversed-phase liquid chromatography, pseudo-RPLC) [13] or with organic-rich eluents (the so-called hydrophilic interaction chromatography) [14]. In recent

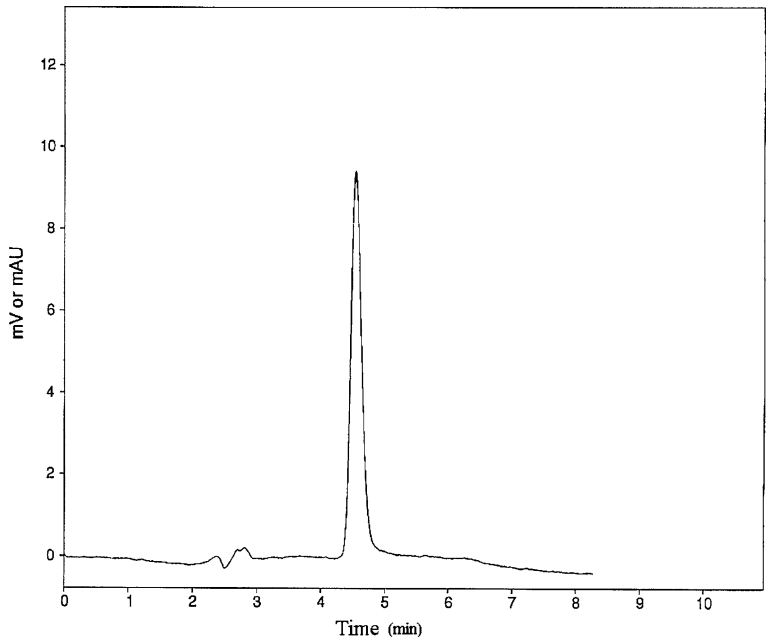

Fig. 1. The chromatogram of atenolol determined in pseudo-RPLC mode. Condition: stationary phase: Kromasil KR100-5SIL, 250 × 4.6 mm I.D. 5 µm; mobile phase: 5% ACN + 95% formate buffer (10 mM, pH = 2.9)

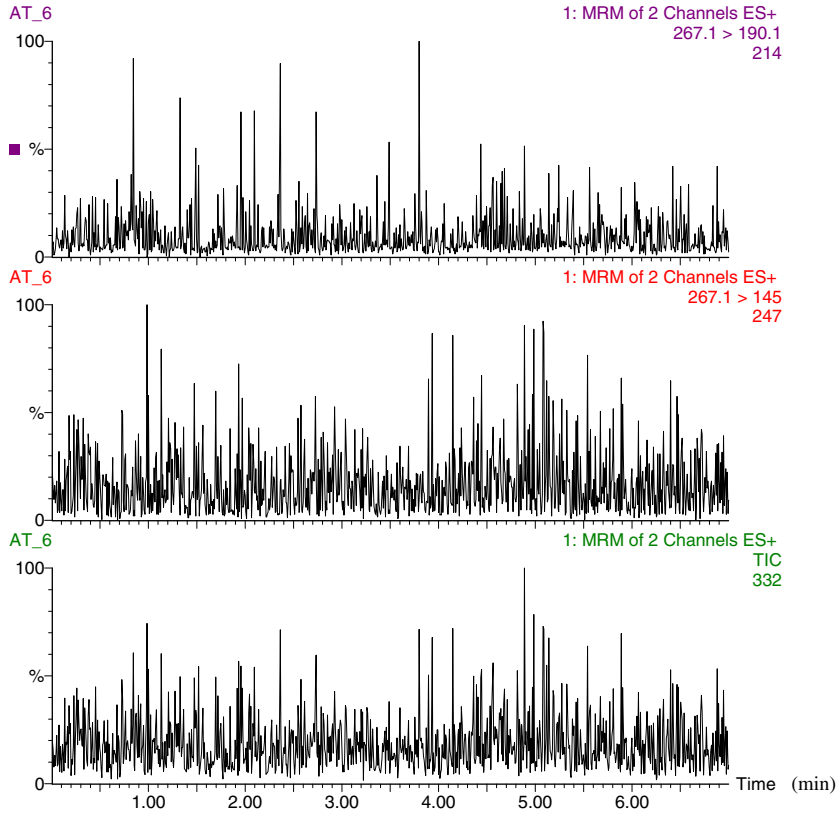

Fig. 2. TIC and MRM HPLC-MS-MS chromatograms of blank human plasma. Conditions are the same as described in the text

years, hydrophilic interaction LC has become an attractive alternative to RPLC for the analysis of some basic solutes although pseudo-reversed phase liquid chromatography (pseudo-RPLC) has received much less attention. In this paper we report, a new assay method using pseudo-RPLC-MS-MS, for the determination of atenolol.

Experimental

Reagents

Acetonitrile (ACN) and formic acid were of HPLC-grade (Tedia Co., USA). All other solvents and reagents were of analytical-reagent grade. Water was purified with a Milli-Q system (Milford, MA, USA). Note that buffer concentrations and pH values refer to the aqueous portion alone. Atenolol was obtained as a gift from Debai Pharmaceutical Inc. (Sichuan, China). Control blank human plasma was supplied by healthy donors.

A stock solution of atenolol was prepared in methanol at a concentration of 4.2 mg mL^{-1}. Working solutions (1–100 ng mL^{-1}) were prepared by appropriate dilution in mobile phase.

Sample Preparation

About 0.2 mL plasma samples spiked with atenolol standards was loaded onto an Oasis HLB cartridge (1 cc/30 mg) provided by Waters (Milford, MA, USA). The cartridge was pre-conditioned with 1 mL methanol and 1 mL deionized water then washed sequentially with 1 mL of 5% methanol and 1 mL of 40% (*v*/*v*) methanol containing 2% ammonium hydroxide. The analytes were eluted from the cartridge with 0.5 mL of 0.5% formic acid-methanol solution. The eluate was evaporated to dryness at 30°C under a gentle stream of nitrogen. The residue was reconstituted in 200 µL mobile phase and vortexed for 20 s. A 10 µL aliquot was injected into the LC-MS-MS system.

LC-MS-MS Analysis

The LC-MS-MS system consisted of an AcQuity™ Ultra Performance Liquid Chromatograph and a Quattro Premier Micromass® Mass spectrometer (Waters/Micromass; Milford, MA, USA). A 5 µm Kromasil KR100-5SIL column (250 × 4.6 mm I.D.) was used for separation. The mobile phase consisted of a binary mixture of 5% ACN and 95% ammonium formate at a flow rate of

1.0 mL min^{-1}. The MS instrument was operated in the positive ion electrospray ionization mode with multiple reaction monitoring (MRM).

The MS parameters for the analysis were: capillary 3.0 kV, source temperature 105°C and desolvation temperature 300°C. The cone and desolvation gas flows were 50 and 60 L h^{-1}, respectively. Optimal collision energy was 25 eV.

All aspects of data acquisition were controlled using MassLynx™ NT 4.0 software with QuanLynx™ program (Waters).

Method Validation

Validation samples at three different concentrations (2.5, 25.0 and 100.0 ng mL^{-1}) were prepared and analyzed to determine extraction efficiency and to evaluate the accuracy and precision of the method in plasma. The extraction recovery was determined by comparing the peak area of the spiked samples with those of unextracted neat solutions at the corresponding concentrations. The precision (repeatability) of the method was obtained by analyzing three replicate samples at 2.5, 25.0 and 100.0 ng mL^{-1} on 3 days. The precision was determined by calculating the relative standard deviation (RSD) for the repeated measurements. The limit of detection (LOD) was defined as the concentration of a signal-to-noise (S/N) ratio of 3.

Results and Discussion

Method Development

Atenolol was tested on bare silica with aqueous-rich eluents (95% formate buffer and 5% ACN). Under these conditions, the presence of a significant amount of water in the mobile phase deactivates the surface silanols and maintains a stagnant, enriched water layer on the surface of the stationary phase into which analytes may selectively partition [12]. Atenolol was well retarded and no peak tailing was observed as shown in Fig. 1.

Since the mobile phase in pseudo-RPLC only consisted of two components (organic solvent and buffer) instead of the three components (acetonitrile or methanol; buffer; ion-pairing reagent or organic amine) required for RPLC, method development is greatly simplified with the added advantage that only a small amount of organic solvent is required. Method development in pseudo-RPLC simply involves the optimization of ACN concentration and the pH value of the buffer. Bare silica with a highly aqueous eluent exhibits the characteristics of reversed-phase packings and the retention of atenolol decreased with increasing percentage of organic solvent. On the other hand, when pH was increased from 2.9 to 6.2, the elution times were gradually prolonged as the separation mechanism becomes predominantly ion-exchange in nature. The efficiency also decreased with higher pHs. The optimum analytical conditions for atenolol by pseudo-RPLC were found to be 5% ACN with 10 mM formate buffer at pH 2.9.

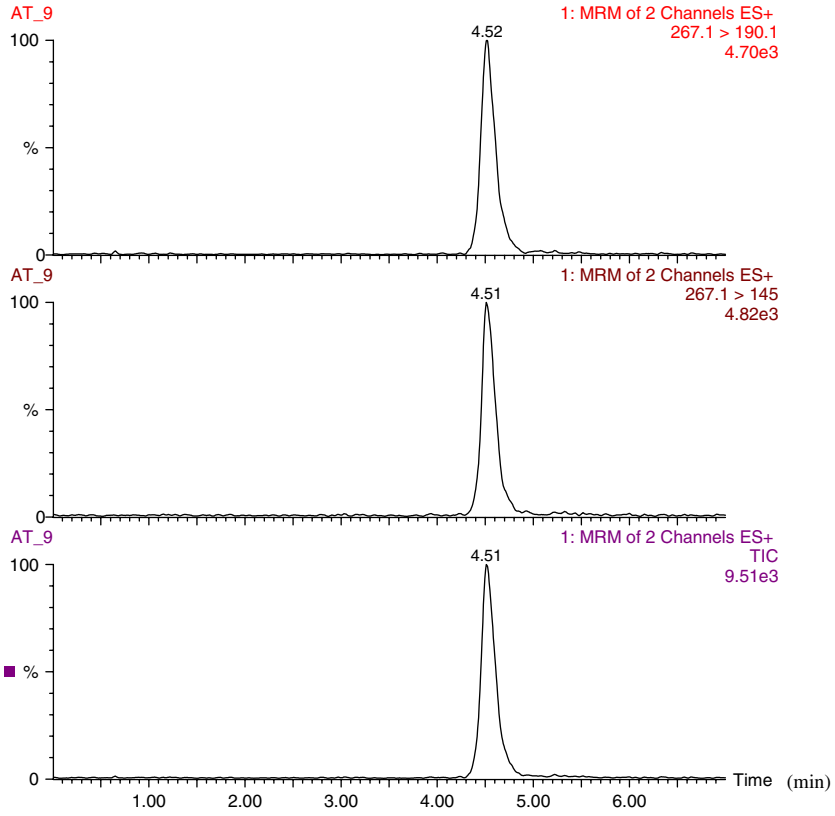

Fig. 3. TIC and MRM HPLC-MS-MS chromatograms of blank human plasma spiked with 25.0 ng mL^{-1} of atenolol. Conditions are the same as described in the text

Detection by MS

Another advantage of pseudo-RPLC is that the mobile phase used is volatile and this is advantageous for ion-spay introduction into the MS, enhancing the sensitivity. Figures 2 and 3 show total ion current (TIC) and MRM chromatograms of blank plasma and spiked samples (25.0 ng mL^{-1}). The ion of the protonated molecule $[M + H]^+$ (m/z 267.1) was used as the precursor ion in MS-MS experiments. The most intensive fragment ions m/z 190 and 145 corresponding to $[M–H_2O–NH_3\text{-isopropyl} + 2H]^+$ and $[190\text{-}CO–NH_3]^+$ were chosen as transition ions for the detection and quantitative analysis. The background from blank plasma was low indicating that SPE efficiently removed endogenous interferences in plasma samples. Thus the MS detection sensitivity was improved to give a LOD of 1 ng mL^{-1} at a S/N of 3.

Validation of the Method

In the linearity assay, atenolol was tested in the range 1–100 ng mL^{-1}. The regression equation was $y = 33.0134x - 11.1157$, $r^2 = 0.996948$ ($n = 5$), where y is peak area and x is concentration. A good linear relationship between the peak area and concentration was observed over the entire range tested for atenolol.

Table 1. Validation data of the method

Concentration added (ng mL^{-1})	Extraction recovery (%)	Intra-day precision (n = 3) RSD (%)	Inter-day study (n = 3)	
			Mean concentration found (ng mL^{-1})	Precision RSD (%)
2.5	90.2	5.2	2.25	9.2
25.0	98.3	4.5	24.59	4.0
100.0	101.4	3.1	101.41	3.5

Condition: mobile phase: ACN/buffer (10 mM pH = 2.9) (5/95, v/v)

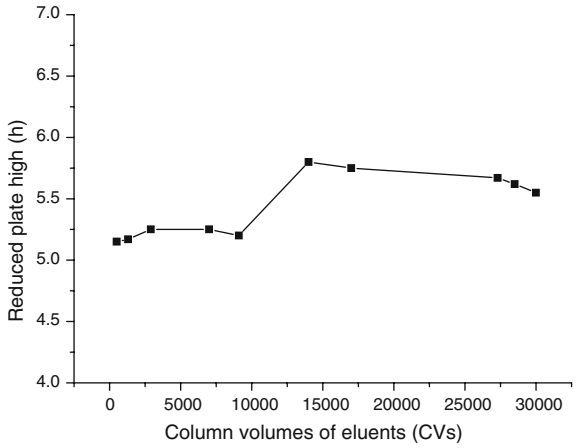

Fig. 4. Column life test. Column: Kromasil KR100-5SIL; mobile phase: ACN + formate buffer

The method validation data (extraction efficiency, intra- and inter-day variation) are summarized in Table 1. Extraction recoveries ranged from 90.2 to 101.4%, while precision values ranged from 3.5 to 9.2% over the three concentrations (i.e. 2.5, 25 and 100 ng mL^{-1}) evaluated over 3 days. The biggest bias corresponded to the lowest concentration. These results indicate that the proposed method has an acceptable accuracy and precision so that it can be used for the determination of atenolol in forensic toxicology.

Column Lifetime

Figure 4 shows the plot of the reduced plate high (h) versus the column volumes (CVs) of eluent. After lasting for 30,000 CVs, the performance of column was basically constant. Even if the column is polluted, after washed with stronger eluents, an acceptable performance can be restored. Silica has an obvious advantage compared with bonded material as there is essentially no significant loss of active surface. When a layer of silica is dissolved a fresh silica surface is exposed [15]. Therefore, the silica column is durable for a long time under the conditions described.

Conclusion

To our knowledge, the method described here is the first procedure for the quantitative determination of atenolol on bare silica in pseudo-RPLC mode. It has several advantages over previously published methods. The bare silica packing is cheaper than bonded phases and method development is rapid. Very little organic solvent is required which reduces disposal problems and again reduces cost. The assay is sensitive with a limit of quantification of 1 ng mL^{-1} for atenolol. The good validation results of the method and the durability of the column allow its use in doping control analysis as well as other long-term applications.

Acknowledgments

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References

1. Braza AJ, Modamio P, Mariño EL (2000) J Chromatogr B 738:225–231
2. International Olympic Committee (IOC), Medical Commission (1998) List of doping classes and methods
3. Sirén H, Saarinen M, Hainari S, Lukkari P, Riekkola ML (1993) J Chromatogr 632:215–227
4. Yee YG, Rubin P, Blaschke TF (1979) J Chromatogr 171:357–362
5. Chiap P, Hubert P, Boulanger B, Crommen J (1999) Anal Chim Acta 391:227–238
6. Giachetti C, Tenconi A, Canali S, Zanolo G (1997) J Chromatogr B 698:187–194
7. Arias R, Jiménez RM, Alonso RM (2001) J Chromatogr A 916:297–304
8. Basci NE, Temizer A, Bozkurt A, Isimer A (1998) J Pharm Biomed Anal 18:745–750
9. Verghese C, McLeod A, Shand D (1983) J Chromatogr 275:367–375
10. Harrison PM, Tonkin AM, McLean AJ (1985) J Chromatogr 339:429–433
11. Olsen BA (2001) J Chromatogr A 913:113–122
12. Naidong W (2003) J Chromatogr B 796:209–224
13. Crommen J (1979) J Chromatogr 186:705–724
14. Alpert AJ (1990) J Chromatogr 499:177–196
15. Bidlingmeyer BA, Henderson J (2004) J Chromatogr 1060:187–193