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A new HPLC method development and validation for simultaneous determination of acetaminophen and codeiene phosphate in bulk and dosage forms

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ABSTRACT

In current research an easy, accurate, reliable, specific and economical HPLC approach is established to analyze acetaminophen and codeine phosphate. Quantification and separation of acetaminophen and codeine phosphate is done with C18 water's column using mobile phase -0.1M, pH 3.5 NaH2PO4: acetonitrile (50; 50). Linearity for acetaminophen was 150µg/ml -450µg/ml and for codeine phosphate was 30µg/ml -90µg/ml. LOD was 2.770µg/ml for acetaminophen and 0.842 µg/ml for codeine phosphate. Precision on was lesser than 2.0% and accuracy was near to 100%.The objective of this research deals with optimization of method conditions to estimate acetaminophen and codeine phosphate also includes method validation, specificity, linearity, precision, accuracy and robustness. This developed procedure can therefore employed in pharmaceutical formulations for intent of quality control.

Keywords: Acetaminophen, Codeine Phosphate, RP-HPLC.

INTRODUCTION

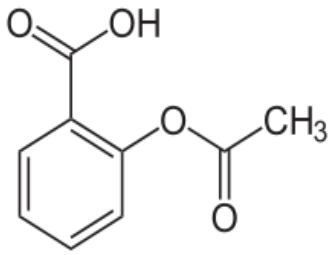
Pain is an uncomfortable sensory experience, like prick, stingling, burning, or ache. It can be sluggish and come and go, or might be continuous. Pain is indeed a notification that anything might be incorrect in the nervous system. Pain can be divided as acute pain and chronic pain. Acute pain normally occurs and exists for a limited duration. Chronic pain continues longer than acute pain and is usually resistant to medical therapy. It is generally linked to a long term disease.

Treatment of slight and moderate pain involves the mixture of acetaminophen (non-salicylate) and codeine (opiate antogonist).

Acetaminophen

Acetaminophen is divided as analgesic, anti pyretic , non steroidal anti inflammatory , cycloxygenase inhibitor. Technical name *is N-(4- hydroxyphenyl) acetamide***.** Half life of the drug is estimated as 2.5 hr and discharged out through urine (90% of the given drug).

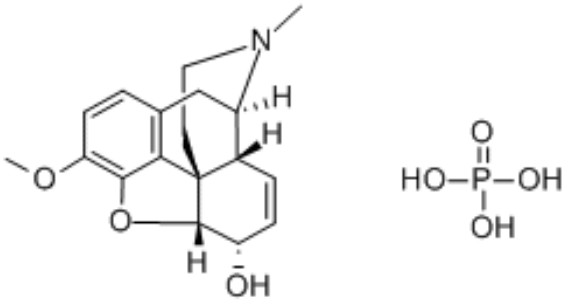
It raises the pain limit by blocking synthesis of two cyclooxygenase isoforms (COX 1 & COX 2). These enzymes produce prostaglandins which are accountable for the intense feeling of pain.



Acetaminophen structure

Codiene phosphate

It is an antagonist for opioid, analgesic drug, antidiarrheal drug, anti tussive drug. Technical name is (4R,4aR,7S,7aR,12bS)-9- methoxy -3-methyl -2,4,4a,7,7a,13-hexahydro-1H-4,12-methanobenzofuro[3,2-e] isoquinolin-7-ol:phosphoric acid .Half life of the drug is estimated as 2.5 hr . It is discharged through urine (90%). It acts by binding to µ-opiod receptors and block their function.µ opioid receptors transmit pain all through the body and the central nervous system. It is used in treating pain of mild to moderate type and cough relief. It is also used in treating irritable bowel syndrome.



Codeine phosphate structure

MATERIALS AND METHOD

Apparatus

Waters HPLC system, photodiode detector with empower software version
Water column C18:5 µm, 4.5 mm and 250 mm dimensions.
Ultrasonicator
Weighing balance
soreson pH meter

Chemicals

Sodium dihydrogen phosphate
Acetonitrile
Phosphoric acid

Conditions to assay drugs:

Flow velocity - 1.0 ml / min
Temperature -25 * c
Vol. injected - 10µl
Detection - 228

Mobile phase

0.1 M NaH_2PO_4 buffer, pH 3.5 and acetonitrile were mixed in 50:50 volume ratios. Same solvent mix was used as diluent for making solutions of drug stock and standard solution.

Codeine phosphate & acetaminophen stock solution

Weighed about 300 mg acetaminophen and 60 mg codeine phosphate was transferred to100 ml

volumetric flask.50 ml diluent was added, mixed well and make up volume to mark by diluent. Concentration of acetaminophen and codeine phosphate stock solution was 3000µg/ml acetaminophen and 600µg/ml codeine phosphate.

Solutions for study of calibration graph

Solution with five concentrations of acetaminophen and codeine phosphate were prepared from stock solution by diluent.

Solution	Acetaminophen in µg/ml conc.	Codeine phosphate in µg/ml conc.
I	150	30
2	225	45
3	300	60
4	375	75
5	450	90

Tablet solution

Tylenol with codeine no.4 (strength: acetaminophen 300 mg and codeine 60mg) tablets are powdered and weighed. Acetaminophen 300 mg and codeine 60mg weight equivalent powder was deported to 100 ml flask. 50 ml diluent was added, 30 min sonicated, filtered through membrane finally make up to 100 ml by diluent. Concentration of stock tablet solution is 3000µg / ml acetaminophen and 600µg /ml codeine phosphate.

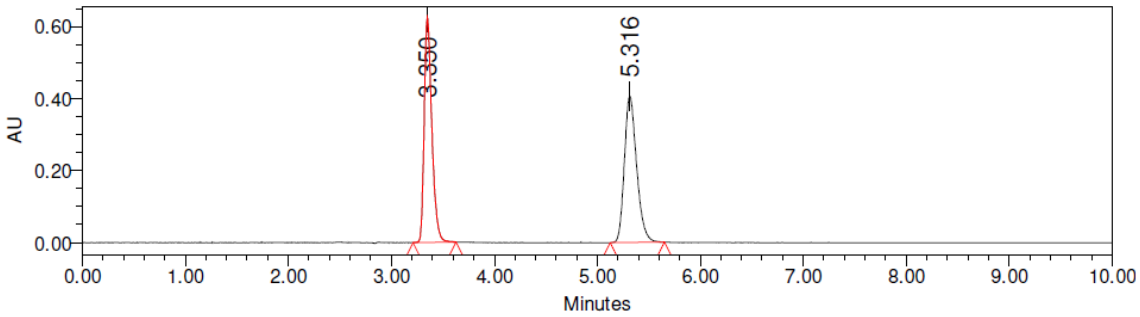
Analysis of codeine phosphate and acetaminophen in tablet

10 microliters of tablet sample for analysis is prepared and is infused to HPLC system. Chromatograms and peak response of acetaminophen and codeine phosphate were noted. Content of codeine phosphate and acetaminophen was determined by using the peak response data.

REULTS

Method development conditions

Mobile phase: NaH_2PO_4: ACETONITRILE (50:50)
Column : WATERS, C18, 250×4.6mm,5µm
Flow rate : 1.0ml/min
Temperature : 25°c
Volume : 10 µl
Run time : 10 min
Detector : 228
pH : 3.5



Retention Time	Area	% Area	USP Resolution	USP Tailing	USP Plate Count
3.350	3222089	48.96	-	1.39	9810
5.316	3359632	51.04	10.90	1.26	9627

Validation

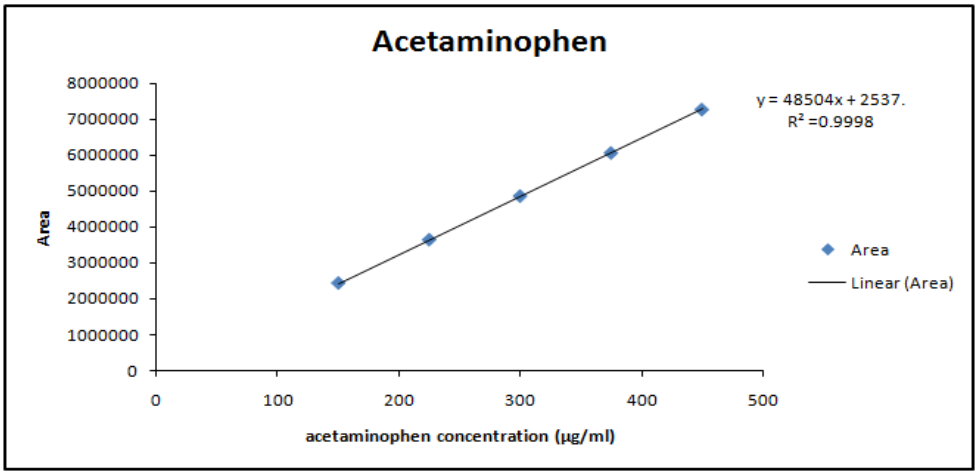
Linearity

Linearity for acetaminophen and codeine phosphate was examined between the range from 150 µg/ml to 450 µg/ml and 30µg/ml to 90 µg/ml. The made five dissimilar concentration solution solutions were infuse to HPLC column.

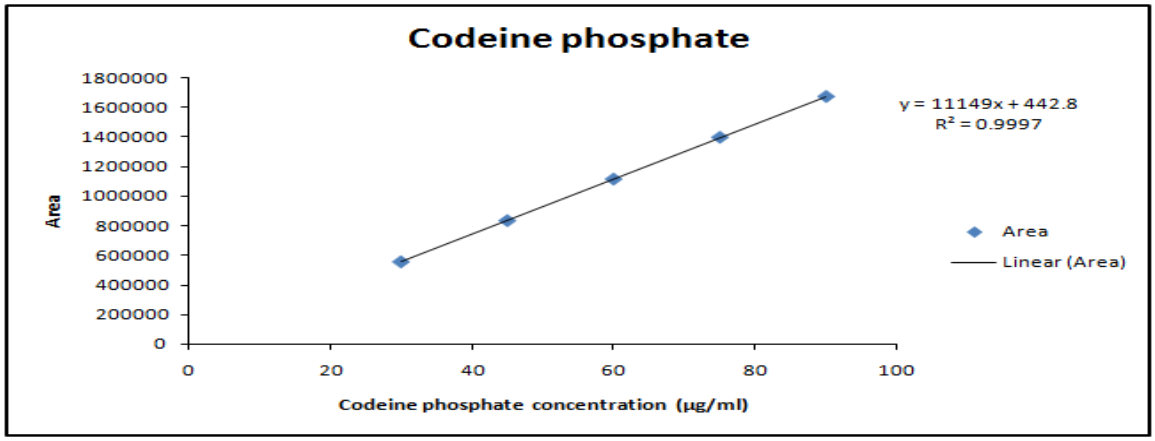
Acetaminophen area response	Acetaminophen in µg/ml conc.	Codeine phosphate area response	Codeine phosphate in µg/ml conc.
2426888	150	557912	30
3638921	225	835834	45
4857571	300	1115288	60
6064280	375	1396662	75
7277269	450	1671158	90

Regression equation:
For acetaminophen:
$y = 48504\,x + 2537$
$R^2 = 0.9998$

For codeine phosphate:
$y = 11149\,x + 442.8$
$R^2 = 0.9997$



Acetaminophen linearity

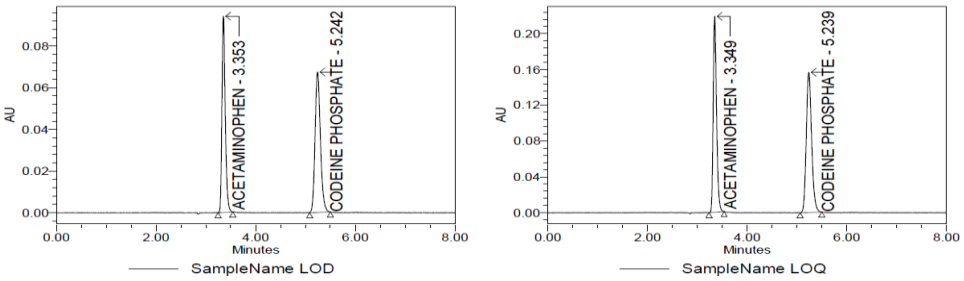


CODEINE PHOSPHATE LINEARITY

Limit of detection and limit of quatitation

To establish detection and quantitation limits, the signal to noise technique was utilized. The concentration of acetaminophen and codeine phosphate giving signal to noise ratio of 3 value could be identified as their LOD and 10 value could be identified as their LOQ.

Acetaminophen LOD - 0.831 µg / ml
LOQ - 2.770 µg / ml
Codeine phosphate LOD - 0.252 µg /ml
LOQ - 0.842 µg /ml.



Peak Name: CODEINE PHOSPHATE

	SampleName	Peak Name	RT	Area	s/n
1	LOD	CODEINE PHOSPHATE	5.242	1259526	3.77
2	LOQ	CODEINE PHOSPHATE	5.239	1451429	10.05

Peak Name: ACETAMINOPHEN

	SampleName	Peak Name	RT	Area	s/n
1	LOD	ACETAMINOPHEN	3.353	1091035	3.24
2	LOQ	ACETAMINOPHEN	3.349	1271651	10.79

Accuracy

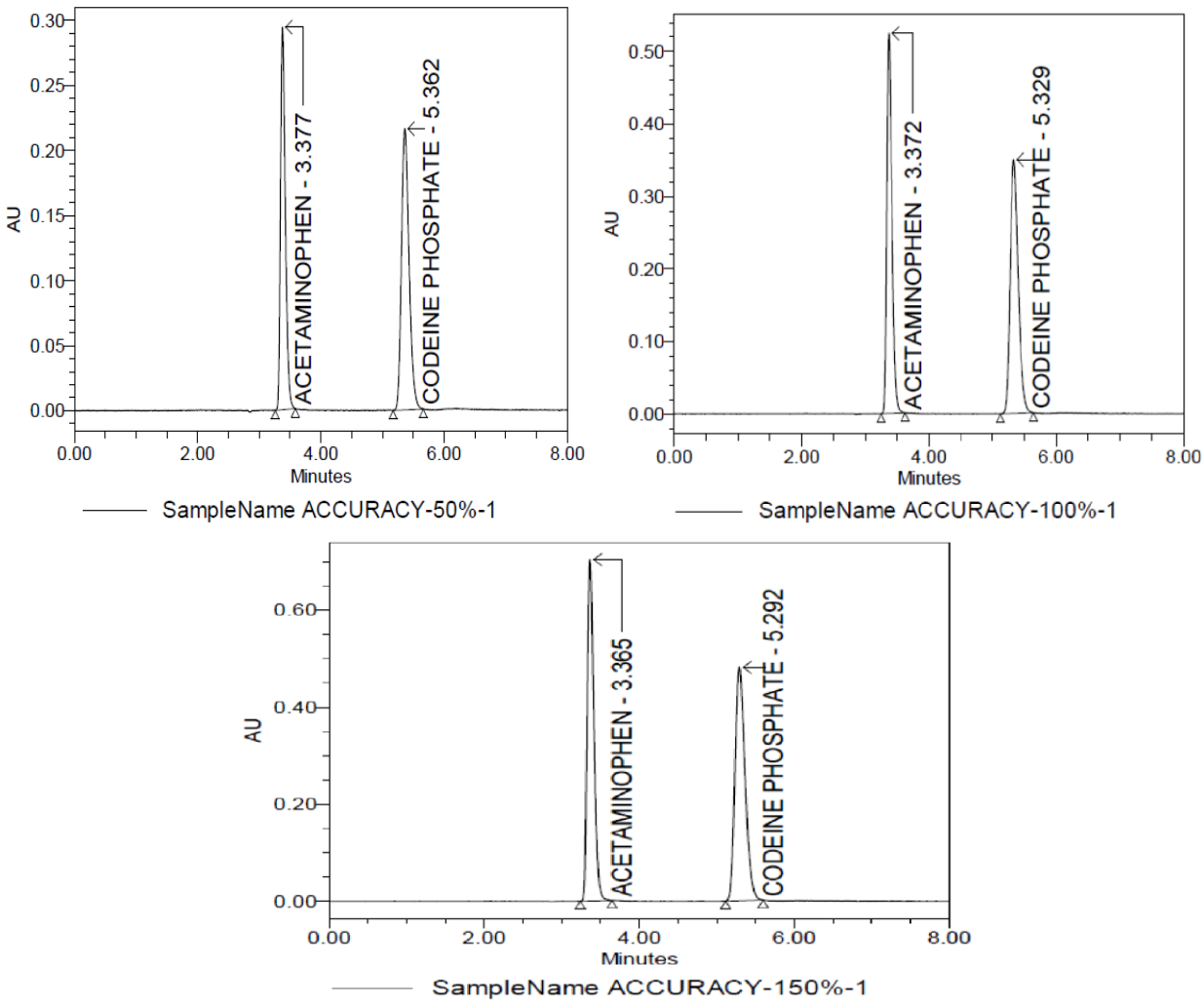
Three samples were prepared at 50%, 100%, and 150% concentration of target test solution by spiking acetaminophen and codeine phosphate standard to tablet solution .The solutions are injects to HPLC column. Calculated the recovered percentage of acetaminophen and codeine phosphate at 50%, 100% and 150% concentration levels.

Area response	µg/ml Conc. spiked	µg/ml Conc. determined	Percent Recover
2422031	150	149.43	99.62
2427451	150	149.77	99.84
2421126	150	149.38	99.58
4856543	300	299.63	99.88
4851560	300	299.33	99.78
4851872	300	299.35	99.78
7279149	450	449.10	99.80
7278892	450	449.09	99.80
7270596	450	448.57	99.68

Acetaminophen accuracy results

Area response	µg/ml Conc. spiked	µg/ml Conc. determined	Percent Recover
557647	30	29.90	99.68
557577	30	29.90	99.66
557254	30	29.88	99.61
1110830	60	59.57	99.28
1113320	60	59.70	99.50
1114393	60	59.76	99.60
1677273	90	89.94	99.94
1677619	90	89.96	99.96
1678429	90	90.00	100.00

Codeine phosphate accuracy



Precision

Six standard solutions were made and inject to HPLC column. Calculated the RSD percentage for peak response of acetaminophen and codeine phosphate .RSD percent was less than 2.0%.

RESULTS OF PRECISION

Solution	Acetaminophen peak response	Codeine phosphate peak response
1	4851947	1111980
2	4852730	1114268
3	4859940	1114734
4	4858078	1113377
5	4859673	1119315
6	4853345	1117554
Average	4855952	1115205
SD	3673.898	2729.539
RSD	0.076	0.245

CONCLUSION

HPLC approach established in current research is easy, accurate, reliable, specific and economical to analyze acetaminophen and codeine phosphate. The validated strategy exhibits adequate results for all the parameters studied. This developed procedure can therefore be employed in pharmaceutical formulations for intent of quality control.

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