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Short Communication

BIOANALYTICAL METHOD DEVELOPMENT OF ATENOLOL IN RAT PLASMA BY ULTRA PERFORMANCE LIQUID CHROMATOGRAPHY AND TANDEM MASS SPECTROMETRY METHOD: APPLICATION TO PHARMACOKINETIC DRUG-HERB INTERACTION STUDY

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ABSTRACT

Objective: To develop a rapid, simple and sensitive ultra-performance liquid chromatography and tandem mass spectrometry (UPLC-MS/MS) method for quantitative estimation of atenolol (ATN) in rat plasma and its application to pharmacokinetic drug-herb interaction study.

Methods: Simple precipitation method was used for the extraction of plasma samples with an aliquot of 25 µl plasma samples extracted using acetonitrile precipitation technique containing internal standard. Chromatographic separation was performed using Phenomenex, Kinetex, C18, 50 x 2.1 mm, 1.7µ by a gradient mixture of 0.1 % formic acid in acetonitrile and 10 mmol Ammonium formate as a mobile phase at the flow rate of 0.7 ml/min. The analyte was protonated in the positive electrospray ionization (ESI) interface and detected in multiple reactions monitoring (MRM) modes using the transition m/z 145.0-267.2.

Results: The developed method had an advantage of fast chromatography run time of 1.8 min with improved sensitivity over existing methods and broader application to use for drug-herb or drug-drug interaction studies in rat pharmacokinetic. Calibration curves have been linear over the wide range of 4.93-5047.00 ng/ml in rat plasma which covers a wide range of plasma concentrations form study samples. ATN is widely used as a biomarker for determination of the drug-herb or drug-drug interactions studies, especially with the drugs that alter the permeability of another administered drug(s) and the developed method can be used to explore drug interaction studies. Especially to evaluate the role of concurrently administered drugs and its impact on permeability enhancer or absorption enhancer drugs following oral dose administration. This method will be significantly useful for the drug-herb pharmacokinetic interaction studies where we cannot quantify the herb because of multiple components in the hydro-alcoholic or aqueous extract of any herb, so choice remained is to quantify ATN and therefore it will be extremely helpful in such scenario.

Conclusion: It was concluded that the developed UPLC-MS/MS method was sensitive linear and rapid; can be used for quantification of ATN in rat plasma for pharmacokinetic drug-herb interaction study.

Keywords: Atenolol, Quantitative estimation, Pharmacokinetic, Drug-drug and drug-herb interaction, Permeability enhancer, UPLC-MS/MS

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ATN is a biopharmaceutical classification system (BCS) class III drug (high solubility, low permeability) and is known as a marker of paracellular permeability. The absorption of ATN after oral administration is rapid but incomplete. Only about 50-60% is absorbed. Peak plasma concentrations of 300-800 ng/ml are seen at between 2-4 h after administration of a 100 mg dose in human. The effect of ATN on heart rate usually begins at one hour, peaks at 2-4 h and persists for 24 h. The antihypertensive effect also usually persists for 24 h. There is a linear correlation of heart rate, but not blood pressure, with plasma ATN concentrations in the range 0.02-200 mcg/ml. ATN distributes readily into most tissues except for brain and cerebrospinal fluid. Approximately 5-15% binds to plasma protein. Elimination of ATN occurs mainly by renal excretion of unchanged medicine. Little or no metabolism occurs in the liver. The plasma half-life is 6-7 h in patients with normal renal function. Considering the absorption, distribution, metabolism and excretion properties of ATN (a BCS class III drug; high solubility, low permeability) is used as a marker of paracellular permeability to evaluate bio-enhancer property of the administered drug or to evaluate drug-drug interactions (DDI) and drug-herb interactions (DHI) in various models [1, 3].

Bioenhancers are such agents, which when combined with an active drug lead to the potentiation of the pharmacologic effect of the drug by improving the blood levels. Such formulations have been found to increase the bioavailability/bio-efficacy of a number of drugs even when reduced doses of drugs are present in such formulations or in presence of the co-administered drug. Evidence has been obtained for such classes of drugs which are (a) poorly bioavailable and/or efficacious, (b) require prolonged therapy, and (c) are highly toxic and expensive. The development of the phytomolecules is based on ancient knowledge of Ayurveda. They augment the bioavailability or biological activity of drugs when administered at low doses. They reduce the dose; shorten the treatment period thus reducing drug-resistance problems. The treatment is made cost-effective, minimizing drug toxicity and adverse reactions. When used in combination with the number of drug classes such as antibiotics, anti-tuberculosis, antiviral, antifungal and anti-cancer drugs they are quite effective. Oral absorption of vitamins, minerals, herbal extracts, amino acids and other nutrients are improved by them. They act through several mechanisms which may affect mainly absorption process, drug metabolism or action on drug-target [4, 5].

Bioanalytical methods of measuring drugs in biologic media are increasingly important to accurately quantitate drugs in biological fluids. Hence considering the wide application of ATN, the present study was planned and executed to describe method development using simple, specific, rapid and sensitive UPLC-MS/MS method for the determination of ATN in rat plasma.

ATN, albendazole, K_2EDTA, formic acid, and ammonium formate were obtained from Sigma-Aldrich (Bangalore, India). Acetonitrile from J. T. Baker (Mumbai, India). All other chemicals used in this study were of analytical grade.

All animal studies had the approval of the institutional animal ethics committee (IAEC) as per approval number MPC/IAEC/02/2015 of "UKA Tarsadia University" (animal house facility) and were in accordance with the guidelines of the CPCSEA, Government of India. Animals were acclimatized in study rooms for at least three days

prior to dosing. Rats were housed in polypropylene cages (1 animals per cage, marked for identification) maintained in controlled environmental conditions (22±3 °C; 40-70% RH) with 12 h light and dark cycles. Rats were given rodent pellet diet, and UV treated filtered water ad libitum. Rat blood samples were collected using a serial sampling design (n=6 per time point; pre-dose, 0.25, 0.5, 1, 2, 4, 6, 8 and 24 h) following oral dose administration to fed Sprague Dawley rats at 10 mg/kg dose.

A standard stock solution of ATN and albendazole were prepared and were stored at 2-8 °C until use. The analytical standard for ATN in acetonitrile over a concentration range of 4.93 ng/ml to 5047.00 ng/ml by serial dilution method and same concentration range for calibration curve were also prepared in blank rat plasma.

An aliquot of 25 µl study sample or spiked calibration standard/ quality control sample was added to individual pre-labeled microcentrifuge tubes followed by 25 µl of IS prepared in water (albendazole, 500 ng/ml) was added except for blank rat plasma, where 25 µl of water was added. Samples were vortexed, followed by 100 µl of acetonitrile was added and samples were vortexed for another 5 min. After centrifugation of the samples at 10000 rpm for 5 min at 4 °C, the supernatant was collected and 5 µl was injected onto the UPLC-MS/MS system.

An Acquity™ UPLC (USA) consisting of flow control valve, vacuum degasser operated in a gradient mode to deliver the mobile phase at a flow rate of 0.7 ml/min. The chromatographic system consisted of phenomenex kinetex column (C18, 50 mm× 2.1 mm, 1.7 µm) and mobile phase consists of 0.1 % formic acid v/v/in acetonitrile (A), 10 mmol ammonium formate in water (B). A gradient UPLC method with 1.8 min run time was employed for analysis. The flow rate was 0.7 ml/min. Separation was achieved using phenomenex kinetex C18 column, maintained at 45 °C employing an injection volume of 5 µl for *in vivo* rat samples.

Mass spectrometric detection was performed on AB Sciex-4000 triple quadrupole LC/MS/MS mass spectrometer equipped with ESI source, the MRM modes was used for data acquisition with Analyst 1.6.2 software. Peak integration and calibration were carried out by using Analyst 1.6.2 software. MS and MS/MS condition for pure standards of ATN and IS albendazole were optimized by continuous infusion at 5 µl/min using syringe pump. The transitions monitored were m/z 267.2 (m/z)>145.0 and 266.1>234.0 for components ATN and IS albendazole, respectively. All analyzes were carried out in positive ion ESI with spray voltage set at 5500 V. The heated ESI temperature was set 550 °C. Nitrogen ion source gas GS1 and GS2 set at 40 and 60 psi, respectively. The collision assisted dissociation (CAD) was used at pressure 6 psi. Total run time for UPLC-MS/MS analysis was 1.8 min.

The method was successfully applied to evaluate the plasma concentration versus time profile of ATN in rat plasma following oral administration at 10 mg/kg dose of ATN. The oral dose volume was 10 ml/kg and dosing was performed using oral gauge needle. The suspension formulation was prepared freshly on the day of dosing. Studies were performed in healthy male Sprague Dawley rats (200-250 g). A serial sampling design was used (n=3 per time point). Approximately, 100 µl of blood samples was collected (K_2EDTA anticoagulant, 20 µl of K_2EDTA solution/ml of blood, 200 mmol) at 0.25, 0.5, 1, 2, 4, 8 and 24 h post-dose. Plasma samples were separated by centrifugation of whole blood and stored below-70±10 °C until bioanalysis.

Pharmacokinetic parameters were calculated using non-compartmental analysis tool of WinNonlin® software (Version 7). The area under the concentration time curve (AUC_{last} and AUC_{inf}) was calculated by linear trapezoidal rule. The peak concentration (C_{max}) and time for the peak concentration (T_{max}) were the observed values.

In order to find most, sensitive ionization mode for the components studied, ESI positive ion mode and ESI negative ion mode were tested with the various combination of mobile phase, i.e., methanol, acetonitrile and water/ammonium acetate buffer (2 mmol)/formic acid (0.1%) in positive and negative ionization mode. It was observed that the signal intensity for $[M+H]^+$ions in ESI positive ion mode were 7-14-fold higher for ATN using 0.1 % formic acid v/v/in acetonitrile and 10 mmol ammonium formate in water, versus experiments run with ESI negative ion mode. The protonated molecular ion of $[M+H]^+$, *m/z* 267.2 were obtained for ATN. No significant solvent adduct ions or fragment ions were observed in the full scan spectra of ATN and albendazole (IS). Thus, it was decided to utilize positive ion mode for detection and quantification of $[M+H]^+$ ions and representative chromatogram showed in the fig. 1 and 2.

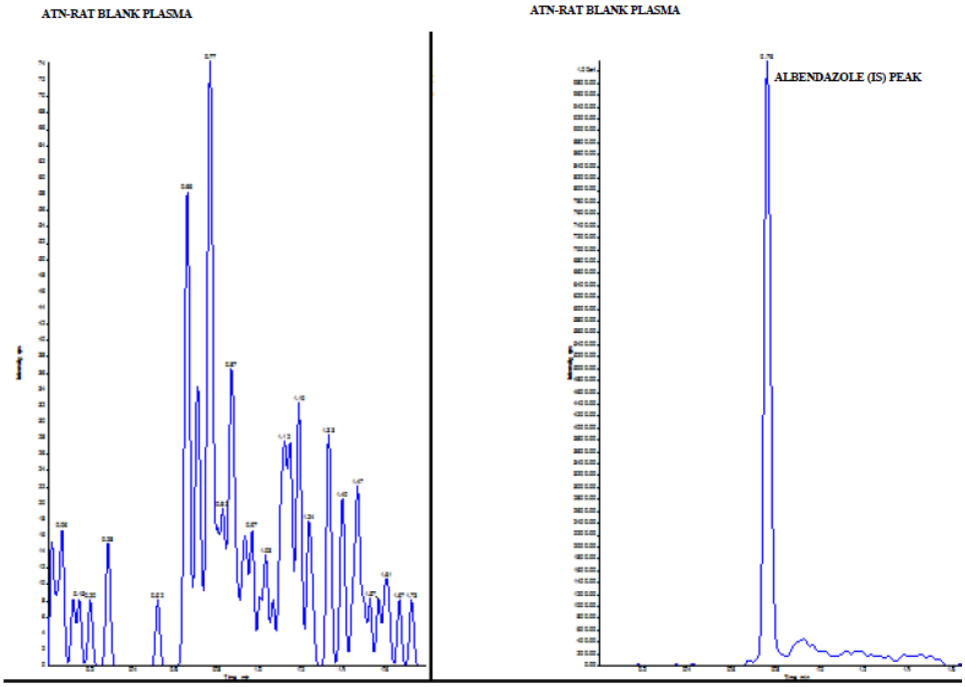

Fig. 1: Representative chromatograms of rat blank plasma using ATN and albendazole LC/MS/MS method

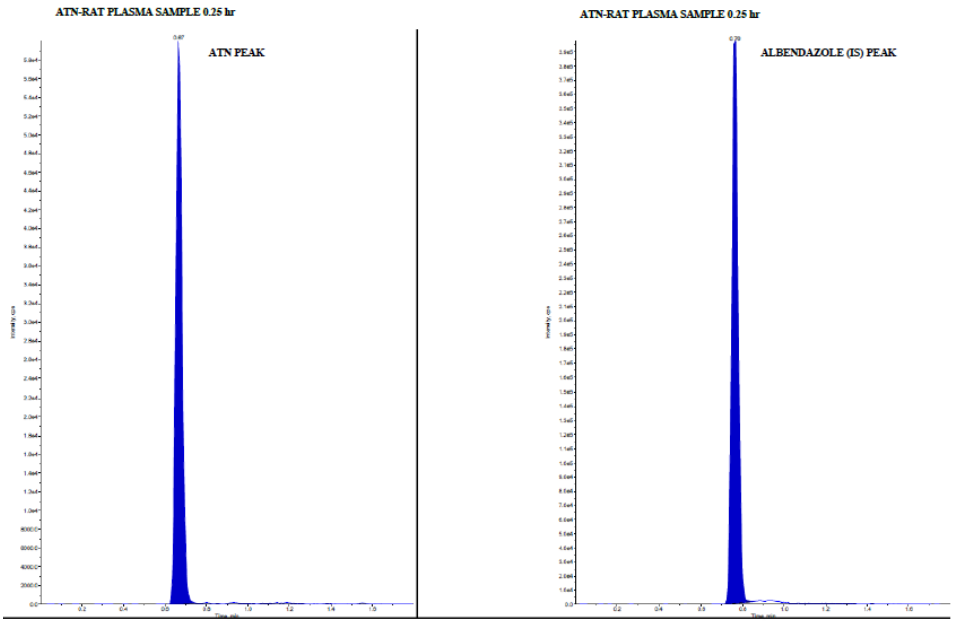

Fig. 2: Representative chromatograms of ATN and albendazole in rat plasma sample

The next step was to develop the simple and efficient sample clean-up devoid of matrix effect and interference from endogenous plasma components for estimation of ATN in rat plasma. Sample extraction method was like liquid-liquid extraction (LLE) was tried using ether and different combinations of hexane and ethyl acetate (80–20%, v/v), n-hexane and isopropyl alcohol (2–5%, v/v) was also tried but none of these was found suitable to give good and consistent recovery for ATN. Finally, precipitation method was tried with 100% acetonitrile and found suitable to give optimum recovery for ATN and internal standard. For determination of matrix effect, control drug-free plasma was extracted using the described method and drug was added in the extracted supernatant. Matrix effect was determined by comparing the analytical response of these samples with that of standard solutions.

The method described above was successfully applied to a pharmacokinetic evaluation of ATN. Following oral administration of ATN at 10 mg/kg oral suspension formulation to male Sprague Dawley rats, plasma concentrations were quantifiable up to 24.00 h (last sampling point) with T_{max} at 6.00 h. The plasma concentration-time profile of ATN is shown in fig. 3 and mean pharmacokinetic parameters are presented in table 4.

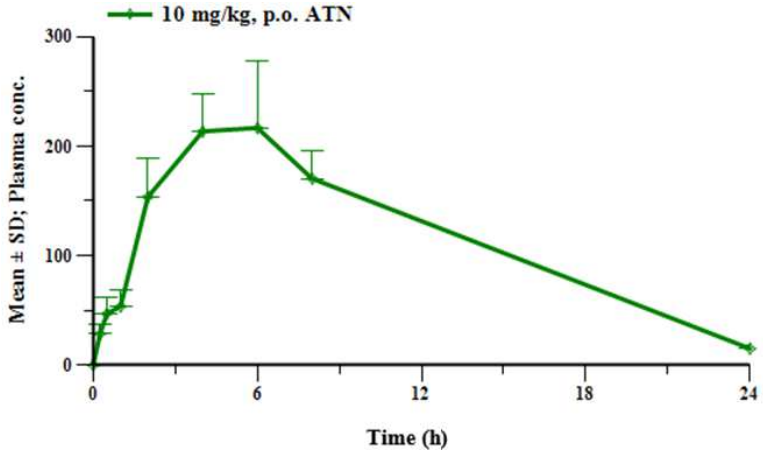

Fig. 3: Mean plasma concentration vs. time following a single dose oral administration of ATN in male sprague dawley rats

Table 4: Mean pharmacokinetic parameters following single oral administration of ATN in male sprague dawley rats

Analyte	T_{max} (h)	C_{max} (ng/ml)	$AUC_{(0-t)}$ (h*ng/ml)	$AUC_{(0-inf)}$ (h*ng/ml)
ATN	6.00±2.00	227.93±46.89	2811.62±120.51	2917.46±123.06

Data of n=6 rats; mean±SD; AUC: area under the concentration-time curve; 0-t: zero to time; 0-inf: zero to infinity; T_{max}: time to reach peak plasma concentrations; C_{max}: peak plasma concentrations

The UPLC-MS/MS bioanalytical method for determination of ATN was developed in rat plasma. The method was sensitive enough to detect low concentration of 4.93 ng/ml for ATN with a short run time of 1.8 min. Recovery of ATN from the spiked control samples were>85% by

using convenient and the rapid precipitation method using acetonitrile. The method was successfully applied to generate the pharmacokinetic evaluation of ATN in rat plasma samples following oral dose administration and can be used to evaluate DHI or DDI interactions studies associated with permeability enhancer properties of herb drugs.

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AUTHORS CONTRIBUTIONS

The work was planned and executed by the corresponding author under the active guidance by the second author. The second author actively involved in the implementation of the thought process as well as the review of the manuscript.

CONFLICTS OF INTERESTS

The authors declare no conflict of interest

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