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RP-HPLC Method Development and Validation of Etodolac and Paracetamol in Tablet Dosage Form

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Abstract: A simple, rapid, selective, reverse phase high performance liquid chromatography (RP-HPLC) was developed for simultaneous estimation of etodolac and paracetamol in its tablet dosage form. The separation was carried out using a mobile phase of methanol and ammonium acetate buffer (adjusted to pH 8.0 by ammonium hydroxide) in the ratio of 80:20 (v/v) pumped at a flow rate of l ml min^{-1} along with 230 nm as a UV detection wavelength. The stationary phase used was C_{18} column (symmetry of 250× 4.6 mm, 5µm). Paracetamol and etodolac were eluted at a retention time of 2.81 min and 5.36 min, respectively. The method was developed and validated as per ICH Q2(R1) guidelines by considering the parameters such as specificity, linearity, accuracy, precision and robustness. The developed RP-HPLC method can be used for routine analysis of paracetamol and etodolac in combinational dosage form.

Key words: Etodolac, Paracetamol, RP-HPLC method, Validation.

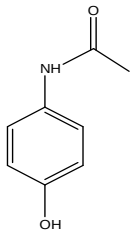
INTRODUCTION

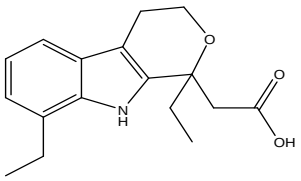
Etodolac is a non-steroidal anti-inflammatory agent (NSAIDs). It is a white crystalline compound insoluble in water but soluble in alcohols and chloroform. Chemically etodolac is 2-{1,8-diethyl-1H,3H,4H,9H-pyrano[3,4-b]indol-1-yl}acetic acid. It is used as anti-inflammatory agent, cyclooxygenase inhibitor, analgesic and anti-pyretic. Etodolac is official in the British Pharmacopoeia and United States Pharmacopoeia.[1-4]

Paracetamol also belongs to the class of NSAIDs and more commonly used as an analgesic and antipyretic agent. It is a white crystalline powder sparingly soluble in water but freely soluble in ethanol and acetone. Chemically paracetamol is N-(4-hydroxyphenyl) acetamide. It is official in Indian Pharmacopoeia [5] and British Pharmacopoeia [6].

The etodolac is used for treatment of osteoarthritis and rheumatoid arthritis. The combination of etodolac with paracetamol is more effective for treating osteoarthritis than etodolac alone. [7]

Based on the literature survey there are few reports on simultaneous estimation of paracetamol and etodolac using UV spectroscopy [8], RP-HPLC [9-10] etc. Hence, the integral aim of our work was to develop a simple, rapid, accurate method for simultaneous estimation of etodolac and paracetamol in tablet dosage form by RP-HPLC which can be routinely used in laboratories.





Etodolac **Paracetamol**

EXPERIMENTAL

Reagents and Chemicals

The drug samples etodolac and paracetamol were obtained from the Lara Drugs limited, Hyderabad, Andhra Pradesh, India. The combination of etodolac and paracetamol in tablet dosage form (i.e., ETOVA-P) manufactured by IPCA Pharmaceuticals Ltd. were purchased from the local market. HPLC grade methanol and ammonium acetate were procured from Rankem India Ltd, while, Milli-Q water was used throughout the analysis.

Instrumentation

The analysis was performed using the Waters HPLC e2695 separation module (Milford, MA, USA). A PDA detector is used for detection of the anayltes. Inertsil ODS (GOD, Hungary) with 250mm×4.6mm (ID), C_{18} reversed phase material of 5µm size was used for the separation studies. The HPLC system was supported by empower version 2 software. The other instruments used were weight balance (Sartorius Ltd, Goettingen, Germany), Sartorius digital pH meter (Sartorius Ltd, Goettingen, Germany) and water bath sonicator (PCI Analytics Pvt. Ltd, Mumbai, India).

Chromatographic Conditions

The separation was achieved by using mobile phase methanol and ammonium acetate in the ratio of 80:20 (v/v) with pH adjusted to 8.0 at a flow rate of 1ml min^{-1} through C_{18} Inertsil ODS column (250mm × 4.6mm, 5µm). The eluted material from column was monitored at 230 nm wavelength and column temperature was 30˚C. The injection volume was 1µl.

Preparation of 0.2M Ammonium acetate buffer

Ammonium acetate was prepared by dissolving 77.08 mg in water and the volume was made up to 500 ml. The pH of the buffer was adjusted to 8.0 using ammonium hydroxide.

Preparation of Stock Solution

325 mg of Paracetamol and 400 mg of Etodolac, was transferred to 50 ml volumetric flask containing methanol. Then an amount of 5 ml was transferred to 25 ml volumetric flask in which water was added to make up the volume.

Preparation of sample solution

An average weight of the 20 tablets was found to be 989.65 mg. The tablets were then crushed from which 989.65 mg was collected and transferred to 50 ml volumetric flask containing methanol and finally the volume was made up by methanol. Then the amount of 5 ml was transferred to 25 ml volumetric flask in which the volume was made up with water.

HPLC Method Development and Validation

The detection wavelength for etodolac and paracetamol was 230 nm, hence it was thought worthwhile to develop a simple and cost effective LC method by using methanol instead of acetonitrile. Various trials were conducted to achieve an adequate separation of both the drugs. The separation was tried using different

proportions of MeOH (A) and ammonium acetate buffer (0.2 M) (B) and also by varying the pH of buffer with ammonium hydroxide.

The developed method was validated with respect to various parameters outlined in the ICH guidelines Q2(R1).

Table 1: System suitability parameters

Parameter	Paracetamol	Etodolac
Linearity range (µg ml^{-1})	650-1959	800-2400
Correlation coefficient	0.9992	0.9994
Slope	5660.3	3678.6
Retention time (min)	2.817	5.366
Resolution factor	-	14.09
Tailing factor	1.20	1.6
USP Plate count	7926	8241

RESULTS AND DISCUSSION

To develop a precise, accurate and suitable RP-HPLC method for the estimation of etodolac and paracetamol, where different mobile phases and columns conditions were tried and proposed chromatographic conditions were found to be appropriate. System suitability parameters are given in Table 1. The paracetamol and etodolac were eluted at 2.817 min and 5.366 min, respectively. The chromatogram showing the separation of paracetamol and etodolac is shown in Fig. 3.

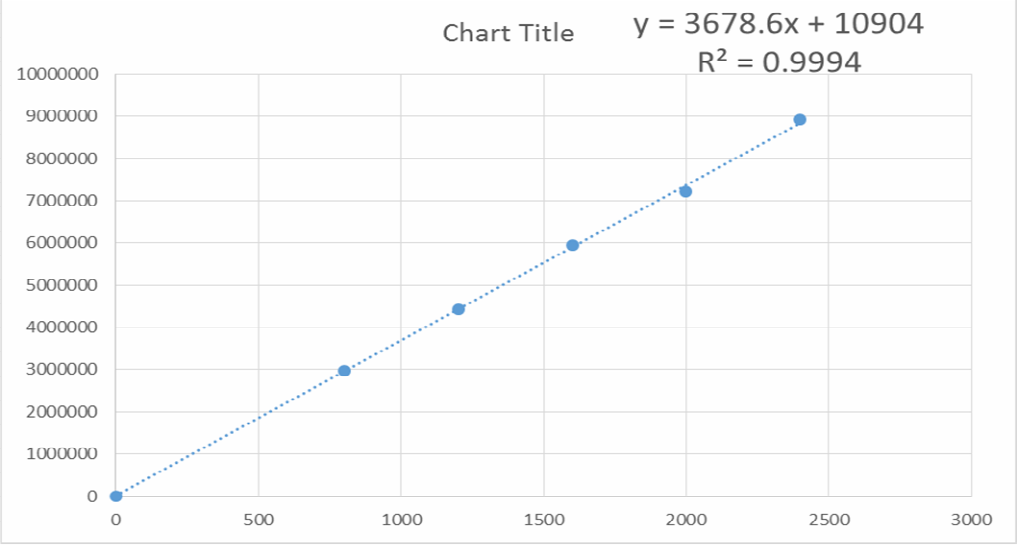


Fig 2: Linearity Graph of Etodolac

Table 2: Linearity observations of Etodolac

Correlation Co-efficient (R^2)	**0.9994**
Slope (m)	**3678.6**
Intercept (b)	**10904**

Table 3: Linearity of Etodolac

Sr. no	Sample name	Injection	µg ml^{-1}	Name	Rt	Area
1	Linearity 50%	1	800	Etodolac	5.386	2974809
2	Linearity 75%	1	1200	Etodolac	5.389	4435514
3	Linearity 100%	1	1600	Etodolac	5.401	5945142
4	Linearity 125%	1	2000	Etodolac	5.400	7222000
5	Linearity 150%	1	2400	Etodolac	5.406	8917025

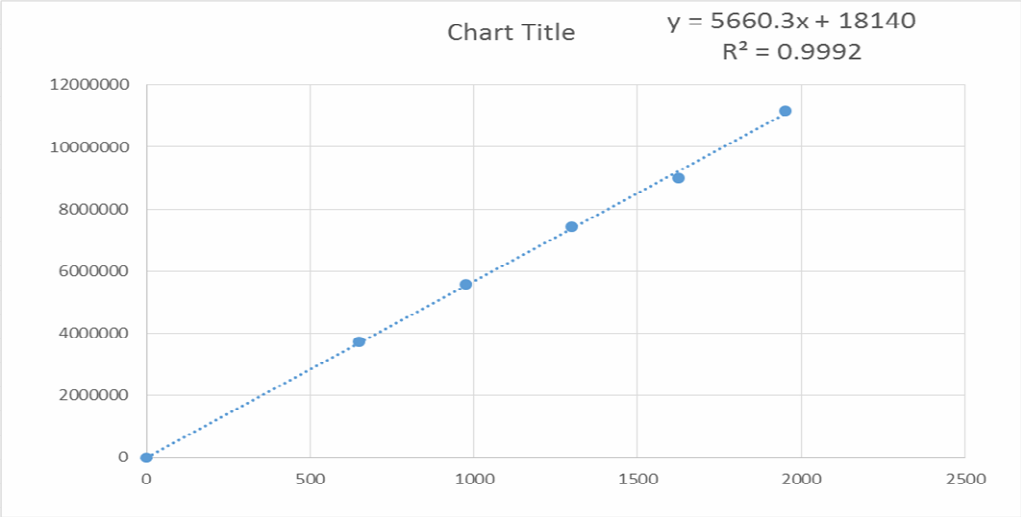


Fig 2: Linearity Graph of Paracetamol

Table 4: Linearity observations of Paracetamol

Correlation Co-efficient (R^2)	**0.9992**
Slope (m)	**5660.3**
Intercept (b)	**18140**

Table 5: Linearity of Paracetamol

Sr. no	Sample name	Injection	µg ml^{-1}	Name	Rt	Area
1	Linearity 50%	1	650	Paracetamol	2.825	3722380
2	Linearity 75%	1	975	Paracetamol	2.829	5565540
3	Linearity 100%	1	1300	Paracetamol	2.832	7443021
4	Linearity 125%	1	1625	Paracetamol	2.831	9006440
5	Linearity 150%	1	1950	Paracetamol	2.835	11163714

METHOD VALIDATION

The developed method was further validated as per ICH guidelines. [11]

Specificity

The peak purity of etodolac and paracetamol were assessed by comparing the retention time of standard etodolac and paracetamol. The co-relation obtained between the sample and standard retention times were good.

Linearity

Linearity was studied by preparing standard solutions at different concentration levels. The linearity was observed at 650-1950 µg ml $^{-1}$ for paracetamol and 800-2400 µg ml^{-1} for etodolac. The regression equation of paracetamol was found to be y = 5660.3x + 18140 and for etodolac it was found out to be y = 3678.6 x + 10904 with a coefficient correlation of 0.9992 and 0.9994.

Accuracy

Accuracy of the method is checked by performing recovery studies in triplicate by standard addition method. A known amount of standard solution was added to pre-analysed samples at three levels of 50%, 100%, 150%. At each levels the recovery studies were carried out and expressed as percent recoveries. The results of accuracy are shown in Table no 7 and 8.

Precision

The precision was evaluated using 100 percent concentration of the sample solution injecting six independent samples from above single formulation. Percentage relative standard deviation was found to be less than 2% which assures method is precise. The results of Precision are shown in Table no 9.

Robustness

The Robustness of developed method was checked by deliberately changing the conditions of an optimized method. The effect of change in flow rate and temperature were studied. Six replicate injections were given and effects of the variations were observed and % RSD was calculated. The results of robustness are shown in Table no 10, 11.

Robustness parameters:

Flow Rate: (-20%) 0.8 ml $^{-1}$ min, (+20%) 1.2 ml min^{-1}.

Temperature: 25˚C and 35˚.

Table 6: Results of analysis of tablet formulation

Parameters	Paracetamol	Etodolac
% estimated	100.08	100.07
Standard deviation	3059.50	2178.93
% RSD	0.041	0.036

Table 7: Results of Accuracy study for Etodolac

Levels	Sets	Area	Drug Added(mcg)	Drug Recovered(mcg)	% Recovery	% Mean Recovery	% RSD
50%	1	2977703	797.88	789.90	99		
50%	2	2970494	797.88	793.09	99.4	99.8	0.86
50%	3	2978381	797.88	805.85	101		
100%	1	5944569	1595.764	1586.18	99.4		
100%	2	5940367	1595.764	1592.57	99.8	100.6	0.67
100%	3	5949507	1595.764	1611.72	101		
150%	1	8917303	2393.646	2345.77	98.2		
150%	2	8919620	2393.646	2369.70	99	99	0.65
150%	3	8911254	2393.646	2388.85	99.8		

Table 8: Results of Accuracy study for Paracetamol

Levels	Sets	Area	Drug Added(mcg)	Drug Recovered(mcg)	% Recovery	% Mean Recovery	% RSD
50%	1	3724181	644.378	634.067	98.4		
50%	2	3723867	644.378	639.22	99.2	99.03	0.46
50%	3	3729343	644.378	641.15	99.5		
100%	1	7446407	1288.756	1277.157	99.1		
100%	2	7441467	1288.756	1286.822	99.8	99.16	0.49
100%	3	7449311	1288.756	1270.713	98.6		
150%	1	11164587	1933.133	1917.667	99.2		
150%	2	11163045	1933.133	1927.526	99.71	99.49	0.21
150%	3	11141587	1933.133	1924.627	99.56		

Table 9: Results of Accuracy study for Etodolac and Paracetamol

Sample preparation	Sample Area (Etodolac)	Sample Area (Paracetamol)	% Assay (Etodolac)	% Assay (Paracetamol)
1	5947205	7441933	100.11	99.95
2	5945140	7445660	100.07	100.03
3	5949041	7446509	100.14	100.01
4	5942852	7440597	100.03	99.93
5	5944962	7449826	99.96	100.59
6	5948565	7446207	100.13	100.01
Mean	5946294	7445122	100.073	100.08
S.D	2178.93	3059.50		
%RSD	0.036	0.041		

Table 10: Results of Robustness study for Etodolac

Sr no	Flow 1	Flow 2	Temperature 1	Temperature 2
1	3625140	3499041	3182852	3254962
2	3626849	3499865	3186754	3254675
3	3626990	3497675	3198763	3267543
4	3628784	3487876	3189712	3298234
5	3628663	3487895	3198342	3245768
6	3627873	3496572	3267427	3298765
Mean	3627383	3494820	3203975	3269991
STD DEV	1245.78	5011.05	28957.52	21129.78
% RSD	0.034	0.143	0.903	0.646

Table 11: Results of Robustness study for Paracetamol

Sr no	Flow 1	Flow 2	Temperature 1	Temperature 2
1	4225660	3756509	3680597	3619826
2	4256478	3789345	3689678	3682354
3	4245345	3789064	3678498	3689457
4	4256734	3789754	3623145	3609256
5	4298650	3719346	3609386	3616095
6	4278905	3789054	3618402	3698915
Mean	4260295	3772178	3649951	3652651
STD DEV	23337.28	26489.92	33396.01	38022.48
% RSD	0.547	0.702	0.914	1.040

Robustness parameters:

Flow Rate: Flow 1: (-20%) 0.8 ml $^{-1}$ min and **Flow 2:** (+20%) 1.2 ml min^{-1}.

Temperature: Temperature 1: 25˚C and **Temperature 2:** 35˚C.

Chromatogram:

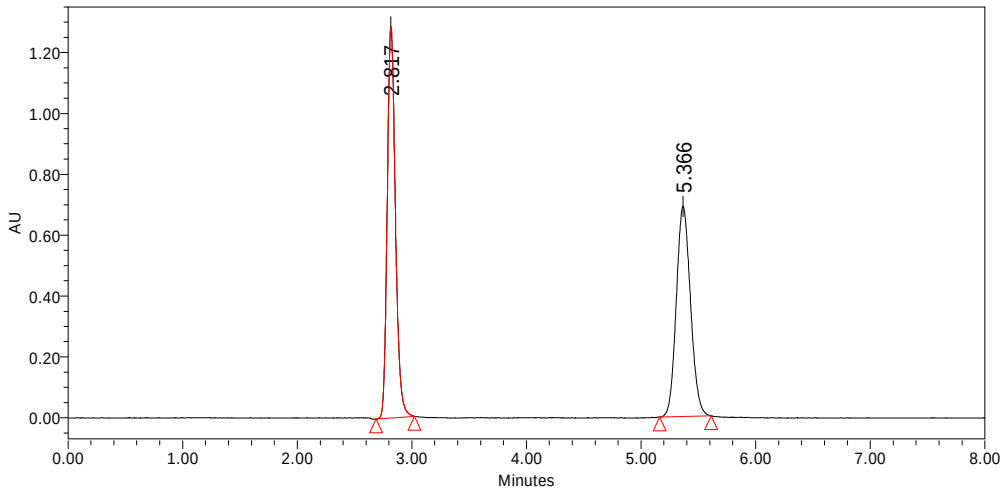


Fig 3: Chromatogram of Etodolac and Paracetamol

CONCLUSION

A simple rapid reverse phase HPLC method was developed and validated according to ICH guidelines for simultaneous determination of etodolac and paracetamol in tablet dosage form. The developed RP-HPLC method was assured to be simple, rapid, selective and robust. The validation report signifies good specificity, accuracy, precision, and reliability of the method. This method can be used for routine practices in the laboratories for the estimation of etodolac and paracetamol in tablet dosage form.

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REFERENCES

1. Drug Bank Open data drug and drug target database [Internet].2005 [updated on 2011 Apr 19] Available from: http://www.drugbank.ca/drugs/DB00749
2. Indian pharmacopoeia 2010. Ghaziabad: The Indian pharmacopoeia commission, Govt. of India Ministry of Health and Family Welfare; 2010. 1318-1322.
3. British Pharmacopoeia 2009. London: The department of health, The stationary office; 2009. 781-784.
4. United State Pharmacopoeia 2007. 30thed. Rockville: USP Convention, INC; 2007. 2129-2131.
5. Indian Pharmacopoeia 1996. New Delhi: The Controller of Publications, Govt. of India; 1996. 554.
6. British Pharmacopoeia 1998. London: Her Majesty's Stationary Office; 1998. 1854.
7. Pareek. A, Chandurkar. N, Ambade. R, Chandanwale A, Bartakke G, Efficacy and Safety of Etodolac-Paracetamol in Patients with Knee Osteoarthritis Flare-Up: A Randomized, Double-Blind Comparative Evaluation. Medical Affairs and Clinical Research Ipca Laboratories Ltd.
8. Balan. P, Carolin Nimila. I, Lakshmi Prasanna. M, Vanaja Rani. M and Rajasekar. S, Simultaneous Estimation of Etodolac and Paracetamol by UV Spectrophotometric Method in Tablet Formulation, Journal of Pharmacy Research, 2011, 4(6), 1663-1665.

9. Balan. P, Carolin Nimila. I, Lakshmi Prasanna. M, Vanaja Rani. M and Rajasekar. S, RP-HPLC Method Development and Validation of Etodolac and Paracetamol in Combined Dosage Form, Asian Journal of Research Chemistry, 2011, 4(7), 1073-1076.
10. Sruthi. A, Thanuja. N, Sai Samhita. M, Sudheer Kumar. D, Sreekanth. G, A Simple RP-HPLC Method for Simultaneous Estimation of Paracetamol and Etodolac in Tablet Dosage Form, Indo American Journal of Pharmaceutical Research, 2013, 3(5), 3742-3751.
11. ICH Guidance for Industry, Validation of Analytical Procedures: Methodology Q2(R1), 2005.
12. Lloyd R. Snyder, Joseph J. Kirkland, Joseph L. Glajch, Practical HPLC Method Development, 2nd Edition.
13. Deepali Gharge*, Prof. Pandurang Dhabale, Simultaneous Estimation of Aceclofenac and Paracetamol in Solid Dosage Form by RP-HPLC Method, International Journal of ChemTech Research, April-June 2010, 2(2), 942-946.
14. V.Sekar* et al., Development and Validation of RP-HPLC Method for the Simultaneous Estimation of Diacerein and Aceclofenac in Dosage Form, International Journal of ChemTech Research, Jan-Mar 2010, 2(1), 68-171.

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