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Research Article

SPECTROPHOTOMETRIC EVALUATION OF NIMESULIDE IMPURITY D: 4-NITRO-2-PHENOXYANILINE USING 8-HYDROXYQUINOLINE AS OXIDATIVE COUPLING REAGENT

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ABSTRACT

Objective: Spectrophotometric investigation of 4-nitro-2-phenoxyaniline (4N2PA), a probable intermediate during the synthesis of anti-inflammatory drug nimesulide, both in pure form and in the presence of nimesulide has been attempted.

Methods: 4N2PA on diazotization followed by coupling with 8-hydroxyquinoline produces a crimson-colored complex which changes to deep violet on diluting with ethanol.

Results: The complex showed maximum absorbance at 560 nm when evaluated spectrophotometrically with a detection limit of 0.005 µg/ml and quantification range of 0.05-3.0 µg/ml. The method has been statistically evaluated with respect to the International Council for Harmonisation guidelines and found to be accurate and precise.

Conclusion: Pure tablet formulations of nimesulide do not respond to the method; however, the presence of minute amounts of 4N2PA in the drug as added impurity could be spectrophotometrically analyzed. Hence, the authors suggest that the reported technique could be a marker test for detecting the presence of 4N2PA in nimesulide formulations.

Keywords: 4-Nitro2-phenoxyaniline, Nimesulide impurity, Spectrophotometry, 8-Hydroxyquinoline.

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INTRODUCTION

4-Nitro-2-phenoxyaniline (4N2PA) is a possible impurity formed *en route* the synthesis of nimesulide which is the first cyclooxygenase-2 (COX-2) selective nonsteroidal anti-inflammatory drug often employed to treat inflammation [1,2]. Nimesulide emerged as a competent anti-inflammatory drug because of its ability to selectively inhibit COX-2 enzyme while being nonreactive to COX-1 enzyme, thereby reducing the chances of gastrointestinal disturbance. Although nimesulide has been banned in many countries due to its alleged side effects of causing liver damage, it is still being used in India for patients aged above fourteen years. Synthesis of nimesulide has been described by Shreenivas *et al.* [3] and at the outset, they had anticipated the idea of using its impurity 4N2PA to treat hypertension in lieu of the drug losartan. Zacharias and Tzanavaras have introduced high-performance liquid chromatography (HPLC) methods for separating nimesulide from its impurities [4]. Reverse-phase HPLC was employed by Tubic *et al.* for concurrent analysis of nimesulide and 4N2PA [5]. The crystal structure of 4N2PA was analyzed by Manjunath *et al.*, which shows two aromatic rings in syn-periplanar (+sp) conformation connected through oxygen atom [6]. Kumar and Bhaskar have performed spectral studies of 4N2PA by both experimental and computational methods [7]. Spectrophotometric estimation of nimesulide using cetrimide was achieved by Florae *et al.* and the technique involved extraction of the reaction mixture into chloroform [8]. Simultaneous estimation of nimesulide and paracetamol using visible spectroscopy was achieved by Charan *et al.* [9] whereas Kirtawade *et al.* employed ultraviolet spectrophotometer for analyzing these two drugs simultaneously [10]. Spectrophotometric assaying of nimesulide in generic and compounding formulations has been reported which also point to the concerns in quality of compounding products [11]. Further, there have been similar studies attempted to enumerate drug impurities and their precursors in fluconazole formulations by LC-tandem mass spectrometry [12].

It is imperative that drugs provide adequate therapeutic value as declared in the formulations. More often than not, constituents other than active pharmaceutical ingredients such as common excipients, coloring agents, stabilizers, possible impurities, and intermediates during commercial manufacturing, etc., also affect the pharmacological action of medicines. At times, these factors could jeopardize the safety of the drug to certain extent. There have been constant demands for fast, reproducible, cost-effective, and eco-friendly methods of analysis for quality control of medicines. No methods have been reported till date for the detection or estimation of 4N2PA either in pure form or presence of nimesulide as an impurity.

This has prompted the authors to develop a simple spectrophotometric method for the determination of 4N2PA for the using 8-hydroxyquinoline as coupling agent for routine quality check.

METHODS

Reaction mechanism

The method is based on the diazotization reaction which converts primary amines into diazonium salts when they are treated with sodium nitrite in the presence of mineral acids at temperature 0-4°C. This diazonium salt is then coupled with 8-hydroxyquinoline to get a crimson-colored complex which changes to deep violet on diluting with ethanol (Fig. 1) [13].

Preparation of reagents

- Sample preparation: 500 µg/ml solution of 4N2PA is prepared by dissolving 50 mg of drug in 2 ml of absolute ethanol and making up to 100 ml with double-distilled water. An aliquot of 25 µg/ml is prepared by suitable dilution of the stock solution
- 8-Hydroxyquinoline: Prepared by dissolving 50 mg in 2 ml of absolute ethanol and making up to 100 ml with double distilled water

- Sodium nitrite: Prepared by dissolving 1 g in 100 ml of double-distilled water
- Sulfamic acid: Prepared by dissolving 2 g in 100 ml of double-distilled water.

Optimized reaction conditions and procedures

A series of preliminary investigations are carried out before reaching the optimized reagent conditions for maximum sensitivity. Optimal conditions were chalked out by carefully varying one parameter while keeping others fixed. The following iteration provided the best color density and stability when added in the given sequence.

Two ml of aliquote is pipetted into a 25 ml standard flask, to which 2 ml of 50 % solution of hydrochloric acid is added, followed by 3 ml of sodium nitrite solution and the mixture is kept in a thermostat for 5 minutes to maintain temperature 0-4°C. To the above mixture, 5 ml of sulfamic acid is added and shaken repeatedly to remove the effervescence. 4 ml of 8-hydroxyquinoline is added followed by 4 ml of sodium hydroxide (5N) solution. On dilution with ethanol, a deep violet color is produced which emerged to be stable for 5 hrs. The colored solution is then scanned between 400 and 660 nm to get an absorption maximum at 560 nm against reagent blank (Fig. 2).

RESULTS AND DISCUSSIONS

The reported method is characterized by trace level limits of detection and quantification (Fig. 3). The method does not demand pH restrictions or use of buffers. The chromogen developed by diazotization appears to be stable for spectrophotometric analysis up to 4-5 hrs. Furthermore, the method is rapid and uncomplicated as it is free from cumbersome extraction steps. Interferences from excipients are negligible as evident from recovery studies (Table 1).

The novelty of this study is evident as this is the first ever method proposed for checking the presence of title molecule as an impurity in drug formulations containing nimesulide. This might also help in quality check of nimesulide-associated formulations in compounding pharmacy industries.

Application in tablet formulations

It is worth noting that nimesulide tablets available in local market did not produce any color when subjected to the reported method. However, violet color started developing on adding 4N2PA in trace amounts onto tablet samples. This trend has been studied by standard addition method, and a calibration curve has been plotted as shown in Fig. 4.

A minimum amount of 0.25 µg/ml of 4N2PA was enough to show characteristic absorbance and this proclivity goes beyond Beer's law range at 4 µg/ml of added impurity. This suggests that the described method shall be used for detecting the presence of 4N2PA as trace level impurity in nimesulide tablet formulations.

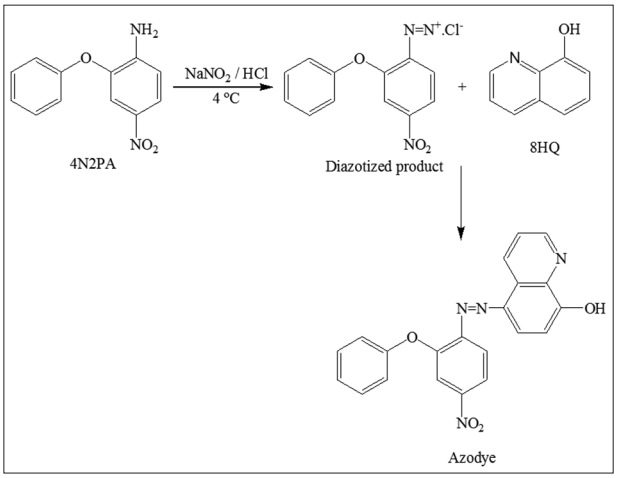

Fig. 1: Proposed reaction mechanism

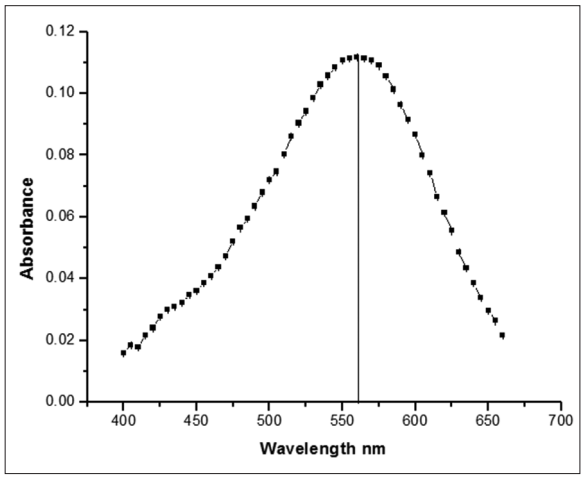

Fig. 2: Absorption maximum of 4-nitro-2-phenoxy aniline at 560 nm

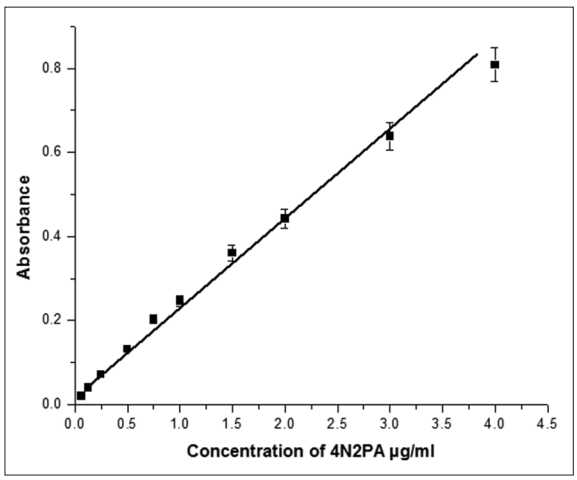

Fig. 3: Calibration curve of 4-nitro 2-phenoxy aniline

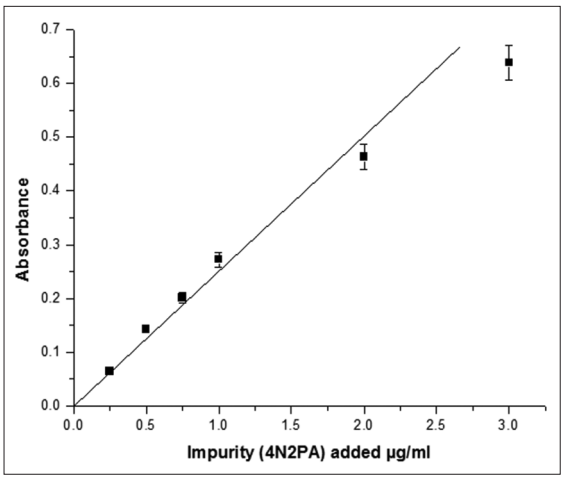

Fig. 4: Beer's law range - standard addition of 4-nitro-2-phenoxy aniline as impurity to nimesulide tablet formulations. A fixed concentration of 20 ppm of tablet solution is used to which varying amounts of 4N2PA aniline added as impurity

Table 1: Effect of common excipients-recovery study

Excipient	Quantity µg/ml	Recovery % mean±SD
Talc	100	99.7±0.93
Starch	150	99.8±0.72
Cellulose	200	100.1±0.53
Aliginate	50	99.6±0.91
Gum arabic	100	99.5±0.65
PVP	50	99.7±0.85
Lactose	100	99.8±0.90

*10 µg/ml of 4N2PA taken for optical measurements, **Average of five replicate determinations, SD: Standard deviation, 4N2PA: 4-nitro-2-phenoxy aniline, PVP: Polyvinylpyrrolidone

CONCLUSION

The method is the first ever reported method for analyzing the presence of impurity (4N2PA) in nimesulide formulations using spectrophotometer. The method appears to be simple, fast, stable, reproducible, and free from extraction steps. Furthermore, it does not necessitate any strict regent conditions, pH constraints, or use of carcinogenic solvents. Authors are hopeful that this method can be useful in routine quality control analysis of nimesulide formulations to check the presence of titled compound as an impurity.

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