under curve is a very simple method and can be employed for routine analysis of these two drugs in combined dosage forms. Once the 'X' values are determined then it requires only determination of area under curve of the sample solution at the selected wavelength range and few simple calculations.

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REFERENCES

1. Lee, H., Shim, H.O. and Lee, H.S., **Chromatographia,** 1996, 42, 39.
2. Farthing, D., Sica, D., Fakhry, I., Pedro, A. and Gehr, T.W.B., **J. Chromatogr. B: Biomed Appl.,** 1997, 704, 374.
3. Soldner, A., Spahn-Langguth, H. and Mutschler, E., **J. Pharm. Biomed. Anal,** 1998, 16, 863.
4. William, R.C., Alsandro, M.S., Fasone, V.L., Boucher, R.J. and Edwards, J.F., **J. Pharm. Biomed. Anal,** 1996, 14, 1539.
5. Bachman, W.J. and Stewart, J.J., **J. Chromatogr. Sci.,** 1990, 28, 123.
6. De Croo, F., Vanden Bossche, W. and De Moerioose, P., **Chromatographia,** 1985, 20, 477.
7. Smahi, Z., Huvenne, J.P. and Traisnel, M., **Ann. Pharm. Fr.,** 1992, 50, 167.
8. Lu, M., Wu, G., Chen, S. and Ruan, Z., **Nanjing Yaoxueyuan Xuebao,** 1983, 1, 1; through **Anal. Abstr.,** 1984, 46, 8D 87.
9. Chandwani, O.D., Dahibhate, P.P., Kadam, S.S. and Dhaneshwar, S.R., **Indian Drugs,** 1996, 33, 401.
10. Bonazzi, D., Gotti, R., Andrisano, V. and Carvrini, V., **Farmaco,** 1996, 51, 733.
11. Pharmacopoeia of India, Vol. I, The controller of Publications, New Delhi, 1996, 370.
12. Kanumula, G.V. and Bhanu, R., **Indian Drugs,** 2000, 37, 38.
13. Bolton, S., In; Pharmaceutical Statistics-Practical and Clinical Application, 2nd Edn., Marcel Dekkar, New York, 1990, 19.

Visible Spectrophotometric and HPLC Methods for Estimation of Rimapril from Capsule Formulation

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Two visible spectrophotometric and one HPLC method have been developed for estimation of rimapril from its capsule formulation.The developed visible spectrophotometric methods were based on formation of chloroform extractable coloured complexes of drug with nitrosonaphthol and with bromophenol blue.The formed coloured complex with nitrosonaphthol showed absorbance maximum at 445.5 nm and Beer's law was obeyed in the concentration range of 50-300 µg/ml of drug while the formed coloured complex with bromophenol blue showed absorbance maximum at 414 nm and Beer's law was obeyed in the concentration range of 20-100 µg/ml of rimapril. Developed HPLC method was a reverse phase chromatographic method using Inertsil C_{18} column and methanol:acetate buffer::80:20 as mobile phase with detection at 230 nm. Loratidine was used as internal standard for the HPLC method. Linearity was observed in concentration range of 10-300 µg/ml of rimapril. Results of analysis for all the methods were validated statistically and by recovery studies.

Rimapril, chemically [2s-[R*(R*), 2α, 3αβ, 6αβ]-1-[2-[(1-ethoxycarbonyl)-3-phenyl]-amino]-1-oxopropyl]-octahydrocyclopenta[b]pyrrole-2-carboxilic acid, is an antihypertensive agent[1]. Few HPLC[2-4] methods are reported for estimation of rimapril from its solution matrix

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and one HPLC[5] method is reported for identification of rimapril and its precursor. However, none of the methods is reported for the estimation of the drug from formulation. An attempt has been made in the present study to develop two simple visible spectrophotometric and one HPLC method for analysis of rimapril from capsules.

A Jasco UV/visible spectrophotometer with 1 cm matched quartz cells and a Shimadzu delivery module LC-10AD with UV SPD-10A detector and a Chromatopac C-R7A integrator were used for present study.

Nitrosonaphthol reagent (1%) was prepared by dissolving 2-nitroso-1-naphthol-4-sulphonic acid in distilled water. Bromophenol blue solution (0.1%) was prepared in acid phthalate buffer of pH 2.6. Both the reagents were extracted several times with chloroform so as to remove chloroform soluble impurities. Standard stock solution of rimapril (500 µg/ml) was prepared in chloroform.

For method I, standard stock solution of drug in chloroform was diluted with the same so as to give several dilutions in the concentration range of 50-300 µg/ml of rimapril. To 10 ml of each dilution taken in a separating funnel, 10 ml of nitrosonaphthol reagent was added. Reaction mixture was gently shaken for 5 min and allowed to stand so as to separate aqueous and chloroform layer. Coloured chloroform layer was separated out and absorbance was measured at 445.5 nm against reagent blank. A calibration curve was prepared.

For method II, standard stock of drug in chloroform was diluted with the same so as to give several dilutions in the concentration range of 20-100 µg/ml of rimapril. To 10 ml of each dilution taken in a separating funnel, 10 ml of bromophenol blue solution was added. Reaction mixture was shaken gently for 5 min and allowed to stand so as to separate aqueous and chloroform layer. Coloured chloroform layer was separated out and absorbance was measured at 414 nm against reagent blank. A calibration curve was prepared.

Contents of 20 capsules were emptied, accurately weighed and average powder weight per capsule was determined. The capsule contents were powdered and powder equivalent to 10 mg of rimapril was accurately weighed and transferred to 100 ml volumetric flask. Chloroform (75 ml) was added to it and the mixture was shaken for 10 min to dissolve the drug. The solution was filtered through Whatman filter paper No. 41 into another 100 ml volumetric flask. The filter paper was washed with chloroform and the washings were added to the filtrate. Final volume of the filtrate was made upto the mark with chloroform.

For method I, 10 ml of this solution was taken in a separating funnel and treated as per the procedure of calibration curve described above. For method II, 5 ml solution of capsule sample (used for method I) was diluted to 10 ml with chloroform and this solution was then taken in a separating funnel and treated as per the procedure of calibration curve for method II. Concentration of drug in sample solution was determined from respective calibration curve. Process was repeated five times with two different brands of capsule formulation and results of analysis are reported in Table 1.

TABLE 1: RESULTS OF ANALYSIS AND RECOVERY STUDIES

Method	Brand	Label Claim (mg/cap of rimapril)	% of Label Claim Estimated*	S.D.	% Recovery**
Spectrophotometric Using Nitroso Naphthol	A	01	99.02	0.976	100.72
	B	01	98.76	0.654	
Spectrophotometric Using Bromophenol Blue	A	01	98.56	0.478	99.76
	B	01	98.73	0.460	
HPLC	A	01	101.23	0.285	99.20
	B	01	101.07	0.097	

* Average of five determinations. ** Average of recovery studies at three different concentration levels.

For HPLC method Inertsil C_{18} ODS 3V (5μ) 250x4.6 mm column and methanol:acetate buffer (3.85 g/l)::80:20 as mobile phase were used. Instrumental conditions were set such that, detection is at 230 nm, flow rate is at 1.0 ml/min, AUFS is at 0.032, attenuation at 5 and chart speed at 1.0 cm/min. Loratidine was used as an internal standard.

Column was saturated with mobile phase for about an hour at the above specified conditions. After the chromatographic conditions were set and the instrument was stabilised to obtain a steady baseline, a mixed standard dilution of pure drugs containing 50 μg/ml each of rimapril and loratidine were prepared in mobile phase, filtered through 0.2 μ membrane filter and loaded in an injector of the instrument fitted with 20 μl fixed volume loop. The solution was injected three times and chromatograms recorded. The mean retention times for rimapril and loratidine were found to be 4.17 and 11.8 min, respectively. A chromatogram of rimapril and internal standard loratidine is reported in fig 1.

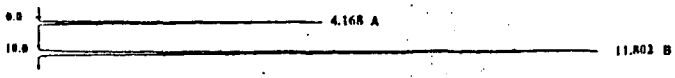

Fig. 1: High Pressure liquid chromatographic pattern of rimapril and internal standard HPLC retention time of rimapril was found to be 4.168 min (A) and that for internal standard loratidine was 11.802 min (B)

Standard stock drug solution of rimapril and loratidine with concentration of 500 μg/ml each separately were prepared in mobile phase. For preparation of drug solutions for calibration curve 0.5, 1.0, 1.5, 2.0 and 2.5 ml stock solution of standard rimapril was transferred to a series of 10 ml volumetric flasks. In each flask 1.0 ml of loratidine standard stock solution was added and volume made up to the mark with mobile phase. Each solution was injected after filtration through 0.2 μ membrane filter and chromatogram was recorded. The calibration curve was plotted between concentration of drug and ratio of peak area of rimapril and loratidine (internal standard). Linearity was found to be in concentration range of 10-300 μg/ml of rimapril.

Powder, content of Capsules equivalent to 10 mg of rimapril was weighed and transferred to a 100 ml volumetric flask containing 75 ml mobile phase. To the same volumetric flask, 10 mg of accurately weighed pure drug sample of loratidine was added. The powder mixture was dissolved in the mobile phase with the aid of sonication. The solution was then filtered through Whatman filter paper No. 41 and the volume made upto the 100 ml with the mobile phase. The solution was then filtered through 0.2 μ membrane filter. Five millilitres of this solution was further diluted to 10 ml with the mobile phase.

The final dilution of solution was loaded in the sample loop of the injection port of the instrument. The solution was injected and chromatogram recorded. The injection was repeated five time and peak area of rimapril and loratidine were recorded. The peak area ratio of drug to internal standard was calculated and amount of drug present in the capsule formulation was determined using calibration curve. The results of analysis are reported in Table 1.

Recovery studies were carried out by addition of known quantity of standard drug solution to pre-analysed sample of capsule formulation at three different concentration levels for all the three developed methods, results for which are reported in Table 1.

In the present work, two visible spectrophotometric and a HPLC methods have been developed for the estimation of rimapril from capsule formulation. The colorimetric methods are based on the formation of chloroform extractable coloured complexes of the drug with nitrosonaphthol and bromophenol blue. Conditions required for the formulation of coloured complex were optimised. The methods were found to be simple, accurate and economical. Mean per cent recovery and standard deviation from capsule formulation using nitrosonaphthol reagent were found to be in the range of 98-100% and 0.60-1.00 respectively for two different brands of capsule formulation. The respective values for method using bromophenol blue were in the range of 98-99% and 0.40-0.50, respectively. The HPLC method is a reverse phase chromatographic method using C_{18} column. The method was developed using loratidine as internal standard. The total run time for the method was just 15 min and difference between the retention times of drug and internal standard was more than 7 min. Per cent recovery of the method was found in the range of 101-102% and standard deviation was in the range of 0-0.3. Developed methods were validated statistically and by recovery studies which gave satisfactory results.

REFERENCES

1. Budavari, S., In; The Merck Index, 12th Edn., Merck and Co. Inc., N.J., USA, 1996, 1394.
2. Ito, M., Kuriki, T., Goto, J. and Nambara, T., **J. Liq. Chromatogr.**, 1990, 13, 991.
3. Barbato, F., Marrica, P. and Quaglia, F., **Farmaco,** 1994, 49, 457.
4. Ranadive, S.A., Chen, A.X. and Serajuddin, A.T., **Pharm. Res.**, 1992, 9, 1460.
5. Aboul-Enein, H.Y. and Bakr, S.A., **Drug Dev. Ind. Pharm.**, 1992, 18, 1013.

Chemical Examination of the Flowers of *Couroupita guianensis* Aubl

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From the flowers of *Couroupita guianensis* Aubl, an aliphatic hydrocarbon and stigmasterol have been isolated. The structure of stigmasterol has been established on the basis of spectral data and by chemical means.

Couroupita is a small genus of trees from the Tropical America and the West Indies[1]. One of its species, *Couroupita guianensis* Aubl is widely cultivated for its large showy flowers and reddishbrown woody capsular fruits upto 20 cm in diameter (cannon balls). The plant belongs to the family Lecythidaceae and is locally known as Kailaspati and in English as cannon ball tree[2]. It is grown in Indian gardens as an ornamental tree. Its leaves are used in skin diseases[2]. We have observed that the petroleum ether extractives of flowers, leaves, bark and fruits of *C. guianensis* possess promising antibacterial activity[3], particularly against *Gram-negative Salmonella typhi* NCTC 786. In all these extractives, MIC of 10 µg/ml was observed which prompted us to undertake the chemical examination of the plant. This communication presents exploratory chemical examination of the flowers of *C. guianensis*.

The flowers of *C. guianensis* (2 kg) were collected from a tree in the Haffkine Institute's garden, Mumbai - 400 012. They were sun-dried, powdered and extracted in a Soxhlet with petroleum ether (60-80°, 6l) and then with methanol (5l) in succession. Petroleum ether extract yielded an orange-coloured oily residue (14 g). Preliminary TLC study using benzene:ethyl acetate (95:5) as a solvent system indicated the presence of four spots. Petroleum ether extract (8 g) was chromatographed over Silica gel G (80-120 mesh, BDH 80) and the column was washed with petroleum ether (60-80°), benzene, chloroform and ethanol in succession. Collected fractions were subjected to rechromatography. Petroleum ether eluted fraction gave a faint yellow-coloured waxy compound. It was crystallized (norit) from ethyl acetate to get a compound, (yield 50 mg), M.P. 79-80°, UV λmax (MeOH) 209nm (ε 1533), IR υmax (KBr), 2917 cm^{-1} and 2849 cm^{-1} (alkyl groups, C-H stretch), 1738 cm^{-1} -1650 cm^{-1} (aliphatic aldehydes/ketones), 1378 cm^{-1}, 1018 cm^{-1}, 887 cm^{-1}, 1018 cm^{-1}, (C-H bending). Mas spectrum of the compound, M.P. 79-80°, showed peaks at 239, 218, 175, 135, 107 and 95 and a molecular ion peak at 257. The compound appears to be an aliphatic long chain hydrocarbon.

The chromatographic column, on further elution with benzene, gave three coloured fractions. One of them was a solid compound. Repeated crystallization (norit) from methanol furnished a compound in fine needles (yield: 285mg), M.P. 170-71°. It analyzed for $C_{29}H_{48}O$ (Found C:84.38%, H:11.95%; requires C:84.4%, H:11.72%). The compound gave positive Liebermann-Burchard test for

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