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Notes

Rapid Spot Test Analysis for the Detection of Dipyrone in Pharmaceutical Preparations

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In June, 1998, the Brazilian people were severely shaken by an astonishing stream of falsification and adulteration concerning commercially available medicines. The first signs were detected with certain brands of contraceptives and prostate cancer medicines, whose active substances were completely replaced by wheat flour (leading to several undesired pregnancies and premature deaths, respectively) as well as antibiotics without any therapeutic action.[1] Soon afterwards it was ascertained that the falsification was of widespread nature, including almost all kinds of pharmaceutical preparations.[1] Even simple formulations (both solid and liquid) comprising drugs widely used as analgesics and antipyretics (*e.g.*, dipyrone-based formulations) were the target of illegal laboratories and some gross drug resellers, in terms of falsification and adulteration.[1] The situation still remains,[2] and there is strong evidence that such anomalies also occur in other countries,[1] including the United States of America and the European Community.[2]

Dipyrone (sodium salt of 1-phenyl-2,3-dimethyl-4-methylaminomethanesulfonate-5-pyrazolone) is marketed in Brazil as such or as the magnesium salt as well as in association with other drugs through 51 and 75 registered tradenames, respectively,[3] making very difficult and time-consuming for the Public Health Service officials to detect falsifications and/or adulterations in dipyrone-based products through the application of the presently available methods for dipyrone identification and determination.[4-6]

From the above considerations, it is clearly apparent that there was an urgency for a quick, simple, inexpensive, sensitive and reliable dipyrone test that could be used routinely for mass screening examinations to detect possible falsifications. Spot-tests[7,8] seem to fulfill all or, at least, most of the aforementioned requirements. In the present work, a spot-test devised by Feigl[7] for pyramidone (a dipyrone analogue; 1-phenyl-2,3-dimethyl-4-dimethylamino-5-pyrazolone) was conveniently adapted and refined, enabling us to establish a simple emergency kit for the efficient and easy performance of dipyrone tests by semiskilled personnel even in the drugstore laboratory (or office) as well as in a mobile screening operation.

Experimental

Reagents

All chemicals were of analytical reagent grade and were used without further purification. Concentrated sulfuric acid (96%) and chromotropic acid (disodium salt, dihydrate) were obtained from "Merck" AG, Darmstadt, Germany. Dipyrone (monohydrate) was purchased from Daichi Seiyaku Co. Ltd., Tokyo, Japan. All pharmaceutical formulations were purchased from utmost reliable drugstores. Doubly-distilled and deionized water was used throughout. The substances used in interference studies, all P. A. or pharmaceutical grade, were purchased from several commercial sources or kindly supplied by pharmaceutical industries.

Procedure

A tiny portion of a solid sample (*ca*. 1 mg) is placed in a micro test tube or in a spot plate depression along with 10 – 15 mg of the disodium salt of chromotropic acid. A drop of concentrated sulfuric acid is added. The resulting suspension is next treated with a drop of water. The mixture instantaneously boils (*i.e.*, a self-heating system is produced) and an immediate color development indicates a positive response. The color ranges from intense bright-blue to faint violet-red, according to the amount of dipyrone present. When slight amounts of dipyrone are involved, it is advisable to run a comparison blank test. For liquid aqueous samples, add a drop of concentrated sulfuric acid to 10 – 15 mg of the disodium salt of chromotropic acid. The mixture is then treated with a drop of the test solution. Identification limit: 125 μg of dipyrone, for both solid and liquid samples.

Results and Discussion

The following commercial pharmaceutical formulations comprising dipyrone in association with several other drugs have been tested:

a) Tablets (tradename; dipyrone content, mg/tablet; manufacturer): 1. (Anador; 500; Boehringer/De Angeli), 2. (Baralgin; 500; Sarsa), 3. (Buscopan; 250; Boehringer/De Angeli), 4. (Cefaliv; 350; Ache), 5. (Conmel; 320; Sanofi-Winthrop), 6. (Doralgin; 300; Dorsay), 7. (Fluviral; 250; Biolab/Searle), 8. (Lisador; 500; Farmasa), 9. (Magnopyrol; 500; Abbott), 10. (Nevralgina; 500; Climax), 11. (Novalgina, 500; Hoechst), 12. (PAR; 500; Sanofi-

This paper is dedicated to the memory of Professors Waldemar Saffioti (1922 – 1999) and Manuel Molina Ortega (1931 – 1999).

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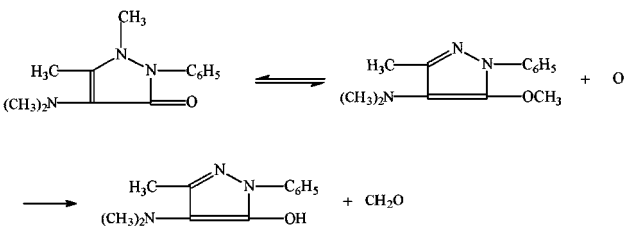
Winthrop).

b) Solutions (tradename; dipyrone content, mg/ml; manufacturer): 1. (Dorflex; 300; Merrel-Lepettit), 2. (Lisador; 333; Farmasa), 3. (Magnopyrol; 400; Abbott), 4. (Neosaldina; 300, Knoll), 5. (Nevralgina; 500, Climax), 6. (Toloxin; 400; Biolab). The results were always positive.

As already mentioned, an earlier version of this test was first described by Feigl[7] for the detection of pyramidone. It is based on a very selective oxidation of that compound in the presence of sulfuric acid, splitting out formaldehyde. The reducing action of pyramidone is related to its tendency to tautomerize;[9] this is a general characteristic of pyrazolones.[9,10] The oxidation probably begins with the isomeric methoxy form[7] of the pyrazolone (Scheme 1):



Scheme 1

The formaldehyde, once formed, is identified by warming it with chromotropic acid, also in sulfuric acid solution, yielding a soluble violet-red color.[7] The nature of this chromogen has never been unambiguously proven, but more recent experimental evidence[11] supports the hypothesis that it has a mono-cationic dibenzoxanthylium structure, formed through a three-step process. Sulfuric acid participates in all steps of this reaction; heating is essential: no color appears at room temperature (20 – 30˚C). This test was first developed into a quantitative spectrophotometric method for dipyrone in this laboratory[4] by using 15 M sulfuric acid and heating the solutions in a boiling water bath for 30 min. For pyramidone testing, Feigl[7] recommends mixing of one drop of the test solution with 2 ml of 6 M sulfuric acid and heating for 10 min in a water bath at 60˚C. In a screening test for formaldehyde in foodstuffs, based on its reaction with chromotropic acid, the resulting system is boiled in a water bath for 10 min.[8] Conventional heating devices (*e.g.*, microburners, water baths, infrared lamps, hair dryers) are certainly a cumbersome addition to an emergency test kit. Thus, a major advantage of the presently proposed test stems from the self-heating system generated by the instantaneous mixing of water (or aqueous solutions) with concentrated sulfuric acid.

Variable parameters affecting dipyrone oxidation and the coupled color reaction with chromotropic acid were investigated. Experimental evidence[4,7] shows, in compatibility with the reactions suggested by Georghiou *et al.*,[11] that the maximum sensitivity is reached with concentrated (18 M) sulfuric acid; on the other hand, tolerance toward interferences is less in that condition, as previously pointed out[4,5] and confirmed in this work.

The sensitivity is reduced both by sulfuric acid dilution and by the significant limitation in self-heating time, as compared with the aforementioned water bath procedures.[4,7,8] The best compromise between sensitivity and selectivity was found by developing the reactions in *ca.* 11 M sulfuric acid, according to the recommended procedure. Lack of interference from the most common excipients and active drugs, especially those often associated with dipyrone formulations, advantageously outweigh sensitivity reduction as dipyrone usually appears in relatively high concentrations as a component of the available solid and liquid dosage forms (*i.e.*, 250 – 500 mg/tablet and 300 – 500 mg/ml).

The possible interference of excipients, additives, diluents, flavoring agents and drugs commonly present in commercial pharmaceutical formulations was investigated. It has been found that talc, starch, gelatin, arabic gum, sodium sulfite, ethanol, magnesium stearate, carnauba wax, white wax, titanium dioxide, polyethyleneglycol, anise essence, polyvinyl pirrolidone, galactose, caffeine, hydrochlorides of adiphenine, papaverine and *N*-phenylpropanolamine, hyoscine hydrobromide, *n*-butylscopolamine bromide, dihydroergotamine mesilate, orphenadrine citrate, homatropine methylbromide, prometazine, isometheptene, ascorbic acid, aspirin, antipyrine, phenylbutazone, oxyphenbutazone, ibuprofen, salicylamide, acetaminophen, phenacetin and acetanilide do not interfere with the proposed test for detecting dipyrone. Lactose (used as excipient), glucose and sucrose (found mostly in sugar-coated tablets) also do not interfere within the amounts often employed in dosage forms[3] (*i.e.*, 50 – 200 mg/tablet or ml).

The following substances have been previously tested[5] for interference, in more drastic experimental conditions, concerning sulfuric acid concentration, heating time and temperature, in comparison with those prevailing in the spot-test proposed in this work: citric acid, hydrochlorides of procaine, chlorpromazine and ephedrine, phenobarbital, meprobamate, butylhyoscine, dexamethasone, vitamin B_1 and codeine phosphate. None of these substances were found to interfere.[5] Interference was noted for pyramidone, urotropine, diazepam and the hydrochlorides of quinine and tetracycline. The test sensitivity is almost the same for pyramidone and dipyrone; urotropine also reacts, displaying high sensitivity. However, the use of pyramidone in pharmaceutical preparations has sharply declined in the last years, being largely replaced by dipyrone; this last drug is cheaper, much more water-soluble and more effective, both as pain-relief and antipyretic agent, in comparison with pyramidone. Urotropine, diazepam, quinine and tetracycline are seldom included in dosage forms comprising dipyrone.[3] Therefore, their potential interference does not bear practical significance.

The lack of interference from aspirin, acetanilide, phenacetin, antipyrine, salicylamide and acetaminophen is noteworthy because all of these drugs are mild analgesics as well as antipyretics, and are cheaper than dipyrone (much cheaper, in the case of aspirin, antipyrine, phenacetin and acetaminophen). Falsifications arising from the total replacement of dipyrone by any one of these drugs can, therefore, be readily detected by the chromotropic acid test.

Adulteration of dipyrone preparations, *i.e.*, the partial substitution of that drug for other inferior material(s) cannot be assessed by the proposed spot-test. In that case, a first stage of sample examination requires the development of, at least, a semiquantitative procedure for the evaluation of dipyrone. As already stated, in the case of a positive reaction a faint violet-red to a bright-blue color is displayed, the intensity of which is proportional to the amount of drug present. The developed color may be compared with a previously prepared color chart. However, to avoid any observers' errors in judging these charts, the introduction of a simple reflectometer capable of quantitating the degree of color change would certainly improve the analytical procedure.[8]

Preliminary experiments have shown that the reactions which form the basis of the spot test proposed in this work can also be successfully carried out on a silica thin-layer plate. Appropriate

heating (aiming at sensitivity increase) has been provided by a hair dryer. This result suggests the possibility of performing quantitative spot test analysis *via* reflectance measurements along the lines recently established by Tubino *et al*.[12] Work in this direction is currently in progress.

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