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SIMULTANEOUS ESTIMATION OF CHLORZOXAZONE, PARACETAMOL AND IBUPROFEN BY UV SPECTROSCOPIC METHOD

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ABSTRACT

A new rapid, accurate, precise, economical and sensitive spectrophotometric method was developed and validated for simultaneous determination of chlorzoxazone (CLZ), paracetamol (PCT) and ibuprofen (IBU) in bulk and pharmaceutical dosage forms. Simultaneous equation method, where CLZ, PCT and IBU were determined at 244.2 nm, 257.2 nm and 221.8 nm. The method shows good linearity for three compounds in the concentration ranges of 5-25µg/ml, 6.5-32.5 µg/ml and 8-40 µg/ml for CLZ, PCT and IBU respectively. The values of %RSD for intraday and interday precision was found to be within limits (2%) The values confirm that those methods are precise. The values of % recovery were 97-103%w/w for this, method which showed that the method was accurate. And free from the interference of excipients used in formulation. The values of % recovery for analysis of formulations are found within 98-102% w/w, which shows that the method are applicable for analysis of marketed formulation. The method was validated according to the ICH guidelines. The results were simple, accurate, sensitive and precise.

INTRODUCTION:

In combination three drugs are act as anti-inflammatory (chlorzoxazone), anti-pyretic (paracetamol) and analgesic (ibuprofen) for effective treatment of fever, inflammation and swallowing by inhibiting the COX-2 synthase[1-2]. Chlorzoxazone is chemically, 5-chloro-2, 3-dihydro-1, 3-benzoxazol-2-one (fig no.1a) which inhibits the mast cells. Paracetamol chemically, N-(4-hydroxyphenyl) acetamide which inhibits both isoforms of cyclooxygenase, COX-1, COX-2, and COX-3 enzymes involved in prostaglandin (PG) synthesis (fig no.1b). Ibuprofen chemically, 2-[4-(2-methylpropyl) phenyl] propanoic acid which act as non-selective inhibitor of cyclooxygenase, an enzyme involved in prostaglandin synthesis via the arachidonic acid pathway[3-4] (fig no.1c).

By extensive literature survey it reveal that very fewer works are reported for simultaneous estimation of chlorzoxazone, paracetamol and ibuprofen i.e. uv spectroscopic methods [5], RP-HPLC method [6-7], supercritical fluid chromatography [8]

Aim of present work is to develop a new, simple, rapid, sensitive, precise accurate and economical method for simultaneous determination of chlorzoxazone, paracetamol and ibuprofen in their pharmaceutical dosage form and to validate the method accordingly to ICH guidelines.

MATERIALS AND METHODS:

Experimental work:

CLZ, PCT and IBU working standards were kindly gifted by MSN Laboratories Pvt. Ltd., Hyderabad, India. Each tablet of (FLEXON -MR)containing of CLZ 250 mg, PCT 325mg and IBU 400 mg was procured from local market and used for analysis of marketed formulation. On basis of solubility studies sodium hydroxide is choice as common solvent (eco-friendly) for the both drugs which is of analytical grade (Merck Chemicals). Spectrophotometer system was of Shimadzu UV-1800 Spectrophotometer with two matched 1cm quartz cells using the following spectral parameters; a single fast scan mode and a slit width (1cm), connected to a computer loaded with Shimadzu UV-Probe 2.34 software and used for all the absorbance measurements and data manipulation.

Standard stock solution preparation:

Standard stock solutions of CLZ stock solution -I (1000µg/ml),PCT stock solution – I(1000µg/ml), and IBU stock solution- I (1000 µg/ml) were prepared by dissolving 25mg of CLZ,PCT and IBU in 25 ml sodium hydroxide(0.1N) separately . CLZ, PCT and IBU working solution stock solutions -II (100µg/ml) was prepared by transferring 2.5 ml from CLZ, PCT and IBU stock solution to a 25ml volumetric flask and diluted up to 25ml with 0.1 M NaOH respectively. Appropriate and accurate volume aliquots of the stock solutions were transferred to 10 ml calibrated flasks and made up to volume with methanol.

Aliquots preparation:

Aliquots of CLZ (0.5, 1.0, 1.5, 2.0 and 2.5mL) stock solution-II (100 µg/mL) were accurately transferred separately into 10 mL volumetric flasks then completed to volume with 0.1N NaOH to prepare concentrations ranging from 5-25µg/mL of CLZ and construct a graph by taking concentration versus absorbance (fig no.2) Aliquots of PCT (0.65, 1.3, 1.95, 2.6, and 3.25mL) stock solution-II (100 µg/mL) were accurately transferred separately into 10 mL volumetric flasks then completed to volume with 0.1N NaOH to prepare concentrations ranging from 6.5-32.5µg/mL of PCT and construct a graph by taking concentration versus absorbance (fig no.3) Aliquots of IBU (0.8, 1.6, 2.4, 3.2, and 4.0 mL) stock solution-II (100 µg/mL) were accurately transferred separately into 10 mL volumetric flasks then completed to volume with methanol to prepare concentrations ranging from 8-40µg/mL of IBU and construct a graph by taking concentration versus absorbance (fig no.4)

Sample preparation:

A total of 5 tablets were accurately weighed and transferred in to a mortar and pestle triturated well to fine powder. An amount (50mg sample) equivalent to one tablet content (Containing 250mg Chlorzoxazone, 325mg Paracetamol and 400mg Ibuprofen) was taken to a calibrated volumetric flask containing 50ml of 0.1 N NaOH and carried out mixing by using VERTEX MIXER upto 20min.After mixing the solution was filtered through Whatmann filter paper number 41& finally dilutes upto 50ml with and make further dilution with 0.1N NaOH. The above solutions were analysed for the content of Chlorzoxazone, Paracetamol and Ibuprofen using the method described in preparation of calibration curve.

SIMULTANEOUS EQUATION METHOD

If a sample containing two absorbing species each of which absorbed at the λmax of the other, it may be possible to determine the both the drugs by the technique of simultaneous equation or Vierodt's method. The ration should be lie outside the range 0.1 to 2.0 for the precise determination of two drugs respectively. This criteria are satisfied only when the λ_{max} of the two components dissimilar. An addition criterion is that the two components do not interact chemically, there by negating the initial assumption that the total absorbance is the sum of the individual absorbance.

C_X = (A1 (ay2az3-az2ay3)-ay1 (A2az3-az2A3) +az1 (A2ay3-ay2A3)/ax1 (ay2az3-az2ay3)-ay1 (ax2az3-az2ax3) +az1 (ax2ay3-ay2ax3)..... (1), C_Y = (ax1 (A2az3-az2A3)-A1 (ax2az3-az2ax3) + az1 (ax2A3-A2ax3)/ax1 (ay2az3-az2ay3)-ay1 (ax2az3-az2ax3) +az1 (ax2ay3-ay2ax3)..... (2), and C_Z = (ax1 (ay2A3-A2ay3)-ay1 (ax2A3-A2ax3) +A1 (ax2ay3-ay2ax3)/ax1 (ay2az3-

az2ay3)-ay1 (ax2az3-az2ax3) +az1 (ax2ay3-ay2ax3)..... (3)

Where, C_X, C_Y and C_Z = concentrations of CHLORZOXAZONE, PARACETAMOL and IBUPROFEN, respectively in mixture and in sample solutions.

A_1, A_2 and A_3 = Absorbance of sample at 244.2, 257.4. And 221.2 nm, respectively, ax_1, ax_2 and ax_3 = Absorptivities of CHORZOXAZONE at 244.2, 257.4. and 221.2 nm, respectively. ay_1, ay_2 and ay_3 = Absorptivities of Paracetamol at 244.2, 257.4. And 221.2 nm, respectively, az_1, az_2 and az_3 = Absorptivities of IBUPROFEN at 244.2, 257.4. And 221.2 nm, respectively

METHOD VALIDATION

The method is validated as per ICH guide lines. The method show good linearity, precision, accuracy. The linearity range of chlorzoxazone, paracetamol and ibuprofen was found to be 5-25 µg/ml , be 6.5-32.5 µg/ml and IBU 8-40µg/ml respectively. The accuracy was determined by three different levels 50%, 100%, 150% of the target concentration of the active ingredient, by adding know amount of concentration for previously analysed sample. Precision was studied to determine the intraday variation by performing nine replicates, %RSD was calculated and also inter-day variation by performing six replicates, %RSD was calculated. The LOD and LOQ were calculated from the standard calibration curves.

The residual standard deviation of the regression line or the standard deviation of the y-intercepts of regression lines was used as the standard deviation. LOD and LOQ were calculated with equations LOD=3.3σ/S and LOQ=10σ/S; where, σ is the Standard deviation of the response and S is the slope of the calibration curve. The proposed method was successful applied for analysis of samples in oral suspension by performing three replicate for ensuring the reproducibility.

RESULTS AND DISCUSSION

The aim of present work was to develop a simple, rapid, precise, accurate UV spectrophotometric method for simultaneous estimation of, Chlorzoxazone, paracetamol and ibuprofen in their pharmaceutical dosage forms, with 0.1 M NaOH and validate accordingly ICH guidelines. UV overlain spectra of both paracetamol and mefenamic acid showed (Figure 5). The concentration of chlorzoxazone, paracetamol and ibuprofen in marketed dosage form in the ratio of 5: 3.25:4. So, the standard calibration curves were constructed and linearity was designed in the range of 5-25 µg/ml,6.5-32.5 µg/ml and 8-40 µg/ml for Chlorzoxazone, paracetamol and ibuprofen respectively. Linearity coefficient and percentage curve fitting were well within the limits as found from the value of 0.9996, 0.9997 and 0.9999 for chlorzoxazone, paracetamol and ibuprofen respectively. Accuracy was determined through recovery study of the drug at three levels ranging from 50-150% and was found to be well within the acceptance limit indicating no interferences of the drug with each other or with the excipients present in the formulation (Table 2). So the proposed method is accurate for the analysis of paracetamol and mefenamic acid in combined dosage form. The % RSD of precision was found to be within limit of 2% (Table 1), indicating high degree of precision and reproducibility.

CONCLUSION

The mentioned spectrophotometric method was validated and successfully applied for simultaneous determination of chlorzoxazone paracetamol and ibuprofen in ternary mixtures and in their available dosage form. The proposed procedure is simple and do not require sophisticated techniques or instruments. This method is simple, rapid, sensitive, accurate, precise, economical and eco-friendly for the routine analysis of chlorzoxazone, paracetamol and ibuprofen in their pharmaceutical dosage forms.

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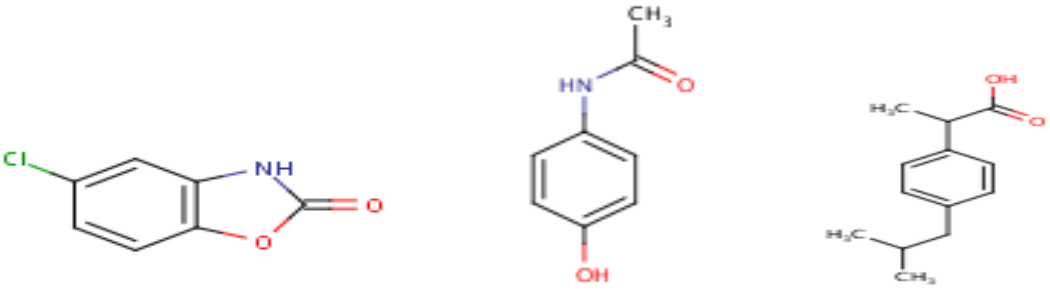

Fig no.1a, 1b, 1c: Chemical structure of chlorzoxazone, paracetamol and ibuprofen

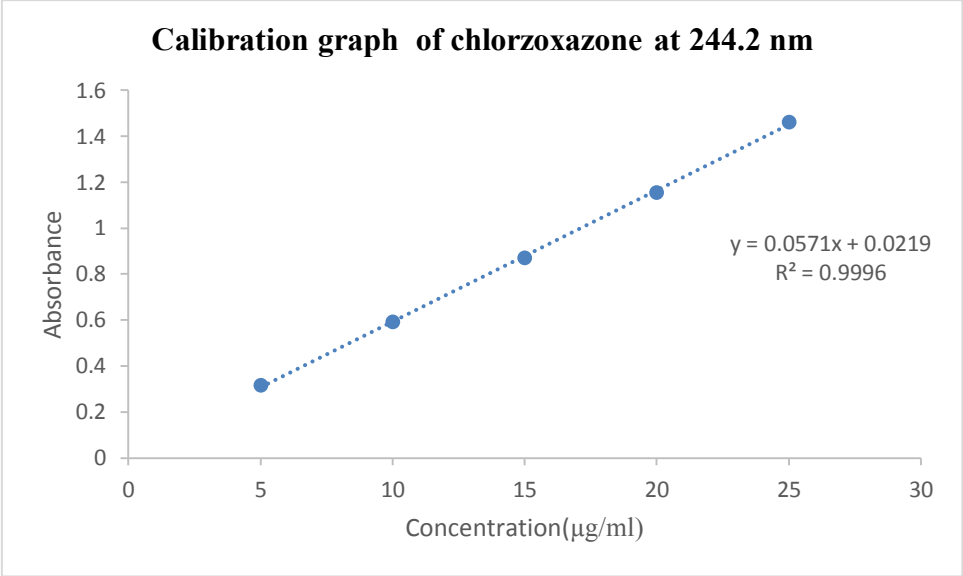

Fig no.2: Calibration graph of chlorzoxazone at 244.2 nm in 0.1N NaOH

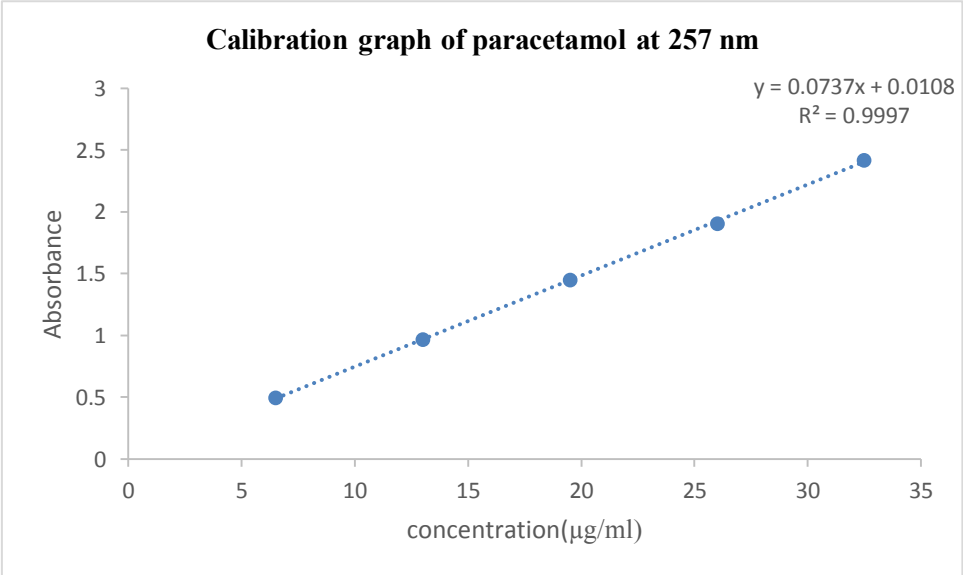

Fig no.3: Calibration graph of paracetamol at 257 nm in 0.1N NaOH

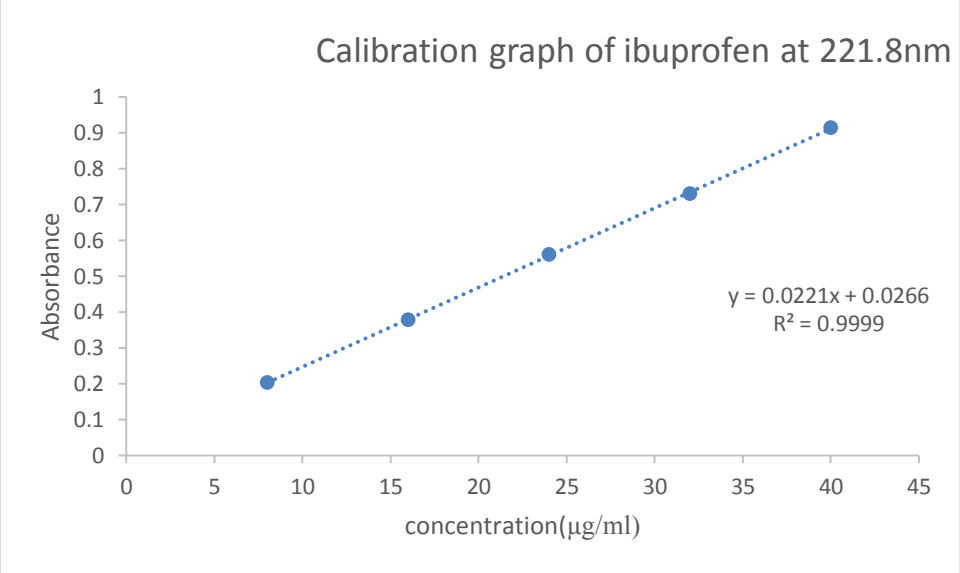

Fig no.4: Calibration graph of ibuprofen at 221.8 nm in 0.1N NaOH

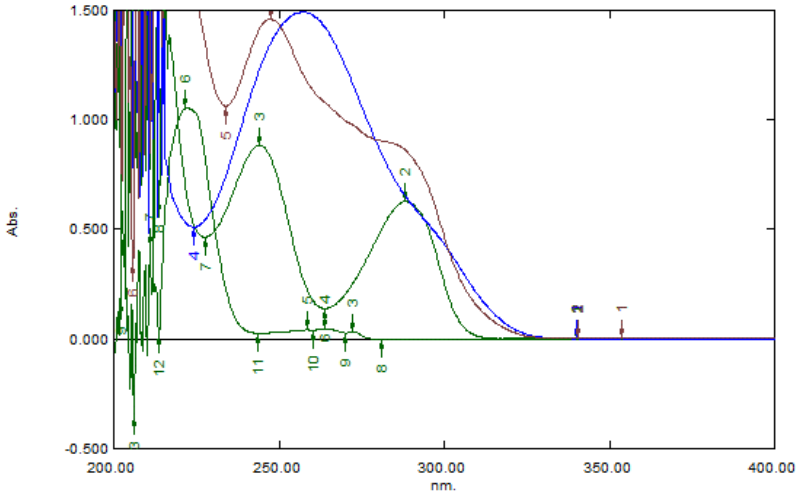

Fig no.5: Overlay Spectra ofclorzoxazone, paracetamol, ibuprofen in 0.1N NaOH

Table No. 1: Validation sheet for proposed method

Validation parameters	Chlorzoxazone	Paracetamol	Ibuprofen
Linearity range	5-25µg/ml	6.5-32.5 µg/ml	8-40 µg/ml
Correlation coefficient	0.9996	0.9997	0.9999
Slope	0.0571	0.0737	0.0221
Intercept	0.0219	0.0108	0.0266
Intraday precision(%RSD)	2.00729	1.003776	1.5477
Inter-day precision(%RSD)	0.653047	1.986	0.775107
LOD(µg/ml)	1.90425	1.051013	2.15567
LOQ(µg/ml)	1.35622	1.00	5.841604

% RSD- Relative standard deviation, LOD- Limit of detection, LOQ-Limit of quantitation

Table No 2: Applicability of formulation

Formulation	Drug	Labelled claim	%Found*(±SD)
FLEXON-MR	Chlorzoxazone	500mg	100.74±0.79%w/v
	Paracetamol	325mg	95.62±0.7%w/v
	ibuprofen	400mg	102.47±0.75%w/v

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