Concurrent analysis of homatropine methylbromide and hydrocodone bitartrate related impurities in reverse phase chromatography by Uplc

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Abstract

A Novel, stability indicating reversed phase Ultra performance liquid chromatography (UPLC) method has been developed and validated for simultaneous determination of Homatropine Methyl bromide (HM) and Hydrocodone Bitartrate (HB) related compounds in pharmaceutical dosage form. This chromatographic separation was carried out on an Acquity UPLC using reversed phase column (100 x 2.1 mm, 1.7 µm), a simple gradient program for 40 minutes used. Mobile phase A (buffer) consists a mixture of 14.5 mM sodium phosphate monohydrate, 12.8 mM sodium 1-octanesulfonate monohydrate and 13.8 mM Triethylamine pH adjusted to 2.0 with phosphoric acid, Acetonitrile used as Mobile phase B and mobile phase flow rate used constantly at 0.45 mL Min^{-1}. The chromatography analysis was monitored at 205 nm with column oven temperature at 45℃ and injection volume as 20 µL. All the components were separated with good resolution in less than 40 minutes. The proposed method has been validated according to ICH guidelines, validation of method showed it to be Specific, Robust, Precise, Accurate and Linear over a range of analysis.

Keywords: UPLC, Homatropine Methyl bromide, Hydrocodone Bitartrate, Related compounds, Validation

Introduction

Combinations of analgesic, decongestant and cough preparations are widely used for cough and pain relief treatments.

Homatropine Methylbromide[(1)] chemically named as 3-{[hydroxyl(phenyl)acetyl]oxy}-8,8-dimethyl-8-azoniabicyclo[3.2.1]octane, it belongs to the group of medicines called anti muscarinic. Homatropine Methylbromide (HM) is employed in treatment of duodenal or stomach ulcers or intestine problems, it can be prescribed along with antacids or other medicine in the cure of peptic ulcer, it can also be used to avoid nausea, vomiting and motion sickness. HM is used on its own or in combination with Hydrocodone Bitartrate. The chemical formula is $C_{17}H_{24}BrNO_3$ and its molecular weight is 370.28 g mol^{-1}. The structure of HM is as given **Fig. 1**.

Hydrocodone Bitratrate[(1)] chemically named (4R,4aR,7aR,12bS)-9-methoxy-3-methyl-1,2,4,4a,5,6,7a,13-octahydro-4,12-methanobenzofuro[3,2-e]isoquinoline-7-one;(2R,3R)-2,3-dihydroxybutanedioic acid, it is a powerful vasoconstrictor, employed as nasal decongestant and cardiotonic agent. The molecular formula is $C_{22}H_{27}NO_9$; the molecular weight is 449.456 g mol^{-1}. The structure of HB is as given **Fig. 1**.

There were few methods proposed for estimation of Homatropine Methyl bromide alone or in combination with other compounds. In some literature survey Homatropine Methyl bromide estimated alone by HPLC,[(3-4)] turbidometric determination,[(6)] colorimetric determination[(7)] and in combination with other compounds including opium alkaloids[(5)] by HPLC. Also, another method reported by UV spectrophotometer.[(8)]

Several HPLC methods were proposed for estimation of Hydrocodone Bitratrate in single[(3)] or in combination of other compounds. In some literature survey, it is estimated in combination with other compounds like Acetaminophen,[(8-14)] another LC-MS-MS method was reported.[(15)]

Ultra performance liquid chromatography (UPLC) has been measured as a novel development in liquid chromatography and it is particularly designed to endure higher pump pressures during chromatographic analysis to enable significant reduction in resolving time and solvent consumption. The UPLC columns packed with about 1.7 µm sized particles provides not only high efficiency but also the ability to work at higher linear velocity without loss of efficiency giving both resolution and speed. Using benefits of UPLC, a variety of applications in different fields including pharmacy,[(16)] clinical analysis, pesticide analysis[(17)] and tetracyclines in human urine[(18)] have reported.

In literature survey, there have been no stability indicating UPLC methods available to estimate related substances of HM and HB simultaneously during analysis in pharmaceutical dosage forms. Due to extensive interference from preservatives in oral solutions the separation and estimation of both analytes and its related impurities in conventional HPLC system is highly time taking and non-productive, hence a rapid stability indicating method is highly in demand. The aim of the current work is the development of a stability indicating and rapid UPLC method for the determination of HM and HB related impurities in the pharmaceutical dosage forms under forced degradation

conditions. The process impurities such as Dihydrocodeine Bitartrate, Dihydrothebainone, 6 beta-Tetrahydrothebaine Bitartrate, Methylmandalate and scopolamine are well controlled in manufacturing process and well separated in present method hence the method validation aims only on Homatropine Hydrobromide and Hydrocodone N-Oxide impurities. The developed method has been validated according to International Conference on Harmonization (ICH) guidelines[(2)] to demonstrate the stability indicating capability of the method.

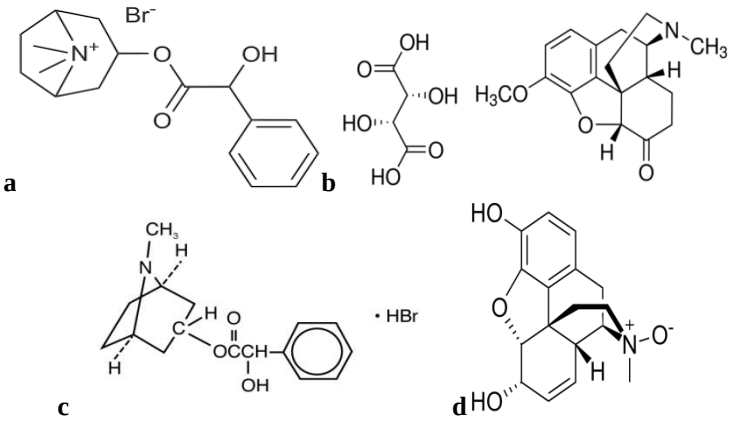

Fig. 1: Chemical structures of compounds; a) Homatropine Methylbromide b) Hydrocodone Bitartrate c) Homatropine Hydrobromide d) Hydrocodone N-Oxide

Materials and Method

Chemicals and Reagents: The reagents and chemicals used in present study are given in this section. HPLC grade of Acetonitrile is from Acrose (New Jersey, USA), Sodium phosphate monohydrate, Sodium 1-octanesulfonate monohydrate acid and Triethylamine is from (Sigma Aldrich) and phosphoric acid is from Labchem (Pittsburgh, PA, USA). HPLC grade water was used for the mobile phase preparation. Active pharmaceutical ingredients Homatropine Methyl bromide, Hydrocodone Bitartrate, Homatropine Hydrobromide impurity and Hydrocodone N-Oxide impurity were procured LGC standards.

Equipment and Chromatographic conditions: The UPLC system used is Waters Acquity (Milford, USA) equipped with binary solvent mangers, Ultra fast auto-sampler and a UV visible Detector was used to make sample injections. A reversed phase Acquity CSH^{TM} Phenyl-Hexyl column with dimensions 100 x 2.1 mm, 1.7µm particle size was used for analysis, column oven temperature used at 45 ºC. Mobile phase-A consist of 14.5 mM sodium phosphate monohydrate, 12.8 mM sodium 1-octanesulfonate monohydrate and 13.8 mM Triethylamine pH adjusted to 2.0 with phosphoric acid and Acetonitrile is used as mobile phase-B and delivered at 0.45 mL/Min. The Sample injection volume used as 20 µL and chromatographic study is monitored at Detector wavelength of 205nm.

Diluent: Diluent consist a mixture of Water and Methanol in the ratio of 94:6 V/V

Standard and Samples solutions

Working Standard solution preparation: Standard solutions at a concentration of about 0.5 µg / mL of Homatropine Methyl bromide 1.0 µg / mL of Hydrocodone Bitartrate was prepared by dissolving the appropriate amount of standard in diluent, filtered the solution through 0.2µm Nylon membrane filter.

Sample solution: Pipetted the sample solution equivalent 150 µg / mL of Homatropine Methyl bromide, 500 µg / mL of Hydrocodone Bitartrate in 100mL volumetric flask, dissolved and diluted to volume with diluent, filtered the solution through 0.2µm Nylon membrane filter.

Impurity standard stock solution: An individual Homatropine Hydrobromide and Hydrocodone N-Oxide impurity standard stock solutions at a concentration of about 1.0 µg mL^{-1} and 2.0 µg mL^{-1} respectively were prepared by dissolving the appropriate amount of standard in diluents, filtered the solution through 0.2µm Nylon membrane filter. These solutions were further diluted based on requirements in method validation parameters.

Method Validation solutions

Linearity solutions: Linearity solutions were prepared by diluting Standard stock solution at six different concentrations levels ranging from 10-150% of standard solution concentration. The responses were measured as peak areas and plotted against concentration.

Specificity solutions: The forced degradation experiments were performed to demonstrate Specificity and stability-indicating ability of the proposed method. The sample and Placebo were exposed to acid (0.5N HCl, 180 min at 60 ºC), base (0.025 NaOH, 60 min at Room temperature), strong oxidation (5% H_2O_2 for 50 min at Room temperature), thermal (105 ºC, 24 hours), and photolytic (1.2million lux h, 200 wh m^{-2}, 2 days)

degradation conditions. Samples were withdrawn at appropriate times and subjected to UPLC analysis after dilution equal to sample solution concentration to evaluate the ability of the proposed method to separate analytes from its impurities and placebo. Photo diode array detector was employed to check and ensure the homogeneity and purity of each analyte peak in all the stressed sample solutions.

Precision samples

Method precision: Method precision samples were prepared by spiking the impurities at standard concentration to the sample solution. Injected six independent sample preparations against working standard and calculated the % RSD for obtained impurity standard concentrations.

Intermediate Method precision (Ruggedness): Intermediate Method precision samples were prepared by second chemist by spiking the impurities at standard concentration to the sample. Injected six independent sample preparations on different day, different instrument and different column against working standard and calculated the % RSD for obtained impurity standard concentrations.

Accuracy solutions: The recovery experiments samples were prepared by spiking the impurity solutions to test sample at 50, 100 and 150% of the standard concentration. Calculated the % Recovery using obtained and spiked amounts.

Robustness solutions: The working standard and spiked sample solutions were used and evaluated variance in each varied condition.

Stability of the solution: Standard and sample solution stability was established by storage of sample solution at refrigeration condition (5ºC) for about 96 hours.

Limit of Quantification (LOQ): The LOQ concentration for analyte peaks considered as 0.05% where the signal to noise ratio found more than 11. The method precision at LOQ was performed by injecting six individual placebo sample solution spiked with impurities at 0.05% level respect to standard concentration.

Filter Study: Filter study was performed to determine the filter suitability for standard and sample filtration and to determine the amount of filtrate to be discarded before a sample solution is collected for analysis. This was performed by comparison of results between centrifuged sample and filtered sample.

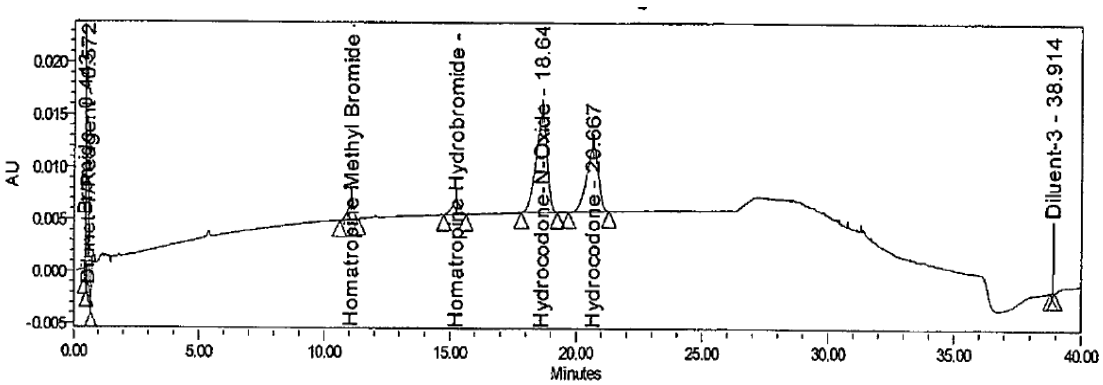

Fig. 2: Typical chromatogram for working standard solution with impurity standards

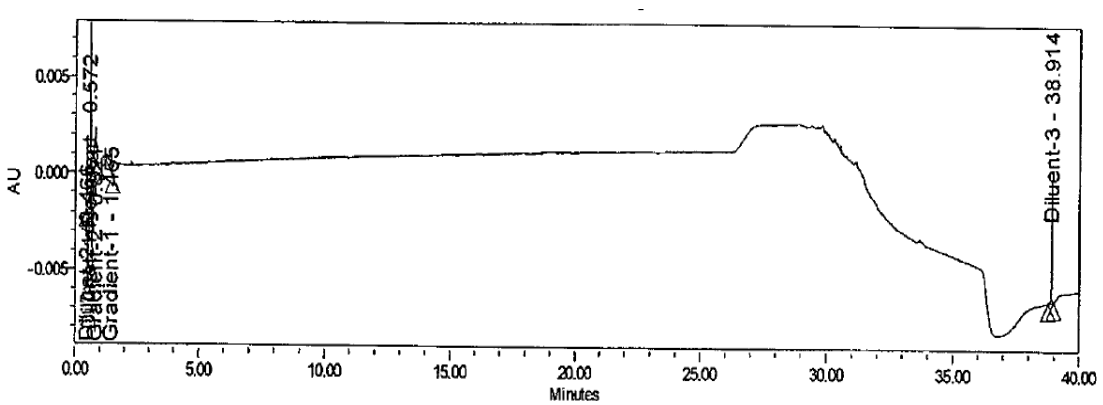

Fig. 3: Typical chromatogram for Blank

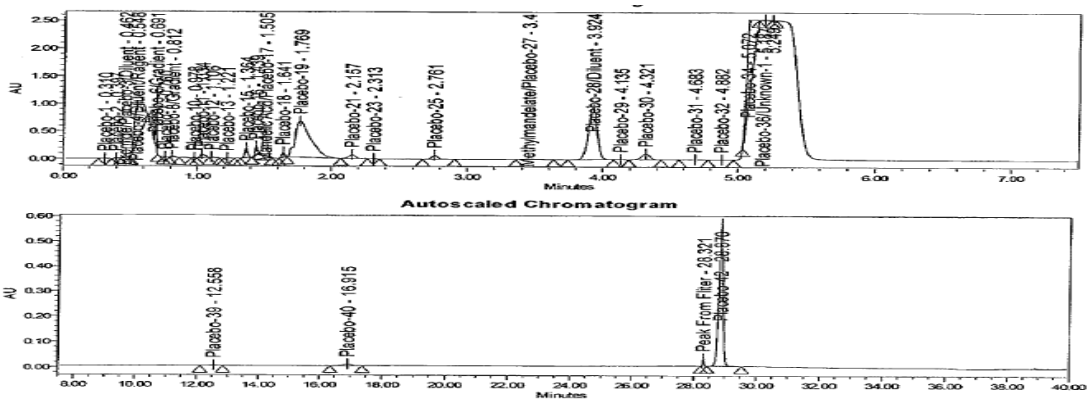

Fig. 4: Typical chromatogram for Placebo

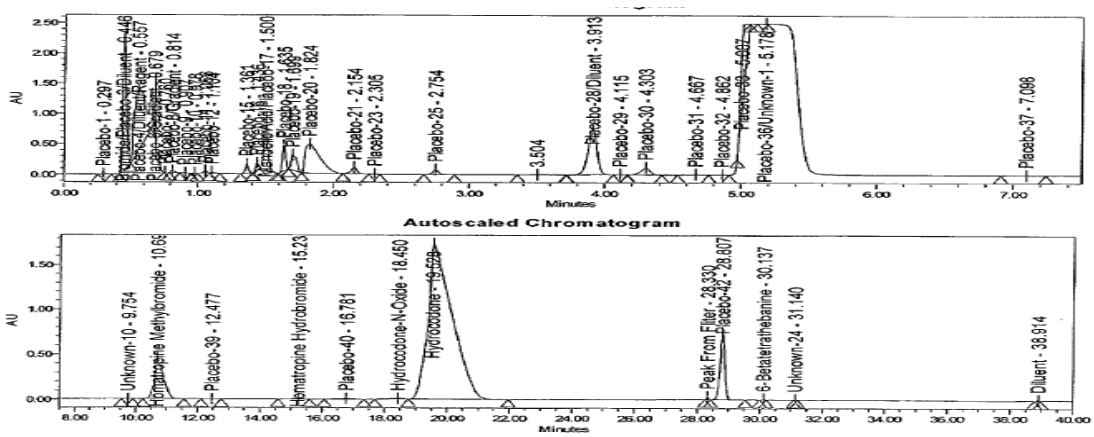

Fig. 5: Typical chromatogram for spiked sample

Note: In Fig. 4 and 5: The chromatogram represented above zoomed from 0-8 minutes for better view.

Result and Discussion

Method development and optimization: During literature survey, there was no stability indicating UPLC Related compound method proposed for simultaneous estimation of Homatropine Methylbromide (HM) and Hydrocodone Bitratrate (HB). The present work was aimed to develop a stability indicating method for proposed compounds by UPLC for determination of Specified and unspecified impurities in pharmaceutical dosage forms. At the beginning, based on physical properties of analyte compounds, the method development process was carried by employing a sample solution contains 500, 150, 0.5 and 1 µg mL^{-1} of HB, HM, Hydrocodone N-Oxide (HN) and Homatropine Hydrobromide (HH) respectively. The separation was performed by reversed phase UPLC on Acquity CSH^{TM} Phenyl-Hexyl 100*2.1mm 1.7µm UPLC column using a gradient mobile phase. Mobile phase A consists buffer with mixture of 14.5mM sodium phosphate monohydrate, 12.8mM sodium 1-octanesulfonate monohydrate pH adjusted to 2.0 with phosphoric acid and Acetonitrile used as mobile phase-B and mobile phase flow rate used constantly at 0.5 mL min^{-1}, the column oven temperature used at 40 ºC. It has been observed that the all placebo peaks and impurity peaks were well separated but Hydrcodone N-oxide impurity is co eluted with parent peak i.e., Hydrocodone Bitartrate peak.

Further, to overcome HN impurity separation from parent peak different parameters were investigated including different mobile phase gradient programs. The decreased organic ratio resulted more tailing to main peaks. To get better separation and peak shape, introduced 'Triethylamine' in mobile phase A and reduced the flow rate to 0.45mL min^{-1}, also increased the column oven temperature from 40 to 45ºC. Finally all the peaks were separated with mobile phase composition having the buffer with mixture of 14.5 mM sodium phosphate monohydrate, 12.8 mM sodium 1-octanesulfonate monohydrate and 13.8 mM Triethylamine, pH adjusted to 2.0 with phosphoric acid and Acetonitrile used as mobile phase-B. A detection wavelength 205 nm was selected where all components exhibit almost a satisfactory response. A typical chromatogram of standard showing the separation represented in Figure 2 and the system suitability data were presented in Table 2.

Analytical parameters and validation

After satisfactory development of method, product was subjected to method validation per ICH guidelines.[2] The method was validated to demonstrate its suitability for intended purpose using the standard procedure and the validation characteristics including System Suitability, Specificity, Accuracy, Precision, Ruggedness, Robustness, LOQ, Linearity and Stability of solution have been evaluated.

System Suitability: The main purpose to perform system suitability was to check suitability of machine to perform method validation. This was established by calculating the percentage Relative Standard Deviation for an average area of six replicate injections of working standard. The % RSD found below 1.1, the Tailing factor for all the analyte peaks found not more than 2.0.

Specificity: There was no interference from the diluent (Blank) and placebo. The forced degradation testing proved that there was no interference from degradation impurities at retention time of targeted peaks. The Purity data for each analyte peak shows the peak is pure and there were no co-eluting peaks. Hence method proved that specific. The typical chromatograms for Blank and placebo are shown in Fig. 3 and 4.

Precision at Limit of Quantitation (LOQ): The concentration with Signal to Noise ratio of at least not less than 10 was taken as LOQ. Injected 0.05% impurity solution and checked the Signal to Noise ratio, found more than 20 and which meets the criteria defined by ICH guidelines[2]. The precision at LOQ was carried out by spiking the impurities at 0.05% of standard concentration to placebo solution and calculated the % RSD for six sample, the results of LOQ concentration and % RSD for each analyte peak are presented in **Table 3.**

Linearity: To demonstrate the linearity of detector response for HB, HN, HH and HM, injected the solutions of concentrations ranging from LOQ to 150% of the working standard concentration of HB and HM. Calculated the correlated coefficient and found to be greater than 0.999 indicated that magnificent correlation between the analyte concentration and peak area. The slope, Y intercept and regression coefficient results were presented in **Table 3.**

Precision: The values of the % relative standard deviation for sample repeatability lie well within 5.0 indicating the sample repeatability of the method is satisfactory. The results are presented in **Table 4**. The typical chromatogram for spiked sample is presented in Fig. 5.

Accuracy: The accuracy results were expressed in terms of mean percentage. The percentage recoveries obtained from triplicate sample found in a range of 95 to 105. The results were presented in **Table 5**. The recovery results indicate that the method is accurate and found that there was no interference due to the presence of excipients in the formulation.

Table 1: UPLC Pump Gradient program

Time	Mobile phase-A	Mobile phase-B	Curve
0.0	90	10	-
25.0	90	10	6
30.0	70	30	6
35.0	70	30	6
35.1	90	10	6
40.0	90	10	6

Table 2: Chromatographic system suitability data

Compound name	Retention time (Min)	Relative Retention Time (RRT)*	Tailing factor	% RSD for six replicates
HM	11.1	0.55	1.0	1.1
HH	15.2	0.78	1.1	1.1
HN	18.6	0.95	1.0	0.5
HB	20.7	1.0	1.0	0.4

*Relative Retention times with respect to 'Hydrocodone' peak.

Table 3: Summery of Linearity and LOQ results

Parameter	HM	HH	HN	HB
Slope	-27.33	356.256	-456.012	-2145
Y intercept	59440	62646	296128	205148
R^2	0.9999	0.9999	0.9999	0.9999
LOQ µg mL-1	0.08	0.08	0.25	0.23
% RSD at LOQ Precision (n=6)	6.2	8.1	2.6	3.4

Table 4: Method precision Results

Sample No	HH	HN
	% impurity	
1	0.242	0.110
2	0.235	0.107
3	0.252	0.109
4	0.243	0.106
5	0.253	0.107
6	0.223	0.109
Mean	0.241	0.108
% RSD	4.6	1.4

Table 5: Accuracy results

Accuracy level	HN	HH
	Average recovery in %	
LOQ	95	103
50%	103	106
100%	105	102
150%	102	99

Table 6: Compiled data of Method Precision and Ruggedness

Sample	HH		HN	
	% Impurity		% Impurity	
	Analyst-1	Analyst-2	Analyst-1	Analyst-2
1	0.223	0.253	0.107	0.102
2	0.235	0.242	0.110	0.110
3	0.253	0.261	0.106	0.109
4	0.243	0.250	0.109	0.108
5	0.252	0.247	0.109	0.109
6	0.242	0.252	0.107	0.106
Mean (n=12)	0.246		0.107	
% RSD (n=12)	4.1		2.1	

Table 7: Filter study results

Sample name	HH		HN	
	Average Area	% Difference	Average Area	% Difference
Centrifuged Sample	23957	-	138526	-
2mL Discarded	22341	6.7	127542	7.9
4mL Discarded	22854	4.6	131024	5.4
6mL Discarded	23154	3.4	135674	2.1
8 mL Discarded	23652	1.3	137423	0.8

Ruggedness: Intermediate precision was determined by performing the test by second analyst using different UPLC system and different column on different day. The % RSD of the sample impurity found less than 6.0%. The results prove that the method is rugged, refer Table 6 for results.

Robustness: The robustness of the method was determined as a measure of the analytical method capability to be unaffected by small deliberate variations in method parameters. Different variations, such as, variation in column temperature by ± 2 ºC and variation in pH of mobile phase A by ± 0.05 were performed. In all robustness conditions the relative standard deviation for replicate standard injections found less than 2.0%. The retention times of spiked sample impurity peaks found well within the 25% from normal condition. So, the method was found to be robust with respect to variability in all robust conditions.

Stability of sample solution: The stored Sample and Working standard solutions at refrigeration condition were analyzed at intervals of 24, 48, 72 and 96 hours using fresh working standard. The difference in area count was compared from initial to 96 hours, the results of solution stability for standard and samples found to be stable up to 96 hours at refrigeration temperature 5 ± 2ºC.

Filter Study: The test sample was prepared by Spiking impurities at about 50% of the working standard concentration and a portion of the sample was centrifuged to get the clear supernatant. The centrifuged sample was used as a control for the filter study. A portion of the supernatant solution was filtered through 0.2 µm Nylon filter by discarding the first 2mL of the filtrate and collected the 2 mL fraction in to an individual UPLC vial, i.e. 2 mL 4 mL and 8 mL fractions and injected.

The difference in area count when comparing filtered and centrifuged sample solutions, the results found that first 6mL of solution should be discarded before collecting for analysis. The results data are shown in the **Table 7**.

Conclusions

A rapid stability indicating gradient UPLC method has been developed for the simultaneous determination of Homatropine Methylbromide and Hydrocodone Bitartrate using UV-VIS detector. The method has validated for Specificity, Accuracy, Precision, Linearity, Robustness, Ruggedness, Solution stability and Filter study per ICH guidelines. The method uses a simple mobile phase composition, easy to prepare. The rapid run time of 40 minutes and relatively low flow rate (0.45 mL Min^{-1}) allows the analysis of large number of samples with less mobile phase that proves to be cost effective. Hence this UV-VIS method can be used for the routine samples.

Method Application

The UPLC method is Novel, rapid and stability indicating for the quantitative determination of Homatropine Methylbromide and Hydrocodone Bitartrate impurities in pharmaceutical formulation.

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