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Gas Chromatographic and Spectrophotometric Determination of Diclofenac Sodium, Ibuprofen and Mefenamic Acid in Urine and Blood samples

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ABSTRACT

Objectives: Non-steroidal anti-inflammatory drugs (NSAIDs) are widely used for the treatment of acute to chronic pain relief. A simple, fast and reliable Gas Chromatographic (GC) method with flame ionization detection (FID) has been developed for the determination of NSAIDs such as diclofenac sodium, ibuprofen and mefenamic acid after derivatization with ethyl chloroformate (ECF).

Materials and Methods: The GC conditions were optimized as elution from column DB-1 (30 m x 0.32 mm id) at column temperature 150°C for 3 min, followed by heating rate 20°C/min up to 280°C for 5 min. Nitrogen flow rate was 2.5 min. For spectrophotometric studies, the absorbance was measured against methanol within wavelength of 200-500 nm.

Results: The calibration curves were linear within 2-10 µg/mL with limits of detection 0.4-0.6 µg/mL of each drug. The derivatization elution, separation and quantitation was repeatable (n=3) with relative standard deviation within 3.9%. The method was applied for the analysis of the drugs from pharmaceutical formulations and the results of analysis agreed with labeled values with RSDs within 0.5-3.9 %. The results were also confirmed by standard addition method. The percent recovery was calculated with spiked deproteinized human blood serum and urine samples and % recovery of the drugs was obtained within 96-98% with RSDs within 3.1%.
**Conclusion:** The validated method proved its ability for the assay of NSAIDs in bulk and dosage form in a short analysis time. The method was also useful for the analysis of biological samples.

Key words: (NSAIDs) drugs, GC analysis, derivatization of NSAIDs drugs

**INTRODUCTION**

Drugs such as diclofenac sodium, ibuprofen and mefenamic acid are commonly used pharmaceuticals in all over the world\(^1\). These drugs belong to the class of non-steroidal anti-inflammatory drugs (NSAIDs) which are used for the treatment of antipyretic, analgesic and anti-inflammatory purposes\(^1\)\(^-\)\(^2\). These types of drugs are generally used as pain killers. People use NSAIDs without any prescription of doctor because of less possibility of side effects. These types of drugs are also very effective on human body for people with arthritis or rheumatic diseases. The women's in pregnant conditions may use NSAIDs by the prescription of their physicians. NSAIDs can cause several side effects if used for longer time such as gastrointestinal bleeding, intestinal ulceration, aplastic anemia, agranulocytosis, kidney injury and cardiovascular risks\(^3\). Diclofenac sodium \([2-(2-6-dichlorophenyl) aminophenyl benzoic acid]\), Ibuprofen \([R S-2(4-(2-methylpropylphenyl) propanic acid] and Mefenamic acid \([2-(2, 3-dimethylphenyl) aminophenylbenzoic acid]\) are non-steroidal anti-inflammatory drugs (NSAIDs) and are commonly used as antipyretic, analgesic and anti-inflammatory purposes. These are also used for the treatment of rheumatic disorders, pain and fever\(^4\)\(^-\)\(^5\).

![Ibuprofen](image1.png)

![Diclofenac sodium](image2.png)

![Mefenamic acid](image3.png)

Ibuprofen  
**Diclofenac sodium**  
**Mefenamic acid**
Many methods have been reported for the determination of diclofenac sodium, ibuprofen and mefenamic acid individually and in mixtures. Diclofenac salts have been determined by spectrophotometer, spectrofluorometry, thin layer high performance liquid chromatography, and gas chromatography in wide variety of samples. Similarly, mefenamic acid has been analyzed by spectrophotometry, spectrofluorometry, electrophoresis and chromatography. In pharmaceutical preparation and biological fluids for the simultaneous determination of NSAIDs chromatographic (GC, HPLC) procedures are used. GC is easy to operate with high resolution efficiency and does not involve the problem of used solvents. The acidic compounds diclofenac, mefenamic and ibuprofen are difficult to elute from GC column and require derivatization before their analysis. GC derivatization can be defined as the procedure used to modify analyte functionality to make it enable for GC separation. Derivatization enables extremely polar substance to become quite volatile so that it can be easily eluted on sensible temperatures. The compounds which attached functional groups for instance -SH, -OH, -NH and –COOH have importance for GC analysis because these groups have tendency of forming hydrogen bonding. These hydrogen bond formations have effects on the volatility, thermal stability and interaction of analyte with column packing material.

The present work examines less expensive ethyl chloroformate (ECF) as derivatizing reagent for GC-FID determination of diclofenac sodium, ibuprofen and mefenamic acid for an aqueous medium from pharmaceutical preparation and biological fluids. In this study, we have developed new GC-FID method which is easy, cheap, fast selective and reliable for the determination of NSAIDs drugs. Furthermore, the spectrophotometric studies have also been performed to optimize all the parameters with and without derivatization for all the non-steroidal anti-inflammatory drugs. The developed method shows better LOD and LOQ with good regression coefficient and applied on real blood and urine samples.

MATERIAL AND METHODS

Chemical and Reagents

All the chemicals used were GR or AR. Ethyl chloroformate (ECF) (Fluka, Buchs, Switzerland), methanol, Chloroform, acetonitrile (Fluka, Buchs, Switzerland), and pyridine (E-Merck, Darmstadt, Germany) were used. Pure standard of Ibuprofen was obtained from Abbott
Laboratories, Karachi, Pakistan and Mefenamic acid from Pfizer Laboratories Karachi, Pakistan as a gift for research. Diclofenac sodium was obtained from Sigma Aldrich (St. Louis, Mo, USA). Ammonium chloride, potassium chloride, boric acid, sodium tetraborate, sodium carbonate, sodium bicarbonate, acetic acetate, ammonium acetate, ammonia solution and hydrochloric acid (37%) for the preparation of buffer solutions were from E-Merck, Darmstadt, Germany.

Buffer solutions (0.1M) from pH 1-12 with 0.5 unit interval were prepared from the following: Potassium chloride adjusted with hydrochloric acid (pH1-2), acetic acid – sodium acetate (pH 3-6), ammonium acetate (pH-7), boric acid- sodium tetra borate (pH7.5-8.5), ammonium chloride-ammonia (pH-10) and potassium chloride- potassium hydroxide (pH 11-12). The standard solutions of ibuprofen, diclofenac sodium and mefenamic acid containing 1mg/mL each were prepared separately in methanol-water (1:1v/v). Further solutions were prepared by appropriate dilutions.

**Instrumentations**

All pH measurements were made with Orion star pH meter (Orion Research Inc. Boston, USA). The spectrophotometric studies were carried out on Hitachi 220 double beam spectrophotometric (Hitachi (Pvt) Ltd, Tokyo, Japan) with dual Quartz cuvettes. Gas chromatographic studies were carried out on an Agilent 6890 model gas chromatograph (Agilent Technologies, CA, USA) connected with the flame ionization detection (FID), split injector, hydrogen generator H2-90 (Parken Hannifer, Haverhill, USA) pure nitrogen (British oxygen company (BOC), Karachi, Pakistan and computer with Chemostation software. Capillary column DB-1(30m x 0.32mm id) with film thickness 0.25 μm (J.W scientific GC column, Willington, NC, USA) was used throughout the study.

**Spectrophotometric procedure without derivatization**

For spectrophotometric study, the stock solutions containing 1mg/mL were prepared in methanol in 25 mL volumetric flask. The solutions were appropriately diluted to 10 μg/mL for ibuprofen, mefenamic acid and of diclofenac sodium separately in 10 mL volumetric flask. The solutions were well mixed, and the absorbance was measured against methanol on spectrophotometer.
Hitachi 220 within wavelength of 200-400 nm. The maximum response was obtained at 240, 260 and 300 nm for ibuprofen, mefenamic acid and diclofenac sodium respectively.

**Spectrophotometric procedure with derivatization**

The spectrophotometric study of the stock solutions containing 1mg/mL were prepared for ibuprofen, mefenamic acid and diclofenac sodium separately and were further diluted to 10 µg/mL in methanol. The solution of 1 mL of 100 µg/mL of each ibuprofen, mefenamic acid and diclofenac sodium was separately transferred to 10 mL volumetric flask and were added (0.5 mL) solvent system of pyridine-methanol-acetonitrile-water (8:42:8:42v/v) and (0.5 mL) buffer of solution pH 9 and (0.4 mL) ECF and sonicated the contents for 20 min at 30˚C room temperature and methanol was added up to the mark in 10 mL volumetric flask, the solutions were mixed and the absorbance was measured against the reagent blank on spectrophotometer within wavelength of 200-400 nm. The maximum responses were obtained at 350, 310 and 350 nm for ibuprofen, diclofenac sodium and mefenamic acid respectively. The reagent blank was prepared with 1mL methanol and following same procedure without addition of the analyte.

**Gas chromatographic procedure**

The solution (0.2-1.0 mL) containing 10 µg/mL of ibuprofen, mefenamic acid and diclofenac sodium separately or in mixture were transferred to well stoppord test tubes. The solution was added methanol-water-acetonitrile-pyridine (42:42:8:8v/v) (0.5 mL) solvent, carbonate buffer solution of pH-9 (0.5 mL), ECF (0.4 mL) and contents were sonicated at room temperature (30˚C) for 15 min. Chloroform 0.5 mL was added and contents were mixed well. The layers allowed separating. The calculated volume of 0.5 mL from organic layer was pipette out in screw capped sample vial. The solution (1µL) was injected to GC at initial column temperature 150˚C for 3.0 min with heating rate 20˚C/min up to 280˚C. The nitrogen flow rate was 2.5mL/min rates for FID were fixed hydrogen 40 mL/min, nitrogen as makeup gas 40 mL/min and air 250 mL/min.

**Analysis of pharmaceutical preparation**

Five tablets of each pharmaceutical formulation; Brufen (Abbott Laboratories, Karachi) containing 200 mg/tablet ibuprofen, Ponstan (Pfizer Laboratories, Karachi) containing 25
mg/tablet mefenamic acid and Qufen (High G. International, Karachi) containing 20 mg/tablet diclofenac sodium were ground to fine powder separately. The powder corresponding to 1 tablet was dissolved in methanol-water (1:1v/v). The solution was filtered, and volume was adjusted to 50 mL. The solution 0.2 mL and 0.4 mL after appropriate dilution was analyzed following GC analytical procedure. The quantitation was made from external calibration curve using linear regression equation \( y = ax + b \).

**Analysis of pharmaceutical preparation by standard addition**

Five tablets of each of the pharmaceutical formulation; Brufen, Ponstan and Qufen were processed as the analysis of pharmaceutical preparations. After appropriate dilution, two solutions of 0.2 mL and 0.4 mL from each of the pharmaceutical preparation were taken in duplicate. A solution was added 0.5 mL of standard drug solution containing 10µg/mL. All the solutions were processed as GC Analytical procedure. The quantitation was done from linear regression equation and from the increase in response (peak height/peak area) with added standard.

**Analysis of biological samples**

The blood and urine samples were collected from the healthy volunteers (students and employees) of Institute of Advanced Research Studies in Chemical Sciences, University of Sindh. The blood samples were collected by vein puncture in EDTA tubes and urine samples were collected in clean plastic bottles. The samples were processed as received. The volunteers were informed the objective of the work and they gave verbal permission to collect their samples.

Each (5 mL) sample was placed at room temperature (30°C) for 30 min and was centrifuged at 4000 rpm for 20 min. The supernatant layer was collected and was added 5 mL of methanol. The contents were mixed well and again centrifuged for 20 min at 4000rpm. The two solutions of supernatant (1mL each) were taken and a solution was added 0.5 mL of standard solution of ibuprofen, mefenamic acid or Diclofenac sodium containing 10µg/mL. The solutions were processed as GC analytical procedure. The quantitation was made from linear regression
equation of external calibration curve. The solution without addition of the standard was treated as blank.

RESULTS AND DISCUSSION

**Spectrophotometric Study without and with Derivatization of Ibuprofen**

The solution of ibuprofen standard was examined on double beam spectrophotometer within the range of 200-500 nm. The absorbance was measured at an interval of 5 nm. The absorption spectra were obtained with and without derivatization which indicates the maximum absorbance at 260nm for ibuprofen without derivatization when measured against blank (methanol), while the absorption spectra after derivatization was obtained which indicates maximum absorbance at 350 nm for ibuprofen (Figure 1. (a and b)).

![Figure 1](image)

**Figure 1** The absorption spectrum (a) shows Ibuprofen without derivatization within concentration range of 10µg/mL in methanol. The absorbance was measured within 200-500 nm (b) after derivatization ECF at concentration 10µg/mL in methanol.

**Spectrophotometric Study without and with Derivatization of Mefenamic Acid**

The mefenamic acid in methanol standards was examined on double beam spectrophotometer within the range of 200-500 nm. The absorption spectrum was obtained which indicated
maximum absorbance at 300 nm for mefenamic acid without derivatization when recorded against reagent blank (methanol) Figure 2 (a). The solution of mefenamic acid standard was also examined after derivatization with ECF within the range of 200-500 nm. The solution of mefenamic acid indicated maximum absorbance at 350 nm and was recorded against as reagent blank Figure 2 (b).

**Figure 2** The absorption spectrum (a) shows Mefenamic acid without derivatization with concentration 10 µg/mL in methanol. The spectrum (b) is absorption spectrum of Mefenamic acid after derivatization with ECF concentration 10 µg/mL in methanol.

**Spectrometric Study without and with Derivatization of Diclofenac Sodium**

The diclofenac sodium solution in methanol was examined on double beam spectrophotometer. The absorption spectra was obtained which indicated maximum absorbance at 240 nm for diclofenac sodium when recorded against as blank (methanol) in Figure 3 (a), while the absorbance measured after derivatization with ECF at 310 nm in Figure 3 (b).
Figure 3 The absorption spectrum (a) of Diclofenac sodium without derivatization within concentration range 10µg/mL in methanol. Spectrum (b) is after derivatization with ECF at concentration 10µg/mL in methanol.

**Effect of pH**

Effect of pH on the derivatization of ibuprofen, mefenamic acid and diclofenac sodium has been analyzed within pH range of 1-10. The reactions were monitored on spectrophotometer against reagent blank at appropriate pH. The absorbance was measured at the wavelength of maximum absorbance at 350 nm for ibuprofen and mefenamic acid, while diclofenac sodium shows absorbance at 310 nm. The maximum absorbances were observed at pH 9 for ibuprofen, mefenamic acid and diclofenac sodium shown in Figure 4.
Figure 4. Effect of pH on the absorbance of ibuprofen, mefenamic acid and diclofenac sodium derivative of ECF on spectrophotometer at 350 and 310 nm

**Effect of ECF on derivatization**

Effect of change in concentration of derivatization reagent ECF was examined between (0.1-0.5 mL at an interval of 0.1 mL) following the analytical procedure. The absorbance was measured at the wavelength of maximum absorbance at 350 nm for ibuprofen and mefenamic acid, while diclofenac sodium shows absorbance at 310 nm. The effect of concentration was not critical and similar response was obtained from 0.2 to 0.5 mL, but for quick response 0.4 mL was selected. The solvent system acetonitrile-water-pyridine-methanol (8:42:8:42v/v) (0.5mL) and carbonate buffer solution pH 9 (0.5mL) were added during derivatization mentioned in Figure 5.
Effect of sonication time on derivatization

The effect of sonication time on the derivatization of ibuprofen, mefenamic acid and diclofenac sodium with ECF were examined within (5 to 25 min at an interval of 5 min) on the absorbance of the analyte shown in Figure 6. Following the analytical procedure, the absorbance was measured at 350 nm for ibuprofen and mefenamic acid while the diclofenac sodium at 310 nm. The sonication time was critical and same response was obtained from 5 to 30 min, but to get reproducible results, sonication time of 20 min was selected. The solvent system acetonitrile-water-pyridine-methanol (8:42:8:42 v/v) of (0.5mL) and carbonate buffer solution pH 9 (0.5mL) were added.
Figure 6. Effects of sonication time on the derivatization of ibuprofen, mefenamic acid and diclofenac sodium

**Spectrophotometric calibration of ibuprofen with derivatization**

The standard solution of different concentration of ibuprofen was measured for the absorbance at 350 nm. Linear calibration curves were obtained which obeyed beers law with in concentration range 20 to 160 µg/mL of ibuprofen. The coefficient of determination \(R^2\) of ibuprofen was obtained at 0.999. The molar absorptivity calculated for Ibuprofen at 350 nm was 43733 Lmol-cm\(^{-1}\). The linear regression equation was obtained for Ibuprofen \(y=0.007x+0.152\).

**Spectrophotometric calibration of mefenamic acid with derivatization**

The standard solution of different concentration of mefenamic acid was measured for the absorbance at 350 nm and linear calibration curves was obtained which obeys beers law with in concentration range 10 to 200 µg/mL of mefenamic acid. The coefficient of determination \(R^2\) of mefenamic acid was obtained at 0.998. The molar absorptivity calculated for mefenamic acid at 350nm was 51152 Lmol-cm\(^{-1}\).The linear regression equation was obtained for Ibuprofen \(y=0.020x+0.076\).

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Spectrophotometric calibration of diclofenac sodium with derivatization

The standard solution of different concentration of diclofenac sodium was measured for the absorbance at 310nm and linear calibration curves was obtained which obeys beers law with in concentration range 20 to 160µg/mL of diclofenac sodium. The coefficient of determination (r²) of diclofenac sodium was obtained at 0.999. The molar absorptivity calculated for diclofenac sodium at 310nm was 67443.348 Lmol·cm⁻¹. The linear regression equation was obtained for Ibuprofen y=0.007x+0.134.

Quantitation of non-steroidal anti-inflammatory drugs by gas chromatography

Non-steroidal anti-inflammatory drugs in GC elution occur after derivatization with ethyl chloroformate. Initially GC conditions were optimized for the elution as symmetrical peaks from the column DB-1(30 m×0.32 mm id). Different temperature programs were examined nitrogen flow rates and reasonable peak shapes were observed at initial column temperature at 150°C for 3.0 minutes, and with heating rate 20°C/min up to 280°C for 5.0 min with total run time 13.2 min and flow rate of nitrogen was adjusted 2.5 mL/min.

The solution (0.2-1.0 mL) of 10 µg/mL of stock solution were taken of each ibuprofen, mefenamic acid and diclofenac sodium and added 0.5 mL of acetonitrile-water-pyridine-methanol with the ratio of (8:42:8:42v/v/v/v), 0.5mL of sodium carbonate buffer solution (pH 9) and 0.4mL of ECF. Sonicated the contents for 20 min at 30°C and 0.5mL of chloroform was added. The mixture was shaken well, and separatory funnel was used to separate the layers. A part of the extract (0.5 mL from 1 mL) was transferred to vial with screw cap and 1µL of the solution was injected to GC.

Quantitation of ibuprofen after derivatization

The linear calibration curve of ibuprofen was obtained by plotting concentration (µg/mL) of ibuprofen against peak height and was obtained within 2-10µg/mL with coefficient of determination (r²=0.996). The linear regression equation was y=0.980x+1.789. The LOD and LOQ were calculated as 3:1 and 10:1 signal to noise ratio with 0.6 µg/mL and 1.8µg/mL respectively.
Quantitation of mefenamic acid after derivatization

The linear calibration curve of mefenamic acid was obtained by plotting concentration (µg/mL) against peak height and was obtained within 2-10 µg/mL with coefficient of determination ($R^2=0.998$). The linear regression equation was $y=0.230x-0.174$. The LOD and LOQ were calculated as 3:1 and 10:1 signal to noise ratio with 0.4 µg/mL and 1.2 µg/mL respectively.

Quantitation of diclofenac sodium after derivatization

The linear calibration curve of diclofenac sodium was obtained by plotting concentration (µg/mL) against peak height and was obtained within 2-10 µg/mL with coefficient of determination ($R^2=0.999$). The linear regression equation was $y=1.070x-1.301$. The LOD and LOQ were calculated as 3:1 and 10:1 signal to noise ratio with 0.5 µg/mL and 1.5 µg/mL respectively.

Table 1. Quantitation results of ibuprofen, mefenamic acid and diclofenac sodium by using Ethyl Chloroformate as derivatizing reagent.

| Drug name          | Limits of Detection (LOD) µg/mL | Limits of Quantitation (LOQ) µg/mL | Calibration Range µg/mL | Co-efficient of determination ($R^2$) | Linear Regression equation |
|--------------------|---------------------------------|-----------------------------------|--------------------------|---------------------------------------|-----------------------------|
| Ibuprofen          | 0.6                             | 1.8                               | 2-10                     | 0.9965                                | $y=0.9809x+1.7892$          |
| Mefenamic Acid     | 0.4                             | 1.2                               | 2-10                     | 0.998                                 | $y=0.2303x-0.1743$          |
| Diclofenac Sodium  | 0.5                             | 1.5                               | 2-10                     | 0.999                                 | $y=1.0705x-1.3018$          |

GC analysis of ibuprofen

Gas chromatography of ibuprofen after derivatization with ECF has been performed. The column of Gas chromatography was carried from column DB-1 (30 m×0.32 mm id). Temperatures were set in different scales and examined flow rate of nitrogen was and
reasonable peak shapes were observed at temperature of column initial set at 150°C for 3 minutes, heating rate followed by 20°C/min up to 280°C and hold for 5 min with whole run time 13.2 min. The flow rate of nitrogen was adjusted 2.5 mL/min.

**GC analysis of mefenamic acid**

Gas chromatography of mefenamic acid after derivatization with EC has been performed. The column used was DB-1(30m×0.32 mm id). Temperatures were set and reasonable peak shapes were observed at temperature of column initial set at 150°C for 3 minutes, heating rate 20°C/min up to 280°C for 5 minutes with whole run time 13.2 minutes. The flow rate of nitrogen was adjusted 2.5 mL/min.

**GC analysis of diclofenac sodium**

Gas Chromatography of diclofenac sodium after derivatization with ethylchloroformate has been performed. The column used DB-1(30m×0.32 mm id). Temperatures were set and reasonable peak shapes were observed at temperature of column initial set at 150°C for 3 minutes, heating rate followed by 20°C/min up to 280°C and hold time for 5 min with whole run time 13.2 min. The flow rate of nitrogen was adjusted 2.5 mL/min.

**GC separation of ibuprofen, mefenamic acid and diclofenac sodium**

Gas Chromatographic separation of ibuprofen, mefenamic acid and diclofenac sodium was carried out after derivatization with ECF. Initially GC conditions were optimized for the elution as symmetrical peaks from the column DB-1(30 m×0.32 mmid). Temperature is set in different scales and examined flow rate of nitrogen and reasonable peak shapes were observed at initial column temperature at 150°C for 3.0 minutes, heating rate followed by 20°C/min up to 280°C for 5.0 min with entire run time 13.2 min. The flow rate of nitrogen was adjusted 2.5 mL/min (Figure 7).
Figure 7. Gas chromatographic separation peaks of (1) Ibuprofen, (2) Mefenamic acid and (3) Diclofenac sodium after derivatization with Ethyl chloroformate from column DB-1 (30m x 0.32mm id).

**Drug analysis of ibuprofen, mefenamic acid and diclofenac sodium with derivatization**

The method developed for the determination of ibuprofen, mefenamic acid and diclofenac sodium after derivatization was applied for the analysis of the active ingredients in Tablets Brufen (ibuprofen), Ponstan (mefenamic acid) and Qufen (diclofenac sodium). At least 5 tablets containing ibuprofen 200mg/tablet, mefenamic acid 250 mg/tablet and diclofenac sodium 20mg/tablet were ground to fine powder and dissolved in appropriate amount in methanol. Then the solution was filtered and volume adjusted of 50 mL and an aliquot of solution after derivatization with ECF was injected on the GC column DB-1 (30mx 0.32mm id) and eluted with mobile phase optimized for GC separation and detection of ibuprofen, mefenamic acid and diclofenac sodium. The quantitation was made from linear regression equation and amount of ibuprofen, mefenamic acid and diclofenac sodium was found to be 156.93 mg/mL, 193.14 mg/mL and 19.48 mg/mL which agreed with amount labeled as 160 mg/mL, 200 mg/mL and 20 mg/mL. The % error was calculated as -1.9, -3.4 and -2.6%. The RSD calculated replicated were analysis (n=3) were within 0.5-3.0%. The percentage recovery of ibuprofen, mefenamic acid and diclofenac sodium was 98%, 96% and 97% respectively.
Table 2. Drug analysis of ibuprofen and mefenamic acid and diclofenac sodium with derivatization

| S. No | Name of standard | Name of tablet | Amount added in the mg/mL | Amount found in the mg/mL | %Error | RSD% | % Recovery |
|-------|------------------|---------------|---------------------------|---------------------------|---------|------|------------|
| 1     | Ibuprofen        | Brufen        | 200mg/mL                  | 157.96mg/mL              | -1.9%   | 0.5% | 98%        |
| 2     | Mefenamic Acid   | Ponstan       | 250mg/mL                  | 193.14mg/mL              | -3.4%   | 3.6% | 96%        |
| 3     | Diclofenac Sodium | Qufen        | 20mg/mL                   | 19.48mg/mL               | -2.6%   | 3.9% | 97%        |

Determination of ibuprofen, mefenamic acid and diclofenac sodium in spiked blood samples

The blood and urine samples 5mL each were collected from healthy volunteers with condition that about a week back they have not taken any medicine. The samples were centrifuged to remove blood cells and precipitate. The blood serum and urine were deprotonized with methanol. The samples (1mL) were spiked with ibuprofen, mefenamic acid and diclofenac sodium within the calibration range of each compound. The derivatization procedure with ECF was followed. The chloroform extract (1µl) was then injected on the GC equipped with column DB-1 (30m x 0.32mm id). The elution was carried out at optimized conditions (Figure 8-13). The quantitation was made from linear regression equation and all results are summarized in Table 3 and 4.
Table 3. GC Analytical result of Ibuprofen, Mefenamic acid and Diclofenac sodium blood samples using Ethylchloroformate

| S.NO | Age/gender | Name of standard | Amount µg/mL added | Amount found µg/mL | %Error | %Recovery |
|------|------------|------------------|--------------------|--------------------|--------|-----------|
| 1    | 25/F       | Ibuprofen        | 50µg/mL            | 49.04µg/mL         | -1.95  | 98%       |
| 2    | 25/F       | Mefenamic acid   | 40µg/mL            | 39.66µg/mL         | -0.80  | 99%       |
| 3    | 25/F       | Diclofenac sodium| 50µg/mL            | 49.0µg/mL          | -0.80  | 99%       |

**CHROMATOGRAM OF IBUPROFEN BLOOD SAMPLE**

![Gas chromatographic elution of ibuprofen in blood sample from column db-1 (30m x 0.32mm id).](image)

**Fig.8.** Gas chromatographic elution of ibuprofen in blood sample from column db-1 (30m x 0.32mm id).
**CHOMATOGRAM OF MEFENAMIC ACID BLOOD SAMPLE**

**Figure 9.** Gas Chromatogram of mefenamic acid in blood sample from column db-1 (30m x 0.32mm id).

**CHOMATOGRAM OF DICLOFENAC SODIUM BLOOD SAMPLE**

**Figure 10.** Gas chromatogram of diclofenac sodium in blood sample from column db-1 (30m x 0.32mm id).
Table 4. GC analytical result of ibuprofen, mefenamic acid and diclofenac sodium from urine samples using ethyl chloroformate

| S.NO | Age/gender | Name of standard | Amount µg/mL added | Amount found µg/mL | %Error | %Recovery |
|------|------------|------------------|--------------------|--------------------|--------|-----------|
| 1    | 25/F       | Ibuprofen        | 60 µg/mL           | 59.14µg/mL         | 1.45   | 98%       |
| 2    | 25/F       | Mefenamic acid   | 50 µg/mL           | 48.36µg/mL         | -2.9   | 98%       |
| 3    | 25/F       | Diclofenac sodium| 40 µg/mL           | 38.83µg/mL         | -3.0   | 97%       |

Figure 11. Gas Chromatogram of mefenamic acid in urine sample from column db-1 (30m x 0.32mm id).
Figure 12. Gas Chromatogram of mefenamic acid in urine sample from column db-1 (30m x 0.32mm id).

Figure 13. Gas Chromatogram of diclofenac sodium in urine sample from column db-1 (30m x 0.32mm id).

**GC determination of ibuprofen with standard addition**

The five tablets of Brufen (ibuprofen) containing 200 mg were processed as above procedure. The solution 0.2 mL was taken in duplicate. A solution was added in 0.2 mL of
ibuprofen containing 14.16 µg and both of the solutions were processed as GC procedure. The quantitation was carried from linear regression equation of external calibration curve and increase in response with added standard (Table 5).

**GC determination of mefenamic acid with standard addition**

The five tablet of Ponstan (mefenamic acid) containing 250 mg were processed as above procedure. The solution 0.2 mL was taken in duplicate. A solution was added in 0.2 mL of ibuprofen containing 17.76 µg and both of the solutions were processed as GC procedure. The quantitation was carried from linear regression equation of external calibration curve and increase in response with added standard (Table 5).

**GC determination of diclofenac sodium with standard addition**

The five tablets of Qufen (diclofenac sodium) containing 20 mg were processed as above procedure. The solutions 0.2 mL was taken in duplicate. A solution was added in 0.2 mL of ibuprofen containing 17.76 µg and both of the solutions were processed as GC procedure. The quantitation was carried from linear regression equation of external calibration curve and increase in response with added standard (Table 5).

Table 5. GC Standard addition of Ibuprofen, Mefenamic Acid and Diclofenac sodium

| S. NO. | Name of standard | Name of tablet | Amount added in µg/mL | Amount found in µg/mL | % Recovery | % Error |
|--------|-----------------|---------------|-----------------------|-----------------------|------------|--------|
| 1      | Ibuprofen       | Brufen        | 14.16µg/mL            | 13.73µg/mL            | 96%        | -3.1%  |
| 2      | Mefenamic Acid  | Ponstan       | 17.76µg/mL            | 17.27µg/mL            | 97%        | -2.8%  |
| 3      | Diclofenac Sodium | Qufen        | 17.76µg/mL            | 17.33µg/mL            | 97%        | -2.4%  |

**Intra day and inter day studies**

Inter and intra day assay was performed by analyzing replicate injections of standard solution to study the repeatability of the method. The intra assay precision (n=5) was performed by the same
analyst on the same day and under the same conditions, with the interval of 2 hours, whereas the inter assay precision (n=3) was performed by the same analyst and under the same conditions, on three consecutive days. The reproducibility of the separation was examined for all analytes in terms of peak area and % RSD values of each are shown in Table 6.

| Table 6. Intra-day study and Inter-day study of NSAIDs Drugs |
|-------------------------------------------------------------|
| **Concentration 10 (µg/mL)**                               |
| **Ibuprofen** | Peak area | %RSD | Peak area | %RSD |
| 1             | 0.01644   | 0.33772 | 0.015473 | 0.362192 |
| 2             | 0.0399    | 0.1564   | 0.02957 | 0.224109 |
| 3             | 0.0544    | 0.35924  | 0.047067 | 0.39546  |
| **Mefenamic acid** |         |        |          |        |
| 1             | 0.01511   | 0.21487  | 0.01511 | 0.214868 |
| 2             | 0.09147   | 0.03365  | 0.09147 | 0.033647 |
| 3             | 0.08417   | 0.05524  | 0.084167 | 0.055236 |
| **Diclofenac sodium** |       |        |          |        |
| 1             | 0.02186   | 0.57062  | 0.019605 | 0.60538 |
| 2             | 0.01432   | 0.13947  | 0.013183 | 0.14222 |
| 3             | 0.08573   | 0.07509  | 0.075233 | 0.01291 |

CONCLUSION

A new analytical procedure has been developed for the Gas Chromatographic and spectrophotometric determination of ibuprofen, mefenamic acid and diclofenac sodium after derivatization with ECF. The LOD and LOQ of ibuprofen was calculated as 3:1 and 10:1 signal to noise ratio as 0.6 µg/mL and 1.8µg/mL, for mefenamic acid as 0.4 µg/mL and 1.2 µg/mL and for diclofenac sodium 0.5 µg/mL and 1.5µg/mL respectively. The method is repeatable and has been successfully applied for the analysis of pharmaceutical preparation and spiked deproteinized serum and urine samples.
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