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BIOANALYTICAL METHOD FOR HMG COA REDUCTASE ENZYME INHIBITORS AND ANGIOTENSIN RECEPTOR BLOCKERS

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ABSTRACT

Here, a bio-analytical technique utilizing RP-HPLC/UV was created and thoroughly verified for the simultaneous measurement of candesartan and rosuvastatin in human and rat plasma using atorvastatin as an internal reference. The material was cleaned up using solid-phase extraction, and its later optimization was done to remove the matrix impact and increase extraction efficiency. Using Design Expert Software, a three-level factorial design was performed to optimize the composition of the mobile phase and evaluate the impact of the mobile phase's pH as part of a quality by design strategy. The new HPLC technique can be employed in an LC-MS/MS experiment and is mass compatible. The current work offers a useful technique for researching drug-drug interactions between statins and sartans; as a result, it can be applied to both clinical pharmacokinetic studies and therapeutic drug monitoring.

Keywords: *RP-HPLC/UV, clinical pharmacokinetic, factorial design.*

1.INTRTODUCTION

Analytical chemistry's subfield of bio-analysis is used to identify and quantify analytes in biological samples, such as blood, plasma, serum, saliva, urine, feces, skin, hair, and organ tissue. The collecting, processing, storing, and analysis of a biological matrix for an analyte are all included in a bio-analytical approach. For bioequivalency, pharmacokinetic, and toxicokinetic investigations to be conducted successfully, a sensitive and selective bio-analytical approach for the quantitative detection of analytes is essential. To produce trustworthy data that can be satisfactorily understood, well-characterized and well validated bio-analytical techniques must be used.[1]

There are now seven statins available for purchase worldwide. Among these, atorvastatin, fluvastatin, pitavastatin, and rosuvastatin are entirely synthetic substances, whereas lovastatin, simvastatin, and pravastatin are inhibitors of HMG-CoA reductase obtained from fungi. The functional distinction between synthetic and natural statins is based on their lipophilicity and capacity to interact with and inhibit the HMG-CoA reductase. Because of their structural features, synthetic statins are known to interact with HMG-CoA reductase more frequently.[2]

Through the cytochrome P450 (CYP) system, losartan is first pass metabolized in the liver to produce its more potent active metabolite, EXP3174. Olmesartan medoxomil, azilsartan medoxomil, and candesartan cilexetil are three prodrugs and must be activated to their active forms in the liver and digestive system. Telmisartan, valsartan, irbesartan, and eprosartan are not prodrugs and do not need metabolic activation. With a mean half-life of 24 hours, telmisartan is the longest-acting angiotensin II receptor blocker, although irbesartan has one of the highest bioavailabilities in this class.[3]

2.METHODOLOGY

Chromatographic Conditions: ACN–5 mM sodium acetate (70:30, v/v; pH adjusted to 3.5 with acetic acid) was used as the mobile phase at a flow rate of 1.0 mL/min in an isocratic elution method for the chromatographic separation of ROS and CAN using a Waters C18 column (250 × 4.6 mm, 5µm) shielded by a pre-column cartridge. A 50 µL injection volume was maintained. At 254 nm, the analytes were measured.[4]

Preparing the Sample: The following solid-phase extraction method was employed to remove the analytes from human plasma: After adding 20 µL of analyte spiking standard and 20 µL of internal standard to 180 µL of blank plasma, the mixture was vortexed for 30 seconds. After adding 500 µL of 1% o-phosphoric acid to the spiked plasma samples, the mixture was vortexed for 30 seconds. The Orochem DVB-LP cartridge (30 mg, 1 mL) was filled with this solution after it had been conditioned (with 1 mL of methanol and 1 mL of triple distilled water)[5].One milliliter of triple-distilled water, one milliliter of 10% v/v methanol in water, and one milliliter of acetonitrile were used to elute it. The eluate was dried off by evaporation under a nitrogen stream at 40°C. 50 µL of the reconstituted solution was put into the HPLC apparatus after the residue was reconstituted using 200 µL of mobile phase.

System appropriateness: By analyzing the values of peak area (% CV), asymmetry, theoretical plate, and retention time of six standard duplicates, the new method's appropriateness for aqueous LQC samples was examined. The six peak areas' percentage CV should be ≥2; however, in order to meet the system appropriateness requirements, theoretical plates must be greater than 2000 and asymmetry must be ≥2 for each of the six standard replicates.Selectivity, linearity, precision, accuracy, carry-over effect, matrix effect, recovery, dilution integrity, and stability studies are among the criteria that were used to validate the developed bio-analytical method in accordance with USFDA recommendations (US Food and the Drug Administration. Guidelines for Industry, Validation of Bioanalytical Methods).[6-9]

Stability Studies: To assess the analyte stability in stock solutions and in plasma samples under various circumstances, stability experiments were carried out. By comparing the area response, the stock solution stability at refrigerated settings (2–8°C for 7 days) was assessed. At LQC and HQC levels, three replicates were used for benchtop stability (25°C for nine hours), processed sample stability (2–8°C for seventy-two hours), freeze-thaw stability (three cycles), auto-sampler stability (25°C for twenty-four hours), and long-term stability (-20°C for thirty days). When a sample's mean percentage difference in concentration was within ±15% of that of freshly processed samples, it was deemed stable.[10-15]

3.RESULTS

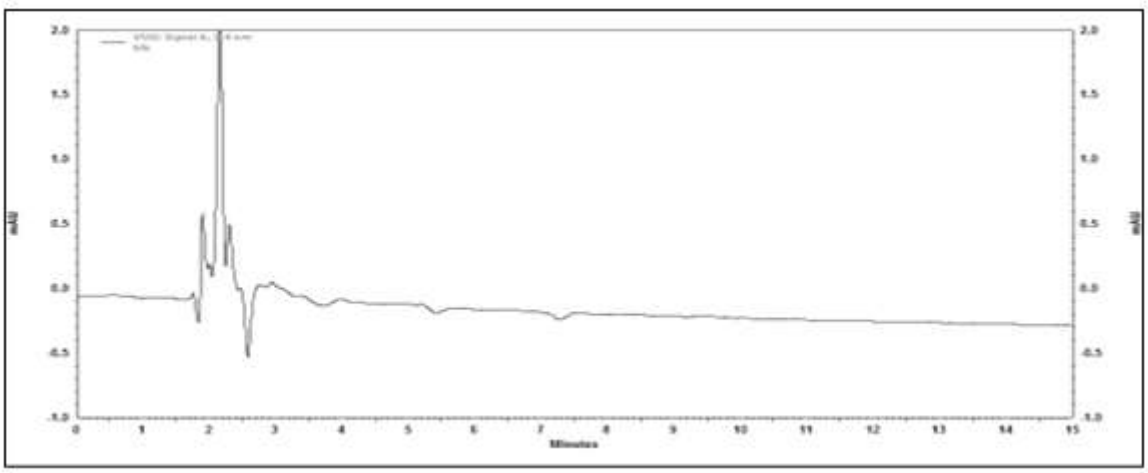

Figure No.1: HPLC chromatogram of blank rat sample

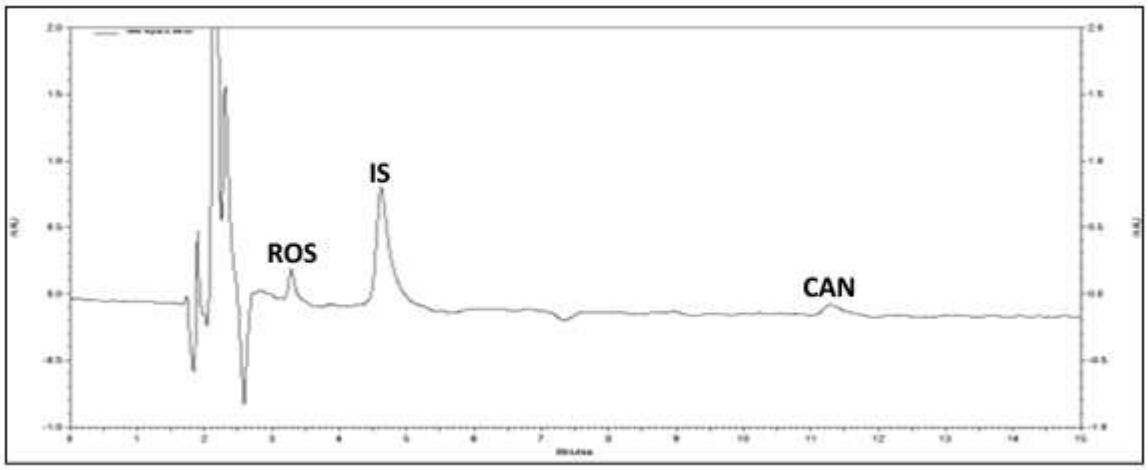

Figure No.2: HPLC chromatogram of ROS and CAN in rat sample

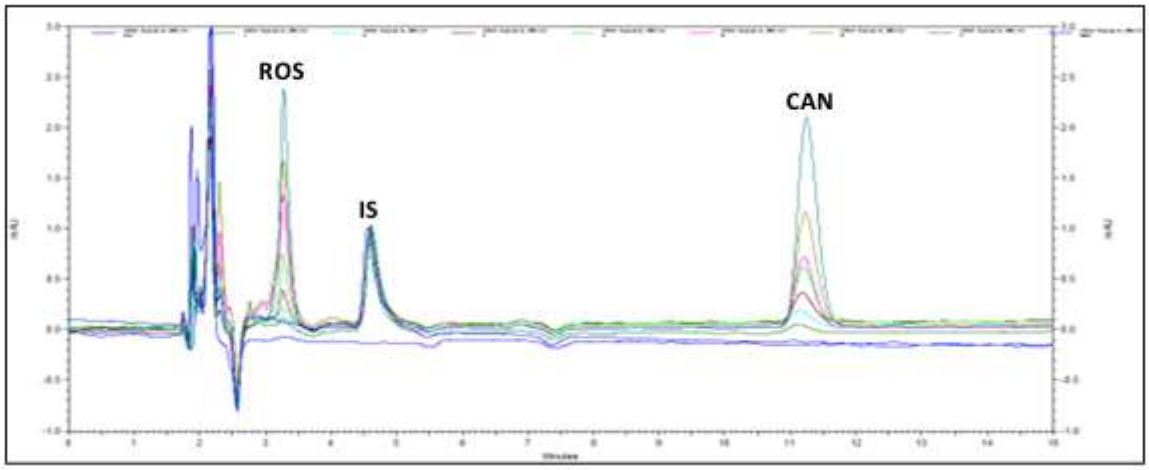

Figure No.3: HPLC chromatogram of calibration curve consisting of 7 non-zero standards along with blank and zero sample for ROS, CAN and IS in rat plasma

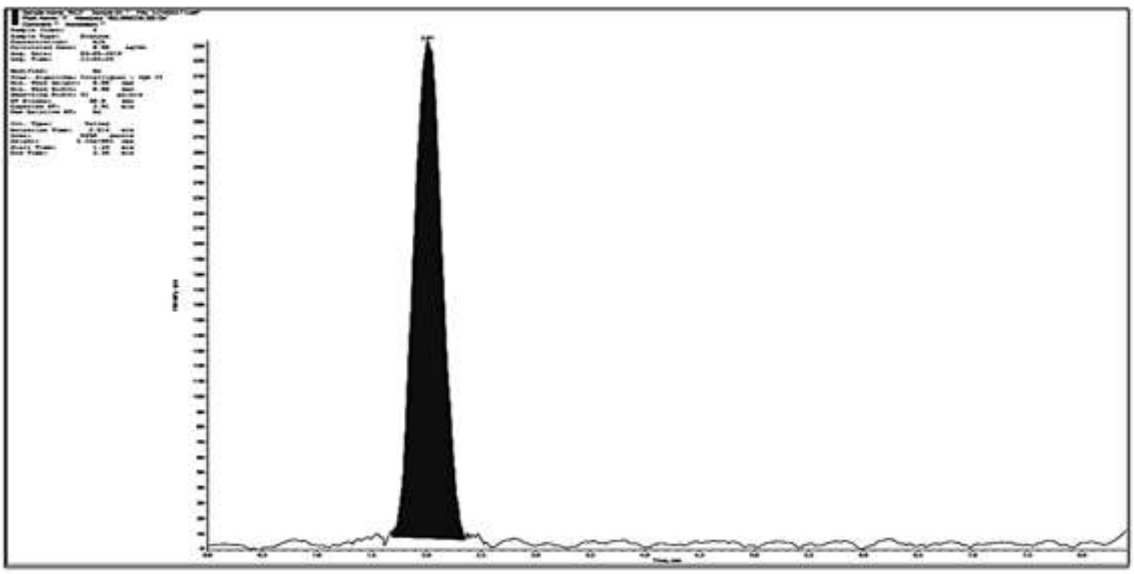

Figure No.4: LC-MS/MS chromatogram of ROS in rat plasma monitored at transition m/z 482.3/258.3

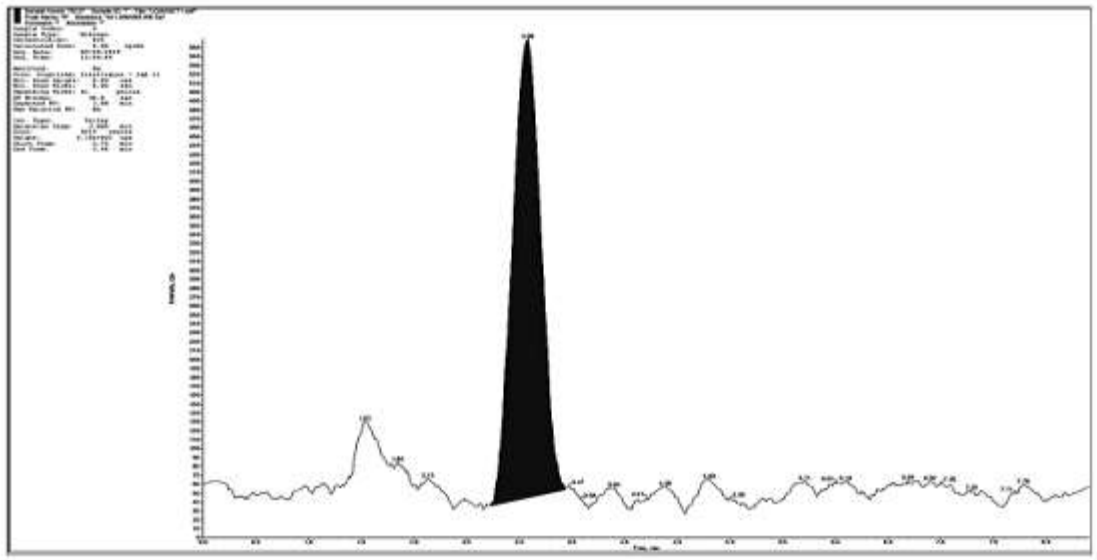

Figure No.5: LC-MS/MS chromatogram of CAN in rat plasma monitored at transition m/z 441.2/263.2

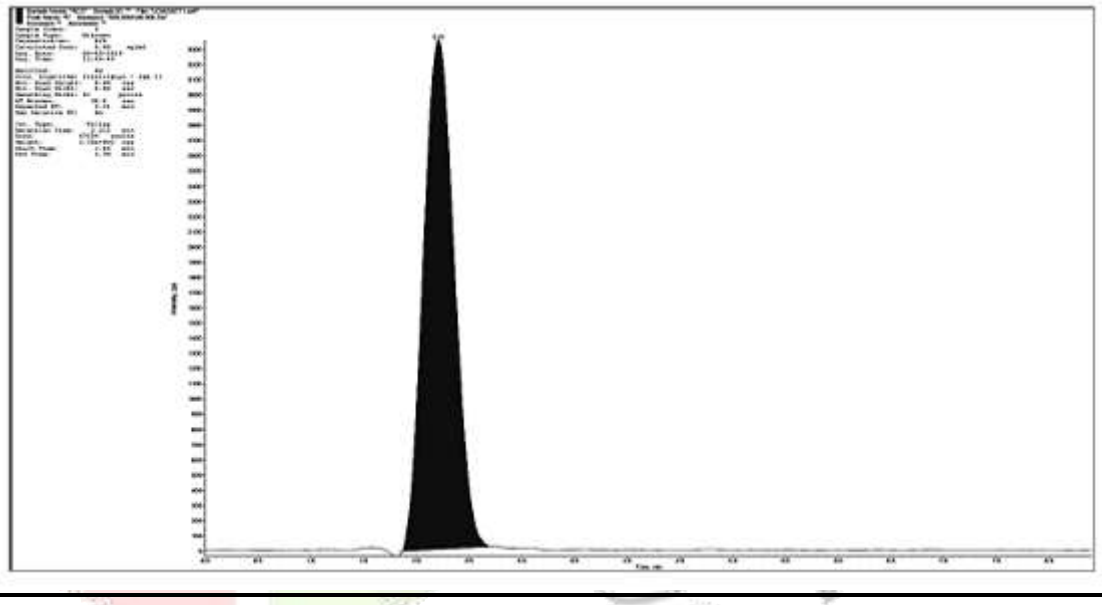

Figure No.6:LC-MS/MS chromatogram of IS monitored in rat plasma at transition m/z 557.4/278.1

For ROS and CAN, calibration standards were made and examined at seven distinct concentrations ranging from 5–150 ng/mL and 10–300 ng/mL, respectively. The ROS and CAN calibration curve showed a strong linearity relationship. For both CAN (y=0.0105x+0.003, r2=0.9995) and ROS (y=0.0106x + 0.0376, r2=0.9996) and the calibration curve data, which include back calculated % Mean nominal concentrations and %CV values of the seven concentrations examined in triplicate. Calibration standards satisfy the necessary requirements since all calibrators do not deviate from nominal concentrations by more than 15% and the percentage CV of all calibrators is less than 15%.

Precision and Accuracy: The outcomes of precision and accuracy both within and between days. Both intra-day and inter-day CV values were less than 15%, suggesting that the suggested approach was accurate. Additionally, the weighted-in QC mean values for both intra-day and inter-day were within 15% of the theoretical value, demonstrating the accuracy of the approach.

4.DISCUSSION

Both human and rat plasma were used to validate the procedure in accordance with US-FDA criteria. All computations were based on the analyte/IS response ratio, and atorvastatin was chosen as the internal standard. There were no notable rises in the retention time of analytes and IS to guarantee the method's selectivity. For both analytes in human plasma, the calibration curve was shown to be linear in the range of 10–150 ng/mL. However, in rat plasma, it was discovered to be linear in the range of 5-150 ng/mL for ROS and 10-300 ng/mL for CAN. At the four QC levels—LLOQ, LQC, MQC, and HQC—accuracy and precision were found to be within acceptable bounds.

For both analytes, a reliable and repeatable recovery of above 85% was discovered. There was no discernible matrix effect, and the carryover effect was considered negligible. It was discovered that the study samples were stable. following three freeze-thaw cycles at -20±5°C and nine hours at room temperature. The stock solution was shown to be stable for seven days at 2-8°C, whereas the processed samples were found to be stable for seventy-two hours. Additionally, the study samples were confirmed to be stable at -20°C for 30 days and in an auto-sampler for 72 hours.

The pharmacokinetic investigation in Wistar rats was successfully conducted using the validated method. The medications were administered both separately and in combination at doses of 1.6 mg/kg of candesartan cilexetil and 4 mg/kg of rosuvastatin. The dosage is equal to 40 mg of rosuvastatin for humans. In healthy wistar rats, the pharmacokinetic profiles of ROS and CAN given as monotherapy were similar to those of combination treatment, indicating that concurrent administration has no effect on individual pharmacokinetic characteristics.

At a dose of 0.4 mg/kg pitavastatin and 1.6 mg/kg candesartan cilexetil (human equivalent dose of 4 mg/kg pitavastatin and 16 mg/kg candesartan cilexetil), the technique was effectively used to examine the pharmacokinetic interaction between PIT and CAN in wistar rats. According to the mean plasma concentration-time curves of PIT and CAN, the pharmacokinetic characteristics of single PIT and CAN are comparable to those of co-administration (P value > 0.05). The pharmacokinetic interaction of PIT and CAN

in rat plasma following a single oral dosage is being studied for the first time using the method described here. Co-administration had no effect on their unique pharmacokinetic characteristics, supporting their clinical reasonable usage.

Our approach offers proof for the clinically sound use of both ROS and PIT with CAN in cardiovascular patients. Therefore, it is safe to investigate both of the aforementioned combinations further as combinational therapy for the treatment of cardiovascular disease.

Additionally, the method has the benefit of using a straightforward mass-compatible HPLC technology to simultaneously determine two clinically significant and often given cardiovascular medications (ROS/CAN and PIT/CAN) in a single chromatographic run. Major clinical laboratories can use this, and it can also be used in pharmacokinetic, bioequivalency, and therapeutic monitoring investigations.

5.CONCLUSION

For the simultaneous measurement of rosuvastatin and candesartan in rat plasma, a straightforward, sensitive, quick, mass-compatible, and economical bio-analytical approach utilizing RP-HPLC/UV was successfully established. Every validation parameter was determined to be within acceptable bounds. The ethical concerns are justified because only 100 µL of rat plasma were needed. The technique showed that individual PK characteristics were notably unaltered by co-administration when it was used to examine the pharmacokinetic interaction of ROS and CAN in Wistar rats. This data supports the clinically sound application of ROS and CAN in cardiovascular patients.

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