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Method development and validation of simultaneous estimation of acetaminophen and behydrocodone in tablet dosage forms

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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 Hibar C18 column and 0.1M K_2HPO_4: methanol (65:35v\v) mixture as stationary and mobile phase, respectively. The elution time values for acetaminophen and benzhydrocodone were 3.692min and 4.956 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 benzhydrocodone. The validation parameters are tested using guidelines of ICH. The validation values obtained are well acceptable. The method proved as suitable procedure for assay acetaminophen and benzhydrocodone in tablet dosage forms with good assay percent values.

Keywords: Acetaminophen, Benzhydrocodone, RP-HPLC.

INTRODUCTION

Acetaminophen

Category	:	Antipyretic, analgesic, Nonsteroidal Anti-inflammatory
IUPAC name	:	N-(4-hydroxyphenyl) acetamide
Molecular formula	:	$C_8H_9NO_2$
Molecular weight	:	151.162 g/mol
Melting point	:	169-171 ^oC
Solubility	:	Soluble in methanol, ethylene dichloride and ethanol; slightly soluble in cool water and soluble in hot water; insoluble in benzene and petroleum ether

Molecular weight	:	151.162 g/mol
Melting point	:	169-171 ^{o}C
Solubility	:	Soluble in methanol, ethylene dichloride and ethanol; slightly soluble in cool water and soluble in hot water; insoluble in benzene and petroleum ether
pKa value	:	9.38
Elimination route	:	80% is excreted in conjugated form through urine and 3% in unconjugated form through urine
Half life	:	1 – 4 hr

Acetaminophen also called as paracetamol, belongs to organic class of comounds, acetanilide. It is used commonly for the relief of fever, headaches and other minor aches & pains [29, 30]. Acetaminophen is used on its own/ in combination with dextromethorphan, chlorpheniramine/ pseudoephedrine/diphenhydramine/ doxylamine/ codeine/oxycodone etc [31]. The mechanism is not clearly known. It is considered that acetaminophen primarily act in central nervous system, increasing the threshold of pain by blocking the isoforms of cyclooxygenase (COX-1, 2 and 3) enzymes which participates in prostaglandin synthesis [32]. The antipyretic activity of acetaminophen is because of its effects on the heat controlling areas in hypothalamus. The result is the peripheral vasodilation, sweating and hence dissipation of heat.

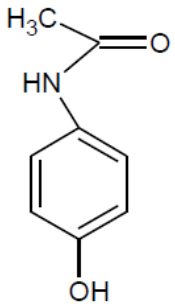

Figure 1: Acetaminophen structure

BENZHYDROCODONE

Category	:	Opioid receptor antagonist
IUPAC name	:	6,7-didehydro-4,5α-epoxy-3-methoxy-17-methylmorphinan-6-yl benzoate
Molecular formula	:	$C_{25}H_{25}NO_4$
Molecular weight	:	403.470 g/mol
Melting point	:	169-171 ^{o}C
Solubility	:	Water solubility is very less; Soluble in chloroform, acetone and ethyl acetate
pKa value	:	8.23
Elimination	:	In urine 90%

The prodrug, benzhydrocodone, is not active pharmacologically. It is metabolized to active hydrocodone by enzymes in the intestine. During this conversion benzhydrocodone undergoes O-demethylation, N-demethylation and 6-keto reduction. Hydrocodone acts like agonist for opioid receptors. It has more affinity for μ-opioid receptor. The correct analgesic mechanism is not known clearly.

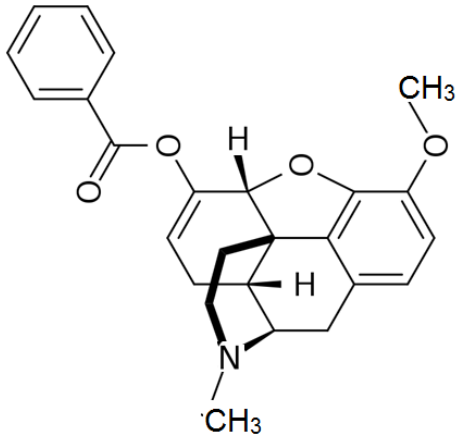

Figure 2. Benzhydrocodone structure

AIM AND OBJECTIVES

- To develop a method by RP-HPLC for the quantification of acetaminophen and benzhydrocodone simultaneously in tablet.
- Through ICH guidelines following, validating the method for system suitability, linearity, precision, selectivity, sensitivity, accuracy, and robustness.

MATERIALS AND METHODS

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 Hibar 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.

Preparation of Mobile phase

- 0.1M Dipotassium hydrogen phosphate and methanol were mixed at ratio 650 ml: 350 ml, respectively. pH is 4.5.

Preparation of acetaminophen and benzhydrocodone standard solutions

325 mg of acetaminophen and 6.12 mg of benzhydrocodone were perfectly weighed and transferred to 100 ml volumetric flask then 30 ml mobile phase was added and sonicated 20 min. Mobile phase was further added to total the volume to 100 ml.

Final Concentration - 3250 µg/ml acetaminophen and 61.20 µg/ml benzhydrocodone). This solution is acetaminophen and benzhydrocodone stock solution.

Acetaminophen and benzhydrocodone solutions for calibration curve are prepared by diluting 0.5, 0.75, 1.0, 1.25 and 1.5 ml of acetaminophen and benzhydrocodone stock solution to ten ml with mobile phase to get following concentrations:

162.5 µg/ml, 243.75 µg/ml, 325.0µg/ml, 406.25 µg/ml and 487.5 µg/ml – acetaminophen. 3.06 µg/ml, 4.59 µg/ml, 6.12 µg/ml, 7.65 µg/ml and 9.18 µg/ml – benzhydrocodone

To study validation contents, acetaminophen and benzhydrocodone solution is made by diluting one ml of acetaminophen and benzhydrocodone stock to ten ml using mobile phase (final concentration: 325 µg/ml acetaminophen and 6.12 µg/ml benzhydrocodone).

Preparation of acetaminophen and benzhydrocodone tablet solutions

Exactly weighed powdered tablet equal to acetaminophen 325 mg and benzhydrocodone 6.12 mg were perfectly weighed and transferred to 100 ml volumetric flask then 30 ml mobile phase was added and sonicated 20 min. Mobile phase was

further added to total the volume to 100 ml (Concentration - 3250 µg/ml acetaminophen and 61.20 µg/ml benzhydrocodone). This solution is acetaminophen and benzhydrocodone tablet stock solution. For analysis, one ml of acetaminophen and benzhydrocodone tablet stock solution is diluting to 10 ml using mobile phase (final concentration: 325 µg/ml acetaminophen and 6.12 µg/ml benzhydrocodone).

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162.5 µg/ml, 243.75 µg/ml, 325.0µg/ml, 406.25 µg/ml and 487.5 µg/ml – acetaminophen.

Method Validation

The method used for the quantification of acetaminophen 325 mg and benzhydrocodone in tablets was validated for system suitability, linearity, accuracy, LOD and LOQ, precision and robustness as formerly described
3.06 µg/ml, 4.59 µg/ml, 6.12 µg/ml, 7.65 µg/ml and 9.18 µg/ml – benzhydrocodone

To study validation contents, acetaminophen and benzhydrocodone solution is made by diluting one ml of acetaminophen and benzhydrocodone stock to ten ml using mobile phase (final concentration: 325 µg/ml acetaminophen and 6.12 µg/ml benzhydrocodone).

RESULTS AND DISCUSSION

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 (^oC), IV-injection volume (µl)

Table 2. Results obtained in different trails

Trail	Drug	RT	PA	RS	PT	PC
1	ACT	3.277	3669715	-	1.88	4787
	BEN	3.613	346413	1.75	3.00	8512
2	ACT	3.227	3729921	-	0.85	2352
	BEN	3.719	724340	1.71	1.25	2698
3	ACT	3.452	3635546	-	1.26	6709
	BEN	3.992	707826	2.50	1.57	4332
4	ACT	4.274	3761134	-	0.99	8845
	BEN	5.010	905965	3.16	1.25	5589
5	ACT	3.692	3893993	-	1.28	11142
	BEN	4.956	974580	6.85	1.31	8135

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

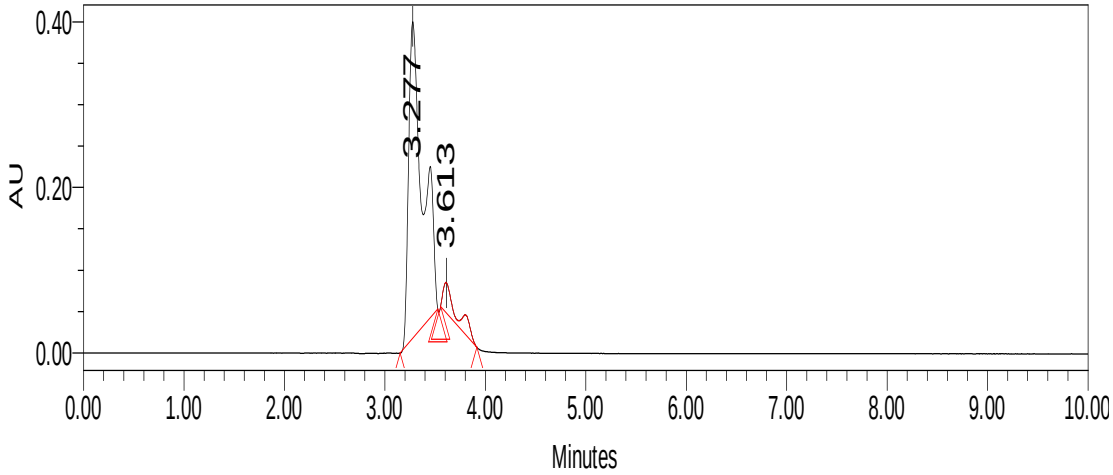

Figure 3. Trail 1 chromatogram

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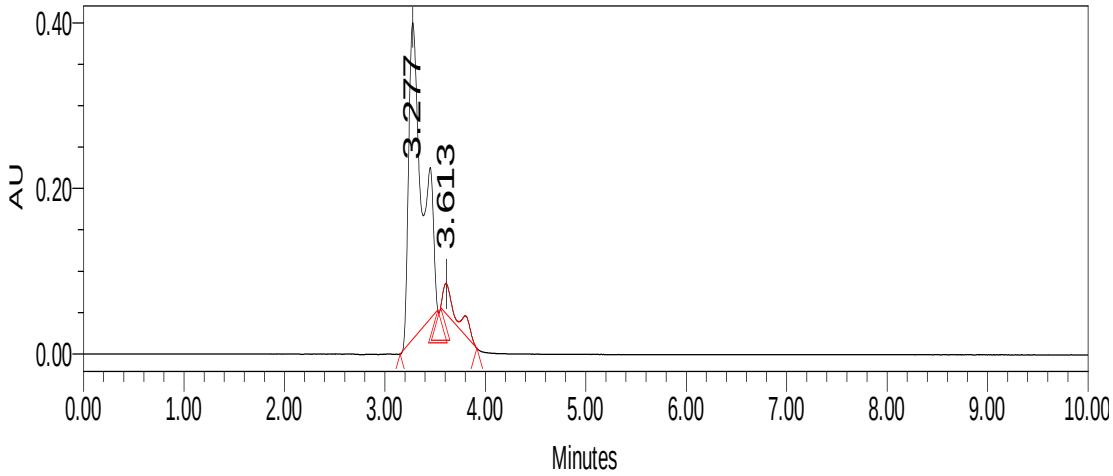

Figure 3. Trail 1 chromatogram

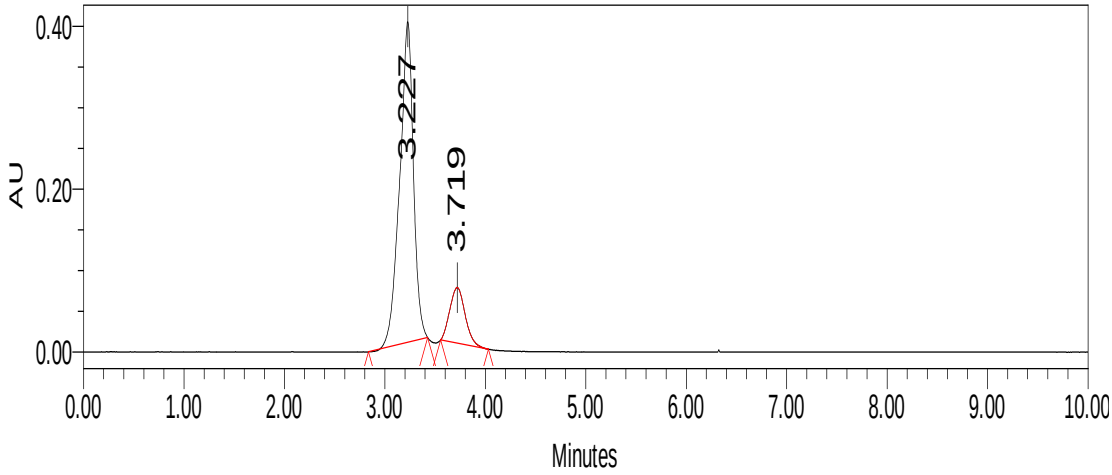

Figure 4. Trail 2 chromatogram

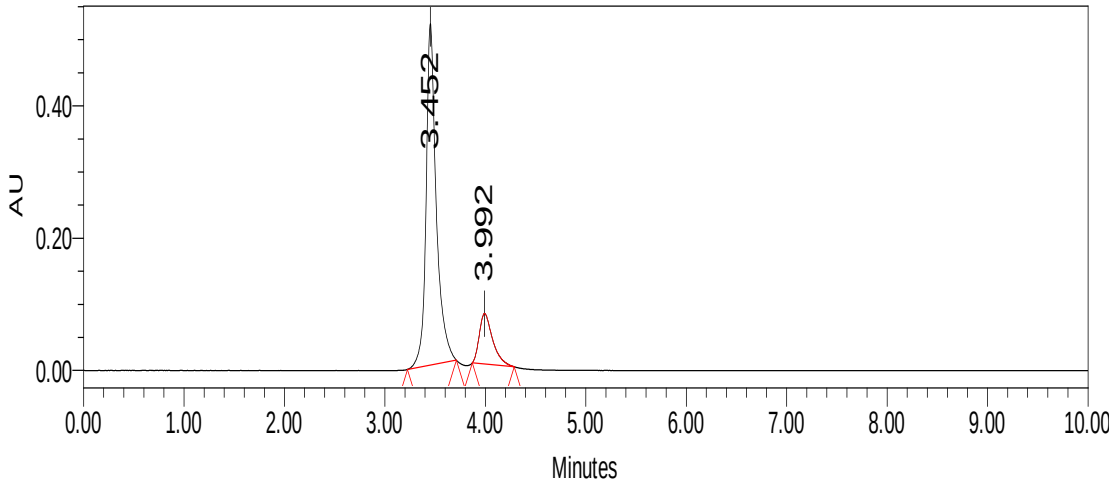

Figure 5. Trail 3 chromatogram

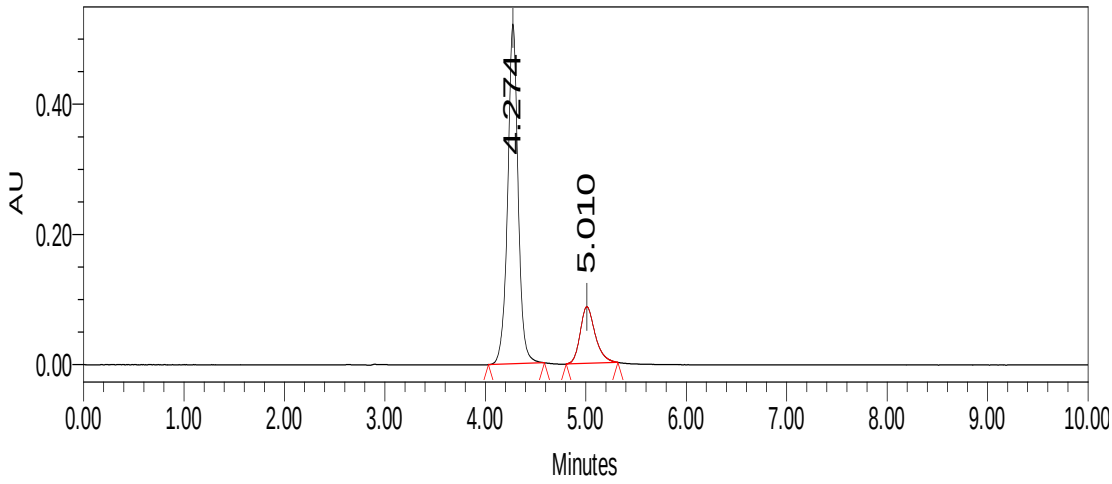

Figure 6. Trail 4 chromatogram

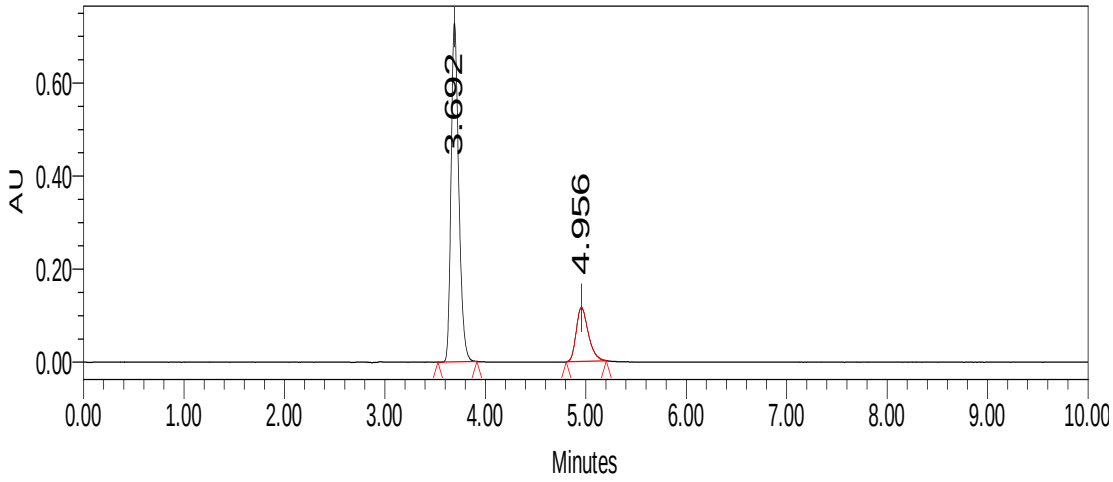

Figure 7. Trail 5 chromatogram

Based on resolution, peak area, plate count and peak tailing, the conditions used in trial 5 was selected as optimized value for assay of acetaminophen and benzhydrocodone simultaneously.

Method validation

System suitability

Acetaminophen (325 µg/ml) and benzhydrocodone (6.12 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

	SampleName	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

	SampleName	Peak Name	RT	Area	USP Plate Count	USP Tailing
1	STD2	ACETAMINOPHEN	3.685	3943605	11175	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		

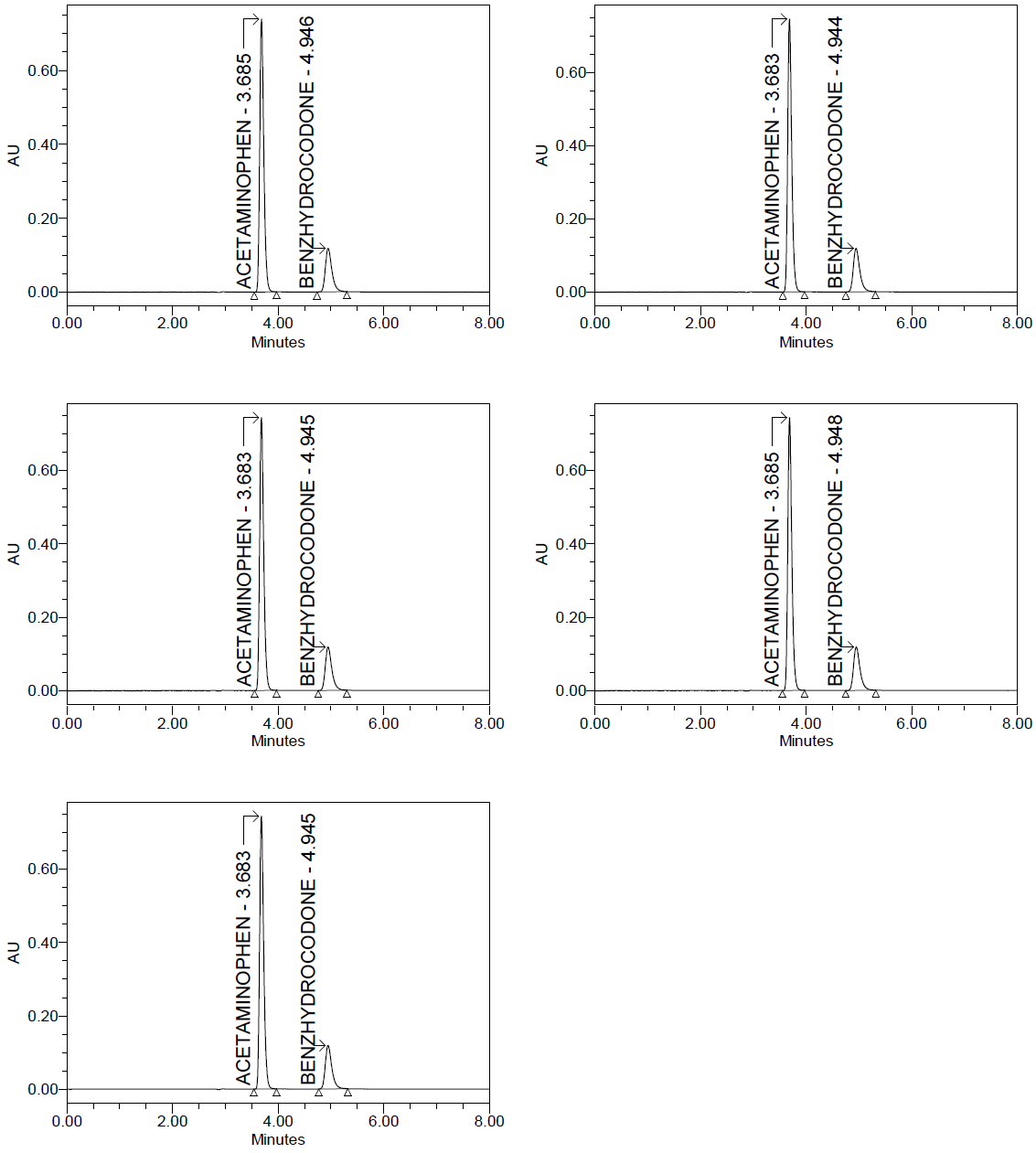

Figure 8. Chromatograms of system suitability

Selectivity

Mobile phase blank, placebo blank, working solution (acetaminophen 325 µg/ml and benzhydrocodone - 6.12 µg/ml) and tablet solution (acetaminophen 325 µg/ml and benzhydrocodone - 6.12 µg/ml) were injected. Checked for interference peaks at the retention times of acetaminophen and benzhydrocodone. No interfering peaks were seen.

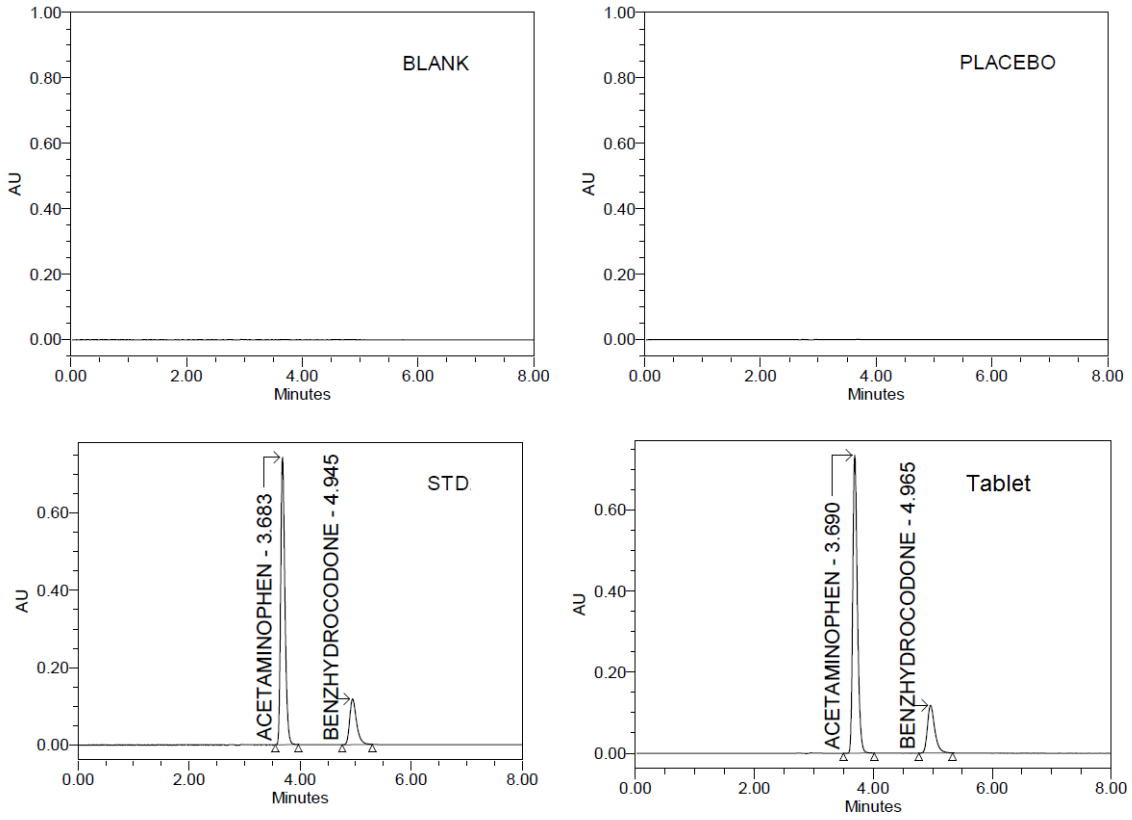

Figure 9: Selectivity chromatograms

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 325 µg/ml (acetaminophen) and 6.12 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

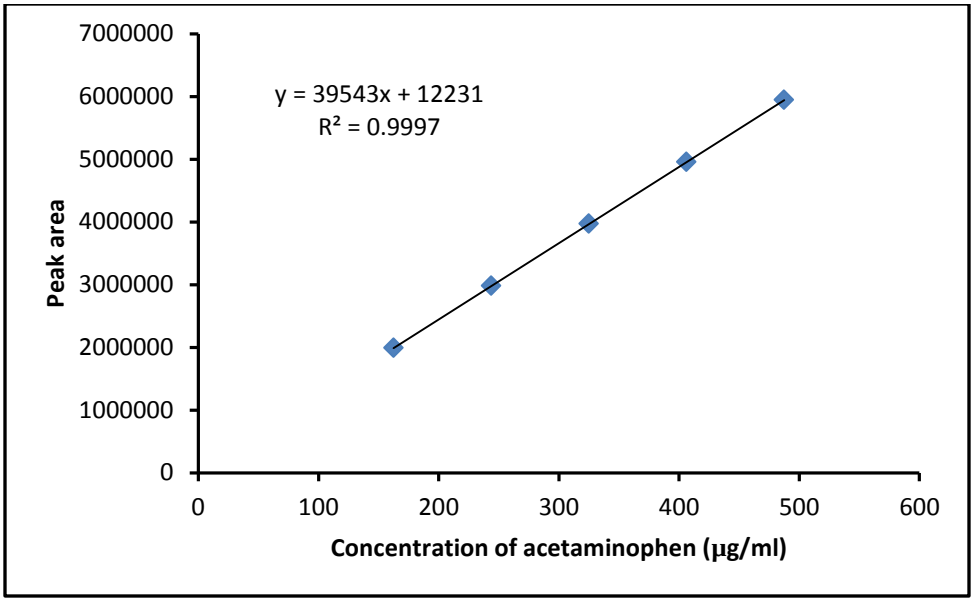

Figure 10. Acetaminophen linearity curve

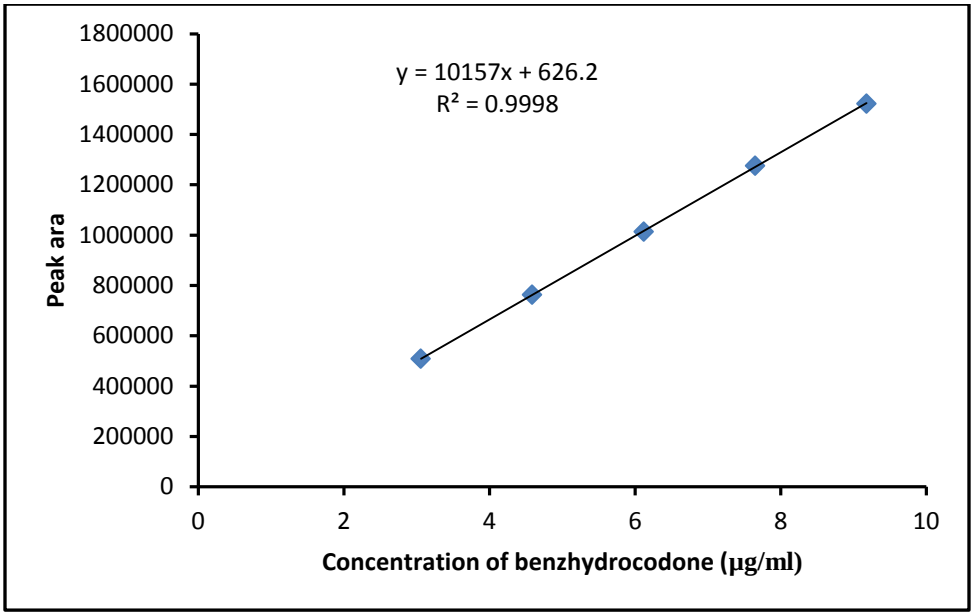

Figure 11. Benzhydrocodone linearity curve

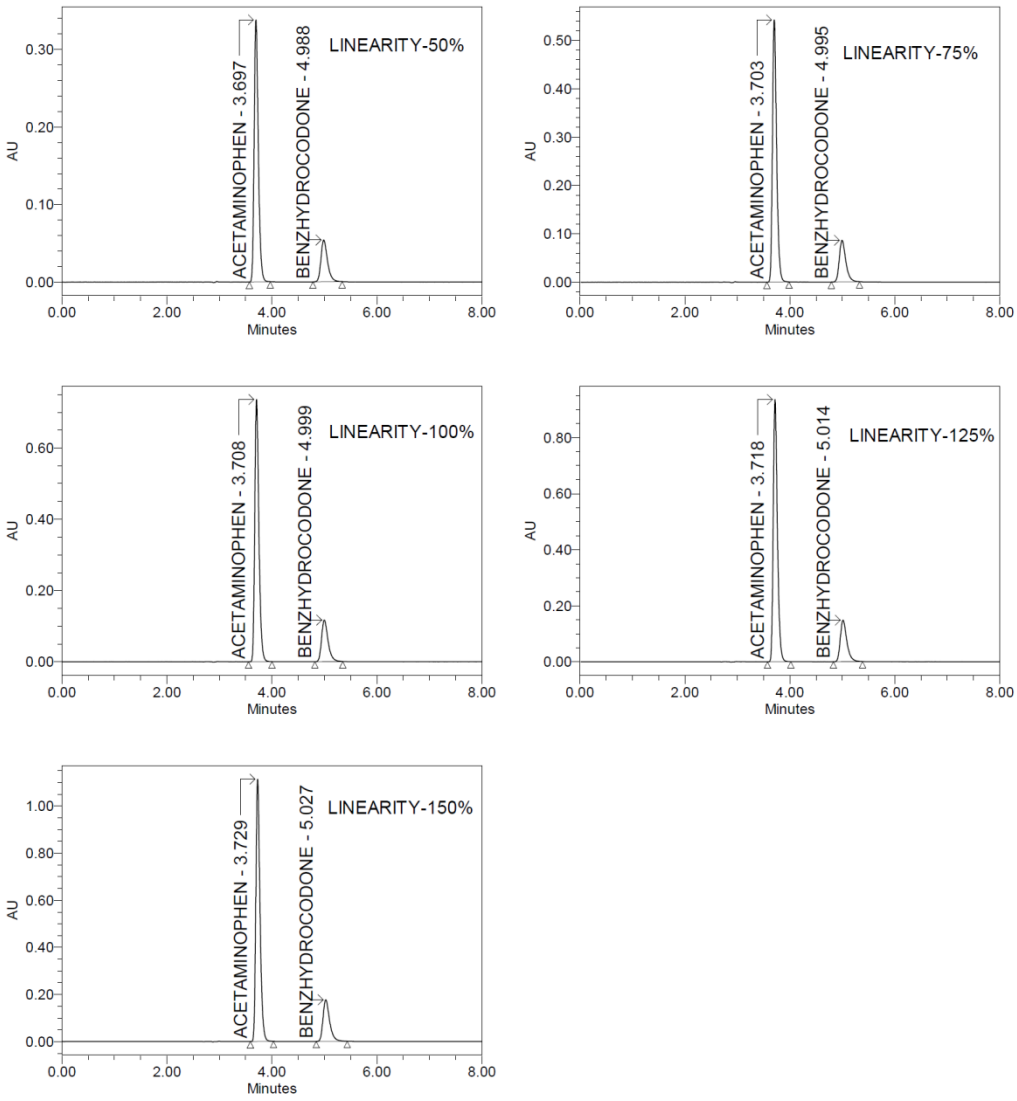

Figure 12. Acetaminophen and benzhydrocodone linearity chromatograms

Precision and accuracy

In this, standard solutions containing 325 µg/ml of acetaminophen and 6.12 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	**2327.929**	**2981.652**	**0.058**	**0.292**
RSD	**0.059**	**0.293**	**0.058**	**0.290**

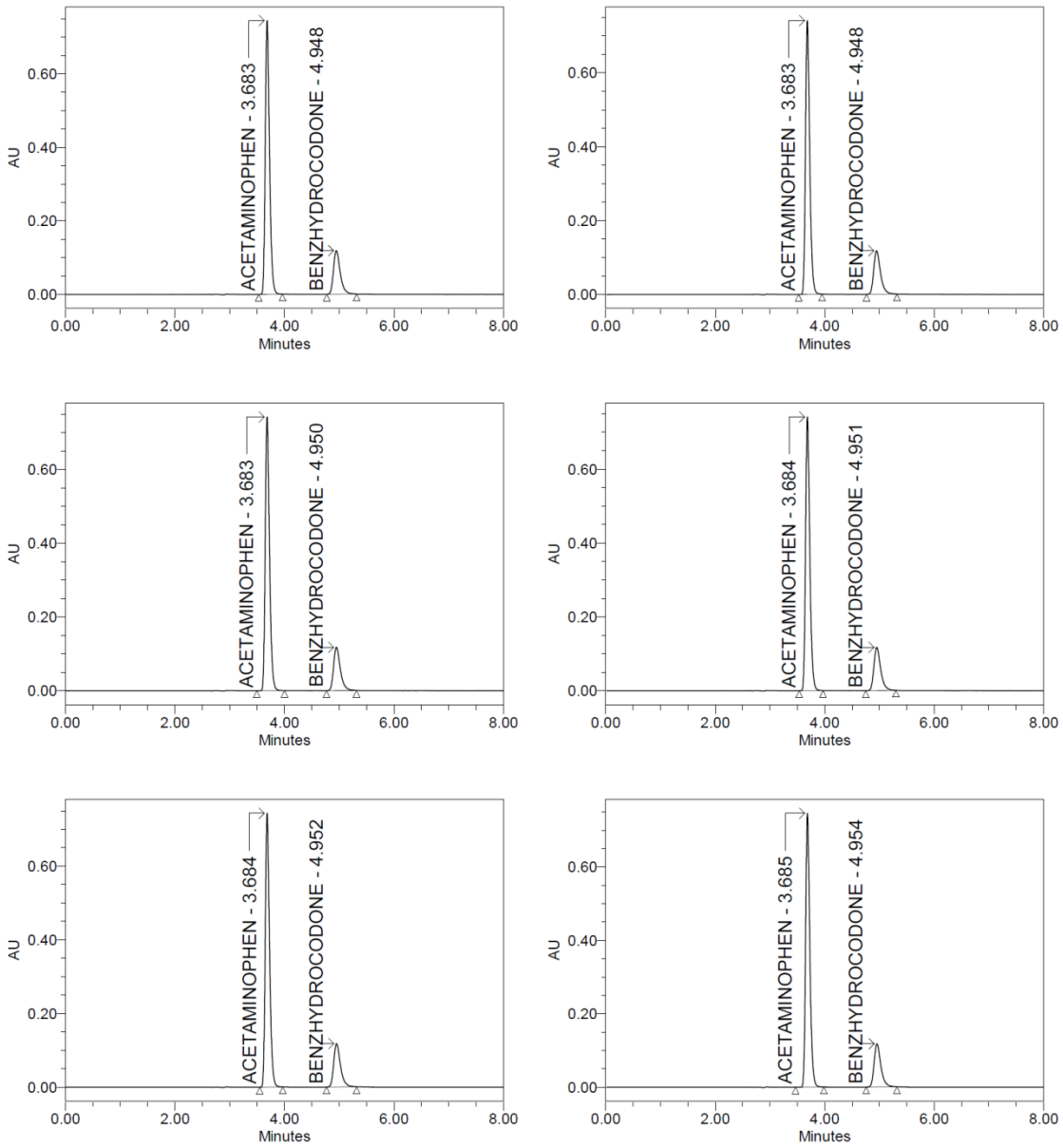

Figure 14. Acetaminophen and benzhydrocodone chromatograms for precision and accuracy testing

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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