Eurasian Journal of Analytical Chemistry
ISSN: 1306-3057 OPEN ACCESS 2024 19 (1):0 211-217

A Stability-Indicating Reverse Phase High Performance Liquid Chromatography Method For Development And Validation Of Meptazinol

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ABSTRACT

Methodology: The UV-Spectroscopic method was developed for the estimation of meptazinol in bulk and tablet dosage form. The solvent selected for the meptazinol UV analysis was methanol, the solution of 10µg/ml was scanned in UV region from 200-400 nm and the λmax value was determined. The RP-HPLC method was developed on HiQ silC18HS (250mm x 4.6 mm, 5µ)column using acetonitrile: methanol [90:10] as mobile phase at flow rate 1.2 ml/min and UV detection at 276 nm. Results: The maximum absorbance was observed at 276 nm. The wavelength 276 nm was selected for further analysis of Meptazinol . The calibration curve was determined using drug concentrations ranging from 10-60 µgm/ml. The system suitability was performed by injecting a standard solution containing 60µg/ml of meptazinol six replicates. For two of them, the peak asymmetric were 2000, and the %RSD of meptazinol was less than 2.

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Keywords: Reverse phase high-performance liquid chromatography; UV-visible spectroscopy, method development; validation, meptazinol ; international conference

INTRODUCTION

Meptazinol, also known as m-3-ethyl-methylhexahydro-1H-aze-pin-3-yl, is an opioid-class analgesic possessing a combination of agonist and antagonist characteristics[1]. In a clinic, it's used to treat moderate to severe pain. Its benefit is that it has less adverse effects, including reduced respiratory depression[2] and psychomimetic effects[3]. Following oral administration, meptazinol is quickly conjugated with glucuronic acid or PAPS (3′-phosphoadenosine-5′-phosphosulfate) and is primarily eliminated through the urinary system[4,5]

A novel opioid-type analgesic with dual agonist/antagonist characteristics is called eptazinol[6] It can be administered intravenously, intramuscularly, or orally. Equianalgesic doses of pentazocine, pethidine, or a combination of dextropropoxyphene and paracetamol were found to produce similar analgesic effectPrior to drawing any judgments on its efficacy in these domains, more research must be done on its preoperative and anesthetic applications. According to a few studies, the start of action was quicker than with the other analgesics, but the duration was less than with pentazocine, morphine, and buprenorphine.

Only patients receiving meptazinol as a premedication or during anesthesia have been reported to experience respiratory depression. Anaesthesia patients or patients having preoperative care have been the only groups to experience any alterations in their hemodynamic status. Like other agonist/antagonist analgesics, meptazinol appears to have a low potential for misuse; however,

conclusive evidence of this can only be obtained through extended therapeutic use. The side effects that have been reported the most frequently include.

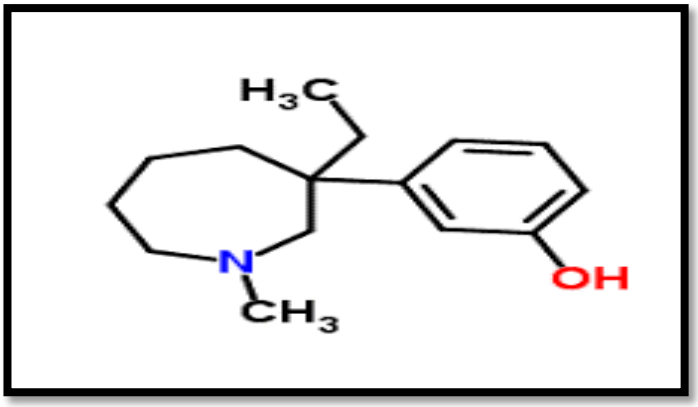


Figure No. 1 Structure of Meptazinol

Attributes	Description
Name of drug	Meptazinol
Functional Category	Opioid- Analgesic
IUPAC and chemical name	3-(3-ethyl-1-methylazepan-3-yl)phenol
Molecular Formula	$C_{15}H_{23}NO$
Molecular weight	233.3492 g/mol
Half life	1.4 – 4hours
Mechanism of Action	Meptazinol is an opioid drug (sometimes called an opiate).It is beneficial to moderate-to-severe pain conditions. It the lot by binding to certain tiny areas, so-called opioid receptors, in brain and spinal cord (central nervous system). This indications to a reduction in pain and reaction of pain.

Table No. 1 Drug Profile of Meptazinol

MATERIAL AND METHOD

Merck Pvt. Ltd. is the supplier of chemicals such as HPLC grade acetonitrile (ACN) and ammonium acetate, whereas Micro Laboratory Private Limited provided a gift sample of meptazinol. We bought Meptid 200 from a nearby drugstore. Most solvents employed in the experiment were of analytical grade.

Instruments

The Jasco HPLC device was utilized to capture the study's chromatograms. To weigh the materials, an electronic analytical balance was utilized.

Selection of Solvent

The choice of Diluent: Methanol was chosen as the diluent due to its ability to produce clear and distinct peaks when used with a mobile phase composition of set .

Standard Solution Preparation

After dissolving 100 mg of Meptazinol in 100 ml of methanol, 1000µg ml-1 was produced. This solution was further diluted in accordance with the needs. Mobile Phase Chromatography Conditions Optimization To find the ideal composition that produces peaks that are crisp, symmetric, and well-resolved, several mobile phase trials were conducted. The ideal chromatographic conditions used in this investigation are shown in Figure 2.

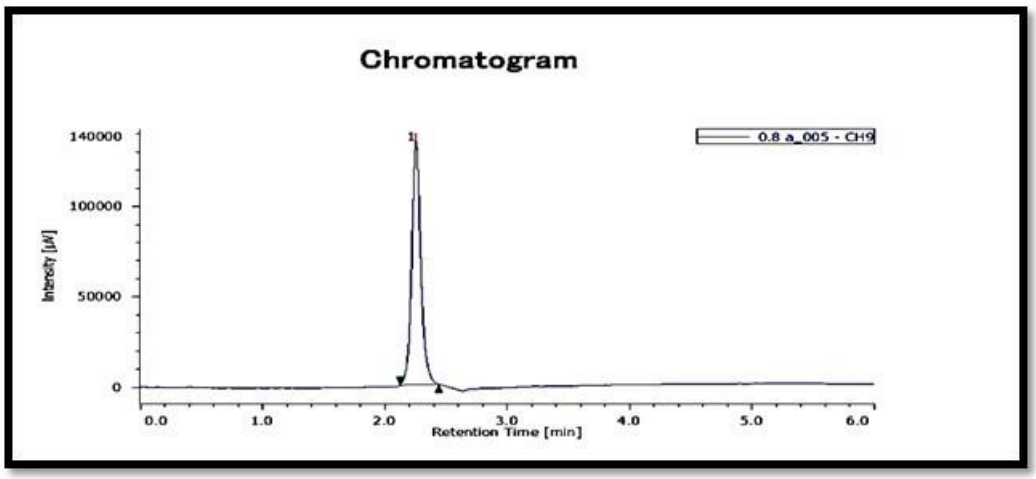


Figure No.2: Chromatogram of meptazinol

Setting up of Calibration Curve

The concentrations in the range of 10 to 60 µg ml-1 were achieved by diluting the reference solution. By conducting the analysis at 276 nm, the calibration curve was created. Every solution's absorbance was measured at its λ max of 276 nm.

Preparation of Tablet Solution[7]

Using 200 mg of MEPPTID, tablet solutions were prepared. Determine the average weight of the pill and the weight of the powder after it was taken in an amount of 96 mg and dissolved in 100 ml of double-distilled water. The resultant solution, which is used for additional analysis, was sonicated and filtered. The formulation's filtered solution was diluted to produce a 100µg ml-1 solution, which was utilized to calculate accuracy or recovery percentage.

VALIDATION OF METHODS[8]

Laboratory tests are used to validate analytical procedures to make sure their performance is appropriate for the intended usage. The suggested spectrophotometric method's linearity, accuracy, precision, LOD, LOQ, robustness, and ruggedness are among the parameters that have been verified.

Linearity

The calibration curve was set using various concentrations of Meptazinol reference standard solutions. The concentration range at which the curve was formed was 1 to 10 µg ml–1. Plotting the concentration of standard solutions against absorbance, which results in a straight line, was used to create the calibration curve. Regression analysis was used to determine the coefficient of determination

Accuracy

The standard addition method was used to calculate the sample recovery percentage in order to assess the accuracy of the test method. 80%, 100%, and 120% solutions were ready. Every solution was made three times, and the mean percentage of recovery was determined from each. At 276 nm, these samples were examined.

Precision

Repeatability within and between days is how precision is achieved. Six distinct preparations with the same concentration, or 60µg ml-1, were examined at 276 nm on the same and different days, with the same experimental setup. For both intraday and interday precisions, the percentage relative standard deviation was determined.

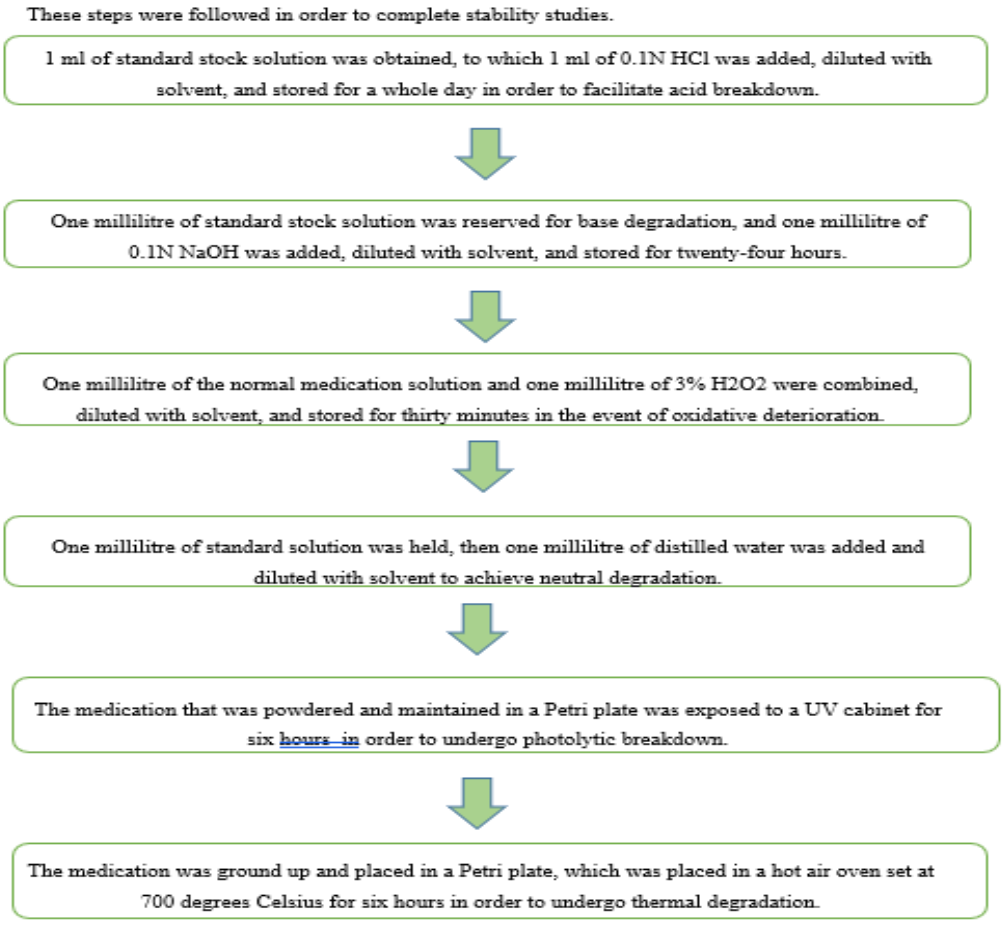
LOD and LOQ

The precision of intraday and interday data was used to compute the detection limit (LOD) and quantification limit. These were computed using the formulas in equations no. (1) and (2),

Where LOD=3.3σ/s... (1)

LOQ = 10σ/s.... (2)

Stability Studies[9]



RESULT & DISCUSSION

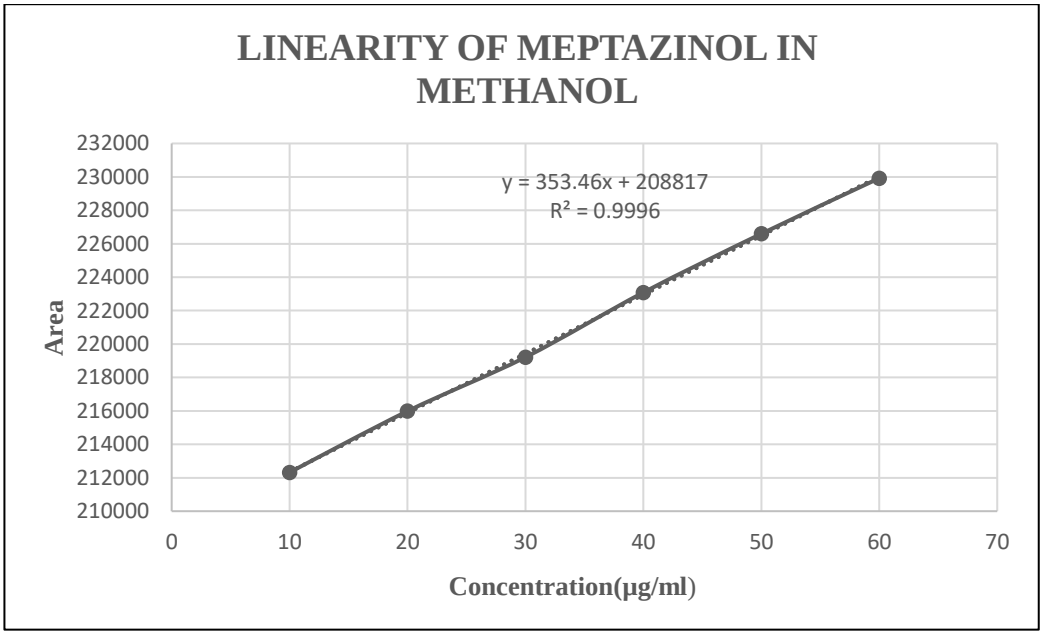
The results of an extensive technique validation investigation for an HPLC method are presented in this article. Key performance characteristics such as linearity, accuracy, precision, limit of detection (LOD), and limit of quantification (LOQ) were used to validate the method. These variables are essential for evaluating the HPLC method's specificity and dependability in quantitative analysis.

Linearity

By utilizing standard solutions with known concentrations to create a calibration curve, the linearity was assessed. There is a strong linear relationship between the observed absorbance and the analyte concentration, as indicated by the correlation coefficient (r2) of 0.9998 that was obtained from the calibration curve. Table 2 and Figure 3 illustrate the method's linearity. This shows that throughout a broad concentration range, the target analyte can be properly quantified using the HPLC method.

Table 2:Calibration Plot Of Meptazinol

Concentration	Peak Area
10	212322
20	215987
30	219213
40	223087
50	226598
60	229923



Accuracy

Analyte concentrations were added to various matrices in percentage recovery tests to evaluate the accuracy of the HPLC procedure. The range of average recovery values, which showed a good degree of accuracy, was 98% to 102%. These findings imply that matrix interferences do not considerably impact the HPLC method, which can yield accurate and dependable results for a wide range of sample types. Table 3 displays the results.

Table 3: Results of Meptazinol Accuracy (n=3 ±SD)

Conc. Level (%)	Sample amount µg/ml	Amount added µg/ml	Area	Mean Area	S.D.	%RSD	CAL(µg/ml	% Recovery
80%	10 10 10	8 8 8	215279 215067 214999	215115	0.06789	0.06789	17.8181	98.9896
100%	10 10 10	10 10 10	216381 216843 216936	216720	0.13716	0.13716	22.3589	101.631
120%	10 10 10	12 12 12	216711 216702 217100	216771	0.14767	0.14556	22.5032	102.287

Precision

The intra-day precision, as assessed by analyzing several aliquots of a single sample in the same day, showed a relative standard deviation (RSD) of less than 2% when repeat analyses of the same sample were carried out. By analyzing the same sample across several days, the inter-day precision was also found to have acceptable variability, with an RSD of less than 2%. The Precision results are shown in Tables 4 and 5. These results demonstrate the great precision and reproducibility of the approach, guaranteeing accurate and consistent measurements.

Table 4: Intraday Precision Results(n=6±S.D)

Concentration	Peak Area	Obtained Concentration	S.D	% RSD
60ppm	235403	79.54223	1.150	1.491
60ppm	235932	77.01295		
60ppm	235403	76.4386		
60ppm	235405	76.5543		
60ppm	235405	75.9944		
60ppm	235406	77.01295		

Table 5: Interday Precision Results(n=6±S.D)

Concentration	Peak Area	Obtained concentration	S.D	RSD
60ppm	236932	15.63810913	0.272	1.76%
60ppm	236038	15.42109696		
60ppm	235835	15.91420917		
60ppm	235876	15.23056623		
60ppm	235678	15.22976354		
60ppm	236038	15.29580607		

Quantification Limit (LOQ) and Detection Limit (LOD)

These were found to be, respectively, 1.3 and 4.02 μg ml -1.

STABILITY STUDIES

In this work, HPLC was used to perform forced degradation tests of meptazinol. These investigations were conducted in order to assess the stability and identify the features of meptazinol's degradation under diverse stress scenarios. Meptazinol was subjected to oxidative, thermal, photolytic, base, and acid hydrolysis among other stress conditions in order to perform forced degradation tests. After that, the samples were examined using UV spectroscopy to track absorbance changes and gauge the degree of deterioration. Table 6 displays the data gained from these investigations.

Table 6: Outcomes of Forced Degradation studies by HPLC spectroscopic technique (n=3)

Sr. No	Degradation condition	Procedure	% Degradation
1)	Oxidation	To 1 ml standard stock solution, 1 ml of 3% H_2O_2 was put into and made up the volume up to mark with diluent	18%
2)	Acid	To 1 ml standard stock solution, 1 ml of 0.1N HCl was put into and made up the volume up to mark with diluent	6%
3)	Base	To 1 ml standard stock solution, 1 ml of 0.1N NaOH was put into and	19%

		made up the volume up to mark with diluent	
4)	Neutral	To 1 ml standard stock solution, 1 ml of distilled water was put into and made up the volume up to mark with diluent	4%
5)	Thermal	Powder form of drug kept in petri plate for 6 hours in hot air oven	6%
6)	Photolysis	Powder form of drug kept in petri plate for 6 hours in UV cabinet.	7.5%

CONCLUSION

The technique validation results for the HPLC method reveal its outstanding linearity, accuracy, precision, sensitivity, and quantification capacity. These results validate the HPLC method's specificity in quantitatively analyzing the target analyte, guaranteeing dependable and solid data collection. The HPLC method is used for precise determination of analyte concentrations in research, quality control, and other analytical application.

Acknowledgment:

Its great pleasure for me to acknowledge all those who helped me and maintained me.I would like to express my sincere gratitude to our principal Dr. Ashish Jain and to the management for their continuous encouragement and providing all necessary facilities. My deepest thanks to my guide Mr. Mukesh S Patil for guiding and making necessary corrections as and when needed. I express my sincere gratitude to PG Incharge Dr. Bhushan Rane for his kind cooperation and guidance.

REFERENCE

1) Bill, D.J.; Hartley, J.E.; Stephens, R.J. and Thompson, A.M.: The antinociceptive activity of meptazinol depends on both opiate and cholinergic mechanisms. British Journal of Pharmacology 79: 191–199 (**1983**)
2) Madhuri A Hinge, Rajvi J. Mahida; Development and Validation of an RP-HPLC Method for Simultaneous Determination of Trimetazidine Hydrochloride and Metoprolol Succinate; International Journal of Chemical and Pharmaceutical Analysis. (**2015);** 2(2);77-83.
3) M. A. Naushad, M. Aqil; Development and Validation of the HPLC Method for the Analysis of Trimetazidine Hydrochloride in Bulk Drug and Pharmaceutical Dosage Forms Journal of Analytical Chemistry (**2008)**, 63(10), 965–97
4) Sreelatha Nagula, Sridhar Thota and Venisetty Raj Kumar; Development and validation of a stability indicating RP-HPLC method for the determination of trimetazidine dihydrochloride, Der Pharma chemica (**2014)**, 6(1), 149-154.
5) Robinson, J. W. Frame, E. M. S. Frame, G. M. II. Undergraduate instrumental analysis, 6 ed.; CRC publisher, 2004, pp. 1-4, 65-67
6) 2 Braun, R D Introduction to Instrumental analysis, 2nd ed.; pharma Med press, 2012, pp. 1-8, 138-152
7) Robinson J. W. et.al. Undergraduate Instrumental Analysis; 6th ed., Marcel Dekker Inc.,:New York, 2005: pp. 3.4,65.
8) Braun. R D Introduction to Instrumental Analysis, 2nd ed., Pharma Med Press, 2012. Pp. 891-892
9) Skoog. D. A. et al, Principles of Instrumental Analysis: 6th ed., Thomson Learning, 2007, pp 1-3.
10) Skoog. D. A. et al: Principles of Instrumental Analysis, 6ª ed., Cengage Learning, 2006, Pg no-1-13.
11) Thomas S., Bharti A.; Sanjeev S.; Ashutosh A, A stability indicating simultaneous dual wavelength UV-HPLC method for the determination of potential impurities in farmprudine active pharmaceutical ingredient J Pharm Biomed Anal 2012, 58111, 136-40
12) Jain M. Srivastava V. Kumar R., Dang: V. Hinyanna S., Kumar A. et al. Devenim of five potential genotoxic impurities in Dalfumpridine using liquid chromatography / Pharm Biomed Anal. 2016,133, 27-31.
13) Snyder. L. R.; Kirkland, J. J. Dolan, J. W. Introduction to modern liquid chromatography, 3rd ed.; Wiley publication, 2010, pp 20-35.
14) Lodba Luxminarayan; Sharma Neha, Viswas Amit, Dr. Khinchi MP. A Review Chromatography Techniques. Asian Journal of Pharmaceutical Research and Development 2017.5(2), 1-08.
15) Snyder. L. R. et al, Practical HPLC Method Development, 2d ed. Wiley India Pvt. Ltd, 2006, pp 21, 59, 40, 234-251.

16) Sethi. P. D. HPLC, Quantitative analysis of pharmaceutical formulation, Volume 1: CHS publishers and Distributors, 2019; pp 11, 14, 28 and 38.
17) Pharmaceutical Guidelines. Analytical Method Validation by Ankut Choudhary: analytical-method-validation.html (Accessed on Mar 05, 2020).
18) G. Lavanya; M. Sunil: M.M. Eswarudu, M. Chinna Eswaraiah, K. Harisudha and R. Naga Spandana Analytical Method Validation: An Updated Review. International Journal of Pharmaceutical Sciences and Research, 2013, 4(4), 1280-1286.
19) Ahuja. S.; Dong. M. W. Handbook of Pharmaceutical Analysis by HPLC, Volume 6 Elsevier/Academic Press, 2005, pp 200
20) Blessy M.; Patel R.D., Prajapati P.N.: Agrawal Y.K. Development of forced degradation and stability indicating studies of drugs A review. J Pharm Anal, 2014, 463), 159-165.
21) Agranat I, Hava C and John C. Putting chirality to work: the strategy of chiral switches. Nature Reviews Drug Discovery 2002; 1: 753–768
22) K.G. Mawuenyega, W. Sigurdson, V. Ovod, L. Munsell, T. Kasten, J.C. Morris, K.E. Yarasheski, R.J. Bateman, Science 330 (2010) 1774.
23) R.T. Bartus, R.L. Dean, B. Beer, A.S. Lippa, Science 217 (1982) 408
24) J. Wyeth and Brother Ltd. (J.F. Cavalla, A.C, White, inv.) Get. Offen. 194 1534 (April 4, 1970) [Chem. Abstr., 3 (1970) 14727 m].
25) C. Rudolphi, G. Blaschke, Journal of Chromatography B, 877 (2009) 3787–3791
26) Rathod K.G. et al. Development and Validation of RP-HPLC Method for Estimation of Dalfampridine, in Bulk and Pharmaceutical Dosage Form, Indo American Journal of Pharmaceutical Research, 2019, 9/17), 614-629.
27) Vivek Jain; Ritu Dehariyal and Geeta Parkhe Method Development and Validation for Estimation of Dalfampridine in Synthetic Mixture by using UV Spectrophotometry and RP HPLC EASI Pharm & Pharmacol. 2019, 1(4), 84-87
28) Dharani NR.: Padmini K.: Suakala S. Stability indicating RP-HPLC method development and validation for estimation of Dalfampridinein bulk and formulation. Int. J Ads. Res. 2016, 4191.184-91
29) Madhumathi CH.: Padmalatha M Snehna 5: Divya C.B: Development and validation of UV spectrophotometery method for the estimation of Dalfampridine in tablets Int J Pharmac Res2014, 2, 1019-26.
30) Vivekkumar, K. Redasani, Gazalal, Shaikh, Sanjay J. Surana Development and validation of spectroscopic methods for the estimation of Dalfampridine in bulk and in tablet formulation Analytical Chemistry- Aw Indian Journal, 2014, 14(1), 37-41.
31) LI, W.; HAO, J.; TANG, Y.; CHEN, Y.; QIU, Z. Structural Comparisons of Meptazinol with Opioid Analgesics1. Acta Pharmacologica Sinica 2005, 26 (3), 334–338.
32) Franklin, R.; Aldridge, A. Studies on the Metabolism of Meptazinol, a New Analgesic Drug. British Journal of Clinical Pharmacology 1976, 3 (3), 497–502
33) P. D. Sethi Quantitative analysis of pharmaceutical formulations High performance thin layer chromatography, 1" ed.; CBS publisher and distributor: New Delhi, 1996, pp. 3-5, 10, 18-20, 24.27.