

could be adopted for routine quality control.

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## Simultaneous Spectrophotometric Estimation of Cefuroxime Axetil and Probenecid from Combined Dosage Form

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**Three simple, accurate and economical procedures for simultaneous estimation of cefuroxime axetil and probenecid in two component capsule formulations have been developed. The methods employ program in the multicomponent mode of analysis of the instrument used, the area under curve method and the graphical absorption ratio method. All these methods utilize 0.05 M NaOH as a solvent. In this solvent system cefuroxime axetil shows maximum absorbance at a wavelength of 278 nm and probenecid shows maximum absorbance at a wavelength of 244.2 nm. The results of analysis have been validated statistically and by recovery studies.**

An extensive literature survey revealed HPLC<sup>1-2</sup>, spectrofluorimetric<sup>3</sup>, and first derivative spectrophotometric and liquid chromatographic determination methods<sup>4</sup> for the

analysis of cefuroxime axetil, whereas HPLC<sup>5-7</sup> and spectrophotometric<sup>9</sup> methods for the analysis of probenecid. Not a single method has been reported for the simultaneous estimation of both these components from a combined dosage form. The objective of this investigation was to devise

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three simple, accurate and economical procedures for simultaneous estimation of cefuroxime axetil and probenecid from a marketed pharmaceutical dosage form. A PC based Jasco V-530 UV/visible recording spectrophotometer was used for the experimental purpose. Freshly prepared 0.05 M NaOH was used as a solvent. Gift samples of cefuroxime axetil and probenecid were procured from M/s Sai Mirra Innopharma Pvt. Ltd., Mumbai.

Sample solutions of cefuroxime axetil and probenecid of the concentration of 10 µg/ml were prepared in 0.05 M NaOH. These solutions were scanned over the range of 220 nm to 320 nm in the multicomponent mode using 244.2 nm and 278 nm as sampling wavelengths. Then mixed standard solution of same concentration was prepared and scanned over the same range. An overlain spectrum of the mixed standards was used to determine the concentration of two drugs in the capsule sample solutions. Twenty capsules were weighed and average weight was calculated. The blend was taken and was crushed to fine powder. The powder equivalent to 10 mg of cefuroxime axetil was transferred to 100 ml volumetric flask. The powder was dissolved in 75 ml of 0.05 M NaOH by intermittent shaking and volume was made up to 100 ml with the same solvent. The solution was then filtered through a Whatmann filter paper No. 41. The solution was diluted with 0.05 M NaOH to obtain 10 µg/ml of cefuroxime axetil and 10 µg/ml of probenecid. This sample was scanned over the range of 320 nm to 220 nm in the

multicomponent mode and the concentration of each component were obtained by the spectral data of the sample solutions with reference to that of the mixed standards. The analysis procedure was repeated five times with capsule formulation available in market. The results of analysis of capsule formulation, statistical evaluation and recovery studies are shown in Tables 1, 2 and 3, respectively.

The X values of the drugs were determined at the selected wavelength range, 235 nm to 255 nm and 270 nm to 290 nm. The X values were determined as, X=area under the curve of component between selected wavelength range/concentration of component in g/l. A set of two simultaneous equations framed using these X values are given below,  $A_1=684.4XC_1+161.05XC_2$  .... (I) and  $A_2=441.46XC_1+626.628XC_2$  .... (II), where,  $C_1$  and  $C_2$  are the concentrations of cefuroxime axetil and probenecid, respectively in g/l in the sample solution.  $A_1$  and  $A_2$  is the area under curve of sample solution at the wavelength range 235-255 nm and 270-290 nm, respectively. The X values were 684.4 and 161.05 at 235-255 nm and 441.46 and 626.628 at 270-290 nm of cefuroxime axetil and probenecid, respectively. The X values reported are the mean of five independent determinations.

By applying the Cramer's rule and Matrices to equation (I) and (II), concentrations  $C_1$  and  $C_2$  can be obtained as,  $C_1=A_1X684.4-A_2X161.05/357767.07$  ..(III) and

TABLE 1: ANALYSIS DATA OF CAPSULE FORMULATION.

Method	Label claim		Amount found (mg/capsule)		% of Label Claim*	
	CA	P	CA	P	CA	P
1	250	250	251.6	252.6	100.6	100.9
2	250	250	245.5	250.5	98.2	100.2
3	250	250	249.1	248.3	99.7	99.3

\* Mean of five readings, CA: Cefuroxime axetil, P: Probenecid.

TABLE 2: STATISTICAL EVALUATION.

Method	Standard Deviation		% Coefficient of variation		Standard error	
	CA	P	CA	P	CA	P
1	0.858	0.659	0.853	0.654	0.383	0.293
2	0.495	0.733	0.504	0.731	0.220	0.326
3	0.141	0.169	0.134	0.170	0.590	0.750

CA: Cefuroxime axetil, P: Probenecid.

$$C_2 = A_2 \times 626.6 - A_1 \times 441.5 / 357767.07 \dots (IV)$$

Capsule sample solutions were made as mentioned in first method. The area under curve of these solutions i.e.  $A_1$  and  $A_2$  were recorded at 235-255 nm and 270-290 nm, respectively, and the concentration of two drugs in the sample were determined by substituting  $A_1$  and  $A_2$  values of sample solution in equation (III) and (IV). The results of analysis of capsule formulations, statistical evaluation and recovery studies are shown in Tables 1, 2 and 3, respectively. The overlain spectrum of cefuroxime axetil and probenecid used for analysis is given in fig. 1.

The graphical absorption ratio method utilizes five mixed concentrations with increasing concentration of cefuroxime axetil and decreasing concentration of probenecid. Here the mixed standard solution of 20  $\mu\text{g/ml}$  was prepared. The  $k$  and  $B$  values of each of the two drugs were determined at the selected wavelengths 259.3 nm and 278 nm in which 259.3 nm is the isobestic point. The  $k$  and  $b$  values were determined from the graph of relative concentrations of individual component and absorbance ratio.

A set of two simultaneous equations framed using these  $k$  and  $b$  values are given below,  $C_1 = [A_2 / A_1] \times [-1.031] + 1.373 \dots (V)$  and  $C_2 = [A_2 / A_1] \times [1.031] - 0.373 \dots (VI)$ , Where  $C_1$  and  $C_2$  are the concentrations of cefuroxime axetil and probenecid, respectively in grams per liter in the sample solution.  $A_1$  and  $A_2$  are the absorbances at 259.3 nm and 278 nm, respectively. The  $k$  and  $b$  values of cefuroxime axetil were -1.031 and 1.373 and the  $k$  and  $b$  values of probenecid were 1.031 and -0.373, respectively. Capsule sample solutions of concentration 20  $\mu\text{g/ml}$  were made as mentioned in first method. By using the  $k$  and  $b$  values previously determined concentration of cefuroxime axetil and probenecid were calculated. The results of analysis of capsule formulations, statistical evaluation and recovery studies are shown in Tables 1, 2 and 3, respectively. The overlain spectrum of cefuroxime axetil and probenecid used for analysis is given in fig. 1.

The proposed methods were found to be simple, accurate, economical and rapid for routine simultaneous estimation of two drugs. All the methods were found to be economical, as they require only 0.05 M NaOH as a solvent. The values of standard deviation, coefficient of variation, standard error were satisfactorily low and recovery studies lying between 98 to 102% (for cefuroxime axetil) and 99 to 101% (for probenecid) were indicative of the accuracy of both the methods. The multicomponent analysis mode of

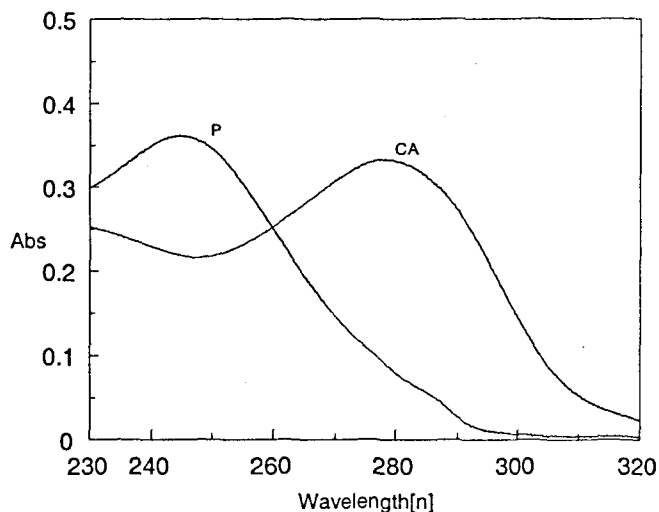


Fig. 1: Overlain UV-spectra of cefuroxime axetil and probenecid.

CA: Cefuroxime axetil, P: Probenecid.

TABLE 3: RECOVERY STUDY DATA.

Method	Amount of drug added to final dilution ( $\mu\text{g/ml}$ )		% Recovery*	
	CA	P	CA	P
1	5	5	98.5	99.5
2	5	5	99.8	100.2
3	5	5	100.6	99.4

\* Mean of five readings, CA: Cefuroxime axetil, P: Probenecid.

the instrument is time consuming in setting up of this method. However, once the data is stored in the memory of the instrument, subsequent analysis becomes faster. The area under curve method involves lot of calculations and it is time consuming, but it gave excellent results. The graphical absorption ratio method can be applied only when any isobestic point is present and it is highly sensitive method. This method also gave excellent result and can be employed for routine analysis of these two drugs in combined dosage form.

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## Purgative Activity of *Cassia tora* Leaf Extract and Isolated Aloe-Emodin

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**From the 90% methanolic extract of the dried leaves of *Cassia tora* Linn., aloe-emodin, 1,8-dihydroxy-3-(hydroxymethyl)-anthraquinone has been isolated and identified. The purgative activity of the methanolic extract as well as that of isolated aloe-emodin from *C. tora* leaves was evaluated in Wistar rats. The extract as well as isolated material showed significant purgative activity in Wistar rats.**

*Cassia tora* Linn. (Family, Caesalpiniaceae) is a well-known plant widely distributed in India and other tropical countries<sup>1</sup>. It is an annual undershrub and grows wild in wasteland. Various parts of the plant have been known possess medicinal value<sup>1</sup>. Several anthraquinones have been isolated from the seeds of *Cassia tora*<sup>2-4</sup>. Sennosides, which are well known for their medicinal importance, have been detected in the leaves of the plant<sup>5</sup>. The extracts of *Cassia tora* have been used as a remedy for various skin ailments, rheumatic disease and as laxatives<sup>6-8</sup>. The extract of *Cassia tora* leaves has been found to possess significant hepatoprotective activity and antiinflammatory activity<sup>9-11</sup>. Isolation of aloe-emodin from the dried leaves of *C. tora* is reported here. The purgative action of the methanolic extract and the isolated aloe-emodin from *Cassia tora* leaves are also reported.

The leaves of *Cassia tora* were collected at the flowering stage in the month of mid August and September and shade dried and powdered. Coarsely powdered leaves (500 g) were extracted with 90% methanol by cold percolation for 24 h. The extract was concentrated and yield of the dried mass was 18.2 g. Aloe-emodin was isolated from the dried extract and the isolated aloe-emodin were dissolved in 2% aqueous w/v Tween 80 solution to evaluate the purgative potential.

The dried methanolic extract was mixed with 50 ml of water and extracted with 200 ml of petroleum ether (60-80°) followed by extraction with 0.5 N potassium hydroxide. The KOH extract was acidified with dilute HCl to Congo red and further extracted with solvent ether. The solvent ether layer contained major anthraquinone pigments which were detected by TLC on silica gel G (benzene:methanol, 90:10)<sup>12</sup>. The TLC revealed one prominent fluorescent spot along with several minor spots under UV (225 nm) which

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