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Simultaneous Estimation of Acetaminophin and Benzhydrocodone by RP-HPLC Method

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ABSTRACT:

A RP-HPLC procedure is developed, validated and applied for simultaneous estimation of Acetaminophen and Benzhydrocodone in tablets. Procedure is based on separation and analysis of acetaminophen and benzhydrocodone in C18 column and 0.1M K2HPO4: methanol (70:30 v/v) mixture as stationary and mobile phase, respectively. The elution time values for acetaminophen and benzhydrocodone were 3.6 min and 4.9 min, respectively. Linear ranges for acetaminophen and benzhydrocodone are 162.5-487.5 µg/ml and 3.06-9.18 µg/ml, respectively. The values of sensitivity were 0.311 µg/ml (LOD) and 1.035 µg/ml (LOQ) for acetaminophen and 0.036 (LOD) µg/ml and 0.121 µg/ml (LOQ) for benhydrocodone. Validation parameters are tested using guidelines of ICH. The validation values obtained are well acceptable. The method proved as suitable procedure for assay of acetaminophen and benzhydrocodone in tablet dosage forms with good assay percent values.

INTRODUCTION:

N-(4-hydroxyphenyl) acetamide is an Acetaminophin. It has possible Antipyretic, Analgesic, Nonsteroidal Activity. It acts by blocking prostaglandin synthesis from Arachidonic Acid by inhibiting Cox1 and Cox2 Enzymes.

6,7-didehydro-4,5α-epoxy-3-methoxy-17-methylmorphinan-6-yl benzoate is Benzhydrocodone.It has possible Analgesic Activity.It is a full agonist of Opioid Receptors with a higher affinity for mucopioid receptor.

Materials:

Reference drug material of acetaminophen and benzhydrocodone was collected from Lara Drugs Private Limited, Telangana, India.

Apadaz tablets: strength - 325 mg acetaminophen and 6.12 mg benzhydrocodone.

Methanol (HPLC grade) from Merck specialties Ltd, India

Dipotassium hydrogen phosphate (Analytical grade) from SD Fine-Chem Limited, India.

Chromatographic conditions for assay:

All analyses were done using an Waters Alliance HPLC system 2695 model, HPLC column C18 (250 × 4.6) mm, (5 µm), column oven and auto sampler were employed all through the analysis by HPLC. Solutions were injected using volumes of 20 µl at flow rate 1.0ml\min and a wavelength of 270 nm.

Method Development:

The conditions for assay were optimized for type of column, mobile phase composition, column temperature, flow rate and wavelength. Detection wavelength was set as ultraviolet absorption maxima shown by acetaminophen and benzhydrocodone (270 nm).

Table 1. Conditions used in different trails

Trail	Column	MP	FR	CT	IV
1	Waters C18	0.1% OPA: Methanol (50:50)	1.0	25	10
2	Inertsil C18	0.1M Na_2HPO_4: Methanol (50:50)	1.0	25	10

3	Zodiac C18	0.1M Na_2HPO_4: Methanol (50:50)	1.0	25	10
4	Hibar C18	0.1M K_2HPO_4: Methanol (55:45)	1.0	25	10
5	Hibar C18	0.1M K_2HPO_4: Methanol (65:35)	1.0	25	10

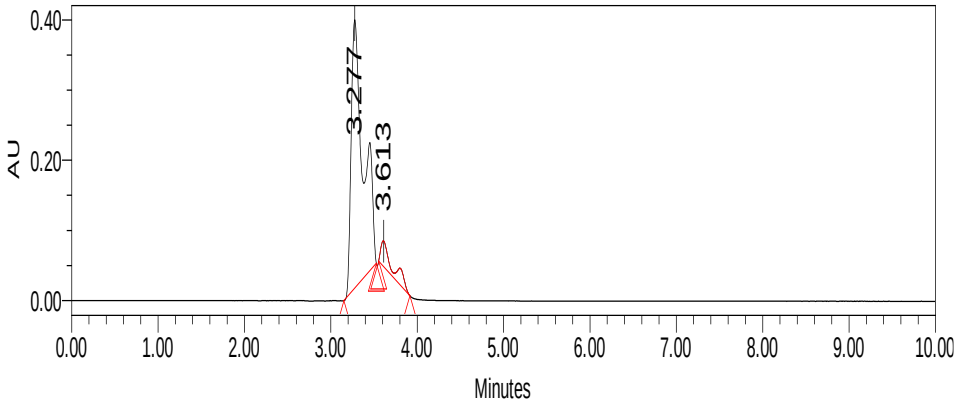
MP-mobile phase, FR-flow rate (ml/min), CT-column temperature (°C), IV-injection volume (µl)

Table 2. Results obtained in different trails

Trail	Drug	RT	PA	RS	PT	PC
1	ACT	3.3	3669715	-	1.88	4787
	BEN	3.6	346413	1.75	3.00	8512
2	ACT	3.2	3729921	-	0.85	2352
	BEN	3.7	724340	1.71	1.25	2698
3	ACT	3.4	3635546	-	1.26	6709
	BEN	3.9	707826	2.50	1.57	4332
4	ACT	4.3	3761134	-	0.99	8845
	BEN	5.0	905965	3.16	1.25	5589
5	ACT	3.7	3893993	-	1.28	11142
	BEN	4.9	974580	6.85	1.31	8135

ACT-acetaminophen, BEN benzhydrocodone, RT-retention time, PA-peak area, RS-resoltuion, PC-plate count, PT-peak tailing

Figure 1:Trail chromatogram



System suitability:

Acetaminophen (330 µg/ml) and benzhydrocodone (6.15 µg/ml) solution injected five times. Criteria used for acceptance of system suitability are:

- Plate count - > 2000
- Resolution - > 2.0
- Peak tailing - ≤ 2.0
- RSD for peak area - ≤ 2.0

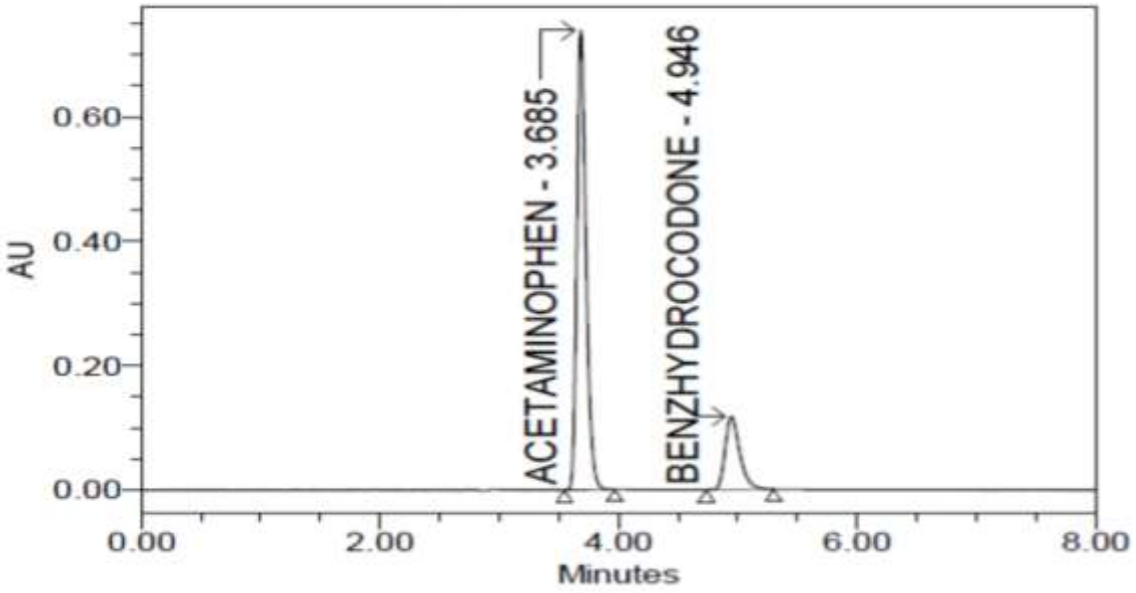
Table 3: Benzhydrocodone data during system suitability

	SAMPLE NAME	PEAK NAME	RT	AREA	USP PLATE COUNT	USP RESOLUTION	USP TAILING
1	STD2	BENZHYDROCODONE	4.946	1013280	8071	6.80	1.36
2	STD2	BENZHYDROCODONE	4.944	1023461	8046	6.82	1.36
3	STD2	BENZHYDROCODONE	4.945	1014063	8022	6.82	1.36
4	STD2	BENZHYDROCODONE	4.948	1017599	8107	6.84	1.37
5	STD2	BENZHYDROCODONE	4.945	1016863	8067	6.84	1.36
Mean				1017053.2			
%RSD				0.4			

Table 4: Acetaminophen data during system suitability

	SAMPLE NAME	PEAK NAME	RT	AREA	USP PLATE COUNT	USP TAILING
1	STD2	ACETAMINOPHEN	3.685	3943605	1175	1.29
2	STD2	ACETAMINOPHEN	3.683	3974661	11233	1.28
3	STD2	ACETAMINOPHEN	3.683	3962823	11251	1.29
4	STD2	ACETAMINOPHEN	3.685	3963022	11259	1.29
5	STD2	ACETAMINOPHEN	3.683	3969532	11231	1.29
Mean				3962728.4		
%RSD				0.3		

Figure 2 :Chromatograms of system suitability



Selectivity:

Mobile phase blank, placebo blank, working solution (acetaminophen 330 µg/ml and benzhydrocodone - 6.15µg/ml) and tablet solution were injected. Checked for interference peaks at the retention times of acetaminophen and benzhydrocodone. No interfering peaks were seen.

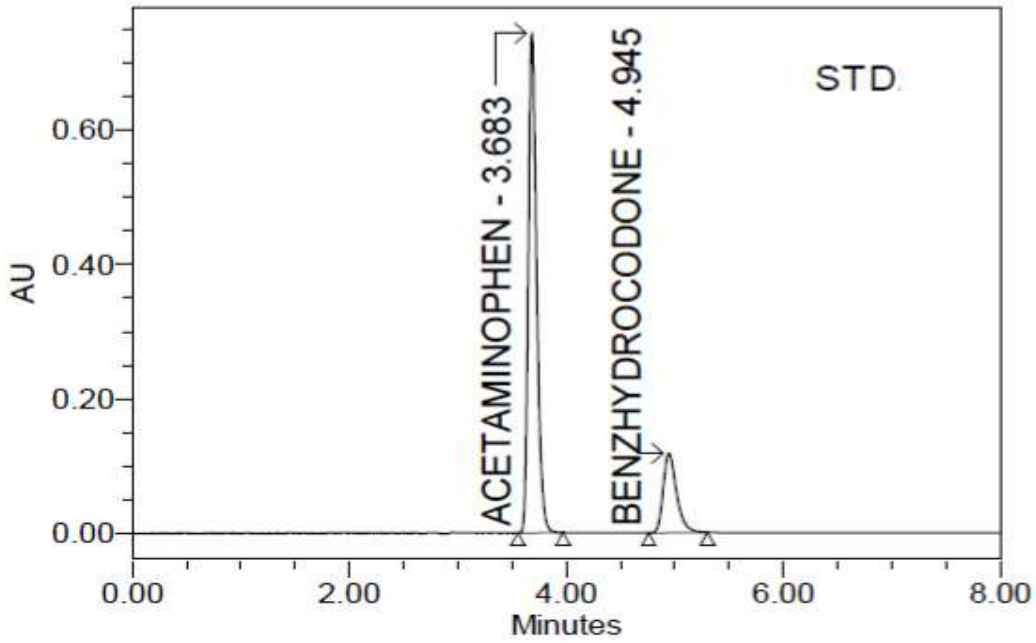


Figure 3 :Selectivity chromatograms

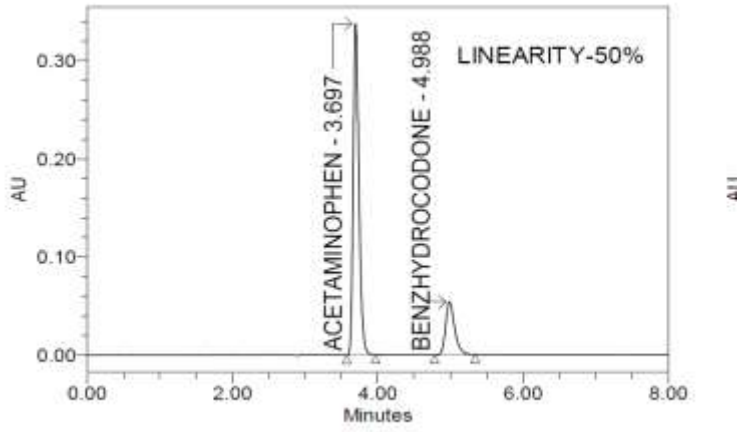
Linearity:

The assay method linearity of acetaminophen and benzhydrocodone were determined in range from 50%, 75%, 100%, 125% and 150% proportional to concentration relative to standard concentration prescribed 330 µg/ml (acetaminophen) and 6.15 µgml (benzhydrocodone). The curves of acetaminophen and benzhydrocodone were linear over 162.5 – 487.5 µg/ml and 3.06 – 9.18 µg/ml, respectively and exhibited a good regression coefficient (R^2 = > 0.9990).

Table 5. Acetaminophen and benzhydrocodone linearity data

Conc %	**Acetaminophen**		**Benzhydrocodone**	
	Peak area	**µg/ml**	**Peak area**	**µg/ml**
50	1987907	162.5	508696	3.06
75	2979936	243.75	762481	4.59
100	3967196	325.00	1013570	6.12
125	4954061	406.25	1274750	7.65
150	5943775	487.5	1522204	9.18

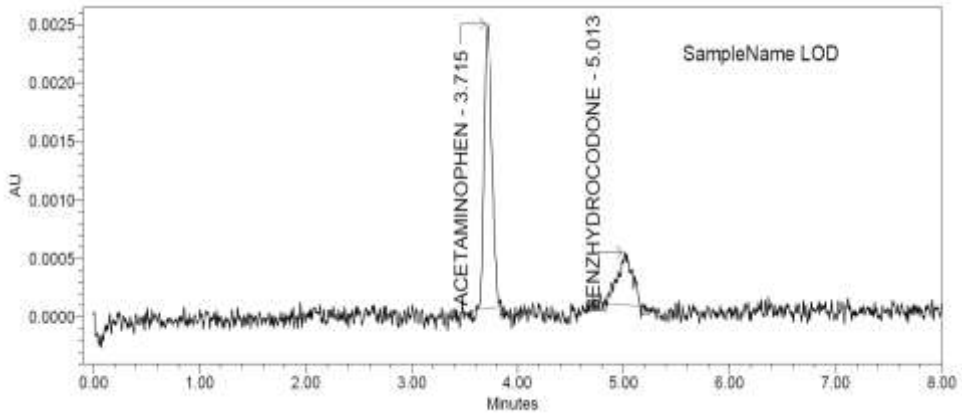
Figure 4: Acetaminophen linearity curve



Limit of detection and limit of quantification:

Limit of detection (LOD) and limit of quantitation (LOQ) calculated as signal to noise ratio 3.1 and 10.1, respectively. LOD was 0.311 µg/ml for acetaminophen and 0.036 µg/ml for benzhydrocodone. LOQ was 1.035 µg/ml for acetaminophen and 0.121 µg/ml for benzhydrocodone.

Figure 5 LOD and LOQ chromatograms



Peak Name: Acetaminophin

	SAMPLE NAME	PEAK NAME	RT	AREA	s/n
1	LOD	ACETAMINOPHEN	3.715	13204	3.50
2	LOQ	ACETAMINOPHEN	3.728	80207	10.29

Peak Name: Benzhydrocodone

	SAMPLE NAME	PEAK NAME	RT	AREA	s/n
1	LOD	BENZHYDROCODONE	5.013	4424	3.95
2	LOQ	BENZHYDROCODONE	5.052	24637	10.39

Precision and accuracy:

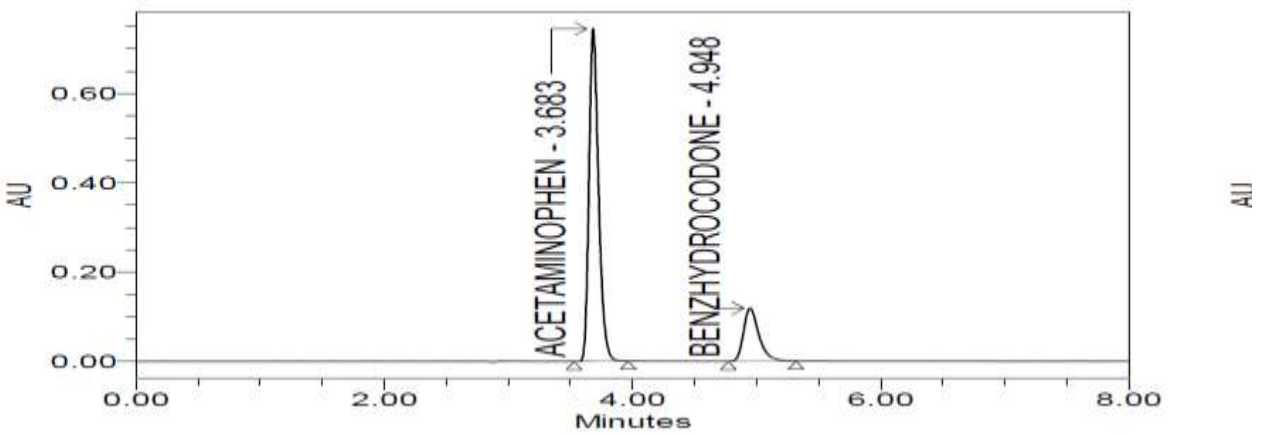
In this, standard solutions containing 330 µg/ml of acetaminophen and 6.15 µg/ml of benzhydrocodone were prepared, and injected 6 times into the HPLC system. Mean of peak areas and % RSD values of peak area and mean percent assay values were calculated to show precision and accuracy, respectively. Acceptable criteria are:

- Precision - %RSD ≤ 2.0
- Accuracy – percent assay 80-120%

Table 6: Acetaminophen and benzhydrocodone precision and accuracy results

Sample No.	**Peak area of acetaminophen**	**Peak area of benzhydrocodone**	**Percent assay of acetaminophen**	**Percent assay of benzhydrocodone**
i	3964203	1017773	99.74	99.67
ii	3968526	1013573	99.85	99.26
iii	3961837	1017638	99.68	99.66
iv	3966868	1019607	99.8	99.85
v	3965045	1012037	99.76	99.11
vi	3964217	1018343	99.74	99.73
Mean	**3965116**	**1016495.17**	**99.762**	**99.547**
SD	**2328**	**2982**	**0.06**	**0.3**
RSD	**0.06**	**0.29**	**0.06**	**0.29**

Figure 6 : chromatograms for precision and accuracy testing



Recovery:

Recovery was tested by spiking 50, 100 and 150% of acetaminophen and benzhydrocodone standards to pre analyzed tablet solution in triplicates. The percent recovery was determined. An acceptance criterion is between 80% – 120% recovery value.

Table 7: Acetaminophen recovery results

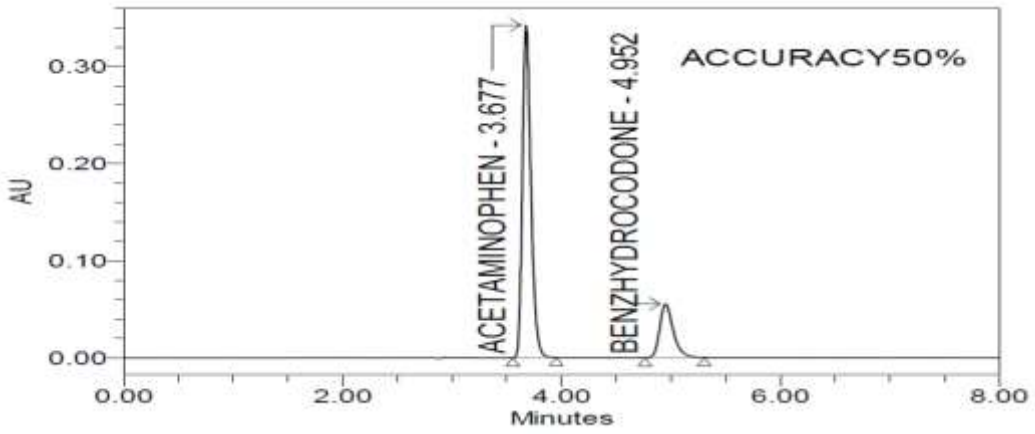
Spiked Percent	**Peak area of ACE**	**µg/ml of ACE added**	**µg/ml of ACE found**	**% of ACE Recovered**	**% Mean**
50%	1985177	162.500	162.32	99.89	99.96
50%	1983911	162.500	162.22	99.83	
50%	1989290	162.500	162.66	100.10	
100%	3965075	325.000	324.22	99.76	99.7
100%	3963716	325.000	324.11	99.72	
100%	3968061	325.000	324.46	99.83	

150%	5944048	487.500	486.03	99.70	
150%	5945780	487.500	486.18	99.73	99.7
150%	5944996	487.500	486.11	99.72	

Table 8: Benzhydrocodone recovery results

Spiked Percent	Peak area of BEN	µg/ml of BEN added	µg/ml of BEN found	% of BEN Recovered	% Mean
50%	508694	3.060	3.05	99.63	
50%	508056	3.060	3.04	99.51	99.6
50%	508299	3.060	3.05	99.56	
100%	1018778	6.120	6.11	99.77	
100%	1012284	6.120	6.07	99.13	99.6
100%	1019727	6.120	6.11	99.86	
150%	1525047	9.180	9.14	99.57	
150%	1525809	9.180	9.14	99.61	99.6
150%	1526563	9.180	9.15	99.66	

Figure 7: chromatograms at 50, 100 and 150% spiked levels



Robustness:

Small deliberate changes are made in the following:

- Ratio of methanol - changed by ±5%
- pH of buffer – changed by ± 0.2 units
- Flow rate - changed by ± 0.1 ml/min
- Column temperature - changed by ± 2 ºC;
- Wavelength – changed by ± 2 nm

In above changed conditions, acetaminophen and benzhydrocodone solution is injected. System suitability parameters determined. Criteria used for acceptance of system suitability are:

- Plate count - > 2000
- Resolution - > 2.0
- Peak tailing - ≤ 2.0
- RSD for peak area - ≤ 2.0

Table 9: Acetaminophen robustness

Conditions	**Value Change**	**Tailing factor**	**Theoretical plate**	**Resolution**
Column's temperature (°C)	23	1.30	9720	-
	27	1.29	10432	-
Flow rate run (ml/min)	0.9	1.31	11691	-
	1.1	1.32	12342	-
Mobile phase pH (units)	4.4	1.30	9720	-
	4.6	1.31	11691	-
Ratio of methanol (%)	30	1.29	11205	-
	40	1.29	11133	-
Wavelength (nm)	268	1.28	11210	-
	272	1.29	11218	-

Table 10: Benzhydrocodone robustness

Conditions changed	**Changed value**	**Tailing factor**	**Theoretical plate**	**Resolution**
Column's temperature (°C)	23	1.33	7214	6.54
	27	1.34	7768	6.86
Flow rate run (ml/min)	0.9	1.38	8478	7.16
	1.1	1.40	9180	7.46
Mobile phase pH (units)	4.4	1.33	7214	6.54
	4.6	1.38	8478	7.16
Ratio of methanol (%)	30	1.36	8073	6.85
	40	1.37	8023	6.82
Wavelength (nm)	268	1.37	7999	6.82
	272	1.37	8068	6.82

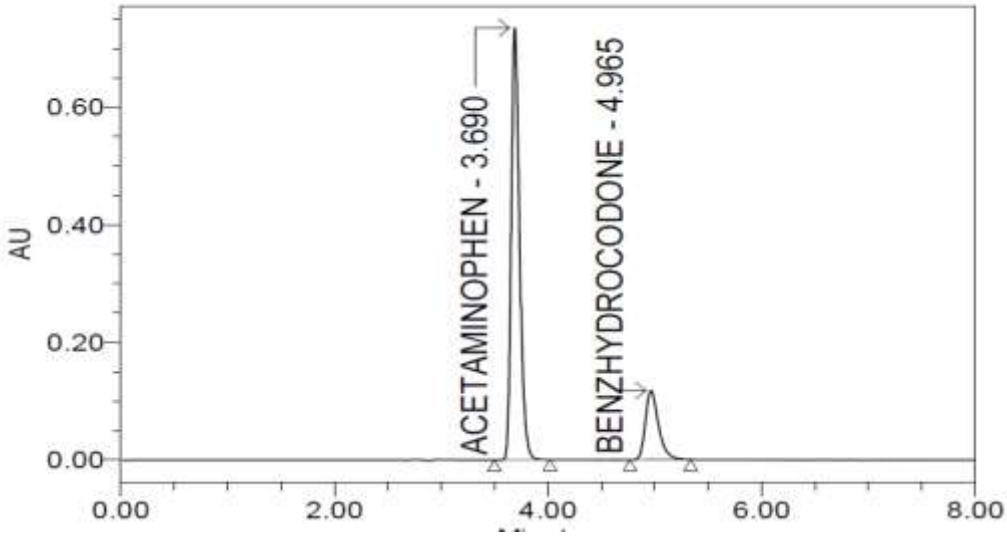
Application of method to assay acetaminophen and benzhydrocodone in tablets:

The content of acetaminophen and benzyhydrocodone was determined in (strength - 330 mg acetaminophen and 6.15 mg benzhydrocodone) by proposed method. The assay percent (nearer to 100%) and relative standard deviation (less than 2%) values are acceptable.

Table 11: Assay of acetaminophen and benzhydrocodone in tablet

Drug content in tablet (mg)	**Drug determined (µg/ml)**	**Drug Assayed (%)**	**Statistical assessment**
Acetaminophen			
330	324.81	99.94	**Mean:** 99.8%
330	324.25	99.77	**RSD:** 0.120%
330	324.06	99.71	-
Benzhydrocodone			
6.15	6.094	99.57	**Mean:** 99.6%
6.15	6.095	99.59	**RSD:** 0.020%
6.15	6.096	99.61	-

Figure 8 : **Chromatograms of tablet assay**



CONCLUSION:

Acetaminophen and benzhydrocodone were simultaneously separated and quantified successfully in the tablets using the developed RP-HPLC method with good precision and accuracy. The RP-HPLC method has adequate sensitivity and selectivity

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