Simultaneous UV Spectrophotometric Method for the Estimation of Cefuroxime Axetil and Probenecid from Solid Dosage Forms

S. V. CHAUDHARI, ASHWINI KARNIK, ANURADHA ADHIKARY, R. S. TANDALE AND P. R. VAVIA*
Pharmaceutical Sciences Division, Mumbai University Institute of Chemical Technology, Nathalal Parikh Marg, Matunga, Mumbai-400 019, India.

A simple, rapid and precise UV spectrophotometric method for simultaneous estimation of cefuroxime axetil and probenecid from combined solid dosage form has been developed. Cefuroxime axetil and probenecid interfere with the UV absorption of each other. However, as they show different absorption maxima, use of modified Beer's law for the simultaneous estimation of both the drugs was explored. As both the drugs have different pharmacopoeial in-vitro dissolution media, simultaneous equations were developed in both the systems. The accuracy and precision of the method was determined and validated statistically. The method showed good reproducibility and recovery with percent relative standard deviation less than 2%. This method was successfully applied for determining the assay and in vitro dissolution of a marketed formulation.

Cefuroxime axetil (CA) is a semisynthetic, broad-spectrum antibiotic for oral administration belonging to second generation of cephalosporins. It is used for the treatment of bone infections, otitis, sinusitis, skin and urinary tract infections[1,2]. Probenecid (PRN) is a uricosuric agent that competes for renal tubular secretion and thereby decreases the renal secretion of a number of drugs like CA and other cephalosporins and penicillins. PRN is therefore used as an adjunct to antibacterial therapy in combination with CA to maintain higher plasma levels of CA for a longer period[3,4].

Several methods like mercurimetric[5] and HPLC[6] have been reported for determination of CA, and similarly, spectrophotometric[7] and HPLC[8] methods for the analysis of PRN. There is no official method for estimation of both the drugs simultaneously. An HPTLC method for simultaneous estimation of these drugs from their formulation has been reported[9].

The present study illustrates the development of a simple, precise, reproducible and economical simultaneous UV spectrophotometric method for the analysis as well as for *in vitro* dissolution of CA and PRN from the same formulation. The pharmacopoeial dissolution study of CA is carried out in 0.07N HCl, whereas for PRN it is done in phosphate buffer pH 7.5, with further dilutions in 0.1N NaOH. Hence the present work involves development of a simultaneous estimation method in both these dissolution systems.

***For correspondence**
E-mail: vaviapradeep@yahoo.com

MATERIALS AND METHODS

Cefuroxime axetil was procured as a gift sample from Elder Pharmaceuticals, Mumbai. Probenecid was gifted by Geno Pharmaceuticals, Mumbai. All other chemicals used were of analytical grade. Demineralised water and calibrated glasswares were employed throughout the work. Jasco double-beam spectrophotometer (Model V-530) with matched quartz cells of 1 cm path length was used. Dissolution studies were carried out using Electrolab TDT 08L dissolution test apparatus (USP).

Standard solutions:
Standard stock solution of CA was prepared in methanol (250 µg/ml). Aliquots of this solution were diluted to known concentrations in 0.07N HCl [I]. Solution of CA was prepared in methanol (2.5 mg/ml). This solution was diluted further in phosphate buffer pH 7.5 to get standard stock solution (250 µg/ml). Aliquots of this solution were diluted to get known concentrations of the drug in 0.1N

NaOH [II]. Similarly standard stock solution of PRN was prepared in methanol (250 µg/ml). Aliquots were diluted to get known concentrations of the drug in 0.07N HCl [III]. Solution of PRN was prepared in methanol (2.5 mg/ml). This solution was diluted further in phosphate buffer pH 7.5 to get standard stock solution (250 µg/ml). Aliquots of this solution were diluted to get known concentrations of the drug in 0.1N NaOH [IV].

CA and PRN interfere with the UV absorption of each other. So it becomes difficult to estimate both drugs from the same formulation by UV spectrophotometry. However, the overlain spectra of both the drugs showed different absorption maxima. Therefore, the modified Beer's law[10,11] was used and simultaneous equations were developed.

The individual drugs as well as their mixtures were checked for their linearity, thereby fulfilling the law of additivity for modified Beer's law. The extinction coefficients - ε (1%/cm) were calculated from the absorbance values using Eqn. 1 and placed in modified Beer's law Eqns. 2 and 3[10,11]. The Eqns. 1, 2 and 3 are as follows: $\varepsilon\ (1\%/cm)=A/C$...Eqn. 1, where ε is the extinction coefficient (absorptivity), A is absorbance and C is concentration (g/100 ml).

$C1=(\varepsilon_2\lambda_2 x A_1-\varepsilon_2\lambda_1 x A_2)/(\varepsilon_1\lambda_1 x\varepsilon_2\lambda_2-\varepsilon_1\lambda_2 x\varepsilon_2\lambda_1)$...Eqn. 2, and

$C2=(\varepsilon_1\lambda_1 x A_2-\varepsilon_1\lambda_2 x A_1)/(\varepsilon_1\lambda_1 x\varepsilon_2\lambda_2-\varepsilon_1\lambda_2 x\varepsilon_2\lambda_1)$...Eqn. 3,

where C1 is the concentration of drug 1 (µg/ml), C2 is the concentration of drug 2 (µg/ml), λ_1 is wavelength maxima of drug 1, λ_2 is the wavelength maxima of drug 2, A_1 is the absorbance of sample at λ_1, A_2 is the absorbance of sample at λ_2, $\varepsilon_1\lambda_1$ is the extinction coefficient of drug 1 at λ_1, $\varepsilon_1\lambda_2$ is the extinction coefficient of drug 1 at λ_2, $\varepsilon_2\lambda_1$ is the extinction coefficient of drug 2 at λ_1, and $\varepsilon_2\lambda_2$ is the extinction coefficient of drug 2 at λ_2. The method was first tried on mixed standard solutions to establish its suitability to marketed formulations. The drugs in the mixtures were taken in proportions covering every possible concentration level of each drug.

Study of Beer-Lambert's law for CA and PRN in 0.07N HCl:

Solutions of CA (20 µg/ml) and PRN (20 µg/ml) in 0.07N HCl buffer were prepared by appropriately diluting stock (I) and (III) respectively in 0.07N HCl and scanned in UV range. From their overlain spectra shown in Fig.1, analytical wavelengths 280 nm (λ_{max} of CA) and 249 nm (λ_{max} of PRN) were selected for development of simultaneous equations. Calibration curves of CA and PRN were plotted at both the selected wavelengths using appropriate dilutions of stock solutions (I) and (III) respectively in 0.07N HCl (n=6). CA and PRN showed linearity individually in the range of 4-24 µg/ml and 5-30 µg/ml respectively and in mixture within concentration range of 4-20 µg/ml (Table 1).

Determination of ε (1%/cm) of CA and PRN at selected wavelengths:

Using Eqn. 1, ε (1%/cm) was calculated for CA and PRN at each of the selected wavelengths (n=6).

Development of simultaneous equations for determination of CA and PRN:

Substituting the values in Eqns. 2 and 3,

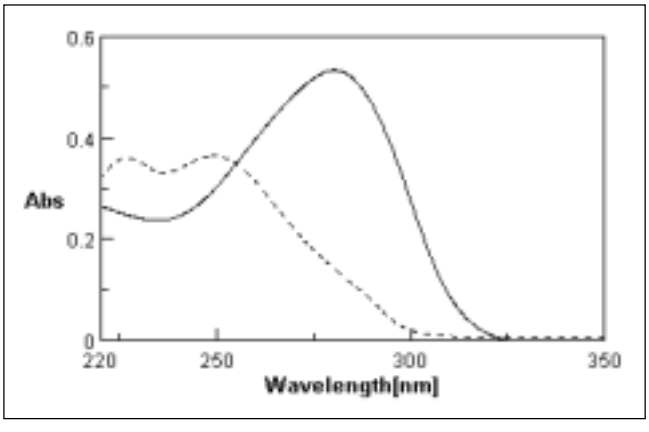

Fig. 1: UV scans of CA and PRN in 0.07 N HCl.
Overlain UV spectra of CA and PRN in 0.07 N HCl in the range of 220 nm to 350 nm (--) UV spectrum of PRN, (-) UV spectrum of CA

TABLE 1: LINEARITY AND RANGE OF DRUGS AND THEIR MIXTURES IN 0.07 N HCL

Drug	Conc. range (µg/ml)	Correlation coefficient		Extinction coefficient at 280 nm (1%/cm)x10^{-3}	% RSD	Extinction coefficient at 249 nm (1%/cm)x10^{-3}	% RSD
		280 nm	249 nm				
CA	4-24	0.9996	0.9994	44.221	0.89	24.872	1.104
PRN	5-30	0.9991	0.9995	13.060	1.20	32.617	0.99
CA + PRN	4-20	0.9994	0.9991	–	–	–	–

Linearity and range of CA and PRN individually and for mixtures with correlation coefficient, extinction coefficient and % RSD at 249 nm and at 280 nm in 0.07 N HCl. (n=6)

C_{CA} =(32.617A_{280}–13.060A_{249})/1.117004...Eqn. 4, and C_{PRN}=(44.205A_{249}–24.872A_{280})/1.117004...Eqn. 5. Eqns. 4 and 5 can be used for the quantitative estimation of CA and PRN in mixtures in 0.07N HCl.

Verification of accuracy and precision of the method:

To verify accuracy of method, solutions of known concentrations of CA and PRN in 0.07N HCl were mixed together to get various concentration mixtures. The concentration of each drug in these mixtures was calculated from Eqns. 4 and 5. Student's t-Test was applied to the set of values and the theoretical and calculated values were not found to be significantly different (Table 2). Precision of the method was determined by carrying out the analysis at different intervals in the same day and on successive days as displayed in Table 5.

Study of Beer-Lambert's law for CA and PRN in 0.1N NaOH:

Solutions of CA (20 µg/ml) and PRN (20 µg/ml) in 0.1N NaOH were prepared by appropriately diluting stock (II) and (IV) respectively in 0.1N NaOH and scanned in UV range. From their overlain spectra (Fig. 2), analytical wavelengths 274 nm (λ_{max} of CA) and 244 nm (λ_{max} of PRN) were selected for development of simultaneous equations.

Calibration curves of CA and PRN in 0.1N NaOH were plotted at both the selected wavelengths using appropriate dilutions of stock solutions (II) and (IV) respectively in 0.1N NaOH, (n = 6). Both the drugs obeyed linearity

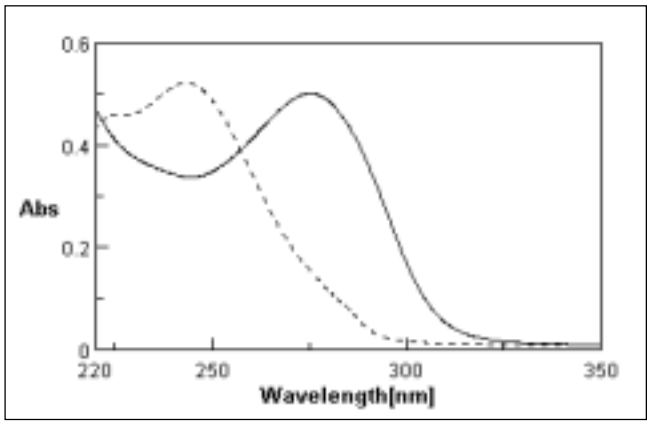

Fig. 2: UV scans of CA and PRN in 0.1N NaOH.
Overlain UV spectra of CA and PRN in 0.1 N NaOH in the range of 220 nm to 350 nm (---) UV spectrum of PRN, (-) UV spectrum of CA

individually and in mixture within concentration range of 4-20 µg/ml (Table 3).

Determination of ε (1%/cm) of CA and PRN at selected wavelengths:

The absorbance (n = 6) of CA and PRN solutions of known concentrations was taken and ε (1%/cm) was calculated using the Eqn. 1 at each of the selected wavelengths.

Development of simultaneous equations for determination of CA and PRN:

Substituting the values in Eqns. 2 and 3, C_{CA}=(36.156A_{274}–11.276A_{244})/1.132944...Eqn. 6, and C_{PRN}=(39.786A_{244}–27.090A_{274})/1.132944...Eqn. 7. Eqns. 6 and 7 can be used for the quantitative estimation of CA and PRN in mixtures in 0.1N NaOH.

TABLE 2: INDIVIDUAL DRUG CONTENT FROM STANDARD MIXTURES IN 0.07 N HCL

CA + PRN (µg/ml)	Theoretical conc. of CA (µg/ml)	Calculated conc. of CA (µg/ml)	$t_{0.1}$	Theoretical conc. of PRN (µg/ml)	Calculated conc. of PRN (µg/ml)	$t_{0.1}$
4 + 20	4	4.04	0.989*	20	20.21	1.231*
16 + 5	16	15.98	1.323*	5	4.98	0.823*
4 + 5	4	4.06	1.101*	5	5.04	1.370*
16 + 20	16	16.09	1.032*	20	19.90	0.903*

Individual concentration of CA and PRN from their combinations in 0.07 N HCl. (n=6) *t values (Student's t Test) at $P < 0.1$ comparing theoretical and calculated concentrations.

TABLE 3: LINEARITY AND RANGE OF DRUGS AND THEIR MIXTURES IN 0.1 N NAOH

Drug	Conc. range (µg/ml)	Correlation coefficient		Extinction coefficient at 244 nm (1%/cm)×10^{-3}	% RSD	Extinction coefficient at 274 nm (1%/cm)×10^{-3}	% RSD
		244 nm	274 nm				
CA	4-24	0.9997	0.9996	27.098	1.058	39.786	1.154
PRN	5-30	0.9998	0.9996	36.156	0.989	11.276	1.025
CA + PRN	4-20	0.9998	0.9995	–	–	–	–

Linearity and range of CA and PRN individually and for mixtures with correlation coefficient, extinction coefficient and % RSD 244 nm and at 274 nm in 0.1 N NaOH (n=6)

TABLE 4: INDIVIDUAL DRUG CONTENT FROM STANDARD MIXTURES IN 0.1 N NAOH

CA + PRN	Theoretical conc. of CA (µg/ml)	Calculated conc. of CA (µg/ml)	$t_{0.1}$	Theoretical conc. of PRN (µg/ml)	Calculated conc. of PRN (µg/ml)	$t_{0.1}$
4 + 20	4	4.06	0.863*	20	19.90	1.111*
16 + 5	16	16.06	1.010*	5	5.08	1.206*
4 + 5	4	4.04	1.40*	5	5.05	0.998*
16 + 20	16	15.89	1.107*	20	20.15	0.743*

Individual concentration of CA and PRN from their combinations in 0.1 N NaOH (n=6) *t values (Student's t Test) at $P < 0.1$ comparing theoretical and calculated concentrations.

Verification of accuracy and precision of the method:

To verify accuracy of the method, solutions of known concentrations of CA and PRN in 0.1N NaOH were mixed together to get various concentration mixtures. The concentrations of individual drugs in these mixtures were calculated from Eqns. 6 and 7. Student's t-Test was applied to the set of values and the theoretical and calculated values were not found to be significantly different (Table 4). Precision of the method was determined by carrying out the analysis at different intervals in the same day and on successive days as displayed in Table 5.

Assay of marketed formulation and Recovery studies of CA and PRN:

Marketed formulation (Altacef LA from Glenmark Pharmaceuticals) contains CA equivalent to 250 mg of cefuroxime and 250 mg of PRN in a capsule dosage form. The contents of 20 capsules were weighed accurately and mixed uniformly. An accurately weighed quantity of powder equivalent to 20 mg of cefuroxime and 20 mg of PRN was taken into a 100 ml volumetric flask and sufficient quantity of methanol was added to it. It was then sonicated for about 10 min and the volume made up with methanol. It was filtered and the filtrate diluted to get a suitable concentration in both 0.07N HCl and 0.1N NaOH. The assay was carried out in triplicate and the results are shown in Table 6. Recovery study was carried out at 3 levels—50%, 100%, and 150%—for both the drugs using Methanol as the solvent (Table 7).

Dissolution study of marketed formulation:

Dissolution of the marketed formulation (n = 6) for CA was carried out in 900 ml of 0.07N HCl medium using USP type II apparatus at 55 rpm. Samples were withdrawn at 15, 30, 45, 60, 90 and 120 min and absorbance was measured at 280 nm and 249 nm. Percent cumulative drug release was determined using Eqn. 4.

Dissolution of the marketed formulation for PRN (n = 6) was carried out in 900 ml of phosphate buffer pH 7.5 medium using USP type II apparatus at 50 rpm. Samples were withdrawn at 15, 30, 45, 60, 90 and 120 min, appropriately diluted in 0.1N NaOH and absorbance was measured at 244 nm and 274 nm. Percent cumulative drug release was determined using Eqn. 7 (Table 8).

RESULTS AND DISCUSSION

Although the drugs interfere with the UV absorption of each other, as the overlain spectra of both the drugs showed different absorption maxima and the peaks were well resolved, it satisfied the criteria for obtaining maximum precision based on additive absorbances. Hence simultaneous equations were developed.

The linear regression data (n=6) showed a good linear relationship for the mixture of both the drugs, CA and PRN, over a concentration range of 4-20 µg/ml, in both 0.07N HCl and phosphate buffer pH 7.5/0.1N NaOH systems. The method for both the drugs at the selected wavelengths was found to be precise and accurate as indicated by inter-day and intra-day analysis, showing percent relative standard deviation (% RSD) less than 2.

TABLE 5: INTRA-DAY AND INTER-DAY PRECISION OF METHOD

Drug	Concentration (µg/ml)	Intra-day % RSD	Inter-day % RSD
CA	5 in 0.07 N HCl	0.958	1.154
	5 in 0.1 N NaOH	0.852	1.068
	20 in 0.07 N HCl	0.812	0.925
	20 in 0.1 N NaOH	1.025	1.254
PRN	5 in 0.07 N HCl	0.658	0.958
	5 in 0.1 N NaOH	0.765	1.017
	20 in 0.07 N HCl	1.0185	1.105
	20 in 0.1 N NaOH	0.855	0.954

Intra-day and inter-day method precision analysis at 5 µg/ml and at 20 µg/ml of CA and PRN in 0.07 N HCl and 0.1 N NaOH with % RSD (n = 3)

TABLE 6: ASSAY OF MARKETED FORMULATION

Label claim	% Drug content in 0.07 N HCl	% RSD	% Drug content in0.1 N NaOH	% RSD
CA 250 mg	99.45	0.881	99.05	0.658
PRN 250 mg	98.95	0.965	99.65	0.789

Assay with % RSD of marketed formulation carried out in triplicate.

TABLE 7: RECOVERY STUDY OF MARKETED FORMULATION

Label claim (mg)	Amount added (%)	Total amount of drug (mg)	Amount recovered (mg ± S. D.)	% Recovery ± S. D.	% RSD
CA 250	50	375	373 ± 0.876	99 ± 0.956	0.963
	100	500	498 ± 0.972	99 ± 1.012	1.015
	150	625	626 ± 0.782	100 ± 1.157	1.154
PRN 250	50	375	374 ± 1.184	99 ± 0.882	0.885
	100	500	500 ± 0.865	99 ± 0.965	0.966
	150	625	625 ± 1.282	99 ± 1.189	1.192

Recovery studies of CA and PRN at 50, 100 and 150 % levels with % RSD (n=6) using methanol as extraction solvent

TABLE 8: PERCENT CUMULATIVE RELEASE OF CA AND PRN FROM MARKETED FORMULATION

Time (min)	% Cumulative release of CA	% Cumulative release of PRN
15	34.90	85.40
30	56.30	91.60
45	69.50	95.60
60	77.90	96.20
90	87.30	98.70
120	92.40	100.04

Percent cumulative release of CA and PRN in 0.07 N HCl and 0.1 N NaOH respectively from marketed formulation (n=6)

The method was validated by analyzing mixtures of standard solutions and Students t-Test was applied to this data. The theoretical and practical values were found not to be significantly different.

Recovery studies of the drugs were carried out at three levels—50, 100 and 150%—and percent recovery of both the drugs was found to be within limits with % RSD less than 2. The assay of both the drugs was found to be within limits. Similarly, the developed method was used for the *in vitro* dissolution studies of these drugs. CA and PRN showed about 92 and 100% release respectively in 2 h.

Thus the developed method is simple, accurate, precise, easy and economical and can be used for routine analysis (assay, dissolution studies) of cefuroxime axetil and probenecid simultaneously from a combined dosage form.

ACKNOWLEDGEMENTS

The authors wish to thank Elder Pharmaceuticals, Mumbai, for providing the gift sample of cefuroxime axetil; and Geno Pharmaceuticals, Mumbai, for providing the gift sample of probenecid.

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Accepted 9 February 2006
Revised 4 June 2005
Received 22 February 2005
Indian J. Pharm. Sci., 2006, 68 (1): 59-63