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Determination of Atenolol, Rosuvastatin, Spironolactone, Glibenclamide and Naproxen in Blood by Rapid Analysis Liquid Chromatography

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A rapid liquid chromatographic (HPLC) method for the determination of atenolol, rosuvastatin, spirnolactone, glibenclamide and naproxen was developed. The atenolol, rosuvastatin, spirnolactone, glibenclamide and naproxen were extracted from the sample with acetonitrile and they were separated on a ZORBAX Stable Bound (4.6 mm × 50 mm, 1.8 μm) C_{18} rapid analysis column with methanol and 0.01 mol L^{-1} of acetic acid (78:22) as the mobile phase. This method provides good reproducibility and sensitivity. The relative standard derivation of overall intra-day variations were less than 2.5 % and the relative standard derivation of inter-day variations were less than 3.0 %. The standard recoveries were ranged from 94-103 %.

Key Words: Atenolol, Rosuvastatin, Glibenclamide, Naproxene, Spironolactone, Rapid analysis liquid chromatography.

INTRODUCTION

The atenolol, rosuvastatin, spirnolactone, glibenclamide and naproxen are the important routine use drug. Atenolol used in the treatment of hypertension, can improve cardiac function, reduce the symptoms of heart failure[1,2]. Rosuvastatin is a member of the cholesterol-lowering drug commonly referred to as statins, used for the treatment of dyslipidemia and other conditions[3,4]. Naproxen has antiinflammatory, fever-reducing and pain-relieving properties. It is recommended that this medication be taken with food to minimize stomach irritation[5,6]. Glibenclamide is widely used for the treatment of non-insulin-dependant diabetes mellitus (NIDDM) diabetes type. This agent has been reported to inhibit the activities of various ion channels and transporters[7,8]. Spironolactone is a potassium-sparing diuretic and is marketed as a competitive aldosterone antagonist. It is indicated for the treatment of oedema and ascites in cirrhosis of the liver and has been widely utilized in the management of congestive heart failure associated with congenital heart disease, bronchopulmonary dysplasia or chronic lung disease and pediatricascites[9,10].

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Several works on the HPLC methods for the quantitation of these drugs in human plasma was reported[11-17]. However, these methods usually need a tedious samples preparation or long time for chromatographic separation. In this paper, a simple, rapid and sensitive HPLC analytical method using ZORBAX stable bound rapid analysis column for the simultaneous determination of the atenolol, rosuvastatin, spirnolactone, glibenclamide and naproxen in blood was developed. Five drugs can achieve baseline separation with 2.0 min. Compared to the routine liquid chromatography method[11-17], 80 % of separation time was saved. It is one of the most rapid methods for chromatographic simultaneous analysis of atenolol, rosuvastatin, spirnolactone, glibenclamide and naproxen.

EXPERIMENTAL

The HPLC analysis was performed on a Waters 2695 Alliance separation system equipped with a Waters 996 photodiode array detector (Waters Corporation, Milford., MA 01757, USA). A ZORBAX stable bound column (4.6 mm × 50 mm, 1.8 μm) (Agilent Technologies Inc, Santa Clara., CA 95051, USA) was utilized.

The atenolol, rosuvastatin, spirnolactone, glibenclamide and naproxen reference standards were obtained from Shanghai Usea Biotech Company (Shanghai, P.R. China). They purities were ≥ 98 %. HPLC grade acetonitrile and methanol were provided by Fisher Scientific Inc (Madison, WI 53711, USA). The ultrapure water used was obtained from a Milli-Q50 SP Water system (Millipore Inc, MA 01730 Bedford). The mobile phase used is methanol and 0.01 mol L^{-1} of acetic acid (78:22) at a flow-rate of 2.0 mL min^{-1}. The sample injection volume is 20 μL. The detection wavelength was 235 nm. The chromatogram of drug standards and blood sample at 235 nm is shown in Fig. 1.

Preparation of sample: Multiple blood samples (10 mL) of 10 healthy non-smoker volunteers (ages ranging from 22-25 years) not involved in any strenuous activity and not taking any other medications were collected in evacuated glass tubes. The blood was then centrifuged at 5000 rpm for 10 min, the plasma separated and deproteinated by acetonitrile. The supernatant obtained was filtered through a 0.45 μm filter. Serum thus obtained was stored at -20 ºC pending drugs analysis.

Preparation of standard solution: To prepare standard solutions, an accurately weighed amount of atenolol, rosuvastatin, spirnolactone, glibenclamide and naproxen which were dissolved in methanol for HPLC. Five concentrations were chosen, with the range 0.05-150 μg mL^{-1}, respectively. The regression equations of the five drugs were established based on the standard samples injected and their peaks area.

RESULTS AND DISCUSSION

To optimize the HPLC conditions for the separation of atenolol, rosuvastatin, spironolactone, glibenclamide and naproxen, the effects of pH, mobile phase composition, the type of column and its dimension and wavelength of detection were investigated.

The first factor examined was the type of the stationary phase. C_8 and C_{18} columns of different dimensions and particle sizes were used. It was found ZORBAX stable bound C_{18} rapid analysis column (4.6 mm × 50 mm, 1.8 μm) gave the most suitable resolution. By this rapid analysis column, the five drugs were separated completely within 2.0 min (Fig. 1). Compared to the previous literature[11-17], this is one of the most rapid methods to simultaneous separation of the five drugs.

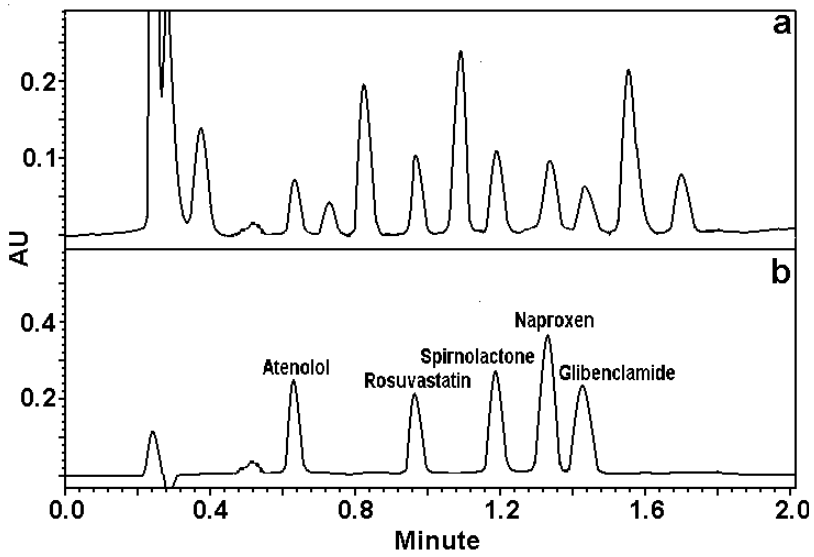

Fig. 1. HPLC chromatogram of drug standards (b) and blood sample (a)

The mobile phase compositions were prepared with appropriate ratios of methanol or water. The adjustment of pH was made using drops of acetic acid, to achieve pH 3.0-4.5. It is observed that the best resolution of all drugs was achieved with a mobile phase composed of methanol and 0.01 mol L^{-1} of acetic acid (78:22, v/v). The use of methanol and water showed an elevated base line and no tailing effect is observed.

The absorption spectrum of atenolol, rosuvastatin, spirnolactone, glibenclamide and naproxen were obtained with 996 photodiode array detector. Results show that the analyts have the strong absorption at 235 nm. Therefore, the 235 nm was selected as detecting wavelength.

Plasma deproteination for the determination of drugs is commonly accepted as the simplest method of sample preparation. Previously developed HPLC procedures for the determination of atenolol, rosuvastatin, spironolactone, gilibenclamide and naproxen in plasma are based on liquid-liquid extraction or solid-phase extraction from plasma samples. The method applied in present study involved the direct injection of the plasma samples after precipitation of protein with acetonitrile. This does not need a complex purification procedure. The sample preparation was greatly simplified.

Under the optimum conditions, the regression equations of five drugs were established based on the standard samples injected and their peaks area. The limits of detection are calculated by the ratio of signal to noise (S/N=3). The results were shown in Table-1. The reproducibility of this method was also examined for 1.0 mg mL^{-1} of the five drugs. The relative standard deviations (n = 9) were shown in Table-1.

TABLE-1
REGRESSION EQUATION, COEFFICIENT AND DETECTION LIMIT

Components	Regression equation C (μg mL^{-1})	Linearity range (μg mL^{-1})	Coefficient	Detect limits (μg mL^{-1})	RSD % (n = 9)
Atenolol	$A = 1.64\times10^5 C + 226$	0.12~100	r = 0.9994	0.02	1.7
Rosuvastatin	$A = 1.24\times10^5 C - 176$	0.10~120	r = 0.9993	0.03	2.1
Spirnolactone	$A = 1.87\times10^5 C + 87$	0.08~120	r = 0.9996	0.015	1.8
Glibenclamide	$A = 1.82\times10^5 C - 204$	0.1~110	r = 0.9995	0.02	1.7
Naproxen	$A = 2.18\times10^5 C + 1.8$	0.05~80	r = 0.9994	0.01	2.0

The recovery test were carried out by adding atenolol, rosuvastatin, spironolactone, gilibenclamide and naproxen to the samples (three different concentrations of markers: 0.5, 1.0 and 5.0 μg). The sample was prepared and injected for HPLC analysis to calculate the amount of the drugs founded. The results shown that the recoveries (n = 5) were ranged from 94-103 %. This method is high recovery.

The measurements of intra- and inter-day variability (determination of the same samples for seven times) were utilized to evaluate the precision of the developed method. The results shown that the relative standard derivation of overall intra-day variations were less than 2.5 % and the relative standard derivation of inter-day variations were less than 3.0 %.

Conclusion

The proposed HPLC method enables simultaneous determination of atenolol, rosuvastatin, spironolactone, glibenclamide and naproxen because of good separation and resolution of the chromatographic peaks within 2 min. Compared to the normal column, more than 80 % of separation time was saved. It is one of the most rapid methods for chromatographic analysis of the five drugs. The sample can directly afford to HPLC analysis after precipitation of protein with acetonitrile. This preparation does not need a complex purification procedure. The sample preparation was greatly simplified. The detector response was found to be linear over a wide concentration range for all drugs. The validity, LOQ and the linearity range of the method makes it acceptable method for clinical studies in patients taking these medications simultaneously. Results are accurate and precise and are confirmed by the statistical parameters and are well within the limits required for bioanalytical assays. The lower limit of quantification permits the use of the method for pharmacokinetic studies.

Thus, this method is rapid, high sensitive and selective and provides good reproducibility and accurateness for the quantification of these five drugs.

REFERENCES

1. E.J. Eichhorn, C.M. Heesch and J.H. Barnett, *J. Am. Coll. Cardiol.*, **24**, 1310 (1994).
2. F. Waagstein, M.R. Bristow and K.J. Swedberg, *Lancet*, **342**, 1441 (1993).
3. W.V. Brown, H.E. Bays, D.R. Hassman, J. McKenney, R. Chitra, H. Hutchinson and E. Miller, *J. Am. Heart.*, **144**, 1036 (2002).
4. A.G. Olsson, F. McTaggart and A. Raza, *J. Cardiovasc. Drug. Rev.*, **20**, 303 (2002).
5. P.A. Todd and S.P. Clissold, *Drugs*, **40**, 91 (1990).
6. O.A. Santini, E.J. Oliveira and P.R. Helena, *J. Braz. Chem. Soc.*, **17**, 785 (2006).
7. U. Panten, M. Schwanstecher and C. Schwanstecher, *J. Exp. Clin. Endocrinol.*, **104**, 1 (1996).
8. H. Annette, L.W. Cornelia and Q. Ulrich, *Br. J. Pharmacol.*, **136**, 995 (2002).
9. R.D. Walker and G.R. Cumming, *J. Can. Med. Assoc.*, **91**, 1149 (1964).
10. L.C. Kao, D. Warburton and M.H. Cheng, *J. Ann. Int. Med.*, **74**, 37 (1984).
11. N. Sultana, M.S. Arayne and B. Iftikhar, *J. Chinese. Chem. Soc.*, **55**, 1022 (2008).
12. M.D. Faulx and G.S. Francis, *Curr. Prob. Cardiol.*, **33**, 703 (2008).
13. M. Pelat, C. Dessy, P. Massion, J.P. Desager, O. Feron and J.L. Balligand, *J. Circ.*, **107**, 2480 (2003).
14. R.K. Trivedi, R.R. Kallem, R. Mullangi and N.R. Srinivas, *J. Pharm. Biomed. Anal.*, **39**, 661 (2005).
15. M. Zakeri-Milani, H. Barzegar-Jalali, Y. Tajerzadeh and V.H. Azarmi, *J. Pharm. Biomed. Anal.*, **4**, 624 (2005).
16. S. Aburuz, J. Millership and J.J. Mcelnay, *J. Chromatogr. B*, **817**, 277 (2005).
17. J.M. Sandall, J.S. Millership, P.S. Collier and J.C. McElnay, *J. Chromatogr. B*, **839**, 36 (2006).

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