International Journal of Chemical Studies

P-ISSN 2349–8528
E-ISSN 2321–4902
IJCS 2015; 2(5): 22-26

Received: 22-12-2014
Accepted: 03-01-2015

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Validated RP - HPLC method for the determination of mepyramine maleate in bulk and pharmaceutical formulation

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Abstract
A simple, selective, linear, precise and accurate RP-HPLC method was developed and validated for rapid assay of Mepyramine Maleate in Bulk and Pharmaceutical Formulation. Isocratic elution at a flow rate of 1.0 ml/min was employed on symmetry µ Bondapak Phenyl 5 µm (3.9 mm x 30 mm) Column at ambient temperature. The mobile phase consisted of Buffer: Methanol: Acetonitrile in the ratio of 620:300: 250 v/v. The UV detection wavelength was 220 nm and 25 µl sample was injected. The retention time for Mepyramine Maleate was 9 min. The method gives linear response from 12.55 – 0.0625 mg/swab of Mepyramine Maleate. The method was validated as per the ICH guidelines. The method was successfully applied for routine analysis of Darunavir in the rapid and reliable determination of Mepyramine Maleate Pharmaceutical formulation.

Keywords: Mepyramine Maleate, RP-HPLC, UV detection, recovery, precise.

1. Introduction

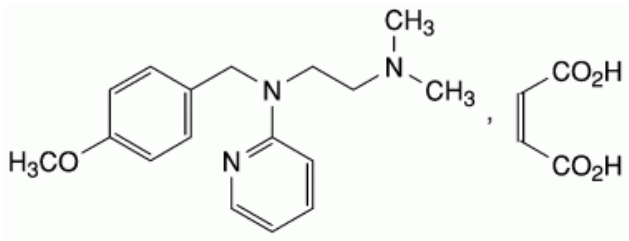


Fig 1: Mepyramine Maleate

The Systematic (IUPAC) name for Mepyramine Maleate is *N*-(4-Methoxybenzyl)-*N*′,*N*′-dimethyl-*N*-(pyridin-2-yl)ethane-1,2-diamine (►*Z*)-butenedioate◄. Mepyramine, also known as pyrilamine, is a first generation antihistamine, targeting the H_1 receptor [1]. However, it rapidly permeates the brain often causing drowsiness. It also has anticholinergic properties. It is used in over-the-counter combination products to treat the common cold and menstrual symptoms [2]. It is also the active ingredient of the topical antihistamine creams Anthisan and Neoantergan, sold for the treatment of insect bites, stings, and nettle rash. G. Nagarajan *et al*. [3] revealed that Chromatographic analysis was performed on an Oyster BDS C18 column (250 x 4.6 mm, 5 µm) column temperature 65 ^{0}C with a mixture of buffer A and buffer B in the ratio 50:50 as mobile phase, at a flow rate of 1.0 mL min-1. UV detection was performed at 208 nm. The method was validated for accuracy, precision, specificity, linearity and sensitivity. The retention times of Enalapril maleate and Ramipril were 4.197 and 5.819 min, respectively. The Limit of detection was 0.571 and 1.090 µg mL-1and the quantification limit was 1.733 µg mL^{-1}and 3.303 µg mL^{-1} for Enalapril maleate and Ramipril, respectively. The accuracy of the proposed method was determined by recovery studies and found to be 98.06% to 100.47%. Pinak. M *et al*., [4] described that Analysis was carried out using acetonitrile : methanol : phosphate buffer (50 : 20 : 30 v/v/v, pH 5.6) mobile phase at 1.0 mL/min flow rate and Sunfire C 18 column (5 µm × 250 mm × 4.6 mm) as stationary phase with detection wavelength of 220 nm. The linearity for chlorpheniramine maleate, ibuprofen, and phenylephrine hydrochloride was in the range of 0.5–2.5 µg/mL, 25–125 µg/mL, and

1.25–6.25 µg/mL, respectively. The % recoveries of all the three drugs were found to be 99.44–101.61%, 99.39–101.79%, and 98.66–101.83%. LOD were found to be 32, 120, and 68 ng/mL for CPM, IBU, and PHE, respectively. Taomin Huang *et al.* [5] developed Optimum chromatographic separations among pheniramine maleate, naphazoline hydrochloride by using an Agilent zorbax eclipse XDB C18 column (150 mm × 4.6 mm, 5 µm) as the stationary phase with a mobile phase consisted of 10 mM phosphate buffer pH 2.8 containing 0.5% triethlamine and methanol (68:32, v/v) at a flow rate of 1 mL min^{-1}. Detection was performed at 280 nm using a diode array detector. Theoretical plates for pheniramine maleate and naphazoline hydrochloride were calculated to be 6762 and 6475, respectively. Regression analysis showed good correlations ($R^2 > 0.999$) for pheniramine maleate in the concentration range of 150–1200 µg mL^{-1} and naphazoline hydrochloride in 12.5-100 µg mL^{-1}. The method results in excellent separation of both the analytes and degradation products. The peak purity factor is ≥980 for both analytes after all types of stress, indicating complete Mukesh Maithani *et al.* [6] described a simple, specific and accurate reverse phase high performance liquid chromatographic method was developed for the simultaneous determination of chlorpheniramine maleate and phenylephrine in tablet dosage forms. A reversed phase C18 column (250 mm × 8 mm) i.d., particle size 10 µm) column with mobile phase consisting of acetonitrile and phosphate buffer 55:45 (v/v) (pH 5.6 ± 0.02, adjusted with triethylamine) was used. The flow rate was 1.0 ml/ min and effluents were monitored at 255 nm. The retention time for chlorpheniramine maleate and phenylephrine were found to be 3.13 min and 4.58 min, respectively.

2. Materials and Methods

2.1 Instrumentation

Peak HPLC containing LC 20AT pump and variable wavelength programmable UV-Visible detector and Rheodyne injector was employed for investigation. The chromatographic analysis was performed on a µ Bondapak Phenyl 5 µm (3.9 mm x 30 mm). Degassing of the mobile phase was done using a Loba ultrasonic bath sonicator. A Denwar Analytical balance was used for weighing the materials.

2.2 Chemicals and Solvents

The reference sample of Mepyramine Maleate (API) was obtained from Jollc. The Formulation Mepyramine Maleate was purchased from the local market. Buffer, Methanol and Acetonitrile used were of HPLC grade and purchased from Merck Specialties Private Limited, Mumbai, India.

2.3 Chromatographic Conditions

The following Chromatographic conditions are followed.
Mobile phase**:** Buffer (620)**:** Methanol (300)**:**
Acetonitrile (250)
P^H: 3.1
Analytical Column: µBondapak Phenyl 5 µm (3.9 mm x 30 mm)
UV Detection wave length: 220 nm
Flow rate: 0.8 ml/min
Injection volume: 25 µl
Temperature: Ambient
Runtime: 9 Min (Mepyramine Maleate)

2.4 Standard solution of the drug:

Accurately weigh 251 mg of Mepyramine Maleate standard into a 5 ml volumetric flask.
Dilute and make up to volume with solvent and mix well (Solution 1). Further dilute 10 µl of this solution to 10 ml volumetric flask and dilute to volume with solvent.

2.5 Preparation of Standard solution (Stock Solution)

Accurately weigh 125.5 mg of Mepyramine Maleate standard into a 100 ml volumetric flask and dissolve and dilute to volume with Methanol (12.55 mg/swab).

2.6 Sample Preparation

Place 10 µl of solution 1 onto a specific surface area of stainless steel plate Swab the surface area Place the swab stick into 10 ml volumetric flask, filled with 10 ml solvent and sonicate for 10 minutes, repeat the process with six different swabs. Filter sample through a 0.45 µm filter.

2.7 Buffer Solution Preparation:

Dissolve 4.0 g of Sodium Perchlorate in 750 ml water. Add 0.3 ml of Ammonia solution and adjust pH to 3.1 with Perchloric acid.

3. Method Development

For developing the method, a systematic study of the effect of various factors was undertaken by varying one parameter at a time and keeping all other conditions constant. Method development consists of selecting the appropriate wavelength and choice of stationary and mobile phases. The following studies were conducted for this purpose.

3.1 Detection of wavelength

The spectrum of 10 ppm solution of the Darunavir in methanol was recorded separately on UV spectrophotometer. The peak of maximum absorbance wavelength was observed. The spectra of Darunavir were showed maximum absorbance at 271 nm.

4. Validation Procedure and Requirements

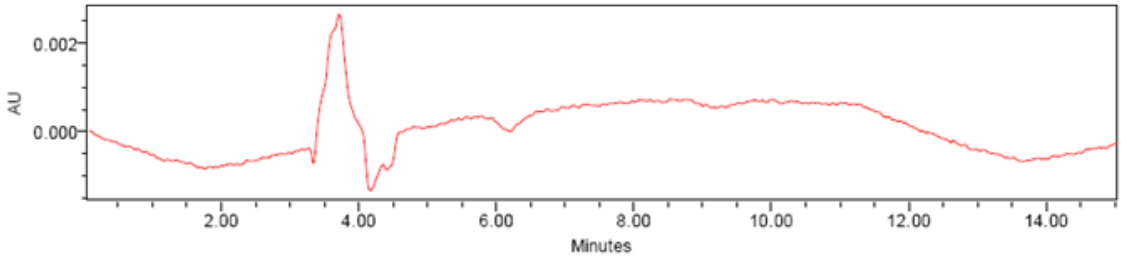
The analytical performance of the method of analysis was checked for specificity, system suitability, detection limit, and method precision.

4.1 Specificity

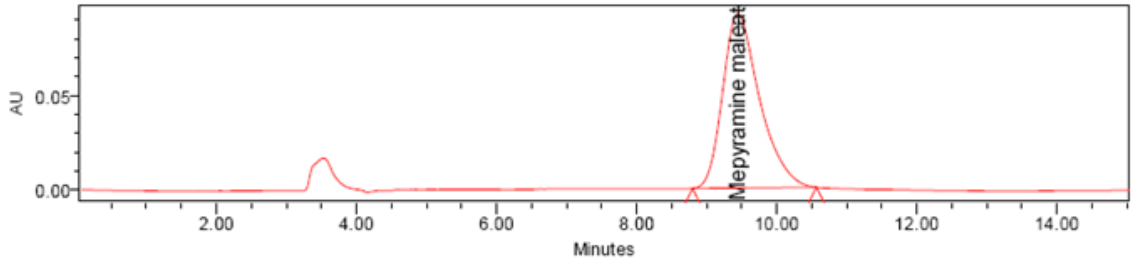
Specificity of an analytical procedure is its ability to assess unequivocally the analyte in the presence of components that may be expected to be present. The solvent and placebo solutions must contain no components, which co-elute with Mepyramine Maleate. The peak purity results from the photo diode-array analysis must show that the Imipramine Hydrochloride peak is pure – i.e. the purity angle (PA) must be less than the threshold angle (TH). The solutions listed below were injected using the conditions specified in the method of analysis

Solvent – No significant peak detected	Chromatogram 1
Peak due to Mepyramine Maleate	Chromatogram 2
Placebo- No significant peak detected at Mepyramine Maleate retention time.	Chromatogram 4

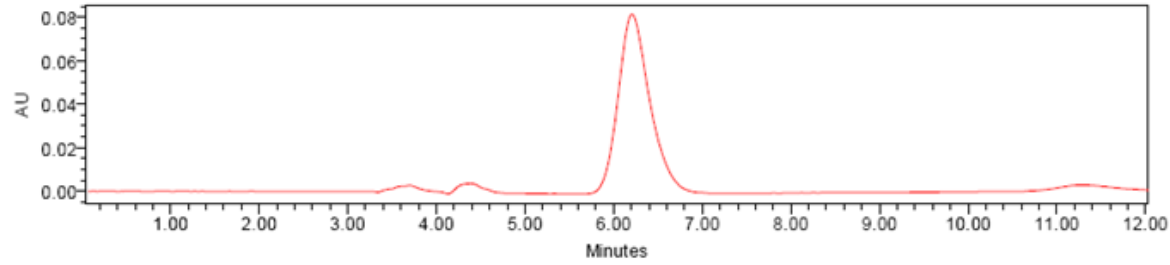
Chromatogram: 1



Chromatogram: 2



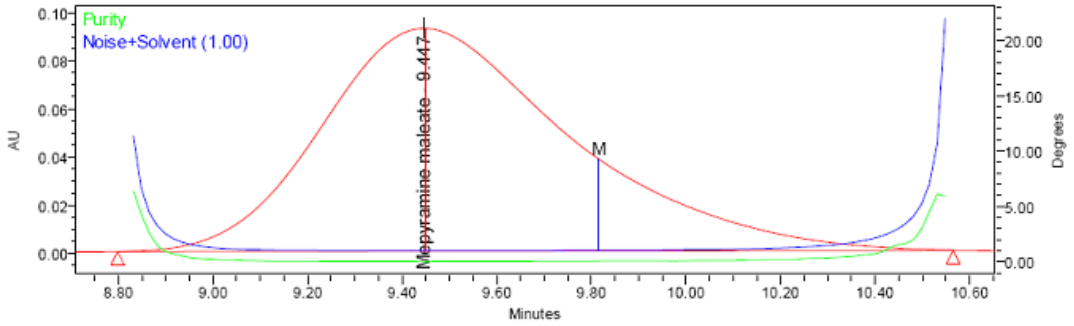
Chromatogram: 3



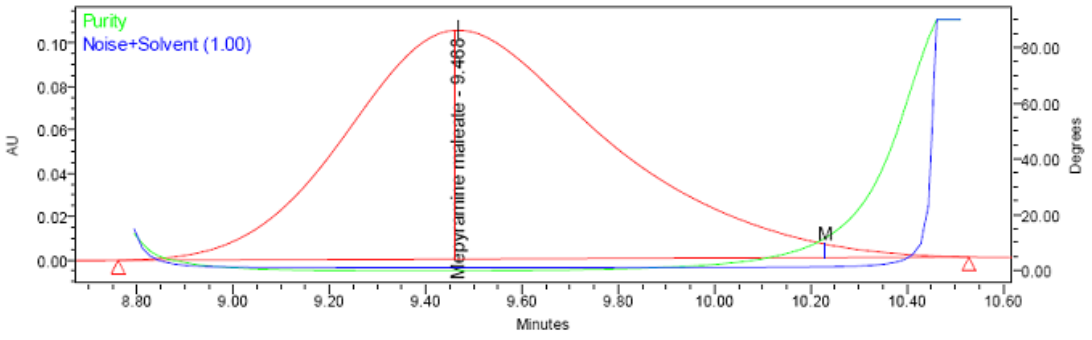
Purity Results

No components are seen to co-elute with the Mepyramine Maleate peak, and the peak Purity results indicate that Mepyramine Maleate peak can therefore be considered spectrally pure. The method employed is specific for the API Mepyramine Maleate in the product. The results are tabulated in the Table:

Peak purity: 1



Peak purity: 2



4.2 System Suitability

System suitability is a measure of the performance and chromatographic quality of the total analytical system – i.e. instrument and procedure. Six replicate injections of API working standard solution were injected according to the method of analysis. The percentage relative standard deviations (% RSD) for the peak responses were determined. The % RSD of the peak responses due to the Mepyramine Maleate for six injections must be less than or equal to 5.0%. The analytical system complies with the requirements specified by the system suitability. The results are tabulated in the table: 1.

Table 1: % RSD Results

Sample	Mepyramine Maleate Area
1	1575565
2	1634733
3	1640255
4	1650620
5	1654595
6	1664841
Mean	1636768
% RSD	1.9

4.3 Limit of Detection

The limit of detection by definition is a parameter of a limit test. It is the lowest concentration of analyte in a sample that can be detected, but not necessarily quantitated under the stated experimental conditions. It merely substantiates that analyte concentration is above or below a certain level. The Detection Limit is determined by the analysis of samples with known concentrations of analyte and by establishing the minimum level at which the analyte can be reliable detected. The detection limit must be capable of detecting the API at 50% MAC. The method gives linear response from 12.55 – 0.0625 mg/swab of Mepyramine Maleate. The results are tabulated in the table: 2.

Table 2: Limit of Detection Values

Conc. mg/swab	INJ – 1	INJ – 2	Average
0.06250000	125805	121735	123770
0.12550000	332705	333831	333268
0.25100000	765688	756402	761045
0.50200000	1675335	1671514	1673425
1.25500000	4155693	4144333	4150013
12.55000000	41786210	41862762	41824486

Preparation of Standard solutions (range)

From (12.55 mg/swab) stock solution the series of standard solutions was prepared. 1.255 mg/swab, 0.6275 mg/swab, 0.502 mg/swab, 0.251 mg/swab, 0.1255 mg/swab, 0.06275 mg/swab, 0.0251 mg/swab, 0.01255 mg/swab. The range of standard solutions above was also injected twice and the average result was shown in graph. Eight solutions containing 12.55, 1.255, 0.502, 0.251, 0.1255, and 0.06275 mg/swab of Mepyramine Maleate, relative to the working concentrations, were prepared and injected according to the method of analysis. A linear regression curve was constructed. Results are shown in the Fig: 2

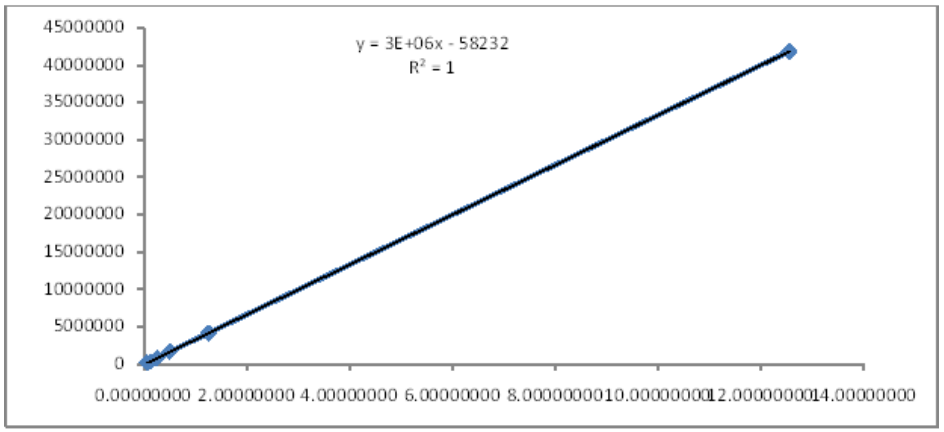


Fig 2: Linearity Results

Table 3: Precision Results

Sample	% Recovery	% Average Recovery
1	101	101
	100	
2	69	69
	69	
3	65	65
	65	
4	104	104
	104	
5	70	71
	71	
6	67	67
	67	
Mean		80

4.4 Precision

10 µl of MAC concentration is placed on a specific surface area (stainless steel) and swabbed as outlined in the Cleaning Validation SOP using the specified solvent and specified material. The precision of the analytical method is determined by assaying the swabs and calculating the % recovery of the API results. The precision will entail repeated testing of six samples prepared and the results are tabulated in the Table: 3

5. Declaration of the Validity of the Method

The Cleaning Validation method is proven to be valid and the validation test results show that the method complies with the validation requirements. The method is therefore acceptable as valid.

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