Formulation, Method Development And Validation Of Active Pharmaceutical Ingredient In Tablet Dosage Form

Pranjali V. Shandilya[1] , S.G.Jawarkar[2]

[1]M pharmacy Quality Assurance, Vidyabharti college of pharmacy Amravati, Maharashtra, India

[2] Professor, M Pharmacy Quality Assurance, Vidyabharti college of pharmacy Amaravati, Maharashtra, India

Abstract*:* Rapid and accurate HPLC method has been developed for the simultaneous estimation of Meloxicam and Rizatriptan in tablet dosage form. Chromatographic separation of both anti-inflammatory drugs was achieved on a Zodiac-100 C18 (150 × 4.6 mm ID, 5 µm) using a mobile phase consisting of 760 mL methanol and 240 mL of buffer (0.2% ammonium acetate in water), at a flow rate of 1.0 mL/min. Detection was carried out at 245 nm. The separation was achieved in less than 10 minutes. The method was validated as per ICH guidelines for parameters including linearity, accuracy, precision, system suitability, specificity, robustness, limit of detection (LOD), limit of quantitation (LOQ), and range. Linearity, accuracy, and precision were found to be within acceptable limits over the range of 0.5–3.0 µg/mL for Meloxicam and 5–30 µg/mL for Rizatriptan. The method proved to be sensitive, specific, and suitable for routine quality control of these drugs in pharmaceutical formulations.

Keywords: *High-Performance Liquid Chromatography, Meloxicam, Rizatriptan, Anti-inflammatory drugs, pharmaceutical formulation, Method validation.*

Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs) are one of the most frequently used classes of medicines in the world, accounting for nearly 5% of all prescribed medications [1]. An inhibitory effect of NSAIDs on cyclooxygenase (COX) activity is responsible for their anti-inflammatory actions because COX is an enzyme essential for the synthesis of prostaglandins (PGs), such as PGE_2, which have a strong capacity to induce inflammation. However, NSAID administration is associated with gastrointestinal complications, such as gastric ulcers and bleeding, which sometimes become life-threatening diseases [2]. About 15-30% of chronic users of NSAIDs have gastrointestinal ulcers and bleeding. In the United States, about 16,500 people per year die as a result of NSAID-associated gastrointestinal complications [3]. Therefore, the molecular mechanism governing NSAID-induced gastrointestinal damage needs to be elucidated in order to develop new NSAIDs that do not have these side effects.

Meloxicam, sold under the brand name **Mobic** among others, is a nonsteroidal anti-inflammatory drug (NSAID) used to treat pain and inflammation in rheumatic diseases and osteoarthritis. It is taken by mouth or given by injection into a vein. It is recommended that it be used for as short a period as possible and at a low dose. Common side effects include abdominal pain, dizziness, swelling, headache, and a rash. Serious side effects may include heart disease, stroke, kidney problems, and stomach ulcers. Use is not recommended in the third trimester of pregnancy It blocks cyclooxygenase-2 (COX-2) more than it blocks cyclooxygenase-1 (COX-1). It is in the oxicam family of chemicals and is closely related to piroxicam. Meloxicam is available in combination with bupivacaine as bupivacaine/meloxicam and in combination with rizatriptan as meloxicam/rizatriptan.

Meloxicam blocks cyclooxygenase (COX), the enzyme responsible for converting arachidonic acid into prostaglandin H_2—the first step in the synthesis of prostaglandins, which are mediators of inflammation. Meloxicam has been shown, especially at low therapeutic doses, to selectively inhibit COX-2 over COX-1. Meloxicam concentrations in synovial fluid range from 40% to 50% of those in plasma. The free fraction in synovial fluid is 2.5 times higher than in plasma, due to the lower albumin content in synovial fluid compared to plasma. The significance of this penetration is unknown, but it may account for the fact that it performs exceptionally well in treatment of arthritis in animal models. [12]

Rizatriptan, sold under the brand name **Maxalt** among others, is a medication used for the treatment of migraine headaches. It is taken by mouth. It can also be applied on the tongue. It is a serotonin (5-HT) 1B/1D receptor agonist (triptan). Common side effects include chest pain, dizziness, dry mouth, and tingling. Other side effects may include myocardial infarction, stroke, high blood pressure, serotonin syndrome, and anaphylaxis Excessive use may result in medication overuse headaches Use is not recommended during pregnancy and breastfeeding is not recommended within 24 hours after taking a dose. Rizatriptan is in the triptan class and is believed to work by activating the $5\text{-}HT_1$ receptor.

Rizatriptan is indicated to treat acute migraine attacks with or without aura. It does not prevent future migraine attacks. Rizatriptan acts as an agonist at serotonin $5\text{-}HT_{1B}$ and $5\text{-}HT_{1D}$ receptors. Like the other triptans sumatriptan and zolmitriptan, rizatriptan induces vasoconstriction—possibly by inhibiting the release of calcitonin gene-related peptide from sensory neurons in the trigeminal nerve.

MATERIALS AND METHODS

Chemicals And Reagent

Meloxicam and Rizatriptan were obtained as complimentary samples from Darshan Pharma Chem Pvt. Ltd.
Plot No: 3609, GIDC Estate, Ankleshwar, Gujarat 393002. High-performance liquid chromatography (HPLC) grade solvents and reagents, including water, methanol, acetonitrile, were used throughout the study ammonium acetate was employed as a modifier in the mobile phase preparation. All chemicals and solvents used were of analytical or HPLC grade and required no further purification before use.

Instrumentation

The high-performance liquid chromatography (HPLC) of Shimadzu SCL-10A_{VP} inbuilt with binary pump (LC-10AT_{VP}), UV detector (SPD-10A_{VP}), Rheodyne 20µl loop capacity manual injector (P/N 77251) was used throughout the analysis. The LC-Solution software was used to interpret the HPLC reports. Zodiac-100 C18 (5µm; 150 x 4.6 mm ID.) and Acclaimed HILIC-1 (5µm; 150 x 4.6 mm ID.) columns, purchased from UltraChrom Innovatives Pvt. Ltd. were used throughout the analysis. Digital weighing balance (ME-204) purchased from Mettler-Toledo (USA), ultra-sonicator Labman® purchased from UltraChrom Ltd, India. Digital pH meter from Mettler-Toledo was purchased from (Mumbai-India). 50 µ micro-syringe was purchased from Hamilton USA. 0.20µ and 0.45µ nylon membrane filters were purchased from Phenomenex® Mumbai, India. Preparation of Mobile Phase.

Preparation of mobile phase

Prepared Homogeneous mixture of 760ml HPLC grade Methanol and 240 ml of Buffer Shake well, filter this solution through 0.2 um membrane filter paper. And sonicate this mobile phase for about 5 minutes.

Preparation of Stock Solution:

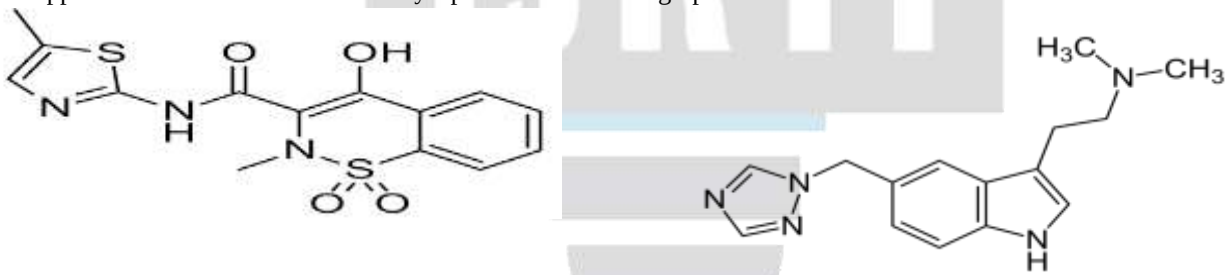
Weigh accurately 15 mg of Meloxicam standard and 10 mg of Rizatriptan standard. Transfer in 100 ml volumetric flask, Add about 90 ml of diluent to the volumetric flask, Shake well and sonicate for 5 min to get completely dissolve shake well and sonicate for 5 min make-up the volume to 100 ml. Pipet-out 2 ml from stock solution and transferred to 20ml flask to volumetric flask.

Standard solution:

Pipette out 2ml from the stock solution and Dilute it into 20ml with the help of Diluent and shake well. Then Sonicate it for 2min and filter it through Used 0.2 um syringe filter before used.

Method Development

After finishing four experimental trials with variations in run time, column and mobile phase the drug observed to be in good peak shape at fourth trial. The % RSD, Tailing Factor and Theoretical plate shows that the drug is within the acceptance criteria. The approach was found to be satisfactory Optimized Chromatographic Condition.



Parameters	Result
Mobile Phase	acetonitrile-methanol-water (40:40:20% v/v/v).
Column	Zodiac-100 C18 (5µm; 150 x 4.6 mm ID)
Flow rate	15 minutes
Injection Volume	20ul
Temperature	Ambient
Wavelength	245
Run time	1.0 ml/mins
Elution Mode	Isocratic
Diluent	Mobile Phase

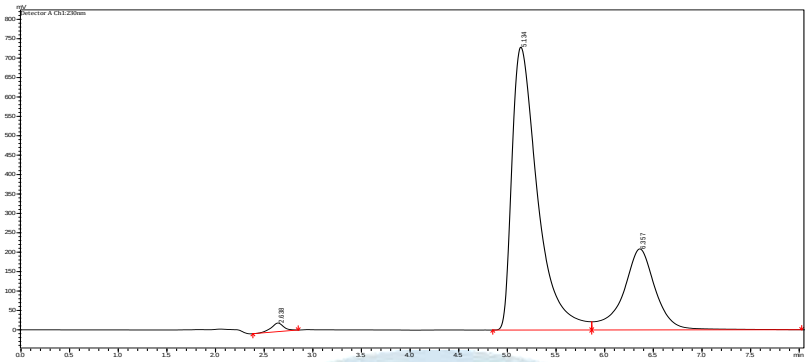


Fig 3: Chromatogram of optimized method for Meloxicam and Rizatriptan

Result and Discussion Specificity

Specificity was evaluated by chromatograms of mobile phase blank, placebo solution, standard solution of Meloxicam and Rizatriptan and its sample solution.

Sr. No	Solution	
1	Blank	0.00
2	Plasma Blank	0.00
3	Meloxicam Standard Sample	4.215
4	Meloxicam Test Sample	4.210
5	Rizatriptan Standard Sample	2.970
6	Rizatriptan Test Solution	2.965

Table 2: Specificity of Meloxicam and Rizatriptan

There is no interference from the blank and placebo at the retention time of Meloxicam chromatographic peak. Retention time for Meloxicam in standard solution, Test solution, individual solution are matching with each other. So this system is specific for the analysis of Meloxicam, hence specificity is justified.

System Suitability

These parameters were shown to be within specified limits. Column efficiency (theoretical plates), resolution factor and peak asymmetry factor, tailing factor, LOD, LOQ are the system suitability parameters. These parameters of the optimized methods were found satisfactory.

Standard Solution in order to conduct the test.

Name	Area	RT (min)	TP(NLP2000)	TF(NMT2)	Resolution (NLT-2)
Standard_Inj_01	925430	5.83	6825	1.09	6.75
Standard_Inj_02	928120	8.52	6840	1.10	6.78
Standard_Inj_03	923890	5.54	6815	1.08	6.74
Standard_Inj_04	926500	5.83	6835	1.10	6.76
Standard_Inj_05	927300	5.82	6850	1.09	6.77
Mean	927648	5.77	6201	1.05	3.75
SD	1580.97	0.0019			
%RSD(NMT2)	0.17	0.05			

Table 3: System Suitability Study for Meloxicam

Theoretical plate ,resolution and Telling factor observed within acceptance criteria, also %RSD of replication injections for area and retention time observed within acceptance criteria , hence system is suitable for analysis of Meloxicam, Hence System suitability is justified.

Name	Area	RT (min)	TP (NKP2000)	TF (NMT2)	resolution(NLT-2)
Standard_Inj_01	845600	3.75	6200	1.05	5.90
Standard_Inj_02	847200	3.74	6220	1.06	5.92
Standard_Inj_03	842800	3.76	6180	1.04	5.88
Standard_Inj_04	846100	3.75	6210	1.02	5.91
Standard_Inj_05	845900	3.74	6195	1.05	5.89
Mean	845520	3.75	6833	1.092	5.77
SD	1480.71	0.0017			
%RSD	0.175	0.06			

Table 4: System Suitability Study for Rizatriptan

Theoretical plate ,resolution and Telling factor observed within acceptance criteria, also %RSD of replication injections for area and retention time observed within acceptance criteria , hence system is suitable for analysis of Rizatriptan Hence System suitability is justified.

Linearity

Linearity for Meloxicam and Rizatriptan The linearity graph of average peak area at each level against the concentration in µg/ml is plotted and found to be straight line graph. This method proved to be linear between µg/ml of Meloxicam and Rizatriptan with a typical Linearity curve of correlation equation.

Meloxicam		
S. No.	Concentration (µg/mL)	Area
1	100	13672647
2	50	6839269
3	25	3469912
4	12.5	1968719
5	6.25	867209
6	3.125	437310
Correlation coefficient (R^2)		0.9996
Std. error of intercept		60796.37386
Slope		148920.0942

Table 5: Calibration Standards Peak Area

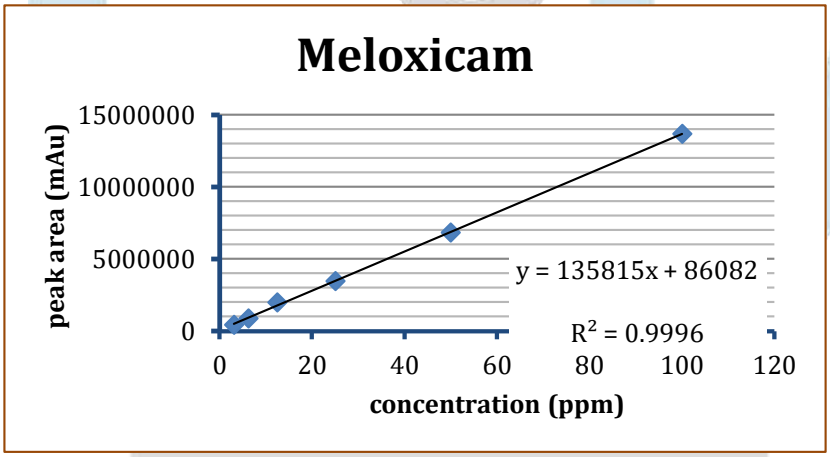


Fig. Linearity curve for Meloxicam

Rizatriptan		
S. No.	Concentration (µg/mL)	Area
1	50	4595001
2	25	2268477
3	12.5	1130647
4	6.25	585007
5	3.125	293171
6	1.56	152287
Correlation coefficient (R^2)		0.9999
Std. error of intercept		9735.645416
Slope		23847.36359

Table 6: Calibration Standards Peak Area

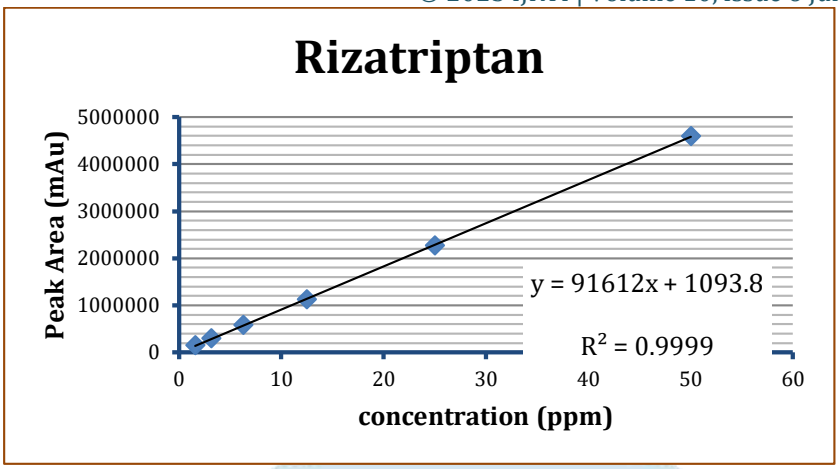


Fig. Linearity curve for Rizatriptan

Correlation coefficient observe within acceptance criteria, hence method is linear and linearity is justified.

LOD and LOQ

The LOD is the lowest concentration of the analyte that can be detected & LOQ is the lowest concentration that can be quantitatively measured based on the steyx and the slope. The LOD and LOQ were calculated using the following formulas: LOD 3.30/s and LOQ-100/%

Conc (ppm or ug/ml)	Area
2.5	365606
3.75	556174
5.0	732686
6.25	911370
7.5	1098819
STEYX	607963
Slope	135815
LOD(ug/ml)	3.29
LOQ(ug/ml)	10.96

Table 7: LOD & LOQ of Meloxicam

Conc (ppm or ug/ml)	Area
2.50	9542310
3.75	2866442
5.00	2458522
6.25	5486235
7.50	2154635
STEYX	9735645
Slope	91612
LOD(ug/ml)	0.78
LOQ(ug/ml)	2.60

Table 8:LOD & LOQ of Rizatriptan

Accuracy

For accuracy studies, samples were prepared at three concentration levels: Low (LQC), Medium (MQC), and High (HQC) Quality Controls. Concentration of each injection was calculated and the standard deviation between the readings is calculated.

Meloxicam			Amount added	Amount recovered	Recovery (90-110)
Name	Preparations	Area	µg/ml	µg/ml	%
Accuracy at 80%	Prep-1	1584013	7.9	7.8	99.0%
Accuracy at 80%	Prep-2	1596012	8.1	8.3	99.7%
Accuracy at 80%	Prep-3	1602533	8.5	8.7	100.1%
Accuracy at 100%	Prep-1	1988012	9.9	9.10	99.4%
Accuracy at 100%	Prep-2	2012311	10.10	9.92	100.5%
Accuracy at 100%	Prep-3	2024061	10.30	11.92	101.2%
Accuracy at 120%	Prep-1	2376012	11.80	12.02	99.0%
Accuracy at 120%	Prep-2	2394321	11.90	12.10	99.8%
Accuracy at 120%	Prep-3	2412013	12.10	12.06	100.5%

Meloxicam	% Mean recovery	SD	%RSD (NMT 2)
Accuracy at 80%	99.6%	0.55	0.56
Accuracy at100%	100.4%	0.91	0.89
Accuracy at120%	99.8%	0.76	0.77

Rizatriptan			Amount added	Amount recovered	Recovery (90-110)
Name	preparations	Area	µg/ml	µg/ml	%
Accuracy at 80%	Prep-1	1984012	7.90	7.02	99.2%
Accuracy at 80%	Prep-2	2011926	8.01	8.06	100.5%
Accuracy at 80%	Prep-3	2025013	8.10	8.04	101.3%
Accuracy at 100%	Prep-1	2485048	9.94	9.08	99.4%
Accuracy at 100%	Prep-2	2508043	10.03	10.13	100.3%
Accuracy at 100%	Prep-3	2520124	10.08	10.15	99.0%
Accuracy at 120%	Prep-1	297016	11.88	12.05	99.9%
Accuracy at 120%	Prep-2	2998045	11.99	12.04	99.9%
Accuracy at 120%	Prep-3	3024012	12.09	12.09	100.8%

Rizatriptan	% Mean recovery	SD	%RSD (NMT 2)
Accuracy at 80%	100.3%	0.55	1.06
Accuracy at 100%	100.2%	0.91	0.57
Accuracy at 120%	99.9%	0.76	0.90

Precision

Precision can be determined by two types: 1) Intraday precision 2) Interday precision

The preparation was injected into HPLC four times and mean peak area was calculated separately for each concentration and from that precision percentage RSD values were calculated.

Table 9: interday precision data of Meloxicam

Name	Preparations	% Assay
Day-1	prep-1	99.97
	prep-2	99.19
Day-2	prep-1	100.30
	prep-2	99.36
Mean		99.71
SD		0.5191
% RSD (NMT 2)		0.52

Table 10: Interday Precision Data Of Rizatriptan

Name	Preparations	% Assay
Day-1	prep-1	99.99
	prep-2	99.77
Day-2	prep-1	99.08
	prep-2	99.12
Mean		99.49
SD		0.4595
% RSD (NMT 2)		0.46

Table 13: Intraday Precision Data of Meloxicam

Name	Preparations	% Assay
Set-1	prep-1	99.97
	prep-2	99.19
Set-2	prep-1	99.39
	prep-2	98.91
Mean		99.37
SD		0.4488
% RSD (NMT 2)		0.45

Table 13:Intraday Precision Data of Rizatriptan

Name	Preparations	% Assay
Set-1	prep-1	99.99
	prep-2	99.77
Set-2	prep-1	98.81
	prep-2	100.63
Mean		99.80
SD		0.7541
% RSD (NMT 2)		0.76

Overall % RSD for Intraday and Interday results were observed within the acceptance criteria. Thus, the developed method is found to be precise, hence precision is justified.

Robustness

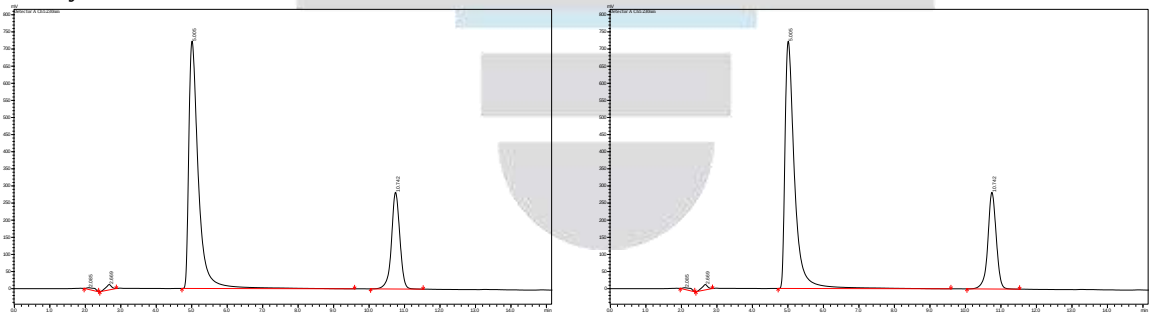
Robustness was attempted by deliberately changing the chromatographic conditions to evaluate the difference in Flow Rate and Buffer concentration. Robustness was studied for Meloxicam and Rizatriptan, results obtained was displayed in following Tables.

Name	Preparations	%Assay
Robustness change in method parameters		
Original method parameters	Test prep-1	99.97
Original method parameters	Test prep-2	99.19
Pump, Flow 1.1 ml/min	Test prep	99.94
Pump, Flow 0.9 ml/min	Test prep	98.88
MeOH:Buffer, 71:29	Test prep	99.51
MeOH:Buffer, 81:19	Test prep	100.48
Mean		99.66
SD		0.5829
%RSD (NMT 2)		0.58

Name	Preparations	%Assay
Robustness change in method parameters		
Original method parameters	Test prep-1	99.99
Original method parameters	Test prep-2	99.77
Pump, Flow 1.1 ml/min	Test prep	100.04
Pump, Flow 0.9 ml/min	Test prep	99.22
MeOH:Buffer, 71:29	Test prep	98.05
MeOH:Buffer, 81:19	Test prep	101.21
Mean		99.71
SD		1.0427
%RSD (NMT 2)		1.05

Overall % RSD of results with change in pump flow rate and change in mobile phase composition observed within acceptance criteria method is robust in terms of slight change in internal method parameters, hence Robustness is justified.

Analysis of Formulation:-



Conclusion

A simple, accurate, precise, and selective isocratic RP-HPLC method was successfully developed and validated for the simultaneous estimation of Meloxicam and Rizatriptan in tablet dosage form. The method was validated as per ICH guidelines and demonstrated satisfactory results for key parameters such as linearity,
accuracy, precision, specificity, LOD, LOQ, and robustness. The use of a cost-effective mobile phase (0.2% formic acid in HPLC-grade water) and detection at 245 nm (isosbestic point) ensured high sensitivity and clear peak resolution within a short retention time. The method is well-suited for routine quality control of formulated anti-diabetic combination tablets containing Meloxicam and Rizatriptan.

References:-

1) Smalley, W.E.; Ray, W.A.; Daugherty, J.R.; Griffin, M.R. Nonsteroidal anti-inflammatory drugs and the incidence of hospitalizations for peptic ulcer disease in elderly persons. Am. J. Epidemiol. **1995**, 141, 539–545.
2) Hawkey, C.J. Nonsteroidal anti-inflammatory drug gastropathy. Gastroenterology **2000**, 119, 521–535.
3) Singh, G. Recent considerations in nonsteroidal anti-inflammatory drug gastropathy. Am. J. Med. **1998**, 105, 31S–38S.
4) Hoshino, T.; Tsutsumi, S.; Tomisato, W.; Hwang, H.J.; Tsuchiya, T.; Mizushima, T. Prostaglandin E2 protects gastric mucosal cells from apoptosis via EP2 and EP4 receptor activation. J. Biol. Chem. **2003**, 278, 12752–12758.
5) Miller, T.A. Protective effects of prostaglandins against gastric mucosal damage: current knowledge and proposed mechanisms. Am. J. Physiol. **1983**, 245, G601–G623.
6) Hoshino, T.; Takano, T.; Tsutsumi, S.; Tomisato, W.; Tsuchiya, T.; Mizushima, T. Effects of prostaglandin E2 on gastric irritant-induced apoptosis. Dig. Dis. Sci. **2002**, 47, 2370–2379.
7) Tsutsumi, S.; Haruna, R.; Tomisato, W.; Takano, T.; Hoshino, T.; Tsuchiya, T.; Mizushima, T. Effects of prostaglandins on spontaneous apoptosis in gastric mucosal cells. Dig. Dis. Sci. **2002**, 47, 84–89.
8) Liang Zhao1, Jessie L.-S. Au1, and M. Guillaume Wientjes1,2 Comparison of methods for evaluating drug-drug interaction. (2010): 241–249.
9) Smith, C.J.; Zhang, Y.; Koboldt, C.M.; Muhammad, J.; Zweifel, B.S.; Shaffer, A.; Talley, J.J.; Masferrer, J.L.; Seibert, K.; Isakson, P.C. Pharmacological analysis of cyclooxygenase-1 in inflammation. Proc. Natl. Acad. Sci. USA **1998**, 95, 13313–13318.
10) Chan, C.C.; Boyce, S.; Brideau, C.; Charleson, S.; Cromlish, W.; Ethier, D.; Evans, J.; Ford, H.A.; Forrest, M.J.; Gauthier, J.Y.; Gordon, R.; Gresser, M.; Guay, J.; Kargman, S.; Kennedy, B.; Leblanc, Y.; Leger, S.; Mancini, J.; O'Neill, G.P.; Ouellet, M.; Patrick, D.; Percival, M.D.; Perrier, H.; Prasit, P.; Rodger, I.; et al. Rofecoxib [Vioxx, MK-0966; 4-(4'-methylsulfonylphenyl)-3-phenyl-2-(5H)-furanone]: a potent and orally active cyclooxygenase-2 inhibitor. Pharmacological and biochemical profiles. J. Pharmacol. Exp. Ther. **1999**, 290, 551–560.
11) Ligumsky, M.; Golanska, E.M.; Hansen, D.G.; Kauffman, G.J. Aspirin can inhibit gastric mucosal cyclo-oxygenase without causing lesions in rat. Gastroenterology **1983**, 84, 756–761.
12) Mukherjee, D.; Nissen, S.E.; Topol, E.J. Risk of cardiovascular events associated with selective COX-2 inhibitors. JAMA **2001**, 286, 954–959.