

## SIMULTANEOUS DETERMINATION AND VALIDATION OF CHLORPHENIRAMINE MALEATE, ACETAMINOPHEN, PHENYLPROPANOLAMINE HYDROCHLORIDE AND CAFFEINE IN TABLET DOSAGE FORM BY USING REVERSE PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (RP-HPLC)

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### ABSTRACT

**Objective:** An accurate, simple, reproducible and sensitive reverse phase liquid chromatographic method for the simultaneous determination of Chlorpheniramine Maleate, Acetaminophen, Phenylpropranolamine Hydrochloride and Caffeine in tablet formulation.

**Method:** Separation was achieved on Inertsil C<sub>18</sub> (250mmx4.6mm, 5µm) analytical column. A mixture of acetonitrile: water: methanol (15:75:10 v/v/v) with buffer solution (pH=2.8) containing triethylamine (0.3 ml) and Hexansulfonic acid sodium salt as ion pair was used as mobile phase. Isocratic elution with program flow rate from 1 ml/min (in 0~5 min) to 1.7 ml/min (after 5 min) till 8 min was used and the detection was at 220 nm wavelength.

**Results:** The retention time of Chlorpheniramine Maleate, Acetaminophen, Phenylpropranolamine Hydrochloride and Caffeine were found to be 3.423, 5.627, 6.052 and 7.690 min, respectively. The validation of the proposed method was carried out for linearity, accuracy, recovery, and precision and limit of detection and limit of quantification. The linear rang of determination of Chlorpheniramine Maleate, Acetaminophen, Phenylpropranolamine Hydrochloride and Caffeine were 1-50 µg/ml, 1-500 µg/ml, 1-200 µg/ml and 1-150 µg/ml, receptively. The percentage recovery of Chlorpheniramine Maleate, Acetaminophen, Phenylpropranolamine Hydrochloride and Caffeine were 101.650-101.640%, 100.305-99.484%, 100.840-100.751% and 98.075-100.461%, respectively. The detection limit and quantification limit for Chlorpheniramine Maleate, Acetaminophen, Phenylpropranolamine Hydrochloride and Caffeine were found to be 0.567 and 1.720 µg/ml, 0.467 and 1.415 µg/ml, 0.512 and 1.552 µg/ml and 0.7514 and 2.277 µg/ml, respectively.

**Conclusion:** The developed method is suitable for routine quality control analysis of titled drugs in combination of tablet formulation.

**Keywords:** Chlorpheniramine Maleate, Acetaminophen, Phenylpropranolamine Hydrochloride, Caffeine and RP-HPLC.

### INTRODUCTION

Chlorpheniramine maleate (CPM), (±) 2-[p-chloro-[2-dimethylamino) ethyl] benzyl] pyridine bimalate (Chlor-Trimeton). Chlorpheniramine (Maleate) is the maleate salt of Chlorpheniramine. Chlorpheniramine is an antihistaminic agent derived from pheniramine. Chlorpheniramine is an anticholinergic antihistamine. It is also effective against nausea and motion sickness, with its primary mechanism of action being its ability to reduce acetylcholine levels in the brain [1]. Chlorpheniramine maleate has been determined alone or in combination using Spectrophotometric method [2,3,4], HPLS-MS [5] and HPLC [6,7,8,9].

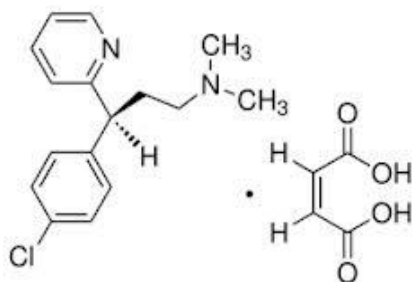


Fig. 1: Chlorpheniramine maleate (CPM)

Acetaminophen (AMP) (Paracetamol), N-(4-hydroxyphenyl)-acetamide (Fig. 1) is a widely used analgesic and antipyretic drug, is frequently used by itself OTC (Panado, Tempra, Tylenol) or in combination with codeine (Tylenol 3), hydrocodone (Vicodin), or oxycodone (Percocet) for the treatment of mild to moderate pain and to reduce fever [10, 11]. Acetaminophen determined alone or in combination with other drugs by Spectrophotometric method [12,13,14,15], chemometric-Spectrophotometric [16], flow injection [17], and HPLC [18,19,20,21].

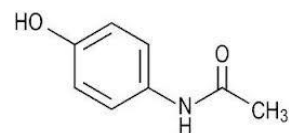


Fig. 2: Acetaminophen (AMP)

Phenylpropranolamine hydrochloride (PPA) (1S, 2R)-2-amino-1-phenylpropan-1-ol is a psychoactive drug of the phenethylamine and amphetamine chemical classes which is used as a stimulant, decongestant, and anorectic agent. It is commonly used in prescription and over-the-counter cough and cold preparations. Phenylpropranolamine acts as a potent and selective releasing agent of norepinephrine and epinephrine, or as a norepinephrine releasing agent (NRA). It also acts as a dopamine releasing agent (DRA) to a lesser extent. It works by mimicking the effects of endogenous catecholamine such as epinephrine and norepinephrine and to a lesser degree dopamine [11,22]. Analytical method for determined (PPA) included Spectrophotometric method [23,24,25] and HPLC [26,27].

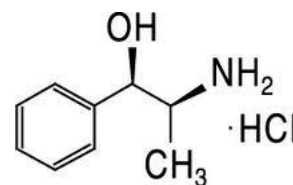


Fig. 3: Phenylpropranolamine HCL (PPA)

Caffeine (CAF) 1,3,7-trimethyl-1H-purine-2,6(3H,7H)-dione has pharmaceutically important chemical properties. Caffeine is weak Brønsted-Lowry base. Caffeine cannot donate a proton from

position 7 and does not act as a Brønsted acid at pH values less than 14. Caffeine does have electrophilic sites at positions 1, 3, and 7. Caffeine in blood is not highly protein bound. Differences in the substituent at the 7-position may be involved. Additionally, caffeine is lipophilic and reputedly achieves higher brain concentrations [11]. Caffeine has been determined using, LC-Spectrophotometric [28], Spectrophotometric method [29,30,31] and HPLC [32,33,34].

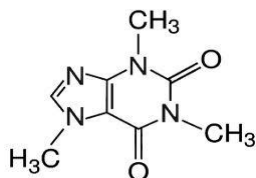


Fig. 4: Caffeine (CAF)

## MATERIAL AND CHEMICALS

### Materials and Chemical

Pure sample of CPM, AMP, PPA and CAF were obtained from The State Company for Drugs Industry and Medical, Iraq. Tablet formulation containing 2 mg CPM, 500 mg AMP, 12.5 mg PPA and 25 mg CAF were obtained commercially. HPLC grade acetonitrile and methanol were procured from Himedia Ltd. All other chemical reagents were of analytical grade. Digital microbalance (Sartorius CPA2P), Digital pH meter (Hanna-pH211), Ultrasonic cleaner (KQ200E) and Double beam Ultraviolet - spectrophotometer (Shimadzu-1800) were employed for the estimation.

### Preparation of Mobile Phase

The mobile phase was a mixture of Acetonitrile: water: methanol (15:75:10 v/v/v) and buffer solution (pH=2.8) containing 0.3 ml triethylamine and 4 ml of Hexansulfonic acid sodium salt as ion pair (14mmol/l).

### Preparation of Buffer solution

A 6.84 ml of concentrated orthophosphoric acid was diluted to 100 ml of water, and the pH was adjusted to 2.8 with orthophosphoric acid.

### Preparation of Standard solution

Standard stock solutions of CPM, AMP, PPA and CAF were prepared by accurately weighing 10 mg from each drug individually and quantitatively transferred into a 10 ml volumetric flask and complete the volume with mobile phase. The mixture was sonicated for 15 min or until the reference standard dissolved completely. From the standard stock solutions, serial dilution in mobile phase were made to prepare standard curves ranging from 1-50 µg/ml for CPM 1-500 µg/ml for AMP, 1-200 µg/ml for PPA and 1-150 µg/ml for CAF.

### Preparation of Sample solution

Twenty tablets, each containing 2 mg CPM, 500 mg AMP, 12.5 mg PPA and 25 mg CAF are weighed and powdered finely. A quantity of powdered which is equivalent to one tablet was weighed accurately and transferred into 200ml calibrated volumetric flask. About 50 ml of diluent was added and ultrasonicated for 15 min; finally the volume adjusted to the mark with mobile phase. The resulting solution obtained was then filtered through 0.45 µm filter for removal of particular matter. 5ml of the filtrate was diluted to 25 ml in the volumetric flask with mobile phase for analysis.

### Instrumentation and Chromatographic condition

A high performance liquid chromatographic system (SHIMADZU Corporation, LC-20 AD double pump) with an auto sampler and Shimadzu SPD-20A UV/VIS detector was used for analysis. Separation was carried out at 25°C, using Inertsil C<sub>18</sub> (250mm x 4.6mm, 5µm) analytical column. The overlain spectrum of CPM, AMP, PPA and CAF is given and the chromatogram of standard solutions is given in Figure (5 and 6).

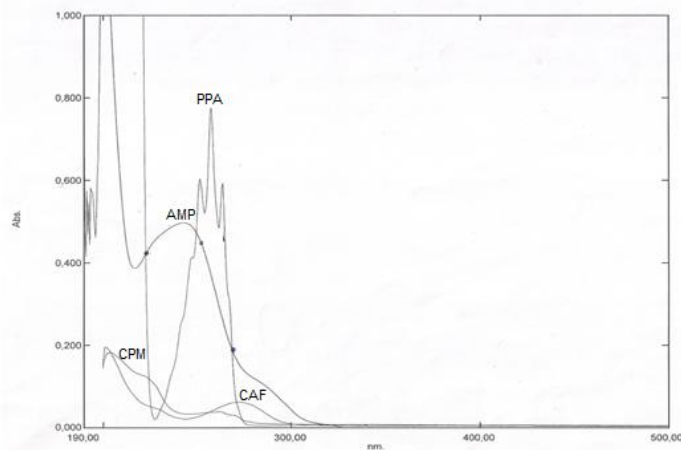


Fig. 5: Overlain spectrum of standard solutions of CPM, AMP, PPA and CAF.

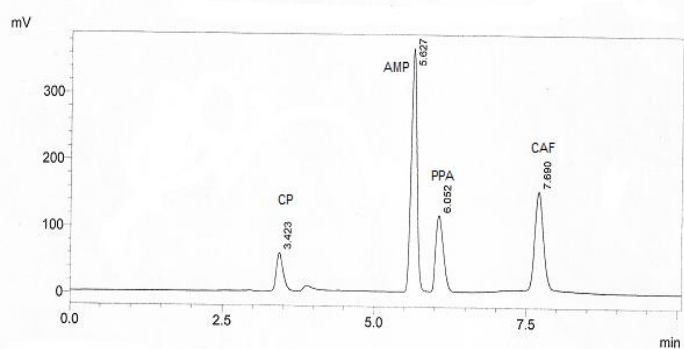


Fig. 6: Chromatogram of standard solutions

**RUSULT AND DISCISSION**

The developed method for determination of CPM, AMP, PPA and CAF was validated by using the following parameter:

**Selectivity**

Selectivity of the current method was demonstrated by good separation of the four active ingredients.

**Linearity**

Standard solutions of CPM (1-50µg/ml), AMP (1-500 µg/ml), PPA (1-200 µg/ml) and CAF (1-150 µg/ml) were prepared in mobile phase. Triplicate 20 µl injections were made for each standard solution to see the reproducibility of the detector at each concentration level the peak area of each drug was plotted against the concentration to obtain the calibration curve (Figures 7-10).

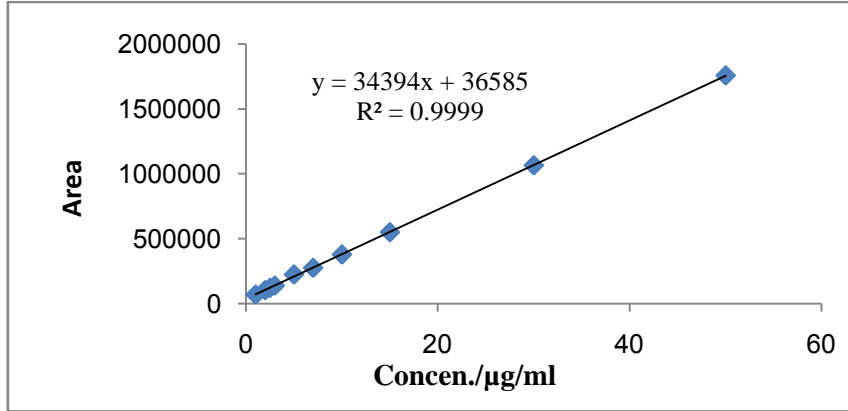


Fig. 7: Linearity graph for Chlorpheniramine maleate

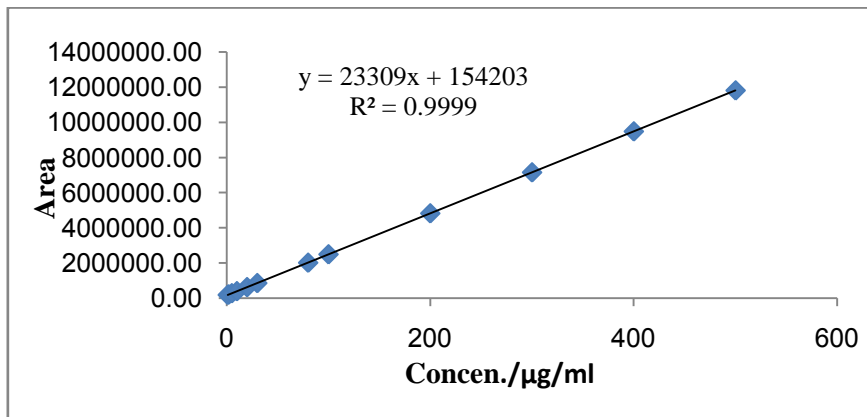


Fig. 8: Linearity graph for Acetaminophen

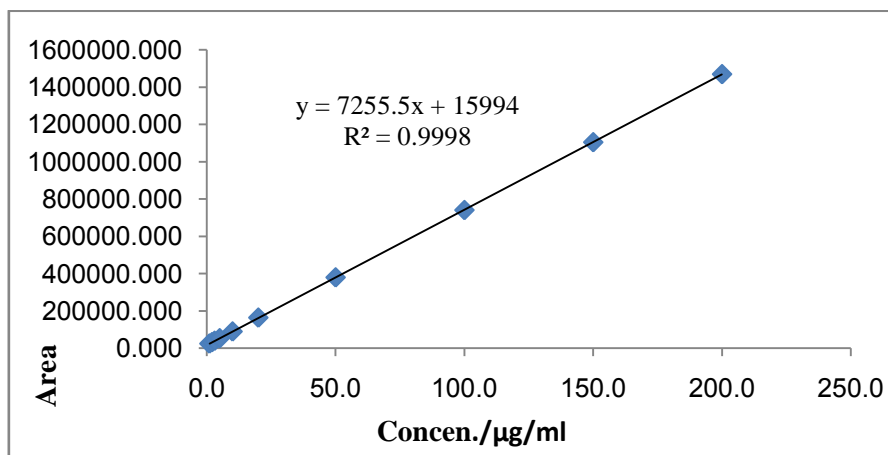


Fig. 9: Linearity graph for Phenylpropanolamine HCL

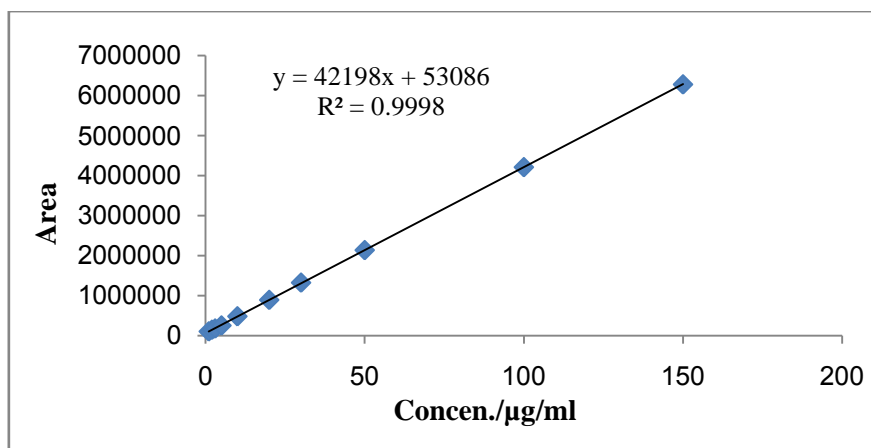


Fig. 10: Linearity graph for caffeine

The results obtained showed that the current method was linear for the four analytes in the range specified above with correlation better than 0.9997.

### Precision

Precision was evaluated by carrying out three different sample preparations for all individual and combination dosage forms. Percentage relative standard deviation (%RSD) was found to be less than 2 as shown in Table 1, which proves that the developed method is precise and reproducible.

Table 1: Result of precision for standard drugs

Drugs	(µg/ml)	R.S.D %
CPM	2	0.200
	10	0.027
	50	0.252
AMP	20	0.207
	300	0.065
	500	0.053
PPA	5	0.406
	30	0.857
	100	0.371
CAF	20	0.020
	50	0.126
	150	0.020

### Limit of Detection and Limit of Quantification

The LOD and LOQ were separately determined on the basis of standard calibration curve. The residual standard deviation of the regression line or the standard deviation of y-intercepts of regression lines was used to calculate LOD and LOQ.

Sensitivity of the proposed method was estimated in terms of Limit of Detection (LOD) and Limit of Quantitation (LOQ).  $LOD = 3.3 SD/S$  and  $LOQ = 10 S.D. /S$ , where S.D. is the residual standard deviation and S is the slope of the line.

The LOD of CPM, AMP, PPA, and CAF were found to be 0.567 µg/ml, 0.467 µg/ml, 0.512 µg/ml, 0.752 µg/ml respectively. The LOQ of CPM, AMP, PPA, and CAF were found to be 1.72 µg/ml, 1.415 µg/ml, 1.552 µg/ml, 2.277 µg/ml respectively.

### Accuracy

The accuracy of method was confirmed by studying recovery at three different concentrations for all samples, by replicate analysis (n=3). Samples of known concentration (reference standard solutions) were analyzed and the measured values, from the respective area counts, were compared with the true values. The results obtained from the determination of accuracy, expressed as percentage recovery, are summarized in Table 2.

Table 2: Result of accuracy for standard drugs

Drugs	Amount added (µg/ml)	Amount found (µg/ml)	Recovery [%]
CPM	2	2.033	101.650
	10	10.583	105.830
	50	50.820	101.640
AMP	20	20.061	100.305
	300	300.301	100.100
	500	497.419	99.484
PPA	5	5.042	100.840
	30	30.240	100.800
	100	100.751	100.751
CAF	20	19.615	98.075
	50	50.749	101.498
	150	150.691	100.461

### APPLICATION

#### Analysis of Marketed formulation

To determine the content of CPM, AMP, PPA and CAF in commercial drugs (Brand name: Dolo Cold, 2 mg CPM, 500 mg of AMP, 12.5 mg of PPA and 25 mg of CAF, India). A 20µl volume of sample solution was injected into HPLC, three times, under the conditions described above. The results for CPM, AMP, PPA and CAF comparable with their corresponding labeled amounts and % R.S.D are shown in Table 3.

Table 3: Percentage recovery data for marketed drug

Drugs	Labeled amount (µg/ml)	Amount found (µg/ml)	Recovery [%]
CPM	2.000	2.066	103.300
AMP	500.000	498.185	99.637
PPA	12.500	12.552	100.416
CAF	25	25.194	100.776

Results in table 3 showed that the estimation of dosage form was accurate within the acceptable level.

### CONCLUSION

An accurate, sensitive and precise HPLC method with UV detection for the simultaneous estimation of Chlorpheniramine Maleate, Acetaminophen, Phenylpropanolamine Hydrochloride and Caffeine was developed and validated for quality control analysis in combined tablets. The proposed method is rapid, where the total analytical run time for four drugs are less than 8 min and shows high degree of accuracy and precision with less than 2 % RSD. It is convenient for laboratory quality control of tablet dosage forms.

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