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A Novel Application of Hydrotropic Solubilization for Simultaneous Estimation and Validation of Acetaminophen, Chlorzoxazone, and Aceclofenac in Tablet Dosage Form

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Abstract

Various organic solvents like methanol, chloroform, alcohol, dimethyl formamide; acetonitrile, hexane, acetone and carbon tetrachloride have been employed for solubilization of poorly water-soluble drugs for spectrophotometric estimations. Drawbacks of organic solvents include high cost and error in analysis due to volatility. Attempting to minimize these drawbacks, three new, simple, accurate, environmental friendly, cost effective, safe, sensitive spectrophotometric methods have been developed for simultaneous estimation acetaminophen, chlorzoxazone, and aceclofenac in tablet dosage form by using 8.0 M urea solution, as a hydrotropic agent. Aqueous solubility of these model drugs were enhanced to a great extent 18, 5 and 10 fold for acetaminophen, chlorzoxazone, and aceclofenac in 8.0M urea solution respectively. Urea solution and additives of tablet did not interfere in analysis, as urea did not show any absorbance above 243nm. In 8.0M urea solution, acetaminophen, chlorzoxazone, and aceclofenac show maximum absorbance at wavelength 244.4nm, 281.6 and 276.0nm respectively. Beer's law was obeyed in the concentration range 0-40µg/ml for three drugs. Method-I is based on simultaneous equation method, method-II is area under curve and method III is multi-component methods of spectrophotometric analysis. Results of analysis for these methods were tested and validated for various parameters according to ICH guidelines, hence can be adopted for the routine analysis of acetaminophen, chlorzoxazone, and aceclofenac in tablet dosage form.

Keywords: Hydrotropic Solubilization, Acetaminophen, Chlorzoxazone, Aceclofenac, Spectrophotometry, ICH guidelines.

INTRODUCTION

Spectrophotometric estimation of poorly water soluble drugs generally requires the use of organic solvents, acid or base. Various organic solvents like methanol, 95%ethanol, cycloheaxne and 1, 4-dioxane etc are generally used for solubilization of poorly water soluble drugs. High cost and error in analysis due to volatility are the drawbacks of organic solvents. These drawbacks can be avoided by the use of hydrotropic solubilizing agents such as sodium salicylate[1], sodium benzoate, sodium lauryl sulphate[2], sodium glycinate[3], sodium gentisate[4], nicotinamide[5], urea[6] sodium acetate, sodium citrate[7] etc. Hydrotropy is a solubilization process where by addition of large amount of second solute results in an increase in aqueous solubility of another solute. By using this solubilization phenomenon various poorly water soluble drugs were analyzed Viz. frusemide[6], cefixime[7], hydrochlorothiazide[8], ketoprofen[9], bulk sample of ketoprofen and salicylic acid[10], norfoxacin in combination with tinidazole[11], and metronidazole[12],

The pH of 8.0M urea solution was 9.2. Therefore in order to check the influence of pH on solubilities of theses drugs, buffer solution of 9.2 pH was made and the solubilities of the drugs were determined. There was nearly no difference in the solubility of theses drugs in distilled water and buffer of pH 9.2. However aqueous solubilities of these model drugs were enhanced to a great extent 18, 5 and 10 for acetaminophen, chlorzoxazone, and aceclofenac in 8.0M urea solution respectively. The study proved that increases in solubility of acetaminophen, chlorzoxazone, and aceclofenac were not due to change in pH but was due to hydrotropic solubilization.

Fixed dose combination tablet containing acetaminophen, chlorzoxazone, and aceclofenac is clinically used for the management of pain and musculoskeletal spasm along with inflammation. Chemically, acetaminophen is 4-hydroxy acetanilide, used as an analgesic and antipyretic drug; chlorzoxazone is 5-choro-2-benzoxazolol is a commonly prescribed muscle relaxant and aceclofenac-2[2, 6-dichlorophenyl) amino] benzoic acid carboxymethyl ester is an analgesic and non-steroidal anti-inflammatory drug. Acetaminophen is official in BP and IP[13,14] chlorzoxazone in USP[15] and aceclofenac in BP[16]. IP and BP suggest titrimetric and UV spectrophotometric assay method for acetaminophen in bulk and tablet formulations. BP suggests a potentiometric assay method for aceclofenac in bulk drugs.

Literature survey revealed that HPLC[17], densitometric[18], spectrofluorimetric[19] and colorimetric[20] methods have been reported for the estimation of aceclofenac in pharmaceutical dosage forms. Also simultaneous estimation of acetaminophen, chlorzoxazone, and aceclofenac in tablet[21] by uv spectrophotometry (using methanol as solvent) and RP-HPLC[22] methods are reported. None of these methods are without their limitations and Though HPLC method is highly precise, it is costly and time consuming experiment so the need was felt to develop new, simple, accurate, environmental friendly, cost effective, safe, sensitive spectrophotometric methods for simultaneous estimation of acetaminophen, chlorzoxazone, and aceclofenac in tablet dosage form by using aqueous solution of 8.0 M urea solution, as a hydrotropic agent.

RESULT AND DISCUSSION

Solubility studies indicated that aqueous solubility of acetaminophen, chlorzoxazone, and aceclofenac were enhanced more than 18, 5, and 10 folds in 8.0 M urea solution as compared to solubility in distilled water and buffer of pH 9.2 respectively.

The Beer- Lambert's concentration range was found to be 0-40 µg/ml for acetaminophen, chlorzoxazone, and aceclofenac at 244.4nm, 281.6nm and 276nm wavelengths and coefficient of correlation were found 0.9999, 0.9999, and 0.9998 respectively.

Percentage estimation of three drugs was found in tablet dosage form were 99.44, 99.76 and 100.8 in method I, 100.10,99.89 and 100.10 in method II and 99.80, 99.84 and 100.43 in method III for acetaminophen, chlorzoxazone, and aceclofenac respectively with standard deviation <2 (Table 1).

The validity and reliability of proposed methods were assessed by recovery studies. Sample recovery for both the methods are in good agreement with their respective label claims, which suggested non interference of formulation additives and hydrotropic solubilizing agent urea in estimation.(Table-2)

Precision was determined by studying the repeatability and intermediate precision. Repeatability result indicates the precision under the same operating conditions over a short interval time and inter-assay precision. The standard deviation, coefficient of variance and standard error were calculated for acetaminophen, chlorzoxazone, and aceclofenac. The results were mentioned in Table 1. Intermediate precision study expresses within laboratory variation in different days. In both intra and inter-day precision study for both the methods %COV were not more than 1.0% indicates good intermediate precision (Table 3).

The value of LOD were 0.4217 µg/ml, 0.0885 µg/ml and 0.0974 µg/ml and LOQ were 1.2779µg/ml, 0.2683µg/ml and 0.2951 µg/ml for acetaminophen, chlorzoxazone, and aceclofenac (Table-3)

MATERIALS AND METHODS

UV-visible double beam spectrophotometer, Shimadzu model 1700 with spectral bandwidth of 1 nm, wavelength accuracy of ± 0.3 nm and a pair of 10 mm matched quartz cells was used. Pure sample of acetaminophen was obtained as gift samples from Zest Pharma, Indore, chlorzoxazone from Uni Drugs Innovative Pharma Technologies Ltd., Indore and aceclofenac from Aristo Pharma Ltd., Mandideep. The commercially available tablets, Micronac-MR (Label claim: acetaminophan-500mg, chlorzoxazone-250mg, and aceclofenac-100mg) was procured from local market.

Preliminary solubility studies of drugs

Solubility of three drugs was determined at 28±1°C. An excess amount of drug was added to three screw capped 30ml glass vials containing different aqueous system viz. distilled water, buffer of pH 9.2, 8.0M urea solution. The vials were shaken for 12 hrs at 28±1°C in a mechanical

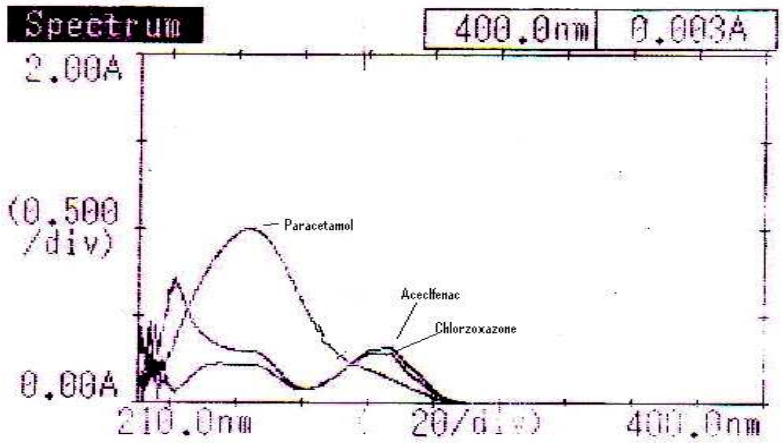
shaker. These solutions were allowed to equilibrate for the next 24 hrs and then centrifuged for 5 minutes at 2000rpm. The supernatant of each vial was filtered through Whatman filter paper No.41. The filtrates were diluted suitably and analyzed spectrophotometrically against corresponding solvent blank.

Preparation of standard stock, calibration curve and binary mixture solutions

The standard stock solutions of acetaminophen, chlorzoxazone,and, aceclofenac were prepared by dissolving 50mg of each drug in 40ml of 8.0M urea solutions and final volume was adjusted with distilled water in 100ml of volumetric flask. From the above solution 10ml of solution was taken and diluted to 50ml with distilled water to get a solution containing 100 µg/ml of each drug.

Working standard solutions of 20µg/ml were scanned in the entire UV range of 400-200 nm to determine the λ max of these drugs. The λ max of acetaminophen, chlorzoxazone, and aceclofenac were found to be 244.4nm, 281.6nm and 276nm respectively (Figure-1). Eight working standard solutions for three drugs having concentration 5, 10, 15, 20, 25, 30, 35, 40µg/ml was prepared in distilled water from stock solution. The absorbances of resulting solutions for three drugs were measured at their respective λ max and plotted a calibration curve against concentration to get the linearity and regression equation of three drugs.

Five mixed standards solutions with concentration of acetaminophen, chlorzoxazone, and aceclofenac in µg/ml of 5:25:30, 10:20:25, 15:15:20, 20:10:15, 25:5:10 were prepared in distilled water by diluting appropriate volumes of the standard stock solutions. The absorbances of resulting solutions were measured at 244.4nm, 281.6nm and 276 nm.



Figuer-1 Overlain spectra of acetaminophen, chlorzoxazone, and aceclofenac

Method-I : Simultaneous equation method

Simultaneous equation method[30] of analysis was based on the absorption of drugs (acetaminophen, chlorzoxazone, and aceclofenac) at the wavelength maximum of the each other. Three wavelengths were selected for the development of the simultaneous equations was 244.4nm, 281.6nm and 276nm λmax of acetaminophen, chlorzoxazone, and aceclofenac respectively. The absorbances of three drugs were measured at 244.4nm, 281.6nm and 276nm. The absorptivity values E (1%, 1cm) determined at 244.4nm, 281.6nm and 276nm for

acetaminophen were 664(ax1), 157(ax2), 134(ax3), for chlorzoxazone 312(ay1), 285(ay2), 301(ay3); for aceclofenac 284 (az1), 314(az2),322(az3). These values were means of six estimations. Thus absorptivity coefficients were determined were substituted in following equations to obtain the concentration of three drugs.

$$A_1 = 664xC_{\text{acetaminophen}} + 312xC_{chlorzoxazone} + 284xC_{aceclofenac} \text{ ……Eqn.1}$$
$$A_2 = 157xC_{\text{acetaminophen}} + 285xC_{chlorzoxazone} + 314xC_{aceclofenac} \text{ ……Eqn.2}$$
$$A_3 = 134xC_{\text{acetaminophen}} + 301xC_{chlorzoxazone} + 322xC_{aceclofenac} \text{ ……..Eqn.3}$$

Where $C_{\text{acetaminophen}}$, $C_{\text{chlrzoxazone}}$ and $C_{\text{aceclofenac}}$ are concentration of acetaminophen, chlorzoxazone, and aceclofenac respectively in g/100mL. A_1, A_2 and A_3 are the absorbance of the mixture at 244.4nm, 281.6nm and 276nm respectively. Absorbance of the sample solution viz.A_1,A_2 and A_3 were recorded at 244.4,281.6 and 276 nm respectively and concentration of three drugs in the sample were determined using Eqn.1, 2 and 3.

Method II: Area under Curve Method (AUC)

AUC method[29] involves the calculation of integrated value of absorbance with respect to wavelength. Area calculation processing item calculates the area of bounded by the curve and horizontal axis. Here horizontal axis represents baseline.

$$(\alpha + \beta) = \int_{\lambda_2}^{\lambda_1} Ad\lambda$$

Where, α = area of portion bounded by curve data and a straight line connecting the start and end point, β= area of portion bounded by a straight line connecting the start and end point on curve data and horizontal axis, λ_1 and λ_2 are wavelengths representing start and end point of curve region.

This method involved calculation of concentration for acetaminophen in the regions of 253-250nm, for chlorzoxazone 271-267 and for aceclofenac 276-273nm, these regions were selected on the basis of repeated observation that plot area calculation of pure sample drug against the concentration.

$$\int_{250}^{253} Ad\lambda = K_1C_1 \text{ …..}\mathbf{Eqn.4} \qquad \int_{267}^{271} Ad\lambda = K_2C_1 \text{ …..}\mathbf{Eqn.5} \qquad \int_{273}^{276} Ad\lambda = K_3C_1 \text{ …..}\mathbf{Eqn.6}$$

$$\int_{250}^{253} Ad\lambda = V_1C_2 \text{ …..}\mathbf{Eqn.7} \qquad \int_{267}^{271} Ad\lambda = V_2C_2 \text{ …..}\mathbf{Eqn.8} \qquad \int_{273}^{276} Ad\lambda = V_3C_2 \text{ …..}\mathbf{Eqn.9}$$

$$\int_{250}^{253} Ad\lambda = A_1C_3 \text{ …..}\mathbf{Eqn.10} \qquad \int_{267}^{271} Ad\lambda = A_2C_3 \text{ …..}\mathbf{Eqn.11} \qquad \int_{273}^{276} Ad\lambda = A_3C_3 \text{ …..}\mathbf{Eqn.12}$$

Where C_1, C_2 and C_3 were concentration of acetaminophen, chlorzoxazone, and aceclofenac respectively in µg/ml and K_1(0.2175), K_2(0.1009), K_3(0.0767), V_1(0.0686), V_2(0.0425), V_3(0.06903) and A_1(0.0706), A_2(0.0436), A_3(0.0760) were constant. In view of that, following

three final equations were developed for estimation of acetaminophen, chlorzoxazone, and aceclofenac.

$$\int_{250}^{253} Ad\lambda = 0.2175xC_{\text{acetaminophen}} + 0.0686xC_{chlorzoxazone} + 0.0706xC_{aceclofenac} \text{ …..Eqn.13}$$

$$\int_{267}^{271} Ad\lambda = 0.100xC_{\text{acetaminophen}} + 0.0425xC_{chlorzoxazone} + 0.0436xC_{aceclofenac} \text{ …….Eqn.14}$$

$$\int_{273}^{276} Ad\lambda = 0.0767xC_{\text{acetaminophen}} + 0.0690xC_{chlorzoxazone} + 0.0760xC_{aceclofenac} \text{ …..Eqn.15}$$

Sample solutions were scanned and area was calculated within the indicated wavelength regions. Concentrations of three components were calculated using Eqn. 13, 14 and 15.

Method III: Multi-component Method

In this method[30] six mixed standards of acetaminophen, chlorzoxazone, and aceclofenac in the ratio of 10:5.2 having concentrations in µg/ml 25:12.5:5, 27.5:13.75:5.5, 30:15:6, 32.5:16.25:6.5, 35:17.5:7, and 37.5:18.75:7.5 were prepared in distilled water by diluting appropriate volumes of the standard stock solutions and scanned in the region of 400 nm to 200 nm. Sampling wavelengths (244.4nm, 281.6nm and 276nm.) were selected on the trial and error basis. The concentration of individual drug was feed to the multi-component mode of the instrument. The instrument collects and compiles the spectral data from mixed standards and concentration of each component were obtained by spectral data of sample solution with reference to that of six mixed standards.

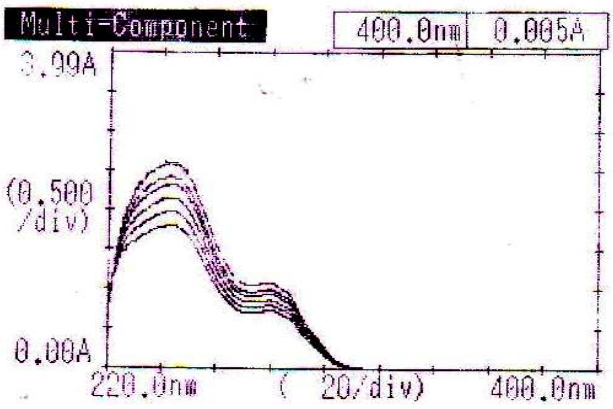


Figure-2 Overlain spectra of mixed standard of acetaminophen, chlorzoxazone, and aceclofenac

Analysis of the tablet formulations

Twenty tablets of marketed formulation were accurately weighed and powdered. A quantity of powder equivalent to 50 mg of acetaminophen was transferred to 100 ml volumetric flask and dissolved in 40 ml of 8.0M urea solution with frequent shaking for 15minutes and final volume was made up with distilled water. The sample solution was then filtered through Whatman filter paper No.41 and first few ml were rejected. From the above solution 10ml of solution was taken

and diluted to 50ml with distilled water to get a solution containing 100 µg/ml of acetaminophen and corresponding concentration of chlorzoxazone and aceclofenac. This solution contains acetaminophen, chlorzoxazone, and aceclofenac in the proportions of 10:5:2.

From above solution 2.5 ml of solution was transferred in 10ml volumetric flask and diluted with distilled water to obtain final concentration of 25µg/ml of acetaminophen, 12.5 µg/ml of chlorzoxazone and 5 µg/ml of aceclofenac Analysis procedure was repeated six times with tablet formulation. The result of analysis of tablet formulation was reported in Table 1.

Table 1: Analysis data of tablet formulation

Method	Drug	Label claim mg/tab	Amount found* mg/tab	Label claim (%)	S.D.*	% COV	S.E*.
I	ACT	500	497.20	99.44	0.5940	0.5971	0.2425
	CHL	250	249.4	99.76	0.4451	0.4459	0.1817
	ACL	100	100.8	100.8	0.8457	0.8384	0.3452
II	ACT	500	500.5	100.10	1.0542	1.0531	0.4303
	CHL	250	249.72	99.89	0.6608	0.6615	0.2697
	ACL	100	100.10	100.10	1.2049	1.2036	0.4919
III	ACT	500	499.00	99.80	0.6509	0.6522	0.2657
	CHL	250	249.6	99.84	0.5611	0.5619	0.2290
	ACL	100	100.43	100.43	1.1826	1.1775	0.4828

ACT: acetaminophen, CHL: Chlorzoxazone, ACL: Aceclofenac, S.D.: Standard deviation, COV:
Coefficient of variation, S.E.: Standard error,*Average of six estimation of tablet formulation**.**

Validation [23, 24]

Accuracy

Accuracy of the developed method was conformed by doing recovery study as per ICH norms at three different concentration levels- 80%, 100%, 120% by replicate analysis (n=3). Here to a preanalysed sample solution, standard drug solutions were added and then percentage of drug content was calculated. The result of accuracy study was reported in Table 2.

From the recovery study it was clear that the method is very accurate for quantitative estimation of acetaminophen, chlorzoxazone, and aceclofenac in tablet dosage form as the statistical results were within the acceptance range i.e. S.D. < 1.0.

Table 2: Result of recovery studies

Tablet Brand	Method	Recovery level (Added amount)	Percent recovery+ SD#		
			ACT	CHL	ACL
(Micronac MR) Label claim: acetaminophen-500mg, chlorzoxazone-250mg, and aceclofenac-100mg	I	80%	99.26 +0.342	101.1+0.866	99.5+0.774
		100%	100.3+ 0.643	100.1+0.127	99.90+0.336
		120%	100.5+0.234	100.5+0.398	100.23+0.456
	II	80%	99.48+0.453	99.60+0.567	100.5+0.876
		100%	100.01+0.876	100.4+0.987	99.65+0.786
		120%	100.06+0.315	100.6+0.870	98.90+0.234
	III	80%	99.42+0.654	99.97+0.986	101.23+0.114
		100%	99.03+0.785	100.38+0.433	99.34+0.145
		120%	99.97+0.123	101.57+0.553	100.45+0.765

ACT: Acetaminophen, CHL: Chlorzoxazone, ACL: Aceclofenac, S.D.: Standard deviation,
Average of three estimation at each level of recovery

Precision

Repeatability

Precision was determined by studying the repeatability and intermediate precision. Repeatability result indicates the precision under the same operating conditions over a short interval time and inter-assay precision. The standard deviation, coefficient of variance and standard error were calculated for three drugs. Repeatability was performed for six times with tablets formulation. The results of statistical evaluation are given in Table 1.

Intermediate Precision- (Inter-day and Intra-day precision)

Intermediate precision was carried out by doing intra and inter day precision study. In intra day study concentration of three drugs were calculated on the same day at an interval of one hour. In inter day study the concentration of drug contents were calculated on three different days study expresses within laboratory variation in different days. In both intra and inter-day precision study for the methods %COV were not more than 1.0% indicates good intermediate precision (Table3).

Linearity

For each drug, appropriate dilutions of standard stock solutions were assayed as per the developed methods. The Beer- Lambert's concentration range was found to be 0-40 µg/ml for these three drugs. The linearity data for both methods are presented in Table 3.

Limit of Detection (LOD) and Limit of Quantitation (LOQ)

The LOD & LOQ of acetaminophen, chlorzoxazone, and aceclofenac by proposed methods were determined using calibration standards. LOD and LOQ were calculated as $3.3\sigma/S$ and $10\sigma/S$, respectively, where S is the slope of the calibration curve and σ is the standard deviation of response. The results of the same are shown in Table 3.

Table 3: Optical characteristics data and validation parameters

Parameters	**Values**		
	ACT	**CHL**	**ACL**
Maximum absorbance (λ max)	244.4nm	281.8nm	276nm
Beer's law limit (µg/ml)	0-40	0-40	0-40
Absorptive E(1%,1cm)*	664	285	322
Correlation coefficient*	0.9999	0.9999	0.9998
Intercept*	0.0014	0.0004	0.0016
Slope*	0.0662	0.0285	0.0321
LOD* (µg/ml)	0.4217	0.0885	0.0974
LOQ*(µg/ml)	1.2779	0.2683	0.2951
Intra-Day* (Precision) (% COV)	0.9424	0.6495	0.5172
Inter-Day (Precision) (% COV**)** n=3	0.5186	0.5190	0.4327

ACT: Acetaminophen, CHL: Chlorzoxazone, ACL: Aceclofenac**,** COV: Coefficient of variation, * Average of six determination.

CONCLUSION

The present paper describes application of hydrotropic solubilization phenomenon for the simultaneous estimation of acetaminophen, chlorzoxazone, and aceclofenac in tablet dosage form by simultaneous equation method, area under curve and multi-component methods of spectrophotometric analysis. All three drugs showed good regression values at their respective wavelengths and the results of recovery study reveled that any small change in the drug concentration in the solution could be accurately determined by the proposed methods and low values of LOD and LOQ indicated good sensitivity of proposed methods. Hence proposed methods are new, simple, accurate, sensitive, and precise and can be adopted for routine analysis of acetaminophen, chlorzoxazone, and aceclofenac in tablet dosage form. Further, as urea does not absorb above 243nm, a large number of drugs having λmax above 243nm can be used for estimation by proposed methods.

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