Development and Validation of Simultaneous Spectrophotometric Methods for Drotaverine Hydrochloride and Aceclofenac from Tablet Dosage Form

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Two simple spectrophotometric methods have been developed for simultaneous estimation of drotaverine hydrochloride and aceclofenac from tablet dosage form. Method I is a simultaneous equation method (Vierodt's method), wavelengths selected are 306.5 and 276 nm. Method II is the absorbance ratio method (Q-Analysis), which employs 298.5 nm as \( \lambda_1 \) and 276 nm as \( \lambda_2 \) (\( \lambda_{\text{max}} \) of AF) for formation of equations. Both the methods were found to be linear between the range of 8-32 µg/ml for drotaverine and 10-40 µg/ml for aceclofenac. The accuracy and precision were determined and found to comply with ICH guidelines. Both the methods showed good reproducibility and recovery with % RSD in the desired range. The methods were found to be rapid, specific, precise and accurate and can be successfully applied for the routine analysis of drotaverine and aceclofenac in their combined tablet dosage form.

Key words: Aceclofenac, absorbance ratio, drotaverine hydrochloride, simultaneous equation

Chemically, drotaverine (DV, fig. 1a) is (1-(3,4-diethoxybenzylidene)-6,7-diethoxy-1,2,3,4 tetrahydroisoquinoline) hydrochloride. It is a benzylisoquinoline derivative\(^1\). It is a highly potent spasmylic agent and has excellent smooth muscle relaxant properties\(^2\). Aceclofenac (AF, fig. 1b) is 2-[(2,6-Dichlorophenyl)amino]benzeneacetic acid carboxymethyl ester\(^3\). It is used as an anti-inflammatory drug. Literature survey revealed that assay of AF in bulk and dosage form is official in Indian Pharmacopoeia 2007\(^4\) and British Pharmacopoeia 2008\(^5\). Several analytical methods have been reported for estimation of DV like spectrophotometry\(^5-7\), HPLC\(^8\), flow injection chemiluminescence analysis\(^9\), thin layer chromatography\(^10,11\) and voltammetry\(^12\). The analytical methods reported for estimation of AF are spectrophotometry\(^13-15\), HPLC\(^16-18\), LC-MS\(^19\) and fluorimetry\(^20\). The present paper describes simple, accurate, specific and precise methods for simultane-

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Simultaneous estimation of DV and AF in their combined tablet dosage form using two UV spectrophotometric methods (a) simultaneous equation method and (b) absorbance ratio method. The proposed methods are optimized and validated as per the ICH guidelines.

A Shimadzu UV/Vis double beam spectrophotometer (model UV-1800) with a pair of 1 cm matched quartz cells was employed in this investigation. All weighing was done on a Shimadzu analytical balance (Model AU-220). Pure samples of DV and AF were obtained as gift samples from Astran Labs, Ahmedabad. Combined tablet formulation (Esnil) was procured from local pharmacy. Methanol AR was used as solvent.

Accurately weighed quantity of DV (80 mg) and AF (100 mg) was transferred to two separate 100 ml volumetric flasks, dissolved in little amount of methanol and diluted to the mark with methanol stock solutions: 800 μg/ml of DV and 1000 μg/ml of AF).

Simultaneous equation method uses the absorbances at two selected wavelengths, both being the λmax of the two drugs. Working standard solutions were scanned in the entire range of 200-400 nm to determine the λmax of both the drugs. The λmax of DV and AF were found to be 306.5 nm and 276 nm respectively (fig. 2). Seven standard solutions having concentrations 8, 12, 16, 20, 24, 28, 32 μg/ml for DV and 10, 15, 20, 25, 30, 35, 40 μg/ml for AF were prepared in methanol. The absorbances of resulting solutions were measured at 306.5 and 276 nm and calibration curves were plotted at these wavelengths. The absorptivity coefficient of these two drugs was determined using the calibration curve equation. Two simultaneous equations were formed using these specific absorbance values.

The concentration of Cx and Cy can be obtained as,

\[ C_x = \frac{(A_2a_{y_2} - A_1a_{y_1})}{(a_{x_2}a_{y_1} - a_{x_1}a_{y_2})} \]
\[ C_y = \frac{(A_1a_{x_1} - A_2a_{x_2})}{(a_{x_1}a_{y_1} - a_{x_2}a_{y_2})} \]

where, \( A_1 \) and \( A_2 \) are the absorbances of mixture at 306.5 and 276 nm respectively, \( a_{x_1} \) and \( a_{x_2} \) are absorptivities of DV at 306.5 and 276 nm respectively, \( a_{y_1} \) and \( a_{y_2} \) are absorptivities of AF at 306.5 and 276 nm respectively, \( C_x \) is concentration of DV, \( C_y \) is concentration of AF.

Absorbance ratio method uses the ratio of absorbances at two selected wavelengths one at iso-absorpive point and other being the λmax of one of the two components. From the overlain spectra of two drugs, it is evident that DV and AF show an iso-absorptive point at 298.5 nm and the second wavelength used was 276 nm, which is the λmax of AF (fig. 3). Seven standard solutions having concentration 8, 12, 16, 20, 24, 28, 32 μg/ml for DV and 10, 15, 20, 25, 30,
35, 40 μg/ml for AF were prepared in methanol. The absorbances at 298.5 nm (isoabsorptive point) and 276 nm (λmax of AF) were measured and absorptivity coefficients were calculated using calibration curve.

The concentrations Cx and Cy of DV and AF, respectively in the sample mixture can be calculated using equations Cx= [(Qm–Qy)/(Qx–Qy)]×A1/ax1 and Cy= [(Qm–Qx)/(Qy–Qx)]×A1/ay1. The Q-values and absorptivities for both drugs were calculated as follows, Qm= [Absorbance of sample solution at 276 nm/absorbance of sample solution at 298.5 nm (A1)], Qx= [Absorptivity of DV at 276 nm/Concentration of DV in g/100 ml], Qy= [Absorptivity of AF at 298.5 nm/Concentration of AF in g/100 ml], where, Qx and Qy are Q values of DV and AF, respectively, ax1 and ay1 are absorptivities at isoabsorptive point for DV and AF, respectively. These values were found to be Qx= 0.511, ax1=194.46, Qy= 2.301, ay1 = 144.64.

Ten tablets were weighed and crushed to obtain a fine powder. An accurately weighed tablet powder equivalent to about 80 mg of DV and 100 mg of AF was transferred to 100 ml volumetric flask and dissolved in 50 ml of methanol. The volume was made up to the mark using methanol as solvent. The resulting solution was filtered through Whatmann filter paper and 10 ml of this filtrate was appropriately diluted to get concentration of 80 μg/ml of DV and 100 μg/ml of AF. This solution was further diluted to get concentration of 16 μg/ml of DV and 20 μg/ml of AF. Absorbance of sample solutions was measured at 306.5 and 276 nm and the concentration of two drugs in the sample were determined using Eqns (1) and (2) (method I). For method II, the absorbance of the sample solution A1 and A2 were measured at 298.5 nm (iso-absorptive point) and 276 nm (λmax of AF) respectively and ratio of absorbance were calculated which was known as Qm. Relative concentrations of two drugs were calculated using equations (3) and (4). The result of analysis of tablet formulation is shown in Table 1.

Aliquots of standard stock solutions of DV and AF were taken in volumetric flasks and diluted with methanol to get final concentrations in range of 8-32 μg/ml for DV and 10-40 μg/ml for AF. This calibration range was prepared five times and absorbances were measured at respective wavelengths for each drug separately.

Precision of the methods was determined by performing interday variation, intraday variation and repeatability studies. In interday variation, the absorbance of standard solutions of DV (8-32 μg/ml) and AF (10-40 μg/ml) were measured on five consecutive days. In intraday variation, the absorbances were measured five times in a day. In repeatability study, three concentrations of both the drugs were analysed in triplicate.

To study the accuracy of the proposed methods, recovery studies were carried out by standard addition method at three different levels. A known amount of drug was added to pre-analyzed tablet powder and percentage recoveries were calculated.

The proposed methods were validated as per ICH guideline. The plot of absorbances versus respective wavelength is shown in Fig. 3.

| TABLE 1: RESULTS OF SIMULTANEOUS ESTIMATION OF DV AND AF IN MARKETED FORMULATION BY METHOD I AND II |
|-------------------------|--------|--------|--------|--------|--------|
| Method                  | DV (mg/Tablet) | AF (mg/Tablet) | % of label claim |
|                         | Label | Label | OBT    | OBT    |
| Method-1 (SE)           | 80    | 100   | 80.29  | 99.05  |
|                         |       |       | 100.37 | 99.05  |
| Method-2 (AR)           | 80    | 100   | 80.40  | 99.65  |
|                         |       |       | 100.50 | 99.65  |

*Average of five determinations; SE - Simultaneous equation; AR - Absorbance ratio; DV - Drotaverine; AF - Aceclofenac; OBT - Obtained.
concentrations of DV was found to be linear in the concentration range of 8-32 μg/ml with correlation coefficient 0.9996 at 306.5 nm and for AF it was found to be linear in the concentration range of 10-40 μg/ml with 0.9990 correlation coefficient at 276 nm for simultaneous equation method (method I). For absorbance ratio method (method II) linearity range was same as for method I with correlation coefficient 0.9995 at 298.5 nm and 0.9990 at 276 nm. Precision was calculated as interday and intraday variations and % RSD was found to be less than 1 for both methods and for both drugs (Table 2). The accuracy of method was determined at 75, 100 and 125% level. The % recovery ranges from 98.23% to 100.49% for both the methods (Table 3). The two methods can be successfully used for simultaneous estimation of DV and AF in their combined tablet dosage form. Marketed tablets were analyzed and results obtained were in the range of 98-102% (Table 1). The proposed methods were found to be simple, accurate and rapid for the routine determination of DV and AF in tablet formulation.

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Role of Butea Frondosa in Ameliorating Gastric Markers in Induced Gastric Lesions of Rats

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Banji, et al.: Butea Frondosa and Its Effect on Gastric Ulcers

The study evaluated the ability of the alcohol extract of Butea frondosa to protect the gastro-duodenal lining from injury inflicted by acetic acid and pyloric ligation in rats. The induced gastric lesions lead to the generation of alkaline phosphatase and pepsin, which serve as important markers of gastric damage. Alcohol extract of Butea frondosa was administered in doses of 10, 100, 300 and 500 mg/kg as a single schedule and for the time dependent studies in a dose of 100 mg/kg for 7, 14, 21 and 28 days, respectively. Our studies reveal a decline in the formation of alkaline phosphatase and pepsin with 300 and 500 mg/kg of the extract and following treatment for 21 and 28 days, respectively. Extract of Butea frondosa produces significant diminution in the formation of gastric markers implying possible gastro-protective action.

Key words: Alkaline phosphatase, Butea frondosa, markers, pepsin

Peptic ulcer disease is a health hazard both in terms of morbidity and mortality. The development of gastric ulcers occurs due to acid pepsin mixture and the breakdown of mucosal defense. Mucus like alkaline secretions, mucosal hydrophilicity, rapid epithelial cell renewal, rich mucosal blood flow, mucosal sulphydryls and increased resistance of gland cells in deep mucosa to acid and peptic activity are the mechanisms involved in mucosal defense. Pepsin is a proteolytic enzyme capable of causing mucosal erosion and ulcerations. Alkaline phosphatase (ALP) catalyses the hydrolysis of various phosphate esters at alkaline pH and can be considered to be a biomarker for gastric damage.

Butea frondosa Koen. Ex Roxb. is used traditionally against ulcers, skin diseases, herpes, acne, while internally it is used against gas colics, worms and piles. Phytochemically, it is rich in flavanoids, terpenoids and lipid constituents. The present study was designed to investigate the impact of

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