Simultaneous Estimation of Atorvastatin Calcium, Aspirin, Ramipril and Metoprolol Tartrate in Bulk and in its Capsule Formulation by First Order Derivative Spectrophotometry

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ABSTRACT

Aim The extensive literature survey revealed that number of methods was reported but there is no method was reported for the simultaneous estimation of Atorvastatin Calcium, Aspirin, Ramipril and Metoprolol tartrate in combination by UV-spectroscopy.

Method The use of first order derivative spectrophotometry allowed simultaneous estimation of Atorvastatin Calcium, Aspirin, Ramipril and Metoprolol tartrate in fixed dose combination products. The absorbance values at 291.5 nm, 247 nm, 242.5 nm and 229.5 nm of first order derivative spectrum was used for the estimation of Atorvastatin Calcium, Aspirin, Ramipril and Metoprolol Tartrate, respectively without mutual interference.

Results and Conclusion This method obeyed Beer’s law in the concentration of 3 – 21 µg/ml, 10 – 70 µg/ml, 10 – 70 µg/ml and 10 – 70 µg/ml of Atorvastatin Calcium, Aspirin, Ramipril and Metoprolol Tartrate, respectively. A t-test indicated that calibration graphs were adequately linear at the evaluated concentration range. The results of analysis have been validated statistically and recovery studies confirmed the accuracy of the proposed method.

Keywords: Atorvastatin Calcium, Aspirin, Ramipril, Metoprolol Tartrate, Methanol and First derivative Spectrophotometry.

INTRODUCTION

Atorvastatin Calcium (ATR) is an oral anti-lipimic agent and chemically it is (βR, 8R)-2-(4-fluorophenyl)-α, δ-dihydroxy-5-(1-methylethyl)-3-phenyl-4-phenyl amino) carbonyl]-1H-pyrrrole-1-heptanoic acid tri hydrate. Aspirin (ASP) is an oral Analgesic; anti pyretic; anti-inflammatory; anti-thrombotic agent and chemically it is 2-(Acetyloxy) benzoic acid. Ramipril (RAM) is an oral anti-hypertensive agent and chemically it is (2S.3aS.6aS) -1-[(S)-N-[(S)-1-Carboxy-3- phenyl propyl] alanyl] octahydro cyclopenta [b] pyrrole-carboxylic acid 1-ethyl ester. Metoprolol Tartrate (MET) is an oral Anti hypertensive; anti angina; anti arrhythmic agent and chemically it is (RS)-1-(isopropylamino)-3-p-(2-methoxyethyl) phenoxypropan-2-ol (2R, 3R)-tartrate [1, 2] all the four drugs are official in IP [3] and The Extra Pharmacopeia [4]. ASP, RAM and MET are official in BP [5]. ASP and RAM are official in USP [6].

The extensive literature survey revealed that number of methods was reported as two component [7-11] and three components [12-16] formulations. A stability indicating UHPLC method was reported for the simultaneous estimation of ATR, ASP, RAM and MET in combined dosage form [17]. But there is no method was reported for the simultaneous estimation of ATR, ASP, RAM and MET in combination by UV-spectroscopy. Hence the purpose of this research work reported here was to develop a simple, accurate,
specific, and precise First derivative UV-spectrophotometric method in bulk and in combined capsule dosage form. The proposed method was validated as per ICH guidelines [18, 19].

EXPERIMENTAL

Materials:
ATR, ASP, RAM and MET were gift samples from Madras Pharmaceuticals, Chennai. The commercial fixed dose combination product (ZYCAD-4® containing 10 mg ATR, 75 mg ASP, 5 mg RAM and 50 mg MET was procured from local market. Methanol AR grade was procured from Qualigens India Pvt Ltd., Mumbai and Distilled Water was obtained from double distillation unit in our laboratory.

Equipments:
A Shimadzu – 1700 Double beam UV-Visible Spectrophotometer (Shimadzu Corporation, Kyoto, Japan) with a pair of 1 cm matched quartz cells and Elico SL 210 UV-Visible Double beam Spectrophotometer (Elico Corporation, Hyderabad, India) were employed in this investigation. All weighing was done on a Shimadzu analytical balance (Model AU-220).

PROCEDURE

Development of the method:
The solutions of ATR, ASP, RAM and MET were prepared separately in methanol and further dilution with distilled water at a concentration of 10 µg/ ml. They were scanned in the wavelength range of 200 – 400 nm. Data were recorded at an interval of 1 nm. The overlain zero order spectra are shown in fig.1. By observing the spectral characters of ATR, ASP, RAM AND MET the methods used for the multi component analysis viz. simultaneous equation method, absorption correction method and absorption ratio method were not applied, because the interference were more. Hence the normal curve was derivitized to first order. From the overlain spectra 291.5 nm, 247 nm, 242.5 nm and 229.5 nm were selected for the simultaneous estimation of ATR, ASP, RAM AND MET, respectively.

Linearity:
Standard stock solution was prepared by dissolving 15 mg of ATR in 10 ml volumetric flask and the volume made up with methanol to get a concentration of 1.5 mg/ ml of ATR. 50 mg of ASP, RAM and MET in 50 ml volumetric flask separately and the volume made up with methanol to get the concentrations of 1 mg/ ml of each drug. From this, suitable dilutions were made with distilled water to get the concentration range of 3 – 21 µg/ ml, 10 – 70 µg/ ml, 10 – 70 µg/ ml and 10 – 70 µg/ml for ATR, ASP, RAM and MET, respectively.

Six replicate analyses were carried out. Absorbance Vs concentration were plotted to obtain the calibration graph.

Limit of Detection and Limit of Quantification:
LOD and LOQ were calculated from the data obtained from the linearity studies (ICH guidelines) [19, 20]. The slope of the linearity plot was determined. For the six replicate determinations, y intercept was calculated and standard deviation of the y intercept was computed. From these values, LOD and LOQ were calculated as follows,

\[ \text{LOD} = \frac{3.3\sigma}{s} \]
\[ \text{LOQ} = \frac{10\sigma}{s} \]

Analysis of Synthetic Mixture:
4 µg/ ml of Atorvastatin Calcium and 30 µg/ ml of Aspirin, Ramipril and Metoprolol Tartrate were prepared individually from their corresponding stock solutions. 1 to 5 ml were pipetted out from each stock solution into a series of six 100 ml volumetric flasks and made up to 100 ml with distilled water to get a mixture of ATR, ASP, RAM, and MET in the concentration range of 4 to 20 µg/ ml for ATR and 30 to 70 µg/ ml for ASP, RAM, and MET. The absorbances of the prepared synthetic mixture were measured at the selected wavelengths. The amount of drugs in the prepared synthetic mixture was calculated.

Analysis of Marketed Formulation:
Twenty capsules were accurately weighed; the capsule containing powder was crushed in to a fine powder. The quantity of the mixed contents of the capsule powder equivalent to 25 mg of ASP was taken in to 25 ml volumetric flask. To this added 15 mg/ ml of RAM and dissolved the drugs in methanol by sonication. Then the solution was made up to volume with methanol and filtered. Further dilutions were made with distilled water so that the theoretical concentrations of 4 µg/ ml, 30 µg/ ml, 20 µg/ ml and 20 µg/ ml of ATR, ASP, RAM and MET, respectively. The absorbances of solutions were measured at 291.5 nm, 247 nm, 242.5 nm and 229.5 nm. The procedure was repeated for six times. The amount of each drug was calculated.

Recovery Studies:
To determine the accuracy of the method, recovery study was performed using the method of standard addition. To the preanalysed marketed capsule formulation powder equivalent to 25 mg of ASP, an accurately weighed quantity of raw material was added at 3 levels viz. 60%, 90% and 120% for ATR, RAM and MET and 80%, 100% and 120% for ASP. The
procedure was repeated as per the analysis of formulation. The amount of drug recovered was calculated.

RESULTS AND DISCUSSIONS

A simple precise accurate first derivative UV spectrophotometric method was developed for the estimation of ATR, ASP, RAM and MET in bulk and in combined capsule dosage form. The UV spectrum of ATR, ASP, RAM and MET were scanned in the wavelength range of 200 – 400 nm (Fig. 1).

By observing the spectral characters of ATR, ASP, RAM and MET, the methods used for the multi component analysis viz. simultaneous equation method, absorption correction method and absorption ratio method were not applied, because the interference were more. Hence the normal curve was derivitized to first order, and overlained. (Fig. 2), from the overlain spectra 291.5 nm, 247 nm, 242.5 nm and 229.5 nm were selected for the simultaneous estimation of ATR, ASP, RAM and MET, respectively. At 291.5 nm, ATR has the absorbance where as ASP, RAM and MET has no absorbance. Hence this wavelength was selected for the analysis of ATR without the interference of other three drugs. At 247 nm ATR and ASP were showed marked absorbance whereas RAM and MET having zero crossing points. The absorbance of ATR was interfered in the analysis of ASP. Hence, the absorbance of ATR was corrected for interference from the total absorbance value. With the help of corrected absorbance the amount of ASP was calculated at 247 nm. At 242.5 nm ATR, ASP and RAM were showed absorbance and MET have zero crossing point. The absorbance of ATR and ASP were interfered in the analysis of RAM. Hence, the absorbance of ATR and ASP were corrected for interference from the total absorbance value. With the help of the corrected absorbance, the amount of RAM was calculated at 242.5 nm. At 229.5 nm ATR, ASP, RAM and MET were showed the absorbance of ATR, ASP and RAM were interfered in the analysis of MET. Hence, the absorbance of ATR, ASP and RAM were corrected for interference from the total absorbance value. The corrected absorbance was used for the analysis of MET at 229.5 nm.

The absorption in first order derivative mode of ATR, ASP, RAM and MET at respective wavelengths 291.5 nm, 247 nm, 242.5 nm and 229.5 nm were linear in the concentration range of 3 – 21 µg/ ml, 10 – 70 µg/ ml, 10 – 70 µg/ ml and 10 – 70 µg/ ml and the correlation coefficient values were found to be 0.9999, 0.9968, 0.9996 and 0.9998 for ATR, ASP, RAM and MET, respectively. Visual observation of the calibration curve gave the impression that they were linear. A student’s t-test was performed to determine whether the experimental intercept (c) was not significantly different from the theoretical zero value. It concerns the comparison of \( t = c/s_C \), where c is the intercept of the regression equation and \( s_C \) is standard deviation of c, with tabulated data of the t-distribution. As the calculated t-value (\( t_{ATR}=0.3277 \), \( t_{ASP}=0.3297 \), \( t_{RAM}=0.1667 \) and \( t_{MET}=0.4437 \)), does not exceed to (0.05%) 2.5335, the intercept of regression equation is not significantly differ from zero. From the above it is observed that though the straight line model is correct for the considered calibration ranges, the intercept of the calibration lines were not significantly different from zero. The t value indicates the obtained intercept value were accurate, thus the calibration graphs were linear (Fig. 3-6).

As per ICH guidelines, LOD and LOQ can be determined using visual evaluation, signal to noise ratio or from the slope of linearity plot and standard deviation. Visual evaluation may be used in non instrumental methods and signal to noise ratio is normally possible with chromatographic methods. Hence, the method based on determination of slope of linearity plot and standard deviation of y intercept of
Fig. 3 Calibration Curve for MET at 229.5 nm

Fig. 4 Calibration Curve for ASP at 247 nm

Fig. 5. Calibration Curve for RAM at 242.5 nm
linearity was used for the determination of LOD and LOQ and found to be 0.6176 µg/ ml and 1.8714 µg/ ml for ATR, 0.1432 µg/ ml and 0.4343 µg/ ml for ASP, 1.6587 µg/ ml and 5.0263 µg/ ml for RAM and 0.1310 µg/ ml and 0.3969 µg/ ml for MET (Table 1).

To study the mutual interference if any, in the simultaneous estimation of ATR, ASP, RAM and MET, synthetic mixtures containing various proportions of ATR, ASP, RAM and MET were prepared and the contents was estimated by the proposed method. The percentage recovery varied from 98.00 - 100.87%, 98.16 - 100.36%, 99.22 - 100.84% and 100.23 – 101.21% for ATR, ASP, RAM and MET, respectively indicating that no mutual interference up to the ratio of 2: 15: 1: 10 for drugs (Table 2). The high percentage recovery indicated that there is no interaction between the drugs present in synthetic mixture and hence the method can be applied for the simultaneous estimation of ATR, ASP, RAM and MET in formulation.

Precision of the method was determined by performing repeatability and intermediate precision. Repeatability of the method was done by the repeated analysis of formulation for six times. The amount of drugs in the formulation was found to be 10.27 ± 1.4218 for ATR, 74.25 ± 1.5833 for ASP, 49.33 ± 0.8496 for MET. The percentage RSD values were found to be 1.3842, 1.5992, 1.7116 and 0.8612 for ATR, ASP, RAM and MET, respectively. Further the ANOVA test confirmed the significance of the results (Table. 3). Intermediate precision of the method was confirmed by intraday and inter day analysis. The analysis of formulation was done three times on the same day and one time on three consecutive days. The precision of the method was confirmed by low %RSD values for Intraday and Inter day analysis. The %RSD for Intraday and Inter day analysis were found to be 0.1528 and 0.0680 for ATR, 0.8116 and 0.3145 for ASP, 1.9063 and 1.1612 for RAM and 1.0513 for MET, respectively.

### Table No.1 – Linear Regression Analysis data

| PARAMETERS                        | ATR* | ASP* | RAM*  | MET* |
|-----------------------------------|------|------|-------|------|
| Beer’s law limit (µg/ml)          | 3 - 21 | 10 – 70 | 10 - 70 | 10 - 70 |
| Molar Absorptivity (L mol⁻¹ Cm⁻¹) | 740.67 | 117.52 | 66.66 | 672.11 |
| Sandell’s Sensitivity (µg/ Cm⁻²/0.001 A.U.) | 1.5960 | 1.0017 | 6.1907 | 0.3742 |
| Correlation Coefficient (r)       | 0.9999 | 0.9968 | 0.9996 | 0.9998 |
| Slope (m)                         | 0.0006 | 0.0009 | 0.0004 | 0.0027 |
| Intercept (c)                     | -0.0001 | -0.0035 | -0.0001 | -0.0016 |
| LOD (µg/ ml)                      | 0.6176 | 0.1432 | 1.6587 | 0.1310 |
| LOQ (µg/ ml)                      | 1.8714 | 0.4343 | 5.0263 | 0.3969 |
| t-test                            | 0.3277 | 0.3297 | 0.1667 | 0.4437 |

*Average of six observations

![Fig. 3 Calibration Curve for ATR at 291.5 nm](image-url)
Table No.2 - Analysis of Synthetic Mixture

| Drug | Concentration prepared (µg/ml) | Amount Found (µg/ml) | Percentage Purity* | Average (%) | SD | RSD | SE |
|------|---------------------------------|----------------------|--------------------|-------------|----|-----|----|
| ATR  | 4                               | 3.92                 | 98.00              |             |    |     |    |
|      | 8                               | 8.07                 | 100.87             |             |    |     |    |
|      | 12                              | 12.06                | 100.50             | 99.78       | 1.1481 | 1.1506 | 0.0459 |
|      | 16                              | 15.89                | 99.31              |             |    |     |    |
|      | 20                              | 20.04                | 100.20             |             |    |     |    |
|      | 30                              | 30.11                | 100.36             |             |    |     |    |
|      | 40                              | 39.60                | 99.00              |             |    |     |    |
| ASP  | 5                               | 49.30                | 98.60              | 99.15       | 0.8646 | 0.8720 | 0.0346 |
|      | 60                              | 58.90                | 98.16              |             |    |     |    |
|      | 70                              | 69.74                | 99.62              |             |    |     |    |
| RAM  | 30                              | 30.16                | 100.53             | 100.07      | 0.7101 | 0.7096 | 0.0284 |
|      | 40                              | 39.77                | 99.42              |             |    |     |    |
|      | 50                              | 50.42                | 100.84             | 100.71      | 0.4135 | 0.4106 | 0.0165 |
|      | 60                              | 60.20                | 100.33             |             |    |     |    |
|      | 70                              | 69.46                | 99.22              |             |    |     |    |
| MET  | 30                              | 30.07                | 100.23             |             |    |     |    |
|      | 40                              | 30.07                | 100.23             |             |    |     |    |
|      | 50                              | 50.43                | 100.86             | 100.71      | 0.4135 | 0.4106 | 0.0165 |
|      | 60                              | 60.73                | 101.21             |             |    |     |    |
|      | 70                              | 70.24                | 100.34             |             |    |     |    |

*Average of six determinations

Table No.3 – Analysis data of formulation

| Drug | Labelled amount (mg/tab) | Amount found (mg/tab) | Percentage Obtained* | Average (%) ± SD | % RSD | SE | CI* | F-value |
|------|--------------------------|-----------------------|----------------------|------------------|-------|----|-----|---------|
| ATR  | 10                       | 10.18                 | 101.80               | 102.71 ± 1.4218  | 1.3842 | 0.039 | 101.22 to 104.20 | 2.234 |
| ASP  | 75                       | 73.94                 | 98.58                | 99.00 ± 1.5833  | 1.5992 | 0.043 | 97.34 to 100.66 | 0.050 |
| RAM  | 5                        | 5.09                  | 101.80               | 99.90 ± 1.7116  | 1.7116 | 0.047 | 98.10 to 101.69 | 0.073 |
| MET  | 50                       | 49.22                 | 98.44                | 98.65 ± 0.8612  | 0.8612 | 0.023 | 97.75 to 99.54 | 0.542 |

*Mean of six observations, CI* = confidence interval (95 %), $F_{tab}(\alpha=0.05; d(1=5, d(2=40))=2.5335$
The ruggedness of the method was validated by using different analysts and different instruments. The percentage RSD for analyst 1 and analyst 2 were found to be 1.3842 and 0.2887 for ATR, 1.5954 and 1.8396 for ASP, 1.7256 and 1.8602 for RAM and 0.8611 and 0.8367 for MET, respectively. The percentage RSD for instrument 1 and instrument 2 were found to be 1.1602 and 1.5153 for ATR, 0.6684 and 0.4462 for ASP, 1.9820 and 1.0782 for RAM and 1.2887 and 0.8223 for MET, respectively (Table 4).

The accuracy of the method was confirmed by recovery studies. ATR, ASP, RAM and MET were added to pre analyzed capsule powder in to three levels. Six replicate analyses were carried out for each level. The percentage recovery was found to be 99.76 -100.45% for ATR, 100.38 -101.08% for ASP, 98.58 -101.64% for RAM and 98.33 -101.69% for MET.

The %bias indicated that the obtained results were in good coordination and %RSD values were found to be 0.3508, 0.3506, 1.5580 and 1.8860 for ATR, ASP, RAM and MET, respectively. The low percentage RSD indicated that there was no interference due to excipients used in formulation (Table 5).

**CONCLUSION**

The method described was found to be simple, precise, accurate, and rugged were confirmed by low %RSD values. High percentage recovery indicates that the excipients used in formulation were not interfering in the analysis of formulation. Hence, the developed method can be effectively applied for the routine quality control analysis of ATR, ASP, RAM and MET in bulk and in combined capsule dosage form.

| S.No | Parameters                      | Experimental | Limit as per ICH guidelines |
|------|--------------------------------|--------------|-----------------------------|
| 1    | Intermediate Precision (%RSD) b | ATR 0.1528   | <2                          |
|      |                                | ASP 0.0680   |                             |
|      |                                | RAM 1.3842   | <2                          |
|      |                                | MET 1.0602   |                             |
| 2    | Ruggedness (%RSD) a            | ATR 1.3842   | <2                          |
|      |                                | ASP 0.2887   |                             |
|      |                                | RAM 1.0602   | <2                          |
|      |                                | MET 1.5153   |                             |

\( a\)- Average of six determinations, \( b\)-Average of three determinations

**Table No.5 – Recovery analysis data**

| Drug | Amount present (µg/ml) | Amount Added (µg/ml) | Amount Recovered (µg/ml) | Mean % Recovery ± SD* | RSD | Bias |
|------|------------------------|----------------------|--------------------------|-----------------------|-----|------|
| ATR  | 4.1333                 | 2.4799               | 2.4911                   | 100.40 ± 0.3513       | 0.3508 | 0.40 |
| ASP  | 29.8667                | 23.8933              | 23.9848                  | 100.70 ± 0.3530       | 0.3506 | 0.70 |
| RAM  | 20.1010                | 12.0606              | 11.8895                  | 100.29 ± 1.526        | 1.5580 | 0.29 |
| MET  | 19.8492                | 11.9095              | 11.7383                  | 99.52 ± 1.8770        | 1.8860 | 0.48 |

* Average of three determinations
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