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DEVELOPED REVERSE PHASE CHROMATOGRAPHIC METHOD FOR ANALYSIS OF PARACETAMOL AND HOMATROPINE METHYL BROMIDE IN RIABASAM TABLET

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Abstract-*A developed chromatographic method has been proved in this work to identification Paracetamol and Homatropine methyl bromide in raw material and riabasam tablets. Chromatographic separation system was accomplished using C18 column (250 mm ×4.6 mm) with particle size 5µm. Isocratic elution of mobile phase prepared by dissolve 1.742 g of 1- pentansulfonic acid sodium salt in 750 ml of water, add 220 ml of acetonitrile then the whole solution was adjusted to a pH 3 with 2 M hydrochloric acid, complete to 1000 ml with water. The mobile phase was pumped at a flow rate of 1 ml/min with UV detector at 225nm at ambient temperature at (25±2 ℃) and injection volume as 100 µL. The method was linear over the concentration range of paracetamol and homatropine methyl bromide were 4–30 µg/mL 10-110 µg/mL respectively. The retention time of Paracetamol and Homatropine methyl bromide were found to be 5.4 minutes and 9.7 minutes respectively. Limit of detection and limit of quantification of paracetamol and Homatropine methyl bromide concentrations were found to be (0.442µg/mL and 0.0885 µg/mL) (1.473 µg/mL, 0.295 µg/mL) respectively. The average percentage recoveries of paracetamol were within 100.20 to 102.79% and homatropine methyl bromide were within 98.82 to 100.15%. The suggested procedure has been ratified according to ICH guidelines, validation of method showed it to be particular, durable, accurate and can be adoptable for quality control analysis of paracetamol and homatropine methyl bromide in riabasam tablets.*

Keywords: *Paracetamol, Homatropine methyl bromide, Method analysis, RP- HPLC.*

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I INTRODUCTION

Paracetamol, N (4hydroxy phenyl acetamides) (figure 1, A) is one of the major analgesics and antipyretic drugs. It is used to treat the pain of fever, headache, cold, arthritis, neuralgia, and diabetic neuropathy. [1]. Also, it is used in the management of acute pains in cutting-edge cancers [2]. In literature the most recent methods for determination of paracetamol include chromatographic, [3]– [6] electrochemical, [7],[8] spectrophotometric, [9]–[13] and fluorescence spectroscopic [14] techniques.

Homatropine Methyl bromide [15] chemically named as 8,8-dimethyl-8-azoniabicyclo [3.2.1] octan-3-yl) 2-hydroxy-2-phenylacetate bromide (Figure 1, B). It plays a significant role in the central nervous system as an anticholinergic medication. It used to avoid stomach problems, puke, and motion sickness. There were few methods proposed for estimation of Homatropine Methyl bromide alone or in combination with other compounds such as capillary zone electrophoresis [16],[17], High performance liquid chromatography [18],19], and turbidimetric determination [16]. In literature survey, there have been no RP-HPLC method available to estimate homatropine methyl bromide and paracetamol simultaneously in combination formulation such as riabasam tablet. The differences in concentration between paracetamol and Homatropine methyl bromide in riabasam tablet is too high (350 mg/tablet of paracetamol and 4 mg/tablet of Homatropine methyl bromide), which make the determination of these two drugs is challenging work, Therefore, the main objective of the current work is the development a new RP-HPLC method for separation of paracetamol and Homatropine methyl bromide in combination formulation. The proposed analytical procedure was successfully used for routine analysis of paracetamol and homatropine methyl bromide in riabasam drug dosage form without any interference from included excipients.

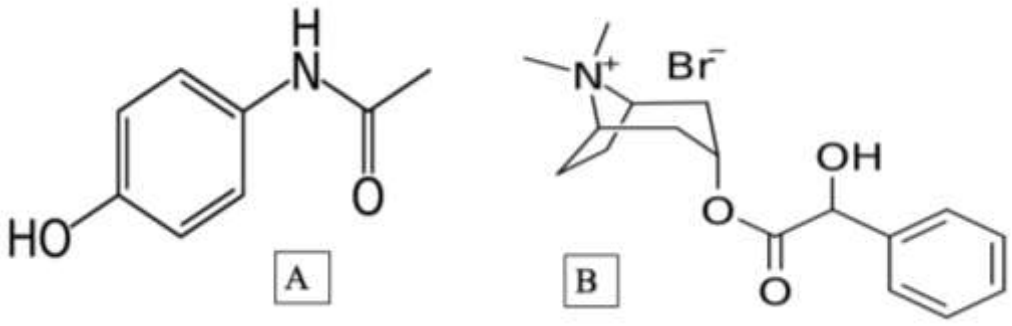

Figure 1: Structure of paracetamol (A) and Homatropine methyl bromide (B).

II EXPERIMENTAL

Instrumentation

The RP-HPLC system was performed on a Shimadzu system with (LC-20A) pump. Analysis was conducted on C18 column (250 mm ×4.6 mm) with particle size 5µmat ambient temperature. Samples injector valve with a 100 µL sample loop. (UPD-20A) variable wavelength UV-visible and LC solutions software. Table (1).

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Table 1: Chromatographic Conditions

RP-HPLC conditions	
Mobile phase	1- pentansulfonic acid sodium salt dissolved, Acetonitrile (78:22 v/v)
Column	Reverse phase C18 column with dimensions (250 mm ×4.6 mm) and particle size5µm.
Flow rate	1 ml/min
Detection	UV-detector, 225 nm
Column temp	Ambient temperature (25±2℃)
Sample injection	100µl for assay and compatibility

Material and chemicals

Reference Standards for Homatropine methyl bromide and Paracetamol are from United state pharmacopeia (USP), 1- pentansulfonic acid sodium salt (BDH), HPLC grade of Acetonitrile is from (Biosolve), and Hydrochloric acid is from (Sigma-Aldrich). Riabasam Tablets were obtained from the state compony for drugs and medical appliances, Samarra, Iraq.

Composition of mobile phase

The mobile phase was prepared by dissolving 1.742 g of 1- pentansulfonic acid sodium salt in 750 ml of water, add 220 ml of acetonitrile then the whole solution was adjusted to a pH 3 with 2 M hydrochloric acid, complete to 1000 ml with water. The mobile phase was ultrasonicated for 15 min.

Preparation of stock and standard solutions

Homatropine methyl bromide standard stock solution was prepared by accurately weighing about 10 mg of Homatropine methyl bromide and transferring to 100 ml volumetric flask the volume filled with distilled water to the mark. Working slandered solution were prepared serially diluted to make (4,8,10,20, and 30 µg/mL). Paracetamol standard stock solution was prepared by dissolving 30 mg in distilled water and filled up to 100 ml volumetric flask. Standard working solutions of paracetamol were prepared individually in water to yield a solution with final concentrations of 10,30,50,70,90, and 110µg/ml.

Samples preparation

Twenty riabasam tablets (each containing 350 mg paracetamol and 4 mg Homatropine methyl bromide) were precisely weighed and grinded into fine powder. A part of powder equal to 4 mg Homatropine methyl bromide was weighed and dissolved in deionized water then transferred to100 ml volumetric flask and complete to the mark by water and the solution shacked it for 5 min subsequently the solution kept in ultrasonic bath for 15 minutes and

filtered with 0.45 µm membrane filter furthermore, further dilutions were made for the analysis of the drug content in the tablet.

III RESULTS AND DISCUSSION

Method Development

1- pentansulfonic acid sodium salt dissolved in a mixture of water and acetonitrile then adjusted to pH 3 with 2 M hydrochloric acid was chosen as a mobile phase after several trials with different mobile phases to obtain the best sensitivity and separation. 1.0 ml/min flow rate provide an optimum signal to noise ratio with a credible resolution time. The chromatogram presents in figure (3) accomplished with using C18 column, the results showed a complete separation with the maximum absorption of paracetamol and Homatropine methyl bromide which recorded at 225 nm. The optimal retention times [paracetamol = 5.4 min and Homatropine methyl bromide = 9.7 min were attained, as a result 225 nm was chosen for the analysis.

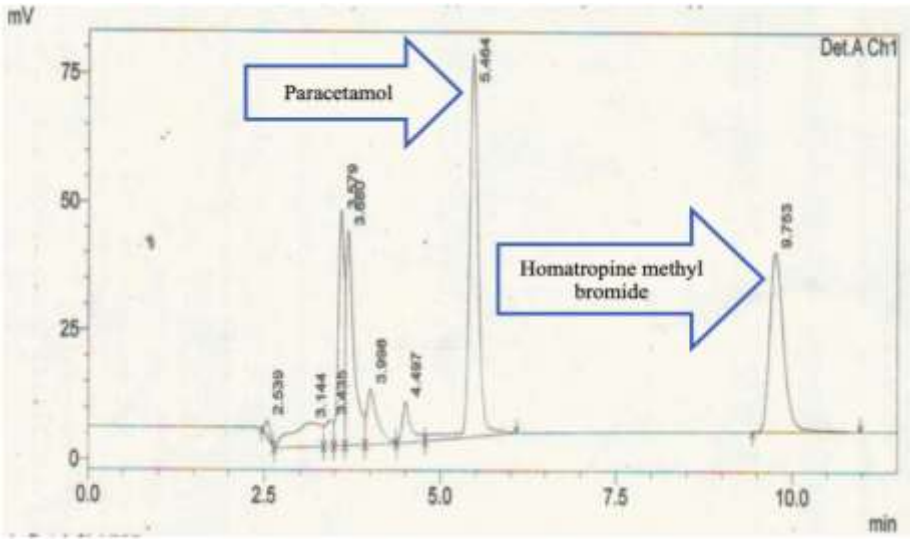

Figure 3: A representative chromatogram of the mixture pure standard paracetamol(10ppm) and Homatropine methyl bromide(30ppm).

Method validation

Specificity and System Suitability

Specificity was checked to assess the ability of the suggested technique to separate the analyte response from blank and placebo under chromatographic conditions. The results proved no impurities interference by the suggested method at retention time of target peaks. According to the chromatogram given in figure (3), it is noted that the paracetamol and Homatropine methyl bromide were entirely separated under the stated chromatographic conditions. The system suitability parameters shown in table (2) such as resolution values and tailing factors

between two peaks were acceptable for these compounds [resolution values =not less than 10 and tailing factor =not more than 2.50]. The typical chromatograms for blank and placebo are shown in figure 4, 5, and 6.

Table 2: System suitability parameters for paracetamol and Homatropine methyl bromide

Compound name	Retention time(min)	Resolution	Plate cunt	Tailing factor
Paracetamol	5.46	-----	5625	2.21
Homatropine methyl bromide	9.75	10.4	9525	1.72

Figure 4: Chromatogram for placebo

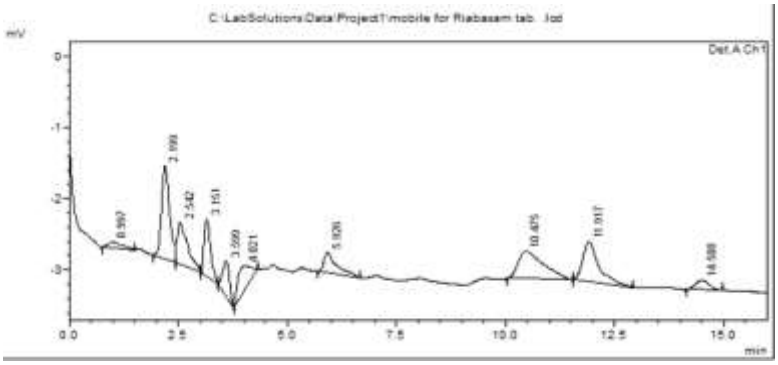

Figure 5: Chromatogram for Mobile phase

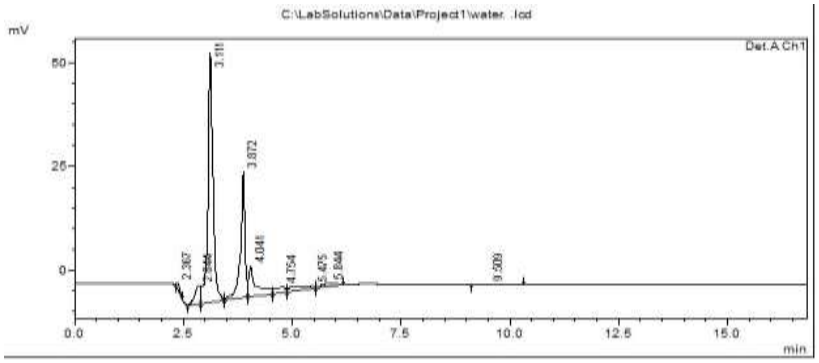

Figure 6: Chromatogram for water.

Linearity, detection limit, and quantification limit

The standard calibration carves were constructed with five concentrations for each one by proposed method. The linearity was proved by the excellent correlation coefficients(r^2) value figure (5). The limit of detection and limit of quantitation were evaluated based on signal to noise ratios, and the following equations: LOD=3σ /S, LOQ=10 σ /S, were used to calculate LOD and LOQ, Where, σ the standard deviation and S the slope. Based on these calculations the LOD and LOQ for paracetamol were found to be 0.442 µg/ml and 1.473 µg/ml and Homatropine methyl bromide 0.0885µg/ml and 0.295 µg/ml respectively, Table (3).

Table 3: Analytical figure of merit of developed HPLC procedure for separation Homatropine methyl bromide and Paracetamol

Parameters	Paracetamol	Homatropine methyl bromide
Concentration range (µg/ml)	10-110	4-30
Intercept	6548.2	3528.3
Slope	289080	49404
Correlation coefficient (r)	1	0.9999
Equation	y=289080x+6548.2	y=49204x+3528.3
Detection limit (µg/ml)	0.442	0.0885
Quantification limit (µg/ml)	1.473	0.295

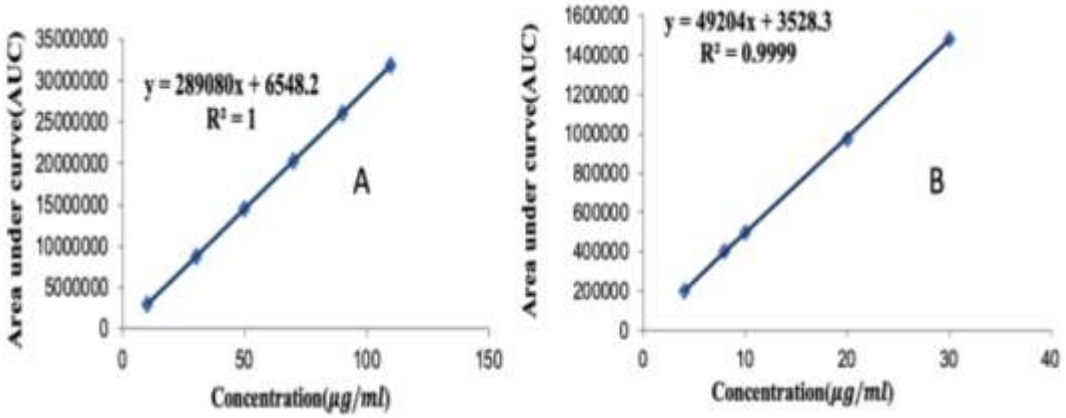

Figure 5: The calibration carves of (A) Paracetamol, (B) for Homatropine methyl bromide under HPLC proposed method.

Precision

The precision of suggested an analytical method is usually assessed as repeatability and reproducibility associated with relative standard deviation (coefficient of variation) of a series of measurements. These experiments were repeated over a 2-day period to evaluate the intra-day and inter-day precision. The %RSD for six replicates was found to be less than 2.0% which provide a good method precision. All the data were within the acceptance criteria and results are presented in Table 4.

Table 4: Precision studies for paracetamol and Homatropine M.B

Drug	% RSD of peak area	
	Day 1	Day 2
Homatropine M. B	1.109	1.377
Paracetamol	0.837	0.954

Accuracy

To check the accuracy of a proposed procedure and assay of a drug formulated product, percentage recovery experiments were determined by adding known amounts of standard drugs at 80, 100, and 120% level to the placebo solution. The amount found, amount added, recovery, and RSD values for Homatropine M.B and paracetamol were calculated. The recovery and RSD% data shown in table (5,6) demonstrated that the method was accurate and no interference from the placebo formulation with the analyte peaks.

Table 5: Accuracy results of Homatropine methyl bromide.

Spiked level	Sample area	Amount added(µg/ml)	Amount found(µg/ml)	Amount recovered (%)	Mean of recoveries%	RSD (%)
80%	397582	8.00	7.972	99.65	99.46	0.57
80%	398664	8.00	7.993	99.92		
80%	394288	8.00	7.905	98.82		
100%	498868	10.00	10.002	100.02	99.92	0.091
100%	497948	10.00	9.984	99.84		
100%	498273	10.00	9.990	99.90		
120 %	597825	12.00	11.996	99.97	100.06	0.090
120 %	598492	12.00	12.009	100.08		
120 %	598903	12.00	12.018	100.15		

Table 6: Accuracy results of Paracetamol.

Spiked level	Sample area	Amount added(µg/ml)	Amount found(µg/ml)	Amount recovered (%)	Mean of recoveries% +RSD (%)	RSD (%)
80%	16309526	56.00	56.076	100.13	101.242	1.38
80%	16742700	56.00	57.56	102.79		
80%	16418366	56.00	56.44	100.81		
100%	20289542	70.00	70.14	100.20	100.203	0.0054
100%	20290978	70.00	70.14	100.21		
100%	20288789	70.00	70.17	100.20		
120 %	24442768	84.00	84.21	100.25		

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120 %	24530892	84.00	84.51	100.61	100.946	0.91
120 %	24864055	84.00	85.66	101.98		

Analysis of Riabasam tablets

The current method was applied for determining the amount of paracetamol and Homatropine methyl bromide in the Riabasam tablet (twenty tablets each one containing 350 mg paracetamol and 4 mg Homatropine methyl bromide). Every sample was determined in three measurements after sample preparation as aforementioned earlier in the experimental part. A typical HPLC chromatogram of riabasam tablet is shown in figure (6) which demonstrate the current method is very precise for synchronous determination of the paracetamol and Homatropine methyl bromide in Riabasam tablet.

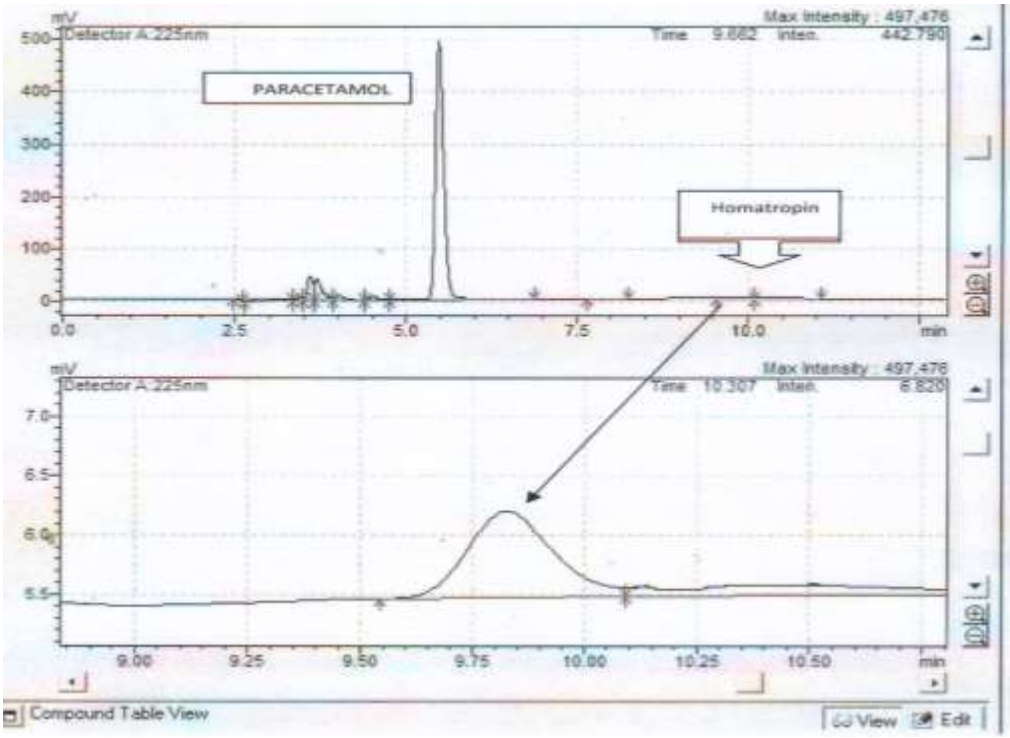

Figure 6: Chromatogram of paracetamol (70 µg/ml) and Homatropine methyl bromide(0.8µg/ml) from Riabasam tablet.

IV CONCLUSIONS

The reversed-phase high-performance liquid chromatographic (RP-HPLC) method was developed in the present work to separate paracetamol and homatropine methyl bromide in standard reference and riabasam tablet using UV-VIS detector. Because of using simple isocratic installation of mobile phase and easy to prepare allows the two compounds separated with good resolution at optimum retention time less than 15 minutes. In the proposed HPLC method no intervene of the placebo and blank with the analyte peaks, which showed to be specific for these

drugs. The results provide excellent percentage of recovery, the analytical calibration curve was linear, reproducible, sensitive, specific, and rugged.

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VI COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interest No conflict of interest was declared

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