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A sulfur-based qualitative test for determining the presence of the secondary alcohol functional group of (-)-quinine and (+)-quinidine

I. V. Vasileva, I. N. Kolev*

Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Medical University "Prof. Dr. Paraskev Stoyanov" – Varna, 84 "Tzar Osvoboditel" Blvd., 9000 Varna, Bulgaria

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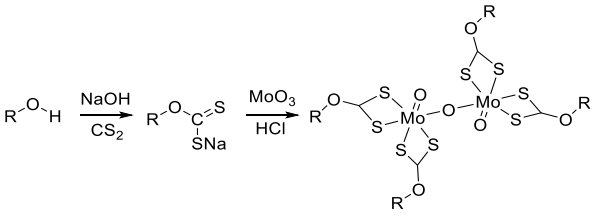
The present study describes a simple analytical qualitative technique for determining the presence of secondary alcohol groups in the composition of the drug enantiomeric pair *(-)-quinine* and *(+)-quinidine*. The analysis is based on the oxidation potential of molten sulfur (S_8) and on the reactivity of the resulting H_2S to $Pb(OAc)_2$. In addition, a methodology for estimating the limit of detection (LOD) of the alkaloids in question was developed. The magnitude of these values for both analytes was established as ~ 0.006 mg (or ~ 6.0 μg).

Keywords: pharmaceutical analysis, quinine, quinidine, secondary hydroxyl group, elemental sulfur

INTRODUCTION

The qualitative analysis of raw drug substances containing secondary alcohol groups is a matter of paramount analytical importance [1, 2]. As a rule, the pharmacopoeial analysis of complexly structured alcohols includes the following two main strategies [3]:

a. implementation of complex-forming reactions; *i. e.* synthesis of distinctively colored mixed-ligand complexes:



and

b. treatment with the *Lucas' reagent* (an equimolar mixture (a solution) of anhydrous $ZnCl_2$ and concentrated HCl).

Although strictly specific, the first strategy requires the use of expensive, non-conventional, and potentially toxic reagents - inorganic salts and oxides; as well as the usage of larger quantities of hazardous solvents - benzene, CS_2, *etc*.

Lucas' reagent, on the other hand, has been usually employed as a "measuring stick" for checking the quality of bottled (the so-called pure commercial) alcohols or as a reagent for the systematic analysis (or even identification) of low-molecular-weight alcohols - saturated mono-functional alcohols having fewer than six or eight carbon atoms [4].

The oxidation-reduction analysis of secondary alcohols (inclusively pharmacologically-active compounds containing 2º alcoholic groups) began early in 1957, when Feigl *et al.* [5] accomplished qualitative analysis of several artificial and natural compounds in the presence of the titled inorganic reagent - elemental S_8. Shortly thereafter, however, this redox test lost its practical value, being substituted by the ones pointed out above and by other analytical methods [3].

Sulfur (S_8) is a non-toxic chemical element – an environment-friendly substance employed in various key industries [6]. Again, it has been used in the medical practice since ancient times as a mild keratolytic and antiseptic agent. In addition, one should take into account that S_8 is also generally recognized as a safe and effective medicine - a very useful cure for the treatment of different skin diseases, including scabies [7, 8].

From the chemical point of view, however, S_8 is principally used as a mild oxidizing agent (in the Willgerodt' reaction [9] and catalytic dehydrogenation of various organic substrates [10]) or as a reagent for the *in situ* formation of carbonyl sulfide [11] (Fig. 1).

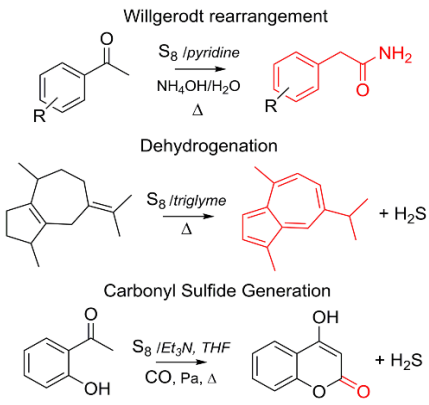


Figure 1. The usage of elemental sulfur in organic synthesis - representative reactions [12].

* To whom all correspondence should be sent:
E-mail: ilian.kolev@mu-varna.bg

Moreover, S_8 is the reagent of choice when performing dehydrogenation of aromatizable substrates (hydrocarbons) (Fig. 2):

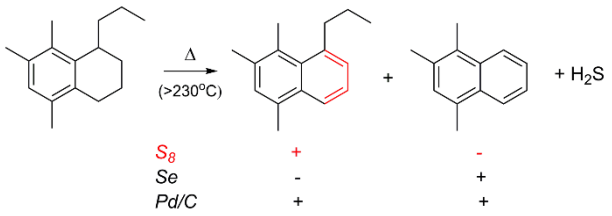


Figure 2. Illustration of the effectiveness of elemental sulfur in dehydrogenating reactions [12, 13].

In the majority of cases, however, when nonvolatile organic samples containing secondary alcohol groups are fused for a short time with sulfur, hydrogen sulfide is split off no matter what other functional groups or elements are present in the analytes' composition [14]. From the analytical point of view, the evolved H_2S gas, in turn, can be readily detected, even in traces, with a piece of filter paper moistened with $Pb(OAc)_2$ (Fig. 3).

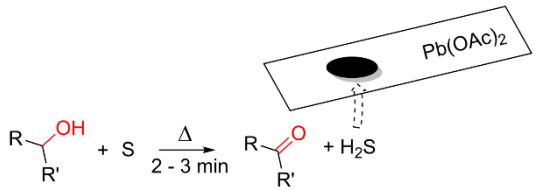


Figure 3. Schematic representation of the oxidation reaction of secondary alcohols in the presence of S_8.

According to Feigl [14] the current redox reaction seems to be especially realizable with analytes that melt at 120÷180°C.

In this context, an analogous pyrolytic splitting out of H_2S gas may occur when long-chain fatty acids (*e.g.*, palmitic, stearic, and oleic acid) and waxes (*non-aromatizable representatives*) are heated to about 250°C along with S_8 [14] by analogy with the ex-mentioned aromatizable hydrocarbons (Fig. 2).

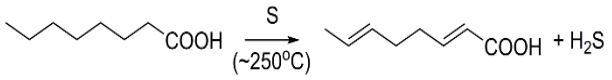


Figure 4. Oxidation of long-chain fatty acids with elemental sulfur

In these and other cases (as shown in Figs. 2 and 4; *cf.*), however, the reaction proceeds so sluggishly that no traceable result can be sensitively (and readily) detected within the first three minutes; even when a lot of S_8 is used. (Even when heated with small amounts of S_8, secondary alcohols rapidly release hydrogen sulfide; unlike other classes of compounds).

A series of positive analytical tests including the pharmacopoeial representatives *Chinidini sulfas* and *Chinini sulfas* was successfully carried out in order to boost the potential of Feigl's test when utilized for pharmaceutical analyses. A strategy for converting both drugs into analytically pure alkaloid bases was also employed. Moreover, the need of introducing S_8 in the form of a CS_2 solution was eliminated by the methodological modification imposed herein. The paper also presents an original microanalytical method for estimating the LOD values of the two alkaloids.

MATERIALS AND METHODS

All chemicals were of analytical grade and used as received without any further purification: quinidine sulfate salt dihydrate (≥ 80% quinidine; ≤ 20% dihydroquinidine, Sigma-Aldrich), quinine hemisulfate salt monohydrate (BioReagent, ≥ 98%, Sigma), sulfur (99.5+%, refined, Acros Organics), $NaHCO_3$ (99.5%, for analysis, Acros Organics), NaI (99+%, extra pure, Fisher Chemicals). The used solvent (acetone in this case) was received from Fisher Scientific. All tests were performed in a well-ventilated hood. To confirm the repeatability of the current analytic procedure, all trials were repeated thrice three days apart.

Procedure of acetone purification

As used herein "commercially available acetone" was purified by the following method [15]: In a 100 mL round-bottom flask, approximately 10 g of finely powdered NaI was dissolved in 50 mL of boiling acetone. Crystals of $NaI.3Me_2CO$ were obtained from the solution thus prepared at cooling to -8°C (in a freezer). The crystals were filtered off and washed thoroughly with "hot" air. Acetone distils off readily on warming.

Conversion of quinidine sulfate to quinidine base

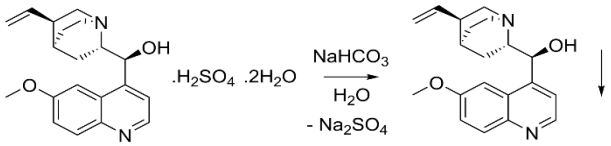


Figure 5. Synthesis of quinidine base from quinidine sulfate.

The conversion of quinidine sulfate to quinidine (Fig. 5) was accomplished by the following protocol: Quinidine sulfate (0.3 g) was dissolved in 30.0 mL of distilled water in a 50 mL beaker under constant magnetic stirring at room temperature. After 10 minutes, 0.6 g (~10 equivalents) of $NaHCO_3$ was added in small portions to the resulting solution still under continuous stirring (300 rpm). After

completing the addition of $NaHCO_3$, the resulting slurry was stirred for additional 30 minutes. The obtained precipitate was removed by filtration (*via* a glass funnel filter with sintered glass disc) and washed repeatedly with precooled distilled water. The crude product thus obtained was kept in a vacuum desiccator charged with P_2O_5 for a period of 48 hours (yield = 95.0%). Melting range (after drying at 120°C for 1 hour): 171.5÷172.4 °C (determined in an open capillary tube with a Krüss Optronic melting point apparatus). Ref. [16]: M.p. 174÷175°C (anhyd.).

Conversion of quinine sulfate to quinine base

As mentioned previously in the case of the conversion of quinidine sulfate to quinidine base the yield = 97.0%. Melting range (after drying at 120°C for 1 hour): 174.5 ÷ 176.8 °C. Ref. [16]: M.p. 176÷177°C (anhyd.).

Spot test procedure

A glass microtest tube (0.1 mL capacity) was used. A little of the solid (~1÷2 mg; quinine-*base* or quinidine-*base*, respectively) was treated with a pinch of sulfur. The mouth of the tube was plugged up with a piece of filter paper moistened with $Pb(OAc)_2$. The tube was then placed in a silicone bath previously heated to 200°C. If necessary, the temperature might be raised to 210°C. If secondary alcohols were present, a black or brown stain (of PbS) appeared on the paper within three minutes.

Limit of detection (LOD)

Glass capillary microtubes with a capacity of 0.045 mL were used to evaluate the analytical sensitivity. The tubes were firstly loaded with a minimum amount of elemental sulfur; so as to obtain a S_8 deposit of about 3.0 mm each in height. Then, by means of a microsyringe, aliquots (2.0 µL) of each test solution were directly injected into the sulfur content of each test tube. For the purpose of the analysis, a set of standard (acetone) solutions with known concentrations of both alkaloids was prepared, namely: 8.0, 6.0, 4.0, 2.0, 0.8, 0.2, and 0.08 mg/mL. The contents of the tubes were then brought to dryness in a drying oven at 40°C.

Next, the open ends of the tubes were stopped with a piece of filter paper moistened with a drop (~ 5.0 µL in volume) of 10% lead acetate solution. The tubes were then immersed a few millimeters deep into a silicone bath pre-heated to 200°C. The appearance of traces of PbS (which we interpreted as a positive response) on the reagent paper revealed the formation of hydrogen sulfide. All tests were performed in triplicate. In addition, when testing acetone solutions, it is advisable to perform a blank test with the solvent used.

RESULTS AND DISCUSSION

To supplement the potential of Feigl's test in the field of the pharmaceutical analysis, we initiated a series of tests for investigating with S_8 the reactivity (reduction one) of the medicines in question (Fig. 6). It should be pointed out that, in the cases where salts of organic bases are analyzed, it is necessary beforehand to release the organic constituent (low-melting organic base) from the inorganic one. That is why, in the current work, the procedures for the synthesis of the relevant alkaloid bases are presented in more details. The chosen strategies for purifying the corresponding bases and solvent (acetone), at large, exclude the possibility of the appearance of artifacts.

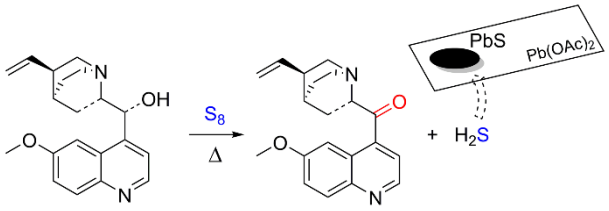


Figure 6. Proposed route of the applied redox reaction between the used Cinchona' alkaloids and elemental S_8.

Actually, as expected, a positive analytical response was observed when an insignificant amount of the examined compounds (powders) was fused with elemental sulfur (Fig. 7A). The appearance of black-colored spots of PbS on the impregnated caps was recorded within one minute. The maximum time required to reach the level of assured analytical perception (maximum intensity), however, was estimated to be 3.0 minutes.

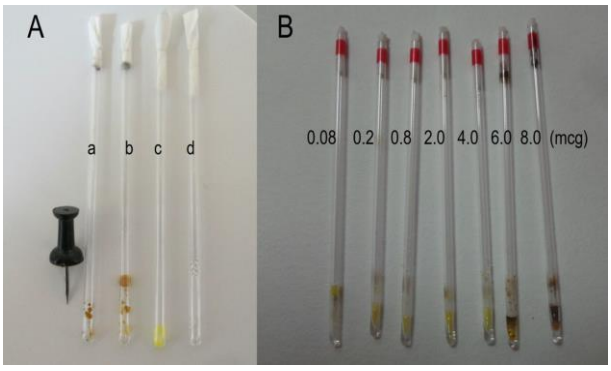


Figure 7. A. A photograph illustrating the appearance of a dark stain (of PbS) on $Pb(OAc)_2$ paper piece when a little of quinidine base was fused with S_8 (tubes **a** and **b**), as well as the absence of a false-positive result from the independent melting of sulfur (**c**) and quinidine (**d**); **B.** A photograph illustrating the result of the LOD determination technique thus applied.

From an analytical point of view, however, the so-formed PbS spots may also be used as a means (analytical marker) for estimation of the so-called "limit of detection" - the numerical expression of the sensitivity (organoleptic one) in the present analytical test (Fig. 7B). So, properly "configured", the present method can also be employed as an analytical tool for detecting traces of the investigated analytes. Though, to register extremely small amounts of the analytes in question it is necessary to select reaction vessels with the smallest possible capacity. For this purpose, glass capillary microtubes with a total capacity of 45 microliters were selected as completely suitable. Regarding the precise and accurate transfer of submicrograms of both analytes in each microtube, the *dried droplet sample deposition technique* was employed in preparing all the samples [17, 18]. Actually, this technique allowed us to examine the two analytes in the form of micro-residues evaporated from acetone (onto the surface of S_8 particles).

Aliquots of each working solution were withdrawn using a 10-µl GC micro-syringe to be delivered exactly where needed, *i.e.* at the bottom of each capillary microtube; and then transferred directly into the volume of S_8 already introduced. The total length of the needle used has, however, a direct bearing on the reproducibility of the test; it must reach the bottom of each capillary microtube. As expected, the analysis revealed that the examined analytes thus deposited into the vessel content, do come into direct contact with the introduced oxidizing agent - S_8. Otherwise, a part of the analyte will be deposited outside the reaction zone, *i.e.* far from the deposited sulfur. As for the analytical sensitivity already achieved, its value will be reduced drastically in this case.

Special precautions must also be observed when evaporating the solvent (acetone) used. Much attention should be paid to avoid localized overheating the samples. Otherwise, significant amounts of the introduced samples can be pushed out of the capillary volume. That is why the resulting suspensions were allowed to evaporate slowly in a drying oven - at 40 °C in a matter of hours. The little that ultimately remains in every capillary must visually resemble the sulfur implemented - the reagent used in excess. All capillaries were then plugged with small pieces of tightly wound filter paper. The latter should be wetted with a specific amount of freshly prepared solution of lead acetate; taking care, here, the droplets deposited not to exceed the sorption capacity of the paper stoppers used. After being thus charged and plugged, the capillaries were placed in a silicone bath preheated to 200°C. The total annealing time of the samples was 3 minutes. During this time, the main analytical (oxidation) reaction took place. Along with this, the resulting melt also changes both its color and its texture. The resulting dark brown color of the melts was preserved even after their cooling/solidifying. Cooled to room temperature, the samples were allowed to stand for another 3 minutes, but in a horizontal position. That allowed the residual H_2S to react exhaustively with the impregnated lead acetate. Thus conducted, the method is able to establish minimal amounts of the analytes studied.

Using the newly developed analytical protocol, we were able to establish accurately the limits of detection (LODs) of the two alkaloids - *quinine* and *quinidine*. The analysis showed that the magnitudes of these values for both analytes were equal to ~ 0.006 mg (or ~ 6.0 µg). Furthermore, the results established here, are very similar to those recorded by Feigl, but for other alkaloids [5, 16].

In order to justify the above-proposed route of the applied redox reaction and exclude a possible presence of false-positive reactions, additional tests were separately performed both with S_8 (Fig. 7A-c) and with quinidine (Fig. 7A-d).

As expected, none of the blank samples used gave a false-positive outcome. A negative response was also registered even at fusing of larger amounts of the high-melting drug salts used - *Chinidini sulfas* and *Chinini sulfas*.

Moreover, the proposed method applied on a much larger scale, may be particularly useful in the production of the alkaloid *quininone* (Fig. 6).

CONCLUSION

The present paper describes yet another analytical strategy for the *quinidine* and *quinine* qualitative analysis - a strategy that selectively registers the presence of hydroxyl groups in the analytes studied. The need of using harmful solvents has also been rejected by the imposed herein modifications of the method.

Being accurate and reproducible, the presented approach appears to be completely applicable and suitable for the routine second identification analysis (pharmaceutical one) of the raw drug substances *Chinidini sulfas* and *Chinini sulfas*.

The benefits of the method are: its employing inexpensive and easily available chemicals – elemental sulfur, acetone, and its being easy to implement by a wide range of researchers and even students.

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