Optimization and validation of RP-HPLC method for the estimation of meloxicam and paracetamol with its genotoxic impurity (p-amino phenol) in bulk and pharmaceutical drug product using PDA detector

Soni L. K.[1] **and Jain Sanjay** [2]
[1.] School of Pharmacy, Devi Ahilya Vishwavidyalaya, Indore (MP), India
[2.] B R Nahata College of Pharmacy, Mandsaur (MP) 458001 , India

Research Article

Article Info:

Received on:01/01/2016
Accepted on: 15/02/2016
Published on: 22/02/2016

QR Code for mobile

Literati Open Access

ABSTRACT :

A simple, accurate, precise, reproducible RP-HPLC method has been developed for simultaneous estimation of meloxicam and paracetamol with its genotoxic impurity (p-amino phenol) in bulk and combined dosage form (tablet). The method was validated in compliance with ICH guidelines[1-2]. The LC separation was achieved on Lichrospher RP-18e (250X4.6mm), 5µm column at 285 nm in isocratic mode using mobile phase composition Methanol: Phosphate buffer (80:20 v/v), pH adjusted to 2.6 by orthophsphoric acid. Flow rate employed was 1.0 ml/min. The retention time for paracetamol, meloxicam and p-amino phenol were found to be 2.28, 3.14 and 6.09 minutes respectively. Linearity ranges were suitable for routine determination(10-120 µg/ml, 1-20 µg/ml 1-10µg/ml) of Paracetamol, Meloxicam and p-Amino phenol with correlation coefficient of 0.9991, 0.9992 and 0.9990 respectively. The % recoveries were in the range of **99.8 ±0.14** for paracetamol, **99.50± 0.52** for meloxicam and 99.4**±0.68** for p-amino phenol impurity with relative standard deviation(RSD) less than 2. The LOD and LOQ were found to be 0.1692 and 0.5073 for Meloxicam, 0.2669 and 0.8007 for Paracetamol, 0.1040 and 0.3120 for p-amino phenol respectively. The proposed method is successfully appplied for the quantification of paracetamol, meloxicam and p-amino phenol impurity in bulk and formulations.

Keywords: Meloxicam, Paracetamol, p-amino phenol impurity, Photodiode array detector.

INTRODUCTION:

Synthesis of drug substances often involves the use of reactive reagents and hence, these reagents may be present in the final drug substances as impurities. Such chemically reactive impurities may have unwanted toxicities, including genotoxicity and carcinogenicity[3-5] and are to be controlled based on the maximum daily dose. Paracetamol, *N*-(4-hydroxyphenyl) acetamide[6-8], also known as acetaminophen, is one of the popular non-steroidal anti-inflammatory drugs widely used for management of pain and fever in a variety of patients including children, pregnant women, the elderly and those with ostheoartheritis, simple headaches and non-inflammatory musculoskeletol conditions. Meloxicam(4-hydroxy-2-methyl-N-(5-methyl-2- thiazoly)-2H-1,2-benzo-thiazine -3-carboxamide- 1,1dioxide) is a potent oxicam derivative having a favorable COX-2 (cyclo oxygenase-2) selectivity. It exhibits anti-inflammatory, analgesic and anti-pyretic activities, especially in various chronic conditions, like osteoarthritis, rheumatoid arthritis and juvenile rheumatoid arthritis [9,10]. p-aminophenol is a degradation product of paracetamol or it may be originated from the synthesis; it is reported to have significant nephrotoxic and teratogenic effects[11], therefore its amount should be strictly controlled. It is limited to a low level of 0.005% in the drug substance by the European and British Pharmacopoeias.[12,13]. Various methods were used for determination of paracetamol either alone or in combination with other drugs[14]. Under the conditions of high temperature and pH, paracetamol undergoes hydrolysis forming p-Aminophenol[15,16]

Paracetamol and meloxicam are frequently associated in pharmaceutical oral formulations. These active compounds have different polarity and, therefore chromatographic method development is cumbersome and is further complicated by the presence of impurities such as p-Aminophenol related to Paracetamol. The dosage forms also contain excipients, some of which may interfere with the analysis of the active ingredients. No single method is reported to determine the active ingredients quantitatively in this combination with a check on p-amino phenol (genotoxic impurity) in formulation

Thus here we have developed an optimal chromatographic condition for the separation and estimation of the Meloxicam and Paracetamol with p-Amino phenol (genotoxic impurity) in formulation.

Experimental

Instrumentation

The LC system consisted of an Waters 600E Controller HPLC system equipped with degasser and coupled to a diode-array detector PDA 2998. The system connected to

***Corresponding author:**
Jain Sanjay,
B R Nahata College of Pharmacy, Mandsaur (MP) 458001 , India
Email: sanjayjain@brncop.com

doi: 10.15272/ajbps.v6i53.770

Conflict of interest: Authors reported none

software Empower 2 for controlling the instrumentation and processing the data to generate the result. The injection volume was set to 20 µl and the separation was carried out on an Lichrosphere RP-18 e (250X4.6mm) with particle size of 5µm. The column temperature was kept constant through a temperature controlled oven.

Chemicals and Reagents

Meloxicam was received as gift samples from Cipla Pharmaceutical Pvt. Ltd. Indore. Paracetamol was received as gift samples from Ipca Laboratories Pvt. Ltd. Ratlam (M.P.) p-amino phenol was purchased from Sigma Aldrich. HPLC grade Methanol, water and acetic acid were purchased from Merck, India, Potassium di hydrogen phosphate (KH_2PO_4) and acetic acid were purchased from Sigma Aldrich labs. The pharmaceutical dosage form used in the study was Melodol, Aristo Pharmaceutical Pvt. Ltd, tablet containing 325 mg paracetamol and 7.5 mg meloxicam was purchased from local drug market.

Preparation of stock solutions

10 mg of paracetamol was taken in 10 ml volumetric flask. This was dissolved in the mixture of methanol and buffer (65:35) and diluted up to the mark to get a concentration of 1000 µg/ml of paracetamol. Similarly stock solutions of 1000 µg/ml of each meloxicam and p-amino phenol were prepared in 10 ml volumetric flask using methanol and buffer.

Preparation of Buffer Solution

20 mM potassium di hydrogen phosphate (KH_2PO_4) buffer solution was prepared in HPLC grade water and pH of buffer solution was adjusted to various pH range with HPLC grade acetic acid solution. The solution is finally filtered through 0.45 µm whatmann filter paper.

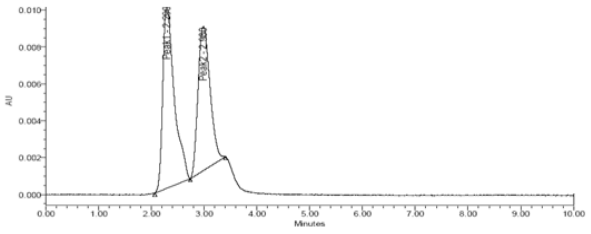
Result and discussion

Optimization of Chromatographic Conditions

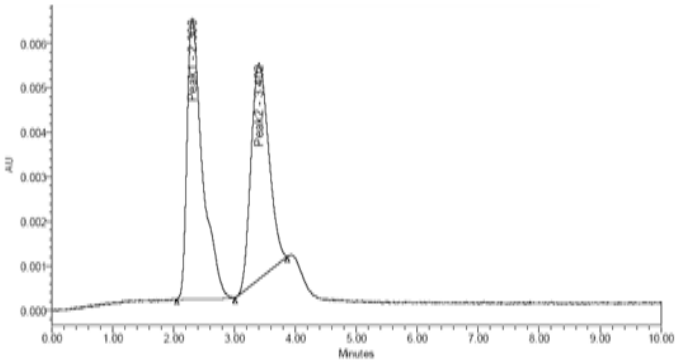
During preliminary investigations of chromatographic behaviour of meloxicam, paracetamol and p-amino phenol, the influence of mobile phase composition (% of methanol, buffer and pH) was investigated. Retention time, capacity factor and resolution were chosen as dependent variable. Mobile phase (methanol:buffer) solution in 2 different volume ratio of 60:40 and 80:20 were used at P^H 2.8, 4 and 6.8. The flow rate was used at 1.0 ml/min and the column temperature was maintained at 30±5 ◦C. The total chromatographic run time is 10 minutes with an additional 10 minutes of column re-equilibration time between each injection. The solution samples were analyzed using a photo-diode array (PDA) detector covering the range of 200–400 nm Because of similar structure of paracetamol and p-Aminophenol (process-related impurity) and similar retention behaviour, capacity factor for those two substances was very poor as well as separation.

The retention and separation of these compounds under the mobile phase ratio of 60:40 at different P^H was not achieved therefore the pH and ionic strength changes of the mobile phase should not have any significant impact on separation of the compounds. At mobile phase ratio of 80:20 and P^H of 2.8 all three compounds showed separation hence, the method development was focused on this condition. Optimization of the compositions of mobile phases, investigating the impact of flow rates, and fine-tuning of the P^H to obtain the final and optimum elution profile of the method was done.

Condition -1



Condition -1b



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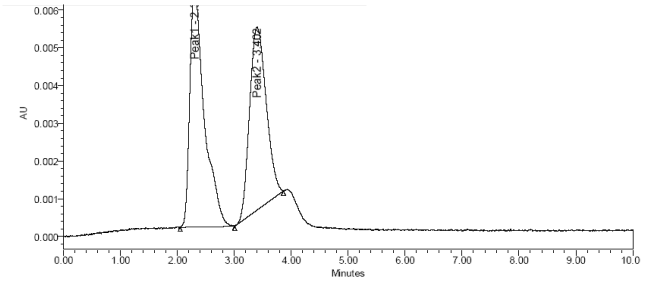

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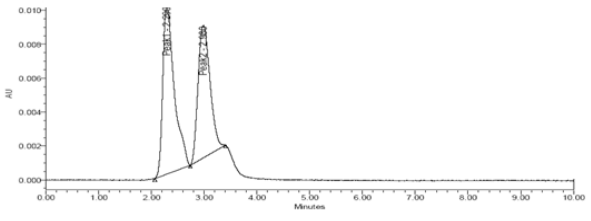

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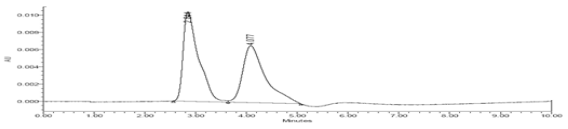

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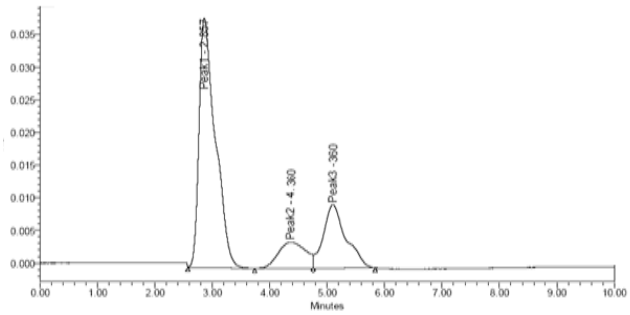
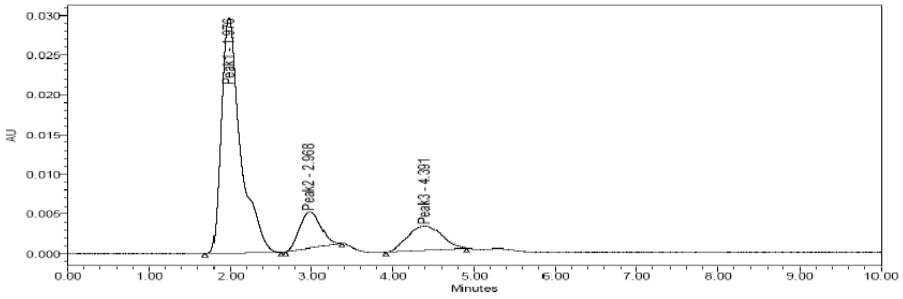


Table 1: Preliminary investigation of condition at different P[H] and solvent ratio

Condition	P[H]	Methanol: Buffer Ratio		RT	K Prime	USP Resolution
1a	6.8	60:40	Peak1 (Paracetamol)	2.284	1.283582	-
			Peak 2 (Meloxicam)	-	-	-
			Peak3(p-amino phenol)	-	-	-
1b	6.8	80:20	Peak1 (Paracetamol)	2.049	1.049406	-
			Peak 2 (Meloxicam)	-	-	-
			Peak 3(p-amino phenol)			
2a	4	60:40	Peak1 (Paracetamol)	2.306	1.307990	-
			Peak 2 (Meloxicam)	3.402	1.049406	2.173935
			Peak 3(p-amino phenol)	-	-	-
2b	4	80:20	Peak1 (Paracetamol)	2.290	1.207290	-
			Peak 2 (Meloxicam)	2.980	2.312720	1.712892
			Peak3 (p-amino phenol)	-	-	-
3a	2.8	60:40	Peak1 (Paracetamol)	2.844	1.844365	-
			Peak 2 (Meloxicam)	4.077	3.077153	1.733685
			Peak3 (p-amino phenol)	-	-	-
3b	2.8	80:20	Peak1 (Paracetamol)	2.848	1.285406	
			Peak 2 (Meloxicam)	4.300	2.144909	2.040190
			Peak3 (p-amino phenol)	5.360	5.098032	4.599652

Optimization of condition for meloxicam , paracetamol and p-amino phenol impurity

Condition-1



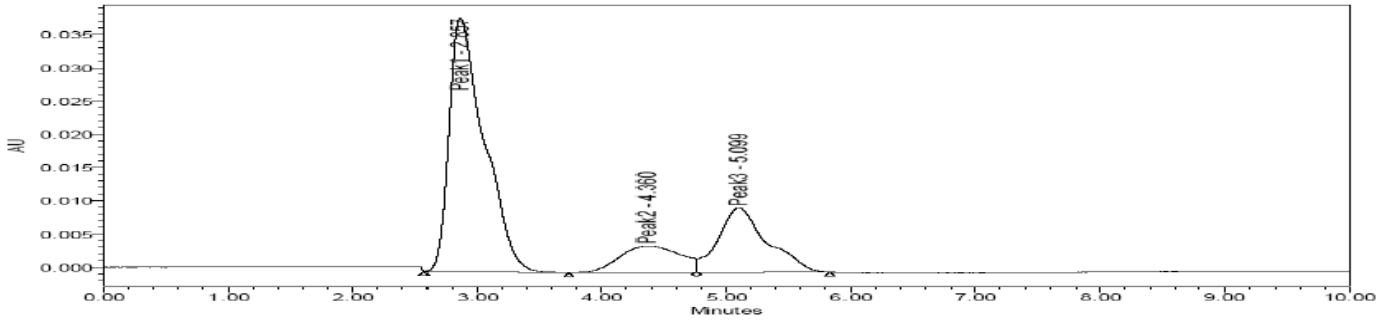
	Peak Name	RT	Area	% Area	Height	USP Plate Count	Symmetry Factor	USP Tailing
1	Peak1	1.976	486371	74.23	29674	419.48	1.71	1.71
2	Peak2	2.968	81780	12.48	4537	594.21	1.08	1.08
3	Peak3	4.391	87091	13.29	3016	503.94	1.02	1.02

	USP Resolution	K Prime
1		0.976386
2	2.195441	1.968259

	USP Resolution	K Prime
3	2.201498	3.390866

Flow rate	pH	Mobile phase	Remark
1.2 ml/min.	3	Methanol : buffer (70:30)	k-prime are less than 1.0

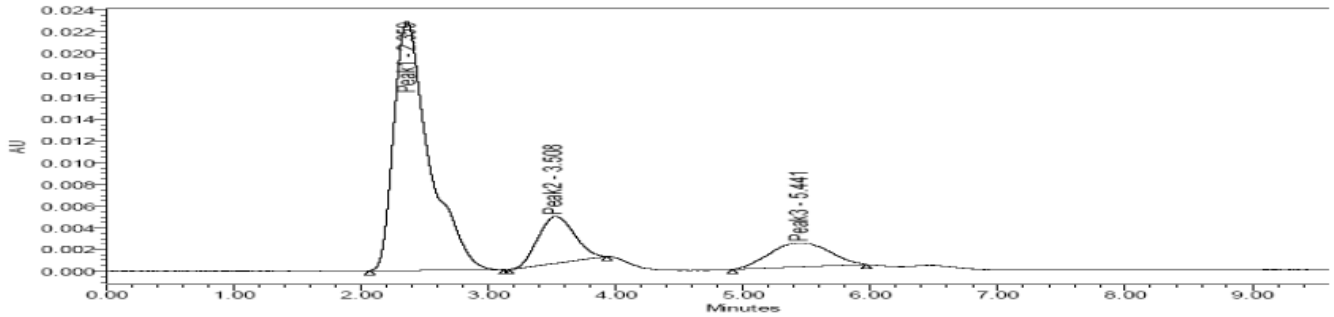
Condition-2



	Peak Name	RT	Area	% Area	Height	USP Plate Count	Symmetry Factor	USP Tailing	K Prime
1	Peak1	2.857	752530	65.42	38202	465.02	1.72	1.72	1.856643
2	Peak2	4.360	132922	11.56	4011	238.65			3.360339
3	Peak3	5.099	264837	23.02	9702	984.85			4.099085

Flow rate	pH	Mobile phase	Remark
1 ml/min.	3	Methanol:buffer(70:30)	Peaks are overlapped

Condition-3



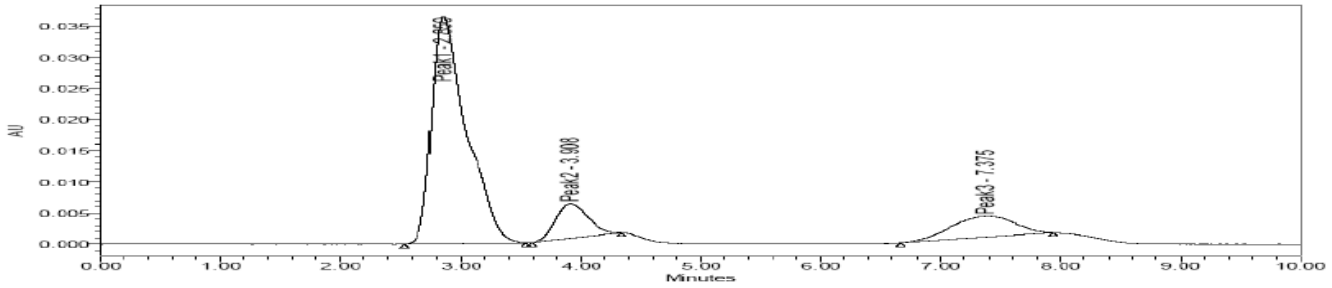
	Peak Name	RT	Area	% Area	Height	USP Plate Count	Symmetry Factor	USP Tailing
1	Peak1	2.359	443029	73.61	22904	408.70	1.69	1.69
2	Peak2	3.508	86963	14.45	4354	660.34	1.10	1.10
3	Peak3	5.441	71850	11.94	2237	636.84	1.01	1.01

	USP Resolution	K Prime
1		1.358643
2	2.219273	2.508220

	USP Resolution	K Prime
3	2.736310	4.440791

Flow rate	pH	Mobile phase	Remark
1.2 ml/min.	2.8	Methanol: buffer (70:30)	All parameter lies in limits

Condition-4



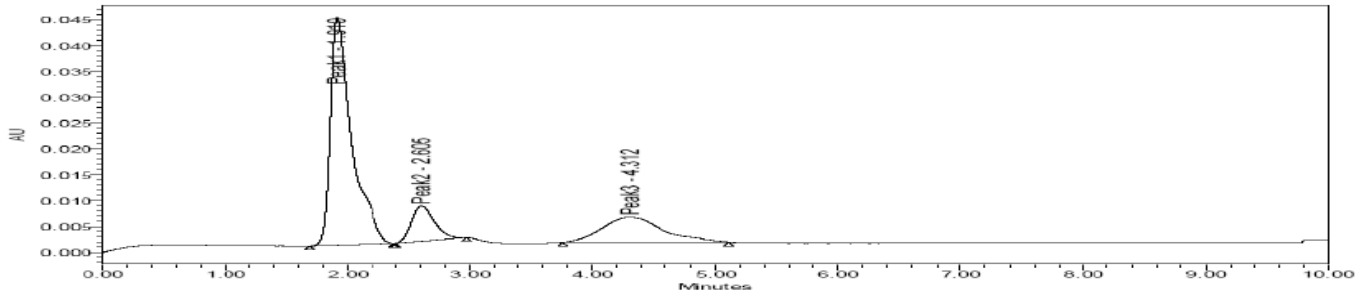
	Peak Name	RT	Area	% Area	Height	USP Plate Count	Symmetry Factor	USP Tailing
1	Peak1	2.850	716110	75.82	36463	543.93	1.72	1.72
2	Peak2	3.908	103617	10.97	5583	981.18	1.09	1.09
3	Peak3	7.375	124743	13.21	3395	866.68	0.89	0.89

	USP Resolution	K Prime
1		1.849618
2	1.970145	2.908191

	USP Resolution	K Prime
3	4.642880	6.374607

Flow rate	pH	Mobile phase	Remark
1 ml/min.	2.8	Methanol : buffer (80:20)	Resolution are less than 2.0

Condition-5



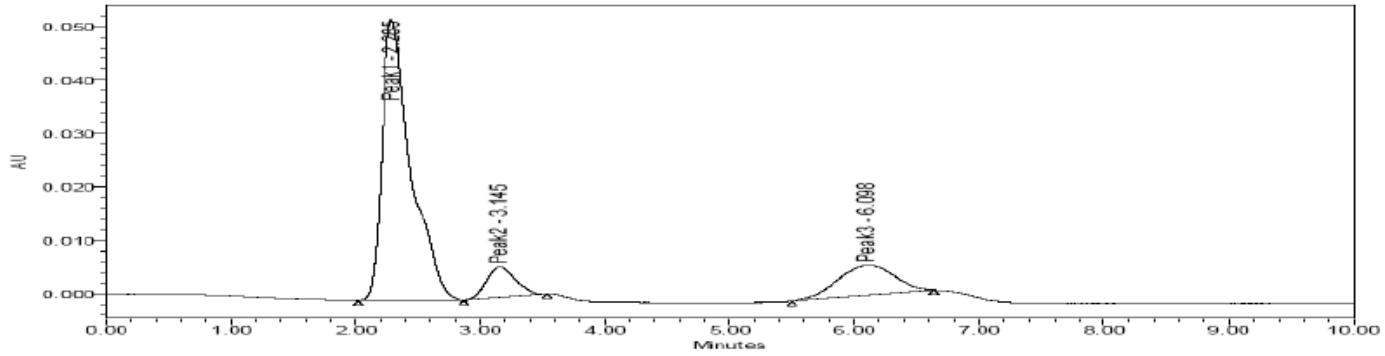
	Peak Name	RT	Area	% Area	Height	USP Plate Count	Symmetry Factor	USP Tailing
1	Peak1	1.910	532261	67.61	44154	729.16	1.95	1.95
2	Peak2	2.605	88062	11.19	6857	930.38	1.18	1.18
3	Peak3	4.312	166927	21.20	4894	386.84	1.22	1.22

	USP Resolution	K Prime
1		0.910000
2	2.148154	1.605064

	USP Resolution	K Prime
3	2.755462	3.312050

Flow rate	pH	Mobile phase	Remark
1.2 ml/min.	2.6	Methanol : buffer (80:20)	Capacity factor are less than 1.0

Conditions-6



	Peak Name	RT	Area	% Area	Height	USP Plate Count	Symmetry Factor	USP Tailing
1	Peak1	2.285	842035	75.81	52499	586.42	1.77	1.77
2	Peak2	3.145	92415	8.32	5796	853.42	1.15	1.15
3	Peak3	6.098	176300	15.87	5684	854.00	0.92	0.92

	USP Resolution	K Prime
1		1.285406
2	2.040190	2.144909

	USP Resolution	K Prime
3	4.599652	5.098032

Flow rate	pH	Mobile phase	Remark
1 ml/min.	2.6	MeoH / buffer (80:20)	All parameters lies in limits

Optimization of method at 6 different conditions involving change in flow rate and P^H were screened for capacity factor, USP resolution, symmetry factor. Condition involving mobile phase ratio (80:20), P^H 2.6 and flow rate of 1ml/min gave most favorable result and all parameters were found in limit.

Table 2: Optimized condition for Estimation of Paracetamol, Meloxicam and p-Amino phenol

S. no.	Parameter	Specification
1	Column	ODS-C18
2	Particle size	5 µm
3	Detector	PDA
4	Wavelength	285 nm
5	Mobile phase	MeOH:Phosphate buffer(80:20)
6	P_H	2.6
7	Flow rate	1ml/min.

The method was validated in compliance with ICH guidelines. Summary of validation parameter is shown in table.

Table 3: Summary of validation parameter

Sr.	Parameter		Results		
1	Specificity		Shows no interference of excipients peaks with analyte peaks.		
2	Linearity range		**Meloxicam** 1-20- µg/ml	**Paracetamol** 10-100 µg/ml	**p-Amino phenol** 1-10. µg/ml
3	Accuracy (% recovery)		**99.8 ±0.14**	**99.50± .52**	99.4±**0.68**
4	Precision (RSD)	Intraday	99.05±**0.48**	98.52±**0.58**	98.32±**0.78**
		Inter-day	101±**0.58**	99.24±**0.78**	98.32±**0.48**
		Repeatability	100.04±**0.08**	99.42±**0.36**	99.62±**0.24**

Tablet analysis

Contents of paracetamol, meloxicam and spiked p-amino phenol in tablet formulation were analyzed by the proposed method.

The low value of RSD indicate the method is precise and accurate.

Table 4: Result of marketed tablet analysis.

Paramters	Paracetamol	Meloxicam	p- amino phenol
% Estimated	99.24	99.08	99.34
Standard deviation	0.74	1.032	0.58

CONCLUSION

Proposed method is a convenient and efficient method for simultaneous determination of Meloxicam, Paracetamol and p-Aminophenol. The developed method does not require using gradient or any procedure of extraction and provides determination (qualitative and quantitative) low levels of the p-Aminophenol in both paracetamol drug substance and dosage forms. The results obtained in this study support that the proposed HPLC method is sufficiently precise, rapid and sensitive to be used for routine analyses.

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