because the concentrations used were not comparative. Curcumin concentrations were kept low in the range of 20 μM because of solubility problems). Of the other compounds tested, PABA showed maximum activity although it was lower in potency compared to 5-ASA. On the other hand, 4-ASA, acetyl salicylic acid, salicylic acid and benzoic acid may even enhance the formation of methemoglobin at a concentration of 0.89 mM (Fig. 2). The absorbance values at 631 nm are significantly above the control values ($P \leq 0.05$).

The results partly corroborte previous reports of antioxidant activity of salicylate[1]. For example, inhition of lipid peroxidation was much higher with 5-ASA than all the other tested derivatives, including sulphasalazine and sulfapyridine[1]. The present investigation requires detailed studies under different experimental conditions especially *in vivo*. It is also important to confirm the results in purified hemoglobin. Since 5-ASA and its structural analogs are prescribed drugs, results have therapeutic significance.

REFERENCES

1. Miles, A.M. and Grisham, M.B., **Methods in Enzymology**, 1994, 234, 555.
2. Doyle, M.P., Pickering, R.A., Dykstra, R.L., Nelson, C.L. and Boyer, R.F., **Biochem. Biophys. Res. Commun.**, 1982, 105, 127.
3. Smith R.C. and Nunn, V., **Arch Biochem Biphys.**, 1984, 232, 348.
4. Unnikrishnan, M.K. and Rao, M.N.A., **Pharmazie.**, 1995, 50, 490.
5. Unnikrishnan, M.K. and Rao, M.N.A., **Mol. Cell. Biochem.**, 1995, 146, 35.

Visible Spectrophotometric and HPLC Methods for the Estimation of Sertraline Hydrochloride from Tablet Formulations

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One visible spectrophotometric and one HPLC method has been developed for the estimation of sertraline hydrochloride from tablet formulations. Developed visible spectrophotometric method is based on the formation of chloroform extractable coloured complex of drug with nitrosonapthol. The coloured complex shows absorbance maxima at 441.5 nm. Beer's law was obeyed in the concentration of 20-100 μg/ml of scrtraline hydrochloride. Developed HPLC method was a reversed phase chromatoraphic method using Intertsil C_{18} column and methanol:acetate buffer (pH 2.8)::80:20 as mobile phase with detection at 220 nm. Caffeine was used as an internal standard and linearity was observed in the concentration range of 10-250 μg/ml of sertraline hydrochloride for HPLC method. Results of anlaysis for both the methods were validated statisically and by recovery studies.

Setraline hydrochloride, chemically (IS-cis)-4-(3,4-dichlorophenyl)-1,2,3,4 tetrahydro-N-methyl)-1-napthalenaeamine hydrochloride, is an antidepressant agent[1]. For the estimation of sertraline hydrochloride from biological fluids, one GC[2] and four HPLC[4-7] methods have been reported. However, none of these methods report the estimation of the drug from formulations. An attempt has been made in the present study to develop a simple visible spectrophotometric and an HPLC method for the analyis of sertraline hydrochloride from tablets.

A Jasco UV/visible recording spectrophotometer with

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1 cm matched quartz cells and Shimadzu delivery module LC-10AD with UV SPD-10A detector and Chromatopac C-R7A integrator were used for present study. Nitrosonapthol reagent was prepared by dissolving 1 g of 2-nitroso-1-napthol-4-sulphonic acid in 100 ml of distilled water and extracting several times with chloroform to remove chloroform soluble impurities.

For colorimetric method, standard drug solution in chloroform (500 µg/ml) was diluted with chloroform to give several dilutions in the concentration range of 20-100 µg/ml of sertraline hydrochloride. To 10 ml of each dilution taken in a separating funnel, 10 ml nitrosonapthol reagent was added. Reaction mixture was shaken gently for 5 min and allowed to stand to separate aqueous and chloroform layers. Coloured chloroform layer was separated and absorbance was measured at 441.5 nm against a reagent blank. A calibration curve was prepared.

For analysis of sample solution, twenty tablets were accurately weighed and average weight of the tablet was determined. The tablets were powdered and powder equivalent to 50 mg of sertraline hydrochloride was accurately weighed and transferred to a 100 ml volumetric flask. Chloroform (75 ml) was added and shaken for 5 min to dissolve the drug. The solution was filtered through a Whatman filter paper no. 41 into another 100 ml volumetric flask. The filter paper was washed with chloroform, the washings were added to the filtrate and the final volume of the filtrate was made with chloroform. Ten milliliters of this filtrate was further diluted to 100 ml with chloroform. Ten millilitres of the final dilution was taken in a separating funnel and treated as per the procedure described for the preparation of calibration curve. Absorbance was measured at 441.5 nm and the concentration of drug in sample solution was determined from calibration curve. Results of analysis are presented in Table 1.

For the HPLC method, Inertsil C_{18} ODS 3V(5µ) 250 x 4.6 nm column and methanol:acetate (80:20) buffer (pH 2.8) as a mobile phase was used. Instrumental conditions selected were : detection at 220 nm, flow rate 1.0 ml/min, AUFS-0.032, attenuation-8 and chart speed-1.0 cm/min. Caffeine was used as an internal standard. Column was saturated with mobile phase for about an hour under the conditions specified. 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 sertraline hydrochloride and caffeine were prepared in mobile phase, filtered through 0.2 µ membrane filter and loaded in the injector of the instrument fitted with a 20 µl fixed volume loop. The solution was injected three times and chromatogram recorded. The mean retention times for sertraline hydrochloride and caffeine were found to be 9.415 and 3.247 min respectively. The representative chromatogram of sertraline hydrochloride and internal standard caffeine is presented in Fig. 1.

Separate stock solutions of sertraline hydrochloride and caffeine each containing 500 µg/ml were prepared in the 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 sertraline hydrochloride was transferred to a series of 10 ml volumetric flasks. In each flask, 1.0 ml of caffeine standard solution was added and the volume was made up to the mark with mobile phase. Each solution was injected after filtration through a 0.2 µ membrane filter and chromatogram recorded. The calibration curve was plotted between concentration of drug and ratio of peak area of sertraline hydrochloride and caffeine (internal standard). Linearity was found to be in the concentration range of 10-250 µg/ml of sertraline hydrochloride.

The tablet powder equivalent to 100 mg of sertraline hydrochloride was accurately weighed and transferred to a 100 ml volumetric flask containing 75 ml of mobile phase. To the same volumetric flask, 100 mg accurately weighed pure drug sample of caffeine was added. The powder mixture was dissolved in mobile phase with the aid of sonication. The solution was then filtered through a Whatman filter paper no. 41 into another 100 ml volumetric flask and the volume was made upto the mark with the mobile phase. The solution was then filtered through a 0.2 µ membrane filter. Five milliliters of this solution was further diluted to 100 ml with the mobile phase.

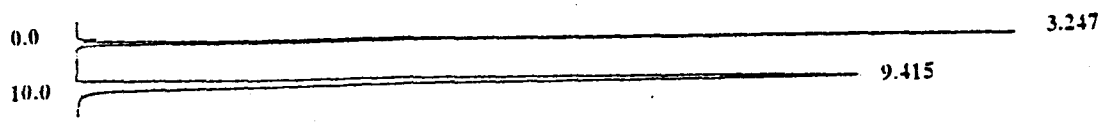

Fig. 1 : Representative Chromatogram of Sertraline hydrochloride and Internal Standard Caffeine
Mean retention time of sertraline hydrochloride was 9.415 min and for internal standard caffeine was 3.247 min.

TABLE 1 : RESULTS OF ANALYSIS AND RECOVERY STUDIES

Method	Batch	Label Claim (mg/cap)	% of Label Claim Estimated*	S.D.	% Recovery**
Spectrophotometric	A	50	97.98	0.456	101.29
	B	50	98.24	0.985	
	C	100	98.02	0.724	
HPLC	A	50	99.50	0.666	99.69
	B	50	98.35	0.518	
	C	100	99.37	0.986	

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

The final dilution of tablet sample 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 times and peak areas of sertraline hydrochloride and caffeine were recorded. The peak are a ratio of drug to internal standard was calculated and the amount of drug present in the tablet formulation was determined using calibration curve. The results of analysis are shown in Table 1.

Recovery studies were carried out by the addition of known quantities of the standard drug solution to pre analysed sample and the determination was repeated using both the developed methods. Results are presented in Table 1.

In the present work, two methods have been developed for the estimation of sertraline hydrocholoride from tablets. The first one is a colorimetric method, which is based on the formation of chloroform-extractable coloured complexes of the drug with nitrosonapthol. Conditions required for formation of coloured complex were optimised. The method was found to be simple, accurate and economical. Percentage recovery using this developed method was found to be in the range of 97-99% and standard deviation was below 1.0. The second is a reversed phase HPLC method using C_{18} column. The method was developed using caffeine as an internal standard. The total run time for the method was just 15 min and the difference between the retention time of drug and internal standard was more than 5 min. Percentage recovery of this method was close to 100 % and the standard deviation was below 1.0. These two methods were validated stastically and by recovery studies which gave satisfactory results. Since none of the above methods is reported for analysis of sertraline hydrochloride from pharmaceutical formulation, these two methods may perhaps be used for the analysis of sertraline from pharmaceutical dosage forms.

REFERENCES

1. Budavari S., In; The Merck Index, 12th Edn., Merck and Co., N.J., USA, 1996, 1455.
2. Patel, J., Spencer, E.P. and Flanagan, R. J., **Biomed. Chromatogr,** 1996, 10, 351, Through **Anal. Abstr.**, 1997, 59, 2G44.
3. Gupta, R.N. and Dziurdzy, S.A., **Clin. Chem.**, 1994, 40, 498, Through **Anal. Abstr.**, 1994, 56, 9G69.
4. McIntyre, I.M., King, C.V., Staikos, V., Gall, J. and Drummer, O.H., **J. Forensic Sci.**, 1997, 42, 951, Through **Anal Abstr.**, 1998, 60, 1G47.
5. Koves, E.M., **J. Chromatogr**, 1995, 692, 103.
6. Levine, B., Jenkins, A.J. and Smialek, J.E., **J. Anal. Toxicol**, 1994, 18, 272, Through **Anal. Abstr.**, 1995, 57, 1G87.