

# HPLC Detection and Quantification of Radiolytic Products of Eight $\beta$ -blockers Irradiated in the Solid State and Hypotheses on Their Origins

Alix Engalytcheff,<sup>1,2</sup> Jean-Paul Vanhelleputte,<sup>1</sup> and Bernard Tilquin<sup>1</sup>

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**Purpose.** The radiolytic products of eight  $\beta$ -blockers were studied in order to understand the mechanisms of irradiation of drugs in the solid state.

**Methods.** The drugs were analyzed by high-performance liquid chromatography coupled to a diode array detector in order to observe the degradation of the main compound after irradiation and in order to study the nonvolatile final products on more concentrated solutions of irradiated drugs.

**Results.** The first test assessed that the main compound was not significantly degraded after gamma irradiation for any of the eight  $\beta$ -blockers. A more complete study, which consisted on separating the nonvolatile products and on quantifying them, indicated first that the radiolytic products could reach the number of 14 and moreover that some could exceed the 0.1% threshold at 30 kGy. Eventually, radiolytic yields were compared with radical yields previously determined.

**Conclusions.** The sensitivity of the first test can be discussed. It seems that, to study the feasibility of the radiosterilization, a complete study of the products of degradation is needed. Moreover, no correlation between radical and final products could be established, which denies that the former would be the precursors of the latter.

**KEY WORDS:**  $\beta$ -blockers; gamma radiosterilization; HPLC-UV; radical mechanisms; radiolytic yields.

## INTRODUCTION

$\beta$ -blockers are antihypertensive drugs. Some of them are given intravenously and must therefore be sterilized before being administered to patients. Some drugs used in this study are only given *per os*, but they were used to enlarge the working panel and hence enabled us a wider comparison. Because they are thermosensitive, the methods using heat (dry heat or autoclaving) cannot be used. Hence, alternative techniques are required, which are time-consuming, expensive, and not as efficient. Radiosterilization does not have these restraints and is considered as a terminal sterilization. This last property has now become requested by the European Agency for the Evaluation of Medicinal Products (EMA) (1). It would be therefore a more advantageous alternative technique. Its interest is all the more obvious in the case of thermosensitive drugs, such as  $\beta$ -blockers. Though radiation treatment of drugs is considered as a possible mean of sterilization (2,3)

and is even considered as the next best choice after the reference methods (steam and dry heat) in decisional trees (1), the mechanism linking the different radiolytic products is not clear yet and therefore must be further investigated.

Radiolysis of solid-state drugs induces indeed the production of new compounds, called radiolytic products, in very small quantities (traces). On one hand, one can observe the production of radicals by using electron paramagnetic resonance (EPR) (4), and on the other hand, there are the final products, which can either be volatile (5) or nonvolatile (6). The latter are analyzed by gas or liquid chromatography, respectively. These products are considered as impurities, and their analysis can be done by following the quality guidelines on impurities from the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) (7).

Our hypothesis on the radiolytic mechanism in solid-state drugs is based on the well-documented production and trapping of free radicals in the solid after irradiation (8–11). They could be the precursors of the final products. A model for quantification of radicals by EPR was proposed, and the determination of the radical yields for eight  $\beta$ -blockers (acetololol, atenolol, esmolol, labetalol, metoprolol, nadolol, pindolol, and propranolol) was achieved (12). The comparison of the radical yields with the ones of the final products is fundamental to validate our hypothesis. Concerning radicals, the given yield corresponds to the sum of all the radicals present and therefore only one parameter can be taken into account. On the contrary, final products can be studied individually because they are separated by high-performance liquid chromatography (HPLC), and so not only the value of the yields but also the amount of the different radiolytic products give information on the radiosensitivity of the drugs.

HPLC enables us to study the effects of irradiation of the drugs by different approaches. First, a simple and rapid analysis allows determination of the degradation of the main compound. The measurement of the loss of the drug does not give much information, and a more detailed work on the nonvolatile products is required in a second step. The detection and the separation of these compounds is delicate because they can be numerous, very close in structure from each other, and in small amounts (traces). Chromatographic parameters must therefore be set and optimized for the analyses of the radiolytic compounds.

## MATERIALS AND METHODS

### Drugs

$\beta$ -blockers were purchased from Sigma (St. Louis, MO, USA) (minimum 99%) except propranolol, which was from Fluka (Buchs, Switzerland), and esmolol hydrochloride, which was kindly provided by Baxter (Nivelles, Belgium). The water content is very low (below 1%). The chemical structure of the eight molecules can be found in Ref. 12.

### Irradiation

Gamma irradiation was performed with a panoramic <sup>60</sup>Co chamber (UCL, Louvain-La-Neuve, Belgium) at room

<sup>1</sup> Laboratory of Chemical and Physicochemical Analysis of Drugs (CHAM), Université Catholique de Louvain, Brussels, Belgium.

<sup>2</sup> To whom correspondence should be addressed. (e-mail : alix.engalytcheff@cham.ucl.ac.be)

temperature and with a dose rate of 417 Gy·h<sup>-1</sup>. This source was calibrated with an alanine dosimetry: alanine pellets were supplied and analyzed by Risø National Laboratory (Denmark). The samples were irradiated in closed vials protected from light and received a single dose of 30 kGy.

### Aqueous Solutions

In the eight cases, the concentration of the main compound for the solutions of the content tests is 0.1 mg/ml. The concentrations of the solutions for the analyses of the final products are different and are given in Table I.

### Liquid Chromatographic System

The HPLC system consisted of a Merck-Hitachi D-6000 equipped with a Rheodyne (Cotati, CA, USA) manual injector with a loop of 20 µl, 2 pumps (L-6200), an oven (T-6300), and a UV-visible diode array detector (L-4500).

The chromatographic separation was performed on a 250 × 4 mm LiChrospher RP-8 Select B column, 5-µm particle size at a flow rate of 1 ml/min or on an Aluspher RP Select B for pindolol (Merck, Darmstadt, Germany).

Six of the impurities separations were achieved via ion-pairing chromatography. Negatively charged ion-pairing agents were used. The mobile phase consisted of a mixture of acetonitrile and an aqueous solution. In the case of atenolol, acebutolol, metoprolol, and propranolol, the aqueous solution was composed of 0.01 M heptane sulfonic acid sodium salt and 0.01 M of potassium dihydrogen phosphate. Phosphoric acid was added to adjust the pH to 2.00. In the case of esmolol, heptane sulfonic acid sodium salt was replaced by pentane sulfonic acid sodium salt and, in the case of nadolol, by octane sulfonic acid sodium salt.

In the case of pindolol, the aqueous solution was composed of 10<sup>-2</sup> M NaOH and 7.5 mM tetrabutylammonium.

In the case of labetalol, the pH of the aqueous solution was simply adjusted with concentrated formic acid in order to reach a value of 2.00. Methanol was used as the organic solvent.

The aqueous solutions were all filtered through an 0.45-µm filter.

Details of the chromatographic conditions (temperature,

wavelength, and concentration of the drug) for the eight β-blockers are given in Table I.

Deionized water was generated from the Milli-Q water purifying system purchased from Millipore Corporation (Bedford, MA, USA). Acetonitrile of HPLC grade was purchased from J.T. Baker (Phillipsburg, NJ, USA). Methanol was distilled in our laboratory. All other reagents were of analytical grade.

### Detection and Quantification

The limit of detection (LOD) and the limit of quantification (LOQ) are considered as 3 and 10 times, respectively, the signal-to-noise ratio.

## RESULTS

### Dose

The analyses show the comparison between the solutions from irradiated and nonirradiated powders of β-blockers. In the pharmacopoeias, the reference dose is considered to be 25 kGy (2, 3). It is now desirable and approved to diminish the dose if the sterility of the drug can still be reached (2). In this study, the drugs were irradiated with 30 kGy, which is a little higher than the reference dose. From the EPR results, β-blockers seemed indeed radio-resistant and a high dose had to be given in order to observe the radiolytic products. The dose was chosen to be compatible with the previous EPR measurements in the scope of a study of the radiolytic mechanisms.

### Preliminary Test (Content Test)

Pharmaceutical firms use a simple preliminary test to determine if irradiation induces changes in the solid. Diluted solutions of the drug powder are analyzed by HPLC coupled to a UV spectrometer. The comparison of the area under the curve (AUC) of the peak of the drug before and after irradiation is analyzed by a Student's *t* test in order to determine if the difference is significant. The results of this test are given in Table II. From this test, no major loss seems to be suffered for any of the β-blockers.

**Table I.** Summary of HPLC Conditions for the Eight β-Blockers Studied

Drug	HPLC type	Solvent partition (Organic:aqueous)	Time <sup>a</sup> (min)	λ (nm)	T (°C)	Concentration (mg/ml)
Acebutolol	Ion-pairing (C7) <sup>b</sup>	17–83	—	254	37	5
Atenolol	Ion-pairing (C7) <sup>b</sup>	7–93 → 12–88 <sup>e</sup>	6–11	232	34	2.5
Esmolol	Ion-pairing (C5) <sup>b</sup>	13–87 → 20–80 <sup>e</sup>	18–25	200	34	2.5
Labetalol	Mass conditions <sup>c</sup>	78–21	—	245	25	5
Metoprolol	Ion-pairing (C7) <sup>b</sup>	20–80	—	225	34	1
Nadolol	Ion-pairing (C8) <sup>b</sup>	9–91 → 17–83 <sup>e</sup>	8–13	200	35	2.5
		17–83 → 22–78 <sup>e</sup>	13–21			
Pindolol	Use of additives <sup>d</sup>	8–92	—	219	28	1
Propranolol	Ion-pairing (C7) <sup>b</sup>	23–77	—	220	33	2

HPLC, high-performance liquid chromatography.

<sup>a</sup> Range of time for the gradient elution.

<sup>b</sup> Ion-pairing HPLC with negatively charged agents: C5, pentane-sulfonate; C7, heptane-sulfonate; C8, octane-sulfonate.

<sup>c</sup> Adjustment of the pH with concentrated formic acid.

<sup>d</sup> Tetrabutylammonium.

<sup>e</sup> Gradient elution.

**Table II.** Results of the Preliminary Test for the Eight  $\beta$ -Blockers

Drug	AUC <sup>a,b</sup> before irradiation	AUC <sup>a,b</sup> after irradiation
Acebutolol	$1.72(5) \times 10^7$	$1.70(5) \times 10^7$
Atenolol	$1.44(2) \times 10^7$	$1.39(2) \times 10^7$
Esmolol	$4.90(6) \times 10^7$	$4.86(7) \times 10^7$
Labetalol	$1.057(6) \times 10^7$	$1.06(2) \times 10^7$
Metoprolol	$1.61(2) \times 10^7$	$1.592(6) \times 10^7$
Nadolol	$9.63(3) \times 10^7$	$9.7(1) \times 10^7$
Pindolol	$2.7(1) \times 10^8$	$2.9(2) \times 10^8$
Propranolol	$3.2(1) \times 10^7$	$3.19(6) \times 10^7$

AUC, area under the curve.

<sup>a</sup> The given AUC is the mean of three trials.

<sup>b</sup> Error on the last digit is given in parentheses.

Previous studies on antibiotics have shown a significant loss of the drug after irradiation for lower doses (13, 14). The observed losses reached, in both works, 4%. In addition, they became yellow and a bad smell was given off after irradiation, which is not in accordance with the pharmacopoeia specifications. These antibiotics are considered to be radiosensitive. As no significant changes are observed for the  $\beta$ -blockers, the test seems to confirm their radio-resistant tendency.

### Study of the Nonvolatile Impurities

A more complete study of the degradation of the main compound is obtained indirectly by the study of all the final products. The comparison of the chromatographic profiles for the eight  $\beta$ -blockers before and after irradiation is given in Fig. 1. In these analyses, the solutions are as concentrated as possible, with respect to the dissolution and the separation, in order to observe the radiolytic products that are in traces. The peak of the main compound saturates. In addition to the concentration, other parameters such as the temperature, the wavelength, and the mobile phase composition must be optimized in order to get the best separation of the final products.

In the chromatograms of the eight drugs before irradiation, some initial impurities are always found. After irradiation, variations with the initial chromatograms can be observed in every case. The changes can either come from the production of a brand new compound or from the increase of a pre-existing impurity. However, there are always radiolytic products and they are numerous, pindolol excepted.

As recommended, calculations are performed using the response factor of the drug substance (7). The products are not identified, and an approximation is thus made to quantify them. Previous studies have indeed shown that the radiolytic products were very close to the main compound (15,16). Their molar absorptivities are therefore considered as similar and the quantification of the radiolytic products is obtained by comparison between their AUC and that of the main compound. In order to compare peaks in equivalent amounts, the AUC of the main compound is determined for very diluted solutions. The concentration of the radiolytic product (RP) is given in the form of a percentage of the main compound (MC), both being normalized by the exact concentration of the solutions:

$$\frac{\frac{\text{AUC}_{\text{RP}}}{\text{Conc}_{\text{RP}}}}{\frac{\text{AUC}_{\text{MC}}}{\text{Conc}_{\text{MC}}}} \times 100$$

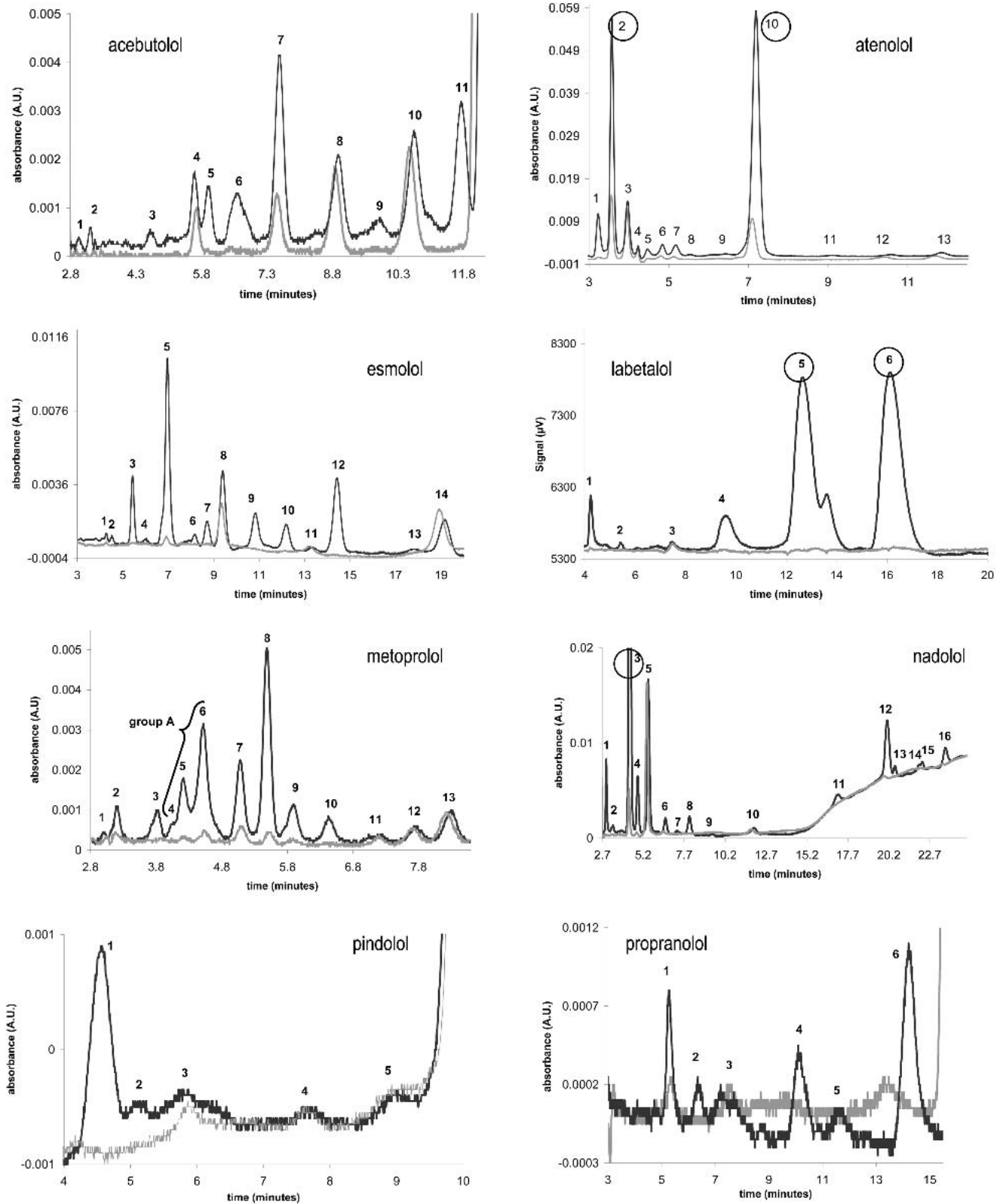
As an example, the concentrations of the impurities of nadolol before and after irradiation are given in Table III. Some concentrations could not be calculated because the AUC of the impurity was below the LOQ. The overview is the same for the eight drugs. Most of the radiolytic products of the  $\beta$ -blockers studied present a concentration below 0.1%. Only five radiolytic products are above this threshold value. They are circled on the chromatograms represented in Fig. 1. Three drugs are concerned: atenolol, labetalol, and nadolol. If we refer to the ICH norms (7), this would mean that an identification of these radiolytic products would be required if one of these drugs had to be radiosterilized. Nevertheless, it must be kept in mind that the given irradiation dose is high. In general, such a dose is not necessary to reach sterility. Hence, in practice, the drug could be sterilized for a lower dose and the concentration of the radiolytic products might not reach 0.1%.

### Radiolytic Yields of the Nonvolatile Final Products

The determination of the radiolytic yields for the non-volatile final compounds is not a necessary step to assess the feasibility of the radiosterilization of a drug. The calculation of the concentration should normally be sufficient. They are nevertheless needed to make the comparison with the radical yields established with EPR (12) in order to understand the relationships between the two types of radiolytic compounds. Thanks to the separation by chromatography, the yields can be obtained for each compound. To compare with the yields for radicals, which are global, the sum of the yields must be taken. These sums are given in Table IV together with the corresponding highest and lowest yields. These results are disparate. Esmolol shows the lowest yields, which are in the same order of magnitude as the yields of acebutolol, pindolol, and propranolol and about 10 times inferior to the yields of atenolol, labetalol, metoprolol, and nadolol. In any case, the highest yield counts for more or less half of the sum.

These results could not have been predicted on the basis of the radical yields obtained by EPR. Only the lowest values are in the same range as the radical yields. The graph of the sum of the yields of the final products as a function of the radical yields is given in Fig. 2. It shows four main different trends. Nadolol is distinguishable because of its rather high radical yield. The others have very close radical yields, and the difference lies in the final product yields. Labetalol and atenolol have very high yields. Acebutolol, esmolol, pindolol, and propranolol have yields in accordance with the radical ones. Metoprolol is in-between.

From these results, it is hard to define one single tendency. Hence, it would mean that different mechanisms can take place. The hypothesis that the radicals are the precursors can not be discarded, but it would not always be the case. In addition, these findings are very interesting because it seems that the mechanisms must be different between drugs that are very similar in structure.



**Fig. 1.** HPLC profiles of the eight  $\beta$ -blockers. The irradiated drugs are represented by the darker chromatograms and the nonirradiated ones by the lighter chromatograms.

**Table III.** Concentration of the Impurities of the Irradiated Nadolol

Impurity number	Retention time (min)	Concentration (%)	
		Before irradiation <sup>a,b</sup>	After irradiation <sup>c</sup>
1	2.9		0.008
2	3.4		0.002
3	4.4	0.013	<b>0.169</b>
4	4.85	0.001	0.009
5	5.5	0.036	0.035
6	6.5		0.004
8	8.0		0.006
10	11.9	0.002	0.003
12	20.1	0.002	0.020
13	20.6		0.002
14–15 <sup>d</sup>	22.3		0.003
16	23.6		0.006

The concentration is given as a percentage of the main compound.

<sup>a</sup> Impurities nos. 1, 2, 7, 8, 9, 11, 13, 14, 15, and 16 are not present in the chromatogram of nadolol before irradiation.

<sup>b</sup> Impurity no. 6 is present but under the LOQ.

<sup>c</sup> Impurities nos. 7, 9, and 11 are not represented as their AUC was under the LOQ.

<sup>d</sup> Co-elution of impurities.

## DISCUSSION

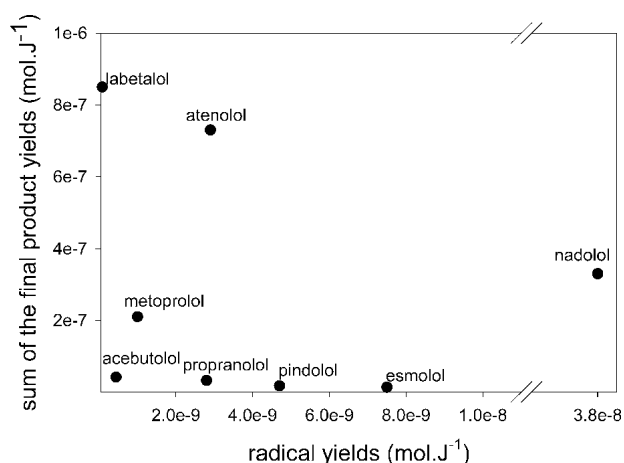
During the past decade, the question concerning the analyses of irradiated drugs has remained open. If, in the first place, the issue was about the detection of radiolytic products and, corollary, the differentiation of irradiated samples vs. nonirradiated ones (17), the debate is now focused on how determining the induced chemical changes.

The content test gives an indication of the degradation of the drug but is not very accurate. It does not show a significant difference between the irradiated and nonirradiated  $\beta$ -blockers, though the study of the impurities shows that numerous radiolytic products can be obtained and even that some are above the 0.1% threshold. Hence, as it is the easiest and quickest method to have a rough estimation, it can still be useful, but the results obtained must be used with caution.

The chromatographic profile is therefore required. In this purpose, the separation of the radiolytic products, which can be detected for highly concentrated solutions of irradiated drugs, has to be determined. Though some authors do not consider these products as unique (18), experiments have proven the contrary: they are new impurities that are not normally found in the drug (19), and therefore the conditions

**Table IV.** Radiolytic Yields of the Nonvolatile Final Products of Eight  $\beta$ -Blockers

	Radical yields ( $10^{-8}$ mol $\cdot$ J <sup>-1</sup> )		
	Sum	Highest	Lowest
Acebutolol	4	1.5	0.07
Atenolol	73	45	0.6
Esmolol	1	0.4	0.01
Labetalol	85	39	0.3
Metoprolol	21	8	1
Nadolol	33	18	0.05
Pindolol	2	2	2
Propranolol	3	2	0.6

**Fig. 2.** Relationship between the radical yields determined by EPR and the sum of the final products yields determined by HPLC.

of the pharmacopoeias do not permit us to detect these products adequately (20). When a study on radiolytic products has to be done, it is first necessary to find the new working conditions.

It is of common thought to say that final products stem from radicals (19). Therefore, investigations of radicals were carried on to understand radiolytic mechanisms (21). In the same belief, some authors have explained reactions with radical intermediaries (22). This fact is indeed true for aqueous systems (23). The chemical changes to the solutes are mostly dependant on the products of the water radiolysis, which are radicals to a greater extent. The first molecules in the solid state that have been deeply investigated were nonpolar, such as alkanes (24), for which this assumption was correct too. Nevertheless, such a relationship has never been proven yet for irradiation in the solid-state drugs, which are polar or polarizable molecules.

The yields of the final products are of an order of magnitude  $10^{-7}$  mol  $\cdot$  J<sup>-1</sup>, which cannot be considered as negligible. These findings are unexpected with regard to the radical yields determined by EPR. The hypothesis based on a correlation between the trapped radicals and the final products seems not to be correct in the case of the  $\beta$ -blockers. These results are hence in opposition with the initial thought. This is in accordance with the qualitative work on captopril (16). Neither for the  $\beta$ -blockers nor for the captopril do the radicals seem to play a key role in the formation of the final products. Up to now, a generalization cannot yet be done. Such studies on correlation between radicals and final products should be pursued in order to give a conclusive explanation of the radiolytic mechanisms in the solid state.

Ionizing radiation is considered as nonselective (23). Any part of any molecule of the system can be excited and therefore loads of types of radiolytic products are possible. This phenomenon is indeed verified by the chromatographic profiles of the irradiated  $\beta$ -blockers, which show numerous products. On the contrary, in EPR, there are several species too but just a few radicals are necessary to explain the main allure of the spectrum (15,25). These few different radicals cannot explain the variety of the final products detected. This is an agreement with the nonobservance of a correlation between the two types of radiolytic products studied.

Thus, this would imply to consider other intermediaries than trapped radicals. Hence, the formation of final products could rather be obtained through ionic transient species or radical mechanisms related to untrapped radicals.

In addition, the radiolytic mechanisms seems to be rather complicated and different, not only between two families of drugs, but even between two drugs very close in structure (25). Hence, such a correlation could not be excluded in certain cases but has not yet been observed to our knowledge. Therefore, neither an EPR test nor a content test could be considered as a general way to prove that a drug withstands radiosterilization. If drugs of the same family (and therefore very similar) react so differently toward irradiation, the question can be raised if such a general test will ever be possible.

The use of liquid chromatography coupled to mass spectrometry (LC-MS) could provide some information on the structure of the impurities (15). Such a complementary study seems necessary in order to identify the impurities above the 0.1% threshold, as requested by ICH. Even though the concentrations will be lowered for a sterilization dose underneath the one used in our study, they might stay close to 0.1%, and it would be better to perform the identification from a viewpoint of absolute safety. In addition, it would go deeper into the study of discrepancies observed in this work.

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#### REFERENCES

1. The European Agency for the Evaluation of Medicinal Products (EMA). *Decisional Trees for the Selection of Sterilization Methods* (CPMP/QWP/054/98), EMA, London, 1999.
2. Sterilization and sterility assurance of compendial articles. In *The United States Pharmacopoeia USP 24*, United States Pharmacopoeial Convention, Rockville, MD, 2000, pp. 2143–2147.
3. Textes généraux sur la stérilité. In: *Pharmacopée Européenne 4ème Edition*, Direction Européenne de la Qualité du Médicament, Strasbourg, 2002, pp. 439–444.
4. S. Colak and M. Korkmaz. Investigation of structural and dynamic features of the radicals produced in gamma irradiated sulfanilamide: an ESR study. *Int. J. Pharm.* **267**:49–58 (2003).
5. L. Hong and H. Altorfer. Characterization of gamma irradiated petrolatum eye ointment base by headspace-gas chromatography-mass spectrometry. *J. Pharm. Biomed. Anal.* **29**:263–275 (2002).
6. Ph. Horsch, L. Bigler, and H. R. Altofer. Influence of radiation sterilization on the stability of trifluorothymidine. *Int. J. Pharm.* **222**:205–215 (2001).
7. Note for Guidance on Impurities in New Medicinal Products. *Topic Q3B, step 4* (CPMP/ICH/282/95), EMA, London, 1996.
8. J.-P. Basly, I. Longy, and M. Bernard. ESR dosimetry of irradiated ascorbic acid. *Pharm. Res.* **14**:1186–1191 (1997).
9. J. Raffi, S. Gelly, L. Barral, F. Burger, P. Piccerelle, P. Prinderre, M. Baron, and A. Chamayou. Electron Paramagnetic resonance of radicals induced in drugs and excipients by radiation or mechanical treatments. *Spectrochim. Acta A* **58**:1313–1320 (2002).
10. H. B. Ambroz, E. M. Kornacka, B. Marciniak, M. Ogrodowczyk, and G. K. Przybytniak. EPR study of free radicals in some drugs  $\gamma$ -irradiated in the solid-state. *Radiat. Phys. Chem.* **58**:357–366 (2000).
11. R. Köseoglu, E. Köseoglu, and F. Köksal. Electron paramagnetic resonance of some  $\gamma$ -irradiated drugs. *Appl. Radiat. Isot.* **58**:63–68 (2003).
12. A. Engalytcheff, Deridder, R. Debuyst, and B. Tilquin. Determination of radical yields in solid-state drugs: radioresistance of beta-blockers. *Radiat. Res.* **160**:103–109 (2003).
13. A.-S. Crucq, C. Slegers, V. Deridder, and B. Tilquin. Radiosensitivity study of cefazolin sodium. *Talanta* **52**:873–877 (2000).
14. M. Gibella and B. Tilquin. Detection of the radiolysis of solid state ampicillin by UV-spectroscopy. *Analisis* **27**:657–662 (1999).
15. N. Barbarin, B. Tilquin, and E. de Hoffmann. Radiosterilization of cefotaxime: investigation of potential degradation compounds by liquid chromatography-electrospray mass spectrometry. *J. Chromatogr. A* **929**:51–61 (2001).
16. A. Engalytcheff, R. Debuyst, G. Vanhaelewyn, F. Callens, and B. Tilquin. Attempts of correlation between the radiolytic species of irradiated solid-state captopril studied by multi-frequency EPR and HPLC. *Radiat. Res.* (in press).
17. N. Barbarin, A.-S. Crucq, and B. Tilquin. Study of volatile compounds from the radiosterilisation of solid cephalosporins. *Radiat. Phys. Chem.* **48**:787–794 (1996).
18. B. D. Reid and B. P. Fairand. Gamma radiation sterilization of pharmaceuticals. In F. M. Nordhauser and W. P. Olson (eds.), *Sterilization of Drugs and Devices Technologies for the 21st Century*, Interpharm Press, Illinois, 1998, pp. 311–392.
19. F. Zeegers, M. Gibella, and B. Tilquin. Analysis of some products from the irradiation of solid chloramphenicol. *Radiat. Phys. Chem.* **50**:149–153 (1997).
20. L. Hong and H. Altorfer. Determination of assay and impurities of gamma irradiated chloramphenicol in eye ointment. *J. Pharm. Biomed. Anal.* **24**:667–674 (2001).
21. T. Mizaki, J. Arai, T. Aneko, K. Yamamoto, M. Gibella, and B. Tilquin. Estimation of irradiation dose of radiosterilized antibiotics by electron spin resonance: ampicillin. *J. Pharm. Sci.* **83**:1643–1644 (1994).
22. N. Barbarin, B. Rollman, and B. Tilquin. Role of the residual solvents in the formation of volatile compounds after radiosterilisation of cefotaxime. *Int. J. Pharm.* **178**:203–212 (1999).
23. J. W. T. Spinks and R. J. Woods. *Introduction to Radiation Chemistry*, Third Ed., Wiley-Interscience, New York, 1990.
24. B. Tilquin, J. Allaert, and P. Claes. A radical post-scavenging study of irradiated cyclic hydrocarbons in the solid state. *Bull. Soc. Chim. Belg.* **86**:777–783 (1977).
25. A. Engalytcheff, M. Kolberg, A.-L. Barra, K. K. Andersson, and B. Tilquin. Multi-frequency EPR techniques to identify the radicals produced in irradiated  $\beta$ -blockers. *Free Radic. Res.* **38**:59–66 (2004).