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"Analytical Method Development And Validation OfSimultaneous Estimation For Ibuprofen And Camylofin Dihydrochloride By Using RP-HPLC"

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ABSTRACT
Objective: A simple, precise and accurate reversed phase high performance liquid chromatography (RP-HPLC) method has been developed and subsequently validated for the simultaneous estimation of Ibuprofen and Camylofin dihydrochloride

Methods: The adequate separation was carried out using SystemConsistingofSunniest,C184.6 mmx 150cm, 5 µcolumn mixture of Acetonitrile and Water adjusted OPA60:40 % v/v as mobile phase of an flow rate of 2.0 ml/minand the effluent was monitored at 260 nm.The retention time of Ibuprofen and Camylofin 2HCl were 4.933 min and 2.825min respectively.

Results: Linearity for Nebivolol HCl and Cilnidipine were found in the range of 20- 150 ppm and 5- 37.5 µg/ml (R2 = 0.9992) respectively. The accuracy of the present method was evaluated at 50%, 100% and 150%. The % recoveries of both drugs were found to be in range of notmorethan 110.0% and notlessthan 90.0% for Ibuprofen and Camylofin 2HCl respectively. Precision studies were carried out and the RSD values were less than two.The Robustnessof tailing factor for Ibuprofen & Camlofin Dihydrochloride was found to be withinthelimits 1.279-1.300 & 2.112 -2.346.
Conclusions: The proposed method was found to be specific, accurate, precise and robust can be used for simultaneous estimation of the Ibuprofen and Camylofin dihydrochloride.
Keywords: Ibuprofen, Camylofin 2HCl, Reversed phase HPLC, Validation

I. INTRODUCTION

HIGH PERFORMANCE LIQUID CHROMATOGRAPHY-

- High performance liquid chromatography is convenient separation technique used for wide types of samples, exceptional resolving power, speed and nano molecular detection levels.
- It is presently used in pharmaceuticals research and development.
- High-performance liquid chromatography (HPLC) is a chromatographic technique that can separate a mixture of compounds and is used in biochemistry and analytical chemistry to identify, quantify and purify the individual components of the mixture.
- Reversed phase chromatography has found both analytical and preparative applications in the area of biochemical separation and purification

Ibuprofen is named chemically as (2RS)-2-[4-(2-methylpropyl] Propanoic acid (Fig.1) is a class of NSID's.
Ibuprofen is the most commonly used and most frequently prescribed NSAID. Cyclooxygenase (COX), which is required for the synthesis of prostaglandins through the arachidonic acid pathway, converts the arachidonic acid to prostaglandin H2 in the body.[(1,2)].

Ibuprofen is supplied as tablets with a potency of 200 to 800 mg. The usual dose is 400 to 800 mg three times a day. It is almost insoluble in water having pKa of 5.3.8 It is well absorbed orally; peak serum concentrations are attained in 1 to 2 hours after oral administration[(2,3,4)].

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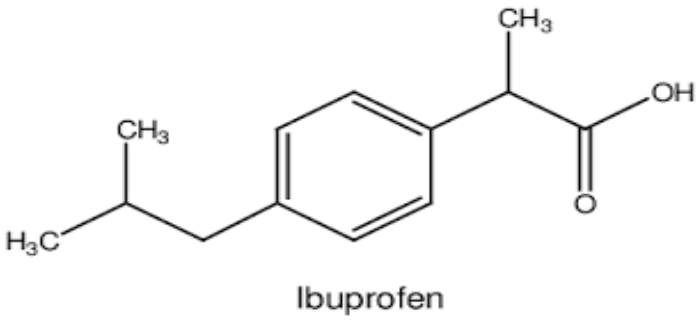

Fig. 1 Structure of Ibuprofen

Camylofin Dihydrochloride 3- methylbutyl 2- (2-diethyl- aminoethylamino)- 2- phenylacetate hydr ochloride (C19H 32N 2O 2,2HCl) Fig 2 is a drug used as an antispasmodic.
Camylofin is a smooth muscle relaxant with both anticholinergic action as well as direct smooth muscle action.
Anticholinergic action is produced by inhibiting the binding of acetylcholine to muscarinic receptors, but the action is less pronounced[5,6]

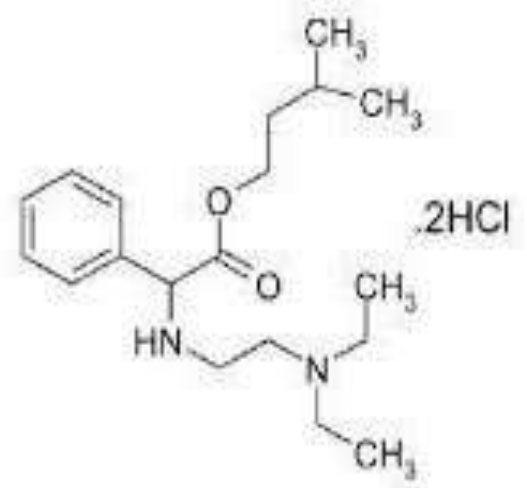

Fig.2 Camylofin dihydrochloride

II. MATERIALS AND METHODS

Equipment's

HPLC– Scimadzu Model LC-2030 PLUS (IND) System Consisting of Sunniest, C18 4.6 mm x 150 cm, 5 µcolumn.,Electronic balance (MettlerToledo),Sonicator (Pci-Analytics),λmax 255nm

Reagents and materials

Ibuprofen and Camylofin 2HCL were obtained as gift samples from Sigma Pharmaceuticals Pvt. Ltd. Goa., Acetonitrile HPLCGrade, Methanol HPLCGrade, Ortho phosphoricAcid, HPLC GradeH_2O

Chromatographic Condition

Flowrate:2.0ml/min, Column:Sunniest,C18 4.6mmx150 cm,5µcolumn, Detectorwavelength:255nm, Columntemp:25ºC, Injectionvolume:10µl, Runtime:8min

Preparation of MobilePhase

H_2O 340ml, Acetonitrile600 mland OPA0.5ml weremixedanddiluteunto1000mlwithH_2O.

PreparationofStandardSolution CamylofinDihydrochloride&IbuprofenStandard Stock:

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50.1 mg Camylofin Dihydrochloride Standard and 200.2mg Ibuprofen was weighed and dissolved in100mlwithDiluent.

METHODVALIDATION

1 SYSTEMSUITABILITY

A Standard solution was prepared by using Camylofin Dihydrochloride 2 µg/ml& Ibuprofen 500 µg/mlworking standard as Pretest method and was injected Five times into the HPLCsystem.

The system suitability parameters were evaluated from standard chromatograms by calculating the % RSD from five replicate injections for Camylofin Dihydrochloride & Ibuprofen, retention times and peak areas as the results shown in Table 1&2 were within acceptable limits.

2 SPECIFICITY

Solutions of standard and sample were prepared as per the test method are injected into chromatographic system.Retention rime of Camylofin 2.783 and Ibuprofen4.958refer as table 3 & 4.

3 PRECISION:

3.1 Repeatability:

a. System precision: Standard solution prepared as per test method and injected 2 µg/ml and 500 µg/ml fivetimes table 5 & 6.

b. Method precision: Prepared six sample preparations individually using single as pertest method and injected each solution table 7 & 8.

The % relative standard deviation of individual Camlofin Dihydrochloride &Ibuprofen from the six units should be not more than2.0%.

The assay of Camylofin Dihydrochloride & Ibuprofen should be not less than 90.0 % andnot more than110.0%.Testresultsareshowingthatthetestmethod isprecise.

4 ACCURACY(RECOVERY)

A study of Accuracy was conducted. Drug Assay was performed in duplicate as per test method with equivalent amount of Camylofin Dihydrochloride & Ibuprofen into each volumetric flask for each spike level to get the concentration of Camlofin Dihydrochloride & Ibuprofen equivalent to 25%, 50%, 75%, 100% 125% and 150% of the labeled amount as per the test method.

The mean % recovery of the Camylofin Dihydrochloride & Ibuprofen at each spike level should be not less than 90.0% and not more than 110.0%.

There recovery results indicating that the test method has an acceptable level of accuracy refer table: 9 & 10.

5 LINEARITY OF TESTMETHOD

A Series of solutions are prepare using Camylofin Dihydrochloride & Ibuprofen working standard at concentration levels for Camlofin Dihydrochloride from 5 ppm to 37.5 ppm of target concentration & concentration levels for Ibuprofen from 20 ppm to 150 ppm of target concentration.Correlation Coefficient should be not less than0.9990.% of y-Intercept should be±2.0.

The line are fit of the system was illustrated graphically. The results are presented in table 11& 12.

6 ROBUSTNESS

a. Effect of variation of flowrate:

A study was conducted to determine the effect to variation in flow rate. Standard solution prepare as per the test method was injected in to the HPLC system using flow rates 1.0 mL /min,1.2 mL/min and 1.4 mL/min. The system suitability parameters were evaluated and found to be within the limits for 1.0 mL /min, 1.2 mL/min and 1.4 mL/min flow.

Camylofin Dihydrochloride & Ibuprofen was resolved from all other peaks and there retention times were comparable with those obtained for mobile phase having flow rates 1.2ml/min.

The tailing factor for Camlofin Dihydrochloride & Ibuprofen was found to be within the limits. As shown in table 13 & 14.

b. Effect of change on mobile phaseComposition:

Camylofin Dihydrochloride & Ibuprofen was resolved from all other peaks and there retention times were comparable with those obtained for mobile phase having Composition H_2O:ACN:OPA (34:60:0.5).

The Tailing Factor of Camylofin Dihydrochloride standards should be NMT 3.0 & Ibuprofen standards should be NMT 2.0 for Variation in Composition.

The tailing factor for Camylofin Dihydrochloride & Ibuprofen was found to be within the limits. As shown in table 15 & 16

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7 LIMIT OF DETECTION AND QUANTITATION (LOD andLOQ):

From the linearity data calculate the limit of detection and quantitation, using the following formula.

$$LOD = \frac{3.3\sigma}{S}$$

σ = standard deviation of the response
S=slope of the calibration curve of the analyte.

$$LOQ = \frac{10\sigma}{S}$$

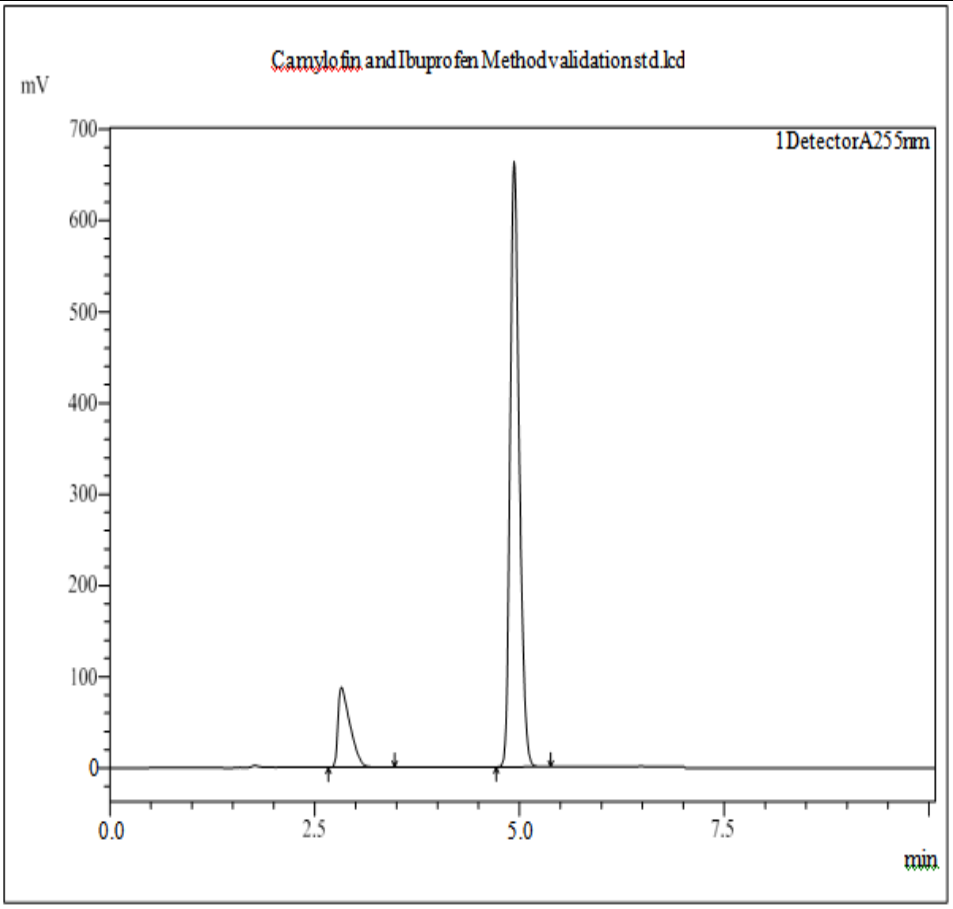
σ = standard deviation of the response
S = slope of the calibration curve of the analysis

III. RESULT AND DISCUSSION

Methoddevelopment:

(TABLE : Optimized Method)

S.NO	Name of the peak	Retention time(min)
1.	**Ibuprofen Standard**	4.933
2.	**Camylofin Standard**	2.825



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Peak No.	Ret. Time	Name	Area	Area%	Tailing Factor	Theoretical Plates
1	2.825	Camylofin	889466	14.709	2.240	10344
2	4.933	Ibuprofen	5157751	85.291	1.264	56265
Total			6047218	100.000		

1 SystemSuitability

TABLE-1:Dataof CamylofinSystemSuitability

	Injection	RT ofCamylofin	Peak area ofCamylofin	TheoreticalPlates	TailingFactor
2 ppmConcentration	1	2.783	2581788	9311	2.339
	2	2.783	2583091	9302	2.333
	3	2.783	2584043	9307	2.337
	4	2.783	2584352	9313	2.349
	5	2.783	2586409	9320	2.364
StatisticalAnalysis	**Mean**	**2.783**	**2583937**	**9311**	**2.344**
	SD	**0.000**	**1705.004**		
	%RSD	**0.00**	**0.07**		

TABLE-2:DataofIbuprofenSystemSuitability

	Injection	RT ofIbuprofen	Peak area ofIbuprofen	TheoreticalPlates	TailingFactor
500 ppmConcentration	1	4.958	5041218	55080	1.304
	2	4.958	5042735	55071	1.300
	3	4.958	5044190	55130	1.297
	4	4.958	5044748	55147	1.296
	5	4.958	5046996	55198	1.304
StatisticalAnalysis	**Mean**	**4.958**	**5043977**	**55125**	**1.300**
	SD	**0.000**	**2174.213**		
46	**%RSD**	**0.00**	**0.04**		

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2Specificity

Table 3 Camylofinstandardat anRT of2.783

S.NO	Nameofthepeak	Retentiontime(min)
1.	Camylofin	2.783

Table 4 Ibuprofenstandardat anRTof4.958

S.NO	Nameofthepeak	Retentiontime(min)
1.	Ibuprofen	4.958

3 Precision:

(a) SystemPrecision:

TABLE-5DataofRepeatability(Systemprecision)

	Injection	RTofCamylofin	PeakareaofCamylofin
2 ppmConcentration	1	2.792	2588498
	2	2.792	2588956
	3	2.792	2589943
	4	2.792	2589677
	5	2.792	2590092
StatisticalAnalysis	**Mean**	**2.792**	**2589433**
	SD	**0.000**	**681.115**
	%RSD	**0.00**	**0.03**

TABLE-6DataofRepeatability(Systemprecision)

	Injection	RTofIbuprofen	PeakareaofIbupro fen
500 ppmConcentration	1	4.958	5050143
	2	4.958	5049508
	3	4.967	5052284
	4	4.967	5052472
	5	4.967	5052131

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StatisticalAnalysis	**Mean**	**4.963**	**5051308**
	SD	**0.005**	**1386.774**
	%RSD	**0.10**	**0.03**

b) MethodPrecision:

TABLE-7:DataofRepeatability(Methodprecision)ofCamylofin

2 ppmConcentration	**Injection**	**Peak area ofCamylofin**	**%Assay**	**Average %Assay**
	1	2599591	101.64	101.61
		2599785	101.58	
	2	2622278	101.00	101.38
		2598053	101.75	
	3	2632514	102.47	101.78
		2605704	101.09	
	4	2656012	102.76	102.16
		2607831	101.56	
	5	2654361	102.73	102.72
		2634858	102.71	
	6	2674825	103.10	103.02
		2647581	102.94	
StatisticalAnalysis	**Mean**	**2627783**	**102.11**	**102.11**
	SD	**26214.213**	**0.747**	**0.647**
	%RSD	**1.00**	**0.73**	**0.63**

TABLE-8:DataofRepeatability(Methodprecision)ofIbuprofen

500 ppmConcentration	**Injection**	**Peak area ofIbuprofen**	**%Assay**	**Average %Assay**
	1	5073149	101.85	101.82
		5073392	101.79	
	2	5188439	102.62	102.30
		5071404	101.98	
	3	5139671	102.73	102.03
		5086410	101.33	
	4	5212093	103.54	102.67
		5090535	101.79	

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	5	5174152	102.82	102.38
		5092475	101.93	
	6	5234856	103.60	102.70
		5098745	101.79	
StatisticalAnalysis	**Mean**	**5127943**	**102.31**	**102.31**
	SD	**59432.084**	**0.735**	**0.347**
	%RSD	**1.16**	**0.72**	**0.34**

4 ACCURACY(RECOVERY)

TABLE-9:DataofAccuracyforCamylofin

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Concentration % of spikedlevel	**Area**	**Amountadded(ppm)**	**Amountfound(ppm)**	**% Recovery**	**Statistical Analysisof % Recovery(Mean)**
25 % Sample1	3220718	2.50	2.48	99.25	**99.35**
25 % Sample2	3226707	2.50	2.49	99.43	
50 % Sample1	3870850	3.00	2.98	99.40	**99.94**
50 % Sample2	3867451	3.00	2.98	99.32	
75 % Sample1	4564498	3.50	3.52	100.47	**100.07**
75 % Sample2	4568793	3.50	3.52	100.56	
100% Sample1	5170949	4.00	3.98	99.59	**99.59**
100%Sample2	5173739	4.00	3.99	99.65	
125% Sample1	5816188	4.50	4.48	99.57	**100.13**
125% Sample2	5815102	4.50	4.48	99.55	
150%Sample1	6534755	5.00	5.03	100.69	**100.70**
150%Sample2	6536968	5.00	5.04	100.72	

TABLE-10:DataofAccuracyforIbuprofen

Concentration % of spikedlevel	**Area**	**Amountadded(ppm)**	**Amountfound(ppm)**	**% Recovery**	**Statistical Analysisof % Recovery(Mean)**

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25%Sample1	6314150	625	6.23.15	99.70	**99.79**
25 % Sample2	6325511	625	624.27	99.88	
50%Sample1	7629727	750	752.98	100.40	**100.28**
50%Sample2	7611273	750	751.16	100.15	
75%Sample1	8820627	875	870.51	99.49	**99.56**
75%Sample2	8834185	875	871.85	99.64	
100%Sample1	10055950	1000	992.43	99.24	**99.25**
100%Sample2	10057486	1000	992.58	99.26	
125%Sample1	11357498	1125	1120.88	99.63	**99.64**
125%Sample2	11358155	1125	1120.97	99.64	
150%Sample1	12672429	1250	1250.65	100.05	**100.07**
150%Sample2	12676441	1250	1251.05	100.08	

5 LINEARITY

TABLE11:Dataof LinearityforCamylofin

Concentration(ppm)	**Area**	**StatisticalAnalysis**
0	**0**	**Linearity Equation=Y= 10101x+35464** **CorrelationCoefficient=0.999**
5	**281834**	
10	**569242**	
15	**810330**	
20	**1099566**	
25	**1316834**	
37.5	**1603789**	

TABLE12:DataofLinearity forIbuprofen

Concentration(ppm)	**Area**	**StatisticalAnalysis**
0	**0**	**Linearity Equation=Y=10253x+21075** **CorrelationCoefficient=0.9992**
20	**281834**	
40	**569242**	
60	**810330**	
80	**1099566**	
100	**1316834**	
150	**1603789**	

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6ROBUSTNESS

a) Changeinmobilephaseflowrate:

TABLE13:DataforEffectofvariationinflowratefor Camylofin:

	StdArea	Tailingactor		StdArea	Tailingfactor		StdArea	Tailingfactor
Flow 1.0ml	3108224	2.347	**Flow 1.2ml**	2581788	2.339	**Flow 1.4ml**	2236716	2.068
	3107902	2.344		2583091	2.333		2236530	2.257
	3114795	2.380		2584043	2.337		2237653	2.086
	3115918	2.286		2584352	2.349		2238616	2.070
	3.116855	2.373		2586409	2.364		2238367	2.081
Avg	**3111710**	**2.346**	**Avg**	**2583937**	**2.344**	**Avg**	**2237576**	**2.112**
SD	**4237.828**		**SD**	**705.004**		**SD**	**941.664**	
%RSD	**0.14**		**%RSD**	**0.07**		**%RSD**	**0.04**	

TABLE14:DataforEffectofvariationinflowratefor Ibuprofen:

	StdArea	Tailingactor		StdArea	Tailingfactor		StdArea	Tailingfactor
Flow 1.0ml	6080160	1.310	**Flow 1.2ml**	5041218	1.304	**Flow 1.4ml**	4371300	1.248
	6077575	1.278		5042735	1.300		4380986	1.309
	6085898	1.261		5044190	1.297		4369981	1.259
	6081688	1.291		5044748	1.296		4373398	1.243
	6082038	1.255		5046996	1.304		4371026	1.249
Avg	**6081472**	**1.279**	**Avg**	**5043977**	**1.300**	**Avg**	**4373338**	**1.262**
SD	**3035.805**		**SD**	**174.213**		**SD**	**4451.509**	
%RSD	**0.05**		**%RSD**	**0.04**		**%RSD**	**0.10**	

b) ChangeinmobilephaseComposition

TABLE15:DataforEffectofvariationinMobilePhaseCompositionforCamylofin:

	StdArea	Tailingactor		StdArea	Tailingfactor		StdArea	Tailingfactor
Buffer:ACN(60:40)	3459775	2.321	**Buffer: ACN(50:50)**	2581788	2.339	**Buffer:ACN(40:60)**	2091673	2.207
	3458818	2.333		2583091	2.333		2091183	2.212
	3456586	2.330		2584043	2.337		2093753	2.212
	3456164	2.319		2584352	2.349		2095152	2.221
	3457200	2.314		2586409	2.364		2093109	2.214
Avg	**3457709**	**2.323**	**Avg**	**2583937**	**2.344**	**Avg**	**2092974**	**2.213**
SD	**1533.417**		**SD**	**705.004**		**SD**	**1602.267**	

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%RSD	**0.04**		**%RSD**	**0.07**		**%RSD**	**0.08**	

TABLE16:DataforEffectofvariationinMobilePhaseCompositionforIbuprofen:

	StdArea	**Tailingactor**		**StdArea**	**Tailingfactor**		**StdArea**	**Tailing factor**
Buffer:ACN(60:40)	6749675	1.291	**Buffer : ACN(50:50)**	5041218	1.304	**Buffer:ACN(40:60)**	4085543	1.283
	6754944	1.299		5042735	1.300		4085006	1.264
	6755559	1.291		5044190	1.297		4085369	1.284
	6755874	1.287		5044748	1.296		4088017	1.293
	6748707	1.286		5046996	1.304		4087703	1.281
Avg	**6752952**	**1.291**	**Avg**	**5043977**	**1.300**	**Avg**	**4086328**	**1.281**
SD	**3466.315**		**SD**	**174.213**		**SD**	**1416.592**	
%RSD	**0.05**		**%RSD**	**0.04**		**%RSD**	**0.03**	

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