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Spectrophotometric and HPLC methods for Simultaneous Estimation of Tizanidine and Valdecoxib from Tablets

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Abstract:

A simple, accurate, economical and reproducible UV spectrophotometric and HPLC method for simultaneous estimation of two component drug mixture of tizanidine and valdecoxib in combined tablet dosage form have been developed. The first developed method employs multiwavelength spectroscopy using six mixed standards and 229.0 nm and 241.0 nm as two wavelengths for estimation. Linearity was observed in concentration range of 0.5-3.0 µg/ml of tizanidine and 5-30 µg/ml of valdecoxib. Developed HPLC method is reverse phase chromatographic method using Hypersil C_{18} column and methanol:acetonitrile:phosphate buffer in ratio of 30:60:10 pH 5.0 as mobile phase. For HPLC method, linearity was observed in concentration range of 2 – 10 µg/ml of tizanidine and 20-100 µg/ml of valdecoxib. Results of analysis were validated statistically and by recovery studies.

Key words: Tizanidine and Valdecoxib

Introduction

Tizanidine 5-chloro-4-(2-imidazolin-2-ylamino)-2, 1, 3-benzothiadiazole (Fig. 1) is α 2 – adrenergic agonist and centrally active myotonolytic skeletal muscle relaxant with a chemical structure unrelated to other muscle relaxants[1-3] . In the literature, a radioimmunoassay method for the quantification of tizanidine hydrochloride has been widely used[4] .Also determination of tizanidine in human plasma by gas chromatography–mass spectrometry has been reported[5,6] .There are very few reports on analytical methods for estimation of tizanidine in bulk and its dosage form. A RP–HPLC method for estimation of tizanidine hydrochloride in combination with nimesulide[7] ,a stability indicating HPLC method[8] ,and a HPTLC method were reported[9] .

Valdecoxib chemically 4-(5-methyl-3-phenyl-4-isoxazoyl) benzene sulfonamide (Fig 2) is a non-steroidal anti-inflammatory agent, a selective inhibitor of cyclooxygenase II (COX – 2) indicated for oral administration for the treatment of osteoarthritis and Rheumatoid arthritis[10-12] .It has been found to be effective analgesic in postoperative pain[13] .So far assay procedures based on solid phase extraction-mass spectrometry in urine sample[14] and HPLC method in human plasma[15] have been reported for the estimation of Valdecoxib.

Developed spectrophotometric and HPLC methods are simultaneous methods of analysis of tizanidine and valdecoxib from combined tablet dosage form. The developed methods were found to be simple, rapid, accurate, reproducible and economical. These methods can be used successfully for quality control testing of the drugs from combined tablet dosage form.

Materials and Methods

Standard bulk drug samples of tizanidine and valdecoxib were provided by Madras Pharmaceuticals Ltd., Chennai. Tablets of combined dosage form were procured from the local market. All other reagents used were of analytical grade for spectrophotometric method and HPLC grade for HPLC method.

Shimadzu UV/Vis Spectrophotometer (model 1601) with 1 cm matched quartz cell was used for spectrophotometric method. Spectra's were recorded using specific program of apparatus, having specifications as follows: Spectral bandwidth 3 nm, Wavelength accuracy ± 0.5 nm, and wavelength readability 0.1 nm increments. For HPLC method, shimadzu delivery module LC-10AD with UV SPD-10A detector was used.

Method I – Multiwavelength Spectroscopy:

Using the overlain spectra of tizanidine and valdecoxib in methanol, the wavelength maxima of both drugs, i.e., 229.0 nm and 241.0 nm, were selected as two sampling wavelengths for this method. Six mixed standards of two drugs in methanol were prepared so as to contain 0.5–3.0 µg/ml of tizanidine and 5-30 µg/ml of valdecoxib. All mixed standard solutions were scanned over the range of 400 nm to 200 nm in multicomponent mode of spectrophotometer using 229.0 nm and 241.0 nm as two sampling wavelengths. The spectral data from these scans were used to determine the concentration of two drugs in the sample solution.

Analysis of Commercial Formulation:

Twenty tablets were accurately weighed and average weight per tablet was determined. Tablets were ground to fine powder, and weighed tablet powder

equivalent to 5 mg of tizanidine was transferred to 50 ml volumetric flask. The powder was dissolved in 20 ml methanol by intermittent shaking and the volume was made up to the mark with methanol. The solution was then filtered through Whatman filter paper no:1. Aliquot of this solution was diluted to get a final concentration 1 µg/ml and 10 µg/ml of tizanidine and valdecoxib respectively. The sample solution was scanned over the range of 400 to 200 nm in multicomponent mode and concentration of each component was estimated by analysis of spectral data of sample solution with respect to that of mixed standards by the instrument. Results of analysis are reported in Table 1.

Method II – High Performance Liquid Chromatographic Method:

HPLC method was developed using Hypersil C_{18} ODS (5µ) 250 × 4.6 mm column. Mobile phase selected for this method contained 60 parts of acetonitrile, 30 parts of methanol and 10 parts of phosphate buffer of pH 5.0 that was filtered through 0.45 micron membrane filter. Flow rate employed was 1.0 ml/min. Detection of eluent was carried out at 241 nm.

Standard Stock Solution:

Standard stock solutions of pure drugs were made separately in mobile phase containing 100 µg/ml of tizanidine and 1000 µg/ml of valdecoxib and filtered through a 0.45 micron membrane filter.

Preparation of Calibration Curve:

For preparation of the drug solution for calibration curves in a series of 10 ml volumetric flasks 0.2, 0.4, 0.6, 0.8 and 1.0 ml of the pure drug standard stock solutions of tizanidine and valdecoxib were transferred. The volume in each flask was made up to the mark with the mobile phase. Each solution was injected and a chromatogram was recorded. Mean retention time for tizanidine was found to be 2.312 min and for valdecoxib 3.021 min. The peak area of tizanidine and valdecoxib were calculated and respective calibration curves were plotted against concentration of drug and peak area of drug.

Procedure for analysis of formulations:

Twenty tablets of the formulation were weighed and average weight per tablet was calculated. Twenty tablets were crushed and ground to a fine powder. Powder equivalent to 10 mg of tizanidine was weighed and transferred to a 50 ml volumetric flask containing 25 ml mobile phase. The powder mixture was dissolved in the mobile phase with the aid of ultrasonication. The solution was filtered through Whatman

filter paper no.41 in to another 50 ml volumetric flask. The filter paper was washed with mobile phase and washings were added to filtrate. Volume of filtrate was made up to the mark with the mobile phase. To another 10 ml volumetric flask, 0.2 ml of this solution was transferred and the volume was made up to the mark with the mobile phase. This solution was filtered through a 0.45 µ membrane filter.

After setting the chromatographic conditions and stabilizing the instrument to obtain a steady baseline, the tablet sample solution was loaded in the 20 µl fixed sample loop of the injection port. The solution was injected and a chromatogram was recorded. The injections were repeated five times and the peak areas were recorded. A representative chromatogram has been given in Fig.1. The peak areas of each of the drugs were calculated and the amount of each drug present per tablet was estimated from the calibration curves. The results of analysis are presented in Table 1.

Table 1: Results of Analysis of Commercial Formulation

Method	Label Claim (mg/tablet)		% of Label claim Estimated*		Standard deviation	
	TZ	VA	TZ	VA	TZ	VA
Method I	2	20	101.5	101.1	0.011	0.419
Method II	2	20	102.5	98.52	0.021	0.444

* Average of five determinations, TZ – Tizanidine and VA - Valdecoxib

Recovery Studies:

To study the accuracy, reproducibility and precision of the above methods, recovery studies were carried out by addition of standard drug solution to pre-analyzed sample at three different levels. Results of recovery studies were found to be satisfactory and are reported in Table –2.

Table 2: Results of Recovery Studies

Method	Concentration added (mg/ml)		% Recovered*	
	TZ	VA	TZ	VA
Method I	0.5	5	98.02	102.1
	1.0	10	102.52	102.4
	1.5	15	99.56	98.51
Method II	1	10	102.61	99.12
	3	30	99.20	98.08
	5	50	98.07	101.02

* Average of three determinations; TZ – Tizanidine; VA- Valdecoxib

Results and Discussion

The proposed method for simultaneous estimation of tizanidine and valdecoxib in combined tablet dosage form were found to be simple, accurate, rapid and economical. The values of coefficient of variance were satisfactorily low and recovery was close to 100 % indicating reproducibility of the methods.

First method involving multiwavelength spectroscopy is specific to instrument having software for provision of such determination. Selection of proper sampling wavelengths and concentration of components in mixed standards is critical. Since calculations are done by the instrument itself, chances of manual error are nil; furthermore, the method is quite rapid.

Second method for simultaneous estimation of two drugs from combined dosage form is reverse phase chromatographic method utilizing C_{18} column and methanol: acetonitrile: phosphate buffer as mobile phase. Detection of eluent was carried out using UV detector. The run time per sample is just 6 min. The excipients in the formulation did not interfere in the accurate estimation of tizanidine and valdecoxib.

Since none of the methods is reported for simultaneous estimation of tizanidine and valdecoxib from combined dosage form, these developed methods can be used for routine analysis of two components without prior separation.

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References

1. P. Koch, D.R. Hirst and B.R. von Wartburg, Xenobiotica ,1989,19 , 1255–1260.
2. F.L.S. Tse, J.M. Jaffe and S. Bhuta, Fundam. Clin. Pharmacol., 1987,1 ,479–485.
3. M.K. Shellenberger, L. Groves, J. Shah and G.D. Novak, Drug Metab. Dispos.,1999, 27 ,201–205.
4. V. Healzlewood, P. Symoniw, P. Maruff and M.J. Eadie, Eur. J. Clin. Pharmacol.,1983, 25, 65–72.
5. J. Lee, J.H. Seo and D.H. Kim, Analyst, 2002,127 , 917–920.
6. L.J. Jackman and T. Jen, J. Am. Chem. Soc.,1975, 97 ,2818.
7. B. Raman and D. Patil, Indian Drugs,2002, 39 ,392–394.
8. M.L. Qi, P. Wang and L. Wang, Anal. Chim. Acta,2003, 478 ,171–177.
9. K.R. Mahadik, A.R. Paradkar, H. Agrawal and N. Kaul, J. Pharm.Biomed. Anal.,2003, 33, 545–552.
10. S. Budavari Eds, In, The Merck index, 12th Edn, Merck & Co, Inc, White house Station, NJ, 1994.
11. J.J. Talley, D.L. Brown and J.S.Carter, J.Med.Chem.,2000, 43, 775.
12. S.E.Daniels, P.J.Desjardins and S.Tawlkar, J. Am. Dent. Assoc.,2002, 13, 611.
13. J.Y.Zhang, D.M.Fast and A.P.Breau, J.Chromatogr. B. Analyst. Technol. Biomed. Life.Sci.,2003, 785 (1) 123.
14. U.Mandal, M.Kumar, M.Jaya Ganesan, S.Nandi, T.K.Pal, M.Chakraborty, A.Roy Chowdhury and T.K. Chatloraj, Indian Drugs,2004, 41 , 91.
15. Indian Pharmacopoeia, Government of India, Ministry of Health and Family Welfare, vol-II, appendix 13, A-147, 1996.
16. Indian Pharmacopoeia, Government of India, Ministry of Health and Family Welfare, vol-II, appendix 13, A-147, 1996.
17. ICH, Q2A, Harmonised tripartite guideline, text on validation of analytical procedures, IFPMA, in: Proceedings of the International Conference on Harmonization, Geneva, March 1994, pp. 1–5.

18. P.D. Sethi, High Performance Thin Layer Chromatography, Quantitative Analysis of Pharmaceutical Formulations, CBS Publishers and Distributors, New Delhi, 1996.

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