samples were analyzed and data of 25 has been presented in Table 2. We observed that PCA (30–35° for 72 h) and R2A (20–25° for 5 d) gave comparable results, with R2A giving slightly higher values in some cases. This equivalency in count is attributed to the initial microbial load of water sample, which may be having low or negligible count of injured or slow growing bacteria. R2A being a low nutrient medium supports the growth of slow growing bacteria or those injured due to variety of reasons that include chlorine treatment and heat treatment[1].

Further, in order to know more about the correlation, results obtained on two media were plotted following the procedure recommended by Altman and Bland[8], as presented in fig. 1 and the results show good correlation between the two. SCDA medium gave low counts, which is in agreement with earlier findings[6].

It is concluded from the study that R2A agar, once compared to PCA can be included or excluded during routine monitoring depending upon the results obtained during comparative validations. If both the media yield equivalent counts, then R2A medium can be excluded during routine monitoring or *vice-versa*. But for this purpose, adequate data has to be generated. It is also concluded that count on R2A medium will depend upon the nature of the microbial load present in the sample as explained above.

Moreover USP[1] clearly states that low nutrient medium like R2A and high nutrient medium like PCA are to be compared during validation of water system and on that basis it has to be decided whether particular system needs to be monitored additionally using low nutrient medium (R2A) on regular basis or the normal medium like PCA is sufficient.

REFERENCES

1. United States Pharmacopoeia, 24th Edn., USP Convention, Inc., Rockville, 2000, 2154.
2. United States Pharmacopoeia, 24th Edn., USP Convention, Inc., Rockville, 2000, 1814.
3. United States Pharmacopoeia, 24th Edn., USP Convention, Inc., Rockville, 2000, 2099.
4. American Public Health Association, Chapter 9215, APHA, Washington, DC, 2001, 9, 31
5. Reasoner, D.J. and Geidreich, E.E., **Appl. Environ. Microbiol.,** 1985, 49, 1.
6. Sundarm, S., Eisenhuth, J., Howard Jr., G. and Brandwein, H. **PDA J. Pharm. Sci. Technol.,** 2001, 55, 65.
7. Van der Linde, K., Lim, BT., Rondeel, J.M., Antonissen, L.P. and de Jong, G.M. **Nephrol. Dialysis Transplant.,** 1999, 14, 2433
8. Altman, D.G. and Bland, J.M., **The Statistician,** 1983, 32, 307.

Spectrophotometric Estimation of Venlafaxine with Folin Ciocalteu Reagent

A. RAJASEKARAN*, M. ARULKUMARAN AND S. KANNANRAJ

Arulmigu Kalasalingam College of Pharmacy, Anand Nagar, KrishnanKoil-626190.

Accepted 25 Sepetember 2003

Revised 1 July 2003

Received 17 September 2002

A simple, rapid, precise, accurate and highly specific spectrophotometric method has been developed for the determination of venlafaxine in its pharmaceutical dosage form. The method is based on the formation of blue colored chromogen due to the reaction of venlafaxine with Folin Ciocalteu reagent in presence of alkali, which exhibits λ_{max} at 730 nm. Beer's law obeyed in the concentration range of 2.5-25 μg/ml. The blue color obtained was stable for more than 24 hours. The method was successfully applied for the quantitative determination of venlafaxine and its pharmaceutical dosage form. The accuracy and reproducibility of the proposed method was statistically validated by recovery studies.

***For correspondence**

E-mail: rsekaran2001in@yahoo.co.in

Venlafaxine [1-2] (VFX) is an orally active serotonin-noradrenaline reuptake inhibitor used for the treatment of major depressive disorders. VFX [3] is chemically described as ±-1-[2-(dimethylamino)-1-(4-methoxyphenyl) ethyl] cyclohexanol. It is not official in any Pharmacopoeia. Literature survey revealed only an extractive spectrophotometric method[4] for the estimation of VFX and its solid formulations. No simple spectrophotometric method is reported so far in the literature for the estimation of VFX and hence a simple, rapid and highly specific spectrophotometric method has been developed for the determination of VFX in its pharmaceutical dosage form.

A Systronics model 118 single beam UV/Vis Spectrophotometer with 1 cm matched quartz cells were used for absorbance measurements. All reagents used were of analytical grade. Solutions of Folin Ciocalteu reagent IN, FCR Loba Chemie, Mumbai) were prepared in distilled water. The stock solution of VFX (100 μg/ml) was prepared in distilled water.

Aliquots of standard solution representing 2.5-25 μg/ml of VFX were transferred into ten 10 ml serially numbered volumetric flasks. One ml of 20% w/v sodium carbonate solution and 1 ml of FCR was added to each volumetric flask. The solution were kept at room temperature for 10 min for completion of reaction and volume was made upto 10 ml with distilled water. The absorbance was measured at 730 nm against reagent blank. Tablet/capsule powder equivalent to 25 mg was weighed accurately and transferred into a 25 ml volumetric flask. The contents were dissolved and made upto 25 ml with distilled water and filtered. Ten millilitre of the filtrate was pipetted out into another 100 ml volumetric flask and diluted to 100 ml with distilled water. Three ml of this solution was pipetted out into a 10 ml volumetric flask. Then 1 ml of 20 % w/v sodium carbonate solution and 1 ml of FCR was added to each volumetric flask. The solutions were kept at room temperature for 10 min for completion of reaction and volume was made upto 10 ml with distilled water. The absorbance was measured at 730 nm against reagent blank. The absorbance of pure VFX at the same concentration was also measured at 730 nm for the calculation of drug content in formulations. The contents in tablet/capsules were also determined by the reported method[4].

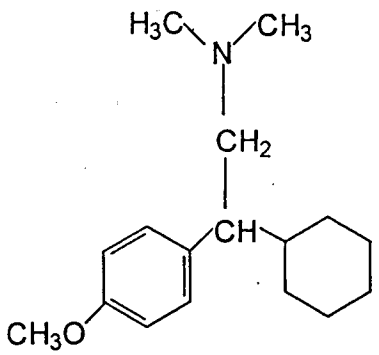
To study the accuracy, reproducibility and to check the interference of excipients in tablet/capsules, recovery study was performed. The recovery of the added standard was studied at four different levels. Each level was repeated three times. From the amount of the drug found, the percentage recovery was calculated using the formula: % Recovery=$N\Sigma XY-(\Sigma X)(\Sigma Y)/N\Sigma X^2(\Sigma X)^2$ where X is the amount of standard drug added, Y is the amount of drug found by the proposed method and N is the number of observations.

The blue colored chromogen formed is due to the reaction of VFX with FCR in presence of alkali, which exhibited λmax at 730 nm. The optimum conditions for the estimation of VFX were established by varying the concentration and amount of sodium carbonate solution[5] Maximum absorbance value was obtained with 1 ml of 20% w/v solution of sodium carbonate. The calibration curve yielded correlation coefficient (r) of 0.9996 over beer's range of 2.5-25 μg/ml. The regression equation was found to be y=0.0186x+0.0043. The molar absorptivity was found to be 5279.39 e/mol.cm. The values of molar absorptivity indicate high sensitivity of the method. The low values of standard deviation and coefficient of variation indicate the proposed method is accurate and highly precise. The percentage recovery values obtained indicate non-interference from

TABLE-1: ANALYSIS OF VENLAFAXINE FORMULATIONS

Sample	Labeled Amount (mg)	Amount found by proposed method	Amount found by reported method[4]	Percentage Recovery	S.D.	C.V.
Venlor	25.00	25.31±0.7	25.04+0.5	99.5	0.4526	1.8045
Veniz-XR	37.50	37.48±0.6	37.52+0.6	99.8	0.0249	0.0663
Venlift-OD	37.50	37.56±0.6	37.58+0.5	99.6	0.0341	0.0908

Each result is the mean of three replicates and for recovery studies 0 mg, 5 mg, 10 mg and 15 mg were added to pharmaceutical preparations.



Venlafaxine

excipients used in the formulations. Phosphomolybdic and tungstic acids in the FCR preparation involve the following chemical species [6-7].

$3H_2O.P_2O_5.13WO_3.5MoO_3.10H_2O$ and $3H_2O.P_2O_5.14WO_3.4MoO_3.10H_2O$

VFX probably effects a reduction of one, two or three oxygen atoms from tungstate and/ or molybdate in FCR thereby producing one or more of the possible reduced species which have a characteristic intense blue color.

In conclusion, the proposed method is simple, rapid, accurate, specific and the reagents used in the method are cheaper. Further, the procedure does not involve any critical reaction conditions like heating and extraction as mentioned in the reported spectrophotometric method. Hence it may be used for the routine analysis of VFX in the pharmaceutical dosage forms.

REFERENCES

1. Reynolds, J.E.F. Eds., In; Matrindale, The Extra Pharmacopoeia, 31st Edn., The Pharmaceutical Society, London, 1996, 337.
2. Krishnan, P.V.V., In; CIMS, Drug Profile, Biogard Pvt Ltd., Bangalore, 2001, 25.
3. Budavari, S., Eds; The Merck Index,12th Edn.,Merck & Co., Whitehouse Station, NJ 1996,1695.
4. Appalaraju, S., Sarasambi, P.S. and Karadi, A.B., **Asian. J Chem.**, 2002, 14, 467.
5. Massart, D.L., Vandegingle, B.G. M., Deming, S.N., Michette, Y. and Kaufman, L., In; Chemometrics, A Text Book, Elseveir, Amsterdam, 1998, 293.
6. Folin, O. and Ciocalteu, D., **J. Biol. Chem.**, 1927, 73, 627.
7. Petreson, G.L., **Anal. Biochem.**,1979, 100, 201.

Transdermal Delivery of Ampicillin Sodium Patch Made from Volatile Vehicle

J. BAGYALAKSHMI* SHEHEERA SHERIFF, SONIA JOHN, T.K.RAVI, P.K. MANNA[1], R. MANAVALAN[1]

Sri RamaKrishna Institute of Paramedical Sciences, College of Pharmacy, Sarojini Naidu Road, Coimbatore-641044.

[1]Institute of Pharamaceutical Technology, Annamalai University, Annamalai Nagar, Chidambaram-608002.

Accepted 26 September 2003

Revised 1 July 2003

Received 30 January 2003

The permeation of ampicillin sodium patch from ethanol/pH4.7 buffer solution containing antinucleant polymers across mouse skin, was investigated. The *in vitro* release of ampicillin sodium was determined under open condition at 25° and 65% relative humidity. Therefore the influence of evaporation of vehicle components on the permeation of ampicillin sodium was examined. Evaporation of the vehicle led to drastic compositional changes leading to supersaturation. However, supersaturation solutions started to crystallize reducing the thermodynamic activity of ampicillin sodium. Antinucleant polymers where used in the preparation of volatile vehicles in order to maintain the increased activity state of the drug.

*For correspondence

E-mail: bags_98@hotmail.com