cumulative release was always on the plus side of the theoretical requirement. SA IV formulations showed the most sustaining effect but its release constant (k=6.09 per h) is slightly lesser than the theoretical requirement (k = 8.19 per h). However, there is scope for correction by making allowance for extra drug (30 mg) or by modification of the formulation parameters.

We conclude that stearic acid and ethylcellulose can be used in combination to produce oral sustained release dosage form of diltiazem hydrochloride.

REFERENCES

1. Culter, N.R., Eff, J.A., Formell, G, Brass, E.P., Archer, S., Chrysant, S.G., Fiddles, R., **J. Clin. Pharmacol.**, 1995, 35, 189.
2. Frishman, W.H., **J. Clin. Pharmacol.**, 1993, 33, 612.
3. Graney, W.F., **Am. J. Med.**, 1992, 93, 565.
4. Bourge, R.C., Kirklim, J.K., Naftel, D.C., Figgs W.D., White, W.C., Ketchum, C., **Am. J. Med.**, 1991, 90, 402.
5. Neumayer, H.H. and Wagner, K., **The Lancet.**,1986, 2,5.
6. Wagner, K. and Neumayer, H.H., **The Lancet**, 1985, 2, 1355.
7. Gordin, A., Pohto, P., Sundberg, S, Nykane, S., Haataja, H., Mannisto, P., **Eur. J. Clin. Pharmacol.**, 1986, 31, 423.
8. The Pharmacopoeia of India, Third Edition (Published by Govt. of India), 1985, 2, A-142.
9. Gohel, M.C., Patel, M.C. and Gaujar, G., **The Eastern Pharmacist** 1996, 39, 133.
10. Cartensen, T.J., In; Theory of Pharmaceutical Systems. Academic Press, Newyork. 1973, 2, 285.
11. The Pharmacopoeia of India, Third Edition (Published by govt. of India), 1985, 2, A-124.
12. Higuchi, T., **J. Pharm. Sci.**, 1963, 52, 1145.
13. Echizem, H. and Eichelbaum, M., **Clin. Pharmacokinet.**, 1986, 2, 425.
14. Lane, E.A., Absolute bioavailability of diltiazem hydrochloride ASHP-Midyear-Clinical Meeting, 1991, 278E.

Visible Spectrophotometric Method for the Determination of Salmeterol Xinafoate

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A simple and sensitive visible spectrophotometric method based on the reaction of salmeterol xinafoate with diazotised dapsone under alkaline conditions to form a stable orange colored chromogen, which can be quantitatively measured at 465 nm, is reported.

Salmeterol xinafoate[1,2] chemically (RS-5-{1-Hydroxy-2-[6(4-phenyl butoxy) hexylamino] ethyl} salicyl alcohol 1-hydroxy-2-naphthoate, is a relatively new long acting antiasthmatic drug. HPLC[3-6] and few spectrophotometric method[7,8] have been reported earlier for the determination of salmeterol in human plasma and in pharmaceutical dosage forms. In the present investigation, the authors have developed a simple, sensitive spectrophotometric method.

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A 1.0 mg/ml solution of salmeterol xinafoate in methanol was prepared. Working standard solution (100 µg/ml) was prepared by further dilution with distilled water. Aqueous solutions of sodium nitrite (3% w/v), sodium hydroxide (1N) and solution of dapsone (0.5% w/v) in 10% hydrochloric acid were prepared in the usual way. An ELICO UV-VIS spectrophotometer model SL-150 with 1 cm matched quartz cells was employed for spectral measurements.

One ml of dapsone solution was pipetted out into a series of 10 ml volumetric flasks, 1 ml of sodium nitrite

Table I - Estimation of Salmeterol Xinafoate

Tablet or Excipient	Tablet Strength (mg)	Amount found		% Recovery
		Proposed method (mg)	UV method+ (mg)	
TAB. I	30	29.76	29.91	99.2[a]
TAB. II	30	29.82	29.94	99.4[a]
Starch	40	39.92	39.97	99.8[b]
Talc	40	40.40	40.75	101.0[b]
Lactose	40	39.84	39.64	99.6[b]

+ As the drug is not official in any pharmacopoeia, official method is not available. Hence UV method is selected for comparison. a: Amount of drug added for recovery is 20 mg. b: Amount of drug added for recovery is 40 mg.

was added to each flask and the contents were mixed well. After 5 min, aliquots of salmeterol solution ranging from 0.2-1.0 ml (20-100 µg) were added followed by 2 ml of sodium hydroxide to each flask and the volume was made upto the mark with distilled water. After 5 min the absorbance was measured at 465 nm against a reagent blank.

Tablet powder equivalent to 10 mg of salmeterol xinafoate was taken and mixed with 25 ml of methanol to dissolve the drug. The solution was then filtered and the filtrate was made upto 100 ml with distilled water. Further analysis was carried out as described above. The tablets were also analysed by UV spectrophotometric method by extraction of the drug into methanol and measuring the absorbance at 254 nm after suitable dilution with distilled water. The results are given in Table-1.

The proposed method is based on the formation of an orange colored chromogen by the reaction of salmeterol with diazotised dapsone under alkaline conditions.

The colored solution exhibited λ max at 465 nm and is stable over a period of 12 h. The colour obeyed Beer's law in the concentration range of 0-10 µg/ml. The regression line was found to be $Y=6.4x10^{-3}X+2.0x10^{-4}$, where X is the concentration of salmeterol in micrograms per ml of dilution and Y is the absorbance at 465 nm. Sandell's sensitivity (µg/Cm^2/0.001 absorbance unit) and molar absorptivity (lit. $mole^{-1}$. cm^{-1}) were found to be 0.0153 and $2.659x10^4$ respectively. When the stock solution containing 75 micrograms of drug was assayed repeatedly (n=5) the percent RSD and range of error (0.05 significance level) were found to be 1.532 and 1.281 respectively. When dosage forms (Tablets) containing salmeterol were analysed the results obtained by the proposed method are in good agreement with the labelled amounts and are comparable with the results of UV spectrophotometric method (Table-1).

To evaluate the validity and reproducibility of the method, known amounts of drug were added to previously analysed samples, which were analysed again using the method developed and the results are shown in Table-1. The usual excipients and other additives present in the formulation were found not to interfere.

Thus, the proposed method appears to be a simple, rapid, sensitive and reproducible one which may be used for the determination of salmeterol in bulk and in pharmaceutical preparations.

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REFERENCES

1. Budavari, S. Eds, The Merck Index, 12th Edn. Merck and Co., Inc., Whitehouse Station, NJ, 1996, 1434.
2. **Martindale the Extra Pharmacopoeia**, 30th Edn., The Pharmaceutical Press, London, 1993, 1257.

3. Brogden, R.N and Faulds, D., **Drug**, 1991, 42, 895.
4. Twentyman, O.P., **Lancet**, 1990, 336, 1138.
5. Shah, V.P. and Midha, K.K., **Eur. J. Drug. Metab. Pharm.**, 1991, 6, 249.
6. Nagesh, B., Naresh, S and Pathak, M.L., **The Eastern Pharmacist**, 1997, 40, 141.
7. Chowdary, K.P.R and Devala Rao, G., **Indian Drugs**, 1997, 34, 605.
8. Chowdary, K.P.R and Devala Rao, G., **J. Inst. Chemists (India)**, 1997, 69k, 170.

Antifungal Activity of *Luvunga scandens* Against Some Keratinophilic Fungi

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The essential oil from the fruits of *Luvunga scandens* Roxb. (Family Rutaceae) has been studies for *in vitro* antifungal activity against four keratinophilic fungi, *Arthroderma benhamiae, Microsporum gypseum, Trichophyton mentagrophytes* and *Ctenomyces serratus* using filter paper disc agar diffusion technique. The oil exhibited very good to moderate inhibitory effect against the fungi. The susceptibility of the oil towards dermatophytes is interesting and can be exploited against dermal infections.

Luvunga scandens Roxb. (Family Rutaceae) is a shrub widely distributed in tropical and subtropical Asia including India and Burma. Dried fruits are available in Indian Bazar (Particularly Bengal) under the name of Kakala or Sugandh-kokila and used for preparing perfumed medicinal oil[1] to cure baldness. The root and berries are cure for exhaustion, biliousness, troubles due to vata, kapha and fever[2]. The essential oil from the fruit pulp has 1:8 cineole (32.3%), methyl cinnamate (14.5%), camphor (9.7%), carene (9.1%) and α-terpineol (5.6%) as the main constituents[3]. The oil has CNS depressant and hypotensive action[4]. The present paper reports the preliminary *in vitro* investigations on the efficacy of the essential oil of *Luvunga scandens* against four keratinophilic fungi.

The dried fruits of *Luvunga scandens* were procured from the local market and identified on the basis of morphological characteristics. The essential oil from the coarsely crushed dried fruit pulp was obtained by hydrodistillation using a Perkin apparatus in an yield of 3.1% (v/w). The test keratinophilic fungi, *Arthroderma benhamiae, Microsporum gypseum, Trichophyton mentagrophytes* and *Ctenomyces serratus* were procured from the Department of Botany, Dr. Harisingh Gour University, Sagar. Filter paper disc agar diffusion technique of Maruzzella and Henry[5] was followed for the evaluation of *in vitro* antifungal activity.

The pathogenic fungal species were subcultured on sterile Sabouraud's nutrient broth. Suspension of subcultured organisms were made following the procedure adopted by Bray[6]. Twenty millilitres of sterilized Sabouraud's dextrose agar medium was taken in each petriplate (15 cm). After the agar had hardened, 4 ml of suspension of subcultured organism was distributed evenly over the surface of the plated medium. Sterilized Whatman filter paper No. 1 discs (6 mm) were thoroughly impregnated with 5 µl neat oil as such and in the dilutions of 1:50, 1:100, 1:200 and 1:1000 using Tween-80. Five test discs along with control disc of griseofulvin dissolved in dimethyl formamide (1000 ppm) were placed on each seeded agar plate and incubated at 28° in cool room for 72 h. The experiments were performed

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