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Research Article

Development and Validation of Vierordt's spectrophotometric method for simultaneous estimation of Drotaverine and Nimesulide Combination

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Abstract

UV spectrophotometric techniques have been developed and validated for the simultaneous determination of drotaverine and nimesulide in synthetic mixture and its combined dosage form. The method was based on UV-spectrophotometric determination of two drugs using Vierordt's simultaneous equation method. This employs formation and solving of mathematical simultaneous equation using 229 nm and 297 nm as the λmax of drotaverine and nimesulide respectively in methanol. The % assay for commercial formulation was found to be in the range 100.07 ± 0.22 % for drotaverine and 99.62±0.19 % for nimesulide by the proposed methods. Recovery was found in the range 99.90±0.11 % for drotaverine and 100.01±0.25% for nimesulide. The methods were validated in terms of linearity, precision, accuracy and ruggedness according to ICH guideline. The methods were successfully applied to pharmaceutical formulation with no interference from excipients. This method can be adapted to routine quality control analysis of drugs in combined dosage form.

Keyword: *Drotaverine, Nimesulide, UV- Spectrophotometric and simultaneous estimation.*

Introduction

In the present developing world, day by day newer and better drugs are developed to provide effective and safe treatment of the disease with minimum side effect. To effectively deliver these advantages to consumer, a rigid check on quality of the marketed drug is essential. The term "Quality" applied to the drug products has been defined as the sum of all factors which contributes directly or indirectly to the safety, efficacy and acceptability of the product. According to various compendia and regulatory requirements, a drug and its formulations used clinically should confirm to a minimum standard of purity. Thus, it becomes essential to develop a sensitive, precise, accurate and reproducible methodology of analysis [1,2].

Drotaverine hydrochloride [DRT], 1-[(3, 4-diethoxy phenyl) methylene]-6, 7-diethoxy-1, 2, 3, 4-tetra hydro isoquinolene is an analogue of papaverine [3]. It acts as an antispasmodic agent by inhibiting phosphodiesterase IV enzyme, specific for smooth muscle spasm and pain, used to reduce excessive labor pain [4]. Drotaverine hydrochloride is official in Polish Pharmacopoeia. A few UV spectrophotometric [5-8] and HPLC [9-11] methods have been reported individually or in combination with other drugs for estimation of Drotaverine hydrochloride. Nimesulide [NIM] is chemically designated as 4-nitro-2-phenoxymethane sulfonilide ($C_{13}H_{12}N_2O_3S$) [12, 13]. It is a new non-steroidal anti-inflammatory drug (NSAIDS) with analgesic antipyretic properties that does not induce gastrointestinal ulceration. It is an inhibitor of prostaglandin synthesis from arachidonic acid and of platelet aggregation. Various UV, HPLC and stability indicating LC methods for NIM have been reported for its estimation individually or in combination with other drugs [16,17].

Determination of components in a binary mixture using Vierordt method was based on the assumption that if the two components do not react or interact in any manner with one another and thus neither affects the light absorbing properties of the

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other, the total absorbance of the two components in the solution is the sum of the absorbances which the two substances would have exhibited individually if the substances were in separate solutions under similar conditions and had the same concentrations as in the mixture [18]. Thus, the method is far more sensitive to wavelength errors because some of the absorbance measurement will have to be made on the slopes of the absorbance curves [19] which require greater care specially with wavelength calibration of spectrophotometer vis-a-vis solutions of known concentrations are required for establishment of numerical coefficients. Present manuscript describes determination of DRT and NSD in tablet dosage form by simultaneous equation method by UV spectroscopy. The proposed methods were validated as per the International Conference on Harmonization (ICH) guidelines.

Materials and Methods

Equipment

Double beam UV-visible spectrophotometer (shimadzu UV-1700) & Shimadzu isocratic liquid chromatographic condition system equipped with liquid chromatogram model LC-10 AD, Rheodyne injector model 7725i with 20µL fixed Rheodyne loop, UV-visible detector model SPD-10A, Knauer eurospher C_{18} column in VAMA Pharma, Nagpur.

Selection of solvent

The methanol was selected as the suitable solvent for estimation of DRT and NSD combination.

Preparation of standard solutions

Solution A (DRT): Accurately weighed quantity of DRT (50 mg) was dissolved in methanol (10 mL) in volumetric flask (50 mL) and volume was made up to the mark with same solvent. A 2.5 mL of resultant solution was diluted to 25 mL with methanol (conc.100 µg/mL).

Solution B (NSD): Accurately weighed quantity, NSD (50 mg) was dissolved in methanol (10 mL) in volumetric flask (50 mL) and volume was made up to the mark with same solvent. A 2.5 mL of resultant solution was diluted to 25 mL with methanol (conc.100 µg/mL).

Solution C (Mixed DRT+NSD): Accurately weighed quantities of DRT (40 mg) and NSD (100 mg) were dissolved in methanol (10-20 mL) in volumetric flask (100 mL) and the volume was made up to the mark. A 2.5 mL of resultant solution was diluted to 50 mL with methanol (conc. DRT-20 µg/mL; NSD-50 µg/mL).

Solution C1: Accurately measured 2 mL portion of solution C was diluted to 10 mL with methanol (conc. DRT-4 µg/mL; NSD-10 µg/mL).

Study of spectra and selection of wavelengths

Aliquot portion (1 mL) of each standard solution A and B were diluted to 10 mL with methanol (conc. 10 µg/mL each DRT and NSD). The solutions were scanned over 400-200 nm range against methanol as a blank. The overlain spectrum of DRT and NSD is depicted in Fig. 1.

Preparation of calibration curve

Aliquot portions of standard solution A and standard solution B (0.1mL, 0.2mL,…3.4mL, 3.5mL) were taken in different volumetric flasks (10 mL) and diluted with methanol to get series of solutions of each drug (conc. 1-35µg/mL).

Similarly, standard solution A and standard solution B were mixed in 4:10 proportions and diluted with methanol in volumetric flask (10 mL) to produce series of solutions of each drug (conc. 1-35µg/mL). The absorbance of each solution was measured at 229 nm and 297 nm, and the graph was plotted as concentration vs absorbance as depicted in Fig. 2.

Determination of A (1%, 1cm) of drugs at selected wavelengths

The standard solution A and solution B (1 mL each) were diluted separately with methanol (10 mL) to have final conc. 10 µg/mL of each drug. The absorbance of each solution was measured at 229 nm and 297 nm against methanol as a blank.

A (1%, 1cm) values were calculated using following formula.

$$A\,(1\%,1\,\mathrm{cm}) = \frac{\text{Absorbance}}{\text{conc.of analyte in g/100 mL}}$$

Estimation of DRT and NSD in standard laboratory mixture

An accurately weighed quantity of DRT (40 mg) and NSD (100 mg) were dissolved in methanol (10-20 mL) in volumetric flask (100 mL). The volume was made up with methanol (conc. DRT-400 µg/mL; NSD-1000 µg/mL). Aliquot portion of resultant solution (2.5 mL) was diluted to 50 mL with methanol. Then, accurately measured, 2 mL portion of resultant solution was diluted

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to 10 mL with methanol to get final solution (conc. DRT-4 µg/mL; NSD-10 µg/mL).

The absorbance of final solutions was measured at 229 nm and 297 nm against blank. The estimation (%) of each drug was determined by using simultaneous equation formula.

$$Cx = \frac{A_2 ay_1 - A_1 ay_2}{ax_2 ay_1 - ax_1 ay_2}$$

$$Cy = \frac{A_1 ax_2 - A_2 ax_1}{ax_2 ay_1 - ax_1 ay_2}$$

Where,

Cx = conc. of DRT in g/100 mL

C_y = conc. of NSD in g/100 mL

A_1 = absorbance of laboratory mixture at 229 nm

A_2 = absorbance of laboratory mixture at 297 nm

ax_1 = absorptivity of DRT at 229 nm

ax_2 = absorptivity of DRT at 297 nm

ay_1 = absorptivity of NSD at 229 nm

ay_2 = absorptivity of NSD at 297 nm

$$\text{Estimation}(\%) = \frac{C \times d}{W} \times 100$$

Where,

C = C_x or C_y = conc. of DRT or NSD in g/100 mL

d = dilution factor

W = weight of drug either DRT or NSD in laboratory mixture

Estimation of DRT and NSD by simultaneous equation method in combined dose tablet formulation

Twenty tablets were weighed and average weight of tablet was calculated. The tablets were crushed to fine powder and mixed thoroughly. Then five samples were prepared using the following procedure.

An accurately weighted quantity of tablet powder (0.3023g) was shaken with 30 mL methanol for 15 min. The volume was made to 100 mL with methanol. The solution was filtered through Whatman filter paper and 2.5 mL of clear filtrate was diluted to 50 mL with methanol. A 2 mL portion of resultant solution was diluted to 10 mL with methanol to get final solution having conc. 4 µg/mL of DRT and 10 µg/mL of NSD. The absorbances of final solutions were measured at 229 nm and 297 nm. The content of DRT and NSD in tablet was calculated by using following formula.

$$\text{Labelclaim}(\%) = \frac{Cx \text{ or } Cy \times d \times Wa}{W \times LC} \times 100$$

Where,

C_x or C_y = conc. of DRT or NSD in g/100 mL

d = dilution factor

Wa = average weight of tablet

W = weight of tablet powder taken

LC = label claim of sample taken

Recovery study

The recovery study was carried out by standard addition method. Standard solution C1 was prepared (conc. DRT-4 µg/mL; NSD-10 µg/mL).

An accurately weighed quantity of preanalysed tablet powder (0.3023g) was transferred to three different volumetric flasks (100 mL). To each of the flask, 30 mL of methanol was added. The flasks were shaken for 15 min. The volumes were made up to the mark with methanol. The solutions were mixed and filtered. A 2.5 mL of each filtrate was diluted to 50 mL with methanol. Then, 2 mL portion of resultant solution was diluted with methanol in three different flask (10 mL) and accurately known amount of standard solution C1 was added to them (Table 4). The absorbances of sample solutions were measured at 229 nm and 297 nm against solvent blank. The recovery (%) was calculated by using following formula.

$$\text{Recovery}(\%) = \frac{A}{B + C} \times 100$$

Where,

A = total amount of drug estimated

B = amount of drug found on preanalyzed basis

C = amount of standard drug added

Validation of proposed analytical methods

Precision

The precision is expressed as SD and RSD of series of measurements. Precision of estimation of DRT and NSD by proposed method was ascertained by replicate analysis of homogeneous samples of tablet powder.

Accuracy

An accuracy of an analytical method is the closeness of test results obtained by that method to the true value. It was ascertained on the basis of recovery studies performed by standard addition method. The

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results of recovery study and statistical data are recorded in table 4.

Ruggedness

The ruggedness studies were carried out for two different parameters that is days and analyst

Intraday study

Intraday study was performed by using the similar procedure as under estimation of drugs in tablet formulation analysis at different times on the same day (5.2.8., p.36). The label claim (%) was calculated using the same formula as for estimation of drugs in tablet formulation analysis.

Interday study

It was performed by using the similar procedure as estimation of DRT and NSD in tablet formulation analysis on different days (5.2.8., p.36). The label claim (%) was calculated using the same formula as for estimation of DRT and NSD in tablet formulation analysis.

Different analysts

The samples were analyzed by three different analysts. The similar procedure was followed and the label claim (%) was calculated.

Linearity and range

Accurately weighed quantities of tablet powder equivalent to 80, 90, 100, 110 and 120 % of label claim were taken. The dilutions were done appropriately to obtain a concentration in the range of 80-120 % and absorbance was recorded at 229 nm and 297 nm.

Results and Discussion

The proposed methods developed for simultaneous analysis of DRT and NSD in combined tablet dosage form were found to be simple, accurate, rapid, economical and sensitive to be applied in routine analysis of tablets. In the described methods there were no additional extractions or separation procedure to extract the drug from the formulation. The method utilizes the spectrum mode of analysis of spectrophotometer. From the overlain spectrum the method utilizes 229 nm and 297 nm as an analytical wavelength for the estimation of DRT and NSD (fig 1). The calibration curve has been obtained for DRT, NSD and laboratory mixture at above selected wavelength and depicted in fig. 2.

Fig. 1. Overlain spectra of DRT and NSD

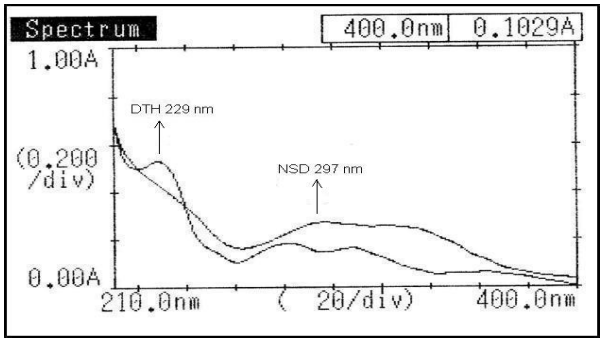
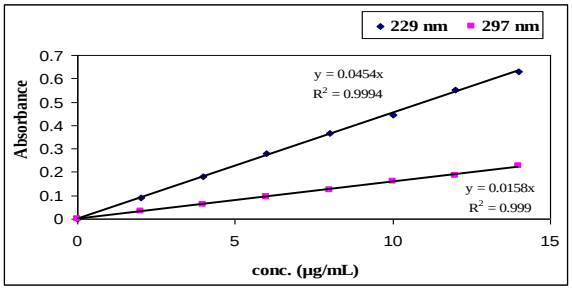
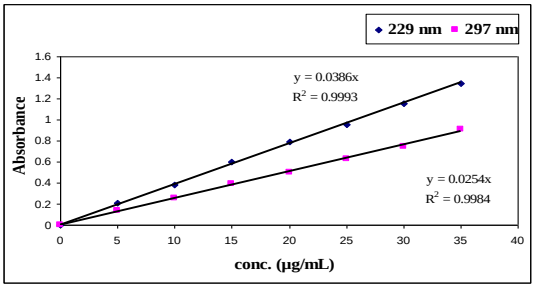


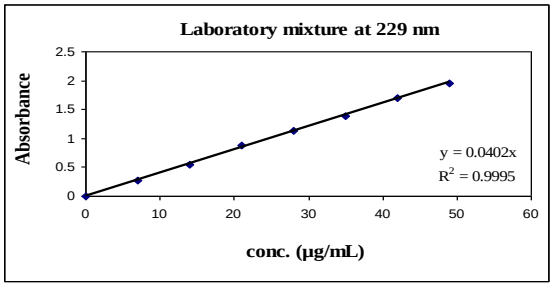
Fig. 2. Calibration plot of DRT, NSD and laboratory mixture at 229 nm and 297 nm



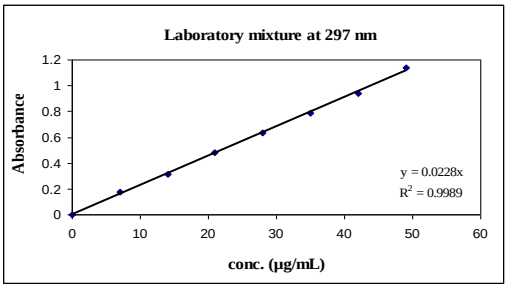
DRT at 229 nm and 297 nm



NSD at 229 nm and 297 nm



DRT and NSD at 229 nm



DRT and NSD at 297 nm

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The method employing simultaneous equation is a very simple method and can be employed for a routine analysis of DRT and NSD. Once the absorptivity values are determined very little time is required for analysis, as it would only require the determination of absorbances of the sample solution at two selected wavelengths and few simple calculations. The results of A (1 %, 1 cm) are shown in Table 1.

Table 1. Determination of A (1 %, 1 cm) of DRT and NSD

SN	Conc. (g/100 mL)	DRT				NSD			
		Absorbance		A (1 %, 1 cm)		Absorbance		A (1 %, 1 cm)	
		229 nm	297 nm	229 nm	297 nm	229 nm	297 nm	229 nm	297 nm
1.	0.0010	0.4440	0.1609	444.0	160.9	0.3804	0.2508	380.4	250.8
2.	0.0010	0.4429	0.1607	442.9	160.7	0.3797	0.2496	379.7	249.6
3.	0.0010	0.4441	0.1615	444.1	161.5	0.3795	0.2504	379.5	250.4
4.	0.0010	0.4434	0.1621	443.4	162.1	0.3806	0.2599	380.6	259.9
5.	0.0010	0.4432	0.1612	443.2	161.2	0.3793	0.2515	379.3	251.5
			Mean	443.52	161.28		**Mean**	379.9	252.44
			SD	0.1164	0.5495		**SD**	0.5701	4.226
			RSD(%)	0.0245	0.3407		**RSD(%)**	0.1500	1.6740
			SEM	0.2311	0.2458		**SEM**	0.2550	1.890

In order to see the feasibility of proposed method for simultaneous estimation of DRT and NSD in tablet formulations, the method was first tried for the estimation of drugs in standard laboratory mixture. The results of estimation of drugs in laboratory mixture are shown in table 2.

Table 2. Estimation of DRT and NSD in standard laboratory mixture

SN	Wt. of standard drug (g)		Absorbance		Estimation (%)	
	DRT	NSD	229 nm	297 nm	DRT	NSD
1.	0.0399	0.1001	0.5542	0.3130	100.06	99.16
2.	0.0403	0.0997	0.5563	0.3143	99.23	100.04
3.	0.0400	0.1001	0.5548	0.3136	99.42	99.50
4.	0.0398	0.0998	0.5553	0.3141	99.59	100.09
5.	0.0401	0.1002	0.5559	0.3138	100.17	99.23
				Mean	99.69	99.60
				SD	0.4067	0.4399
				RSD(%)	0.4079	0.4416
				SEM	0.1819	0.1967

DRT and NSD also estimated by simultaneous equation method in combined dose tablet formulation. The results of estimation of drugs in tablet formulation are shown in Table 3.

Table 3. Estimation of DRT and NSD in tablet

NOBEL SPAS				Average weight = 0.3023g	
SN	Weight of tablet powder (g)	Absorbance		Label claim (%)	
		229 nm	297 nm	DRT	NSD
1.	0.3020	0.5547	0.3133	99.97	99.46
2.	0.3024	0.5566	0.3140	100.65	99.11
3.	0.3022	0.5558	0.3137	100.52	99.40
4.	0.3018	0.5562	0.3128	99.83	100.04
5.	0.3023	0.5549	0.3142	99.41	100.11
			Mean	100.07	99.62
			SD	0.5104	0.4332
			RSD(%)	0.0510	0.4348
			SEM	0.2282	0.1937

To study the accuracy i.e. % recovery, for both developed methods, recovery studies were carried out by the addition of standard drug solution to preanalysed tablet sample with proper dilution at three different concentration levels within the range of linearity for

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both the drugs. The result of recovery studies were found to be satisfactory and are reported in table 4.

Table 4. Recovery study of tablet

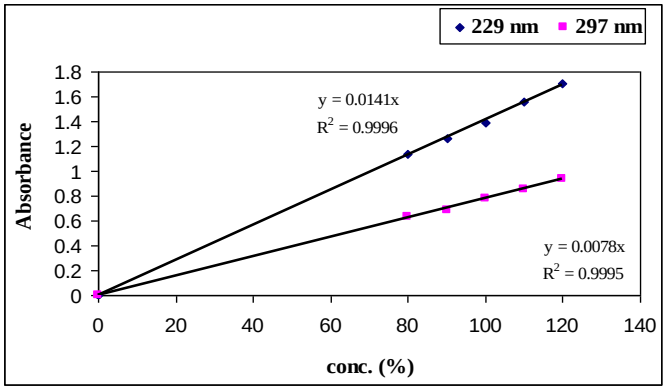
SN	Wt. of tablet powder taken (g)	Conc. of tablet + standard solution added (µg/mL)		Absorbance		Recovery (%)	
		DRT	NSD	229 nm	297 nm	DRT	NSD
1.	0.3034	4 + 2	10 + 5	0.8735	0.4718	100.13	99.53
2.	0.3026	4 + 4	10 + 10	1.1523	0.6229	99.76	100.09
3.	0.3021	4 + 6	10 + 15	1.3951	0.7942	99.83	100.42
					Mean	99.90	100.01
					SD	0.1966	0.4499
					RSD(%)	0.1967	0.4498
					SEM	0.1135	0.2598

A validated vierodt's simultaneous spectrophotometric method for quantitative analysis of DRT and NSD in pharmaceutical preparation was developed. The method gives results of high accuracy and high recovery of 99.90 ± 0.19 and 100.01 ± 0.44 respectively at good precision. % R.S.D. values were found to be less than 2 indicate reproducibility of the method. Validation parameter such as accuracy, precision, ruggedness, linearity and range has been studied and the results are depicted in table 5 and fig 3. By observing the validation parameters, the method was found to be specific, accurate, precise, repeatable and reproducible.

Table 5. Study of Ruggedness parameter

SN	Intraday study			Interday study			Different analysts		
	Time	Label claim (%)		Day	Label claim (%)		Analyst	Label claim (%)	
		DRT	NSD		DRT	NSD		DRT	NSD
1.	Morning	100.09	99.97	1	99.13	99.09	1	99.21	100.34
2.	Afternoon	100.02	100.10	2	100.73	99.07	2	100.20	99.29
3.	Evening	100.42	99.52	3	99.05	99.23	3	99.45	98.87
Mean		100.17	99.86	**Mean**	99.63	99.13	**Mean**	99.62	99.50
SD		0.2136	0.3044	**SD**	0.9477	0.0871	**SD**	0.5164	0.7572
RSD(%)		0.2132	0.3048	**RSD(%)**	0.9512	0.0878	**RSD(%)**	0.5183	0.7610
SEM		0.1233	0.1757	**SEM**	0.5472	0.0503	**SEM**	0.2982	0.4371

Fig. 3. Plot of linearity and range for DRT and NSD



Conclusion

The method was proved to be convenient and effective for the simultaneous estimation of DRT and NSD in their combined pharmaceutical dosage form. The proposed methods are applicable, prompt and specific for the simultaneous estimation of DRT and NSD in their commercial pharmaceutical formulation. The results of the analysis of pharmaceutical formulation by the proposed methods were highly reproducible and reliable and it is in good agreement with the label claim of the

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drug. This method can be adapted to routine quality control analysis of drugs in combined dosage form.

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Conflict of interest

Declared None

References

1. Skoog DA, Holler FJ and Nieman TA. Principles of Instrumental Analysis. 5th ed. A division of Thomson Learning, Inc.; 2004, 1.
2. Mendham J, Denney RC, Barnes JD, Thomas MJK. Vogel's Textbook of Quantitative Chemical Analysis. 5th ed. Singapore: Pearson Education Ltd.; 2003, 5-10.
3. The Merck Index – An Encyclopedia of chemicals, Drugs, and biological, Merck Research Laboratories, Whitehouse station, New Jersey: 2001, 13, 6, 609-610.
4. Sethi S., Textbook of Pharmacology. Elsevier, New Delhi: 2004, 831-840.
5. Dahivelkar PP, Mahajan VK, Bari SB, Shirkhedkar AA, Fursule RA, Surana SJ. Simultaneous derivative and multi-component spectrophotometric determination of drotaverine hydrochloride and mefenamic acid in tablets. Indian J. Pharm. Sci. 2007, 69, 812-814.
6. Dahivelkar P, Surana S., Spectrophotometric method for simultaneous estimation of drotaverine hydrochloride and mefenamic acid in bulk and tablet formulation. Indian Drugs. 2006, 43, 896-900.
7. Metwally FH, Abdelkawy M, Naguib IA. Determination of nifuroxazide and drotaverine hydrochloride in pharmaceutical preparation by three independent analytical methods; Journal of AOAC International. 2006, 89(1), 78 –81.
8. Ayad MM, Youssef NF, Abdellatif HE, Soliman SM. A comparative study on various spectrometries with thin layer chromatography for simultaneous analysis of drotaverine and nifuroxazide in capsules. Chem. Pharm. Bull. 2006, 54, 807-813.
9. Panigrahi D, Sharma R. Development and validation of an RP-HPLC method for simultaneous analysis of Drotaverine and Omeprazole in a tablet dosage form. Journal of Acta Chromatographica. 2008, 20, 439- 450.
10. Mezei J, Kuttel S. A new method for highperformance liquid chromatographic determination of drotaverine in plasma. J. Pharm. Sci. 1984, 73, 1489-1491.
11. Dahivelkar PP, Bari SB, Bhoir S, Bhagwat AM. High Performance Liquid Chromatographic Estimation of Drotaverine Hydrochloride and Mefenamic Acid in Human Plasma. Iranian J. Pharm. Research. 2009, 8, 209-215.
12. Martindale - The Complete Drug Reference, RPS Publishing, Great Britain, 36, 2009, 14-15.
13. Indian Pharmacopoeia, Indian Pharmacopoeia Commission, Ghaziabad. 2007, 2, 63-64.
14. Nikam AD, Sampada SP, Gandhi SV. Estimation of paracetamol and aceclofenac in tablet formulation by ratio spectra derivative spectroscopy. Indian J.pharm.Sci. 2008, 70, 635-637.
15. Shah R, Magdum Patil SK, Chougule DK and Naikwade N, Validated Spectroscopic Method for Estimation of Aceclofenac from Tablet formulation. Research J. Pharm. and Tech. 2008, 1, 430-432.
16. Musmade P, Subramanian G., Srinivasan KK. High performance liquid chromatography and pharmacokinetics of aceclofenac in rats. Analytica Chimica Acta. 2007, 585, 103–109.
17. Momin MY, Yeole PG, Puranik MP, Wadher SJ. Reverse phase HPLC method for determination of Aceclofenac and Paracetamol in tablet dosage form. Indian J. Pharm. Sci. 2006, 68, 387-389.
18. Reilley CN and Sawyer DT. Experiments for Instrumental Methods, McGraw Hill Book Company, New York and London.
19. Chatten LG. Pharmaceutical Chemistry, Vol. II, Marcel Dekker, New York and London 1969.

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