

# REPLACEMENT EXHIBIT 2050

ISSN 0014-827 X

FRP SAX pag. 381-456  
Vol. 49, No. 6, June 1994

MR  
10

W1 FA826F  
NO. 6 1994  
-----SEQ: SR0065843  
FARMACO 08/04/94



# IL FARMACO

An International Journal of Medicinal Chemistry

Published by  
Società Chimica Italiana  
Viale Liegi 48 - I 00198 Roma, Italy

00005	FARMACO
1994 VOLUME 49 ISSUE 6	
SISAC	
0014-827X(1994)49:6;1-Q	
W1 FA826F	
S045093/01	

00006096712024  
00628700

Spedizione in abbonamento postale /50

“TAXE PERCUE” “TASSA PAGATA” PADOVA C.M.P.

This material was copied  
at the NLM and may be

# IL FARMACO

---

Thanks are due to

Consiglio Nazionale delle Ricerche (C.N.R., Roma) for financial support of the journal

Supporting Subscribers:

Ordem dos Farmaceuticos - Soc. Farmaceutica Lusitana Rua da Sociedade Farmaceutica, 18 - LISBONA

Boehringer Ingelheim Italia S.p.A. - Via Serio 15 - 20139 MILANO

Glaxo S.p.A. - Biblioteca Scientifica - Via Fleming 2 - 37100 VERONA

Menarini Ricerche Sud S.p.A. - Via Tito Speri 10 - 00040 POMEZIA (Roma)

---

## ABBONAMENTO SUBSCRIPTION FEE 1994

Italia .....	Lit. 300.000
Abbonamento sostenitore .....	Lit. 1.600.000
Europe and Mediterranean Countries .....	Lit. 450.000
(postage included)	
Transoceanic Countries .....	Lit. 500.000
(postage included)	

Payment to be made to:  
**Società Chimica Italiana**  
Viale Liegi, 48 - 00198 ROMA

---

**PUBLISHED BY SOCIETÀ CHIMICA ITALIANA**

Viale Liegi, 48 - 00198 ROMA

---



Rivista associata all'Unione Stampa Periodica Italiana

RIVISTA PUBBLICATA MENSILMENTE e distribuita prevalentemente e gratuitamente ai Soci.

Direttore responsabile GIOVANNI RODIGHIERO - Iscr. Trib. Roma N. 438/90

---

Stampa LA PHOTOGRAPH - Tel. 049/8625690 Albignasego (PD) - LASER fotocomp. - Tel. 049/609358 PD

This material was copied  
at the NLM and may be

Contents

ORIGINAL ARTICLES

- D. BONAZZI, V. ANDRISANO, A.M. DI PIETRA, V. CAVRINI - Analysis of trimethoprim-sulfonamide drug combination in dosage forms by UV spectroscopy and liquid chromatography (HPLC) 381
- V. ANDRISANO, R. GOTTI, A.M. DI PIETRA, V. CAVRINI - Comparative evaluation of three chromatographic methods in the quality control of fatty alcohols for pharmaceutical and cosmetic use 387
- C. ALTOMARE, S. CELLAMARE, A. CAROTTI, M. FERAPPI - Linear solvation energy relationships in reversed-phase liquid chromatography. Examination of RP-8 stationary phases for measuring lipophilicity parameters 393
- M.G. QUAGLIA, E. BOSSÙ, C. DESIDERIO, S. FANALI - Use of capillary electrophoresis for testing the stability of a drugs mixture in perfusional solution 403
- F. BARBATO, P. MORRICA, S. SECCIA, M. VENTRIGLIA - High performance liquid chromatographic analysis of quinolone antibacterial agents 407
- V. FERIOLI, G. GAMBERINI, C. RUSTICHELLI, F. VEZZALINI - Direct determination of non-UV-absorbing compounds by high-performance liquid chromatography 411
- G. GAMBERINI, V. FERIOLI, C. RUSTICHELLI, F. VEZZALINI - Calorimetric and infrared spectrophotometric study of ethambutol dihydrochloride 415
- V. FERIOLI, C. RUSTICHELLI, F. VEZZALINI, G. GAMBERINI - Determination of azelaic acid in pharmaceuticals and cosmetics by RP-HPLC after pre-column derivatization 421
- S. TIRENDI, T. LANCETTA, E. BOUSQUET - Estrogens determination in urine by RP-HPLC with UV detection 427
- G. BOCCARDI - Autoxidation of drugs: prediction of degradation impurities from results of reaction with radical chain initiators 431
- R. FICARRA, P. FICARRA, S. TOMMASINI, M. CARULLI, D. COSTANTINO, M.L. CALABRÒ - Chromatographic investigations on brotizolam 437
- E. MARIANI, A. BARGAGNA, M. LOMGOBARDI, E. BRUSCHI - Gas chromatographic determination of chlorinated pesticides and PCBs in complex cosmetic matrices 441
- E. BÜYÜKBİNGÖL, S. SÜZEN, G. KLOPMAN - Studies on the synthesis and structure-activity relationships of 5-(3-indolal)-2-thioindantoin derivatives as aldose reductase enzyme inhibitors 443
- N. ERGENÇ, G. ÇAPAN - Synthesis and anticonvulsant activity of new 4-thiazolidone and 4-thiazoline derivatives 449
- G. PIFFERI, R. NIZZOLA, A. CRISTONI - Synthesis and muscle relaxant activity of cyclic baclofen analogues 453

This material was copied  
at the NLM and may be

## AUTOXIDATION OF DRUGS: PREDICTION OF DEGRADATION IMPURITIES FROM RESULTS OF REACTION WITH RADICAL CHAIN INITIATORS (\*)

GIOVANNI BOCCARDI

Sanofi Recherche, Centro Ricerche Sanofi-Midy S.p.A,  
via Piranesi 38, 20137 Milan, Italy.

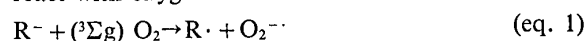
*Summary* — In the study of the degradation of drug substances by molecular oxygen, their specific reaction mechanisms must be taken into account. The rate-determining step is usually the reaction of the substrate with a radical chain initiator, which is often an unknown impurity. The reactivity and selectivity of autoxidation can be controlled better by using a radical chain initiator, such as AIBN, than by changing the temperature or the oxygen pressure. In this paper the products profiles of four pharmaceutical substances in a simple oxidation test with AIBN are compared with the results of long term natural stability tests or with already established stabilities.

Stress testing is the basis of all studies of the stability of a new drug substance. The first aim of this kind of investigation is to discover the chemical and physical factors that can affect the stability of the molecule adversely, in order to design stable formulations. The second aim is to obtain samples of the drug contaminated with all possible and significant degradation impurities, in order to validate the analytical methods for the long term stability studies and to isolate the main impurities. While standard experimental conditions for the study of accelerated and long term stability are defined in all the regulatory guidelines, the protocol for the reactivity study must be fit to the particular chemistry of the molecule being examined. For investigation of the hydrolytic pathway of degradation, the general protocol is to study the effect of acids or bases at elevated temperatures on the stability of aqueous solutions of the drug substance, because it is well known that hydrolysis reactions are catalyzed by acids and bases. Oxidation is a more complex reaction, and the pharmaceutical literature describes stress testing with various oxidizing agents, such as hydrogen peroxide, heavy metal ions, acids, bases, high oxygen pressure, high temperature and, in some instances, strong oxidants such as potassium permanganate and chromic anhydride. Very often this literature emphasizes the poor predictiveness of this kind of stress testing. One reason for this poor predictiveness is that the operating mechanisms of the oxidation with the above reagents are completely different from the radical chain mechanism of autoxidation. Long term, room temperature degradation of an organic chemical is better simulated by using a radical chain initiator to accelerate the rate-controlling step of autoxidation. Use of this approach in the reactivity study has been described in the recent

pharmaceutical literature<sup>1-4</sup>. In this paper the experimental conditions for use of some radical chain initiators and the predictivity of this kind of reactivity test for four examples will be discussed.

In the electronic structure of molecular oxygen<sup>5</sup>, the highest occupied molecular orbitals are two degenerate  $\pi^*$  orbitals in which there must be two electrons. The ground state, according to the Hund rule, is the state in which each of these two orbitals is occupied by one electron, and the spins are parallel: this is the triplet ground state ( $^3\Sigma_g$ ) of the atmospheric molecular oxygen. Triplet dioxygen can be excited, both chemically and photochemically, to the first excited state with spin multiplicity 0, the singlet state  $^1\Delta_g$ , 22 kcal higher than the ground state<sup>5</sup>. The triplet ground state is the state of dioxygen involved in autoxidation. The reactivity of triplet dioxygen toward organic molecules can be summarized as follows.

Electron-rich molecules such as pyrroles<sup>6</sup>,  $\alpha,\beta$ -unsaturated enamines<sup>7</sup>, carbanions<sup>8</sup>, 9,10-cyclopentane-4a,4b-dihydrophenanthrene<sup>9</sup>, strained cycloalkenes<sup>9</sup> and, under more drastic conditions, tertiary amines, sulfoxides, alkenes and alkynes<sup>10</sup> can react with oxygen in an electron-transfer reaction:



In addition, triplet oxygen reacts very fast with organic radicals:



and this reaction is very important in propagation of radical chains.

However the vast majority of organic molecules are in the singlet state, and their reaction with triplet dioxygen:



is spin forbidden. For this reason, a great many organic molecules, in spite of the large negative value of the Gibbs free energy of oxidation, are kinetically inert

(\*) Presented at the V Convegno su recenti Sviluppi ed Applicazioni nell'Analisi Farmaceutica, Alghero, October 13-16, 1993.

# Explore Litigation Insights

Docket Alarm provides insights to develop a more informed litigation strategy and the peace of mind of knowing you're on top of things.

## Real-Time Litigation Alerts



Keep your litigation team up-to-date with **real-time alerts** and advanced team management tools built for the enterprise, all while greatly reducing PACER spend.

Our comprehensive service means we can handle Federal, State, and Administrative courts across the country.

## Advanced Docket Research



With over 230 million records, Docket Alarm's cloud-native docket research platform finds what other services can't. Coverage includes Federal, State, plus PTAB, TTAB, ITC and NLRB decisions, all in one place.

Identify arguments that have been successful in the past with full text, pinpoint searching. Link to case law cited within any court document via Fastcase.

## Analytics At Your Fingertips



Learn what happened the last time a particular judge, opposing counsel or company faced cases similar to yours.

Advanced out-of-the-box PTAB and TTAB analytics are always at your fingertips.

## API

Docket Alarm offers a powerful API (application programming interface) to developers that want to integrate case filings into their apps.

## LAW FIRMS

Build custom dashboards for your attorneys and clients with live data direct from the court.

Automate many repetitive legal tasks like conflict checks, document management, and marketing.

## FINANCIAL INSTITUTIONS

Litigation and bankruptcy checks for companies and debtors.

## E-DISCOVERY AND LEGAL VENDORS

Sync your system to PACER to automate legal marketing.