ISSN 2249-3875

International Journal of Pharmaceutical Erudition

**Review Article**

# A Review on Development and Validation of RP-HPLC Method to Detect Impurity Profiling of Methylthioninium Chloride in Pharmaceutical Dosage Form

**Himani tiwari*, Pradeep K. Goyal, Bhupendra Vyas, Anju Goyal**

B. N. Institute of Pharmaceutical Sciences, Udaipur (Raj.)313001

Impurity is defined as any substance coexisting with the original drug, such as starting material or intermediates or that is formed, due to any side reactions. According to the International Conference on Harmonization of Technical Requirements for the Registration of Pharmaceuticals for Human Use (ICH) guideline on impurities in new drug substances, an impurity is defined as 'any component of the new drug substance that is not the chemical entity defined as the new drug substance'. There are different methods for detecting and characterizing impurities with TLC, HPLC, and HPTLC etc. Methylthioninium chloride (INN, or methylene blue,) is an investigational drug being developed by the University of Aberdeen and TauRx Therapeutics that has been shown in early clinical trials to be an inhibitor of Tau protein aggregation. The drug is of potential interest for the treatment of patients with Alzheimer's disease. In vitro studies suggest that methylene blue might be an effective remedy for both Alzheimer's and Parkinson's disease by enhancing key mitochondrial biochemical pathways. It can disinhibit and increase complex IV, whose inhibition correlates with Alzheimer's disease.

**Key Words: Alzheimer's disease,** International Conference on Harmonization (ICH), Parkinson's disease, Methylthioninium chloride, Impurity.

## INTRODUCTION[1-6]

Pharmaceuticals impurities are the unwanted chemicals that remain with the active pharmaceutical ingredients (APIs) or are developed during formulation or upon aging of both API and formulated APIs to medicines. According to the International Conference on Harmonization of Technical Requirements for the Registration of Pharmaceuticals for Human Use (ICH) guideline on impurities in new drug substances, an impurity is defined as 'any component of the new drug substance that is not the chemical entity defined as the new drug substance'. Methylene blue is a heterocyclic aromatic chemical compound with the molecular formula $C_{16}H_{18}N_3SCl$. It has many uses in a range of different fields, such as biology and chemistry. At room temperature it appears as a solid, odorless, dark green powder, that yields a blue solution when dissolved in water. The hydrated form has 3 molecules of water per molecule of methylene blue. Methylene blue should not be confused with methyl blue, another histology stain, new methylene

***Address for Correspondence**
heenatiwari31@gmail.com

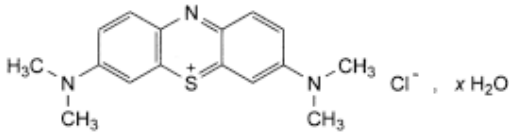
blue, nor with the methyl violets often used as pH indicators. As an experimental pharmaceutical drug, the International Nonproprietary Name (INN) of methylene blue is methylthioninium chloride. Methylene blue was first prepared in 1876 by German chemist Heinrich Caro (1834-1910). It is on the World Health Organization's List of Essential Medicines, a list of the most important medication needed in a basic health system.

**Types of Impurity:**

**According to USP[7,8]:**

The United States Pharmacopoeia (USP) classifies impurities in various sections (A) Impurities in Official Articles (B) Ordinary Impurities. This found in bulk significance on biological activity of the

**Table no. 1 Drug Profile of Methylthioninium chloride[8,9,10]**

| Parameters | Description |
|---|---|
| Drug name | Methylthioninium Chloride |
| IUPAC name | 3,7-Bis(dimethylamino)phenothiazin-5-ylium chloride (methylene blue) |
| Molecular formula | $C_{16}H_{18}ClN_3S,xH_2O$ |
| Molecular weight | 319.9g/mo |
| CAS No. | 61-73-4 |
| Structural formula |  |
| Sulfated ash | Maximum 0.25 per cent, determined on 1.0 g. |
| Category | Antiseptic agent. Antidote in methemoglobinemia |
| Characteristic | Dark blue, crystalline powder with a copper-coloured sheen, or green crystals with a bronze-coloured sheen |
| Solubility: | Soluble in water, slightly soluble in ethanol (96 per cent) |
| pKa | 0-1 |
| Melting point | 190° C |
| Mechanism of Action | Reduce tau levels in vitro and in vivo and several different mechanisms of action, lowers methaemoglobin levels in the RBCs through activation of reductase enzyme |
| Action and use | Reducing agent; antidote to methaemoglobinaemia |
| Content | 95.0 per cent to 101.0 per cent (dried substance). |
| STORAGE | In an airtight container, protected from light. |
| Loss on drying | 8.0 per cent to 22.0 per cent, determined on 1.000 g by drying in an oven at 105 °C. |

drug substance. These impurities may arise out of the synthesis, preparation or degradation of chemical.And (C) Organic Volatile Impurities-Organic volatile chemicals are produced in the Manufactureof drug substances or excipients or the preparation of drug products; they are volatile in nature and by themselves get removed out at time of storage or processing.

**According to ICH Guideline[9,10,11]:**

The new drug substance specifications should include, limits for-

i) Organic Impurities

- Each specific unidentified impurity at or above 0.1%
- Any unspecific impurity, with limit of not more than 0.1%
- Total impurities

ii) Residual solvents

iii) Inorganic impurities

**According to Literature [12, 13]**

**Organic impurities:**

They are the most common impurities found in every API unless proper care is taken throughout the multistep synthesis. Although the end products are always washed with solvents, there is always a chance that the residual unreacted starting material remain, unless the manufactures are very careful about the impurities.

It can be any of following:

a. Starting Material-Example: In PCM Bulk, there is a limit test for p-amino - phenol, which could be starting material or intermediate for synthesis.

b. By product-Example: In the case of paracetamol bulk, diacetylated+ paracetamol may be formed as a byproduct.

c.Intermediates

d. reagents

The spectroscopic studies (NMR, IR, MS etc.) conducted to characterize the structure of actual impurities present in the drug substance above an apparent level of 0.1% (e.g., calculated using the response factor of the drug substance) should be described. All recurring impurities above an apparent level of 0.1% in batches manufactured by the proposed commercial process should be identified of these studies.

**Inorganic impurities:**

They may also derive from the manufacturing processes used for bulk drugs. They are normally known & identified & include the Reagents, Ligands, Catalysts, Heavy Metals, Filter aids, Charcoals etc. Inorganic impurities are normally detected and quantified using Pharmacopeial or other appropriate standards. Carryover of catalysts to the drug substance should be evaluated during development.

**Residual solvents:**

Residual solvents are organic volatile chemicals used during the manufacturing processes or generated during the production. Some solvents that are known to cause toxicity should be avoided in the production of the drugs.

Depending on the possible risk to humans, residual solvents are divided into 3 classes,

Class 1: Human carcinogens.

Class 2: Non genotoxic.

Class 3: Lower risk to human health.

**Genotoxic impurities:**

These are the impurities that damage DNA by mutation of genatic code. Example: Alkylation.

**High Performance Liquid Chromatography (HPLC)[1,2,3]**

HPLC is fastest growing analytical technique for analysis of drugs. Its simplicity, high specificity and wide range of sensitivity makes it ideal for analysis of many drugs in both dosage forms and biological fluids.

**a) Normal Phase Chromatography[22, 23]:**

Normal phase chromatography is a particular type of adsorption chromatography in which the adsorbent stationary phase is polar and the mobile phase is relatively non polar Normal-phase

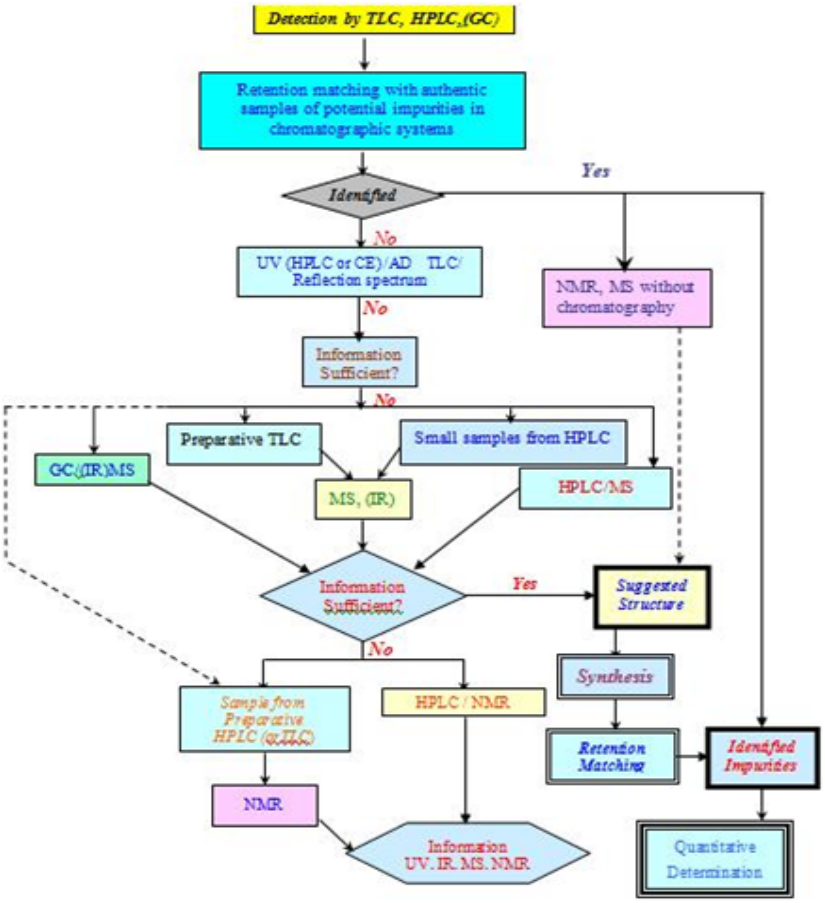



Fig.2: **General Scheme for Drug Impurity Profiling**[21]

partition chromatography uses a polar stationary phase and a nonpolar organic solvent, such as n-hexane, methylene chloride, or chloroform, as the mobile phase. The stationary phase is a bonded siloxane with a polar functional group.

**b) Reversed Phase Chromatography[22]:** Reverse phase chromatography is also a type of adsorption chromatography in which the adsorbent stationary phase is non polar and the mobile phase is relatively polar. Reverse-phase partition stationary phase and a polar mobile phase. chromatography uses a relatively nonpolar The most common bonded phases are n-octyldecyl (C18) and n-decyl (C8) chains, and phenyl groups. Retention increases exponentially with the alkyl chain.

**Table . 2 : List of commonly used buffers in the HPLC[11,12]**

| **Buffer** | **pKa** | **Usable pH Range** | UV cutoff (A>0.5) |
|---|---|---|---|
| Trifluoroacetic acid | << 2 | 1.5 -2.5 | 210 nm (0.1 %) |
| KH2PO4 / Phosphoric acid | 2.12 | 1.1 - 3.1 | <200 nm (0.1%) |
| Tri-K-Citrate / hydrochloric acid 1 | 3.06 | 2.1 - 4.1 | 210 nm (10 mM) |
| Potassium formate / formic acid | 3.8 | 2.8 - 4.8 | 210 nm (10 mM) |
| Tri-K-Citrate / hydrochloric acid 2 | 4.7 | 3.7 - 5.7 | 230 nm (10 mM) |
| Potassium acetate / acetic acid | 4.8 | 3.8 -5.8 | 210 nm (10 mM) |
| Tri-K-Citrate / hydrochloric acid 3 | 5.4 | 4.4 - 6.4 | 230 nm (10 mM) |
| Ammonium formate | 3.8 & 9.2 | 2.8-4.8 & 8.2-10.2 | - |
| Bis-tris propane HCl/Bis-tris propane | 6.8 | 5.8 - 7.8 | 215 nm (10 mM) |
| Ammonium acetate | 4.8 & 9.2 | 3.8-5.8 & 8.2-10.2 | 205 (10 mM) |
| KH2PO4 / K2HPO4 | 7.21 | 6.2 - 8.2 | <200 nm (0.1%) |
| Tris HCl / Tris | 8.2 | 7.3 - 9.3 | 205 nm (10 mM) |
| Bis-tris propane HCl/Bis-tris propane | 9.0 | 8.0 - 10.0 | 225 nm (10 mM) |
| Ammonium hydroxide / Ammonia | 9.2 | 8.2 - 10.2 | 200 nm (10 mM) |
| Borate (H3BO3 / Na2B4O7.10H2O) | 9.24 | 8.2 – 10.2 | - |
| Glycine.HCl / glycine | 9.8 | 8.8 - 10.8 | - |
| 1-methylpiperidine.HCl/ 1-methyl piperidine | 10.1 | 9.1 - 11.1 | 215 nm (10 mM) |
| Diethylamine.HCl / diethylamine | 10.5 | 9.5 – 11.5 | - |
| Triethylamine.HCl / triethylamine | 11.0 | 10.0 - 12.0 | <200 nm(10 mM) |
| Pyrollidine.HCl/pyrollidine | 11.3 | 10.3 – 12.3 | - |

Reverse-phase chromatography is the most common form of liquid chromatography, primarily due to the wide range on analytes that can dissolve in the mobile phase.

**HPLC Analytical Method Development[4]**

The analytical technique of High Performance Liquid Chromatography (HPLC) is used extensively throughout the pharmaceutical industry. It is used to provide information on the composition of drug related samples. The information obtained may be qualitative, indicating what compounds are present in the sample or quantitative, providing the actual amounts of compounds in the sample. HPLC is used at all the different stages in the creation of a new drug, and also is used routinely during drug manufacture. The aim of the analysis will depend on both the nature of the sample and the stage of development. HPLC is a chromatographic technique; therefore it is necessary to have a basic understanding of chromatography to understand how it works. **Method Requirements**

The goals or requirements of the analytical method that need to be developed are considered and the analytical figures of merit are defined. The required detection limits, sensitivity, linearity, range, accuracy and precision are defined[28,29].

**Literature Search and Prior Methodology**

The literature for all types of information related to the analyte is surveyed. For synthesis, physical and chemical properties, solubility and relevant analytical methods. Books, periodicals, chemical manufacturers and regulatory agency compendia such as USP publications are reviewed[26].

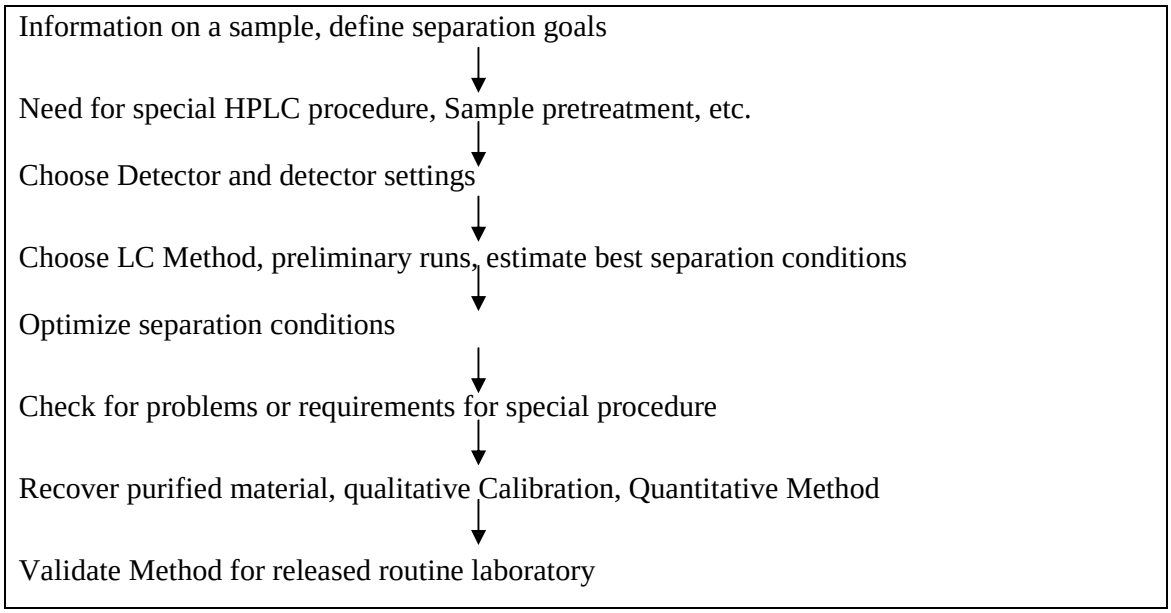



**Fig. 2: Steps involved in Method Development[5,6,7,8]**

**Choosing A Method**

Using the information in the literatures and prints, methodology is adapted. The methods are modified wherever necessary. Sometimes it is necessary to acquire additional instrumentation to reproduce, modify, improve or validate existing methods for in house analyzed and samples[26].

If there are no prior methods for the analyte in the literature, from analogy, the compounds that are similar in structure and chemical properties are investigated and are worked out. There is usually one compound for which analytical method already exist that is similar to the analyte of interest[26].

**Instrumental Setup And Initial Studies[19,20]**

The required instrumentation is setup. Installation, operational and performance qualification of instrumentation using laboratory standard operating procedures (SOP's) are verified. Always new consumables (e.g. Solvents, filters and gases) are used for e.g. Method development is never started on HPLC column that has been used earlier. The analyte standard is a suitable injection/introduction solution and in known concentration and solvents is prepared. It is imp. To start with an authentic, known standard rather than with a complex sample matrix. If the sample is extremely close to the standard (e.g., bulk drug), then it is possible to start work with the actual sample.

**Optimization[22]**

During optimization one parameter is changed at a time, and set of conditions are isolated, rather than using a trial and error approach. Work has been done from an organized methodical plan, and every step is documented (in a lab notebook) in case of dead ends.

**Documentation of Analytical Figures of Merit[22]**

The originally determined analytical figures of merit limit of quantitation (LOQ), limit of detection (LOD), linearity, time per analysis, cost, sample preparation etc., are documented.

**Evaluation of Method Development with Actual Samples[22,23]**

The sample solution should lead to absolute identification of the analyte peak of interest apart from all other matrix components.

**Determination of Percent Recovery of Actual and Demonstration of Quantitative Sample Analysis[31]**

Percent recovery of spiked, standard analyte into a sample matrix that is shown to contain no analyte is determined. Reproducibility of recovery (average +/- standard deviation) from sample to sample and whether recovery has been optimized has been shown. It is not necessary to obtained 100% recovery as long as the

results are reproducible and known with a high degree of certainty. The validity of analytical method can be verified only by lab. Studies. Therefore documentation of the successful completion of such studies is a basic requirement for determining whether a method is suitable for its intended applications.

**Chemical and Instruments[34,35]**

**Materials**

- Analytical pure sample of methylthioninium chloride was obtained as a samples from macsen laboratory (Udaipur, Raj.)
- Formulation A- Methylthioninium Injection
- Formulation B- Powdered dosage form
- Contains labeled amount of 5mg of MTC, was procured from local market.

**Reagents**

- Methanol (A. R. grade)
- Methanol (HPLC grade)
- Acetonitrile (HPLC grade)
- Ortho phosphoric acid (A. R. grade)
- Di-potassium hydrogen phosphate (A. R. grade)
- Trifluoro acetic acid
- HPLC water

Weigh and transfer 60 mg of methylthioninium chloride working standard into a 100ml volumetric flask, add 5ml Acetonitrile and sonicate to dissolve. Make up the volume upto 100ml with diluent and mix.

**Table no. 2 : List of Equipment[32]**

| S.No. | Name of Equipments | Model |
|---|---|---|
| 1. | Digital weighing balance (Analytical) | Sartorius |
| 2. | UV-Visible Spectrophotometer- | Shimazdu 1800<br>Software – UV Probe version 2.33 |
| 3. | Karl-Fischer Auto Titrator | Metrohm<br>Software – Tiamo |
| 4. | pH Meter | Mettler Toledo |
| 5. | Sonicator | Toshniwal |
| 6. | Milli Q (water System for HPLC) | Sr 0429 Millipore 0.22µm |
| 7. | Chromatography (HPLC)<br>(a.) Make : Agilent Technology<br>Detector : PDA<br>Software : Empower Pro<br>(b.) Waters :<br>Detector : 2996 PDA Detector<br>2487 Dual Wavelegth absorbance dectector<br>2489 uv Visible Detector<br>Software : Empower Pro | Agilent<br><br>waters |

- Glacial Acetic Acid (HPLC grade)

**Preparation of standard solutions :**

**Diluent Preparation :**

Water : Methanol (50:50) is used as a diluent.

**Selection of wavelength for Determination :**

The working standard solution of methylthioninium chloride was scanned in the range of 200-400 nm.

The responses of standard solution were measured and the wavelength at 237 nm was selected for the RP-HPLC method.

**Selection of column temperature**

An inclusion of column temperature (40c) has minimized day-to-day variation of retention time due to fluctuations in the ambient temperature; along with this peak sharpening and shortening of run time were observed.

**Selection of mobile phase**

From the literature search, HPLC method available showed use of buffer, acetonitrile or methanol as mobile phase and hence, they were selected for initial runs and different composition of them were tried at different flow rate as mention in table.

**Validation of Analytical Methods**

Method validation is a process used to confirm that the analytical procedure employed for a specific test is suitable for its intended use. Results from method validation can be used to judge the quality reliability and consistency of analytical results; it is an integral part of any good analytical practice.

Analytical method need to be validated or revalidated:

- Before their introduction into routine use;
- Whenever the conditions changed for which the method has been validated (e.g. an instrument with different characteristics or sample with a different matrix ); and
- Whenever the method is changed and the change is outside the original scope of the method.

**Analytical Method Validation Parameters[29,34]:**

According to ICH Q2 R1, typical analytical performance characteristics that should be considered in the validation of the types of methods are:

**Accuracy:**

The accuracy of an analytical procedure expresses the closeness of agreement between the value that is accepted either as a conventional true value or as an accepted reference value and the value found. Accuracy for the area percent method should be established from 50% of the ICH reporting limit to the nominal concentration of drug substance in the sample solution. A method is said to be accurate if it gives the correct numerical answer for the analyte. The method should

be able to determine whether the material in question conforms to its specification (for example, it should be able to supply the exact amount of substance present). However, the exact amount present is unknown, which is why a test method is used to estimate the accuracy[34].

**Precision:**

Repeatability: Repeatability expresses the precision under the same operating conditions over a short interval of time. Repeatability is also termed intra assay precision. Repeatability of a method can be determined by multiple replicate preparations of the same sample. This can be done either by multiple sample preparations (n = 6) in the same experiment or by preparing three replicates at three different concentrations. Precision means that all measurements of an analyte should be very close together. All quantitative results should be of high precision - there should be no more than a ±2% variation in the assay system. A useful criterion is the relative standard deviation (RSD) or coefficient of variation (CV), which is an indication of the imprecision of the system. According to the ICH, precision should be performed at two different levels - repeatability and intermediate precision. Repeatability is an indication of how easy it is for an operator in a laboratory to obtain the same result for the same batch of material using the same method at different times using the same equipment and reagents. It should be determined from a minimum of nine determinations covering the specified range of the procedure (for example, three levels, three repetitions each) or from a minimum of six determinations at 100% of the test or target concentration[31].

**Selectivity and Specificity:**

Specificity is the ability to assess unequivocally the analyte in the presence of components that may be expected to be present. Most related substance methods will be used in a stability study, and therefore they have to be stability indicating. Stability indicating means that the method has sufficient specificity to resolve all related substances and the drug substance from each other. The selectivity of an analytical method is its ability to measure accurately and specifically the analyte of interest in the presence of components that may be expected to be present in the sample matrix. If an analytical procedure is able to separate and resolve the various components of a mixture and detect the analyte qualitatively the method is called selective. On the other hand, if the method determines or measures quantitatively the component of interest in the sample matrix without separation, it is said to be specific[31].

**Quantitation limit and Detection limit:**

The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample that can be determined quantitatively with a suitable precision and accuracy. The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample that can be detected but not quantitated as an exact value. The limit of detection is the parameter of limit tests. It is the lowest level of analyte that can be detected, but not necessarily determined in a quantitative fashion, using a specific method under the required experimental conditions. The limit test thus merely substantiates that the analyte concentration.is above or below a certain level[29]

Several approaches for determining QL & DL are:

- Visual evaluation.
- Signal to noise ratio approach.
- Standard deviation of the response and slope.

**Linearity and range:**

The linearity of an analytical method is its ability to elicit test results that are directly (or by a well defined mathematical transformation) proportional to the analyte concentration in samples within a given range. Linearity usually expressed in terms of the variance around the slope of regression line calculated according to an established mathematical relationship from test results obtained by the analysis of samples with varying concentrations of analyte.

**Robustness:**

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small but deliberate

**Table 3: Validation Parameters Acceptance Criteria[33,34,35]**

| S. No. | Parameter | Acceptance criteria |
|---|---|---|
| 1. | Accuracy | Recovery (98-102%) |
| 2. | Precision: repeatability<br>Intermediate precision | RSD<2% |
| 3. | Specificity/Selectivity | Interference <0.5% |
| 4. | Detection limit | NMT>2or3 |
| 5. | Quantitation limit | NMT>10, RSD<2% |
| 6. | Linearity | Correlation coefficient (r)>0.999 |
| 7. | Range | 80-120% |

variations in method parameters and provides an indication of its reliability during normal use. The robustness of an analytical method is a measure of its capacity to remain unaffected by small but deliberate variation in method parameters and provides an indication of its reliability during normal usage. The determination of robustness requires that methods characteristic are assessed when one or more operating parameter varied[29].

**Conclusion**

In this progress report attempts were made to develop analytical method for the estimation of methylthioninium chloride by RP-HPLC. HPLC method has several advantages like speed, rapidity, greater sensitivity and ease of automation. Also a wide choice of chromatographic separation methodologies and a whole range of mobile phases using isocratic or gradient elution techniques are available. The real goal of validation process is to challenge the method and determine the limits of allowed variability for the condition needed to run the method. Impurity profiling of a substance under investigation gives maximum possible account of impurities present in it. The establishment of guidelines for impurity levels in drug substances and products provides the quality criteria for manufacturers. Beginning with limit tests for impurities, this field of impurity identification and quantitation has progressed. Isolation and characterization of impurities is required for acquiring and evaluating data that establishes biological safety which reveals the need and scope of impurity profiling of drugs in pharmaceutical research.

**REFERENCE**

1. Gorog S, Identification and Determination of Impurities In Drugs, Elsevier Science Publishing Company,Amsterdam,published:May-2000,154.

2. Sanjay B. Bari, Bharati R. Kadam, Yogini S. Jaiswal, Atul A. Shirkhedkar, Impurity profile: Significance In Active Pharmaceutical Ingredient, Eurasian Journal of Analytical Chemistry, Volume2,Number1,2007,ISSN:1306-3057

3. Renu Solanki, Impurity Profiling Of Active Pharmaceutical Ingredient And Finished Drug Product, International Journal Of Drug Research And Technology,2012,vol-2(3),231-238,ISSN-2277-1506

4. Atamna H, Nguyen A, Schultz C, Boyle K, Newberry J, Kato H, Ames BN. "Methylene blue delays cellular senescence and enhances key mitochondrial biochemical pathways". FASEB J. 22 (3): Pg. No. 703–712; 2008

5. Rose, David . "New drug Rember brings 'unprecedented' Alzheimer's treatment advance". The Times. Retrieved; 2008.

6. Zelicia Gerald, Waldemar Ockert "Alzheimer's disease market: hope deferred". Nature Reviews Drug Discovery 12 (19-20); January 2013

7. Wischik C, Bentham P, Wischik D, Seng K . "Tau aggregation inhibitor (TAI) therapy with rember arrests disease progression in mild and moderate Alzheimer's disease over 50 weeks". Alzheimer's and Dementia 4 (4): T167–T167; July 2008

8. ICH, Q2 (R1) Validation of Analytical Procedure: Text and Methodology, International Conference on Harmonization, Geneva, Switzerland; 2005.

9. International Conference on Harmonization, Draft Revised Guidance on Impurities in New Drug Substances. Federal Register Q3A(R), 2000, 65 (140): 45085.

10. ICH guideline, Q3Avalidation of analytical procedure: Methodology, 1996,6,11.

11. Michael E; Ira S. Krull; Analytical method development and validation, 1992,269.

12. United National drug control program, V.01-83778-May2001, P.No-65.

13. United State Pharmacopeia, The National Formulary, Asian Edition. 2004.

14. International Conference on Harmonization, Draft Revised Guidance on Impurities in New Drug Products. Federal Register Q3B(R), 2000, 65 (139): 44791.

15. International Conference on Harmonization, Impurities, Q3C-Guidelines for Residual Solvents, Q3C. Federal Register, 1997, 62(247): 67377.

16. International Conference on Harmonization, Specifications, Q6A: Test Procedures and Acceptance Criteria for New Drug Substances and New Drug Products. Chemical substances, 1999,65(146):67488.

17. Yogesh Thorat, Significance of Impurity Profiling: A review, International Journal of Drug formulation&Research,Vol-2,Issue-4,Jul-Aug2011,186.

18. Dorottya Barots,Sandor gorog,Recent Advances In Impurity Profiling Of Drug, Current PharmaceuticalAnalysis,Vol-4,Issue-4,P.No:215-230(16),ISSN-1573-4129.

19. United States Pharmacopoeia XXIV, United States Pharmacopoeial Convention, INC, Rockville; 2000.

20. Talluri N K , Impurity Profiling of Drugs and Pharmaceuticals, New Analytical Methods- Development and Validation, LAP Lambert Academic

Publishing, 2011, 14

21. Keval D. Parmar, Dr.N.M.Patel, Dr.P.M.Patel A Review: Impurity Profile Study, J.of Pharmacy Research, 2011; 4(8).

22. Mr.Anand M. Kudal, impurity profile of active pharmaceutical ingredient: A review, latest reviews, vol. 4 issue 5, pharmainfo.Net, pharmaceutical information, articles and blogs, 2006.

23. Alsante K M, Hatajik T D, Lohr L L and Sharp T R, Isolation and Identification of Process Related Impurities and Degradation Products from Pharmaceutical Drug Candidates. Part 1, American Pharm. Review,2001; 4(1):70.

24. Peter J S; Ahmed A; Yan W, Journal of Pharmaceutical Biomedical Analysis, 2006, 41, 883.

Radhakrishna T, Satynarayana J and Satynarayana A, Determination of Methylthininium chloride and its Related Impurities by HPLC. Indian Drugs, 2002, 39 (6): 342.

25. Skoog D.A., West D.M. and Holler J.F., Analytical Chemistry-An Introduction, 6th edition,

Sharma B.K., "Instrumental Method of Chemical Analysis", 18th edition, Goel Publishing House, Meerut, 1999, Page No. 1-33, 56-81, 175-203.

26. Beckett, A.H. and stenlake, J.B. Eds., Practical Pharmaceutical Chemistry, 4th edition, part 2nd, CBS publication, 2004, page no. 275-276.

27. Braun R.D., Introduction to Instrument Analysis, Pharma Book Syndicate, Hyderabad, 2005, page no. 261

28. Sethi P. D., Introduction to HPLC: Quantitative Analysis for Phamraceutical Formulation, 1st edition, CBS Publishers and Distributors, New Delhi, 2001, Page no. 51-66

29. Willard HH, Merrit LL (Jr), Dean JA, Settle FA (Jr). Instrumental methods of analysis. 6th ed. New Delhi: C.B.S. Publishers and Distributors; 1986.

30. Radhakrishna T, Satynarayana J and Satynarayana A (2002) HPLC method for the Degradation of Methylthininium chloride and its Related Impurities. Indian Drugs, 2002, 40(3): 166.

31. Zawilla N H; Li B; Hoogmartens J; Adams E, Journal of Pharmaceutical Biomedical Analysis, 2006, 42, 114.

32. Lohr L L; Sharp T R; Alsante K M ; Hatajik T D ,American Pharmaceutical Review, 2001, 4(1), 130-139.

33. British Pharmacopoeia, the Department of Health, Social Services and Public Safety, 2004.

34. Halmos Z, Szantay C, Brlik J J, Csehi A, Varga K, Horvath P, Kislaki M, Domani G, Nemes A and Gorog S, Estimation of impurity profile of drugs and related materials Part 15. Identification of

minor impurities in Methylthininium chloride. J Pharm Biomed Anal, 1996; 15:1.

35. Kelly S, Padraig M G, Stuart J M and David H G , An impurity in a Methylthininium chloride product identified is 5-(Hydroxymethyl)-2-furfural, 10- Hydroxymorphine and 10-Oxomorphine. J Pharm Biomed Anal 2003; 92:485