Procaine as a New Coupling Agent for the Spectrophotometric Determination of Fibrates by Diazotization Coupling Reaction

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Abstract

A sensitive, selective, rapid, simple and accurate of the spectrophotometric determination of fibrates in bulk and in dosage forms. This method depend on diazotization of primary amine group of procaine hydrochloric acid with sodium nitrate and hydrochloric acid followed by coupling with fibrates in aqueous mildly acidic medium to form azo dye stable orange – yellow. Showed obeyed beers law between 0.2- 14 ppm, with molar absorptivity 3.01*10^4L.mole^-1.cm^-1 at 385nm. Sandells sensitivity 0.006mg.cm-1, limit of detection (LOD) 0.018ppm and limit of quantitication (LOQ) 0.058 ppm. This method has been successfully applied to determination of fibrates in bulk and in pharmaceutical preparation, tablet with a good recoveries 99.63-100.25 % 

Keywords: Procaine hydrochloric acid, Fibrates, Diazotization-coupling, Spectrophotometry.

Introduction

Fibrates I are a class of amphipathic carbonylic acid. Its used for a range of metabolic disorders, accessory therapy in many forms of the hypercholesterolemia when combination with statin[1]. Fibrates reduce the number of the non fatal heart attacked, but not improve all cause mortality therefore indicated only in those not in tolerant to statins[2-3].

Side effects occure when Fibrates cause increase in risk of idiosyncratic destruction of muscle tissue, rhabdomyolysis and leading to renal failure because combination with statin. Drug toxicity includes acute kidney injury[4-5].
Fibrates used since 1930 s[6], but the mechanism of action discovered in 1990 s that Fibrates activate preoxisome proliferator activated receptors (PRAR), Fibrates area substrate of metabolized by CYP3A4[7].

Fibrates have been shown to extend lifespan in the roundworm C elegans[8].

Various method used of determination of Fibrates such as chromatography[9], spectrophotometric[10], volumetry[11] and polarography[12].

The aim of this research to develop simple, selective and sensitive spectrophotometric method based on diazotization – coupling reaction with procaine to determination of Fibrates in pure and pharmaceutical preparation.

2- Experimental

2.1 Instruments: All absorbance and spectral was carried by double – beam Uv-Visible spectrophotometer (EMC 11 and T 80 Germany) with quartz cell of 1cm path length.

2.2 Reagents: All chemicals were grade purity. Standard referencie procaine and fibrates were obtained from (SAFA pharmaceutical Industries Co, Iraq). Pharmaceutical preparation containing fibrates obtained from the commercial market.

2.3 Solutions: All aqueous solution were prepred by distill water .100 ppm of fibrates was prepared by dissolving 0.01g of fibrates then complete to the mark by distill water.

Working standard solutions prepared freshly by diluting the stock solution by distill water to obtain the appropriate concentration. Sodium nitrate (BDH) 0.01M. hydrochloric acid (BDH) 1M. procaine 100 ppm (Sigma-Aldrich) prepared by dissolved 0.01g in 100 mL distill water.
2.4 Procedure and calibration curve: Transfer volumes of procaine solution, covering the range 0.2-15 ppm, into series of 10 mL volumetric flask. Add 0.5 mL of 1M HCl and mixtures are shaken. Then 1.5 mL of 0.01M NaNO2 solution added and the mixture lives about 2 minutes. After that 1.5 mL of 0.2 M sulphamic acid solution was added and the mixture allowed for 2 min. After that 3 mL of 0.01M fibrates were added, then completed to the mark with distilled water. After 10 minutes measure the absorbance against reagent blank.

2.5 Procedure for dosage forms: Take 0.01g from the fenofibrates and dissolved in 20 mL distilled water then transferred to the 100 mL volumetric flask, shaken and completed to the mark by distilled water.

3 Results and discussion

3.1 Choosing of coupling reagent:

Several coupling reagent such as procaine, metochlobramide and thiozon, were used in this study the useful analytical results were obtained with procaine. This reagent give a stable water azo dye with fibrates. Therefore this reagent was selected and optimum condition of this reaction with fibrates was further studied.

3.2 Spectral Characteristics: Absorption spectrum of yellow-Orange azo product with maximum absorption at 385 nm shown in Fig(1)

![Figure](1): A: Absorptions spectra of fibrates. B: Absorptions spectra of fibrates with procain.
3.3 optimization of reaction conditions

The effect of the various parameters on absorption intensity of the azo were studied and the condition were optimized.

3.3.1 Effect of acid: Different volumes (0.05 – 4 mL of 1M) of different acids have been examined. 0.5 mL of 1M HCl gives a good results as shown in table (1)

Table(1) effect of different acids on absorbance

| Acids 1M | Absorbance | Volume of acid(mL) |
|---------|------------|-------------------|
|         |            | 0.5 | 1 | 2 | 3 | 4 | 5 |
| HCl     |            | 0.931 | 0.986 | 0.887 | 0.832 | 0.743 | 0.742 |
| HNO₃    |            | 0.721 | 0.711 | 0.691 | 0.662 | 0.661 | 0.662 |
| H₂SO₄   |            | 0.792 | 0.681 | 0.671 | 0.692 | 0.711 | 0.721 |
| CH₃COOH |            | 0.314 | 0.492 | 0.481 | 0.392 | 0.342 | 0.373 |
| CH₂O    |            | 0.521 | 0.511 | 0.421 | 0.451 | 0.411 | 0.502 |

3.3.2 Effect of Sodium Nitrate Concentration and Time: The NaNO₂ effect of concentration was study by using different volumes (0.1 – 3.5 mL) of 0.01 M NaNO₂ solution. 1.5 mL of NaNO₂ and 3 minutes gives a maximum absorbance shown in table (2).

Table(2) Effect of nitrate concentration

| Volume of 0.01 M NaNO₂ mL | Absorbance |
|---------------------------|------------|
| 0.5                       | 0.531      |
| 1.0                       | 0.732      |
| 1.5                       | 0.982      |
| 2.0                       | 0.981      |
| 2.5                       | 0.982      |
| 3.0                       | 0.982      |
| 3.5                       | 0.982      |
3.3.3 Effect of Sulfamic Acid Concentration and Time: In order to remove the excesses of nitrous acid, used different volumes (0.1 – 5.0 mL) of 0.2 M sulfamic acid solution, the maximum absorbance were with 1 mL of sulfamic acid and 2 minute shown in table (3) therefore used 1 mL of sulfamic acid in this study.

Table (3) effect of sulfamic acid concentration

| Volume of sulfamic acid in mL | Absorbance |
|------------------------------|------------|
| 0.5                          | 0.621      |
| 1.0                          | 0.941      |
| 2.0                          | 0.921      |
| 3.0                          | 0.901      |
| 4.0                          | 0.901      |
| 5.0                          | 0.901      |

3.3.4 Effect of Reagent Concentration: Table (4) shows different volumes (1.0 – 4 ml) of 0.01 M procaine used to testing on reagent concentration, the results showed that 2.5 mL of reagent is sufficient to produce the maximum colourintensity.

Table (4) Effect of reagent concentrations

| Volume of reagent 0.01 M mL | Absorbance |
|-----------------------------|------------|
| 1.0                         | 0.631      |
| 1.5                         | 0.741      |
| 2.0                         | 0.902      |
| 2.5                         | 0.935      |
| 3.0                         | 0.935      |
| 4.0                         | 0.935      |
| 4.5                         | 0.935      |

3.3.5 Effect of Time: Table (5) shows different times which are used to investigate the formation of the azo product, the maximum absorption obtained after 5 minute
### Table (5) Effect of time on absorbance

| Time (min) | Absorbance |
|-----------|------------|
| 1.0       | 0.521      |
| 5.0       | 0.932      |
| 10.0      | 0.932      |
| 20.0      | 0.932      |
| 30.0      | 0.931      |
| 40.0      | 0.932      |
| 50.0      | 0.932      |
| 3 days    | 0.931      |

#### 3.3.6 Effect of Temperature:

Table (6) shows different temperatures (15 – 80 °C) used to investigate that the temperature between (15 – 30 °C) give the maximum absorbance. At higher temperature the absorbance value decreased, which is probably due to the dissociation of the product.

### Table (6) Effect of temperature on absorbance

| Temperature °C | Absorbance |
|----------------|------------|
| 15.0           | 0.931      |
| 20.0           | 0.934      |
| 30.0           | 0.932      |
| 40.0           | 0.893      |
| 50.0           | 0.885      |
| 60.0           | 0.712      |

#### 3.4 Calibration Curve and Sensitivity

Under optimum conditions above, standard calibration curve of the fibrates – procaine azo product showed in Fig(2).
curve for fibrates which is determined using procaine as coupling reagent.

Various parameters of analytical performance of the proposed method in are shown in table (7).

**Table (7) Analytical feature of the procedure developed to the determine of the fibrates.**

| Parameter                        | Proposed Method           |
|----------------------------------|----------------------------|
| Regression equation             | $Y = 0.084X - 0.001$       |
| Slope                           | 0.997                      |
| Correlation coefficient          | $R^2 = 0.997$              |
| Linear range (ppm)              | 0.2 – 14                   |
| Molar absorptivity(L.mol$^{-1}$.cm$^{-1}$)[13] | $3.01 \times 10^4$        |
| Limit of detection(LOD)(ppm)[14] | 0.018                      |
| Limit of quantitative (LQD)(ppm)[14] | 0.057                      |
| Sandells sensitivity, S (µg.cm$^{-2}$)[13] | 0.011                      |
| Recovery(%)[14]                 | 99.63                      |

3.5 **Nature and Stability Constant of the Product:** Stoichiometric ratios were determined by Jobs method[15] shown in Fig(3). The results showed a 1:1 fibrates and procaine. The formation of product occur as in scheme 1. The Stability constants of the product is found to be $3.24 \times 10^8$. 
M⁻¹ according to the equation cited[16] table (8). This result refer to stable products are formed between fibrates and procaine.

![Figure(3) Jobs method](image_url)

Figure(3) Jobs method
Scheme 1 : Proposed mechanism of the reaction between fibrates and procaine.

Table (8) Stability constant of the product

| Drug      | Conc. of drug M | Absorbance with quantitative conc. (Aₐ) | Absorbance with increasing in conc. of reagent (Aₘ) | α   | Kst. L.mole⁻¹ |
|-----------|----------------|----------------------------------------|---------------------------------------------------|-----|---------------|
| Fibrates  | 1.0×10⁻⁴       | 0.470                                  | 0.478                                             | 0.008 | 1.55×10¹²     |
3.6 Interference Study: In the beginning study we must determine which excipients found in the fibrates drugs, the study done by taking 10 ppm of fibrates with excess amount of excipients then measuring the absorbance. An error of the 5% in the absorbance readings was considered tolerable, none of these excipients interfered seriously.

Table (9) Interference effect on Fibrates

| Interference    | (10 ppm) Fibrates | Conc. | E%  | Rec% |
|-----------------|-------------------|-------|-----|------|
| Tween 80        |                   | 10.14 | +1.4| 101.4|
| PVP             |                   | 10.04 | +0.4| 100.4|
| Acacia          |                   | 10.18 | +1.8| 98.2 |
| NaCl            |                   | 9.97  | -0.3| 99.7 |
| Mennitol        |                   | 10.03 | +0.3| 100.3|
| Talc            |                   | 9.96  | -0.4| 99.6 |
| Benzoic acid    |                   | 10.14 | +1.4| 98.6 |
| Lactose         |                   | 10.12 | +1.2| 98.8 |
| Sucrose         |                   | 10.19 | +1.9| 98.1 |

3.7 Pharmaceutical Applications

The proposed method were applied to analysis for three different dosage forms contain fibrates in order to evaluate the analytical usefulness of the spectrophotometric method. Good results with good recoveries and reproducibility were obtained when determined three different concentration of each pharmaceutical preparation tablet, therefore the proposed method successfully to the analysis.

Table (10): Application on Fibrates in Fenofibrates tablet

| Fenofibrate tablet | Concentration | E%  | Rec% |
|--------------------|---------------|-----|------|
|                    | Prepared ppm  | Measured ppm |       |
| 5                  | 5             | 5.16 | +3.2 | 96.8 |
| 7                  | 7             | 7.13 | +1.8 | 101.8|
| 10                 | 10            | 9.91 | -0.9 | 99.1 |
4 Conclusion

The proposed method is found to be rapid, simple, selective and highly sensitive than most of spectrophotometric methods available in the literature, the recovery study data indicate the reproducibility and accuracy of method. This method can be adopted as excellent spectrophotometric method.

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