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SIMULTANEOUS HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC ANALYSIS OF OXICAMS IN PHARMACEUTICAL FORMULATIONS

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ABSTRACT

A highly sensitive, accurate and rapid HPLC analytical method for the determination of Tenoxicam (TEN), Piroxicam (PIR) and Meloxicam (MEL) in a finished dosage form was developed and validated. C18 reversed phase column was used. The mobile phase was composed of methanol and aqueous buffer solution (disodium hydrogen phosphate dihydrate mixed with citric acid) (60:40),at pH of 5.8. The flow rate was 1.2 ml/min and the applied analytical wavelength was 360 nm. The calibration curve was linear over the range 1.010- 3.030, 1.513-4.538 and 1.765-5.295 mg/L for TEN, PIR and MEL, respectively. The lower limit of detection and the lower limit of quantification for TEN were 0.06 and 0.18 mg/L, while they were 0.09 and 0.28 mg/L, for PIR, and 0.12 and 0.38 mg/L, respectively for MEL. The peaks resolution was tested under several conditions and found that 5% increase of organic mixture or a 0.5 increase in the pH buffer would decrease the peak resolution between PIR and MEL. In conclusion, such method may successfully replace the classical analytical methods of Oxicams.

Keywords: HPLC, Tenoxicam, Piroxicam, Meloxicam, Validation

INTRODUCTION

Tenoxicam, Piroxicam and Meloxicam are the representative drugs belonging to the oxicam group. They are nonsteroidal antiinflammatory drugs which display a potent analgesic activity and effective in the treatment of rheumatoid arthritis, oesteoarthritis and other jointdiseases. The pharmacological actions of these oxicams are related to inhibition of cyclo-oxygenase (Cox), a key enzyme of prostaglandine biosynthesis at the site of inflammation[1]. Most nonsteroidal anti-inflammatory drugs are weak acids, with a pKa of 3-5. They are absorbed well from the stomach and intestinal mucosa. They are strongly protein-bound in plasma (typically >95%), usually to albumin, so that their volume of distribution typically approximates to plasma volume. Most NSAIDs are metabolized in the liver by oxidation and conjugation to inactive metabolites which are typically excreted in the urine, although some drugs are partially excreted in bile. Metabolism may be abnormal in certain disease states, and accumulation may occur even with normal dosage [2].

There are many published methodologies for determining each of the oxicams in drug dosage forms and biological fluids using spectrophotmerty [3-7], pology [8, 9], chromatography [10-14] , electro-chemical [15, 16], spectrofluorometry [17, 18], and liquid chromatographic tandem mass spectrometry (LC–MS/MS) [19]. The most standard methods, however, are published in British and USA Pharmacopoeia. Therefore, it was considered very useful to develop a simple, rapid and sensitive method for the

determination of these drugs simultaneously in pharmaceutical preparations. Not only such method reduces the cost and time, but also it facilitates studying the stability the oxicam drugs after exposing into various vigorous degradation conditions (acidic, basic, heat and oxidative), which would be of interest for the quality control and clinical monitoring laboratories.

MATERIALS AND METHODS

Reagents: The following reagents were used: acetonitrile HPLC grade (TEDIA), avicel (Across), citric acid (BWR), disodium hydrogen phosphate dihydrate buffer (Merck), lactose (Merck), methanol HPLC grade (Fisher), magnesium sterate (Merck), phosphoric acid (Merck), sodium hydroxide (Scharlau), starch (Across), and water HPLC grade (TEDIA).

Apparatus and Chromatographic Conditions: The following equipments were used: Thermo (HPLC) (Surveyor, LC 6000) pump and degasser connected to a PDA plus-5 detector. Injections were performed using auto-sampler type (Surveyor), 100 µL sample loop and Chromo-Quest Computing integrator software.

Mobile Phase and Standard Preparations: The buffer solution was prepared by dissolving 11 g of disodium hydrogen phosphate dihydrate and 7.7g citric acid in 1000 mL of HPLC- grade water. Then 400 mL of buffer solution were mixed with 600 mL of methanol, and the pH was adjusted to 5.80 $\pm$ 0.05 using phosphoric acid. The mobile phase was filtered through a 0.45 µm membrane filter and degassed by sonication**.** A stock solution of each of TEN, PIR and MEL was prepared by dissolving about 40, 60 and 70 mg (highly pure material > 99.7%), respectively in 100 mL of 0.1 M NaOH. Each stock solution was further diluted in the mobile phase to final concentrations of about 2, 3 and 3.5 mg/L were obtained, respectively**.**

A placebo was prepared based on the excipients present in tablet samples, namely (Starch 15%, Lactose 15%, Mg-sterate 3% and Avicel 67%) without any active-ingredients. A placebo solution was prepared by addition of 620 mg in 100 mL 0.1 M NaOH.

Procedure: UV-VIS scan (Cary) from 250-500 nm was applied for each solution of TEN, PIR and MEL. A maximum absorbance was observed for each drug in a range of 355-362 nm. A wavelength at 360 nm was selected for HPLC analysis. A Hypersil ODS-3 C18 with 5.0 µm particle size (250 cm x 4.6mm) column was used to separate the oxicam drugs at 20 ^{o}C. The flow rate was 1.2 mL/min. The mobile phase was kept flowing until baseline became stable at 360 nm wavelength. The injection volume was fixed at 100 µL.

Statistical Analysis: The data was assessed by one-way ANOVA to define the significant changes in the data obtained by the proposed method. $P<0.05$ is considered significant.

RESULTS AND DISCUSSION

Chromatogram and precision: When the three Oxicams, TEN, PIR, and MEL were introduced together in one sample into the HPLC using the conditions mentioned above, the peaks show an excellent resolution (Fig. 1). This led us to further study the system and method precisions. The RSD values were well below 1.10% indicating a high system precision (Table 1). The precision of the method was performed also by analyzing six preparations of the drugs at a target concentration. The RSD values were well below 1.10% indicating a good method precision (Table 2).

Linearity: After analyzing each preparation in duplicate, a linear regression analysis was performed on the average peak areas versus the concentrations of the levels studied. LOD and LOQ were calculated. For TEX, the method is linear over the range between 1.010-3.030 mg/L with 0.06 and 0.18 mg/L LOD and LOQ, respectively (Fig. 2, Table 3). For PIR, the method is linear over the range between 1.513-4.538 mg/L with 0.09 and 0.28 mg/L LOD and LOQ, respectively (Fig. 3, Table 4). As for MEL, The method is linear over the range between 1.765-5.295 mg/L with 0.12 and 0.38 mg/L LOD and LOQ, respectively (Fig. 4, Table 5).

Accuracy: In order to estimate the accuracy, three samples at three different concentration levels 50, 100 and 150% were analyzed. Analytical concentration at level 100% is analyzed in triplicate preparations at each level (with duplicate readings) against a standard. The average% recovered was between 98-101% and with no significant differences (Tables 6-8).

Stability of analytical solutions: This was performed by determining the concentrations of the analytical solutions (standard and sample) which were stored for three days at 4C and at room temperature in comparison to a freshly prepared standard solution.

The results showed acceptable limits of stability which were within 98.0%-102.0% of the spiked amount with no significant differences.

Robustness of the analytical method: Variations in the wavelength, mobile phase and pH have been made to the analytical method in order to evaluate and measure the capacity of the method to remain unaffected by such variations. Analytical concentration at level 100% was analyzed by preparations at each level (with duplicate readings) against a standard solution. The results show that the RSD% is less than 2.0% (Table 9, Fig 5-6).

Furthermore, slight variations in composition of mobile phase have been made to the analytical method in order to evaluate and measure the capacity of the method to remain unaffected by small variation. Analytical concentration at level 100% is analyzed by preparations at each level (with duplicate readings) against a standard solution (Table 10, Fig 7-8).

When the mobile phase mixture changed to 65:35, the time needed for all Oxicams to be measured is less but with less resolution especially between PIR and MEL. This could be explained by increasing MeOH in the mobile phase which increases polarity and thus accelerates elution of PIR and MEL.

Slight variations in pH have been made to the analytical method in order to evaluate and measure the capacity of the method to remain unaffected by small variation. Analytical concentration at level 100% is analyzed by preparations at each level (with duplicate readings) against a standard solution and sample solution. It is noted that the developed method is robust for TEN, PIR and MEL when a pH variation of + 0.5 was performed (Table 11, Fig. 9). In contrast, after a pH variation of – 0.5, the assay % could not be calculated for PIR and MEL due to the overlapping between these components (Table 12, Fig. 10). The reduction of pH decreases the difference in polarity between PIR and MEL that leads in overlap elution.

CONCLUSION

The method finalized is clear, accurate, fast, economical and easy to apply. Such method may successfully replace the classical analytical methods of Oxicams. Furthermore, the results obtained in this work open new prospects for the quantitative determination of the Oxicams that can be applied to chemical laboratories, as well as to drug industry.

Table 1: The system precision of TEN, PIR, and MEL in the simultaneous assay method of determination

Parameters	TEN	PIR	MEL
Average area (RSD %)	2131772 (0.701)	3317783 (0.672)	4119413 (0.670)
Asymmetry	1.165	1.109	1.112
Theoretical plates	3354	3988	4029
Resolution	0.000	5.430	2.322
Initial retention time	3.857	5.528	6.398
Final retention time	3.877	5.568	6.460

Table 2: The precision of TEN, PIR and MEL in the simultaneous assay method of Oxicams

	Assay%		
Sample #	TEN	PIR	MEL
1	99.92	99.09	100.83
2	101.29	100.76	99.75
3	101.36	100.02	99.26
4	99.95	100.32	100.98
5	99.27	99.29	99.71
6	99.45	99.98	100.30
Average	100.21	99.91	100.14
RSD%	0.904	0.627	0.680

Table 3: The linearity of TEN assay in the simultaneous assay of Oxicams

Level # (of the label claimed)	Concentration (mg/L)	Average area	RSD %
50	1.010	1204008	0.921
80	1.616	1825138	0.288
100	2.020	2270347	0.108
120	2.424	2743320	0.238
150	3.030	3356370	0.135

Table 4: The linearity PIR assay in the simultaneous assay of Oxicams

Level # (of the label claimed)	Concentration (mg/L)	Average area	RSD %
50	1.513	1967208	1.204
80	2.420	2970161	0.225
100	3.025	3689570	0.114
120	3.630	4459266	0.201
150	4.538	5454767	0.063

Table 5: The linearity of MEL assay in the simultaneous assay of Oxicams

Level # (of the label claimed)	Concentration (mg/L)	Average area	RSD %
50	1.765	2477679	0.607
80	2.824	3758746	0.250
100	3.530	4644805	0.149
120	4.236	5643028	0.322
150	5.295	6872035	0.134

Table 6: The accuracy of measuring TEN in the simultaneous assay of Oxicams

Level # (of the label claimed)	Area	Recovered%	Average recovered% (RSD %)
50%	1035581	100.00	100.14 (0.309)
	1060327	101.88	
	1020522	98.55	
100%	2007881	98.67	100.11 (0.059)
	2083080	100.38	
	2073765	101.29	
150%	3083665	99.36	100.74 (0.061)
	3220348	101.23	
	3204311	101.62	

Table 7: The accuracy of PIR assay in the simultaneous assay of Oxicams

Level # (of the label claimed)	Area	Recovered%	Average recovered% (RSD %)
50%	1599577	99.14	98.94 (0.309)
	1636777	99.05	
	1639462	98.63	
100%	3188801	98.13	98.71 (0.033)
	3208997	98.45	
	3251375	99.55	
150%	4871249	100.23	100.02 (0.151)
	4924672	100.13	
	4893368	99.69	

Table 8: The accuracy of MEL assay in the simultaneous assay of Oxicams

Level # (of the label claimed)	Area	Recovered%	Average recovered% (RSD %)
50%	1874681	98.67	99.12 (0.456)
	1997153	99.00	
	1997153	99.68	
100%	3727382	98.15	99.90 (0.136)
	3919624	100.41	
	3983048	101.14	
150%	6070122	101.28	100.95 (0.094)
	6077153	101.05	
	6097683	100.53	

Table 9: Wavelength (±3 nm) effect on the asymmetry, %RSD and resolution of the simultaneous assay of Oxicams

Parameter	Wavelength **(nm)**								
	375			360			363		
Material	TEN	PIR	MEL	TEN	PIR	MEL	TEN	PIR	MEL
RSD %	0.568	0.551	0.613	0.701	0.672	0.670	0.449	0.436	0.441
Theoretical plates	3362	4077	4191	3354	3988	3988	3346	4000	4085
Asymmetry	1.177	1.120	1.126	1.165	1.109	1.112	1.179	1.117	1.117
Resolution	0.000	5.407	2.643	0.000	5.430	2.322	0.000	5.405	2.659

Table 10: Organic mixture (±5%) effect on the asymmetry, %RSD and resolution of the simultaneous assay of Oxicams

Parameter	Mobile phase 55:45 (Methanol : Buffer)			Mobile phase 60:40 (Methanol : Buffer)			Mobile phase 65:35 (Methanol : Buffer)		
Material	TEN	PIR	MEL	TEN	PIR	MEL	TEN	PIR	MEL
RSD %	0.307	0.331	0.251	0.701	0.672	0.670	0.353	0.431	0.424
Theoretical plates	3163	4016	4149	3354	3988	3988	3618	4200	4255
Asymmetry	1.147	1.084	1.098	1.165	1.109	1.112	1.206	1.141	1.140
Resolution	0.000	7.057	3.382	0.000	5.430	2.322	0.000	3.931	1.897

Table 11: Mobile phase pH (+5%) effect on the %RSD of the simultaneous assay of Oxicams

Sample #	Assay %		
	TEN	PIR	MEL
1	101.51	98.70	100.15
2	101.23	98.37	99.89
3	101.56	98.75	100.28
Average	101.43	98.61	100.11
RSD%	0.175	0.209	0.198

Table 12: Mobile phase pH (-5%) effect on the %RSD of the simultaneous assay of Oxicams

Sample #	Assay %		
	TEN	PIR	MEL
1	101.11	-	-
2	101.72	-	-
3	101.93	-	-
Average	101.59	-	-
RSD%	0.419	-	-

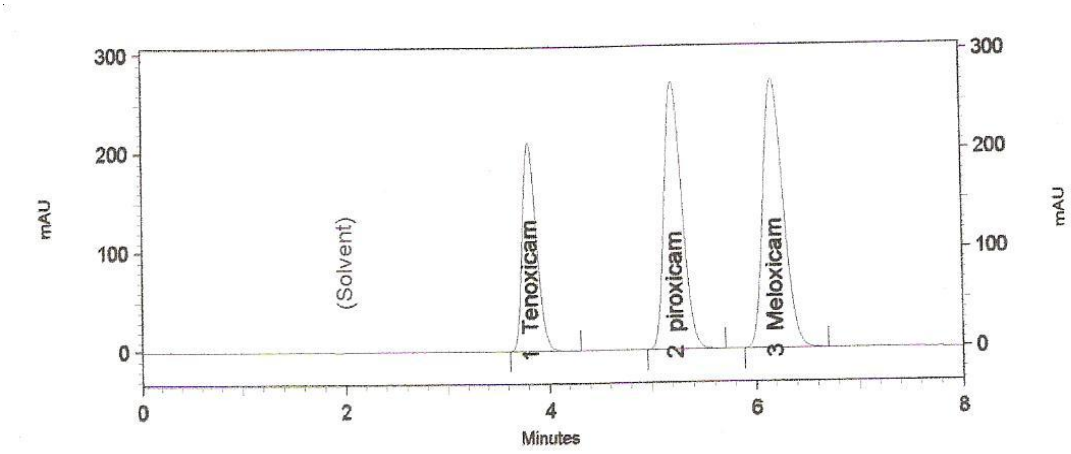

Figure 1: A chromatogram of TEN, PIR and MEL when administered together in one sample

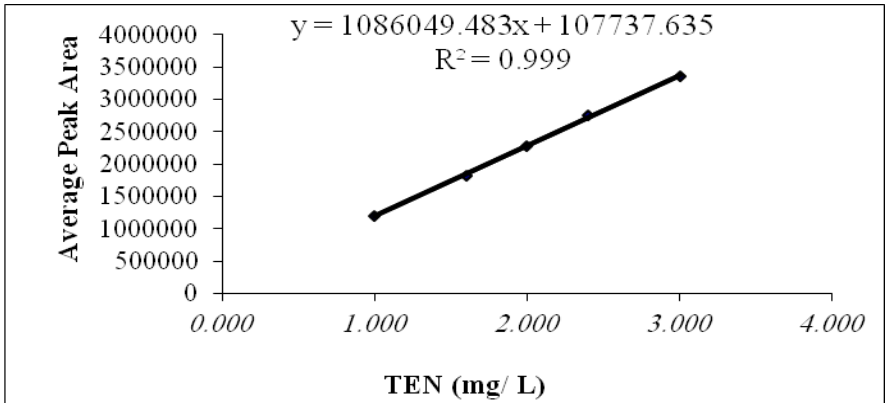

Figure 2: Calibration curve of peak area versus concentration (mg/L) of TEN

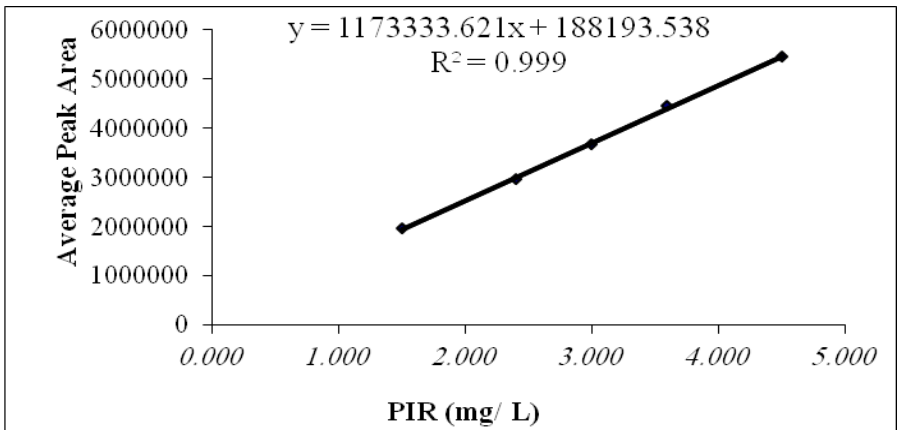

Figure 3: Calibration curve of peak area versus concentration (mg/L) of PIR

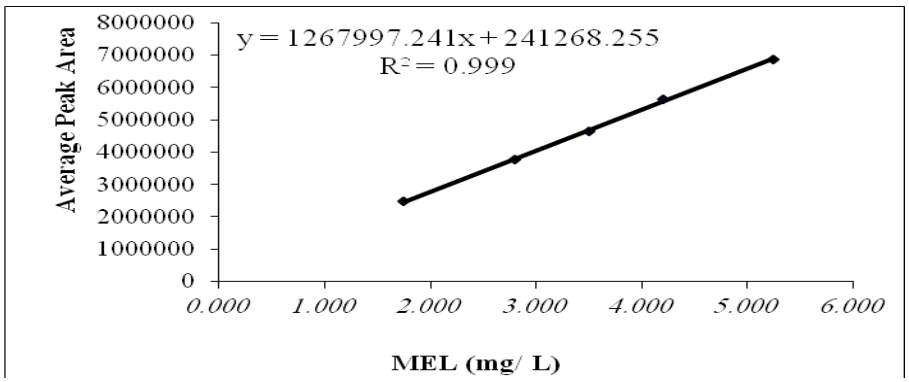

Figure 4: Calibration curve of peak area versus concentration (mg/L) of MEL

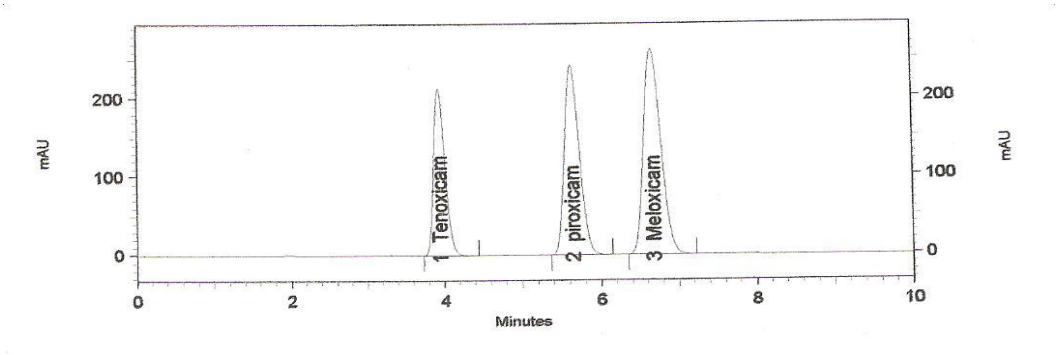

Figure 5: A chromatogram of Oxicams in relation to a change in the wavelength (+3 nm) for a standard solution

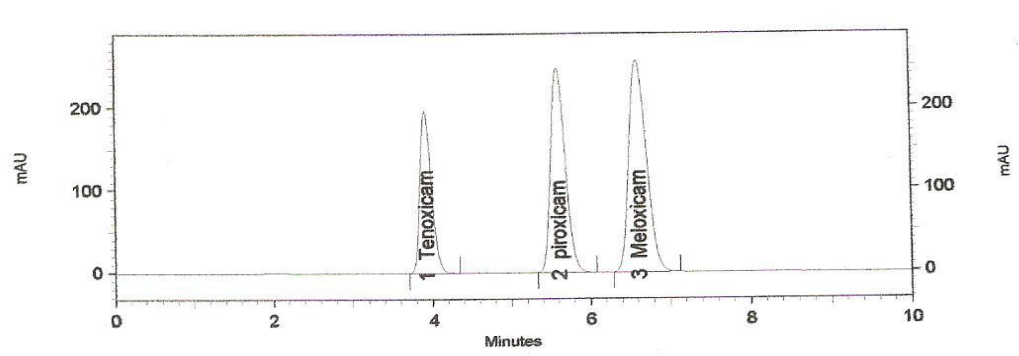

Figure 6: A chromatogram of Oxicams in relation to a change in the wavelength (−3 nm) for a standard solution.

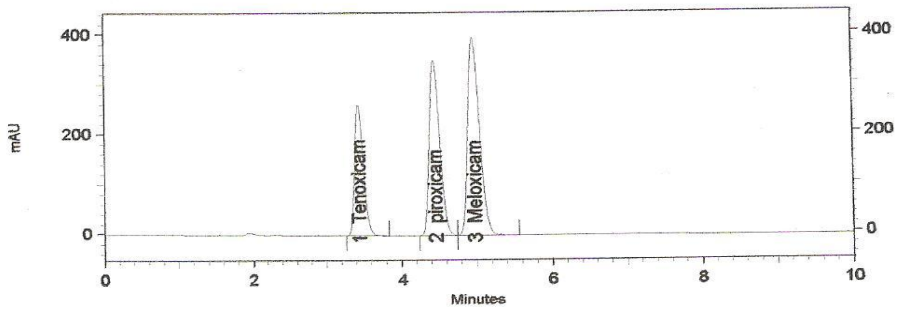

Figure 7: A chromatogram of Oxicams in relation to a change in organic mixture +5% for a standard solution

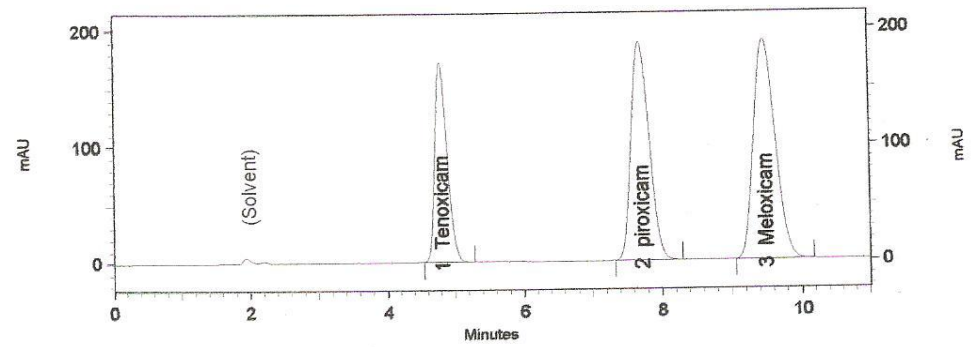

Figure 8: A chromatogram of Oxicams in relation to a change in organic mixture −5% for a standard solution

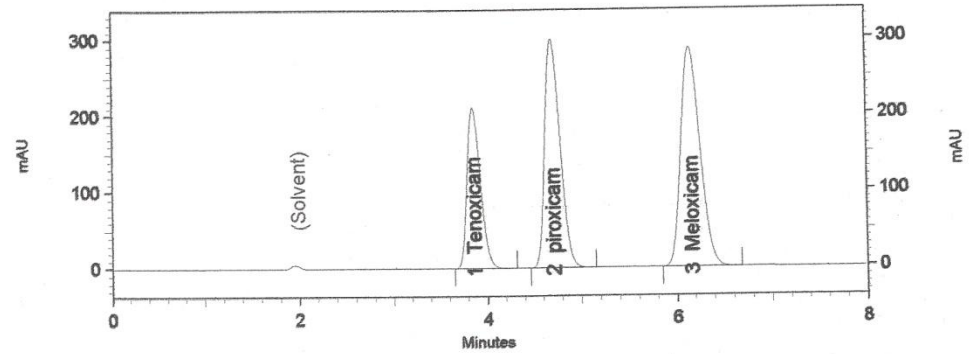

Figure 9: A chromatogram of Oxicams in relation to a change in the mobile phase pH + 0.5 for a standard solution

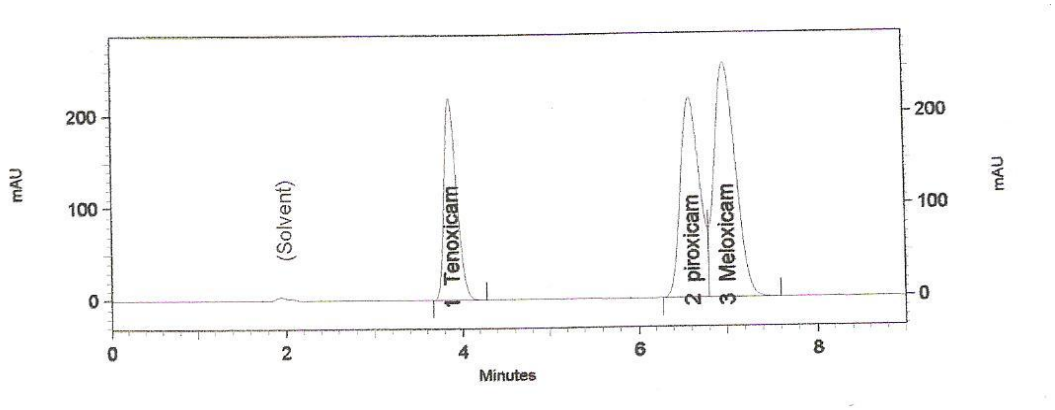

Figure 10: A chromatogram of Oxicams in relation to a change in the mobile phase pH -0.5 for a stand

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