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RAPID CHROMATOGRAPHIC AND SPECTROPHOTOMETRIC DETERMINATION OF CITALOPRAM IN RELEVANCE TO PHARMACEUTICAL ANALYSIS

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ABSTRACT

This paper describes the study to measure the sensitivity and accuracy of two techniques, chromatography and spectrophotometry for the direct determination of citalopram in pharmaceutical formulations. Chromatography was performed on SE-30 column (1 m × 3 mm i.d) using Nitrogen as a carrier gas (flow rate of 40 ml/min), having retention time of 12.8 min. Calibration curve was found linear in the range of 200-400 µg/ml. LOD and LOQ values were found as 1.1726 µg/ml and 3.5535 µg/ml respectively and the recoveries were in the range of 98.36–100.07%. In spectrophotometry method the absorption spectra were measured at 240 nm for standard and tablets form. Calibration curve was found linear in the range of 2-4 µg/ml. LOD and LOQ values were found as 40.59 ng/ml and 123.0 ng/ml respectively. Recoveries were in the range of 98.53–100.78%. The experimental results obtained indicate that the above proposed methods are specific, sensitive and accurate for the determination of citalopram in pharmaceutical formulations. These methods were also compared statistically and found that there was no significant difference between the two methods in terms of accuracy and precision. Whilst, the chromatographic method developed will applicable for its detection in biological matrices also.

INTRODUCTION: Citalopram (CIT) [(RS)- 1- [3-(dimethyl amino)propyl]-1-(4- fluorophenyl)-1, 3-dihydroiso benzofuran-5-carbonitrile] (**Fig. 1**) is a widely used antidepressant comes under the class of selective serotonin reuptake inhibitors (SSRIs) having broad spectrum of therapeutic activity against depressive disorders [1]. It exerts effect through a selective inhibition for reuptake of neurotransmitter serotonin by the pre-synaptic receptors. It is prescribed in the treatment of related disorders, such as obsessive-compulsive disorder, anxiety, social phobia, and posttraumatic stress, but has many concentrations dependent adverse effects also [2]. Thus it is worthy to develop a sensitive, rapid and reliable method for their determination in pharmaceutical formulations.

Citalopram is comparable to the tri-cyclic antidepressants in their clinical efficacy, but because of its favourable pharmacological profile, considered safe, potent and well-tolerated drug [3]. This makes it most widespread prescribed antidepressant all over the world. Thus, it is worthy to develop a sensitive, rapid and reliable method for their determination in pharmaceutical formulations.

The aim of this paper is to validate sensitive, selective, and cost effective methods for the quantitative measurement of CIT in pharmaceutical formulations.

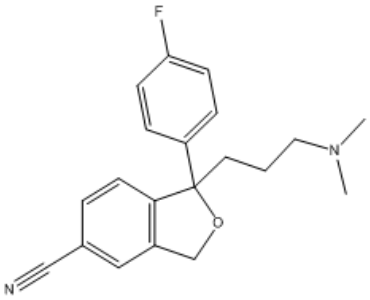

FIG. 1: CHEMICAL STRUCTURE OF CITALOPRAM

Several methods have been used for determination of citalopram in biological matrices like gas chromatography (GC) [4, 5], high performance liquid chromatography (HPLC) [6, 7], liquid chromatography tandem mass spectrometry (LC-MS/MS) [8, 9], capillary electrophoresis (CE) [10], micellar electrokinetic capillary chromatography [11], etc. But, these methods offer the analysis of citalopram in biological matrices like blood and urine.

Thus, the purpose of this work is to develop simple, rapid and sensitive methods for the determination of citalopram in pharmaceutical formulations using chromatography as well as spectrophotometry technique. The statistical approaches have also been applied in order to yield exact values that can be successfully applicable for the determination of citalopram.

MATERIALS AND METHODS:

Chemicals and Reagents: Citalopram hydrochloride was provided by Zydus Cadila Pharmaceutical (Ahmadabad, Gujarat, India). Methanol was purchased from Abhishek Enterprises (Ahmadabad, Gujarat, India) and Tablets were provided by the Pharmaceutical Companies {Formulation 1 is C-PRAM (Unichem, Zirakpur, India) 10mg; Formulation 2 is C-TALO [Alkem Pentacare, Hyderabad, India)] 10mg}**.** Whatmann filter paper no. 42 was used to filter the solutions.

Chromatographic Conditions: The GC-chromatograms were recorded using Shimdzu 2010-GC at Retention Time (RT) of 12.8 min. Samples were injected at a temperature of 260 C and analytes separation was achieved on SE-30 column (1 m × 3 mm i.d, at 250^{o}C). Nitrogen was used as carrier gas with 40 ml/min of flow rate. Detection was done through flame ionisation detector (FID) at a temperature of 290^{o}C. Data acquisition and analysis was performed using standard software supplied by the manufacturer (**Fig. 2**).

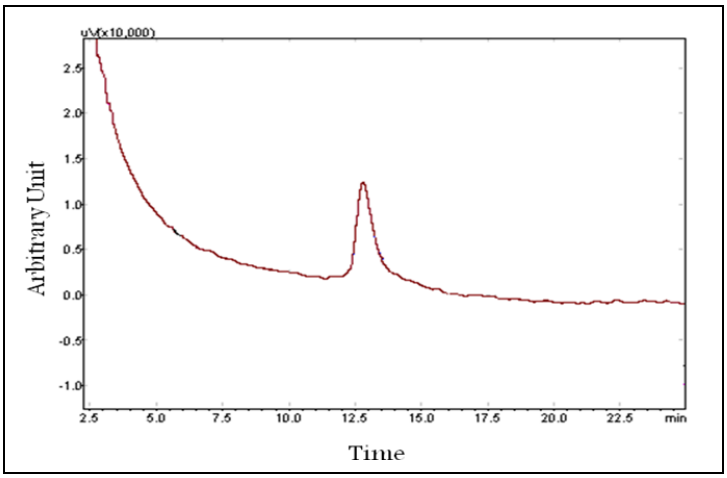

FIG. 2: GC CHROMATOGRAM OF CITALOPRAM [400 ppm, RT=12.8 min]

Sample and Standard Preparation: Standard solution of citalopram was prepared by dissolving 10 mg in 10 ml of Methanol which formed 1000 µg/ml of solution in order to obtain working solutions of 100, 200, 250, 300, 350 and 400 µg/ml. RT = 12.8 min was noted against respective reagent blanks to plot the calibration curve. The regression analysis data prepared from these solutions were given in **Table 1**.

TABLE 1: REGRESSION ANALYSIS DATA OF CALIBRATION CURVES BY GC

Concentration (µg/ml)	Area Mean ± S.D.*	C.V.
200	78823.14 ± 381.7412	0.484301
250	163562.1 ± 731.3232	0.447123
300	257520.4 ± 695.1399	0.269936
350	361253.4 ± 797.2854	0.2207
400	469289.6 ± 883.5706	0.188278

*Six replicate samples

Estimation of Citalopram (CIT) in Tablet Dosage Form: Analyses of tablet formulation 1 and 2 of citalopram were obtained by using twenty tablets of each. The tablets were crushed to furnish a homogeneous powder and a quantity equivalent to 10 mg was weighted in a 10 ml of volumetric flask and dissolved in methanol. The solution was filtered through Whatmann filter paper No. 42. The filtrate was diluted appropriately with methanol to give 300 µg/ml of working concentration and was analyzed on GC against reagent blank.

Spectrophotometric Conditions: The UV-spectra were recorded using Jasco V-630 spectrophotometer with 1 cm quartz cell used for the measurement of absorbance. The spectra were obtained on same and different days from the same solution (**Fig. 3**).

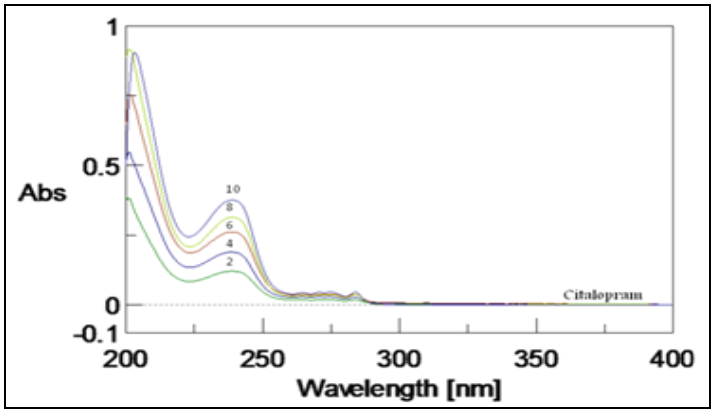

FIG. 3: UV SPECTRUM OF CITALOPRAM (λ_{max}=240 nm)

Sample and Standard Preparation: Standard solution of citalopram was prepared by dissolving 10mg in 10 ml of Methanol which formed 1000 µg/ml of solution. Further it was diluted to obtain 100 µg/ml of solution as a final Stock solution. Working solutions of 2, 4, 6, 8 and 10 µg/ml were made from above stock solution. Absorbance at 240 nm was noted against respective reagent blanks to plot the calibration curve. The regression analysis data prepared from these solutions were given in **Table 2.**

TABLE 2: REGRESSION ANALYSIS DATA OF CALIBRATION CURVES BY UV

Concentration (µg/ml)	Absorbance at 240 nm Mean ± S.D.*	C.V.
2	0.1259 ± 0.001227	0.974344
4	0.1892 ± 0.000206	0.108746
6	0.2613 ± 0.000391	0.149649
8	0.3139 ± 0.000365	0.11633
10	0.376244 ± 0.000345	0.91596

*Six replicate samples

Estimation of Citalopram in Tablet Dosage Form: Analyses of tablet formulation 1 and 2 of citalopram were obtained by using twenty tablets for each. The tablets were crushed to furnish a homogeneous powder. Now weigh powder equivalent to 10 mg of citalopram was transferred to 10 ml of volumetric flask and dissolved in methanol. The solution was filtered through Whatmann filter paper No 42. The filtrate was diluted appropriately with methanol to obtain 6 µg/ml and was analyzed on UV spectrophotometer against reagent blank.

RESULT AND DISCUSSION: Various analytical methods have been described for determination of citalopram in biological matrices, but very few methods have been described for its selective determination in pharmaceutical formulations. The method developed on the gas chromatography is simple, rapid and reliable which does not need any kind of derivatization step during analysis of samples [12-14]. Also, as compare to other reported procedures the spectrophotometry method developed for the determination of citalopram is simple, accurate and having low cost as does not require expensive chemicals or extraction procedures [15, 16]. Validation studies were performed with both techniques in terms of linearity, accuracy and other validated data summarised in **Table 3**.

TABLE 3: OPTICAL CHARACTERISTICS AND VALIDATION PARAMETERS FOR UV AND GC

Parameters	Values	Values
λ_{max} (nm)	240	-
Beer's Law limit (µg/ml)	2-10	-
Molar absorptivity (lit/mol/cm)	3.10 x 10^2	-
Regression Equation (Y[a])		
Slope (b)	0.031	1956.2
Intercept(c)	0.061	320787
Correlation coefficient (r)[b]	0.997	0.9976
Recovery%	98.33– 101.8	98.0– 101.1
Repeatability (RSD, n=6)	0.149649	0.269936
Precision(CV)		
Intra-day [b]	0.52– 0.65	0.65– 0.92
Inter-day [b]	0.40– 0.86	0.50– 1.32
Limit of Detection [c]	40.59 ng/ml	1.1726 µg/ml
Limit of Quantitation [d]	123.0 ng/ml	3.5535 µg/ml
Specificity	Specific	Specific
Solvent suitability	Suitable for 4 months.	Suitable for 4 months.

[a]Means Y=bX + c, where 'X' is concentration in µg/ml and Y is absorbance unit. [b]Means six replicate samples. [c] Limit of Detection (LOD) =3.3xS.D/slope, [d]Limit of Quantitation (LOQ) =10xS.D/slope

Accuracy of the methods was determined by recovery studies in the tablet formulations of citalopram (**Table 4 and 5)**. Recovery studies were carried out for both formulations by addition of known quantities of standard drug solution to pre-analyzed sample at three different concentrations for both techniques (**Table 6, 6, 7, 8 and 9)**. Also, the experiment was repeated three times in a day to determine intra-day precision and on three different days to determine inter-day precision. The coefficient of variance (CV) was calculated at each concentration level. The reproducibility was confirmed and carried out at variable conditions.

TABLE 4: RESULTS OF ANALYSIS OF CITALOPRAM FORMULATION IN GC

Amount of drug in Tablet powder taken (mg)	Amount found (mg)	Percentage estimated	Amount found (mg)	Percentage estimated
	Formulation 1	Formulation 1	Formulation 2	Formulation 2
10	9.930	99.30	9.887	98.87
10	9.821	98.21	9.973	99.73
10	9.986	99.86	9.894	98.94

Formulation 1 is C-PRAM 10 mg (Unichem, India); Formulation 2 is C-TALO 10mg [Alkem (Pentacare), India]

TABLE 5: RESULTS OF ANALYSIS OF CITALOPRAM FORMULATION IN UV

Amount of drug in Tablet powder taken (mg)	Amount found (mg)	Percentage estimated	Amount found (mg)	Percentage estimated
	Formulation 1	Formulation 1	Formulation 2	Formulation 2
10	9.920	99.20	9.934	99.34
10	9.871	98.71	9.865	98.65
10	9.990	99.90	9.978	99.78

Formulation 1 is C-PRAM 10 mg (Unichem, India); Formulation 2 is C-TALO 10mg [Alkem (Pentacare), India]

TABLE 6: RESULTS OF RECOVERY STUDY THROUGH GC OF FORMULATION 1

Labelled amount (mg)	Amount of drug added (mg)	Amount of drug obtained [1] (mg)	Percentage recovery [2]	Standard deviation	Coefficient of variation
10	5	15.15	101.0	0.0123	1.82
10	10	19.80	99.0	0.0101	1.70
10	15	25.65	102.6	0.0097	0.62

[1] Average of three determinations. [2]Recovery of amount added to the pharmaceutical formulation 1 (average of three determinations)

TABLE 7: RESULTS OF RECOVERY STUDY THROUGH GC OF FORMULATION 2

Labelled amount (mg)	Amount of drug added (mg)	Amount of drug obtained [1] (mg)	Percentage recovery [2]	Standard deviation	Coefficient of variation
10	5	14.70	98.0	0.0134	1.76
10	10	20.22	101.1	0.0104	1.68
10	15	24.91	99.64	0.0086	0.80

[1] Average of three determinations. [2]Recovery of amount added to the pharmaceutical formulation 2 (average of three determinations)

TABLE 8: RESULTS OF RECOVERY STUDY THROUGH UV OF FORMULATION 1

Labelled amount (mg)	Amount of drug added (mg)	Amount of drug obtained [1] (mg)	Percentage recovery [2]	Standard deviation	Coefficient of variation
10	5	14.89	99.26	0.0131	1.62
10	10	20.05	100.25	0.0346	1.07
10	15	24.76	99.04	0.0012	0.86

[1] Average of three determinations. [2] Recovery of amount added to the pharmaceutical formulation 1 (average of three determinations)

TABLE 9: RESULTS OF RECOVERY STUDY THROUGH UV OF FORMULATION 2

Labelled amount (mg)	Amount of drug added (mg)	Amount of drug obtained [1] (mg)	Percentage recovery [2]	Standard deviation	Coefficient of variation
10	5	14.75	98.33	0.0171	1.52
10	10	19.85	99.25	0.0246	1.47
10	15	24.45	101.8	0.0016	0.66

[1] Average of three determinations. [2] Recovery of amount added to the pharmaceutical formulation 2 (average of three determinations)

A comparison of the two methods show that the results obtained from the UV method were in good agreement with those obtained from the GC method (**Table 10**). The results obtained from the two methods were compared statistically by Student's t-test and Fisher test. The calculated t and F values were less than the theoretical values, indicating no significant difference between the mean content of citalopram and the precision obtained by the two proposed methods.

TABLE 10: STATISTICAL COMPARISON OF TWO METHODS IN TERMS OF ACCURACY AND PRECISION

Values	UV	GC	UV	GC
	Formulation 1		Formulation 2	
Amount labelled (mg)	10	10	10	10
Amount found (mg)	9.927	9.916	9.926	9.922
Student's t-test	*$t_{calculated}$ = 0.18	$t_{experimental}$ = 2.91	*$t_{calculated}$ = 0.093	$t_{experimental}$ = 2.91
Fisher test	*$F_{calculated}$ = 3.85	$F_{experimental}$ = 4.45	*$F_{calculated}$ = 4.0	$F_{experimental}$ = 4.45

(*at p=0.05)

Thus, the validation of proposed methods is confirmed statistically by low values of standard deviation, percent coefficient of variation and standard error. Both methods are simple, rapid and precise. They do not suffer from any interference due to common excipients of tablets such as glucose, starch, talc, lactose, sucrose, etc. Therefore, it is evident from the above-mentioned results that both these methods will be useful for routine analysis of citalopram in bulk drug and tablet dosage forms.

CONCLUSION: The present study described the development of simple and rapid chromatography and spectrophotometry method for the accurate determination of citalopram in bulk and tablets. The methods validated are also free from experimental variables such as heating or extraction step.

Furthermore, all the analytical reagents are inexpensive, have excellent shelf life, and are available in any analytical laboratories easily. The method developed on GC gives the further possibility of its detection in biological matrices that too without complicated pre-analytical processing, while the analysis of citalopram by UV is considered the most convenient analytical technique because of its inherent simplicity, low cost, and wide availability in most quality control laboratories.

Both methods are simple, cost effective and do not contain any stringent experimental variables which affect the reliability of the results. Moreover, the statistical analyses of results indicate that the methods yield proper values, as properly designed methods will provide high degree of assurance that every step, process and change has been properly evaluated.

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