NSAID Analysis Using Chromatographic and Spectrophotometric Methods

Vina Awallina Diroh,*a Rio Gusraya Unaldi a, Mutiara Wayu Puspasari a and Muhammad Muzhil Aslam a

a Department of Pharmacy, Faculty of Health Sciences, Universitas Alma Ata Bantul, 55183, Yogyakarta, Indonesia
*vinaawallina@gmail.com

The purpose of this review article to evaluate the content of Nonsteroidal anti-inflammatory drugs (NSAID) using chromatographic and spectrophotometric methods. This journal review uses seven analytical methods on NSAID, namely high performance liquid chromatography (HPLC), fourier transform infrared (FTIR), gas chromatography (GC), thin layer chromatography (TLC), and spectrophotometry methods. The outcomes of every method have been elaborated in this paper and all of them can be used to detect the NSAID compounds.

Keywords: NSAID, lemon, yogyakarta

Introduction

Non-steroidal anti-inflammatory drugs or known as NSAIDs are used worldwide for their analgesic, anti-inflammatory, and antipyretic effects. NSAIDs (Figure 1) are one of the most commonly prescribed drug classes worldwide and account for approximately 5-10% of all drugs prescribed each year [1-4]. The main therapy on musculoskeletal patients is the use of non-steroidal anti-inflammatory drugs (NSAIDs) either as monotherapy or in combination with drugs of the same class or pain relievers from other groups. The use of more than one drugs have potentially caused drug-drug interactions that can affect to patient [5-8].

Pharmacodynamic effects of NSAIDs according to several reports by many researchers [9-13] aspirin-like drugs are antipyretic, analgesic and anti-inflammatory. In contrast to paracetamol (acetaminophen) which is a weak anti-inflammatory. The following are the pharmacodynamic effects of NSAIDs such as:

a) Analgesic effect. Only effective for treating pain of low to moderate intensity, such as headache, myalgia, arthrgia and other pain originating from the integument (skin), especially pain related to inflammation.

b) Antipyretic effect. Only used to lower body temperature or only used when fever.

c) Anti-inflammatory effect. Most of the new drugs are used as a treatment for musco-lethal disorders, such as rheumatoid arthritis, osteoarthritis and ankylosing spondylitis.

d) Side effects of NSAIDs. A number of side effects of NSAIDs occur on gastric-intestinal, kidney and platelet function. The frequency of side effects varies for each drug and the amount of dose given and the duration of its use except for its effect on platelets [14-19].

Analytical Methods of NSAIDs

Methods applied

The application of various instruments have presented in Table 1.

1. HPLC

Methods: IBP determination was carried out using an HPLC instrument supplied with a C18 column (250 mm × 4 mm; 4.6 µm particle size) and a UV detector at 214 nm. The mobile phase was composed of a mixture of water adjusted to pH 2.5 with phosphoric
acid and acetonitrile (40/60, v/v). To examine the method, a formulation containing IBP was prepared and compressed, and the amount of IBP adhered to punch faces was determined [19-21].

2. FTIR

FTIR method have been applied to identify the functional groups of various compounds of NSAID. Pure performance inflow test analysis (PITA) and the physical mixture of PITA with co-processed excipients were subjected to FT-IR analysis using the potassium bromide (KBr) pellet method. A mortar and pestle were used to grind 3 mg of sample and 300 mg of KBr. A small amount of the mixture was placed under a hydraulic press and compressed at 10 kg/cm, forming a transparent pellet. The pellet was kept in the sample holder and scanned from 4000 to 500 cm⁻¹ in FT-IR [22-24].

3. GC

There several steps to analyse NSAID using GC, where the solution of chemical compounds (0.2 – 1.0 ml) containing 10 µg/ml ibuprofen mefenamic acid, and diclofenac sodium separately or in mixture were transferred to well stoppered test tubes. To the solution were added methanol: water: acetonitrile: pyridine (42:42:8:8 v/v) (0.5 mL) solvent, carbonate buffer solution of pH 9 (0.5 mL), and ECF (0.4 mL) and the contents were sonicated at room temperature (30 °C) for 15 min. Chloroform (0.5 mL) was added and the contents were mixed well. The layers were allowed to separate. The calculated volume of 0.5 mL from the organic layer was pipetted out into a screw capped sample vial. The solution (1 μL) was injected into the GC instrument at an initial column temperature of 150 °C for 3.0 min with a heating rate of 20 °C/min up to 280 °C. The nitrogen flow rate was 2.5 mL/ min, while the rates for FID were fixed hydrogen 40 mL/min, nitrogen as makeup gas 40 mL/min, and air 250 mL/min [25-27].

4. Ultraviolet-visible spectrophotometry

Several studies have been reported the application of UV spectrophotometry where the Shidmadzu UV-160 and UV-1800 UV/Vis spectrophotometer were used with 1 cm matches quartz cell, CP224S analytical balance (Sartorius) and ultra-sonic cleaner (Fisher scientific FB15061) were used. Micropipette of Variable volume 10-1000 μL (Capp Ecopipette single channel) and Digital balance (Mettler Toledo XP 105). Naproxen (Ezo life sciences) (CAS 22204-53-1) was supplied by Sigma Aldrich. Naprosyn® USP gel (RPG Life sciences, India) was purchased from local market. All other chemicals and solvents used were of HPLC grade [28-30].
5. Ultraviolet spectrophotometry

The proposed method of UV spectrophotometry have been validated well according to the ICH guidelines that related to linearity, limit of detection and limit of quantification, precision and accuracy. Linearity the constructed calibration curve was found to be linear in the range 2.5-15 µg/ml with correlation coefficient 0.998 and linear regression equation y=0.6535x-0.0166. This indicated an excellent correlation existed between the absorbance and concentration of ketoprofen. The obtained values of LOD and LOQ were 0.778 and 2.35µg/ml, respectively.

Table 1. NSAIDs analysis using various methods

<table>
<thead>
<tr>
<th>Sample</th>
<th>Method</th>
<th>LOD (µg/ml)</th>
<th>Linear Range (µg/ml)</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ibuprofen</td>
<td>HPLC</td>
<td>1.7</td>
<td>6.1-200</td>
<td>[19]</td>
</tr>
<tr>
<td>Lornoxicam</td>
<td>FTIR</td>
<td>0.2</td>
<td>-</td>
<td>[22]</td>
</tr>
<tr>
<td>Diclofenac Sodium</td>
<td>GC</td>
<td>0.6</td>
<td>2-10</td>
<td>[25]</td>
</tr>
<tr>
<td>Mefenamic Acid</td>
<td>Ibuprofen</td>
<td>0.4</td>
<td>2-10</td>
<td>[25]</td>
</tr>
<tr>
<td>Naproxen</td>
<td>UV-Vs</td>
<td>1.54</td>
<td>10.60</td>
<td>[28]</td>
</tr>
<tr>
<td>Lornoxicam</td>
<td>UV-Vs</td>
<td>-</td>
<td>-</td>
<td>[29]</td>
</tr>
<tr>
<td>Mefenamic acid</td>
<td>Ibuprofen</td>
<td>UV</td>
<td>216</td>
<td>[31]</td>
</tr>
<tr>
<td>Naproxen</td>
<td>TLC</td>
<td>-</td>
<td>-</td>
<td>[34]</td>
</tr>
<tr>
<td>Ketoprofen</td>
<td></td>
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<tr>
<td>Flubiprofen</td>
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</tbody>
</table>

The outcomes of various approaches

1. HPLC

The method was developed and validated based on United States Pharmacopeia (USP) assay procedure with the objective to enhance the method sensitivity to allow measurement of small changes in the amounts of ibuprofen adhered to punch faces during compression. It was sought to develop a simple and easy-to-apply method that can be used for routine analysis in pharmaceutical industry laboratories. Initially, the mobile phase composition of the USP assay method (400:600; chloroacetic acid solution in water 0.01 g/mL: acetonitrile) was modified by replacing chloroacetic acid solution with water adjusted to pH 2.5 using diluted phosphoric acid. While chloroacetic acid comes as a solid material that must be dissolved prior to use, phosphoric acid is available as liquid making it easier to use. The pH value (pH 2.5) was selected in order to maintain IBP carboxylic group in its unionized form; ibuprofen has a pKa value of 4.5. To select the optimal mobile phase ratio, several water (adjusted to pH 2.5 using phosphoric acid) to acetonitrile volumetric ratios (80:20, 60:40, 50:50, 40:60, 20:80) were evaluated by checking IBP peak as well as its sharpness and retention time. Optimum conditions were achieved with water: acetonitrile 40:60 (v/v) [10].

2. FTIR

The PITA-ODTs showed acceptable physical properties in accordance with pharmaco-special standards. PITA-ODT prepared with Pharmaburst® (F2) had significantly (p<0.05) the fastest DT (6.66±1.52 s) and highest Q10 min (79.07±2.02%) and was chosen as the best formula. The in-vivo pharmacokinetic study of M1 formula showed higher percent relative bioavailability (%RB) of 286.7% and 169.73% for PITA and LORNO, respectively, compared with the marketed products [22].

3. GC

Quantitation of NSAIDs by GC has been performed generally after derivatized with a specific reagent such as ECF. Initially GC conditions were optimized for the elution as symmetrical peaks from the DB-
column (30 m × 0.32 mm id). Different temperature programs were examined with nitrogen flow rates and reasonable peak shapes were observed at initial column temperature at 150 °C for 3.0 min, and with a heating rate of 20 °C/min up to 280 °C and a hold time of 5.0 min with a total run time 13.2 min and the flow rate of nitrogen was adjusted to 2.5 mL/min. Solution (0.2-1.0 mL) of 10 μg/mL stock solution was taken of each of ibuprofen, mefenamic acid, and diclofenac sodium and 0.5 mL of acetonitrile: water: pyridine: methanol (8:42:8:42 v/v/v/v), 0.5 mL of sodium carbonate buffer solution (pH 9), and 0.4 mL of ECF were added. The contents were sonicated for 20 min at 30 °C and 0.5 mL of chloroform was added. The mixture was shaken well, and a separatory funnel was used to separate the layers. A portion of the extract (0.5 mL from 1 mL) was transferred to a vial with a screw cap and 1 μL of the solution was injected into the GC apparatus.

Drug analysis of ibuprofen, mefenamic acid, and diclofenac sodium with derivatization reagent. 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 brufen (ibuprofen), ponstan (mefenamic acid), and qufen (diclofenac sodium) tablets. At least 5 tablets containing ibuprofen 200 mg/tablet, mefenamic acid 250 mg/tablet, and diclofenac sodium 20 mg/tablet were ground to a fine powder and dissolved in an appropriate amount in methanol. Then the solution was filtered and volume adjusted of 50 mL and an aliquot of solution after derivatization was injected into the DB-1 GC column (30 m × 0.32 mm id) and eluted with mobile phase optimized for GC separation and detection of ibuprofen, mefenamic acid, and diclofenac sodium. The quantitation was done from the linear regression equation and the amounts of ibuprofen, mefenamic acid, and diclofenac sodium were found to be 156.93 mg/mL, 193.14 mg/mL, and 19.48 mg/mL, respectively, which agreed with the amounts labeled, i.e. 160 mg/mL, 200 mg/mL, and 20 mg/mL, respectively. The % error was calculated as -1.9%, -3.4%, and -2.6%. The RSD calculated from replicated analysis (n=3) was within 0.5-3.0%. The percentage recovery of ibuprofen, mefenamic acid, and diclofenac sodium was 98%, 96%, and 97%, respectively [7].

4. UV-Visible Spectrophotometry

The analysis of NSAIDs using UV-Vis spectrophotometry should be based on the wavelength vs. concentration. Furthermore, the validation study must be applied in this analysis such as the linearity and accuracy. The linearity of the analytical method was its ability to elicit test results which are directly proportional to analyte concentration in samples within a given range. To establish the linearity of the proposed method, various aliquots of the standard solution of the drug were prepared from stock solution and analysed. Each solution was analysed after filtration through 0.45 μm membrane filter after discarding first 2 mL. The prepared linearity dilutions were then analyzed in series by UV Spectrophotometer and their respective absorbance were recorded at the λmax a graph was plotted between absorbance and theoretical concentration. The drug showed linearity in the range of 10-60μg/mL with correlation coefficient of 0.9984 as maximum absorbance in methanol 331 nm was obtained [8].

5. Ultraviolet Spectrophotometry

Development of analytical method is crucial to counteract for limitation of official methods in terms of cost, sensitivity and accuracy in order to assure delivery of drugs with adequate quality and safety to the patient. This study is an attempt to develop a simple, accurate, precise and cost-effective spectrophotometric method for estimation of ketoprofen tables/capsules dosage forms using simple solvent system and available UV-visible spectrophotometer.

Ketoprofen has a carboxylic functional group in its chemical structure making it soluble in aqueous solution of alkali hydroxides and carbonates. Ketoprofen is soluble in NaHCO₃ in a reaction producing carbon dioxide and sodium salt of the drug, which is soluble in water. Therefore, this reaction has advantage of solubilizing the drug as well as identification test of the drug depending on the carboxylic functional group. In addition, Ketoprofen with its extended chromophore system is considered a good candidate for assay using UV-spectrophotometry with high sensitivity. Sodium hydroxide and sodium carbonate are strong bases that have high solubilizing property for acid like ketoprofen; however, using these salts as solvent for tablets materials can lead to dissolving unwanted materials with tablets/capsules matrixes which can lead to interference in the assay. That is why sodium hydrogen carbonate was chosen as solubilizing agent in this work as it is a weak base that has less solubilizing properties than these two strong bases. It worth noting that the useful method developed for the assay of ketoprofen used three mixed hydrotrophy reagents for solubilizing the drug before the assay. This system has advantages over the other reported methods as it utilizes a nontoxic solvent and does not involve tedious sample preparation or
necessitates the provision of a critical reaction conditions [10].

Conclusions

In this analysis, NSAID drugs are the drugs that are often consumed by some patients, especially, when the patient experiences pain. Among the methods that can be used are Chromatography methods such as gas chromatography, HPLC, thin layer chromatography and also spectrophotometry such as UV-Visible spectrophotometry, UV spectrophotometry, FT-IR spectrophotometry. However, this method is often time consuming and also requires considerable skill to use it.

The drawback of this method is the requirement of HPLC grade quality organic solvents, where the costs for their purchase and disposal must be considered. Another weakness of the spectrophotometric instrument is that absorption is affected by the pH of the solution, temperature and the presence of interfering substances and the cleanliness of the cuvette, can only be used in the ultra violet region with a wavelength of 185 nm, use only on functional groups containing valence electrons with low excitation energy, light used must be monochromatic.

The advantages of spectrophotometric instruments are that they can be used to analyze many organic and inorganic substances, are selective, have high accuracy with a relative error of 1%-3%, analyzes can be carried out quickly and precisely, and can be used to determine very small quantities of substances. In addition, the results obtained are quite accurate, where the read numbers are directly recorded by the detector and printed in the form of digital numbers or graphs that have been regressed. The limitations of each technique have been reviewed. From the discussion above, none of these methods can become routine methods in daily practice because each method has its own advantages and disadvantages. Therefore, the use of this spectrophotometric method is more effective and also innovative.

Conflicts of interest

There are no conflicts to declare.

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References

11. Zambakjian. C, Sakur. AA, A new gas chromatographic method development and


21 Kristoffersen, L, Oiestad, EL, Opdal, MS, Krogh, M, Lundanes, E, Christophersen, AS. Simultaneous determination of 6 beta-blockers, 3 calcium-channel antagonists, 4 angiotensin-II antagonists and 1 antiarrhythmic drug in post-mortem whole blood by automated solid phase extraction and liquid chromatography mass spectrometry: Method development and robustness testing by experimental design, *Journal of Chromatography B*, 2007, 850, 147.


