of either sex weighing about (100-250 g) in each group were used. The paw volume was measured in a plethysmograph by $KMnO_4$ solution displacement. Ibuprofen was used as a standard and 0.5 % gum acacia mucilage used as a control group and rest of the groups were used for the test drugs. The % inhibition of paw edema volume was calculated by the formula, % inhibition=$(1-V_t/V_c)x100$, where Vt is the mean volume of the test drug, Vc is the mean volume of the control and the results were given in Table 4.

All compounds were in conformity with the structures envisaged. The structures were proved on the basis of spectral data. All titled compounds were found to be active at 10 µg/ml concentration against H_{37} RV strain of *Mycobacterium tuberculosis* .All the compounds except 4c, 4i and 4j showed almost same inhibitory action against Coagulase positive *Staphylococcus* but less than that of standard penicillin. 4i and 4j were also moderately active against *P. aeruginosa* in comparison to standard. Only compound 4 g was moderately active against *C. albicans,* when compared with the standard. Results also indicate that 4e showed higher and 4j and 4l showed equal antiinflammatory activity in comparison to that of standard ibuprofen.

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Spectrophotometric Estimation of Total Alkaloids from *Rauwolfia* Root Powder and Formulation.

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Total alkaloids (calculated as reserpine) from various samples of *Rauwolfia serpentina* root powder and its marketed formulation were estimated by spectrophotometric method using ion-pair complexation of acid dye (methyl orange) with the alkaloids. The complex was selectively ex-

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tracted in to chloroform and by treatment with hydrochloric acid, the dye was liberated from the complex. The pink colour of the liberated dye is proportional to the amount of alkaloids which was measured at 530 nm. The method adopted for extraction of alkaloids from samples has the advantage of extraction of mainly the alkaloids and not the other interfering substances. All the alkaloids extracted in this method were found to form complexes with the dye under the experimental conditions. Beer's law was obeyed between 5-40 µg/ml. The method was found to be simple, sensitive and accurate. It could be applied to formulations containing *Rauwolfia* and found suitable for routine quality control purposes.

Roots of *Rauwolfia serpentina* (Family: Apocynaceae) known as *Sarpagandha* in India, have been used to treat several diseases. The therapeutic value of this plant is mainly due to indole alkaloids present in it[1]. Reserpine and recinnamine are the two principle alkaloids from reserpine group and they are known to have potent tranquilizing, sedative and anti hypertensive actions.

Many methods that include high performance liquid chromatography[2,3], gas chromatography[4], voltametry[5], polarography[6], room temperature phosphometry[7], spectroflouromertry[8,9] and spectrophotometry[10] have been reported for the determination of reserpine. But most of these methods deal with analysis of pure reserpine and do not include reserpine and related alkaloids from the raw herb and its preparations. Further, the methods are complex, expensive and time consuming. We have reported applicability of ion-pair complexing for determination of total alkaloids from various herbs[11-13]. The spectrophotometric technique is simple, sensitive and economically viable. Hence, we thought it worthwhile to see whether alkaloids from *Rauwolfia* could be analyzed by application of the acid dye technique which involves ion-pair complexation between the dye and the nitrogen of the alkaloids. In the present paper, we have developed spectrophotometric method for determination of total alkaloids from crude *Rauwolfia* root powder and its formulations.

Reserpine reference standard was obtained as a gift from M/S. Vinkem Labs Private Limited, Kakkalur, Tiruvallur. The samples of *Rauwolfia* root powder were obtained from Junagadh, Ahmedabad and Mumbai and authenticated in our Pharmacognosy and Phytochemistry Department. One herbal formulation, *Sarpagandha* tablet (Shree Baidyanath Ayurveda Bhawan Pvt. Ltd., Jhansi) was purchased from local market. All the chemicals and reagents used were of analytical grade. Reagents include acetate buffer of pH 4.6 (5.4 g of sodium acetate and 2.4 g of glacial acetic acid in 100 ml of double distilled water), 0.05% methyl orange solution in double distilled water and 1 M hydrochloric acid.

For preparation of the linearity curve, standard solution of reserpine (1 mg/ml) was prepared by dissolving 100 mg reserpine in 10 ml of chloroform and making the volume to 100 ml with methanol. The spectrophotometric analysis carried out is as follows: 10 ml of each 5, 10, 15, 20, 25, 30 and 35 µg/ml concentration of reserpine was made by proper dilutions of standard solution with chloroform. It was taken in to a separating funnel, 5 ml of acetate buffer and 3ml of 0.05% methyl orange solutions were added and the contents were shaken well. The complex formed was extracted thrice with chloroform (10+10+5 ml). The pooled chloroform containing the complex was transferred to another separating funnel containing 25 ml of 1 M hydrochloric acid. The dye liberated in to hydrochloric acid from the complex was measured against a blank at 530 nm using spectrophotometer (Elico, Hyderabad). Blank was prepared by the same method described above without addition of reserpine. The absorbance values were plotted against their respective concentrations of reserpine to obtain a linearity curve.

For the extraction of alkaloids from the samples, weighed quantities (1 g of *Rauwolfia* root powder/1.197 g of tablet powder corresponding to 1 g of drug powder) were taken, moistened with 10 % ammonia (2 ml), dried and refluxed with chloroform (50 ml) for 1 h. This mixture was filtered, filtrate concentrated and volume was adjusted to 25 ml with chloroform. Measured volume (0.5 ml) of this extract was taken and diluted to 10 ml with chloroform in a volumetric flask This was treated with reagents as described above. The amounts of total alkaloids from sample solutions were calculated from the calibration curve and represented as reserpine. All experiments were carried out in triplicates.

The proposed method is based on the formation of ion pair complex between alkaloids and methyl orange at pH 4.6 which can be extracted in to chloroform, followed by release of the dye from the chloroform in to hydrochloric acid. The intensity of the pink colored dye in hydrochloric

TABLE 1: METHOD VALIDATION PARAMETERS

Parameters	Results
Linearity rang (µg/ml)	5-40
Correlation coefficient	0.999
Slope	0.0153
Intercept	+0.0023
Accuracy (%)	99.96

TABLE 2: ESTIMATION OF TOTAL ALKALOIDS OF RAUWOLFIA ROOT POWDER

Sample	Total alkaloids *(%w/w)
Root powder	
A	0.62±0.048
B	0.34±0.035
C	0.31±0.032
Sarpagandha Tablet	0.19±0.028

A, B and C are *Rauwolfia* root powders obtained from Junagadh, Ahmedabad and Mumbai respectively, *Sarpagandha* Tablet (Shree Baidyanath Ayurved Bhavan Pvt. Ltd.., Jhansi). *Denotes that each value is the mean and standard deviation of three determinations.

acid was measured at 530 nm. Various parameters involved in color development such as effect of pH, amount of acetate buffer, amount of methyl orange solution, amount of hydrochloric acid and time involved at various stages of reaction were optimized. Maximum color intensity was obtained with 3 ml of 0.05% methyl orange, 5 ml of acetate buffer and 25 ml of 1 M hydrochloric acid. The pink color produced was stable for more than 24 h.

TABLE 3: PERCENT RECOVERY STUDY

Amount of total alkaloids present (µg)	Amount of reserpine added (µg)	Amount of total alkaloids found (µg)*	% Recovery*	Average % recovery
345	100	444.3±3.05	99.84±1.94	99.88
345	200	544.6±2.95	99.92±1.05	

* Each value is the mean and standard deviation of three determinations.

The method of analysis was validated for precision, accuracy, linearity range, slope, intercept and correlation coefficient (Table 1). The method was validated for precision by repeating the experiment five times with the same quantity of reserpine. Percentage relative standard deviation was found to be ±1.05. In the present study we found that the samples from Junagadh, Ahmedabad and Mumbai had 0.62, 0.34 and 0.31% w/w of total alkaloids respectively. The herbal tablet (Shree Baidyanath Ayurved Bhavan Pvt. Ltd.) contained only 0.19% w/w of total alkaloids (Table 2). The accuracy of the method was determined by performing recovery study at two levels by adding known amount of reserpine to already analyzed powder sample. The average percentage recovery found was 99.88% (Table 3).

In *Rauwolfia*, strongly basic, weakly basic and intermediate basic alkaloids are present and all of them show maximum solubility in chloroform. Hence, powder was moistened with ammonia, dried and extracted with chloroform. The alkaloid profiles of the extracts were studied by TLC along with the standard reserpine in mobile phase: toluene-ethyl acetate-diethyl amine (7:2:1) and Dragondroff's reagent for visualization. The samples showed five major alkaloids with R_f 0.93, 0.64 (reserpine), 0.50, 0.40 and 0.29. However, reserpine was found to be absent in the formulation though other four alkaloids were present. All the alkaloids, when isolated by preparative TLC, formed color complex with the dye.

This method for estimation of total alkaloids of *Rauwolfia* is simple, sensitive, precise and accurate. This method can be used for routine analysis of total alkaloids of *Rauwolfia* from crude root powder and its formulations.

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Simultaneous Spectrophotometric Estimation of Amoxycillian and Cloxacillin from Tablets

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A new spectrophotometric method for the simultaneous and individual estimation of amoxycillin and cloxacillin in binary tablet formulations has been described.The method based on the estimation of one drug in presence of another drug by absorbance difference method.

The combination formulations of amoxycillin and cloxacillin have been marketed for the treatment of respiratory tract infections, urinary tract infections and throat infections. The literature describes various methods for the analysis of amoxcillin[1-3] and cloxaxillin[4,5] as individual drug products. Only one spectrophotometric method[6], for simultaneous analysis of amoxycillin and cloxacillin have been cited. No method for the simultaneous analysis of amoxycillin and cloxacillin in binary tablet formulations has been reported by absorbance difference method. The objectives of the present investigation is to develop a simple, rapid, precise, reproducible and economical method for the simultaneous analysis of the binary drug formulations by using absorbance difference method without any interferences from each other.

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A spectronic 1001, spectrophotometer with 1 cm quartz cells were used for absorbance measurements. All the chemicals used were of analytical grade. AR. Grade methanol was used as solvent and 1:10 ammonia:water solution was prepared by usual manner.

Pure amoxycillin (50 mg) was dissolving in 50 ml methanol. This stock solution was further diluted with methanol to get working concentration of 50 µg/ml. Fifty milligrams of pure cloxacillin was dissolved in 50 ml methanol and further diluted to obtain the working concentration of 30 µg/ml. Four standard mixture solutions of 5 ml were prepared from 4ml, 3ml, 2ml and 1ml of amoxycillin standard solution by diluting with cloxacillin solution of respective quantity. Various aliquots (5, 6, 7 and 8 ml) of amoxycillin solution were transferred into a series of 10 ml standard flasks. To each flask, 1 ml of 1:10 ammonia: water solution was added and volume was adjusted to 10 ml with distilled water. The absorbance of these solutions was scanned over the range of