Qualitative Determination of Citalopram and Escitalopram in whole human blood using liquid-liquid extraction and GC/GC-MS

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Abstract
Citalopram is an antidepressant drug, generally prescribed for the treatment of major depression. It comes under the SSRI’s class (selective serotonin reuptake inhibitor). It is frequently used off-label to treat anxiety, panic disorder, obsessive compulsive disorder, premenstrual dysphoric disorder.

As per forensic point of view citalopram which is non tricyclic antidepressant is generally prescribed for treating depression because of its undesirable side effects than classical tricyclic antidepressants so they are nowadays more commonly used as the drugs of first choice and therefore the chance of detecting it in the biological materials analyzed in toxicology division is high.

Present study has been structured with an aim to test citalopram, present in blood and the comparison of citalopram and escitalopram which are the enantiomers. For extraction of citalopram from blood, sodium tungstate method was used followed by liquid-liquid extraction.

The alkaline extract of blood obtained from Sodium Tungstate was used for identification by color tests. Citalopram is basic in nature, but it does not respond to commonly used color test for benzodiazepines/alkaloids. Hence various color tests were performed which were reported in literature out of which marquis & mandelin gives positive result. Cobalt thiocyanate has also given positive test for presence of citalopram which can be used in future for testing citalopram.

Further the identification of the drug was carried out by thin layer chromatography by various solvent systems. In search for different solvent systems various combinations of solvents were tried and reproducible results were produced. For the separation of citalopram & Escitalopram solvent system Toluene: Acetonitrile: methanol has given the good Rf values and reproducible.

Results:
Other instrumentation technique, like UV visible spectroscopy, Gas liquid chromatography (GLC), GC-MS was also used for the qualitative analysis.

Keywords: Citalopram, Escitalopram, Extraction, Tricyclic antidepressants.

Introduction
Citalopram is a basic drug, generally prescribed for the treatment of anxiety and major depression. It is also used successfully for hypersexuality in early Alzheimer Disease.

Citalopram its chemical name is (3(Dimethylamino) propyl) 1(4-fluorophenyl) 1,3 dihydroisobenzofuran 5carbonitrile hydrobromide. ITS IS AN optically active molecule. The drug citalopram is a combination of two enantiomeric forms, \((R)\) \((-\) citalopram & \((S)\) \((+)\) citalopram. The former one is pharmacologically inactive and later is pharmacologically active and hence it is responsible for the drug, showing its antidepressant activity later one is known as Escitalopram.

Citalopram along with its Metabolites are racemic compounds \((+)\) enantiomer of citalopram is active & plays role in inhibition of serotonin reuptake & its accounts for 24% to 49% of total plasma citalopram level, but R \((-)\) form do not play any role in inhibition of serotonin.

Common Name: citalopram
Brand Names: Akarin (Denmark, Nycomed)
C Pram S (India)
Celapram (Australia.¹ New Zealand),
Celexa (U.S. and Canada, Forest Laboratories, Inc.)
Celica (Australia)
Ciazil (Australia, New Zealand)
Cilate (South Africa)
Cilift (South Africa)
Cimal (South America, by Roemmers and Recalcine)
Cipralex (South Africa)
Cipram (Turkey, Denmark, H. Lundbeck A/S)
Cipramil (Australia, Brazil, Belgium, Finland, Germany, Netherlands, Iceland, Ireland, Israel, Norway, Sweden, United Kingdom, New Zealand, South Africa, Russia)  
Cipraned, Cipanen (Greece)  
Ciprapine (Ireland)  
Molecular Formula: C₃₉H₃₂BrFN₄O  
Molecular Mass: 405.311 g/mol  
Half Life: 3 hours  
Action and use: used as Antidepressant, antianxiety, in treatment of Insomnia, Panic Disorder.

Symptom of Overdose: Most common symptom following mild overdose include tiredness, confusion, dizziness, stomach pain, sweaing, nausea, sinus tachycardia, tremors & convulsions, withdrawal symptoms: There is anecdotal evidence that citalopram may cause symptom nausea, vomiting, insomnia, somnolence, dizziness, asthenia, headache.

Metabolism: It is metabolized in the liver mostly by CYP2C19 (CYTOCHROME P450 2C19) an enzyme, it is a liver enzyme. Its metabolites are desmethyj citalopram & Didesmethyl citalopram Metabolised in Liver by 2N Demethylation steps.  
Citalopram to Demethylcitalopram (DCT) in presence of CYP2C19 than from DCT it is converted to Didemethal citalopram (DDCT). Oxidation occurs by Monoamine oxidases A & B and aldehyde oxidases, which leads to formation of Propionic acid derivative and citalopram N-oxide,  
Citalopram is at least 4 times more potent than DCT and 13 times more potent than DDCT in inhibiting the serotonin (5HT). Citalopram is a serotonin reuptake inhibitor (SRI), it is a type of drug that act as a reuptake inhibitor of 5-Hydroxy tryptamine (5HT) a neurotransmitter serotonin. This leads to an acute increase in extracellular 5-HT levels in animals following citalopram 10mg/kg twice daily.

Pharmacokinetics: The peak plasma levels occur 2 to 4 hours after single or multiple dose, citalopram absorption is unaffacted by food, the oral bioavailability is 80% approx. & this peak plasma concentration follow daily dose of 40mg is 311nmol/L.  
Elimination: The distribution time occurs for 10 hour and the citalopram follows a biphatic elimination.  
Half life for citalopram -30 to 35 hour  
Half life for DCT- 50 HOUR  
Half life for DDCT- 100 HOUR Upto 23% of dose citalopram is excreted unchanged in urine.

Case Study  
A 54-Year-old woman presented to emergency unit with altered consciousness and under the influence of alcohol, she had a medical history of psychotic depression treated with citalopram 20mg/day and zopiclone 7.5mg/day. Rapid urine toxicology screen was negative for tricyclic antidepressants, benzodiazepines, cocaine or opiates using a HPLC/UV method 1st serum citalopram level was 5.88mg/l (High concentration responsible for unstable clinical situation)  
Activated charcoal was administered 5g & monitoring on citalopram level was started.

Objective  
1. Extraction of citalopram blood  
2. Detection of citalopram in blood by color test & tlc by new solvent system.  
3. Comparison of citalopram and escitalopram

Methods and Materials  
1) Requirement of glassware’s Glasswares  
2) Stopped borosil  
3) Test tubes (10ml)  
4) borosil test tubes 10ml,  
5) borosil beakers (100ml,250 ml)  
6) Glass rod,  
7) Funnel,  
8) Conial Flask (250ml)  
9) Glass drooper,  
10) Glass plates (10* 10)  
11) Solvent chamber  
12) Silica Crucible,  
13) Borosil Measuring cyclinder (10ml,100ml),  
14) spray Bottle (atomizer),  
15) spot plate, capillary tubes etc.

Chemicals  
Acetic acid, ethanol, Methanol, dichloromethane, diethylether, ammonia, sodium tungstate, sulphuric acid, hydrochloric acid, nitric acid, silica gel, Cobalt-thiocyanate, Formaldehyde Bismuth nitrate, potassium iodide, butanol, ethylacetate, Toluene.

Methods  
Preparation of Sample  
Description of Exhibit  
II. Blood Sample  
Description of Tablet (Spiked Drug)  
I. Description about Pack of CITALOPRAM & ESCITALOPARM Tablet  
CITALOPRAM  
Brand name CPRAMS  
Color: Pink

Fig. 2: Cpram S Quantity: 10 TABLETS (10Mg)  
Escitalopram
Brand name: NEXITO 5  
Color: Yellow  
Quantity: 5mg (10 tablets)

**Fig. 3: Spiking Of drug into Exhibit**

I. Drug Spiked into Samples & method of Spiking.  

**Preparation of Standard**  
I. Description of Standard  

**Extraction Method**  
**From Blood:** Sodium Tungstate method  

**Identification Method**  
5.2.4.1 Preliminary Identification  
I. Color test  

5.2.4.2 Confirmatory Identification  
I. Chromatographic techniques  
1) Thin Layer Chromatography (TLC)  
II. Spectroscopic technique  
1) UV Visible spectroscopy  
III. Hyphenated technique  
1) GC  
2) GC-MS  

**Preparation of Sample:** Description of samples  
The following Drug samples “citalopram” & Escitalopram was provided from, Indian pharmacopeia commission.  
CPRAM S 10mg – 10 tablets  
ESCITALOPRAM (5mg) 10 tablets  

**Spiking Of drug into Exhibit**  
Drug Spiked into Samples & method of Spiking: The blood sample was collected from “Jaipur Golden Hospital”. Carefully transferred 10 ml of blood in beaker of 250 ml and 30 mg of citalopram tablet was spiked by after crushing it into powered form.  

**Preparation of Standard:** Preparation of standard of Drugs.  
30 ml of 1000ppm solution of each of the provided drug were prepared by dissolving 30mg of drug in 30 ml of ethanol i.e. 30mg/30ml.

| Standard drugs | Concentration (ppm) |
|----------------|---------------------|
| Citalopram     | 1000                |
| Escitalopram   | 1000                |

**Extraction Method**  
**From Blood:** Sodium Tungstate method  
Liquid-Liquid extraction  

**Identification Method**  

**Preliminary Identification**  
I. Color test  

**Confirmatory Identification**  
1) Thin Layer Chromatography (TLC)  
2) UV Visible spectroscopy  
3) GC-MS  

**Extraction of Drug From Blood**  
**Sodium Tungstate Methods:** The 10ml was taken into a 250 ml beaker. 1 gram of sodium tungstate was added to it. Few drops of sulphuric acid was added The mixture is then heated in boiling water bath for 3 hours. After 3 hours there is complete digestion and lysis of RBC and clear straw color liquid will be seen and lysed cells would be settled down.  
The Mixture is cooled slightly and filtered through filter paper. The filtrate was taken into 250 ml separating funnel.

**Liquid Liquid Extraction**  
To the 250 ml of separating funnel 10-15 ml of filtrate was added and 20-50ml of diethyl ether was added and shaken for 5 minutes and separated, again 50 ml of diethyl ether is added to the acidic layer shaken for 5 minutes and separated. The ether layers are combined.  
The aqueous solution remaining in separating funnel after separation of ether layer was made alkaline by addition of ammonium hydroxide. The aqueous layer was taken into 250 ml of separating funnel and 50 ml of dichlormethane is added & shaken for 5 minutes. The organic layer is separated. The extraction is repeated thrice. The organic layer after separation are combined. The extract was collected and passed through the anhydrous sodium sulphate for absorbing excess moisture and then a clear filtrate was collected in china dish. Now the china dish was putted on water bath, evaporated the organic layer and residue was remain.  
After evaporation of solvent. the residue was dissolved in 10 ml of ethanol & subjected to analysis for confirmation of drug.

![Fig. 3: Spiking Of drug into Exhibit](image1)

**Fig. 4: Liquid –Liquid Extraction**

**Preliminary Examination:** It is a preliminary step in analysis of any compound. It is frequently used to determine the broad category of groups under which our sample can be placed.

**Table 1**

| Standard drugs | Concentration (ppm) |
|----------------|---------------------|
| Citalopram     | 1000                |
| Escitalopram   | 1000                |
Color tests
Marquis Test
Reagent: Dissolve 1 volume of formalin in 9 volume of concentrated sulphuric acid
Procedure
1 ml of blood extract was taken into a spot tile and added 2 drops of marquis reagent and observed the color.
Observation: Yellow color observed
Positive for Citalopram
Mandelin Reagent: Dissolve 0.5 -1 g of ammonium vanadate in 100 ml of concentrated sulphuric acid.
Procedure
1 ml of extract was taken in a spot tile and added 1 drops of mandelin reagent the color was brownish green observed.
Positive for Citalopram
Frohde: Test: Preparation of reagent: 0.5 gm Ammonium Molybdate is dissolved in 100 mL distilled water.
Method: To the dried extract, add the reagent, mixed thoroughly and color was observed.
Observation: No color observed.

Results
Negative for Citalopram
Dragendorff Test: Preparaton of reagent: Dissolve 1 gm Bismuth Subnitrate in 3 mL Conc. HCl and diluted to 20 mL using distilled water. 1 gm Potassium Iodide was then added to above solution.
Method: To the dried extract, add the reagent, mixed thoroughly and color was observed.
Observation: Orange yellow color was observed.

Results: Positive for Citalopram
Ferric chloride Reagent Test
Preparation of Reagent: Dissolve 4.50 g of ferric chloride hexahydrate and 2.5 mL of hydrochloric acid in water, and dilute to 100 mL with water.
Method: To the dried extract, add the reagent, mixed thoroughly and color was observed.

Table 2

| Compound          | Colour Observed          | Reaction time |
|-------------------|--------------------------|---------------|
| Citalopram        | Blue                     | Instantaneous |
| Escitalopram      | Blue                     | Instantaneous |
| Sample            | Blue                     | Instantaneous |

Fig. 5

Cobalt Thiocyanate reacts with amines and gives colour according to the concentration of compound, various amines were tested with the reagent.

Table 3

| S. No | Compound       | Colour Observed                        |
|-------|----------------|-----------------------------------------|
| 1     | Diethylamine   | BLUE Precipitate than solution turn brown |
| 2     | Isopropylamine | Blue green precipitate                  |
| 3     | Dethanolamine  | BLUE Precipitate than solution turn brown |
| 4     | Ethylenepentamine | Blue precipitate than solution turn brown |
| 5     | Ethylenediamine | Blue precipitate than solution turn brown |
| 6     | Tetraethylene pentamine | Blue precipitate than solution turn brown |
| 7     | Diphenylenepentamine | Pink solution (color of reagent) |
| 8     | Triethylamine  | Blue green color                        |
| 9     | Water          | Pink solution ( reagent color)          |
| 10    | Samples        | Blue color                              |
| 11    | Standard       | Blue color                              |
Thin-Layer Chromatographic Technique: Thin layer chromatography or TLC is a chromatography technique used to separate non volatile mixture into its constituents. Thin layer chromatography or TLC, is a solid-liquid form of chromatography where the stationary phase is normally a polar absorbent and the mobile phase could be a single solvent or combination of solvents like binary solvents. Ismailof and Schraiber in 1938 discovered TLC. Technique developed by Meinhard and hall 91949), Kirchner, Miller and Keller (19510,Mattier (1952) and Egon Stahal (1958). TLC is performed on a sheet of glass, plastic, or aluminium foil, which is coated with a thin layer of adsorbent material, mainly silica gel, aluminium oxide cellulose. The layer of adsorbent is known as stationary phase, that’s why it is called as Thin layer chromatography.

Principle: TLC function on the same principle as all chromatography a compound have different affinities for the mobile and stationary phases and this affects the speed at which it migrates. Different compounds in sample of mixtures travel at different rates due to differences in their attraction to the stationary phase, because of differences in solubility in the solvent, by changing the solvent, or by varying the ratios of solvents, the separation of components measured by the Rf value could be adjusted.

Preparation of TLC Plates: TLC plates were prepared by Dissolving silica gel G and distilled water in 1:2. the slurry was poured on glass plates in one motion. plates were allowed to dry at room temperature and then kept in oven at 90°C for 1-2 hour.

Spotting of samples and standard on TLC Plates: The 10µl of standard solution of drugs were spotted on TLC plate along with the extracted blood sample by using micropipette. The spotting was done just above the 2 cm from the base edge of TLC plates.

| Mobile Phase | Standard Rf | Blood Extract Rf |
|--------------|-------------|-----------------|
| Ethylacetate: (13:3:2) butanol: Ammonia | 0.878 | 0.878 |
| Ethylacetate: (60:35:5) methanol: formic acid | 0.658 | 0.647 |
| Methanol: (60:40) Butanol | 0.41 | 0.41 |
| Ethylacetate: (13:7) Butanol: | 0.68 | 0.68 |
| Toluene (3:5:3.5): Acetonitrile: Methanol | 0.57 | 0.694 |
| Ethylacetate: benzene ammonia (7:5:3) | NIL | NIL |

After the complete development, the plate was taken out and allowed to dry it at room temp. Till evaporation of solvent completely. After complete dryness, the plate was visualized under UV at 254nm followed by spraying of following spraying reagent Plate in solvent system (ethylacetate: methanol: ammonia)
Plate In Solvent System- Toluene: Acetonitrile: Methanol.

Visualization of TLC Plates
1) UV- Light Source: Observed the developed TLC plate under UV light source at 254 nm. There is Green background with light color spots.
2) Iodine Fuming Chamber: 10g Sublimed Iodine in a beaker covered with aluminium foil kept for 15 minutes, for saturated the iodine vapours in the chamber after that kept the developed TLC plate on clean & dried Iodine Fuming Chamber. Then covered the chamber with its lid and observed the spots on TLC plates after 5 minutes.

Observation: A dark yellow color spot was observed.
3) Dragen-dorff’s Reagent: 1 g of bismuth subnitrate is dissolved in 3ml of 10m of hydrochloric acid. It is diluted to 20ml. 1 g of potassium iodide is dissolved in it. If black precipitate of bismuth tri-iodide separates, It is dissolved in 2M hydrochloric acid.

Observation – orange color spot observed

6.9g of sodium nitrite was dissolved in 1000ml of distilled water.
After spraying the plates with dragen-dorff’s plates were sprayed with sodium nitrite spray
Observation orange color spots become little intense

For comparison of citalopram & escitalopram: The standard solutions of drugs of citalopram and escitalopram were spotted on TLC plates and run on different combinations of solvents in order to get the good separation of these two enantiomers. After the development of TLC plates which were run upto 10 cm from its base were removed and dry it at room temperature

Table 5

| S.NO | Ratio       | CITALOPRAM | ESCITALOPRAM |
|------|-------------|------------|--------------|
| Ethylacetate: benzene | 6:6 | 1 | 0.88 |
|       | 6:6 | 0.99 | 0.87 |
|       | 6:6 | 0.88 | 0.89 |
|       | 6:6 | 0.996 | 0.88 |
|       | 6:6 | 1 | 0.88 |
|       | 6:6 | 0.99 | 0.99 |
|       | 6:6 | 0.99 | 0.88 |
|       | 6:6 | 0.87 | 0.85 |
|       | 6:6 | 0.75 | 0.89 |
|       | 6:6 | 0.79 | 0.99 |
| Methanol: Butanol | 60:40 | 0.4 | 0.4 |
|       | 60:40 | 0.39 | 0.22 |
|       | 60:40 | 0.55 | 0.41 |
|       | 60:40 | 0.38 | 0.35 |
|       | 60:40 | 0.3 | 0.4 |
|       | 60:40 | 0.41 | 0.42 |
|       | 60:40 | 0.399 | 0.412 |
|       | 60:40 | 0.412 | 0.44 |
|       | 60:40 | 0.41 | 0.39 |
|       | 60:40 | 0.29 | 0.3 |
| Ethylacetate: | 60:35:5 | 0.658 | 0.647 |
| Solvent System | Conditions | Rf Values |
|---------------|------------|-----------|
| methanol: formic acid | | 0.559 0.646 |
| | | 0.549 0.55 |
| | | 0.64 0.66 |
| | | 0.58 0.61 |
| | | 0.66 0.63 |
| | | 0.61 0.63 |
| | | 0.59 0.62 |
| | | 0.57 0.55 |
| | | 0.66 0.63 |
| Ethylacetate: butanol: Ammonia | 13:3:2 | 0.878 0.880 |
| | | 0.75 0.825 |
| | | 0.85 0.75 |
| | | 0.458 0.51 |
| | | 0.5 0.49 |
| | | 0.77 0.85 |
| | | 0.79 0.86 |
| | | 0.83 0.85 |
| | | 0.9 0.825 |
| | | 0.86 0.85 |
| Ethylacetate: Butanol: Ammonia | 75:15:5 | 0.95 0.98 |
| | | 0.94 0.93 |
| | | 0.89 0.9 |
| | | 0.97 0.99 |
| | | 0.91 0.92 |
| | | 0.934 0.99 |
| | | 0.9 0.98 |
| | | 0.97 0.96 |
| | | 0.96 0.95 |
| Ethylacetate: Benzene: Ammonia | 7:5:3 | NIL NIL |
| Ethylacetate: Methanol: Ammonia | 85:10:5 | 0.822 0.696 |
| | | 0.81 0.7 |
| | | 0.82 0.68 |
| | | 0.8 0.699 |
| | | 0.799 0.68 |
| | | 0.833 0.696 |
| | | 0.834 0.71 |
| | | 0.88 0.71 |
| | | 0.810 0.7 |
| Hexane: Benzene :methanol: ethylacetate :ammonia | 2:2:2:3:1 | nil nil |
| Cyclohexane: propanol: ammonia | 5:4:1 | 1 1 |
| Methanol: | 4:2:4 | 0.87 0.87 |
ormic acid : butanol

|          |           |           |
|----------|-----------|-----------|
|          | 0.86      | 0.85      |
|          | 0.87      | 0.86      |
|          | 0.87      | 0.87      |
|          | 0.87      | 0.87      |
|          | 0.85      | 0.84      |
|          | 0.79      | 0.82      |
|          | 0.79      | 0.83      |
|          | 0.86      | 0.86      |
|          | 0.81      | 0.83      |

Toluene: Acetonitrile: Methanol 3:3.5:3.5

|          |           |           |
|----------|-----------|-----------|
|          | 0.517     | 0.694     |
|          | 0.516     | 0.666     |
|          | 0.511     | 0.683     |
|          | 0.516     | 0.611     |
|          | 0.518     | 0.688     |
|          | 0.517     | 0.694     |
|          | 0.516     | 0.688     |
|          | 0.517     | 0.694     |
|          | 0.516     | 0.693     |
|          | 0.544     | 0.699     |

On the basis of above result and Rf it can be seen that good separation is achieved in solvent system toluene: acetonitrile: methanol (3:3.5:3.5) ethylacetate: Butanol: Ammonia(13:3:2)

Table 6

| Mobile phase                  | Run time (Min) | Mean Rf Citalopram | Mean Rf Escitalopram | Standard deviation Citalopram | Standard Deviation Escitalopram |
|-------------------------------|----------------|--------------------|----------------------|-------------------------------|--------------------------------|
| Toluene: Acetonitrile: Methanol | 20 (min)       | 0.5188             | 0.681                | 0.00863                       | 0.02779                         |
| Ethylacetate: Butanol: Ammonia | 25 (min)       | 0.758              | 0.76                 | 3.195                         | 0.1144                          |

On the Basis of above Rf value and reproducible results, mean value, standard deviation, run time, it was find out that the Solvent system toluene: acetonitrile: methanol(3:3.5:3.5) is the better solvent than others for detection of citalopram by TLC and for separating the citalopram and escitalopram by TLC.

**UV-VIS SPECTROSCOPY**

**Basic Principles:** UV-VIS Spectrometry in the UV – VIS REGION is considered to be one of the oldest physical methods used for quantitative analysis and structural elucidation. The UV region extends from 100–400 nm. Further divided into far ultra violet region which is below 200 nm also known as vacuum UV another is near ultraviolet region which is 200-400nm UV- Vis region extends from 400-750 nm. UV-VIS SPECTROPHOTOMETER plots a graph between the wave length versus absorbance.

**LAMBERT’S LAW:** According to this law, the part of incident radiation absorbed by a homogenous medium is independent of the intensity of incident radiation.

**Beer’s Law:** According to this law the amount of incident radiation absorbed by the homogeneous
medium is directly proportional to the concentration of molecules in it...

\[
\log \frac{I_1}{I_2} = A = abc
\]

$I_1$ IS THE INTENSITY OF Incident radiation, $I_2$ is the intensity of radiation transmitted Through the sample solution. $A$ is the absorbance or optical density, $c$ is the concentration of solute (mol/l) and $b$ the path length in cm.

**Sample preparation:** From the standard solutions 1000 ppm of citalopram and escitalopram. 1 ml was taken and it was diluted to 10ml with ethanol. Than 10 ml solution of each citalopram and escitalopram was taken in test tube and covered it with aluminium foil.

1. Firstly, checked the calibration of UV-Visible Spectrometer by indexing it as per the norm of the protocol manual.
2. Then setup the instrument range on absorbance mode with the wavelength.
3. range between 200-400 nm by using Range- mode.
4. After that both the reference solvent was poured on the Quartz Cuvettes and placed it to the sample holder then set-up the Base-line w.r.t. corresponding solvents. [i.e.ethanol]. 
5. After set-up the base-line, scan the prepared standards individually over the range of 200-400 nm and recorded their corresponding spectra.
6. Compared the both of the spectrum w.r.t. their $\lambda_{max}$ & pattern of spectra recorded.

**Qualitative Analysis**

![Fig. 12 Sample - Escitalopram](image)

![Fig. 13 Sample - Citalopram](image)

**Table 5**

| Samples (standard) | Wavelength (nm) | Absorbance |
|-------------------|-----------------|------------|
| Escitalopram      | MAX 238.0       | 0.6941     |
|                   | MAX 203.0       | 1.7118     |
ESCITALOPRAM Shows maximum absorption at wavelengths 203.0, 238.0,284.0 with corresponding absorbance 1.7118, 0.6941,0.0940
CITALOPRAM shows maximum absorption at wavelengths 209.0,238.0,284.0 with corresponding absorbance 2.8765,1.8438,0.2669.

**Gas liquid Chromatography (GLC):** Gas chromatography involves separation of a mixture of volatiles by partitioning between a solid or liquid stationary phase and a gaseous mobile phase.
Gas chromatography-specifically gas liquid chromatography-involves a sample being vapourised and injected onto the head of chromatographic column. The sample is transported through the column by the flow of inert, gaseous mobile phase. The column contains a liquid stationary phase, which is adsorbed onto the surface of an inert solid. On passing through the column volatile components impart different affinities to stationary and mobile phases and separated in fraction.

Gas liquid chromatography the stationary phase consists of solid adsorbent on which liquid material is sorbed.

**Instrumental components**
Carrier Gas. The carrier gas must be chemically inert. Commonly used gases include nitrogen, helium, argon and carbon dioxide. The choice of carrier gas is often dependent upon the type of detector used.

**Sample injection port:** For optimum column efficiency the sample should be not too large, and should be introduced onto the column as a “plug” of vapour. Slow injection of large samples causes band broadening and loss of resolution. The most common injection method is on where a micro syringe is used to inject sample through a rubber septum into a flash vapouriser port at the head of the column. The temperature of the sample port is usually about 50 degrees above the boiling point of the least volatile component of the sample. The injector can be used in two modes split or splitless. Split injection is used for volatile compounds or for diluting the samples, It is used in headspace method to reduce injection time. In forensic toxicology, split injection is largely used for analysis of medications, drugs of abuse and powders.

**Columns:** There are two general types of column, packed and capillary. Packed columns contain a finely divided, inert slid support, material coated with liquid stationary phase. Most packed columns are 1.5-10m in length and have an internal diameter of 2-4mm. Capillary columns have an internal diameter of few tenths of millimeter. These may be WCOT(Wall coated open tubular) or SCOT(support coated open tubular)

**Column Temperature**
This is one of the controlling factors in gas chromatographic separation. For precise work, column temperature must be controlled to within one tenths of a degree. The optimum column temperature is dependent upon the boiling point of the sample.

**Detectors**
There are many detectors, which can be used in gas chromatography. Different detectors will give different types of selectivity. A non-selective detector respond to all compound except the carrier gas, a selective detector responds to a range of compounds with a common physical or chemical property and a specific detector responds to a particular class compounds under the chemical class. detectors can also be grouped into concentration dependent detectors and mass flow dependant detectors.

The effluent from the column is mixed with hydrogen and air, and ignited. Organic compounds burning in the flame produce ions and electrons, which can conduct electricity through the flame. A large electrical potential is applied at at he burner tip and a collector electrode is located is located above flame. The current resulting from the pyrolysis of any organic compounds is measured, FID is mass sensitive rather than concentration sensitive, which gives the advantage that changes in mobile phase flow rate do not affect the detector’s response. FID is a useful general detector for the analysis of organic compounds.

**Details of instrument**
Instrument- Gas Liquid Chromatography (GLC) 
Manufacturer- Thermofisher 
Column- 2.5% SE 30 on 80 100 mesh Chromosorb G 
Detector- FID 
Column Temperature-100˚ C-300’
Carrier gas nitrogen 45 ml/min 
Detector Temperature – 290˚
Sample Preparation
The extracted Blood samples and Standard of citalopram were filtered through the what mann filter paper and the injection of 1µl is used for injecting the samples into the injector.

|          | MAX 284.0 | 0.0940 |
|----------|-----------|--------|
| Citalopram| MAX 209.0 | 2.8765 |
|          | MAX 238.0 | 1.8438 |
|          | MAX 284.0 | 0.2669 |

Graphs 1: Gas Chromatogram Of Standard Of citalopram
Graph 2: Gas chromatogram of blood extract

Table 7: Tabular representation of gas chromatogram of standard citalopram

Table 8: Tabular representation of gas chromatogram of blood extract
Gas liquid chromatography shows comparable retention time for standard of citalopram as well as for the Blood extract which is at 21.020 & 21.330. This further confirms that the extracted drug was citalopram.

**Gas Chromatography – Mass Spectroscopy**

It is one of the hyphenated technique combining two technique wherein GC that separates chemical mixtures and a very sensitive detector (MS) with a data collector (the computer component) which identify the mass ions which separate. The two instruments are joined by interface.

Once the sample solution is introduced into the GC inlet it is vaporized immediately because of high temperature (250) and swept onto the column by the carrier gas and the components are separated which pass through the MS for identification of mass ions. Thus separation and identification cum quantification are achieved simultaneously.

**Sample preparation:** Instrument type- GC-MS

Model No.- 6890 N Network GC System & 5975 insert XL

Mass –Selective Detector

Manufacturer- Agilent Technologies

U.I - FSL / Delhi/ UI/ Chem/ 07

Year of Purchase- 2007

Detector - MS-FID

Software used- MSD CHEM Station

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**Table 9**

| Samples           | Retention time observed for citalopram (min) | Comparable Peaks obtained at (min) | Area (Mv. S) |
|-------------------|--------------------------------------------|----------------------------------|-------------|
| Standard citalopram | 21.020                                     | 1.7609, 3.33, 17.680, 15.110, 24.457, 26.240, 30.333 | 39234.396   |
| Blood extract     | 21.330                                     | 1.763, 9.313, 17.530, 23.153, 24.635, 18.787 | 6103.034    |

**Operating Condition of GC-MS for citalopram Drug in sample exhibits**

1. Model: Agilent 6890 N Network GC system & 5975 insert XL Mass Selective Detector
2. Column: SLB-5MS (30 m x 0.25 mm)
3. Detector: MSD / FID
4. Mode: Split mode Column
5. Oven Temp: 150-280OC
6. Detector Temp: 300 OC
7. Carrier Gas: N2
8. Hydrogen gas: 40 ml/min

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Fig. 10
Graph of Citalopram Standard

![Graph of Citalopram Standard](image1)

Graph of Escitalopram Standard

![Graph of Escitalopram Standard](image2)
Table 6

| Samples             | Retention Time (MIN) | Other Peaks Observed at (MIN) | Compound Present              | Quantity Matches with standard Library % | Area % |
|---------------------|----------------------|-----------------------------|-------------------------------|-----------------------------------------|--------|
| Citalopram (sample A) | 15.292               | 15.291,15.82                | Escitalopram                 | 97                                      | 94     |
|                     |                      |                             | Citalopram                   | 94                                      | 53     |
|                     |                      |                             | Dothiepin                    | 53                                      |        |
| Escitalopram (Sample B) | 15.263               | 4.769,5.356,5.783, 6.862,14.656, 15.824 | Escitalopram                 | 97                                      | 94     |
|                     |                      |                             | Citalopram                   | 94                                      | 59     |
|                     |                      |                             | Dothiepin                    | 59                                      |        |

On the basis of GC-MS graph it can be seen that both the enantiomers, citalopram, & escitalopram shows same mass spectra which are very much similar they shows similar retention time 15.292 and 15.263. Both of them contain three compounds escitalopram, citalopram, dothiepin with the matching probability of above 95% with the GC-MS library.

Results and Discussion

1. **Color Test:** Various color test were performed for the citalopram, but only marquis, mandelin, dragen-dorff, gave the positive result for citalopram.

a. Apart from that cobalt thiocyanate also gave positive result for citalopram. This suggest that this can be used for the analysis of citalopram because there is no specific test mention in literature for
citalopram however this test is also given positive by cocaine and some benzodizepenes also.

b. Cobalt thiocyanate reacts with tertiary amines present in the drug and various amines were tested (result was summarized in table no) with cobalt thiocyanate and all gave positive results depending on their concentration.

2. TLC Analysis: Various solvent system were tried in order to find new solvent system for the detection of citalopram. New solvent systems were also tried for separating the two enantiomers citalopram and escitalopram, the solvent system which shows good separation of these enantiomers were considered their Rf was calculated and then their mean and standard deviation were calculated. And on the basis of above calculations only these two solvent systems gave optimum results if we consider the criteria like size of spot, reproducibility, less standard deviation and acceptable Rf and also a good separation between citalopram & escitalopram.

Toluene: acetonitrile: Methanol (3:3.5:3.5)
Ethylacetate: butanol: Ammonia (13:3:2)

For the analysis of citalopram in biological fluid or any other matrix these solvent systems can be used. Rf value of extracted blood sample matches with the standard citalopram in these solvent system which confirms the presence of citalopram in blood extract.

UV-VIS Spectrophotometer: On comparing the wavelength (maxima) and absorbance of citalopram with the escitalopram it is observed that citalopram show maximum absorbance 2.876 at 209.0 and escitalopram shows maximum absorbance 1.7118 at 203.0.

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### Table 7

| Sample       | Wavelength(nm) | Absorbance |
|--------------|----------------|------------|
| Escitalopram | MAX 203.0      | 1.7118     |
|              | MAX 238.0      | 0.6941     |
|              | MAX 284.0      | 0.0940     |
| Citalopram   | MAX 209.0      | 2.8765     |
|              | MAX 238.0      | 1.8438     |
|              | MAX 284.0      | 0.2669     |

1. **Gas Liquid Chromatography(GLC):** On basis of retention time and comparable peaks it can be confirmed that the recovered drug from the blood extract was citalopram.

2. **Gas Chromatography-Mass Spectroscopy:** Samples of citalopram and escitalopram were analyzed by GC-MS. Both the samples have shown comparable retention time 15.292 min and 15.693 min for citalopram and escitalopram, Both (CPRAM S & NEXITO) or (citalopram and escitalopram) having combination of escitalopram, citalopram, and dothiepin. Sample B Escitalopram has shown some extra peaks which were not present in sample A. From the above it can be stated that for the comparison of citalopram and escitalopram GC-MS technique is not very much helpful because both the enantiomers were having same molecular formula and that can be the reason for having similar retention time.

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