WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 6.805
Volume 5, Issue 4, 1501-1508. Research Article ISSN 2277– 7105

DEVELOPMENT AND VALIDATION OF UV SPECTROSCOPIC METHOD FOR ESTIMATION OF MILRINONE IN FORMULATION

Ranjith Kumar D.*, Mohan S., Vanathi P., Prakash G., Sasikala M., Karpakavalli M.

Associate Professor, Karpagam College of Pharmacy, SF No. 762, Othakkalmandapam, Coimbatore–641 032.

Article Received on
08 Feb 2016,
Revised on 29 Feb 2016,
Accepted on 20 Mar 2016
DOI: 10.20959/wjpr20164-5993

***Correspondence for Author**
Ranjith Kumar D.
Associate Professor, Karpagam College of Pharmacy, SF No. 762, Othakkalmandapam, Coimbatore–641 032.

ABSTRACT

Simple, specific, sensitive, reproducible, precised and cost effective UV spectroscopic method for Milrinone in single dosage form has been developed. Milrinone is used in the treatment of congestive heart failure. In the estimation of Milrinone by UV spectroscopy, the λ_{max} was found to be 271nm for Milrinone in solvent methanol. The calibration curve was obtained for Milrinone in the range 1.0-10.0µg/ml. The slope, intercept and correlation coefficient values were found to be 0.0594, 0.0672 and 0.9989 respectively. The 50% and 100% recovery studies were carried out for formulation 1 and formulation 2 by adding known amount of standard drug. The related standard deviation (%RSD) was <2%. The stability studies were carried out and the drug was found to be stable more than 12 hours in solution form. Thus, the developed validated UV spectroscopic method can be used for the routine analysis of Milrinone from its single dosage form in research institutions, laboratories and educational institutions.

KEYWORDS: Milrinone, UV spectroscopy, methanol, validation, % RSD, injection.

INTRODUCTION

Milrinone is chemically 1-6-Dihydro-2-methyl-6-oxo-(3,4-bipyridine)-5-carbonitrile and is used in the treatment of congestive heart failure. The development of UV spectroscopic method for the estimation of Milrinone from its injection dosage form by using calibration curve and the method validation based on ICH guidelines has been followed.

MATERIALS AND METHODS

Drug Sample

Milrinone was obtained as a gift sample from Sanofi- Sythe lab Mumbai.

Chemical and solvent

- Methanol AR grade

Instruments used

- Jasco V-530 UV/VIS Spectrophotometer,
- Elico LI 120 pH meter,
- Shimadzu Digital Electronics Balance – BL220H.

Selection of Solvent

Solubility of the drug was tried in different solvents. Milrinone was found to be soluble methanol and showed a good spectrum with excellent stability. So methanol was selected as the solvent of choice. (Fig. 1).

Preparation of Standard Stock Solutions

Stock solution of 10mg in 100ml was prepared. From this 1ml was taken in a 10ml standard flask and made up to the volume with methanol to get a concentration of 100µg/ml.

Selection of Wavelength

From the stock solution 8µg/ml of Milrinone was prepared separately using methanol. The solution was scanned between 200-400nm and spectra was recorded. Milrinone exhibited maximum absorbance at 271nm (Fig. 2) Hence 271nm was selected for the proposed study.

Preparation of Standard Curves:

Aliquot dilutions were prepared from standard stock solution to get a concentration ranging from 1-10µg/ml using methanol. Absorbance of these solutions was measured at 271nm (Table 1). The measured absorbances were plotted against concentrations. From the graph it was found that Milrinone showed the linearity range between 1-10µg/ml. (Fig. 3).

Analysis of Formulation

Ampoules containing 10mg in 10ml of Milirinone was taken from this quantity equivalent to 1ml was taken and transferred to 10ml standard flask to get concentration range of 100µg/ml. From this 0.5ml and 0.6ml were taken in a 10ml standard flask separately and made up to 10ml with methanol to get concentration range of 5µg/ml and 6µg/ml respectively. The

resultant solutions were scanned in the wave length range of 200-400 nm and the absorbance were measured (Fig. 4). The amount was calculated using single point standardisation method. (Table 2).

Method Validation

The method developed was validated in terms of linearity and accuracy.

Linearity

Milrinone was found to be linear in a range of 1-10µg/ml. The absorbances of these solutions were measured at 271nm, and a calibration graph was plotted using concentrations Vs absorbances. The slope, intercept and correlation coefficient values were found to be 0.0594, 0.0672 and 0.9989 respectively. (Fig. 3).

Accuracy

The accuracy, specificity, suitability and validity of the present method were studied by conducting percentage recovery studies. A known quantity of the pure drug was added to the pre-analyzed sample formulation at 50% and 100% levels. The percentage recovery and standard deviations were calculated. (Table 3).

Precision

Intra-day assay

Intra-day precision was studied by measuring the absorbance of the standard drug solutions repeatedly on the same day. Solutions of 5µg/ml and 6µg/ml were used for the study. %RSDs were calculated which is shown in Table 4.

Inter-day assay

Interday precision was studied by measuring the absorbance of the standard drug solutions repeatedly on different days. Solutions of 5µg/ml and 6µg/ml were used for the study. % RSDs was calculated. (Table 5)

Stability Studies

Stability studies for the drugs were carried out and they were found to be stable at room temperature up to 12 hours which is shown (table 6).

RESULTS

Table 1: Linearity range

Concentration (µg/ml)	Absorbances (271nm)
1	0.1293
2	0.1816
3	0.2446
4	0.3002
5	0.3562
6	0.4402
7	0.4920
8	0.5382
9	0.6063
10	0.6520

Table 2: Analysis of formulation

Drug	Formulation	Amount (mg/amp)		%Label claim ±SD*
		Label claim	Estimated amount	
Milrinone	Formulation 1	1	0.951	95.1± 0.43
	Formulation 2	1	0.945	94.5 ± 0.51

*Mean of six observation

Table 3: Recovery Studies

Drug	Formulation	Amount recovered (mg)/ amp)		% RSD *	
		50%	100%	50%	100%
Milrinone	Formulation 1	98.06	97.99	0.14	0.16
	Formulation 2	98.41	98.5	0.15	0.17

*Mean of six Observations

Table 4: Intraday assay

Drug	Formulation	Concentration [µg/ml]	Absorbance	% RSD *
Milrinone	Formulation 1	5	0.3472 0.3552 0.3434 0.3420	1.7071
		6	0.4485 0.4562 0.4432 0.4412	1.4979
	Formulation 2	5	0.3321 0.3312 0.3301 0.3300	0.3007
		6	0.4268	0.9046

			0.4234 0.4201 0.4182	

*Mean of four determinations.

Table – 5 Inter-day assay

Drug	Formulation	Concentration (µg/ml)	Day	Absorbance	% RSD *
Milrinone	Formulation 1	5	1st	0.3562 0.3550 0.3521 0.3482	1.0088
			2nd	0.3432 0.3431 0.3420 0.3391	0.5593
		6	1st	0.4486 0.4521 0.4462 0.4454	0.6714
			2nd	0.4432 0.4401 0.4312 0.4286	1.6024
	Formulation 2	5	1st	0.3381 0.3354 0.3301 0.3286	1.3376
			2nd	0.3234 0.3224 0.3212 0.3201	0.4445
		6	1st	0.4281 0.4273 0.4240 0.4208	0.7763
			2nd	0.4202 0.4183 0.4182 0.4104	1.0432

Table – 6 Stability Studies

Drug	Formulation	Concentration (µg/ml)	Time in hours	Absorbance
Milrinone	Formulation 1	6	0 2 4 6	0.4412 0.4402 0.4400 0.4398

			12	0.4301
	Formulation 2	6	0 2 4 6 12	0.4268 0.4234 0.4201 0.4198 0.4101

Figures

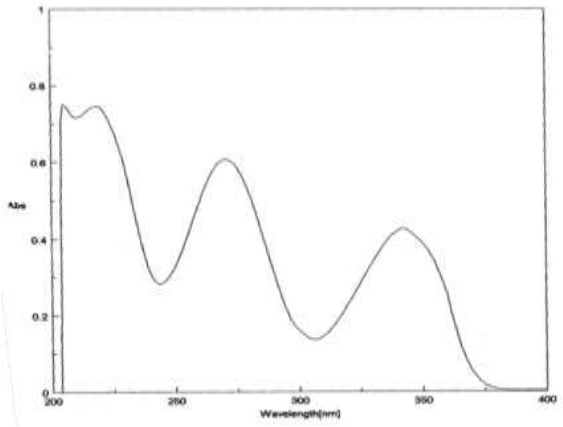

Fig. 1 selection of solvent

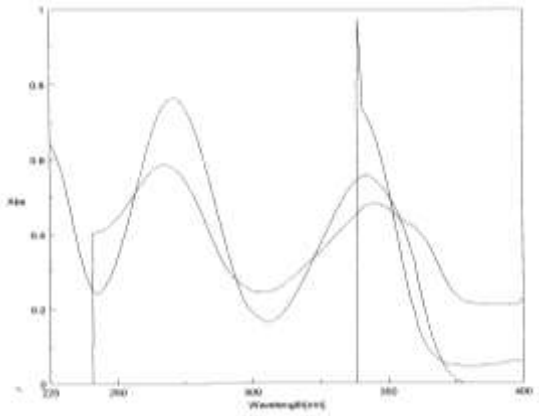

Fig. 2 selection of wavelength

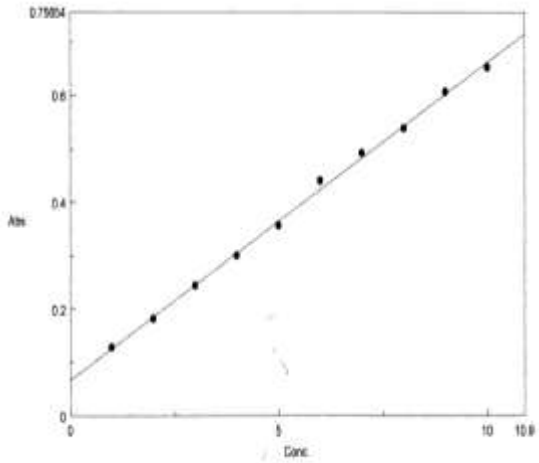

Fig. 3 standard graph of milrinone

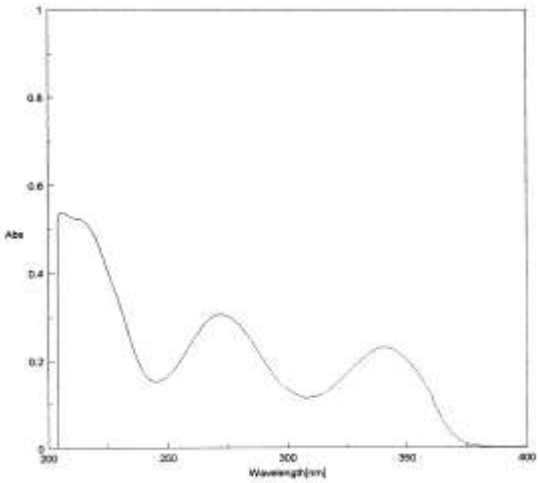

Fig. 4 spectrum of formulation

DISCUSSION

The proposed method is simple, accurate and rapid and can be employed for the routine analysis. This method can be applied for substances obeying Beer's law where a reference standard of adequate purity is available. The low standard deviation and good percentage recovery indicates the reproducibility and accuracy of the method.

CONCLUSION

The developed simple, specific, sensitive, reproducible, precised and cost effective UV spectroscopic method for Milrinone in single dosage form can be used for the routine analysis of Milrinone from its single dosage form in research institutions, laboratories and educational institutions.

ACKNOWLEDGEMENT

We are thankful to the Management of Sri Ramakrishna Institute of Paramedical sciences, Coimbatore for providing all the facilities to carry out the mentioned research work successfully. We take a great opportunity to thank the Management and the Principal of Karpagam College of Pharmacy, Coimbatore for supporting us to publish our research paper in this article.

REFERENCES

1. Sasikala M, Bojja Aswini, Gowthami CL, S Nithya, Jamuna KM, Kiran Kumar A. Extraction, isolation, characterization of phytoconstituents from Curcuma caesia Roxb. By various analytical methods. International Journal of Research in Pharmaceutical and Nano Sciences, 2012; 1(2): 257-268.
2. Sasikala M, Bojja Aswini, Gowthami CL, S Nithya, Jamuna KM, Kiran Kumar A. Application of UV spectroscopic method for drug interaction studies of Mefenamic acid with Ofloxacin. International Journal of Research in Pharmaceutical and Nano Sciences, 2012; 1(2): 274-280.
3. The Merck Index. 13th ed., Merck & co. Inc., NJ, U.S.A, 2001; 3466.
4. Sharma BK. Instrumental Methods of Chemical Analysis. 24th ed., Goel Publishing House, Meerut, 2005; 68-176: 286-319.
5. Ashutoshkar, Pharmaceutical Drug Analysis, 1st ed., Minerva Press, Vasant Vihar, New Celhi, 2001; 55-57.
6. Satinder A, Stephen S, Hand Book of Modern Pharmaceutical Analysis., 3: 1-2.
7. Skoog DA. Principles of Instrumental Analysis. 3rd ed., CBS publishing & Sons, 1988; 40-58.
8. Beckett AH, Stenlake JB. Practical Pharmaceutical Chemistry-Part B. 4th ed., CBS publishers and Distributors, New Delhi, 2002; 275-315; 85-157.
9. Sethi PD. Quantitative Analysis of Drugs in Pharmaceutical Formulations. 3rd ed., CBS Publishers and Distributors, 1997; 1-72.

10. Berry IR, Nash RA. Pharmaceutical process validation. 2nd ed., Marcel Dekker Inc, New York, 2000; 411.
11. Parfitt K, Martindale. The complete Drug Reference. 32nd ed., the Pharmaceutical Press, London, 1999; 862.
12. Munson JW. Pharmaceutical Analysis: Modern methods, Part–A. International medical book distributors, Mumbai, 2001; 230-231: 239-262.
13. Mendham J, Denney RC, Barnes JD, Thomas MJ. Vogel's Textbook of Quantitative Chemical Analysis. 6th ed., Pearson education, Delhi, 2003; 1-11: 251-261, 617.
14. Munson JW. Pharmaceutical Analysis: Modern methods - Part B. International medical book distributors, Mumbai, 2001; 15-30: 155-173.
15. International Conference on Harmonization. Draft Guideline on Validation of Analytical procedures; Methodology, Federal Register, 1996; 1-8.