

Spectrofluorometric Determination of Rofecoxib and Mosapride Citrate in their Individual Dosage Form

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Two new specific, selective, simple, rapid and inexpensive spectrofluorometric methods have been developed for the determination of rofecoxib and mosapride citrate in their individual dosage forms. Spectra of rofecoxib in 0.1 N sulphuric acid and mosapride in methanol showed excitation wavelength to be 282 nm and 331 nm while emission wavelength to be 406 nm and 360 nm respectively. Method for rofecoxib was found to be linear over an analytical range of 10-50 ng/ml with correlation coefficient of 0.9990. Limit of detection and limit of quantitation were found to be 1.71 ng/ml and 5.69 ng/ml, respectively for the same method. Method for mosapride was found to be linear over an analytical range of 2-10 ng/ml with correlation coefficient of 0.9996. Limit of detection and limit of quantitation were found to be 0.32 ng/ml and 1.05 ng/ml, respectively for the same method. Both the methods were validated and found to be suitable for estimation of rofecoxib and mosapride from their individual pharmaceutical formulations. Satisfactory recovery from the spiked samples of standard drugs suggests no interference of any excipients present in the formulations.

Rofecoxib¹ (ROF) is comparatively a new non-steroidal antiinflammatory drug², which is active at a low dose³ and chemically it is 4-[4-(methylsulfonyl) phenyl]-3-phenyl-2(5H)-furanone. ROF is not official in any of the pharmacopoeia. Various methods for estimation of ROF reported in literature were HPLC with post-column photochemical derivatization^{4,5}, HPLC⁶⁻¹⁰, HPLC with tandem mass spectrometry^{11,12}, micellar electrokinetic capillary chromatographic (MEKC)¹³, LC¹⁴, LC-MS¹⁵⁻¹⁷, monolithic silica LC¹⁸, difference spectroscopy¹⁹, derivative spectrophotometry²⁰ and UV/Vis spectrophotometry^{10,21,22}. Mosapride citrate²³ (MOS), a successor to cisapride, is a selective 5HT₄ receptor agonist²⁴ and has been introduced as citrate salt for its pharmacokinetic actions. Chemically it is enantiomeric mixture of \pm 4-amino-5-chloro-2-ethoxy-N-[[4-(4-fluorobenzyl)-2-morpholinyl] methyl] benzamide citrate (AS-4370)^{25,26}. Few HPLC²⁷⁻³⁰ methods have been reported in literature for determination of MOS in serum, bulk drug and pharmaceutical formulations. Since spectrofluorometric methods

are highly sensitive, selective and often less expensive than HPLC, the objective of this study was to develop accurate, precise, sensitive, selective, reproducible and quick spectrofluorometric methods for determination of ROF and MOS.

MATERIALS AND METHODS

A Shimadzu Spectrofluorometer (Model RF-540 with DR-3 data recorder), equipped with a 1 cm fluorescence free quartz cell having all transparent sides was used for all spectral and fluorescence measurements. Glassware used in each procedure were soaked overnight in a chromic mixture (K₂Cr₂O₇+concentrated H₂SO₄), rinsed thoroughly with double distilled water and dried in dust-free air. Whatman filter paper No. 42 was used to filter the solution of different formulations to separate them from the solvent immiscible formulation excipients.

An authentic working standard for ROF was kindly gifted by Torrent Research Centre, Gandhinagar, and the same for MOS dihydrate was obtained as a gift sample from Intas Pharmaceuticals, Ahmedabad. Double distilled water and analytical-reagent grade chemicals were used. Metha-

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nol and sulphuric acid were purchased from Allied Chemicals Corporation, Vadodara. 0.1 N H₂SO₄ was prepared and standardized as per IP-1996³¹. Tablets and suspensions were procured from local drug store. (Zyrof® tablet of Zydus Cadila containing 12.5 mg of ROF, Roflam® tablet of Micro Nova Pharmaceuticals Pvt. Ltd. containing 12.5 mg of ROF, Torrox® suspension of Torrent Pharmaceuticals Ltd. containing 12.5 mg/5 ml of ROF, Mosa® tablet of Intas Pharmaceuticals Pvt. Ltd. containing 5 mg of MOS and Mozasef® tablet of Sun Pharmaceutical Industries Ltd. containing 5 mg of MOS).

Preparation of standard solution for calibration curve:

ROF is highly photosensitive and can degrade to even more fluorescent product in the presence of light so the solutions were prepared freshly and protected from laboratory light by covering them with aluminum foil. Stock solution of 100 µg/ml of ROF was prepared in methanol from which a working stock solution containing 0.2 µg/ml was prepared in 0.1 N H₂SO₄. Suitable aliquots of the working stock solution were taken in to 10 ml volumetric flask and volumes were made up with 0.1 N H₂SO₄ to prepare a series of standard solution (10-50 ng/ml) for calibration curve. MOS was dissolved in methanol to prepare a stock solution of 100 µg/ml. Series of standard solutions (2-10 ng/ml) were prepared from above stock solution by taking suitable aliquots in to 10 ml volumetric flasks and diluting with methanol. All the stock solutions and working standard solutions of ROF and MOS were protected from day light by wrapping in aluminum foil.

Preparation of sample solution:

Twenty tablets were weighed, powdered and tablet powder equivalent to 2.5 mg of ROF was taken and stirred with 15 ml methanol on magnetic stirrer for about 30 min at 40°. ROF from the syrup was extracted in the same manner by taking the suspension equivalent to 2.5 mg of ROF in place of tablet powder. Then it was filtered through Whatman filter paper No. 42 into 25 ml of volumetric flask. Filter paper was rinsed thrice with 2 ml of methanol and volume was made up with methanol to prepare 100 µg/ml. From above solution suitable aliquots were taken and diluted with 0.1 N H₂SO₄ to prepare the sample solution in the range of calibration curve.

Twenty tablets were weighed, powdered and powder equivalent to 10 mg of MOS was taken and stirred with 50 ml methanol on magnetic stirrer for about 30 min. Then it was filtered through Whatman filter paper No. 42 into 100 ml of volumetric flask. Filter paper was rinsed twice with

5ml of methanol and volume was made up with methanol to prepare 100 µg/ml. From the above solution suitable aliquots were taken and diluted with methanol to prepare the sample solution in the range of calibration curve.

Assay procedure:

Standard solution of ROF was scanned in the range of 200-320 nm for determination of excitation wavelength and it was found to be 282 nm. Same solution was scanned for determination of emission wavelength in the range of 350-450 nm taking 282 nm as excitation wavelength and it was found to be 406 nm.

Similarly standard solution of MOS was scanned in the range of 250-350 nm for determination of excitation wavelength and it was found to be 331 nm. Same solution was scanned for determination of emission wavelength in the range of 300-450 nm taking 331 nm as excitation wavelength and it was found to be 360 nm.

RESULTS AND DISCUSSION

Under the established conditions, the ROF and MOS obey Beer's law over the concentration range of 10-50 ng/ml and 2-10 ng/ml at 406 nm and 360 nm as shown in figs. 1 and 2, respectively. Other parameters like excitation wave-

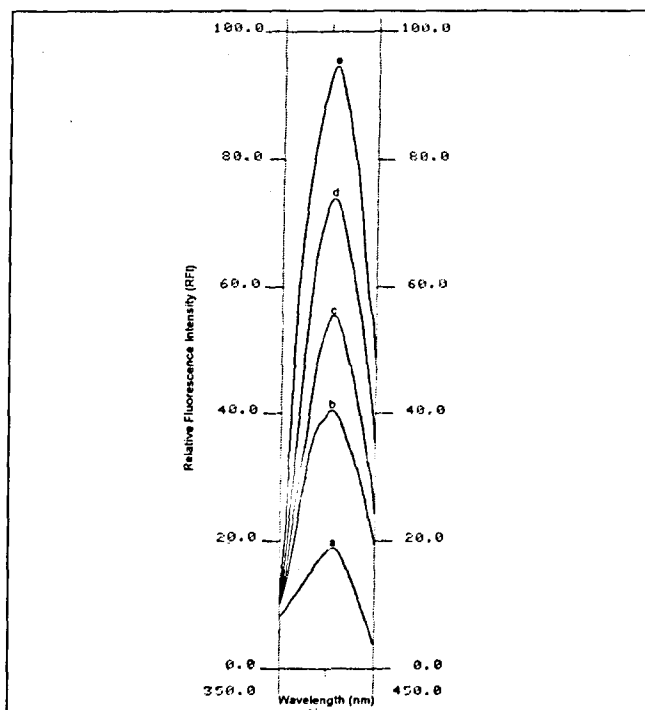


Fig 1: Emission fluorescence spectra of ROF at 406 nm. (a) 10 ng/ml, (b) 20 ng/ml, (c) 30 ng/ml, (d) 40 ng/ml and (e) 50 ng/ml.

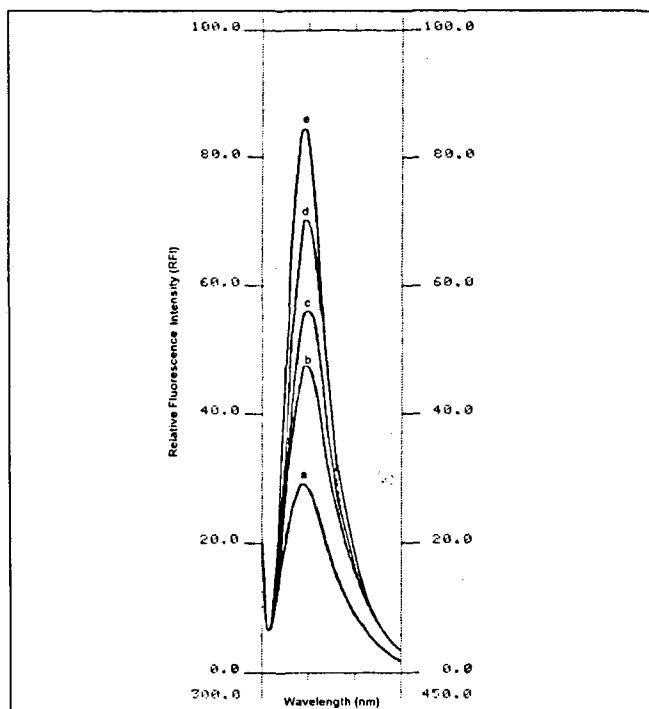


Fig 2: Emission fluorescence spectra of MOS at 360 nm. (a) 2 ng/ml, (b) 4 ng/ml, (c) 6 ng/ml, (d) 8 ng/ml and (e) 10 ng/ml.

length, emission wavelength, regression equation, coefficient of determination (r^2), correlation coefficients (r), limit of detection (LOD), limit of quantitation (LOQ), etc are enumerated in Table 1. Calibration curve for both the drugs were repeated for five time and RSD at each concentration level was found to be less than 1%, which indicates that methods can be used for analysis of bulk drug samples. The correlation coefficient values obtained are highly significant for both the methods. Recovery studies were carried out by adding known amount of standard drug to the previously analyzed samples at three levels. Result of recovery studies are given in Table 2, and they were more than 98% for all formulations. Satisfactory recovery indicated no interference of excipients on extraction efficiency of drugs from the sample matrices.

Developed methods were subjected to analytical validation and analytical parameters such as accuracy, precision, linearity, limit of detection (LOD) and limit of quantitation (LOQ). Inter-day and intra-day RSD were also found out to ascertain precision and accuracy of the developed methods. For both the methods the accuracy was greater than 98% and RSD did not exceed 2% in any case. The low values of standard deviation and coefficient of variation establish the precision of the proposed methods. Stan-

TABLE 1: OPTICAL CHARACTERISTICS AND ANALYTICAL DATA

Parameter	Rofecoxib (ROF)	Mosapride Citrate (MOS)
Excitation wavelength, λ_{Ex} (nm)	282	331
Emission wavelength, λ_{Em} (nm)	406	360
Linearity range (ng/ml)	10-50	2-10
Regression equation (Y^a)	$Y=1.8965x+1.1646$	$Y=6.725x-0.29$
Slope (b)	1.8965	6.725
Intercept (a)	1.1646	-0.29
Coefficient of determination (r^2)	0.9981	0.9993
Correlation coefficient (r)	0.9990	0.9996
Limit of detection, LOD (ng/ml)	1.71	0.32
Limit of quantitation, LOQ (ng/ml)	5.69	1.05
Inter-day % RSD	<1.84%	<1.91%
Intra-day % RSD	<1.76%	<1.85%
Accuracy	>98.74%	>98.96%

^a $Y=a+bx$, where x is the concentration (ng/ml). $LoD=3\sigma/S$, $LoQ=10\sigma/S$, where σ is standard deviation of blank, and S slope of calibration.

TABLE 2: RECOVERY STUDY OF PHARMACEUTICAL FORMULATIONS

Formulation	Standard Drug added (mg)	Drug found*±SD (mg)	% Recovery
ROF T-1	12.5	12.48±0.16	99.86
ROF T-2	12.5	12.39±0.13	99.13
ROF S-1	12.5mg/5ml	12.5±0.17	100.2
MOS T-1	5	4.99±0.08	99.97
MOS T-2	5	4.96±0.10	99.24

*Average of three determinations at three levels. ROF T-1 tablet contain 12.5 mg of ROF, ROF T-2 tablet contain 12.5 mg of ROF, ROF S-1 suspension contain 12.5 mg/5 ml of ROF, MOS T-1 tablet contain 5 mg of MOS and MOS T-2 tablet contain 5 mg of MOS.

Standard stock and working standard solutions of ROF and MOS did not show significant change in relative fluorescence intensity and hence were stable for up to 6 h when wrapped in aluminum foil.

From the discussion above, it is clear that the developed methods are accurate, precise, repeatable, reproducible, linear, quicker, inexpensive, sensitive and simple. In absence of any reported method for estimation of ROF and MOS by spectrofluorophotometry, proposed methods make them useful in the routine and quality control analysis of bulk drug and pharmaceutical formulations.

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