A NEW VALIDATED THIRD ORDER DERIVATIVE SPECTROSCOPIC METHOD FOR SIMULTANEOUS ESTIMATION OF METOPROLOL SUCCINATE AND RAMIPRIL IN TABLET DOSAGE FORM

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ABSTRACT

Objective: The objective of the present work is to develop and validate a new UV derivative spectrophotometric method for simultaneous estimation of metoprolol succinate and ramipril in methanol: water (50:50v/v).

Methods: “Zero crossing technique” was chosen for quantitative determination. The zero-crossing points (ZCP’s) were found to be 209 nm where metoprolol succinate was quantified and 211 nm where ramipril was quantified. This method was then subjected to accuracy, linearity, sensitivity and reproducibility according to ICH guidelines to ensure and confirm its validity.

Results: The method was found to be obeying Beer’s law in the range of 10-50 µg/ml and 5-25 µg/ml for metoprolol succinate and ramipril, respectively. The % recoveries were observed between the range of 99.2-100.2 for metoprolol succinate and 99.57-99.86 for ramipril. The intra-day and inter-day results showed reproducibility.

Conclusion: It can be concluded that the developed third-order UV derivative spectroscopic method for the simultaneous determination of metoprolol succinate and ramipril can be recommended for routine quantitative analysis.

Keywords: Third order, UV derivative spectroscopy, Metoprolol succinate, Ramipril, Validation, Zero crossing technique

INTRODUCTION

Metoprolol succinate is chemically known as 1-[4-(2-methoxyethyl)phenoxy]-3-(propan-2-ylamino)propan-2-olbutanedioate (fig. 1). It competes with adrenergic neurotransmitters such as catecholamines for binding at β1-adrenergic receptors in the heart that result in a decrease in heart rate, blood pressure and cardiac output [1, 2].

Ramipril as shown in (fig. 2) is chemically (2S,3aS,6aS)-1-[(2S)-2-[(2S)-1-ethoxy-1-oxo-4-phenylbutan-2-yl]amino]-3,3a,4,5,6,6a-hexahydro-2H-cyclopenta[b]pyrrole-2-carboxylic acid, acts by inhibiting angiotensin-converting enzyme (ACE), thereby lowers the production of angiotensin II and also decreases the breakdown of bradykinin. The decrease in angiotensin II results in lowering total peripheral resistance, widening the blood vessels and hence decreases the blood pressure [3]. Commercial brands of metoprolol succinate and ramipril are available and have been prescribed to the patients who are suffering from myocardial infarction, nephropathy, angina and congestive heart failure [4].

Literature survey revealed that metoprolol succinate and ramipril can be estimated either individually or combinedly by different methods like HPLC [5-7], LC [8], RP-UPLC [9], UV-Spectroscopy [10, 11]. However, simultaneous estimation of these drugs by derivative spectrophotometric method was not reported till date. Hence a third order derivative spectrophotometric method was developed for the simultaneous estimation of these drugs for the first time. The developed UV derivative method was validated according to the ICH guidelines.

MATERIALS AND METHODS

Instrument

A double beam Agilent Cary UV spectrophotometer with Cary WinUV software (version 5.0.0.999) having a wavelength range of 190-1100 nm and 1 cm quartz cell was used for all the spectral studies. A calibrated Shimadzu BL 220H weighing balance was used for accurate weighing of the chemicals. The statistical calculations were done by using Microsoft excel 2010 version.

Chemicals and reagents

Analytically pure metoprolol succinate and ramipril from Yarrow chemicals, Mumbai were used. PROLOMET R 50 tablets (in the dose of 50 mg metoprolol succinate and 5 mg ramipril) manufactured by Sun Pharma were procured and used for the investigation.

Preparation of metoprolol succinate standard stock solution

25 mg of metoprolol succinate was accurately weighed and transferred into a 25 ml volumetric flask and made up with methanol and water in the ratio of 50:50 v/v to get 1000 µg/ml solution. From the above stock solution different working standards in the range of 10-50 µg/ml were prepared.
Preparation of ramipril standard stock solution

25 mg of ramipril was accurately weighed and transferred into a 25 ml volumetric flask and made up with methanol and water in the ratio of 50:50 v/v to get 1000 µg/ml solution. From the above stock solution different working standards in the range of 5-25 µg/ml were prepared.

Method development

For quantitative estimation, UV derivative spectroscopic method using zero crossing technique was chosen [12]. The working standard solutions containing 30 µg/ml of metoprolol succinate and 15 µg/ml of ramipril were scanned in the wavelength range of 200-400 nm using methanol and water solvent (50:50 v/v) as reference in derivative mode at bandwidth of 2 nm and a scan speed of 400 nm/min. It showed wavelength maxima at 215 nm for metoprolol succinate (fig. 3) and 210 nm for ramipril (fig. 4). The isobestic point was found to be 216 nm as shown in (fig. 5). The obtained zero order spectra were converted to first order (fig. 6), second order (fig. 7) and third order spectrum (fig. 8).

Method validation

The developed method was validated in terms of linearity, accuracy, intra-day and inter-day precision studies, detection limit and quantification limit according to ICH guidelines [13, 14].
Linearity

For the third order derivative method, 10-50 μg/ml of metoprolol succinate and 5-25 μg/ml of ramipril were scanned at the working wavelengths of 209 nm and 211 nm for metoprolol succinate and ramipril respectively. Calibration curves were constructed by plotting concentration against the absorbance values and correlation coefficients were calculated from the regression line equations.

Accuracy

To check the accuracy of the developed method, recovery studies were carried out by applying standard addition method. A known amount of standard metoprolol succinate and ramipril corresponding to 80, 100 and 120% of the label claim was added to pre-analyzed sample of the tablet. The recovery studies were carried out at each level three times and the average amount was calculated.
Precision

The precision of the developed method was carried out by intermediate precision. The intraday and interday precisions of the third order derivative method were determined by analyzing responses in triplicate on the same day and on 3 different days over a period of 1 w of metoprolol succinate (50 µg/ml) and ramipril (5 µg/ml).

Sensitivity

The LOD and LOQ values were calculated from the linearity data using Microsoft excel 2010 version. Standard deviation of the analytical response and the slope of the calibration curve method was followed according to the equations, LOD = 3.3σ/S and LOQ = 10σ/S, where σ is the standard deviation of the sample and S is the slope.

Assay of commercial brand

Twenty tablets of PROLOMET R 50 were taken, weighed and finely powdered. The weight equivalent to the labelled claim (55 mg) was taken, 20 ml of solvent (methanol and water 50:50v/v) was added in a 100 ml volumetric flask and sonicated in an ultrasonic bath for 15 min. This solution was then made upto 100 ml using the same solvent, filtered through a 0.45 µm filter and this filtrate was used to prepare sample solution (55 µg/ml). The mixture solution was scanned at the wavelengths of metoprolol succinate (209 nm) and ramipril (211 nm) using a UV-Visible spectrophotometer. These absorbance values were then substituted in the regression equations to calculate the concentration.

RESULTS AND DISCUSSION

Few chromatographic methods have been mentioned in the literature to date for the simultaneous estimation of metoprolol succinate and ramipril in their binary mixtures [6, 8, 15]. Also one spectrophotometric method, based on simultaneous equation has been reported for combination of metoprolol succinate, atorvastatin calcium and ramipril [16]. However, no spectrophotometric method has been reported for metoprolol succinate and ramipril in their fixed dose combination.

Third order derivative method

From the zero order overlain UV spectra of Metoprolol succinate (30 µg/ml) and ramipril (15 µg/ml), it was found that the spectra are overlapping each other, exhibiting the complexity in measuring these drugs by direct UV absorption method in a binary mixture. The UV derivative method has advantage that it removes the spectral interference from one of the two drugs while estimating the other drug at zero crossing point. The first order spectra did not show any zero crossing points (ZCP), while the second order spectra was not informative enough to carry out further experimentation. Hence in the present investigation, third order derivative spectrophotometric techniques was described.

The third order spectrum showed zero crossing points (ZCP) at 211 nm for metoprolol succinate and 209 nm for ramipril. Hence working wave lengths of 209 nm for metoprolol succinate and 211 nm for ramipril were selected.

Table 1: Linearity characters of the developed method

| Statistical parameters | Metoprolol succinate at 209 nm | Ramipril at 211 nm |
|------------------------|-------------------------------|-------------------|
| Linearity (n=5)        | 10-50 µg/ml                   | 5-25 µg/ml        |
| Correlation coefficient (r²) | 0.998                      | 0.997             |
| Regression equation: y=mx+c | y = 0.021x+0.016        | y = 0.063x+0.769 |
| Slope (m)              | 0.021                        | 0.063             |
| Intercept (c)          | 0.016                        | 0.769             |

Fig. 9: Calibration curve of metoprolol succinate

Fig. 10: Calibration curve of ramipril
metoprolol succinate and ramipril in tablet dosage form. The developed method was said to be effective for routine analysis of the drugs. The result of the assay was found to be within the acceptable limits. Hence, the developed method was validated according to the ICH guidelines and was applied to the pharmaceutical dosage form. The result of the assay was found to be effective for routine analysis of metoprolol succinate and ramipril. The developed method was validated according to the ICH guidelines and was applied to the pharmaceutical dosage form. The result of the assay was found to be within the acceptable limits. Hence, the developed method was said to be effective for routine analysis of metoprolol succinate and ramipril in tablet dosage form.

**Validity of the method**

The linearity was found in the range of 10-50 µg/ml and 5-25 µg/ml for metoprolol succinate and ramipril. The calibration curves were shown in (fig. 9 and fig. 10) and the correlation coefficient (r) value was found to be 0.998 and 0.997, respectively. The r² values for both the drugs were found to be less than or equal to 1. Hence the developed method was found to be linear. The % recoveries were found to be 99.2%, 100.02% and 99.18% for metoprolol succinate and 99.5%, 99.4% and 99.81% for ramipril respectively. As the % RSD values were found to be 0.754 for metoprolol succinate and 0.1008 for metoprolol succinate and 95.5% for ramipril.

**CONCLUSION**

A simple, precise, economical and sensitive UV derivative spectroscopic method was developed for the simultaneous estimation of metoprolol succinate and ramipril. The developed method was validated according to the ICH guidelines and was applied to the pharmaceutical dosage form. The result of the assay obtained was found to be within the acceptable limits. Hence, the developed method was said to be effective for routine analysis of metoprolol succinate and ramipril.

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**Table 2: Accuracy of the developed method**

| Spiked level | Amount of sample taken (mg) | Amount of pure drug added (mg) | Amount recovered (mean±SD) | % Recovery±SD
|--------------|----------------------------|-------------------------------|---------------------------|-------------------|
| Metoprolol succinate | Ramipril | Metoprolol succinate | Ramipril | Metoprolol succinate | Ramipril | Metoprolol succinate | Ramipril |
| 80% | 10 | 5 | 8 | 4 | 17.88±0.01 | 8.96±0.01 | 99.32±0.05 | 99.57±0.17 |
| 100% | 10 | 5 | 10 | 6 | 20.1±0.01 | 9.94±0.02 | 100.53±0.07 | 99.4±0.26 |
| 120% | 12 | 6 | 12 | 8 | 21.82±0.02 | 10.98±0.03 | 99.19±0.11 | 99.81±0.27 |

**Table 3: Precision data of the developed method**

| Parameter | Metoprolol succinate (50 µg/ml) | Ramipril (5 µg/ml) |
|-----------|---------------------------------|--------------------|
| n=3       | mean±SDa                         | % RSDb             |
|           | mean±SDa                         | % RSDb             |
| intra-day | 1.1152±0.07 (0.72)               | 1.1142±0.07 (0.72) |
|           | 1.098                           | 1.078              |
| inter-day | 1.1139±0.09 (0.72)               | 1.1139±0.09 (0.72) |
|           | 0.02693                         | 0.02693            |

**Table 4: LOD and LOQ results of the developed method**

| Statistical parameter | Metoprolol succinate (µg/ml) | Ramipril (µg/ml) |
|-----------------------|-------------------------------|-----------------|
| LOD                   | 3.18                          | 1.56            |
| LOQ                   | 9.62                          | 4.68            |

**Table 5: Assay of commercial brand**

| Marketed formulation | Metoprolol succinate | Ramipril |
|----------------------|----------------------|----------|
| PROLOMET (Sun Pharma)| Labeled claim (mg)    | Amount recovered (mg) | % Recovery |
| 50                   | 50                   | 50.8     | 101.6%    |
|                      | Labeled claim (mg)    | Amount recovered (mg) | % Recovery |
|                      | 5                    | 4.94     | 98.8%     |

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**AUTHORS CONTRIBUTIONS**

All authors have equal contribution.
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