lines were obtained at 95% confidence interval using least square method. Correlation coefficient 'r' values (n=3) for all three drugs were ≥0.999. Accuracy of method was determined by recovery studies (n=3). The concentration of standard spiked to the sample was 0.07-0.13 μg/ml for TIZ, 1.75-3.25 μg/ml for DCL and 11.4-21.1 μg/ml for PAR. Recovery data from the study are reported in Table 1. The mean % recovery was found to be 99.0 % for TIZ, 99.3 % for DCL and 98.6 % for PAR. The content of the drugs in the commercial dosage form was found to be 99.1 % of TIZ, 99.5 % of DCL and 99.6 % of PAR per tablet by this method. The estimated amount was within the acceptable limits of the labeled claim of the formulation.

The developed RP-HPLC method provides a convenient and efficient method for the separation and estimation of TIZ, DCL and PAR in combined dosage form. There was no interference from the excipients used in the tablet formulation and hence the method is suitable for analysis of tablets. The results of validation showed that the proposed method is simple, linear, precise, accurate and selective and employed in routine assay of TIZ, DCL and PAR in tablets.

REFERENCES

1. Raman, B. and Patil, D., **Indian Drugs**, 2002, 39, 392.
2. Tuncel, M. and Dogrukol, D., **Anal. Lett.**, 1992, 25, 437.
3. Bouklough, A.A., El Jameel, A., Vire, J.C. and Patriarche, G.J., **Anal. Chim. Acta.**, 1992, 257, 41.
4. Bhoir, I.C., Raman, B., Sundareshan, M. and Bhagwat, A.M., **Anal. Lett.**, 1998, 31, 1533.
5. YU, J.Q., Luo, S.D., Lei, J.C., and Cai, H.S., **J. China Pharm. Uni.**, 1997, 28, 282.
6. Raman, B. and Patil, D., **Indian Drugs**, 2000, 37, 437.
7. Chawla, J.L., Sodhi, R.A. and Sane, R.T., **Indian Drugs**, 1996 171.
8. Vasudevan, M., Ravishankar, S., Ravibabu, T. and Nagarajan J.S.K, **Indian J. Pharm. Sci.**, 2000, 62, 122.
9. Sodhi, R.A., Chawla, J.L., and Sane, R.T., **Indian Drugs**, 1996 33, 280.
10. Ravishankar, S., Vasudevan, M., Gandhimathi, M. and Suresh, B., **Talanta**, 1998, 46, 1577.
11. Shingare, M.S., Naidu, K.R., and Kale, U.N., **Indian J. Pharm. Sci.**, 2003, 65, 315.
12. International Council on Harmonization, Guidance for Industry In: Q2B Validation of Analytical Procedure: Methodology, 1996, 2.

Stability Evaluation of Beetroot Colour in Various Pharmaceutical Matrices

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A red pigment was obtained from dried juice of *Beta vulgaris* Linn, which exhibited a λmax of 533 nm and 484 nm indicative of the presence of betacyanins (red) and betaxanthins (yellow), respectively. The influence of temperature and pH on the stability of the red pigment in pharmaceutical liquid oral bases was investigated. The colour was also evaluated for its stability in its adsorbed form on microcrystalline cellulose, lactose and dextrose. The colour was found to exhibit highest stability in adsorbed form with microcrystalline cellulose.

Colours form an integral part of pharmaceutical additives to enhance the organoleptic properties and patient acceptance. The use of certain synthetic colours has been banned, as they are well known to produce toxicity in animals, e. g. auramine found to inhibit growth and lead to liver and kidney dysfunction and so many others known for hypersensitivity and carcinogenic reactions[1]. Hence a lot of attention has been given to develop natural pigments as colourants with higher safety margins. In caryophyllales the

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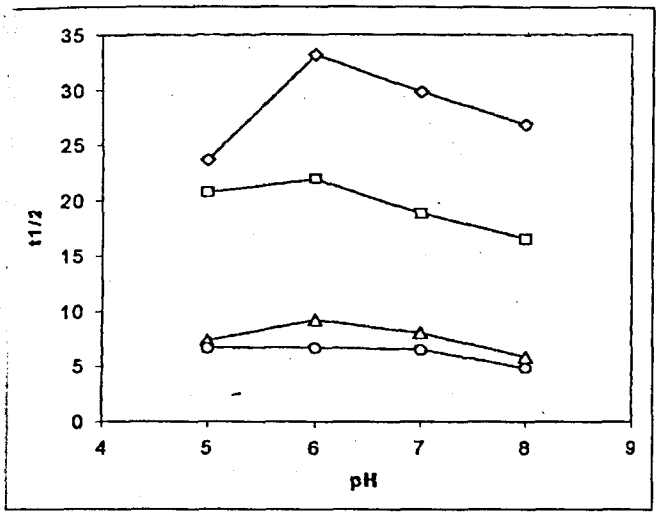

Fig. 1: Stability profile of beetroot colour in syrup and elixir bases.

Syrup at room temperature (-✧-), syrup at 40°, 75% RH (-□-), elixir at room temperature (-△-) and elixir at 40°, 75% RH (-○-).

pigmentation of reproductive and vegetative organs is produced by betalains, a group of nitrogenous plant pigment. Betalains are classified into two families of compounds; red betacyanins and yellow betaxanthins[2]. Among the red pigments, betalains have the potential to be developed as pharmaceutical red colourants. The red beetroot (*Beta vulgaris* Linn., Family Chenopodiaceae) is an excellent source of betacyanins, the red pigments. They typically contain 0.4 to 1.0 % of pigment expressed as betanin[3]. Besides being inexpensive, beet root powder is permitted as colourant under 1960 colour additive Ammendment[4].

The present study was taken up with an objective of investigating red beetroot colour for its possible application as a pharmaceutical adjunct. The study involved exploring the pH and temperature dependent stability of colour in various pharmaceutical vehicles like syrup and elixir bases. Stability of this colour in its adsorbed form with commonly used pharmaceutical additives for solid dosage forms like dextrose, lactose and microcrystalline cellulose (MCC) has also been carried out.

Beet roots were collected from the local market in Mumbai. After appropriate cleaning, they were blanched at temperature 80-90°. The coarse grinding of blanched red beets was carried out in a mixer. The expressed juice was dried under vacuum and dried extract was stored in a dessicator till further use. The dried extract when reconstituted in water and analysed using a Shimadzu UV/vis spec-

TABLE 1: PH AND TEMPERATURE DEPENDENT STABILITY OF BEETROOT COLOUR IN SYRUP AND ELIXIR BASES.

Base	Temp	Degradation rate constant (k_1) and half life ($t_{1/2}$)				
		pH	5	6	7	8
Syrup	R.T.	K_1 (h^{-1})	2.92×10^{-2}	2.09×10^{-2}	2.32×10^{-2}	2.57×10^{-2}
		$t_{1/2}$ (h)	23.70	33.15	29.79	26.87
	40°	K_1 (h^{-1})	3.34×10^{-2}	3.16×10^{-2}	3.68×10^{-2}	4.17×10^{-2}
		$t_{1/2}$(h)	20.75	21.96	18.81	16.62
Elixir	R.T	K_1 (h^{-1})	9.44×10^{-2}	7.53×10^{-2}	8.61×10^{-2}	1.17×10^{-1}
		$t_{1/2}$ (h)	7.34	9.20	8.04	5.90
	40°	K_1 (h^{-1})	1.03×10^{-1}	1.03×10^{-1}	1.05×10^{-1}	1.43×10^{-1}
		$t_{1/2}$ (h)	6.72	6.72	6.54	4.83

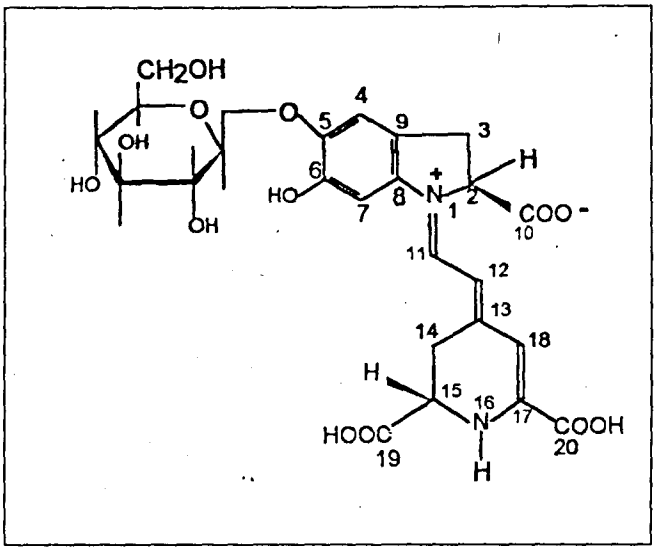

Fig. 2: Structure of betanin.

trophotometer (1601) exhibited a characteristic spectrum showing λmax at 533 and 484 nm indicating presence of betacyanins and betaxanthins, respectively[5]. MCC, lactose and dextrose were of pharmaceutical grade and procured from local sources. Syrup (85% w/v) and elixir (65% w/v sucrose and 12% absolute alcohol) were prepared in a pH range of 5 to 8 using standard buffer solutions as per standard procedures[6,7].

Stability evaluation at various temperature (room temperature 30-33° and 40°, 75%RH) and pH conditions using syrup and elixir bases was carried out after withdrawing aliquots at one hour intervals upto 4 h. The colour intensity was monitored spectrophotometrically at 533 nm. For evaluation of colour stability in its adsorbed form with MCC, lactose and dextrose, aliquots were analysed for its colour intensity at one week intervals upto seven weeks by monitoring optical densities at 533 nm.The change in colour intensity in both the above cases was studied by determining the decrease in betacyanins and calculating percent of betacyanins using the formula, % colour retained=optical density at time t/optical density at time t_0. Graphs of log % colour retained Vs time were plotted for various stability conditions and degradation rate constants were calculated after linear regression (k_1=-slope x 2.303, fig. 1).

TABLE 2: STABILITY OF BEETROOT COLOUR IN ADSORBED FORM

Adsorbent	$K_1(w^{-1})$	$t_{1/2}$ (w)
Dextrose	7.44×10^{-2}	9.32
Lactose	2.31×10^{-1}	3.00
MCC	6.05×10^{-2}	11.45

Red pigment of beetroot comprising of 95% of betanin has been reported to undergo first order degradation kinetics[8]. The present study has confirmed this phenomenon. The degradation rate constant (K_1) for the stability of the colour in various pharmaceutical matrices and at different pH and temperature conditions are depicted in Table 1 for the syrup and elixir bases and Table 2 for the colour in adsorbed form. The stability experiments on beetroot colours revealed that hue of betacyanins was not affected in pH range 4 to 8. However, below pH 3, the intensity of the purple red colour of beetroot decreased and in concentrated hydrochloric acid the colour was violet. Above pH 8 the colour was blue and it changed to yellow form on addition of concentrated sodium hydroxide. This is probably because of release of betalamic acid due to alkaline hydrolysis of betanin[9]. The colour exhibited higher stability at room temperature in pH range between 6 and 7 especially in the syrup base. The colour was found to be less stable in the elixir base containing alcohol.

When the stability experiments were carried out on colour in its adsorbed form, highest stability was observed by use of MCC as an adsorbent. The stability of beet root colour in presence of lactose as an adsorbent was found to be appreciably lower as can be seen from higher K_1 values (Table 2) probably due to participation of lactose in Maillard reaction with amine containing compounds. Betanin is a red coloured compound with an amine group in its nucleus (fig. 2). Earlier investigators have demonstrated the ability of lactose to undergo condensation reactions with compounds containing amino group[10]. The present study has confirmed this as lactose has caused a pronounced effect on stability of beetroot colour.

It can be further stated that this natural red pigment is better suited for solid dosage forms and products to be reconstituted, because of its higher stability in adsorbed form. A further investigation into physicochemistry of betalains is necessary if these natural colourants are to be more competitive and viable alternatives for synthetic colourants currently used.

REFERENCES

1. Bhat, R.V. and Mathur, P., **Current Science,** 1998, 74 , 198.
2. Martinez – Parra, J. and Munoz, R., **J. Agric. Food chem.,** 2001, 49, 4064.

Pasch, J.H., vonElbe, J.H. and Dinesen, N., **Food Product Development**, 1975, 9, 38.
Pasch, J.H. vonElbe, J.H., **J. Food Sci.**, 1975, 40, 1145.
Dreiding, A.S., In; Mabry, T.J., Eds., Recent Advances in Phytochemistry, Vol. 1, Appleton-Century-Crofts, New York, 1968, 147.
Ansel, H.C., Allen, L.V., Popovich, N.G., In; Pharmaceutical Dosage Forms and Drug Delivery systems, 17th Edn., Lippincott Williams and Wilkins, New York, 1999, 318.
United States Pharmacopoeia 25, National Formulary 20, U.S. Pharmacopoeia Convention Inc., 12601, Twinbrook Parkway, Rockville, MD 20852, 2002, 2340.
VonElbe, J.H., Maing, Y. and Amundson, C.H., **J. Food Sci.**, 1974, 39, 335.
Jackman, R.L. and Smith, J.H., In; Hendry, G.A. F., Eds., Natural Food Colourants, Blackie and Son Ltd., London, 1992, 222.
Kibbe, A.H., Eds., In; Handbook of Pharmaceutical Excipients, 3rd Edn., American Pharmaceutical Association, 2000, 283.

RP-HPLC Estimation of Rofecoxib and Tizanidine in Combination Tablets

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A reverse phase high performance liquid chromatography method was developed for the simultaneous estimation of rofecoxib and tizanidine in tablet formulations. The separation was achieved by Luna C_{18} column and methanol : phosphate buffer pH 3.5 (55:45 v/v) as eluent, at a flow rate of 1 ml/min. Detection was carried out at 240 nm. Valdecoxib was used as an internal standard. The retention time of rofecoxib and tizanidine was found to be 4.53 and 5.92 min, respectively. The method has been validated for linearity accuracy and precision. Linearity for tizanidine and rofecoxib were in the range of 0.6-1.4 μg/ml and 7.5-17.5 μg/ml, respectively. The mean recoveries obtained for tizanidine and rofecoxib were 98.73% and 99.70%, respectively. The developed method was found to be accurate, precise, selective and rapid for simultaneous estimation of rofecoxib and tizanidine in tablets.

Tizanidine HCl, 5-chloro-4-[2 imidazolin-2-yl amino]-2,1,3-benzothiadiazole, which is used as a centrally acting muscle relaxant[1]. Rofecoxib is 4-[4-(methylsulfonyl)-phenyl]-3-phenyl-2 (5H)-furanone, a specific cyclooxygenase-2 inhibitor[2]. It is indicated for the treatment of spasm and pain associated with musculoskeletal disorders. A tablet formulation containing 2 mg of tizanidine and 25 mg of rofecoxib is available (Sioxx-MR, Sysmed Labotarories). A survey of literature revealed that HPLC methods are reported for the determination of rofecoxib in tablets[3-4] and biological fluids[5-6]. A HPTLC method for estimation of rofecoxib has also been reported[7]. RP-HPLC method was reported for the simultaneous estimation of tizanidine and nimesulide in tablets[8]. Methods based on electroanalytical techniques[9-10] and supercritical fluid chromatography[11] have also described in the literature for the estimation of tizanidine. However, no HPLC method for the simultaneous estimation of rofecoxib and tizanidine in combined dosage forms has so far been reported. The present work describes the development of a simple, precise and accurate reverse phase HPLC method for simultaneous estimation of rofecoxib and tizanidine in tablets.

The drug samples, rofecoxib and tizanidine were obtained as gift samples from the Sun Pharmaceutical Industries, Vadodara. HPLC grade methanol was supplied by Merck Co. Mumbai. Water of HPLC grade was collected from a Milli-Q system. Sodium dihydrogenorthophosphate AR and phosphoric acid AR were purchased from S. D.

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