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Stability indicating HPTLC method development and validation for estimation of Methocarbamol and Diclofenac sodium in combined dosage form

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Abstract
A specific, accurate, precise and robust HPTLC method was developed and validated for estimation of Methocarbamol and Diclofenac sodium in combined dosage form. For HPTLC, Hamilton microlitre syringe (Linomat syringe 659.0014, Hamilton-Bonaduz Schweiz, Camag, Switzerland) was used. The solid phase containing cylinder specifications are given bellow here. UV chamber (Camag, Switzerland), Twin trough chamber (20 × 10 cm; Camag, Switzerland), Linomat 5 sample applicator (Camag, Switzerland) and TLC scanner 4 (Camag, Switzerland) operated by win CATS version 1.4.6 software (Camag, Switzerland) were used in the study. All drugs and chemicals were weighed on an electronic balance (AUW 220, Shimadzu Corp., Japan). All data calculations were performed using Microsoft Excel 2010 software (Microsoft Corporation, USA).

Keywords: HPTLC, methocarbamol, diclofenac sodium

Introduction
Methocarbamol is centrally acting muscle relaxant and Diclofenac sodium is NSAIDS class anti-inflammatory agent. Combination of these drugs is used in treatment for the relief of pain and inflammation associated with Rheumatoid arthritis, osteoarthritis, ankylosing spondylitis, low back pain and other acute musculoskeletal disorders.
This NSAIDS are available in market with various brand names but unique, responsive, quick, sharp, and cost-effective HPTLC method for their analysis in combine dosage form have not been reported.

Methodology
Selection of column (solid phase containing cylinder) for HPTLC
It took robust literature survey for selection of that provides better resolution, with peak shape and Rf. The selection specifications are,

Solid phase containing cylinder for HPTLC:
Precoated silica gel aluminium plate 60 F254
Manufacturer   :   E. Merck, Darmstadt, Germany
Dimensions   :   20 × 20 cm, 100-μm thickness

Selection of wavelength for detection for HPTLC
On scanning of both the drugs in range of 200-400 nm three iso absorptive point were found which are 238 nm and 274 nm. Amongst all 274 nm having higher absorbance compared to others which is beneficial for estimation of drugs in lower concentration. So the optimum wavelength for detection was set at 274.0 nm, which was obtained by scanning in the range of 200-400 nm against methyl alcohol as a blank. Sample spotted of MET 10,000 ng/band and DICLO 1000 ng/band.
On scanning of drugs by HPTLC, Methocarbamol shows three degraded products but Methocarbamol itself having distinct peak with Rf 0.63 which is recordable. On scanning of both the drugs by HPTLC, they shows 2 degraded product but Methocarbamol and Diclofenac itself having distinct peaks with Rf 0.23 and 0.73 which is recordable. So, drug can be easily identified and estimated amongst it degraded product which means that proposed method is used to estimate drugs in acid stress condition without any significant problem.

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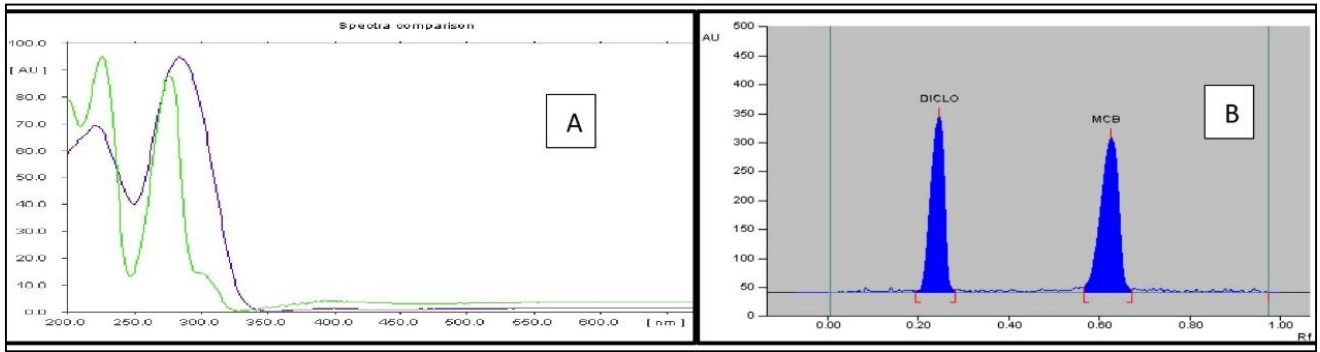Research Scholar at JJT
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Fig 1: Overlay UV Graphical response of MET and DIC
Fig 2: Optimized chromatogram of MET & DIC

Stress study (Stability study)
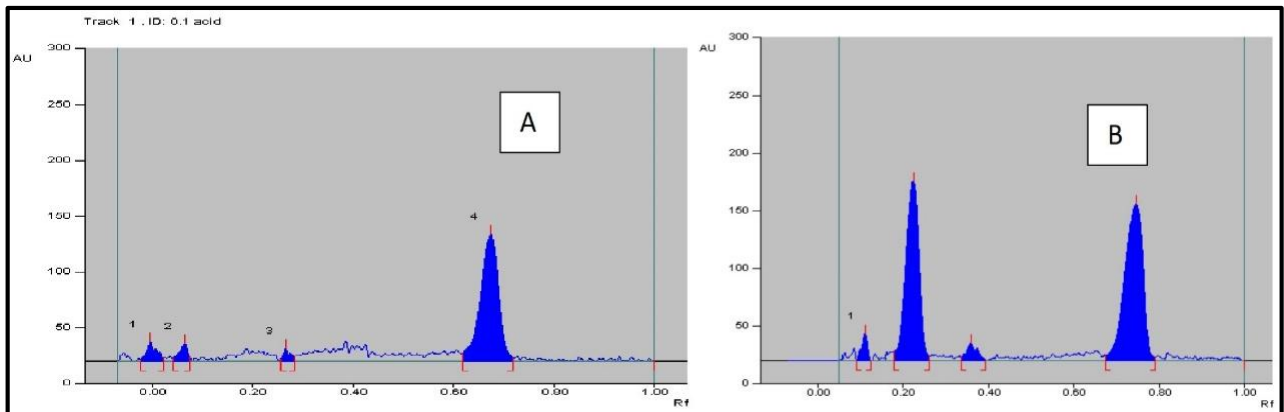**1. Acid stress study (Stability study)**

Fig 3: Chromatogram of MET in acid stress condition
Fig 4: Chromatogram of MET and DIC test in acid stress condition
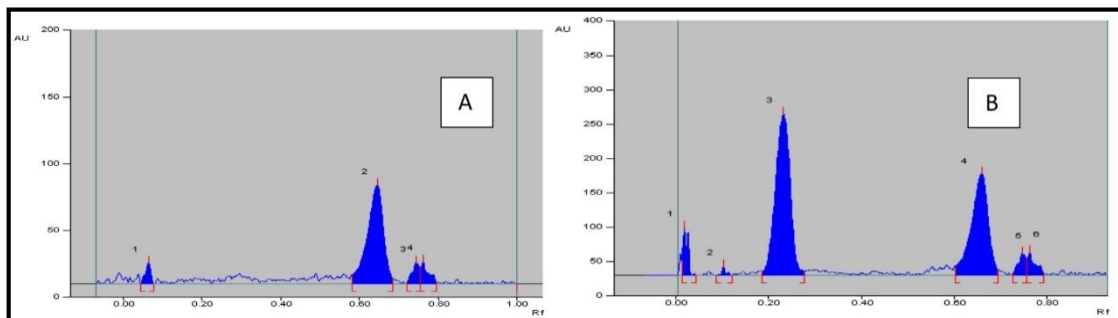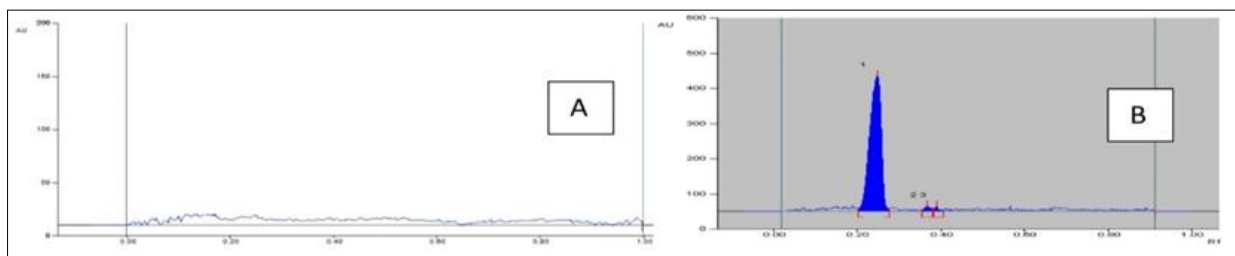
2. Base stress study (Stability study)

Fig 5: Chromatogram of MET in base stress condition
Fig 6: Chromatogram of MET and DIC test in base stress condition

On scanning of drugs by HPTLC, Methocarbamol shows 4 degraded product but Methocarbamol itself having distinct peak with Rf 0.65 which is recordable. So, drug can be easily identified amongst it degraded product.

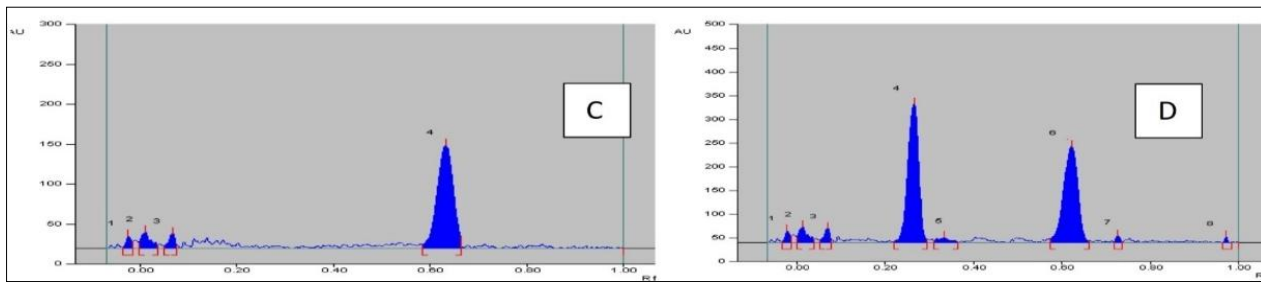**3. Oxidative stress study (Stability study)**

Fig 7: Chromatogram of Oxidative blank
Fig 8: Chromatogram of DIC in Oxidative stress condition
Fig 9: Chromatogram of MET in Oxidative stress condition
Fig 10: Chromatogram of MET and DIC test in Oxidative stress condition

On scanning of both the drugs by HPTLC, they shows 4 degraded product but Methocarbamol and Diclofenac itself having distinct peaks with Rf 0.68 and 0.23 and which is recordable. So, drug can be easily identified and estimated amongst it degraded product which means that proposed method is used to estimate drugs in base stress condition without any significant problem on scanning of drugs by HPTLC, Diclofenac shows 2 degraded product but Diclofenac itself having distinct peak at 3.2 min which is recordable. So drug can be easily identified amongst it degraded product.

On scanning of drugs by HPTLC, Methocarbamol shows 3 degraded product but Methocarbamol itself having distinct peak with Rf 0.64 which is recordable. So drug can be easily identified amongst it degraded product.
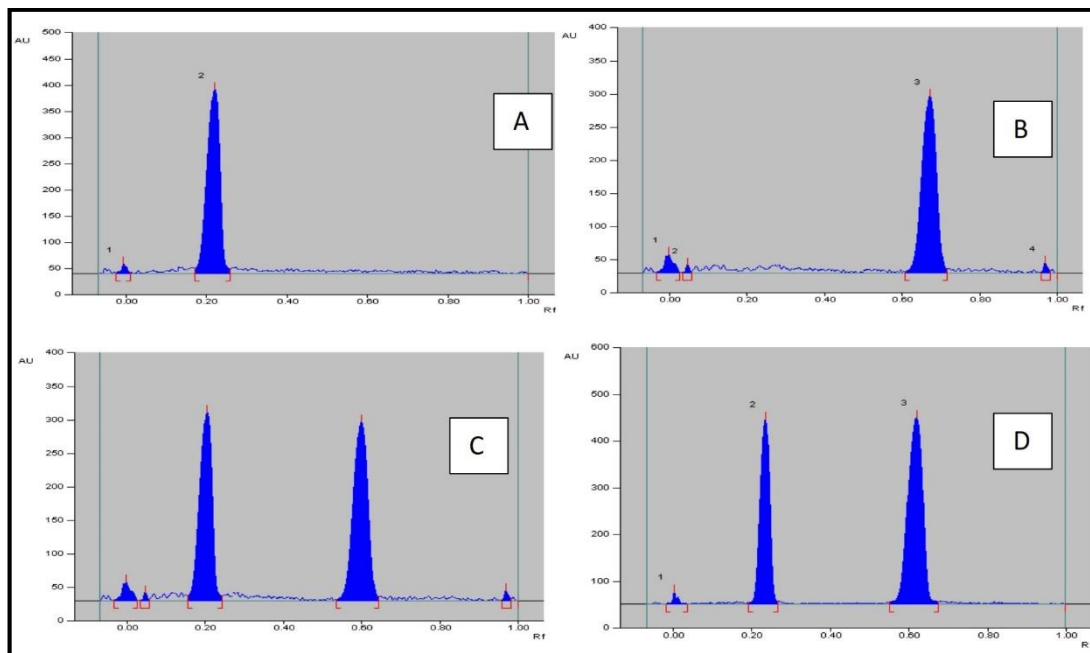
4. Photo stability study

Fig 11: Chromatogram of DIC in Photo stress condition
Fig 12: Chromatogram of MET in Photo stress condition
Fig 13: Chromatogram of MET and DIC test in Photo stress condition
Fig 14: Chromatogram of MET and DIC test in Thermal stress condition

On scanning of both the drugs by HPTLC, they shows 6 degraded product but Methocarbamol and Diclofenac itself having distinct peaks with Rf 0.62 and 0.23 which is recordable. So drug can be easily identified and estimated amongst it degraded product which means that proposed method is used to estimate drugs in Oxidative stress condition without any significant problem.

5. Thermal stress study (Stability study)

On scanning of both the drugs by HPTLC, they show 1 degraded product but Methocarbamol and Diclofenac itself having distinct peaks with Rf 0.59 and 0.22 which is recordable. So, drug can be easily identified and estimated amongst it degraded product which means that proposed method is `problem. The said study reported in figure no 14.

Result and Discussion

Table 1: Stability study data for Methocarbamol and Diclofenac by HPTLC

S. No	Degradation	% Recovery			Avg	SD	% RSD	% Degradation
		1	**2**	**3**				
				DICLO				
1.	Acid	79.9	80.91	79.78	80.21	0.49	0.62	19.78
2.	Base	92.8	92.70	94.18	93.24	0.66	0.71	6.75
3.	Oxidation	95.5	97.77	96.35	96.57	0.90	0.93	3.42
4.	Thermal	95.5	101.7	97.38	98.21	2.60	2.65	1.78
5.	Photolytic	99.1	97.92	102.1	99.76	1.78	1.78	0.23
				MET				
1.	Acid	90.8	89.31	88.62	89.59	0.92	1.03	10.40
2.	Base	86.7	87.53	87.86	87.39	0.44	0.51	12.60
3.	Oxidation	82.3	85.97	84.42	84.23	1.50	1.78	15.76
4.	Thermal	97.5	98.01	98.78	98.10	0.52	0.53	1.89
5.	Photolytic	99.2	100.3	99.77	99.80	0.47	0.47	0.19

Assay was performed by using marketed dosage form by proposed method and instrument. % Assay was found to be 100.59 and 101.61 for MET and DIC respectively. % assay found more than 90 % indicates method can be used for marketed dosage form for given drugs. So method can be used for used dosage form and any other similar dosage form.

All developed methods have their own distinct advantages and works on different unique principle thus actually one cannot compare all of them on single platform. Although the effort was made here to compare their accuracy to test % drug content.

For Methocarbamol Results are as stipulated here. The Average Assay was 100.59 % with standard deviation (SD) 0.69 and % RSD obtained was 0.69 with number of repetitions, n was 3.

Results of Diclofenac Sodium, Average Assay was 101.61% with standard deviation 0.31 and % RSD was 0.3 where number of repetitions, n was 3.

The results comparison clearly shows that all methods shows nearly 100% assay determination and <2 % RSD for both the drug but the HPTLC method shows lower % RSD for MET and lowest for DIC determination indicating most significant method.

The proposed methods signify high accuracy, selectivity and reproducibility. These advantages recommend the usage of the planned approaches in routine and quality control analysis without interference of commonly encountered pharmaceutical preparation additives.

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