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Simultaneous Determination of Rofecoxib and Tizanidine by HPTLC

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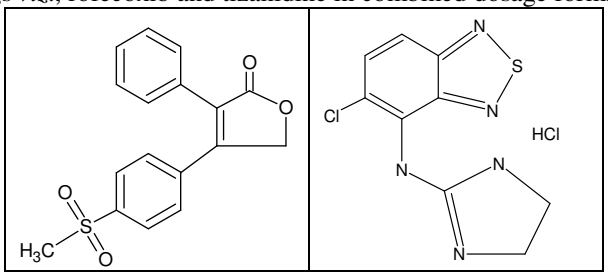
Abstract: An innovative high performance thin layer chromatography method was developed and validated for simultaneous determination of rofecoxib and tizanidine from tablet dosage form. Rosiglitazone maleate was used as an internal standard. The separation was achieved using HPTLC plates (Merck #5548) precoated with silica gel $60F_{254}$ on aluminum sheets and a mobile phase comprising of toluene: ethyl acetate: methanol: triethyl amine in volume ratio of 6:3:0.5:0.1 (v/v/v/v), with chamber saturation of 15 min. The plate was developed up to 8 cm and air dried. The plate was then scanned and quantified at 235 nm. The linearity of rofecoxib and tizanidine were in the range of 3.75 µg/spot to 11.25 µg/spot and 0.30 µg/spot to 0.90 µg/spot respectively. The limit of detection for rofecoxib and tizanidine was found to be 45.00 ng/spot and 30.00 ng/spot respectively. The limit of quantification for rofecoxib and tizanidine was found to be 135.00 ng/spot and 90.00 ng/spot respectively. The percentage assay was found between the range of 99.58% to 103.21% for rofecoxib and 98.73% to 101.55% for tizanidine respectively, whereas recovery was found between 99.97% to 100.43% for rofecoxib and 100.00% to 101.00% for tizanidine by standard addition method. The proposed method is accurate, precise and rapid for the simultaneous determination of rofecoxib and tizanidine in dosage form.

Keywords: HPTLC, Rofecoxib, Tizanidine, Tablet.

Introduction

Pharmaceutical analysis is an integral part of pharmaceutical chemistry. In special drug laboratories the responsibilities of performing analysis is delegated entirely to the staff members. Whenever a new molecule is delivered to have therapeutically beneficial effects; research on the molecule starts as also developing new methods for assay. Today the importance is not only based on the assay of the compound but also on the role played by the analyst in quality control or quality assurance of the product. Thus common research in the pharmaceutical analysis is a continuous process as the synthesis and discovery of a new drug. Not to be outdone one method, which is reported need not spell the end of such an assay. Hence a search is required for a better method where the throughput/unit time is higher and where method is more precise. Rofecoxib[1-3], described chemically as 4-4'-(methyl sulphonyl phenyl)-3-phenyl-5*H*-furan-2-one is a non-steroidal anti-inflammatory drug. Its molecular formula is $C_{17}H_{14}O_4S$ and molecular weight is 314.36. Molecular structure of rofecoxib is given Figure 1 (a).

The mechanism of action of rofecoxib is believed to be due to inhibition of prostaglandin synthesis *via* inhibition of cyclo-oxygenase-2 (COX-2)[4]. Tizanidine hydrochloride[1-3] is chemically described as 4-chloro-*N*-(4,5-dihydro-1*H*-imidazol-2-yl)-8-thia-7,9 diazabicyclo[4,3,0]nona-2,4,6,9-tetraen-5-amine, centrally acting alpha 2-adregenic drug, widely tested and documented for the treatment of spastic condition associated with muscles. Its molecular formula is $C_9H_8ClN_5S$. HCl and molecular weight is 469.61. Molecular structure of tizanidine is given Figure1 (b). Its major site of action is in the spinal cord. It preferentially inhibits polysynaptic mechanism responsible for extensive muscle tone, mainly by reducing the excitatory amino acid from interneutrons[4]. Use of rofecoxib and tizanidine in combination is effective against inflammatory pains[4]. Fixed dose containing rofecoxib (25 mg) and tizanidine (2 mg) is available in the market as tablet form. In this present work a new HPTLC method is developed, optimized and validated[5-7] for the assay of two drugs *viz*., rofecoxib and tizanidine in combined dosage forms.



(a) Rofecoxib (b) Tizanidine

Figure 1. Structure of rofecoxib and tizanidine

In the literature, only one HPTLC[8] method and few HPLC[9-10] methods are reported for the simultaneous determination of rofecoxib and tizanidine in dosage form. Although the described HPTLC[8] method gave reproducible results, however the method was relatively less sensitive (LOD for rofecoxib and tizanidine was found to be 20 ng and 200 ng respectively whereas LOQ was found to be 0.4 mg and 2.0 mg respectively) and it is without internal standard. Along with these, several analytical methods[11-21] like spectrophotometry, spectroflurimetry and HPLC have been reported for the estimation of rofecoxib and tizanidine from their formulations as well as in biological fluid. In this communication, we

report a new simple, rapid and sensitive HPTLC method for simultaneous determination of rofecoxib and tizanidine in combination dosage form, which can be used for the routine analysis in ordinary laboratories. Method is developed, optimized and validated accordingly.

Experimental

Reference standards and chemicals

The pure reference standards rofecoxib, tizanidine and rosiglitazone were obtained from TDM Labs. Sion, Mumbai as gift reference standard with certificate of analysis. The formulation, tablets sample with combination of rofecoxib (25 mg) and tizanidine (2 mg) is available in market. toluene, acetonitrile, methanol and formic acid used were of analytical grade. All dilutions were performed in standard volumetric flasks.

Preparation of standard stock solutions

Accurately weighed 251.2 mg of rofecoxib (99.54 %) was taken in a 25.0 mL volumetric flask. This was dissolved in minimum quantity of acetonitrile and diluted up to the mark with acetonitrile to get 10,000 µg/mL of rofecoxib (Sol. A).

Accurately weighed 172.4 mg of tizanidine hydrochloride (98.99 %) was taken in a 25.0 mL volumetric flask. This was dissolved in minimum quantity of acetonitrile and diluted up to the mark with acetonitrile to get 5,000 µg/mL of tizanidine (Sol. B).

Accurately weighed 167.5 mg of rosiglitazone maleate (99.19 %) was taken in a 25.0 mL volumetric flask. This was dissolved in minimum quantity of acetonitrile and diluted up to the mark with acetonitrile to get 5,000 µg/mL of rosiglitazone maleate (Sol. C).

Preparation of working solution

Further, the mixture of working solution was prepared by diluting 12.50 mL of rofecoxib (10,000 µg/mL) and 2.00 mL of tizanidine (5000 µg/mL) with 0.30 mL of rosiglitazone, internal standard (5000 µg/mL) stock solution in 50.0 mL volumetric flask with methanol to get strength of 2500.00 µg/mL of rofecoxib and 200.00 µg/mL of tizanidine.

Preparation of sample solution

Twenty tablets were (Torroxx-TX®) weighed and average weight was calculated. These tablets were crushed, powdered and taken in a 10 mL volumetric flask weight equivalent to one tablet and dissolved in minimum amount acetonitrile. To this flask 0.3 mL of stock solution of internal standard (5000 µg/mL of rosiglitazone) was added and diluted up to the mark with methanol and sonicated for 30min. This solution was then filter through Whatman no. 41.The filtrate was collected in the flask and used as sample solution.

Instrument

Camag, Linomat IV sample applicator was used. Camag Twin trough glass chamber (20 x 10 cm) was used for development of plates and Camag TLC scanner III equipped with cats 3.0 version software was used for interpretation of data.

Optimized chromatographic conditions

The experiment was performed on silica gel $60F_{254}$ HPTLC plates pre-coated on aluminum sheet using mobile phase, comprising of toluene: ethyl acetate: methanol: triethyl amine in the volume ratio (6.0:3.0:0.5:0.1). The plate was pre-washed by methanol and activated in an oven at 110^0C for 1 h before use. 3.0 µL sample solutions were applied on the HPTLC plate as sharp bands of 5 mm width with the help of Camag Linomat IV sample applicator at

the distance of 15 mm from the edge of the HPTLC plate with the speed of 10 sec/µL. Ascending development to distance of 8cm was performed in saturated 20 cm x 10 cm camag twin trough chamber for 15 min at room temperature. The developed TLC plate was air dried and then scanned between 200 and 400 nm using Camag TLC Scanner III. The wavelength chosen for further quantification was 235 nm. The overlay UV spectra for Rofecoxib and Tizanidine are shown in Figure 2.

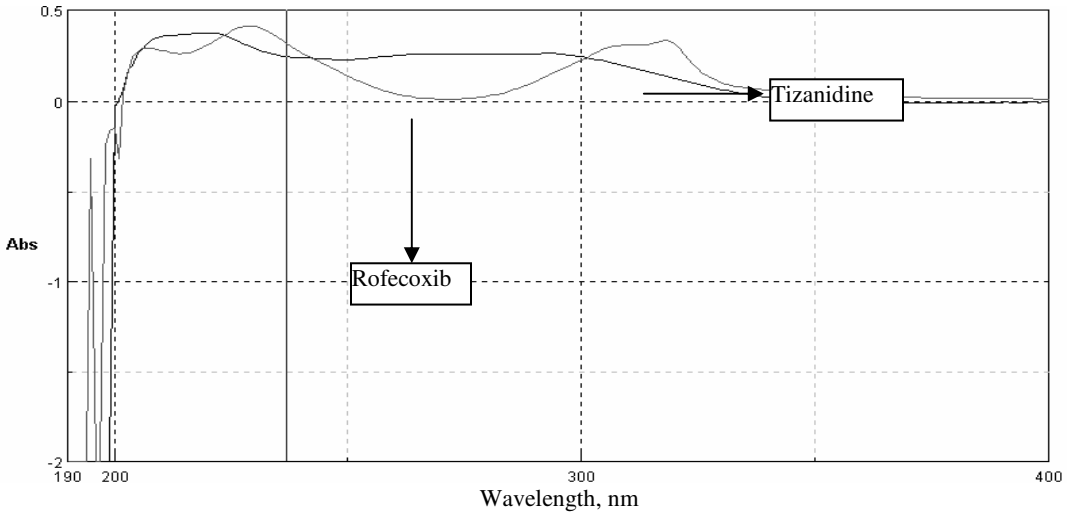


Figure 2. Overlay UV spectra of a) Rofecoxib and b) Tizanidine in methanol

Validation Procedures

System suitability test

A system-suitability experiment was performed before determination of rofecoxib and tizanidine in unknown samples by spotting 3 µL solution of 7.50 µg/spot of Rofecoxib and 0.60 µg/spot of Tizanidine solution. The coefficient of variation (CV) for peak area ratio of value for both the drugs was less than 2.0% for six replicates measurement of the same sample. This shows that the method and the system both are suitable for determination of rofecoxib and tizanidine.

Linearity

Validation of the method is a process by which it is established by laboratory studies that the performance characteristics of the method meet the requirements for its intended application. To demonstrate that the proposed method is adequate for its intended use, the method was subjected to statistical validation to determine accuracy and precision. The linearity of a method is a measure of how well a calibration plot of detector response *vs*. concentration approximates a straight line. Solution containing mixture of rofecoxib and tizanidine of eight different concentrations were prepared in 5.0 mL volumetric flasks from stock solution in acetonitrile and 0.75 mL of 1000 µg/mL of Rosiglitazone (internal standard solution) was added to each flask. The concentration range for each of the two pharmaceuticals in the working standard solutions was 1250.00 to 3750.00 µg/mL for Rofecoxib (i. e. 3.75, 4.50, 5.25, 6.25, 7.50, 9.00, 10.50 and 11.25 µg/spot) and100.00 to 300.00 µg/mL (i. e. 0.30, 0.36, 0.42, 0.54, 0.60, 0.72, 0.84 and 0.90 µg/spot) for Tizanidine. To a pre-washed and activated thin layer chromatographic plate, 3 µL of the above solutions of different concentrations were applied and the experiment was carried out thrice using instrumentation and

chromatographic conditions mention above. The plate was then scanned and quantified at 235 nm. The chromatograms were recorded and the peak area ratios (analyte/internal standard) were computed using Camag TLC scanner III equipped with cats 3.0 version software. A typical HPTLC chromatogram is shown in Figure 3. A linear relationship between peak area ratio versus concentration was observed for rofecoxib and tizanidine in the range of 3.75 µg/spot to 11.25 µg/spot and 0.30 µg/spot to 0.90 µg/spot respectively. This range was selected as linear range for analytical method validation of two components. The data were analyzed by linear regression least-squares fitting. The statistical data obtained are given in Table 1

Table 1. Analytical performance data (Linear regression data for calibration curve)

	Rofecoxib	Tizanidine
Linear working range (LWR), µg/spot	3.75 -11.25	0.30-0.90
Std Err of Y Estimate	0.9708	0.0479
Correlation Coefficient	0.9976	0.9946
X Coefficient(s)	6.5520	2.7256
Constant	-0.2511	0.0437
Std Err of Coefficient.	0.1324	0.0817

Assay

3µL of sample solution was spotted along with same concentration of working solution (2500.00 µg/mL of rofecoxib and 200.00 µg/mL of tizanidine) on to the plate under the optimized chromatographic conditions. The procedure was repeated seven times. The peak area ratio values of rofecoxib and tizanidine to the internal standard were calculated. The densitometric responses from the standard and sample were used to calculate the amounts of the drug in the tablet. The results obtained are as shown in Table 2.

Table 2. Results of assay experiment

Observation No.	Amount of rofecoxib found in mg/tablet	Percentage assay	Amount of tizanidine found in mg/tablet	Percentage assay
1	25.34	101.38	2.01	100.39
2	24.90	99.62	2.00	99.92
3	25.24	100.95	1.98	98.80
4	25.80	103.21	1.97	98.73
5	24.90	99.58	1.99	99.45
6	25.47	101.88	1.97	98.75
7	25.26	101.03	2.03	101.55
Mean	25.27	101.0922	1.99	99.66
S.D.	0.3174	1.2695	0.02	1.05
%CV	1.26	1.26	1.06	1.06

Limit of detection and limit of quantitation

The Limit of Detection (LOD) was found to be 45.00 ng/spot for rofecoxib and 30.00 ng/spot for tizanidine. Limit of quantitation (LOQ) for rofecoxib and tizanidine were determined experimentally by spotting six replicates of each drug at LOQ concentration. The LOQ of rofecoxib and tizanidine were found to be 135.00 ng/spot and 90.00 ng/spot respectively. The signal to noise ratio is 3 for LOD and 10 for LOQ.

Recovery studies

Recovery experiments were carried out to check for the presence of positive or negative interferences from excipients present in the formulation, and to study the accuracy and precision of the method. Recovery experiment was performed by the standard addition method. The recovery of the added standard was studied at three different levels *viz* 110%, 120% and 130% of the estimated amount of the drug. Each set of recovery of added standard was calculated. The results of recovery experiment are tabulated in Table 3.

Table 3. Results from recovery analysis for rofecoxib (n=7)

Level	Amount of rofecoxib added in, mg	Total amount of rofecoxib found, mg*	SD	%CV	%Recovery
0.0%	(25.0) + 0.00	25.04	0.1824	0.71	100.14
10%	(25.0) + 2.50	27.51	0.0654	0.21	100.03
20%	(25.0) + 5.00	30.13	0.3932	1.31	100.43
30%	(25.0) + 7.50	32.49	0.0752	0.22	99.97
				% Average Recovery	100.14

Table 4. Results from recovery analysis for tizanidine (n=7)

Level	Amount of Tizanidine added in, mg	Total amount of Tizanidine found, mg*	SD	%CV	%Recovery
0.0%	(2.0) + 0.00	2.01	0.1023	0.0021	100.50
10%	(2.0) + 0.20	2.21	0.0264	0.0006	101.00
20%	(2.0) + 0.40	2.42	0.0356	0.0009	101.00
30%	(2.0) + 0.60	2.59	0.0261	0.0007	100.00
				% Average Recovery	100.62

**Each value is average of seven determinations*

Results and Discussion

The method was a normal phase HPTLC method. It makes use of a silica gel $60F_{254}$ stationary phase precoated on aluminium sheet. The mobile phase comprises toluene: ethyl acetate: methanol: triethyl amine in the volume ratio of (6.0:3.0:0.5:0.1) which gives good separation between rosiglitazone maleate (IS) (R_f =0.27), tizanidine (R_f=0.49) and rofecoxib (R_f=0.68). Figure 3 shows a typical densitogram showing well resolution between (1) rosiglitazone (I.S.), (2) tizanidine and (3) rofecoxib. The linearity range for the rofecoxib and tizanidine were found to be 3.75-11.25 µg/spot and 0.30-0.90 µg/spot respectively. The correlation coefficient was found to be 0.9976 and 0.9946. That means a good relationship was observed between the concentration ranges of both the drugs. The limit of detection for rofecoxib and tizanidine was found to be 45.00 ng/spot and 30.0 ng/ spot respectively. The limit of quantification for rofecoxib and tizanidine was found to be 135.00 ng/spot and 90.00 ng/spot respectively. It proves the sensitivity of method for the drugs. The recovery for rofecoxib and tizanidine was found in the range of 99.97 – 100.43% and 100.00 – 101.00% respectively which indicates that the method is free from interference from excipients present in the formulation. The low values of standard deviation and coefficient of variation at each level of the recovery experiment indicate high precision of the method.

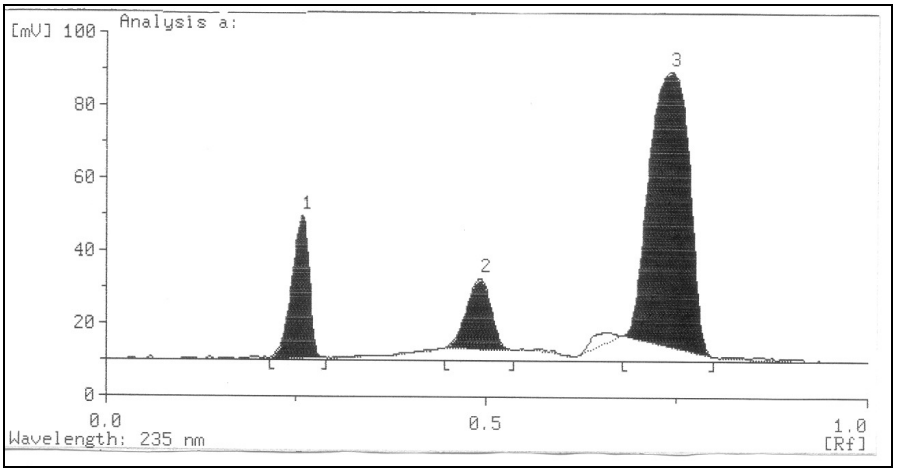


Figure 3. Typical chromatogram Peak, (1) Rosiglitazone maleate (internal standard), (2) Tizanidine and (3) Rofecoxib

Conclusion

The data given above reveal that the proposed method is simple, accurate and sensitive with good precision and accuracy. This encourages their successful use in routine quality control analysis of rofecoxib and tizanidine from their fixed dosage form as well as raw materials.

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References

1. George Lunn, HPLC methods for recent approved pharmaceutical analysis, 2003, **2**, 562, 566, 641.
2. Susan Budavari, Maryadele J O' Neil, Ann Smith, Patricia E Heckelman and Joanne F Kinneary, The Merck Index, 12th Ed., 1996, Merck, USA. (monograph no. 9624) 1618.
3. Susan Budavari, Eds, In, The Merck Index, 13th Ed., Merck & Co., Inc., Whitestation, NJ, 2001, 1481, 1691.
4. Parfait K, (Ed.) Martindale- The complete Drug reference, 33rd Ed., Pharmaceutical press, 1 Lambeth High Street, London SEI 7 JN, UK, 1993, 768.
5. ICH, Text on validation of analytical procedures, ICH harmonized tripartite guidelines, adapted 27 Oct 1994.
6. Touchstone J C, John Wiley, Practice of thin layer chromatography, Third Edition, Published by and sons, Inc., USA, 1992.
7. Sethi P D, High performance thin layer chromatography Quantitative analysis pharmaceutical formulations, First Edition, CBS Publishers and distributors, India, 1996.
8. Ravi T K, Gandhimathi M, Sireesha K R and Jacob S, *Indian J Pharm Sci.***,** 2006, **68(2)**, 234.

9. Subramanian G, Pandey S and Udupa N, *Indian J Pharm Sci.*, 2004, **66 (5)**, 699.
10. M Gandhimathi, T K Ravi and Susheel John Varghee, *Pharm Biomed Anal.***,** 2005, **37(1)**, 183.
11. Mathew C Z and Halpin R A, *Drug Metab Despo.,* 2002, **28**, 1244.
12. Prascit P and Wong Z, *Bioorg Med Chem Lett*., 2003, 176.
13. Raman B and Pattel D, *Indian Drugs.***,** 2002, **30**, 63.
14. Billen D, Boens N and Deschryvet F C, *J Chem Soc.***,** 2003, 101.
15. Wang H, Handa A K, Suedbalkar V P, Patrick J and Jansen, *Int J Pharm*., 2001, 45.
16. Woolf E, Ful and Matuzeweski B, *J Chromatogr B Biomed Sci Appl.,* 1999, **730**, 221.
17. Chevez-Eng C M, Constanzer M L and Matuzeweski B K**,** *J Chromatogr B Biomed Sci Appl.,* 2000, **748 (1)**, 31.
18. Ajithadas Aruna, Anusuya P and Kumar Pavan A R, *Indian Drugs,* 2001, **38 (10)**, 523.
19. Bhoir I. C, Raman B and Sundaresan M, *Anal Lett*., 1998, **31**, 1533.
20. Kauffman J M, Lopez R B, Fernandis G M and Patriarch G J, *J Pharm Biomed Anal.,* 1992, **10**, 763.
21. Sujata K, Chitra K, Dhanusihka S H, Krishna M V and Janardhan V, *Indian Drugs,* 2002, **39 (10)**. 548.