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ORIGINAL ARTICLES

PHENOTHIAZINE DYE LABELING TEST FOR QUINOTOXINE

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ABSTRACT

INTRODUCTION: Quinotoxine is an alkaloid that was originally obtained synthetically by Pasteur. Its presence in quinine bark was subsequently established. However, numerous biological tests with this alkaloid dispelled all suspicions about its toxicity. Despite this, the suffix -toxin in its trivial name has remained unchanged. In the 1970s, the appearance of this alkaloid was registered on the pharmaceutical market. Over the years, a number of its derivatives, which featured a powerful antibacterial effect, also appeared. Whether it has been regarded as a synthetic precursor or a drug, the potential of the alkaloid in question is undeniable. Therefore, every single aspect of its analysis is fully justified.

AIM: The aim of this article is to provide a much handier method for the qualitative analysis of the alkaloid quinotoxine.

MATERIALS AND METHODS: A strategy developed by Kehrmann was applied for the qualitative analysis of quinotoxine; this strategy targets the alkaloid's inherent piperidine residue. All tests were conducted using standard laboratory glassware. The authenticity of the studied alkaloid was confirmed by infrared and ultraviolet visible (UV-Vis) analysis.

RESULTS: Immediately following the introduction of the studied alkaloid to the bromine-phenothiazine solution, the appearance of a characteristic blue coloration was registered. To rule out the possibility of a false-positive result, the alkaloid quinine was also analyzed in parity. No indication of any analytical reflex to the quinuclidine-containing alkaloid was observed. The main characteristic of this test, the limit of detection (LOD), was also established. Its value was determined to be 7.5 micrograms.

CONCLUSION: This communiqué can be perceived as a first attempt to analyze the piperidine residue of the alkaloid quinotoxine. Given that other available methods target the quinoline residue, which is common to this alkaloid class, the presented test may represent an optimal choice for achieving the intended goal. Moreover, given the feasibility of the presented test, it can be easily implemented and can be performed by student pharmacists.

Keywords: *quinotoxine, phenothiazine, qualitative method*

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INTRODUCTION

Quinotoxine (quinicine; Fig. 1) is an alkaloid that was first obtained by Pasteur in 1853 by heating quinine with dilute hydrochloric acid (1,2). Shortly after, in 1871, the alkaloid was also isolated from quinine bark. The name *quinotoxine* was given by Hesse (3).

Quinotoxine (IUPAC: 1-(6-methoxyquinolin-4-yl)-3-(3-vinylpiperidin-4-yl)propan-1-one) is a yel-

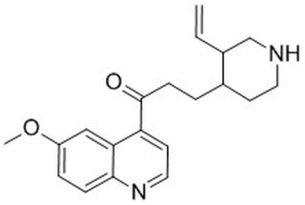


Fig. 1. Molecular structure of quinotoxine.

low oil with $[\alpha]_D$ + 38.6° ($CHCl_3$). It is easily soluble in chloroform, ethanol, methanol, and diethyl ether. It is purified through its easily crystallized oxalate[1] and tartrate[2] salts. The base itself has a very bitter taste.

The suffix *-toxin* in the trivial name of this alkaloid has long been disputed (4). Detailed studies have proven the absence of suspected toxicity (5,6). Moreover, in the form of hydrochloride and the designation *viquidil* (*viquidil hydrochloride*), the alkaloid entered the pharmaceutical market as a cerebral vasodilator, and it remained classified as such for nearly a decade (7). Several European countries put it into production under different trade names: *Desclidium* (producer Spret, France 1972; Rorer, Italy 1973 and Badische, West Germany 1979), *Chinotoxin* (Badische, West Germany), and *Permiran* (Lab. Franc. Therap., France) (8). In addition to being a cerebral vasodilator, *viquidil hydrochloride* has also been used as an antispasmodic agent, with indications approaching those of the alkaloid *papaverine* (9). No information is, however, found in the literature regarding the reason for the discontinued production of this medication. It is assumed that it has been replaced by others with much higher selectivity.

Over the last few years, data on the appearance of several of its derivatives can be found in scientific periodicals. Its reduced analog, RP80082A, is patented as a topoisomerase IV inhibitor with an IC_{50} of 1 μM (Fig. 2) (10).

Based on RP80082A, two more compounds (AVE6971, AVE4221; Fig. 3) with significantly higher antibiotic activity than that of *linezolid*, *vancomycin,* and *quinupristin/dalfopristin* were further designed (10).

[1]The oxalate crystallizes with nine molecules of crystal water (in prisms with m.p. 166÷167°C). The salt is hardly soluble in water (1:120), but recrystallizes from chloroform and/or ethanol.

[2]The acid tartrate crystallizes with six molecules of water, which it loses gradually when heated at 50°C and 140°C.

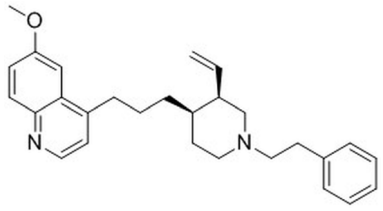


Fig. 2. RP80082A molecular structure.

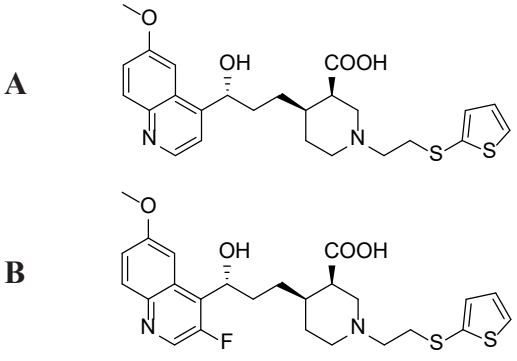


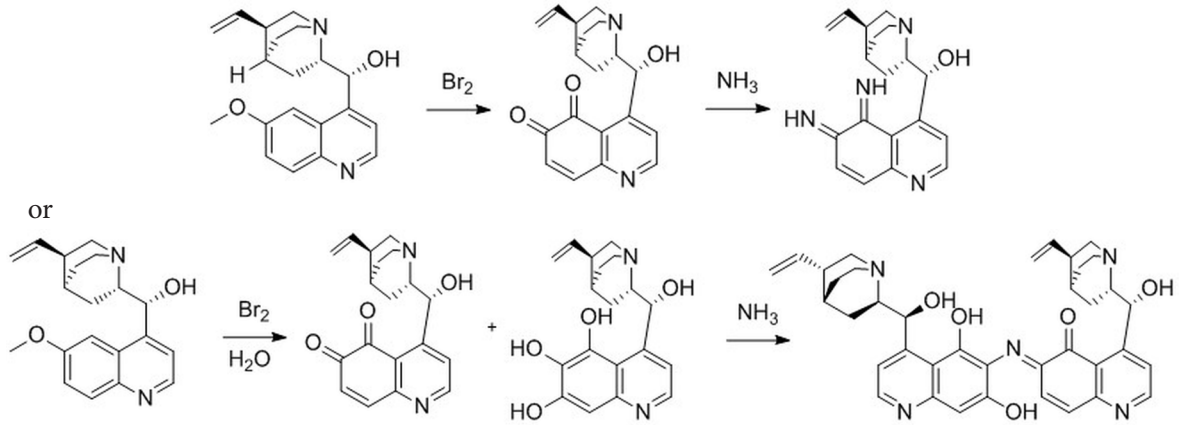
Fig. 3. The molecular structures of the registered quintoxine derivatives AVE6971 (A) and AVE4221 (B).

Given all that has been said thus far, it is clear that the alkaloid in question can be used with full justification as a basis for many scientific studies.

As for the qualitative analysis of this compound, extremely scarce information can be found in the scientific literature (11). The leading author in the field has listed the following tests as being entirely applicable to the alkaloid in question: the Lipkin's and Ball's tests, as well as those using the diazobenzenesulfonic acid[3] and dinitrothiophene, respectively. The author has also postulated that "*The phenylhydrazine and nitrous acid tests of Ganassini were tried and discarded as unsatisfactory.*" In addition, the Lipkin's test is still used today, but it is now referred to as the *Thalleoquin test* (Scheme 1).

When investigating the literature on the qualitative analyses of the piperidine residue, nothing has been identified to date. Therefore, the present work is focused precisely on the qualitative analysis of this structural residue—a residue that distinguishes this alkaloid from its synthetic precursor, *quinine*.

[3]Also known as Pauly's reagent.



Scheme 1. Reaction routes illustrating the putative development of the Thalleoquin test.

AIM

The aim of this article is to provide a much handier method for the qualitative analysis of the alkaloid quinotoxine in terms of its piperidine residue.

MATERIALS AND METHODS

All chemicals were of analytical grade and used as received: quinine hemisulfate salt monohydrate (BioReagent, ≥98%; Sigma-Aldrich Co., St. Louis, MO, USA), methanol (99.9%, extra dry, AcroSeal®; Acros Organics, Veneto, Italy), potassium bromide (99+%, FTIR grade; Sigma-Aldrich Co.), potassium bromate (99+%, extra pure; Acros Organics), sulfuric acid, 25% solution in water (for analysis; Acros Organics), and phenothiazine (99%; Acros Organics). Double distilled and deionized water was used throughout the study.

Attenuated total reflectance (ATR)-FTIR spectra were recorded using an FTIR spectrometer (model Tensor II; Bruker, Ettlingen, Germany), equipped with an ATR module.

The spectra were collected within the middle infrared (IR) range.

The ultraviolet-visible spectroscopy (UV-Vis) absorption spectrum was measured in 1.0 cm quartz cuvette using a UV-Vis spectrophotometer, T60, from PG Instruments (Leicestershire, UK). The analyte was assayed in the form of 0.01 mmol solution in 0.1 M HCl.

Preparation of 2% Methanolic Solution of Bromine

$KBrO_3$ reacts with KBr in dilute sulfuric acid solution to produce Br_2, according to equation 1, Fig. 4.

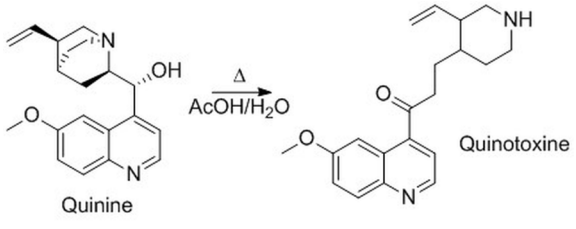
The separated bromine was taken to a bubbler filled with anhydrous methanol; as shown in Fig. 4.

$$KBrO_3 + 5KBr + 3H_2SO_4 \longrightarrow 3Br_2 + 3K_2SO_4 + 3H_2O \quad (1)$$

Fig. 4. A photograph of the experimental setup used for the production of bromine. In the same photograph, reaction equation 1 is also presented.

Preparation of Quinotoxine (Scheme 2)

Employing the method used by Biddle (12), 2.2 grams of quinine sulfate, 10 mL of glacial acetic acid, and 1.0 mL of water were heated to boiling for approximately 45 hours. The cooled solution (dark brown in color) was then treated with an excess of NaOH. The separated dark-brown oil was treated/



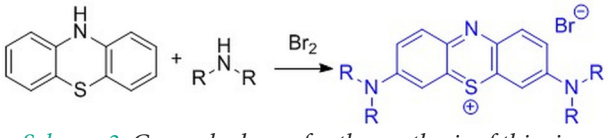
Scheme 2. Synthesis of quinotoxine.

extracted with diethyl ether. The desired product was isolated by evaporation of the accompanying solvent (the used diethyl ether). The identity of the latter was established by FTIR and UV-Vis analysis.

Spot-Test Procedure (Scheme 3)

A drop of a 0.1% methanolic solution of phenothiazine was mixed in a glass beaker (low form and 10 mL capacity) with a drop of a 2.0% methanolic solution of Br_2; then, a drop of the test solution (0.1% methanolic solution of quinotoxine) was added. A blue color signaled the presence of a secondary amine.

All tests were performed in a well-ventilated hood. To confirm the repeatability of the analytic test, all trials were repeated thrice.



Scheme 3. General scheme for the synthesis of thiazine dyes.

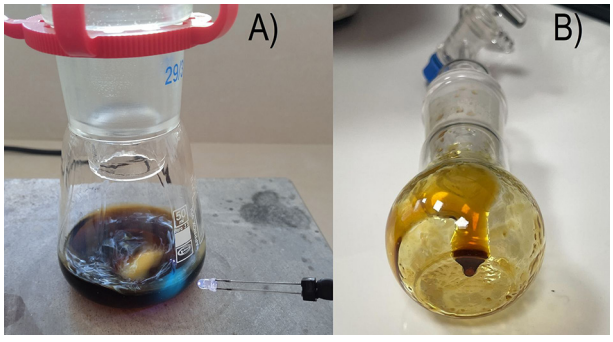


Fig. 5. Flasks containing (A) the UV-monitored reaction solution and (B) quinotoxine oil.

The authenticity of the thus obtained product was established by ATR-FTIR and UV-Vis analysis (Fig. 6).

The purity of the product was determined by thin-layer chromatographic analysis[5], where the presence of no additional/side reaction product was witnessed.

As for the main analytical method, the strategy developed by Kehrmann (14) for the analysis of secondary amines was applied. Although the method was subsequently developed by Bröll and Fischer (15) for the analysis of cyclic amines, it has not yet found application in the analysis of natural compounds. Therefore, the aim of the present work was to establish the applicability of this test in the analysis of the alkaloid quinotoxine—a compound containing a secondary amino group within its piperidine moiety (Scheme 4).

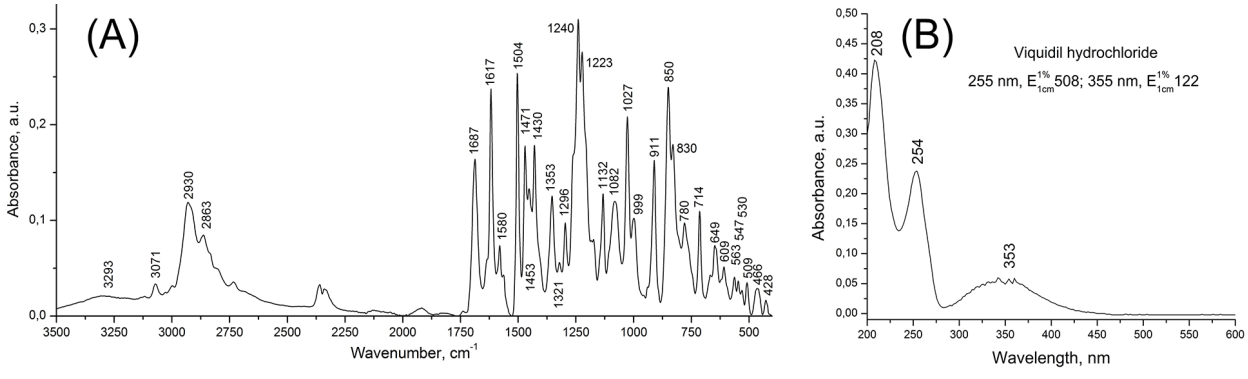


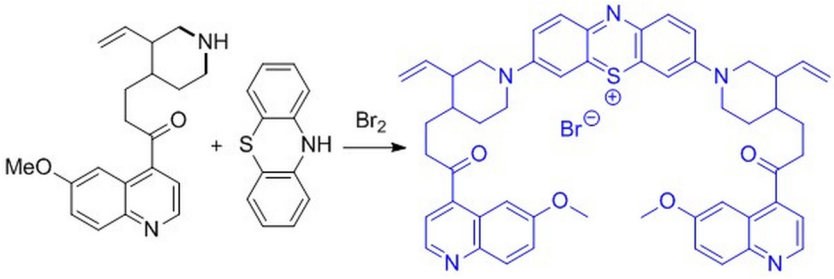
Fig. 6. ATR-FTIR (A) and UV-Vis (B) spectra of the alkaloid quinotoxine.

RESULTS AND DISCUSSION

The synthesis of quinotoxine was carried out according to the methodology of Biddle (13). A minor modification was made regarding the selective isolation of the toxin from its concomitant impurities, representing an adapted concept from the manufacturing patent of *viquidil hydrochloride* (8). Furthermore, the overall duration of quinotoxine synthesis was also increased. To this end, the reaction mixture was monitored periodically with an external UV source[4] (Fig. 5).

[4]Exhaustion of the introduced quinine was evident by the loss of the fluorescent properties of the reaction medium.

[5]Using the fluorescent properties of the chromatographic plaque used.



Scheme 4. Proposed route of the applied spot-test reaction.

De facto, the expected analytical response was registered immediately upon the introduction of the tested alkaloid to the bromine–phenothiazine solution (Fig. 7). By presumption, the appearance of the so-called methylene blue color was considered as an irrefutable positive analytical sign for this test. The expressivity of the test can also be taken as an additional measure of the inertness of the remaining quinotoxine functional residues with respect to the specified reaction (piperidine) center.

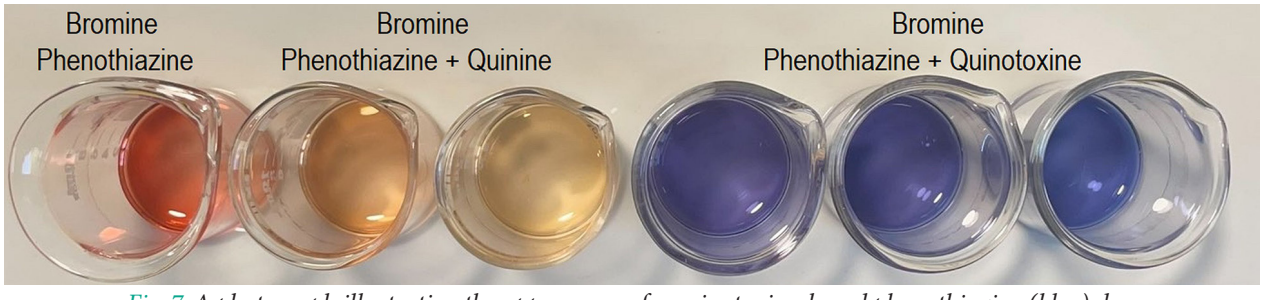


Fig. 7. A photograph illustrating the appearance of a quinotoxine-based phenothiazine (blue) dye.

To rule out the possibility of a false-positive result, the alkaloid quinine was also analyzed in parity. No indication of any analytical reflex to the quinuclidine-containing alkaloid was observed (Fig. 7).

Actions were also taken to establish the limit of detection (LOD) of the specified test. The analysis showed that the magnitude of this value was equal to ~0.0075 mg (or ~7.5 μg). Moreover, the results were very similar to those recorded by Bröll and Fischer, but for other artificial compounds (15).

CONCLUSION

The presented communiqué can be perceived as a first attempt in the analysis of the piperidine residue of the alkaloid quinotoxine. Given that other available methods target the quinoline residue, which is common to this alkaloid class, the presented test has the potential to be the approach of choice to achieve the intended goal. Moreover, since this test is feasible, it can be easily implemented and possibly performed by student pharmacists.

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