*Invited Article*

# Green Pharmaceutical Chemistry for the Sustainability

Theerasak Rojanarata*

*Department of Pharmaceutical Chemistry and Pharmaceutical Development of Green Innovations Group (PDGIG), Faculty of Pharmacy, Silpakorn University, Nakhon Pathom 73000, Thailand*

** Corresponding author. E-mail address: teerasak@su.ac.th*

**Abstract**

As a result of current environmental crises, the awareness and cooperation from all sectors are needed to help prevent and solve the problems. In pharmaceutical chemistry discipline, attempts have been made to use the processes with the least adverse impacts on the environment. In this article, an overview of the "6-R" approach applicable to the development of greener methodologies for drug synthesis and analysis is presented and the paradigms of works are illustrated. It is also anticipated that the article will create the environmental conscience and promote the social responsibility for the development and use of eco-friendlier processes, leading to the sustainability.

***Key Words:*** Green; Environmentally friendly; Pharmaceutical chemistry; Sustainability

In the era where the environment issues are increasingly concerned, two terms universally mentioned about are inevitably "green" and "sustainability". Green chemistry, coined in 1991, is defined as "the design of chemical products and processes that reduce or eliminate the use and generation of hazardous substances". This approach has shifted the previously used chemical methods to become more environmentally acceptable. Later, a variety of greens e.g. green energies, green vehicles, green buildings and so forth are commonly heard. The other term; sustainability is defined as: "…meeting the needs of the present without compromising the ability of future generation to meet their own needs". At present, "green" and "sustainable" are inseparable and have been used interchangeably because it has been accepted that green philosophy has become both a culture and a strategy for achieving the sustainability. It is not surprising that the number of scientific researches and inventions on greener processes and products have grown enormously, intending to find a more sustainable way of life.

While the awareness and cooperation are urgently called for from all sectors of the society, this movement has also driven the practice and research in pharmaceutical chemistry to the greener ways. In the past, a lot of pharmaceutical processes were developed and used without delicate ecological concerns, leading to undesirable environmental

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consequences. The goal of the new drug investigation was primarily to obtain the most pharmacologically active molecules with the highest stability. Hence, when they end up their functions leaching into the environment, some drugs persist and tend to accumulate in soil, water as well as terrestrial and aquatic organisms. In a similar way, organic chemists are customarily taught to maximize the yield of a synthesis by exhaustively incorporating reagents, operating conditions and energies into the reactions. Although this is a reasonable goal and an effective measure of the efficiency of a particular reaction, it may not be a good achievement index in the aspect of atom economy and sustainability. In the pharmaceutical analysis, the development of analytical methods has paid attention on the optimization of critical parameters e.g. accuracy, precision, sensitivity, simplicity, cost and speed. However, other aspects concerning operator safety and environmental impact of the methods are not commonly considered. Accordingly, it is a fact that in some circumstances the chemicals employed for the analysis are even more toxic than species being determined. By the aforementioned reasons, the development of pharmaceutical processes by merging suitable technologies with environmental safety is more concerned nowadays and is one of the key challenges of the millennium.

Among various green strategies to move towards sustainability, our group namely "Pharmaceutical Development of Green Innovations Group (PDGIG)" has established the ***"6-R"*** approach for the development of pharmaceutical processes. According to this rationale, the research and development should be ***"relevant"*** to the real problems and should employ ***"reachable"*** technologies; in other words, technologies that can be practically implemented by the operators. In this sense, it can be seen that many academic researches do not meet industrial interests and applications. Too sophisticated technology with high cost of instrument acquisition, operation and maintenance may become deterrents which hinder the adoption of greener methods coming from the research base. To break these barriers down, collaborative research between researchers and practitioners will become a solution. Also, simplicity not complexity of the method should be born in mind to help bridge the gap between academia and industry and to facilitate the technology transfer.

In technical aspect, toxic chemicals or harmful procedures should be ***"replaced"***. This can be done by the development of new safer methodologies or the modification of existing processes using safer reagents. However, the complete substitution of toxic reagents is always not an easy task. The ***"reduction"*** of the reagent use and waste generation should be considered. In this respect, downscaling of the methods, the use of modern analytical techniques with breakthroughs in microelectronics, miniaturization and the combination with chemometrics allow the development of the assays with reagent-saving features. This strategy not only benefits the environment and operators as a result of reduced risk of exposure to the hazardous reagents, but also saves costs on chemical purchase and waste management. Furthermore, this approach is encouraged and useful in the context of education. Substitution of old large scale experimental practices by attractive downsized procedures will result in the immediate reduction of wastes as well as developing the environmental conscience essential for our students in the future.

Once eco-friendlier methods have been proposed, it must be ascertained that they ***"remain"*** satisfactory in performance. For example, the accuracy and precision of the analytical methods must meet the prerequisites when the analysis is miniaturized. The extraction efficiency should not be lowered after previously used extractants are substituted by alternative benign solvents. Lastly, the method should be ***"responsible"*** for the environment. Answers must be ready for a simple question such as "Can we safely release the wastes

from the process to the environment?" Basically, the guidance for the waste management should be provided together with the protocol of developed method. More efficiently, additional efforts may be made by the recovery of reagents towards achieving zero emission or by on-line decontamination of wastes.

We illustrate here some examples of greener alternatives for the pharmaceutical processes which have been developed by our group.

**Enzyme-based pharmaceutical processes**

Enzyme catalysis has several characteristics that are relevant to the green chemistry. Enzymes speed up the rates of reactions with high degree of chemo-, stereo- and regio- specificity. Therefore the enzyme-based drug synthesis and analysis offer faster throughput, possibility of asymmetric synthesis and satisfactory analyte selectivity. In addition, their water solubility and natural operation under mild to moderate conditions offer the opportunity for the reactions to take place in aqueous medium without the use of organic solvents or extreme pH, temperature, etc. Because of these advantages over non-catalyzed and chemically catalyzed reactions, we have exploited D-phenylglycine aminotransferase, an enzyme originally isolated from soil bacteria in Thailand, for the synthesis of D-phenylglycine, an unnatural amino acid used as a side chain for the anti-infective drugs ampicillin and cephalexin (Rojanarata et al., 2004). The developed method was straightforward, requiring one-step reaction and inexpensive precursors. Toxic chemicals e.g. hydrogen cyanide which are employed in the traditional racemic resolution were no longer used. Importantly, the satisfactory yield with high enantiopurity was obtained.

For the drug analysis, we have applied this enzyme for the development of a new assay for amoxicillin in the pharmaceutical preparations (Rojanarata et al., 2010). In this method, the drug was selectively converted by penicillin acylase and D-phenylglycine aminotransferase to the product 4-hydroxybenzoylformate with strong ultraviolet light absorption (Figure 1). The amount of amoxicillin was thus determined as an increase of absorbance by using spectrophotometers which were commonly available in the laboratories. The assay was found to be linear and sensitive. The accuracy, precision and specificity of the assay were satisfactory and comparable to the pharmacopoeial method which was based on high performance liquid

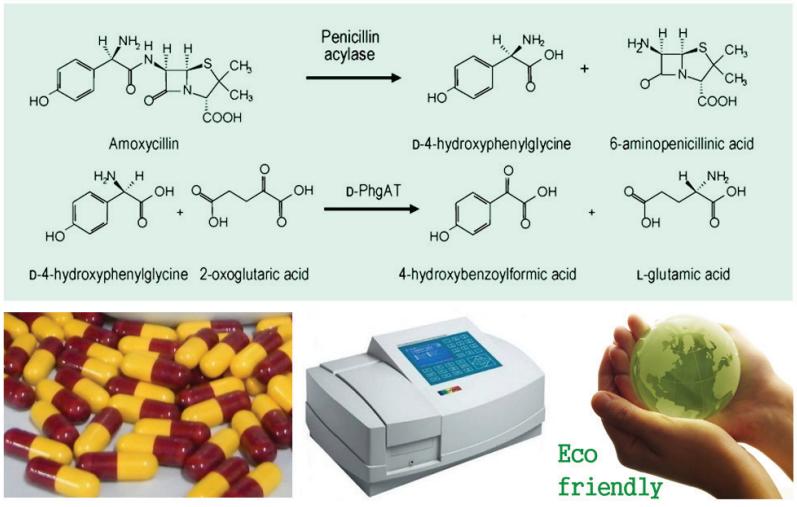


**Figure 1** Novel assay for amoxicillin in the pharmaceutical preparations based on enzymatic reactions and spectrophotometry

chromatography (HPLC). In the environmental view points, all procedures were free from the use of organic solvents or hazardous chemicals which were detrimental to the environment and had a low consumption of reagents. In addition, the antimicrobial activity of drug samples was enzymatically inactivated prior to waste disposal, therefore the inappropriate release of the antibiotic into the environment and the occurrence of drug resistance were lower. Thus, the method was safe for the operators and eco-friendly.

To further broaden the application of enzymes to the isolation of natural products, we have employed plant cell wall-degrading enzymes i.e. cellulase, hemicellulase and pectinase for the enzyme-assisted extraction of active constituents from Thai herbal plants such as the extraction of oil from Plai (*Zingiber Cassumunar* Roxb.) (Figure 2) (Chuchote et al., 2009). The results showed that the incorporation of enzymatic treatment of plant materials prior to the extraction process yielded a higher content of α-terpinen-4-ol, the


**Figure 2** Enzyme-assisted extraction of Plai oil using plant cell wall-degrading enzymes

pharmacologically active compound in Plai oil, than that obtained from the ethanol extraction method without the enzymatic treatment. This finding demonstrates that the enzyme helps break down the cell walls and facilitates the release of oil, leading to the enhanced extraction efficiency.

**Chromatographic methods using greener mobile phases**

Organic solvents and volatile organic compounds (VOCs) are currently used in almost all pharmaceutical processes e.g. drug synthesis, extraction, recrystallization, dissolution of solids and chromatographic separation. Besides the adverse health effects such as dizziness, irritation and carcinogenicity, the main environmental concerns related to these solvents especially VOCs are their ozone depletion properties, the global warming potential resulting from the production of photochemical smog and the environmental persistence. For these reasons, a number of strategies for solvent replacement have been investigated including the use of benign non-volatile organic solvents, water under superheated conditions, supercritical fluids, renewable solvents, ionic liquids, etc.

Acetonitrile is ranked as a toxic chemical as in liquid or vapor and waste has to be detoxified through special chemical treatment, leading to high to very high disposal cost. Methanol is also toxic to humans and causes adverse effects on aquatic life. Since both solvents are most commonly used in reversed phase HPLC, we have developed an alternative method for the stability-indicating assay

of prednisolone in tablets using eco-friendlier ethanol-water mobile phase (Rojanarata, 2011). In the environmental considerations, ethanol is acknowledged as green because of its biomass origin e.g. from agricultural feedstock and its biodegradability. Additionally, it is less harmful to human as well as the environment and requires less expensive and easier waste management. From our study, the chromatographic analysis of prednisolone was achieved on the C18 column at 50 ˚C, using a 30:70 ethanol-water as the mobile phase with the flow rate of 0.8 mL $min^{-1}$. There was no interference from the background absorption of the mobile phase as well as the problems related to the back pressure generated in the system. The peak of prednisolone was well resolved from various degradation products as well as the tablet excipients at the retention time of about 10 min. In addition, statistical analysis confirmed that the assay results obtained from the proposed method were not significantly different from those obtained from the British Pharmacopoeia method which used methanol and water (42:58) as the mobile phase. Therefore, the proposed method was proven an effective alternative assay in the aspect of both analytical performance and sustainable viewpoint.

**Miniaturized titrations for the analysis of sodium chloride and basic drugs as hydrochloride salts**

Classical methods such as titrations are still the methods of choice for the assays of many pharmaceutical bulk materials and some preparations because of their simplicity, cost and speed of operation. However, one of the drawbacks is the large size of the analysis, resulting in a large amount of reagents consumed and wastes generated. To solve these problems, we have designed a miniaturized titration using Volhard's agentimetric titration for the assay of sodium chloride as a model (Rojanarata et al., 2011a). The reactions were downscaled to less than 2 mL and were carried out in microcentrifuge tubes using micropipettes for the transfer of reagents. The assay started with the precipitation of chloride with a measured excess of silver nitrate. The unreacted silver nitrate left in the supernatant was separated from the precipitate by centrifugation, transferred to a new set of tubes and then titrated with different volumes of standard ammonium thiocyanate solution. The equivalence point was determined based on a photometric titration by the absorbance measurement at 450 nm to diminish human visual errors, using microplate reader which quickened multi-sample measurements

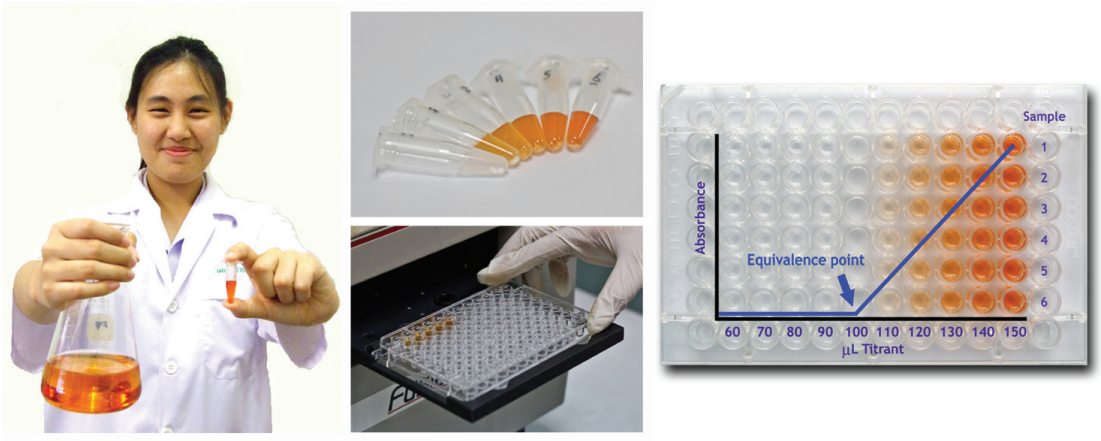


**Figure 3** Miniaturized titration for the assay of sodium chloride which saves the reagent consumption and reduces waste generation while remaining the satisfactory analytical performance

(Figure 3). After testing with NaCl raw material, NaCl tablets, NaCl intravenous infusion and NaCl and glucose intravenous infusion, the downsized method showed good accuracy comparable to the British Pharmacopoeial large-scale method and gave satisfactory precision (intra-day, inter-day, between-analyst and between-pipette model) with the relative standard deviation of less than 1%. In addition, the method was found to be faster in the case of multi-sample analysis. The amount of the reagents consumed in the miniaturized titration was significantly reduced by 25 - 215 folds, while the release of solid wastes was drastically reduced at about 25 fold. The use of noxious and environmentally harmful dibutyl phthalate was absolutely eliminated in the proposed method.

Based on the same principle of chloride determination, the developed method was further applied for the assay of basic drugs which were often prepared as hydrochloride (HCl) salts. Nowadays, most pharmacopoeial assays of their bulk materials are acid-base titrations in non-aqueous solvents in which harsh, unsafe chemicals such as glacial acetic acid, acetic anhydride and mercury(II) acetate are usually employed. Using phenylpropanolamine HCl and metformin HCl raw materials as model analytes, it was found that the miniaturized photometric Volhard's method gave accurate and precise analytical results (Rojanarata et al., 2011b). Importantly, it was safe for the analysts by the elimination of undesirable or dangerous chemicals and was friendly to the environment by lower the release of toxic wastes.

## Conclusions

Reaching to this point, it is anticipated that the reader will have concluded that green philosophy is not a new branch of sciences, but it is an approach that strengthens all of disciplines including pharmaceutical chemistry. In addition, it has inseparable technological, environmental, economic and societal goals. Therefore, it is indeed hopeful that the ***think green culture*** will happen in all professions and generations and the environmental awareness is created among the mass of people, taking all of us to the sustainable future.

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