Comparison of Bulk and Precipitation Polymerization Method of Synthesis Molecular Imprinted Solid Phase Extraction for Atenolol using Methacrylic Acid

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ABSTRACT
Objective: Atenolol is one of beta-blocker are prohibited as doping based on World Anti-Doping Agency (WADA). The purpose of this study was to the synthesis of molecular imprinted polymer (MIP) for extraction of atenolol from the sample. Method: This research compared the two of the method, bulk and precipitation polymerization. The MIP was successfully prepared from methacrylic acid as a functional monomer, ethylene glycol di-methacrylate as a crosslinker, benzoyl peroxide as an initiator, butanol as a porogen solvent with atenolol as a template molecule. Result: The result showed that the bulk polymerization method produces sorbents that have good adsorption capacity and small particle compare to the precipitation polymerization. Both methods were selective for atenolol. Conclusion: Generally, the MIP solid phase extraction is an alternative method for extraction atenolol from the sample.

Key words: Atenolol, Molecular Imprinted Polymer, Solid Phase Extraction.

INTRODUCTION
Doping refers to prohibited drug, substance or material that used by athlete to improve their performance. Various types of doping are used for reduce anxiety, increase muscle mass, reduce weight or to cover another drug during health check. Doping can cause harmful effect for human include myocardial infarction, hyperlipidemia, hypertension, thrombosis, heart failure and sudden death. Atenolol is one of beta-blocker group that usually used as doping by athlete to reduce anxiety, tremor and low heart rate. Doping analysis can be determined through metabolite or specimens examination. It requires sensitive instruments with pure samples and completely separated from the matrices. Numerous analytical method are used to determine atenolol such as High Performance Liquid Chromatography (HPLC). Gas Chromatography-Mass Spectrometry (GC-MS) and diffuse reflectance spectroscopy. Recently, solid phase extraction (SPE) based on molecular imprinting polymer (MIP) has been developed as a separation technique is expected to be low cost, practical and applicable and has a high recovery percentage. Sorbents with molecular imprinting techniques have a recognizable binding sites that can bind with specific drug targets, thereby being able to separate drugs with complex matrices. Preparation of molecular imprinted polymer consist of monomer, crosslinker, initiator and porogen. Monomer must be able to interact with the template form a specific complex donor-receptor in polymerization. Methacrylic acid is an universal monomer that usually used in MIP. This monomer increase imprinting effect through dimerization reaction. Synthesis of MIP-SPE atenolol based on non-covalent bonding using methacrylic acid result the good sorbent with acetonitril or mix acetonitril as a porogen. Porogen that usually used in non-covalent bonding MIP is a solvent that has low dielectric constanta, tend to non-polar solvent, because polar solvent can interfere the hydrogen form. In this research, MIP-SPE atenolol was synthesized using methacrylic acid as functional monomer and butanol as a porogen by bulk and precipitation polymerization method.

MATERIALS AND METHODS
Materials
All of material used is analytical grade. Atenolol, metoprolol tartrate hydrochloride and propanolol hydrochloride were obtained from Tokyo Chemical Industry. Methacrylic acid and ethylene glycol dimethacrylate (EGDMA) were purchased from Sigma Aldrich. Acetone, alcohol 95% and acetic acid 96% were purchased from Brataco. Acetoniirle and methanol were obtained from Fischer Scientific. Butanol, benzoyl peroxide and potassium bromide were purchased from Merck. The absorbance measurement was recorded by UV-visible spectrophotometer (Analytical Jena Specord 200 using a 1.0 cm quartz cell). Identification of functional group was analyzed by Fourier Transform Infrared (FTIR) IR (Prestige-21 Shimadzu).

Methods
Determination of the Association Constant of Monomer-Template Complex using UV Titration Method
Determination of the association constant can describes the interaction of monomer and template. Stock solution of atenolol in butanol was prepared in 2 x 10⁻⁵ M and methacrylic acid was 5 x 10⁻³ M. Atenolol solution was measured by UV-visible spectrophotometer then methacrylic acid was added gradually until the absorbance tend to stable. The association constant was calculated by Benesi-Hildebrand equation.

\[
\frac{1}{\Delta Y} = \frac{1}{Y\Lambda_{HG}} Ka[G] + \frac{1}{Y\Lambda_{HG}}
\]

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$$\Delta Y = \text{Absorbance complex} - \text{Absorbance of } H;$$

$$K_a = \text{Association constant}; \quad [G] = \text{Concentration of guest (monomer)}.$$

## Synthesis of Molecular Imprinted Polymer by Precipitation Polymerization Method

Methacrylic acid (4 mmol) as a monomer was added into atenolol solution in butanol (1 mmol) as a template in 350 ml solvent. The mixture was sonicated for 5 mins in a closed vial. Then, 20 mmol EGDMA as crosslinker was added and continued to sonicate for 20 mins. 1 mmol benzoyl peroxide was added into solution as initiator, sonicated for 5 mins, then oven at 70°C for 2 hrs. The solution moves to water bath shaker at 70°C for 18 hrs. Subsequently, the solution was centrifuged and the precipitation was washed by methanol and water. The polymer was dried in oven at 60°C for 18 hrs. To verify the MIP results, the Non Imprinted Polymer (NIP) was also synthesized using this steps but without template.

## Synthesis of Molecular Imprinted Polymer by Bulk Polymerization Method

Atenolol was dissolved in butanol (1 mmol) in closed vial then 4 mmol methacrylic acid was added and sonicated for 5 mins. EGDMA (20 mmol) as cross linker was added and continued to sonicate for 20 min. Then, benzoyl peroxide (1 mmol) as an initiator was added to the solution. The solution was moved to oven at 70°C for 2 hrs and then to the water bath shaker at 70°C for 18 hrs. The polymer was mashed and filtered using a mesh size of 60. Afterward, the polymer was rinsed with methanol and water then dried in oven at 70°C for 18 hrs. The Non Imprinted Polymer (NIP) was also synthesized using this steps but without template.

## Adsorption Capability Evaluation

Evaluation of adsorption capability was carried out in methanol, acetonitrile, acetonitrile: methanol (1: 1) and acetonitrile: methanol (1: 9). Sorbent of MIP (20 mg) was dissolved in atenolol solution of 5 ppm (in different solvent) and allowed to stand for 24 hrs. Filtrate from the mixture was measured by UV-Vis Spectrometry. The adsorption capability was calculated by the difference between the initial atenolol concentration and the free atenolol concentration in the filtrate. The NIP sorbent was also evaluated by the same procedure.

## Adsorption Capacity Evaluation

Evaluation of adsorption capacity was carried out by varying the concentration of atenolol solution of 1, 2.5, 5, 7.5 and 10 ppm. A 5 ml atenolol solution from each concentration was added into 20 mg of MIP sorbent. The mixture was shake and allowed to stand for 24 hrs. The filtrate was measured by UV-Vis spectrometry. NIP sorbent was also evaluated by the same procedure. The adsorption capacity was calculated by using Freundlich isotherm adsorption curve.\(^{12-13}\)

## MIP Selectivity Evaluation

Evaluation of MIP selectivity was determined by calculating the coefficient of distribution of atenolol, metoprolol and propranolol solution at 5 ppm. A 5 ml of each solution was added into 20 mg of MIP sorbent. The mixture was shake and allowed to stand for 24 hrs. The filtrate was measured by UV-Vis spectrometry. NIP sorbent was also evaluated by the same procedure. The distribution coefficient was calculated by the following equation:\(^{14}\)

$$K_d = \frac{C_p}{C_s}$$

where $$K_d$$ is distribution coefficient, $$C_p$$ is concentration of substrate in polymer (mol/g) and $$C_s$$ is concentration of substrate in solution (mol/g). The ratio of $$K_d$$ of MIP and $$K_d$$ of NIP was calculated as imprinting factor value.\(^{12}\)

## RESULT

### Determination of the Association Constant of Monomer-Template Complex using UV Titration Method

Interaction of monomer-template can be analyzed by determination of the association constant. Association constant was calculated based on slope and intercept on Bennesi-Hildebrand equation. Based on Figure 1, $$K_a$$ of atenolol and methacrylic acid was 9.24 x 10\(^{-1}\) M\(^{-1}\).

### Comparison of Physical Characterization of MIP by Precipitation and Bulk Polymerization Method

Physical characterization of MIP was analyzed by using FTIR and SEM. FTIR to describe the functional group on the compound and SEM (Scanning Electron Microscope) to describe the morphology of the polymer. Table 1 and Table 2 show the FTIR analysis of MIP and NIP sorbent. The SEM analysis show in Figure 2 and Figure 3.

### Adsorption Capability Evaluation

The results of adsorption capability of the MIP sorbent are shown in Figure 4 for the bulk polymerization and Figure 5 for precipitation polymerization.

![Figure 1](image)

**Figure 1:** Graph of association constant of atenolol and methacrylic acid.

| Table 1: FTIR analysis of MIP and NIP sorbent by bulk polymerization. |
|---|---|---|---|
| Sorbent of MIP before extraction | Sorbent of MIP after extraction | Sorbent of NIP | Functional Group |
| 3580.91 | 3563.55 | 3594.41 | -OH stretching |
| 3461.32 | - | - | N-H stretching |
| 2347.71 | 2974.29 | 2974.29 | C-H stretching |
| 1735.00 | 1734.04 | 1733.07 | C=O stretching |
| 1635.00 | 1633.74 | 1634.70 | C=C stretching |
| 1407.85 | 1466.89 | 1467.86 | C=O bending |
| 1100.20 | 1160.20 | 1162.13 | C-O stretching |
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Adsorption Capacity Evaluation

The adsorption capacity was calculated by using Freundlich isotherm adsorption curve. The result show in Table 3.

MIP Selectivity Evaluation

Evaluation of MIP selectivity was determined by calculating the coefficient of distribution and imprinting factor. Table 4 show the selectivity of MIP and NIP Sorbent by bulk polymerization and Table 5 show the selectivity of MIP and NIP Sorbent by precipitation polymerization.

DISCUSSION

Interaction of methacrylic acid as a monomer and atenolol as a template can be analyzed by determination of the association constant. Generally, the better and stronger interactions that occur, the better of the imprinting effect and the more stable of the complex during polymerization. Therefore, the interaction of monomer-template must be tested by non-covalent imprinting stoichiometry study. In this study, the interaction was determined based on association constant value (Ka). If the Ka is around 10^3 M^{-1}, the complex is stable and the binding site has a good performance with recovery value more than 90%.

Based on Figure 1,
Table 5: Selectivity of MIP and NIP Sorbent by precipitation polymerization (n=3).

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Atenolol</th>
<th>Propranolol</th>
<th>Metoprolol</th>
</tr>
</thead>
<tbody>
<tr>
<td>KD</td>
<td>MIP</td>
<td>Propranolol</td>
<td>Metoprolol</td>
</tr>
<tr>
<td>KD</td>
<td>325.4±3.1</td>
<td>44.51±0.4</td>
<td>207.6±2.2</td>
</tr>
<tr>
<td>NIP</td>
<td>78.2±2.1</td>
<td>68.79±0.5</td>
<td>191.3±0.3</td>
</tr>
<tr>
<td>Imprinting Factor</td>
<td>4.16±0.2</td>
<td>0.65±0.3</td>
<td>1.09±0.2</td>
</tr>
</tbody>
</table>

In the bulk polymerization, MIP has the m value close to 1 means that MIP is more homogenous than NIP. The value of a describes the adsorbent capacity in absorb the analyte. In bulk polymerization, MIP has able to absorb up to 7.804 mg/g compare to the NIP. It is indicates the binding site of the sorbent is complement with the shape and size of the template. However, this value is still relatively small because the amount of analyte that can be absorbed is small, so it takes a large amount of sorbent to absorb more analytes. In the precipitation polymerization, MIP less homogenous than the NIP because NIP has the m value close to 1. Besides that, MIP has the less adsorption capacity compare to the NIP. It can be conclude that adsorption capacity of MIP from bulk polymerization is better than from precipitation polymerization.

The selectivity of MIP was carried out by using metoprolol and propranolol, as a same beta blocker group with atenolol. The selectivity was determined by calculating distribution coefficient distribution that describes the number of analytes absorbed to the concentration of analytes in solution. In the bulk and precipitation polymerization, KD of atenolol is higher than others. It indicates that MIP sorbent is selective for atenolol. MIP was synthesized using atenolol as a template so the cavity of the MIP was formed the cavity for the atenolol. Imprinting factor is also calculated to see the ratio between MIP and NIP. It is describes the performance of MIP. Both of method has higher imprinting factor of atenolol compare to the others and precipitation polymerization has the higher imprinting factor of atenolol than bulk polymerization. It is indicates that selectivity of MIP form precipitation method is better than bulk method, however both of method has the good performance compare to NIP.

**CONCLUSION**

The sorbent of MIP can be synthesized by using methacrylic acid as a monomer, butanol as a porogen and EGDMA as a crosslinker with ratio 1:4:20. The sorbent of MIP can be synthesized by using both of method, bulk and precipitation polymerization method. The result show that polymer from precipitation polymerization have a surface with relatively larger particles compare to the bulk polymerization. The sorbent from bulk polymerization has the higher adsorption capacity (7.804 mg/g) compare to the precipitation polymerization method (2.95 mg/g). Both of method are selective for atenolol but MIP form precipitation method is more selective than bulk method.

**ACKNOWLEDGEMENT**

Financial support from the Ministry of Research and Higher National Education of Indonesia through Penelitian Terapan Unggulan Perguruan Tinggi (PTUPT) research scheme in 2017-2018 are greatly acknowledged.

**CONFLICT OF INTEREST**

The authors declare no conflict of interest.

**ABBREVIATIONS**


**REFERENCES**


