Radiolysis of Solid-State Drugs and the Analytical Tools Applicable to this Study

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Abstract

The use of ionizing irradiation is a technique recommended by the Pharmacopoeias to sterilise solid-state drugs. Its high efficiency (Security Assurance Level of 10^{-6}) combined to a limited rise of temperature make it the next best choice after heat techniques. Its main drawback lies in the production of unique impurities in small quantities. These products have been investigated through various analytical techniques, which are described and compared in this review. On the contrary of the irradiation of food, no protocols are defined yet. Analyses are though necessary either to determine the feasibility of the radiosterilization or to control the quality of the irradiated drug and common procedures should be established. In this perspective, we will therefore conclude by characterising the most appropriate methods.

Résumé

La méthode de la radiostérilisation est aujourd'hui proposée pour les substances thermosensibles; la radiolyse produit de nouveaux composés assez diversifiés mais en très faible quantité. Aucune pharmacopée n'est adaptée à cette nouvelle technologie car la quantité de produits radiolytiques est dans les limites acceptables. Une revue critique des méthodes analytiques à introduire dans les nouveaux protocoles est proposée.

1. Introduction

Ionising radiation is a mean of sterilising material such as drugs. Its application is wide and has notably a major impact in the pharmaceutical field. Sterilisation of drugs can indeed be required. It concerns specific routes of administrations (parenteral solutions, ophthalmic preparations and sterile topical products). Several means of sterilisation are recognised and described by the European or the US Pharmacopoeias [1,2], the reference books for the pharmacist. Radiosterilisation is one of them. This ionising treatment can either be achieved by gamma rays or by electron beams, each method having its own advantages and drawbacks [3]. The European Agency for the Evaluation of Medicinal Products (EMEA) has edited decision trees for the selection of sterilisation method for pharmaceuticals [4], which was established by the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH). In the decision tree concerning the solid-state drug, radiosterilisation ranks in second position, among all the accepted sterilisation techniques. Furthermore, the adaptation of the irradiation dose is considered.

Up to now, the reference dose is 25 kGy (Gy stands for Gray and corresponds to Joule/kg.) [1,2]. Nevertheless, it is permitted to reduce it when the prove was given that the lower dose chosen insures the sterility of the product [2]. The dose given depends notably on the bioburden of the product to be sterilised. Hence, the choice of the dose relies on the quality assurance and is a parameter of sterilisation that must be defined for each application.

The effects of irradiation on many different types of substances are studied since numerous decades. During the last ones, a more pharmaceutical point of view has been taken [5,6,7] provided that it could become a efficient method of sterilisation. Analyses were undertaken on drugs either in solution or in powder. The results for liquid solutions were deceiving, showing a very large degradation of the main compound [7]. There are indeed major differences between the radiolysis in the pure solid or in diluted aqueous solutions, which explain the discrepancies of the radio-induced effects [8].

In the case of aqueous solutions, reactive species of the "activated" solvent destroy the drug. This is reflected in the recommendations of ICH: radiosterilisation is not represented in the decisional tree for the method of selection of aqueous solutions [4]. So, the research essentially focuses now on the radiosterilisation of drugs in the solid-state. In the same viewpoint, we will limit the scope of this review to this state.

Though the aim of the investigations concerning the irradiation of pharmaceuticals were miscellaneous, some common facts can be extracted. First, irradiation might have an effect on the organoleptic properties of the drug (e.g. colour, odour ...). In addition, it produces a slight degradation of the main compound into impurities, called, in this case, radiolytic products.

As a matter of fact, the radiolysis studies have indirectly enabled the analytical techniques to be screened. In our opinion, it is very important to compare these techniques, to classify them as a function of their scope and to point out the most efficient. Moreover, the monographs of the Pharmacopoeia are poorly adapted to the analysis of radiosterilized drugs. Even though, protocols are defined in the Pharmacopoeias to analyse the related substances of drugs, they are not suitable to analyse the impurities of a radiosterilized drug since the impurity profile is for some extent different after an ionising treatment.

In a radiosterilisation viewpoint, the analytical techniques could be classified as a function of:

(a) the type of information
(b) the use of this information

Two major kinds of information can indeed be distinguished. On one hand, the results of the analyses can give an overview of the drug and, for instance, bring the information whether the drug was irradiated or not. Though this is a very simple piece of information, it can be very useful. Indications on the pre-treatment of batches of raw material is indeed necessary for pharmaceutical firms. As the question of detection of the irradiation of a substance was raised in an early stage of the investigations, the techniques permitting to answer to this question are now well-defined. On the other hand, the analyses can be more accurate and give details on the radiolytic products in order to define radiolytic mechanisms. In this case, the choice of the analytical technique will depend on the radiolytic product or mechanism to be studied.

In addition, some techniques are very efficient and bring useful information but, which would be of weak interest in a study of feasibility. We have chosen to focus on the development of protocols in view of a determination of feasibility.

2. Overall study

Detecting whether a drug was irradiated or not has to be determined to give an overview of the drug. In addition, the quantitative aspect is given by the determination the amount of the degradation of the drug (chemical potency).

Suitable methods for the detection of irradiation must be based on the effects of the irradiation common towards all molecules, *e.g.* the production of ionic species [9] or radicals [9]. These effects can be measured respectively by Electron Paramagnetic Resonance (EPR) and thermoluminescence (TL), which makes them adequate for detection. For the present, these techniques are officially used in order to detect the irradiation of food [11]. Within the frame of the regulations on food, they are involved in appropriate methods with well-established protocols [12,13,14]. No regulations concerning the detection of irradiated drugs are available yet.

TL studies have shown that non-irradiated drugs already present peaks and that there are slight variations in the TL profile after irradiation [9]. Hence, a careful study of comparison has to be done to distinguish the non-irradiated from the irradiated pharmaceutical. In contrast, drugs do not present an EPR signal intensity before irradiation apart from a few exceptions [15]. Thus, the apparition of the signal is an evidence of an sterilisation treatment. Considering the easier distinction between irradiated and non-irradiated drugs, EPR seems to be more recommended than TL.

Some other techniques (infra-red spectroscopy, nuclear magnetic resonance, pH measurements, *etc* ...) were tried but have seldom shown significant differences between irradiated and non-irradiated products [16]. This used to be misinterpreted into feasibility. Actually, the changes due to the radio-induced degradation products are drown in the signal of the main compound. Considering the results about the radiolytic species, it seems clear that the concentration of the products are indeed too small to make a significant difference after irradiation. In conclusion, these methods must be used with caution in order to avoid an erroneous conclusion.

A more widespread test consists in the comparison of the absorption of the irradiated and the non-irradiated drug solutions with an UV spectrophotometer. However, this possibility seems hazardous. It implies, just as in the previous paragraph, that the changes must be in a sufficient amount not to be drawn in the main signal. In addition, it also assumes that the UV spectrum of the main compound is different from the ones of the radiolytic impurities. For most of the drugs, this last hypothesis is not true for most of the radiolytic impurities [17]. It seems more reasonable to proceed to a separation of the compounds in order to isolate the main compound and to use its own UV response [18].

High performance liquid chromatography (HPLC) is an analytical tool, that is sometimes used in the context of the detection of irradiation. Up to now, studies of irradiated solid state drugs using HPLC are not as abundant as it could be expected. This separation technique can be of multiple use. Different approaches to understand the effect of radiation on the drug can indeed be undertaken. One can either focus on the main compound or on the impurities with regard to the initial concentration used. The former work may sometimes be referred as a "test of potency" in the literature. An alternative appellation would be "test of content".

The work on the main compound has to be achieved with low concentration in comparison to the work on non-volatile final products, which will be dwelt on later. It consists of a comparison of areas under the curve between the peaks of the active compound before and after irradiation. The area under the curve of the non-irradiated compound is considered as 100%. The concentration of the irradiated compound is given in the percentage of the non-irradiated one. This easy test should give an overall quantitative information on the degradation of the main compound. It is used in various studies [18,19,20].

However, we think that the use of the test of content, even if it integrates a technique of separation, must be restricted or, at least, taken with circumspection. It was indeed shown that non-volatile radiolytic species were produced though the test of content did not reveal any significant differences between the non-irradiated and the irradiated samples [18].

Hence, only few techniques permits to give an overview of the drug. Besides, results must be taken with caution in order to avoid a misinterpretation or an erroneous conclusion of feasibility.

3. Study of the radiolytic species

Investigations concerning the radiolytic species have a very different aim from the overall studies. These products are due to the radio-induced degradation of the drug, as it is schematised in Figure 1, and they can be numerous. Their quantity is variable ranging from few ones to extreme and marginal values around 40. Thus, this requires preferably the use of techniques of separation. The choice of the technique depends on the properties of the products of radiolysis.

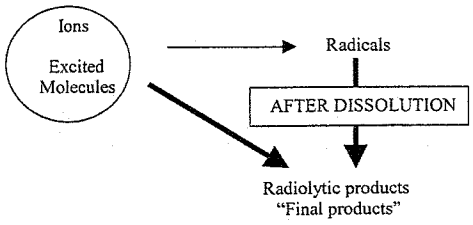

Figure 1 : Relationships between the different radiolytic species formed after the irradiation of an solid-state drug.

Different products of the radiolysis can be distinguished : radicals and final products. Radicals cannot be investigated by a technique of separation. As proposed previously, their analysis can be achieved by EPR. Concerning the final products, two types must be considered : the volatile ones, on one hand, and the non-volatile on another hand. As their name indicates their properties, it is obvious that the first ones might be studied by gas chromatography and the others by liquid chromatography. Figure 2 summarises the main analyses possible after the irradiation of a solid sample in a sealed vial.

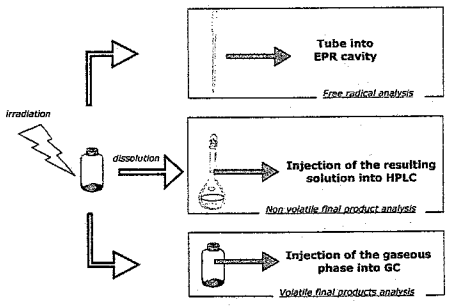

Figure 2 : Recapitulative scheme of the analyses possible after the irradiation of a solid-state pharmaceutical.

The products of the radiolysis of drugs have already been widely investigated. The detailed studies have indirectly improved the use of the techniques in the frame of the analyses of radiolytic products. Moreover, with the ongoing development of the techniques, it is nowadays possible to analyse all the radiolysis products of a drug. The challenge lies essentially in the detection of the products, which are in very small amounts, indeed even traces.

3.1. Study of the radicals

Radicals are paramagnetic species and can be studied by Electron Paramagnetic Resonance (EPR). This technique is also called Electron Spin Resonance (ESR). After solid-state radiolysis, it is well-known that radicals can be trapped in the solid matrix [8]. Their observation can still be possible after several years. In contrast, the non-irradiated drug do not present radicals. This very sensitive tool is therefore proposed in order to detect if a drug has been treated or not to irradiation [21].

EPR as been one of the most used tool to study the effects of the irradiation in solid-state drugs. Among the first studies undertaken by EPR, the evolution of the amount of radicals is dealt with in numerous works. A systematic work was undertaken on various drugs [*e.g.* 22]. It focused on the decay of radicals with regard to the time. For most drugs, the decay is in two steps: the first one is the more rapid and takes place in several days. It is then followed by a slow step, which corresponds roughly to a steady-state. Some authors also focused their interest on the effect of the storage conditions on the decay [23]. The production of the radicals as a function of the dose was also investigated systematically [24].

The quantification of the radicals is a fussy topic. Its perfection is still in progress [21,25,26]. Several works have focused on this topic. Their inter-comparison is difficult since the methods of quantification are variable (see Table I) [15,21,27]. In our opinion, it is therefore necessary to develop a simple method, which involved the use of an easy-available reference. Lately, we have proposed the use of alanine, for which the radio-induced effects are well-described [28]. This radio-sensitive amino-acid has two major advantages based on its similarity with the drugs studied. First, it is in the solid-state. In addition, the radicals present have to be produced by irradiation. This second advantage has the utmost importance since it allows the systematic error on the determination of the irradiation dose to be cancelled.

Table I

Some different references chosen for the EPR quantification of radicals

drug	Reference for quantification	reference
ampicillin	Hexyl radicals due to the radiolysis of n-hexane	21
ascorbic acid	DPPH powder	23
miscellaneous drugs	DPPH in a benzene solution	15

Up-to-now, few works have dealt with the identification of the radicals [24,29]. Two major complications have indeed to be overcome. On one hand, the analysis of drugs gives powder spectra since spins are randomly orientated. This means that the spectral features are broad and it is difficult to extract the EPR parameters useful in the case of an identification. On the other hand, there might be several types of radicals present in the same drug [22,24]. The resulting spectrum is therefore a sum of different types of spectra. Since EPR is not a separating technique, the components of the different spectra are all mixed up.

Thus, different attempts were made in order to simplify the EPR spectra. A systematic work on the experimental parameters has shown to give more information on the spectra but is still very limited [22,24]. Therefore, as the customary methods could not enable to resolve the EPR spectra of drugs, complementary techniques have to be considered. All investigations used the most common technique, X-band EPR, whose frequency is ca. 9 GHz. As a matter of fact, High Field (and High Frequency) EPR (HF-EPR) has proved its great efficiency in the case of intricate spectra. When the working frequency is increased, the separation of the anisotropic features is indeed enlarged (see Figure 3). This is of interest since it could highlight new lines that could be hidden in the compact X-band spectrum. Furthermore, Very HF-EPR (VHF-EPR) might resolve g-anisotropy by enhancing it. Different frequencies were tried recently on drugs. Q-band (ca.

34 GHz) EPR was shown to be helpful in the radical identification of irradiated captopril [31] whereas VHF-EPR (285 GHz) was useful in the case of esmolol [32]. They both show that HF-EPR is a very promising technique in order to better understand the radical effects of the irradiation of drugs.

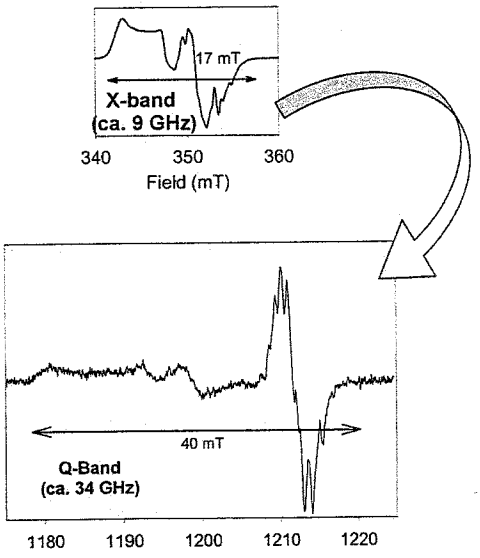

Figure 3 : EPR spectra of irradiated captopril. The anisotropic signal is extended from 17 mT to 40 mT by using Q-Band frequency (ca. 34 GHz) instead of X-Band (ca. 9 GHz).

Although the analyses of the trapped radicals might be very interesting for fundamental research, these pieces of information would not have the same impact on a determination of the radiosterilisation feasibility. The concentration of the radicals is very low. Moreover, they are supposed to recombine when the product is dissolved and are therefore not present in drug solution, which is administered to the patient. For these reasons and in view of a feasibility study, there is no reason to deal with a radical study in the drug powder. In addition, the radical identification requires rare techniques and a very specific knowledge [24]. Concerning the quantification, the results of the previous investigations have not outlined a correlation between the radical concentration and the drug degradation [18,31]. Hence, it has no concrete application up-to-now. It possesses though several advantages (rapidity, weak sample preparation, sensitivity...), which would make it a very practical test.

3.2. Study of the final products

3.2.1. Study of the non-volatile final products

The study of the non-volatile final products is similar to one of the pharmacist's task, which consists in the study of the related substances present in the drug and is required in the monographs of the Pharmacopoeias. The technique generally recommended is HPLC.

On the contrary of the previously described test of content, which also calls for the use of liquid chromatography, the work on the non-volatile final products has to be undertaken with high concentration of the main compound. The impurities are in general in such small amounts that it is necessary to saturate the peak of the main compound to observe them. One limiting factor in the observation of these products is therefore the solubility of the drug studied into the solvent.

In most of the studies, the liquid chromatography is used for the identification and / or the quantification of the radiolytic impurities. The aim of the work induces the choice of the detector. UV was certainly the detector the most coupled to LC in the previous radiosterilisation investigations. Its use enables one to quantify the products studied and, to a certain extend, to gain information on their structure. This second application is limited. A deeper investigation of the impurity structures has become possible with the improvement of the analytical techniques and, particularly, with the development of the on-line coupling of HPLC and mass spectrometry.

The first studies involved the coupling of LC and UV-spectrophotometer. The comparisons between the profiles of the non-irradiated drugs and the irradiated drug highlighted major facts. These profiles are indeed different. The number of non-volatile final products increases with the irradiation treatment and goes up to a dozen [18,33]. Some radiolytic products are typical degradation products. As a consequence, the concentration of the impurity is increased after irradiation. In addition, one specific effect of the radiation treatment is the production of unique radiolytic products [34]. This was observed for most of the drugs studied. Besides, the production of isomers often occurs [19]. From a more general point of view, LC-UV is essentially useful in the quantification of the impurities.

Radiosterilisation investigations through LC-MS are more recent and few yet. They confirmed some results of LC-UV showing that the non-volatile radiolytic products are close in structure with the active compound and with each other [35]. Its use was considered as very promising for a better understanding of the mechanisms of degradation of the drugs. Up to now, it has allowed authors to identify impurities for small active compounds [36]. In the case of larger drugs such as cefotaxime, the work is more tedious [33]. For instance, as the impurities are very close in structure, they show the same mass spectrum and are hard to distinguish. MS^n is required to try differentiate daughter ions.

Because of the presence of unique impurities, adapted protocols have to be defined in the case of radiosterilised drugs. The investigations of the impurities might require a very different method than the one proposed in the Pharmacopoeia. In addition, the close structure of the impurities, their amount and their presence in traces makes the separation even more complicated to be achieved.

In accordance with ICH norms [37], it is recommended to identify any impurity if it is above a defined quantitative threshold. This threshold varies as a function of the maximum daily dose (MDD) of the drug that can be administered to the patient. It goes from 0.1 % for high MDD up to 1 % for low MDD (see Table II).

Table II

Identification thresholds for degradation products as a function of the maximum daily dose of the drug, that can be administered to the patient.

Maximum Daily Dose	Identification Threshold
< 1 mg	1.0%
1 mg – 10 mg	0.5%
> 10 mg – 2g	0.2%
> 2 g	0.1%

It is noteworthy that the identification of the impurities is not required for any impurity, which influences the quantification. If the impurity is not identified, the choice of a reference remains open. Thus, ICH guidelines recommend to use the active compound as the reference [38]. The concentration of the impurity is given as a percentage of the active compound. As the quantification is performed by LC-UV, this hypothesises that the absorptivity of the impurity is close to the one of the active compound. Most of the radiolytic products are indeed very similar to the initial compound and the hypothesis is therefore true.

Hence, the investigations of the impurities has to be taken into two steps. The first step is the quantification of the impurities by HPLC-UV. The second step is the identification and is necessary for impurities with concentration above the threshold defined by the ICH norms. As this second step depends on the results of the quantification of the impurities, the first step should take into account that an identification might have to take place. The use of LC-MS implies restriction in the choice of the buffer and the additives used in the mobile phase, which should already be taken into consideration at an early stage of the investigation.

Lastly, it is worth mentioning that the coelution of impurities is highly probable because of their very close properties. Thus, it can be difficult to achieve a complete separation of all the impurities. In some cases, it might be interesting to have more information on the coeluting compounds. The use of different detectors can be helpful, DAD (diod array detector) and MS being more useful in these types of works. As evoked above, radio-induced impurities are sometimes isomers. Chiral columns can therefore be helpful by overcoming the coelutions due to the presence of enantiomers.

3.2.2. Study of the volatile final products

Drugs sometimes give off a smell after irradiation. Sulphur-containing drugs (such as cephalosporins or captopril) are especially concerned by this drawback. In order to better understand the reasons of the odour of irradiated products, GC (Gas Chromatography) experiments were undertaken. The results showed that some volatile products could be taken for responsible of the smell [38].

Generally, the analyses of organic volatile compounds (OVCs) involve the use of a specific injector, the headspace (HS). This injector enables one to analyse directly the powder. This permits to avoid time consuming and complicated sample preparation and to work on trace compounds. The static version of this sampler extracts the volatile compounds by incubating a sealed vial at a fixed temperature until the volatile analyte has equilibrated between the vapour phase and the solid sample. In the end, only the volatile phase is injected in the chromatographic system.

The gas chromatograph can be coupled to various detectors. In most of the radiosterilisation studies, a coupling to MS is used. The aim of the investigations requires indeed the identification of the products. In the viewpoint of a quantification, a coupling to a Flame Ionisation Detector (FID) is preferred. In addition, the sample should be dissolved into an adapted solution.

OVCs can be found in non-irradiated drugs. They are solvents used in the drug synthesis and were not totally removed afterwards. For this reason, their appellation in the Pharmacopoeia is residual solvents. In general, their analysis do not figure in each monograph of the Pharmacopoeia but is considered in the general monograph entitled "Substances for pharmaceutical use" [39]. It defines the protocol to be followed [40], which requires the use of HS. In addition, the Pharmacopoeia reproduces an ICH text [41] in its General Texts [42] to fix the tolerated levels of residual solvents. The classification considers four different types of risk (see Table III). For each class, a list of solvents is given and the levels are defined. Some must be avoided (*e.g.* benzene). Some must stay below threshold concentrations.

Table III

The ICH classification of the residual solvents in pharmaceuticals as a function of their risk assessment

Class number	Risk assessment
Class 1	Solvents to be avoided
Class 2	Solvents to be limited
Class 3	Solvents with low toxic potential
Class 4	Solvents for which no adequate toxicological data was found

HS-GC analyses have showed that the gas chromatogram obtained after irradiation of a drug generally presents additional peaks due to volatile radiolytic products. Hence, the distinction between the chromatograms before and after irradiation is obvious. A deeper investigation proposed the residual solvents, initially present in the non-irradiated drug, to be the source of the volatile final products [43]. This demonstration was done on cephalosporins. This radiolytic scheme seems to be the principal one for the cephalosporin-belonging drugs tested in that study. Other patterns of radio-induced degradation are possible. For instance, the wide research concerning the feasibility of irradiation of chloramphenicol eye ointment [*e.g.* 44] explained the production of some volatile radiolytic species by the dismutation of the active compound. This pathway was proposed in the case of the chloramphenicol itself or of some of the constituents of the ointment. Some of these latter consist of long carbon-chain, which give birth to shorter alkanes or alkenes after irradiation.

Works on other families of drugs were also performed. The β–blockers showed a contrast between the non-irradiated and the irradiated GC profile as all the materials we have previously mentioned. Although, numerous radiolytic products could be detected after irradiation, the non-irradiated chromatogram did not show the presence of initial volatile compounds. This was due to the sensitivity of the static headspace injector. The detection could indeed be improved with the use of a purge and trap injector.

In conclusion, the irradiated drugs present volatile radiolytic products. It is noteworthy that these radiolytic products are derived from the drug. They can be deemed as related substances in contrast to the volatile products normally found in the drug. Their detection requires the use of a specific injector. The use of a static headspace is recommended in the Pharmacopoeias [41] but other techniques of injection could resolve intricate cases. In view of the pharmaceutical guidelines, the presence of most volatile products can be tolerated but must remain under defined limits. Anyway, it is also clearly stipulated that "since there is no therapeutic benefit from residual solvents, all residual solvents should be removed to the extend possible to meet product specifications, good manufacturing practices, or their quality based requirements" [42]. In order to adapt these recommendations to the viewpoint of the radiosterilised drugs and on the basis of the

results of Barbarin [43], this would imply to avoid materials containing initial residual solvents. When possible, limited residual-solvent-containing materials should be preferred.

4. Conclusion

In view of the latest investigations in the field of the analysis of irradiated drugs and their conclusion, it seemed necessary to remind the studies to perform to assess the feasibility of radiosterilization. Radiosterilization has indeed intrinsic effects, which requires specific analytical protocols. Because of the radio-induced effects, the monographs of the Pharmacopoeia might not be adapted for the study of irradiated pharmaceuticals.

The determination of the radiosterilisation feasibility of a drug requires information concerning the radiolytic products. If an EPR study of the radicals is superfluous, the investigation of the final products is a must. Its principle stays close from a regular drug impurity profiling strategies [45]. In regard to the non-volatile final products, an adapted monograph might have to be established. This protocol involves the detection and the quantification of the products. Then, if their concentration is above defined thresholds, the products have to be identified. On the opposite, the volatile final products have to be identified in the first place. If some must be avoided, others are likely to be present but should remain under given concentrations. Hence, their quantification must be achieved.

These steps correspond to the analytical aspect of a feasibility study. Other data might be necessary to complete the study; for instance, in the toxicological field. Besides, sterilisation parameters must be defined and validated.

Throughout the article, we did not consider the irradiation dose used for the experiments. It is indeed very variable. Anyway, the dose influences poorly the choice of the analytical technique. As the dose is increased, the degradation of the drug will be more important and so will be the radiolytic products. Therefore, the radio-induced effects will be easier to observe. The techniques might be less sensitive in these cases. However, it would require very high doses to obtain such an influence.

In addition to the analytical protocols, the Pharmacopoeia definition of the drug characters might have to be adapted as well. After irradiation, the organoleptic properties of the drugs can change. A coloration of the drug (into yellow) is often reported. If it is proved that the irradiated drug remains safe for the human use, the coloration should not be considered as a limiting factor.

Lastly, it seems that no test are appropriate to give an accurate overview of the radio-induced effects on the drug. In general, these effects are indeed too weak to have an impact on the degradation of the main compound. Hence, most of the direct tests on this main compound are not sensitive enough and induce a huge risk of misinterpretation. Two tests remain useful, such as the detection of the irradiation of a product, which should be successfully achieved by EPR. In addition, a test of content involving HPLC-UV can be considered in order to give a rough estimation of the importance of the degradation at a preliminary stage of the radiosterilisation study.

In the future, the use of radiosterilisation might spread due to the growing utilisation of proteins as drugs or of polymer-made delivery systems. Actually, these latter are the research topics that are being developed recently [46,47].

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