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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

206 to 276 nm. Similarly, cloxacillin solutions prepared and were scanned over the range of 206 to 276 nm. Randomly two wavelengths 226 and 258 nm are selected for amoxycillin, at these two wave lenghts the absorbance values are almost zero and in case of cloxacillin at the same wavelength 226 and 258 nm have maximum absorbance difference. A calibration curve was drawn between the absorbance difference values of cloxacillin and the amount of cloxacillin in µg/ml. The amount of cloxacillin present in the sample was estimated from calibration curve. Similarly two wavelengths 212 and 222 nm were selected for cloxacillin, at these two wavelengths the absorbance difference was almost zero and in case of amoxycillin having maximum absorbance difference values at the same wavelengths 212 and 222 nm. The amount of amoxycillin present in the sample was estimated from calibration curve.

Various aliquots of mixture (5 ml) of amoxycillin and cloxacillin solutions in diffferrent proportions were prepared. The absorbance values were measured at two wavelength 212 and 222 nm for estimation of amoxycillin and two wavelenghts 226 and 258 nm for estimation of cloxacillin. A calibration curve was drawn between the abosrbance difference values of amoxycillin and the amount of amoxycillin present in µg/ml. A calibration graph was drawn between the absorbance difference values of cloxacillin and the amount of cloxacillin present in µg/ml. The amount of amoxycillin and cloxacillin was computed from their respective calibration curves.

Twenty tablets (Novclox-marketed by Cipla, Tormoxin Plus–marketed by Torrent, Trasmox–marketed by Sarabhai, Natoclox–marketed by Sun Pharma) were weighed and powdered. A quantity of powder equivalent to 50 mg was transferred in 50 ml volumetric flask, shaken thoroughly with 25 ml methanol and filtered. The filtrate was diluted to 50 ml with methanol and further diluted as in standard solution preparation. One milliliter of this solution containing 50 µg/ml amoxycillin and 50 µg/ml cloxacillin. Different aliquots of solutions were taken and analysed by using the procedure described earlier. The amount of amoxycillin and cloxacillin was computed from their respective calibration curves. The results are represented in Table 1.

The method developed in the present investigation may perhaps be used for the analysis of amoxycillin and cloxacillin from tablets. The results obtained by proposed method are in good agreement with label claim of the tablets. The additives and excipients usually present in tablets do not interfere under these conditions. As a check on accuracy of the method, recovery experiment was performed and percent recovery was found to be close to 100%. The statistical analysis was studied by proposed method. The values of standard deviation and coefficient of variation values were satisfactorily low, indicates accuracy and the reproducibility of the method (Table 2). Student 't' test show that the calculated 't' values are less than 't' theoretical value 2.78 with 4 degree of freedom at 5% level of significance indicate that there is no significant difference between proposed and official method.

The results indicate that the proposed absorbance difference method was found to be simple, rapid, precise, accurate and less time consuming and can be used for the routine analysis of amoxycillin and cloxacillin in pharmaceutical formulations.

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TABLE 1: ESTIMATION OF AMOXYCILLIN AND CLOXACILLIN FROM TABLETS

Sample	Label Claim (mg/tab)		"Amount found by proposed method (mg)		% Recovery by proposed method*	
	†AX	◇CX	†AX	◇CX	†AX	◇CX
Capsule 1[a]	250	250	249.2	249.6	99.6	99.8
Capsule 2[b]	250	250	249.9	250.33	98.8	99.6
Capsule 3[c]	250	250	249.5	250.2	99.9	100.4
Capsule 4[d]	250	250	250.16	249.23	100	99.9

*Average of five determinations. †amoxycillin, ◇cloxacillin "determination of amoxycillin and cloxacillin in combined dosage form pharmaceutical preparations by proposed method. [a]Tresmox marketed by Sarabhai, Vadodara. [b]Novaclox marketed by Cipla, Mumbai. [c]Tormoxine plus marketed by Torrent, Ahmedabad. [d]Natoclox marketed by Sun Pharm, Mumbai.

TABLE 2: STATISTICAL ANALYSIS OF ESTIMATION OF AMOXYCILLIN AND CLOXACILLIN

Sample	*S.D		**C. V		$^{a}t_{cal}$	
	[b]AX	[c]CX	[b]AX	[c]CX	[b]AX	[c]CX
Capsule 1	0.9533	0.6236	0.3824	0.2498	1.380	1.0277
Capsule 2	0.6798	0.2054	0.2719	0.0821	0.1783	1.940
Capsule 3	1.0801	0.8498	0.4329	0.3397	0.8017	0.3261
Capsule 4	0.6236	0.6649	0.2492	0.2669	0.4450	2.006

*Standard deviation, **coefficient of variation [a]caluclated 't' value by proposed method, theoretical values at 95 % confidence limit, 't' 2.57. [b]amoxycillin, [c]cloxacillin

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Antimicrobial Activity of *Cocculus hirsutus* (Linn)

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The *in vitro* antibacterial activity of the extracts and isolates of *Cocculus hirsutus* (root) has been studied against *Bacillus subtilis* and *Escherichia coli*. The total methanol extract, total alkaloid mixture and the two isolates have shown significant activity against the organisms used, almost comparable with the standard antibiotics, benzyl penicillin and streptomycin. This *in vitro* testing also resulted in activity guided isolation of two antibacterial principles from the root.

Cocculus hirsutus belonging to the family Menispermaceae is a semi-woody climber growing wild across our country[1]. It is highly valued in the indigenous system of medicine. Leaf juice is a soothing application in certain skin diseases and also indicated in gonorrhea, leucorrhoea and menorrhagia. Root infusion is a refrigerant and an antiperiodic[2]. Phytochemical and pharmacological studies have been conducted on the whole plant, stems and leaves. Ginnol, β-sitosterol, trilobine, isotrilobine, coclaurine, magnoflorine, jamtinine, shaheenine, cohirsitine, hirsutine, cocsuline-N-2-oxide, cohirsinine and hirsudiol have been reported from this plant[3-8]. The roots have not been studied extensively for chemical constituents. This communication reports the antibacterial activity

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