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Analytical Method Development And Validation Of Acetaminophen, Caffeine ,Phenylephrine Hydrochloride And Dextromethorphan Hydrobromide In Tablet Dosage Form By Rp- Hplc

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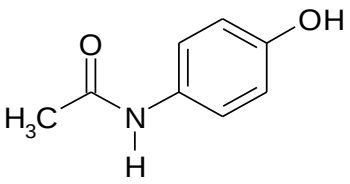
ABSTRACT: *A simple, selective, rapid, precise and economical RP HPLC method has been developed for the determination of Acetaminophen, Caffeine, Phenylephrine Hydrochloride and Dextromethorphan Hydrobromide in tablet formulation. The analysis was resolved by using a gradient mobile phase (Sodium salt of heptane sulphonic acid buffer solution and acetonitrile) at a flow rate of 1ml/min on an gradient consisting of Shimadzu LC 2010 HPLC system on variable wavelength UV detector using, Inertsil C8 (4.6 mm x 15 cm, 5 µm) column at a wavelength of 214 nm. The retention time were found to be Acetaminophen (5 min), Caffeine (6 min), Phenylephrine HCl (10 min), Dextromethorphan HBr (20 min). The percent recovery of Acetaminophen, Caffeine, Phenylephrine Hydrochloride and Dextromethorphan Hydrobromide were found to be in between 98% to 102%. The developed method was simple, precise, accurate and reproducible and therefore suitable for routine analysis of drugs in tablet dosage form*.

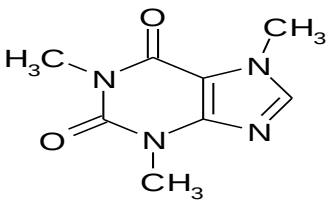
Keywords: *Acetaminophen, Caffeine, Phenylephrine HCL, Dextromethorphan HBr*

I. INTRODUCTION

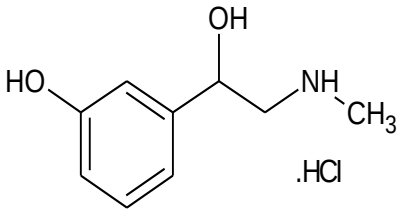
Acetaminophen designed chemically as {N-(4-Hydroxyphenyl) acetamide}, it is an analgesic, antipyretic agent. Caffeine designed 1,3,7-Trimethyl-3, 7-dihydro-1H-purine-2,6-dione, it is a Central nervous system stimulant. Phenylephrine Hydrochloride designed chemically as (R)-1-(3-hydroxyphenyl)-2-methylaminoethanol hydrochloride, it is a decongestant. Dextromethorphan Hydrobromide chemically designed as {ent-3-methoxy-9a- methyl morphinan Hydrobromide monohydrate}, it is a cough suppressant.



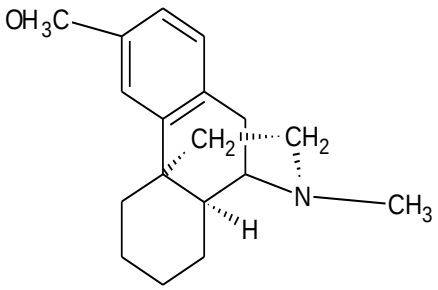
Acetaminophen



Caffeine



Phenylephrine Hydrochloride



Dextromethorphan Hydrobromide

Various HPLC method were reported for Acetaminophen, Caffeine, Phenylephrine Hydrochloride and Dextromethorphan Hydrobromide. But no HPLC method was reported for simultaneous determination of Acetaminophen, Caffeine, Phenylephrine Hydrochloride and Dextromethorphan Hydrobromide in combination.

II. EXPERIMENTAL

Chemical and Reagents

Acetaminophen, Caffeine, Phenylephrine Hydrochloride and Dextromethorphan Hydrobromide working standards and marketed drugs formulation SANATUSIN DAY TABLET were procured from Okasa Pharma Pvt. Ltd., Satara, (Maharashtra). Acetonitrile(Runa) and Water used was of HPLC grade, 1-Heptane Sulphonic acid Sodium Salt (Molychem) was used in mobile phase A and Orthophosphoric acid was used for P^H adjustment.

Analytical conditions

Instrument used in present study were Agilent 1200 series & Shimadzu LC-2010$A_{HT.}$ Liquid chromatographic
system equipped with UV-Vis detector and analyzed by using Chromeleon 6.2 version software.

Chromatographic Condition

Chromatographic separation was performed on an Inertsil C8 (4.6 mm x 15 cm, 5 µm) column. The analysis was resolved by using a mobile phase (Sodium salt of heptane sulphonic acid buffer solution and acetonitrile) at a flow rate of 1ml/min for gradient program shown in (Table No.1). The injection volume was 20µl and ambient at temperature. The mobile phase was filtered through a 0.45 µ membrane filter and sonicated. Detection was carried out a 214 nm. The retention time were found to be Acetaminophen (5 min), Caffeine (6 min), Phenylephrine HCl (10 min), Dextromethorphan HBr (20 min), within run time of 30 mins.

III. PREPARATION OF SOLUTIONS

Diluent: 0.1 N Hydrochloric acid.

Preparation of Standard solutions:

Weighed accurately and transfered about 25 mg of Acetaminophen working standard into a 50 ml volumetric flask. Added 35 ml of diluent. Sonicated to dissolve, cool and diluted upto the volume with diluent (Solution A1). Weighed accurately and transfered about 25 mg of Caffeine working standard into a 100 ml volumetric flask. Added 70 ml of diluent. Sonicated to dissolved, cooled and diluted upto the volume with diluent (Solution B1). Weighed accurately and transfered about 25 mg of Phenylephrine hydrochloride working standard into a 50 ml volumetric flask. Added 35 ml of diluent. Sonicated to dissolved, cooled and diluted upto the volume with diluent (Solution C1). Weighed accurately and transfered about 25 mg of Dextromethorphan hydrobromide working standard into a 50 ml volumetric flask. Add 35 ml of diluent, sonicated to dissolved, cooled and diluted upto the volume with diluent (Solution D1). Added 10 ml of solution A1, 2 ml of solution B1 & 5 ml of solution C1 & 10 ml of solution D1 into 50 ml of volumetric flask. added 35 ml of diluent,.sonicated to dissolved, cooled and diluted upto the volume with diluents.

Sample solution:

Transferred 2 intact tablets of sample into a 200 ml volumetric flask. Added 100 ml of diluent. Sonicated for 30 mins, cooled and diluted upto the volume with diluent. Filtered through Sartorius filter paper no.389 (For Phenylephrine hydrochloride and Dextromethorphan hydrobromide)
Further diluted 2 ml into 100 ml with diluent. (For Acetaminophen and Caffeine).

Preparation of mobile phase:

Mobile Phase A- Dissolved 1gm of sodium salt of heptane sulphonic acid in 1000 ml of purified water. Adjust pH to 3.0 with orthophosphoric acid. Filter through 0.45 µm membrane filter.
Mobile Phase B- Acetonitrile (Degassed).

IV. RESULTS AND DISCUSSION

Optimization of chromatographic conditions:

Analytical method used for assay of Acetaminophen, Caffeine, Phenylephrine Hydrochloride and Dextromethorphan Hydrobromide used in Sanatusin Day Tablet by using High performance liquid chromatography technique was validated. Validation was carried out on Shimadzu LC 2010A HPLC System and Agilent-1200 HPLC System with Chromeleon software (6.2).The validation of the method was assessed by establishing validation criteria such as Specificity and System Suitability, Linearity and Range,Precision (repeatability & intermediate precision), Accuracy, Solution Stability and Robustness study.

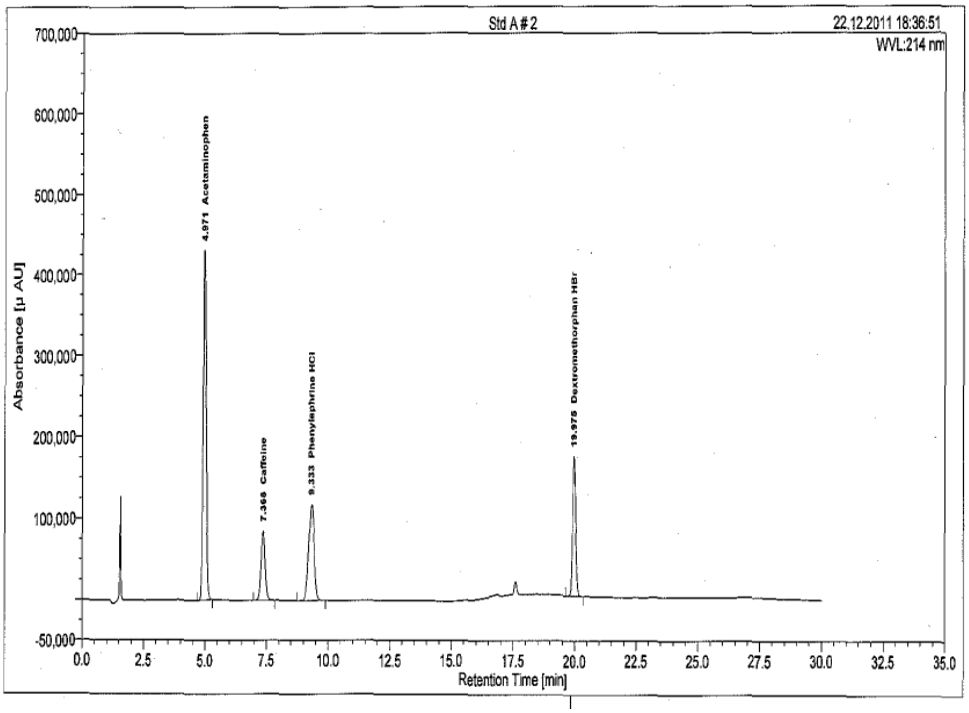


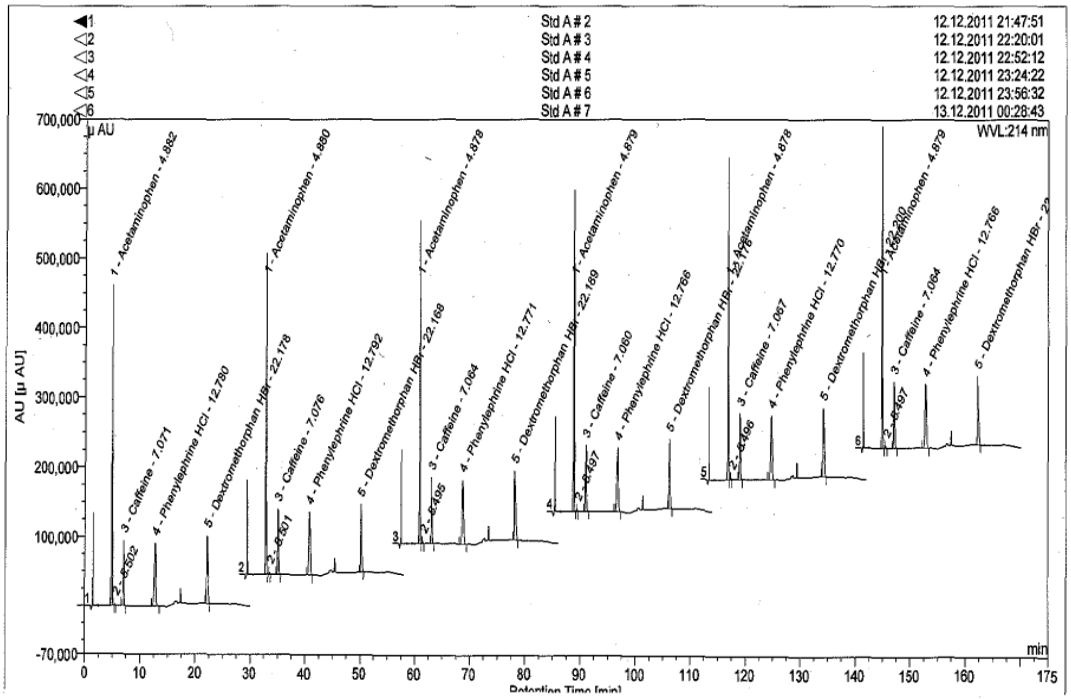
Figure 3: A typical chromatogram of Acetaminophen, Caffeine, Phenylephrine Hydrochloride And Dextromethorphan Hydrobromide

V. METHOD VALIDATION

Specificity:

Specificity was carried out to monitor interference from blank and to monitor system suitability. Standard solutions were injected into the chromatograph in six replicates. The % RSD for peak area response and retention were found within limit (Not more than 2.00% for peak area response and not more than 1.00% for retention time).The system suitability parameters like theoretical plates and tailing factor were found within limits.

Parameter	Acceptance Criteria	Acetaminophen	Caffeine	Phenylephrine hydrochloride	Dextromethorphan hydrobromide
Theoretical Plates	**NLT 2000**	**8206**	**8993**	**11075**	**47113**
Tailing Factor	**NMT 2**	**1.1**	**1.1**	**1.0**	**1.7**
Similarity Factor	**0.98 to 1.02**	**0.98**	**1.0**	**1.00**	**0.98**
%RSD of STD A for Area	**NMT 2**	**0.1**	**0.2**	**0.2**	**0.4**
%RSD of STD A for RT	**NMT 1**	**0.1**	**0.1**	**0.1**	**0.0**



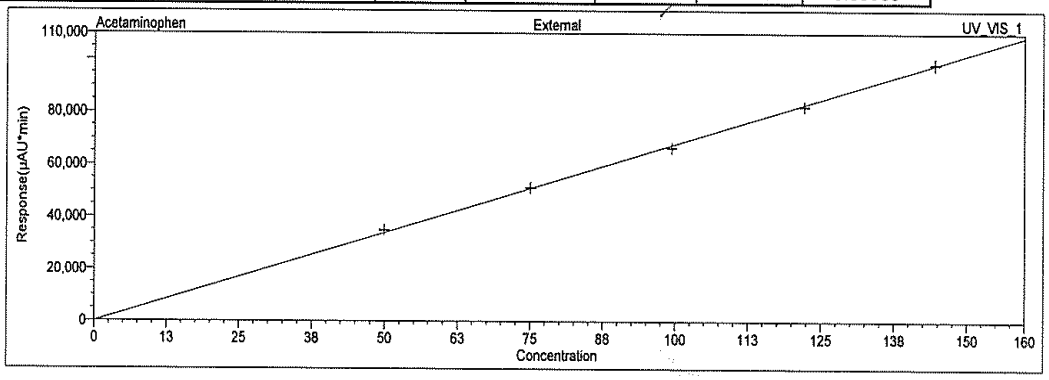
Linearity study

Linearity and Range were carried out over a range of 50 to 150% of working level concentration. The linearity regression correlation coefficient, % Y-intercept and % RSD for peak area response and retention time for lower and higher range were calculated. The linearity regression correiation coefficient for the component was found within limit (Not less than 0.999). The % Y-intercept for the component was found within the limit (Not more than +2.0).

Name	**%Y Intercept**	**Correlation Coefficient**	**Response Factor (%RSD)**
Acetaminophen	**1.5**	**1.00**	**1.8**
Caffeine	**-1.7**	**0.999**	**2.2**
Phenylephrine Hydrochloride	**1.6**	**1.00**	**1.3**
Dextromethorphan Hydrobromide	**1.5**	**1.00**	**1.4**
Acceptance Criteria	**-2 to +2**	**NLT 0.99**	**NMT 3%**

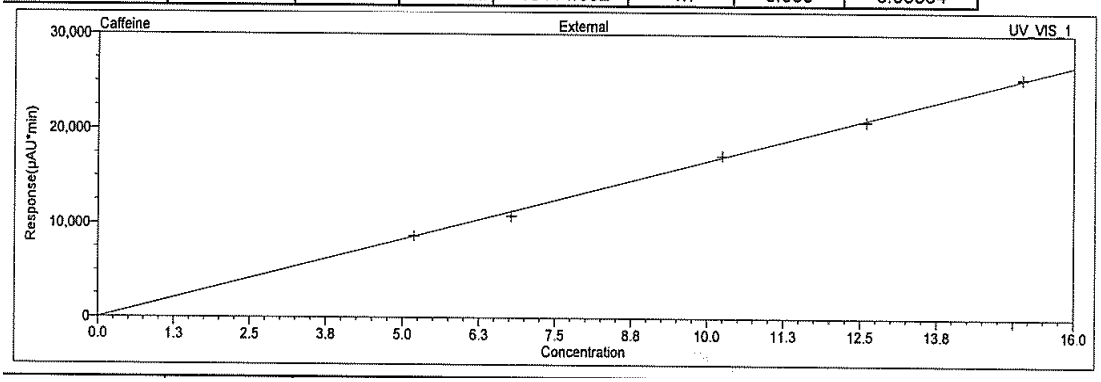
Linearity of Acetaminophen:

Peak Name Acetaminophen UV_VIS_1	o. of Calibratio Points Acetaminopher UV_VIS_1	Calibration Type cetaminophe UV_VIS_1	Slope	Y intercept	% Y Intercep	Corelation Coefficient	R Square
Acetaminophen	18	Lin	40068.568	59542.163	1.5	1.000	0.99909



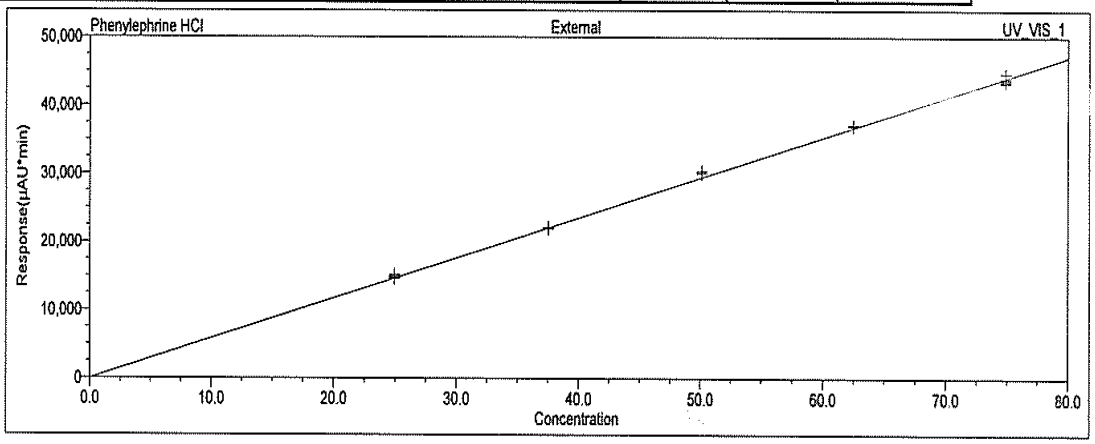
Linearity of Caffeine:

Peak Name Caffeine UV_VIS_1	o. of Calibratio Points Caffeine UV_VIS_1	Calibration Type Caffeine UV_VIS_1	Slope	Y intercept	% Y Intercep	Corelation Coefficient	R Square
Caffeine	18	Lin	########	-18114.692	-1.7	0.999	0.99864



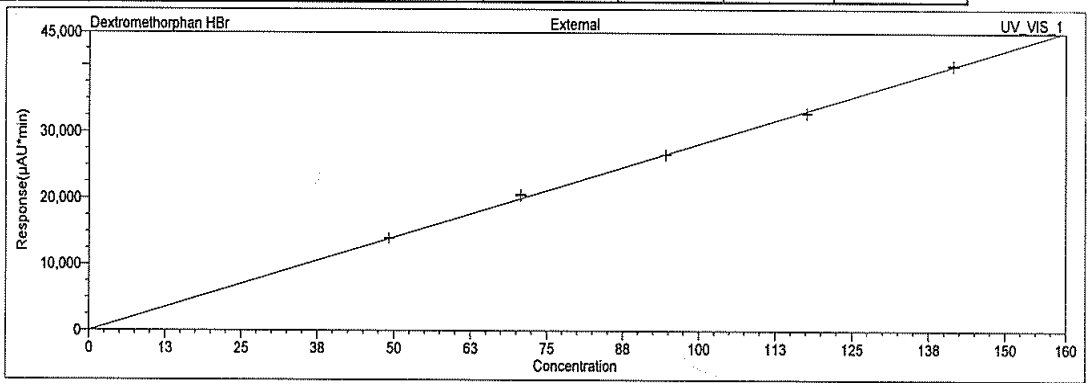
Linearity of Phenylephrine hydrochloride:

Peak Name Phenylephrine HCl UV_VIS_1	o. of Calibratio Points henylephrine H UV_VIS_1	Calibration Type nylephrine UV_VIS_1	Slope	Y intercept	% Y Intercep	Corelation Coefficient	R Square
Phenylephrine HCl	18	Lin	34947.224	29606.781	1.6	1.000	0.99900



Linearity of Dextromethorphan hydrobromide:

Peak Name extromethorphan H UV_VIS_1	o. of Calibratio Points tromethorphan UV_VIS_1	Calibration Type omethorpha UV_VIS_1	Slope	Y intercept	% Y Intercep	Corelation Coefficient	R Square
extromethorphan H	18	Lin	16734.751	23443.968	1.5	1.000	0.99915



Accuracy/Recovery:

Accuracy levels were prepared by 50, 100, & 150 % of working level concentration, prepared in triplicate for each levels and the percentage recovery were calculated for each levels separately. The percentage recoveries observed for the levels were found well within the limit set for the accuracy study (Not less than 98.0% and not more than 102.0%), shown that the content was recovered and hence is accurate.

Parameter	Acceptance Criteria	Acetaminophen	Caffeine	Phenylephrine HCl	Dextro-methorphan HBr
Recovery 50%	98% to 102%	99.0	100.0	99.5	101.3
Recovery 100%	98% to 102%	99.0	98.4	100.3	102.0
Recovery 150%	98% to 102%	98.4	98.2	99.6	100.8
%RSD of Recovery	NMT 2	0.2	0.1	0.3	0.7
Similarity Factor	0.98 to 1.02	1.01	0.99	1.01	0.99

Precision:

The precision of the method was demonstrated by inter-day and intra-day variation studies. In the intra-day studies, six injections of standard solution were injected into the chromatographic system in different time interval within a day. In the inter-day variation studies, six injections of standard solution were injected at different days. % RSD werecalculated and were found < 2 (Table 4).

Parameter	Acceptance Criteria	Acetaminophen	Caffeine	Phenylephrine HCl	Dextromethorphan HBr
%RSD of Assay	NMT 2	2.0	1.3	2.0	1.8
% Variation	NMT2	1.0	0.1	1.9	2.1
Similarity Factor	0.98 to 1.02	1.00	1.01	1.01	1.00

S.D: Standard deviation*, RSD: Relative standard deviation,**

Ruggedness

Ruggedness of the method was studied by different analysts and on different make of instruments (LC-2010 C HT liquid chromatography SHIMADZU). % RSD values were found <2 (Table 5) thus indicating excellent ruggedness of method.

Robustness:

The robustness of method was carried out by changing the different chromatographic conditions (one at a time) such as:

1. Change in temperature of autosampler from 25 to 23
2. Change in temperature of autosampler from 25 to 27
3. Change in pH from 3.0 to 2.8
4. Change in pH from 3.0 to 3.2

Solution stability:

The solution stability was monitored to check the stability of solution. A sample solution was preserved at 2-8^{o}C. Solution stability checked at after each time interval (2, 4, 8, 12, 16, 20 and 24 hrs) and analyzed after the specified time interval.

VI. CONCLUSION

The optimized and validatedHPLC method was shown to be simple, sensitive, precise and accurate and hence can be used for the routine analysis of Acetaminophen, Caffeine, Phenylephrine Hydrochloride and Dextromethorphan Hydrobromide in bulk and pharmaceutical preparation. The sample recoveries from all formulations were in good agreement with their respective label claims, and sensitivity of this method is within the range.

ACKNOWLEDGEMENT

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