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Identification by CI-mass spectrometry of an unexpected benzodiazepine degradation product

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Abstract

The French Military Health Service (SSA) has developed an innovative drug product, as a treatment against neurotoxic organophosphate poisoning (NOP). It contains three drug substances: an anticholinergic, an anticonvulsant and a cholinesterase reactivator. Testing stability study, in normal conditions, over 18 months, for this speciality, has given unexpected results. Indeed, one of the drug substances, avizafone (pro-drug of diazepam), breaks down partially into a compound which migrates into the plastic container where this degradation product is demethylated after absorption. Mass spectrometry with negative chemical ionisation (negative CI-MS) was used, to monitor decomposition of the drug substance. This method first showed migration of the degradation product and has been used to monitor its evolution during the stability testing study. The demethylation seems to be due to an additive product present in the plastic. The degradation products remain trapped in the container holding the pharmaceutical formulation.

Keywords: Degradation products: Negative ionisation; Avizafone; Mass spectrometry; Plastic cartridge

1. Introduction

Fighting chemical agents which may be military vectors or used by terrorists is a permanent concern of governments. Among these aggressive agents, organophosphates play a particularly important part.

For this purpose, the SSA has developed an original way of administrating an antidote to these organophosphorous compounds. The treatment itself is composed of three drug substances, which include atropine, pralixodime and avizafone. The first one is an anticholinergic, broadly used for different treatments [1]. Pralixodime is a cholinesterase reactivator [2], the main target of organophosphates. Finally, avizafone, an amide prodrug of diazepam, is soluble in water whereas diazepam is not soluble. Following amidase action, the new form of diazepam may exert its anticonvulsivant effects [3]. This treatment is self-administered intramuscularly. To cope with the urgency of the situation created by organophosphate poisoning, the Pharmacie Centrale des Armées (PCA, Orléans, France) has developed this innovative treatment, combining an injection mechanism and active molecules. This type of a treatment has already been studied [4].

For this study, the three drug substances were simultaneously inserted into a polypropylene cartridge in the form of either an aqueous solution, or freeze-dried; the cartridge was then inserted in the injection device. The PCA studied the stability of these products in the cartridge. The study procedure followed the conditions specified by the International Conference on Harmonization (ICH).

A mass spectrometry study by direct insertion was performed on the plastic cartridge in our laboratory. It was found to turn yellow under certain storage conditions. Several analytical conditions were used to underscore the migration and absorption of the degradation products of the antidote from the aqueous solution or freeze-dried form by the plastic packaging.

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Table 1
Analysis times

	T_0 + 3 months	T_0 + 6 months	T_0 + 9 months	T_0 + 12 months	T_0 + 18 months
5 °C	X	X		X	X
25 °C 60%RH	X	X		X	X
40 °C 75%RH	X	X	X	X	

2. Experimental

2.1. Chemicals

Avizafone (pro-diazepam or 2-benzoyl-4-chloro-*N*-methyl-*N*-lysylglycin anilide), MACB (2-methyl-amino-5-chlorobenzophenone) and carbostyril (3-amino-6-chloro-1-methyl-4-phenyl carbostyril), i.e., Roche (London, England); diazepam (7-chloro-1,3-dihydro-1-methyl-5-phenyl-2H-1,4, benzodiazepine-2-one), i.e., European Council (Strasbourg, France); ACB (amino-5-chlorobenzophenone), i.e., Promochem (Molsheim, France). Water used was purified with a Milli Q System (Millipore, S^t Quentin-en-Yvelines, France). Polypropylene was provided by Atofina (Seneffe, Belgium). All the other chemicals used were of analytical grade. The lyophilized drug with three drug substances (atropine, avizafone and pralidoxime) was produced at PCA (Orléans, France).

3. Stability study programme

The stability study was performed over 18 months, under ICH conditions: 5 °C, 25 °C 60%RH (relative humidity) and over 12 months at 40 °C 75%RH [5,6]. All analysis times are listed in Table 1.

4. Mass spectrometry

The mass detector was a simple quadrupole MS (Thermo Electron MD 1000, France). Spectra were obtained by electron ionisation (EI), under positive (CI^+) and negative (CI^-) chemical ionisation conditions. The source conditions for EI were 70 eV electron energy and the temperature was programmed from 60 to 600 °C at a rate of 65 °C min^{-1}. The reference temperature was 300 °C. Data were collected over a mass range of 15–500 uma using a 0.90 s scan time and a 0.10 s interscan delay. For CI, methane was used as the reagent gas and typical source conditions were the same as EI. Probe spectra were obtained using both EI mode and CI conditions. Samples were analysed with a direct inlet probe. The software used on the Thermo Electron instrument for data processing is Mass Lab Version 1.4.

4.1. Sample preparation

Three drug product cartridges were analysed for each study condition (5 °C, 25 °C 60%RH and 40 °C 75%RH)

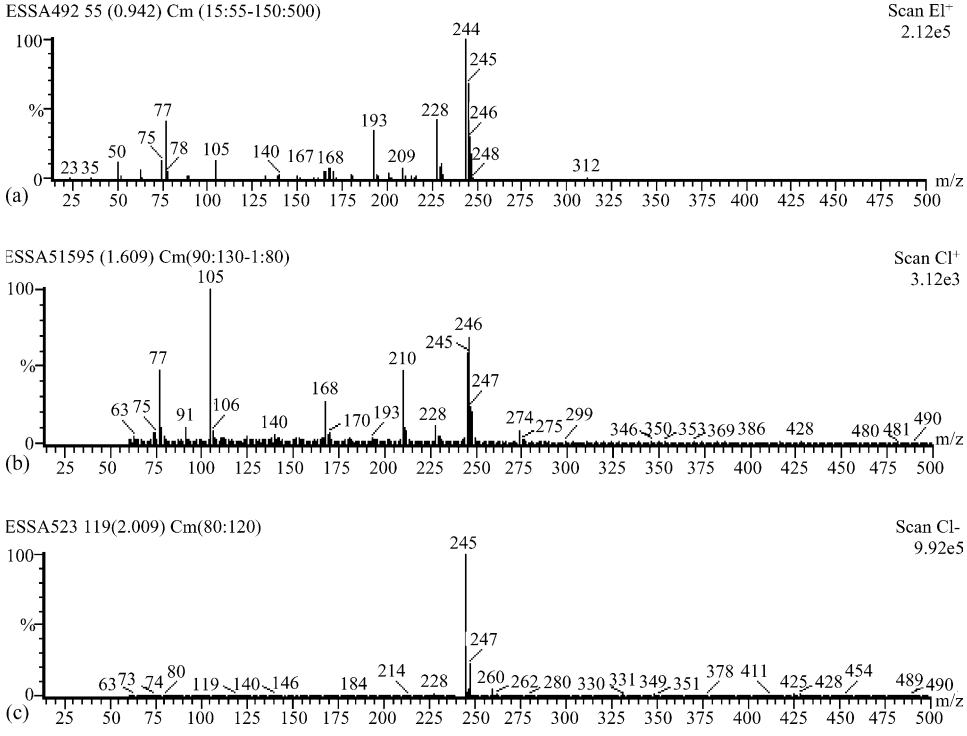

Fig. 1. Mass spectrum of MACB in positive EI-MS (a), positive CI-MS (b) and negative CI-MS (c) at 150 °C.

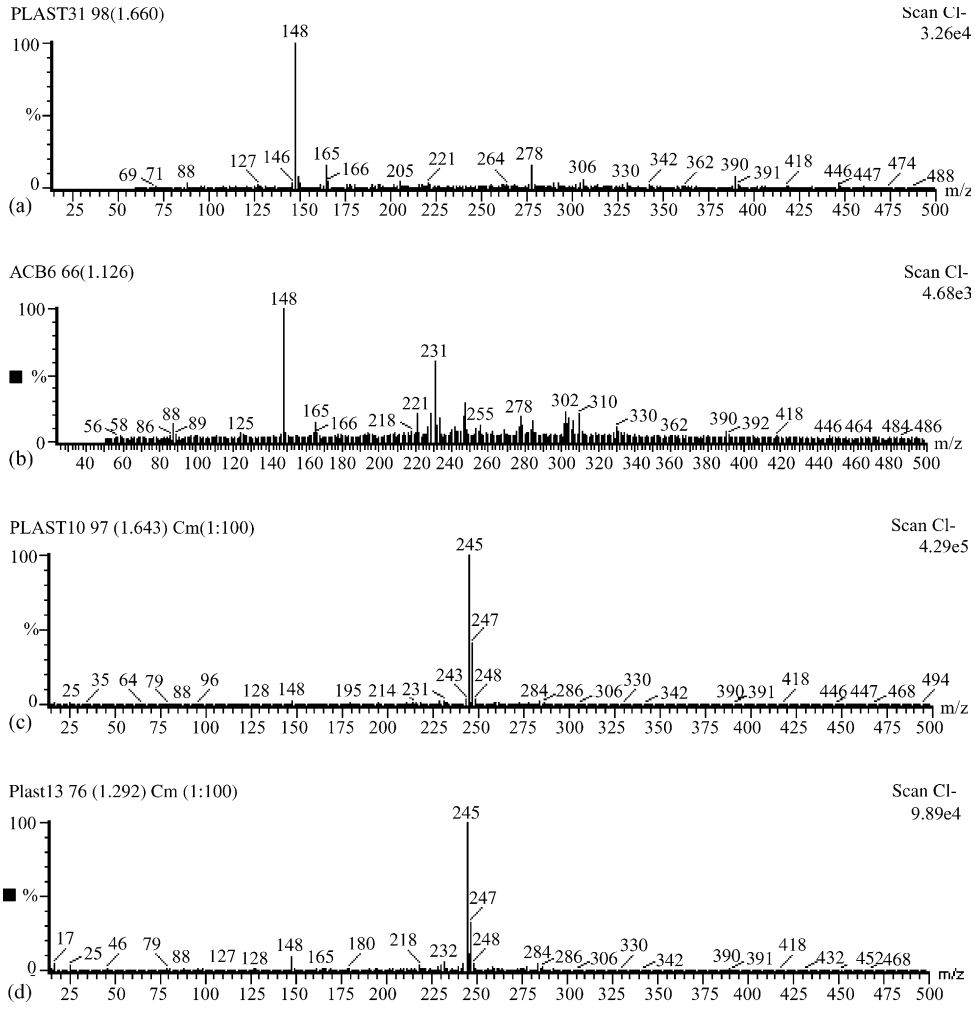

Fig. 2. Mass spectrum of polypropylene in negative CI-MS at T_0 (a), T_0 + 18 months 5 °C (b), T_0 + 3 months 40 °C 75%RH (liquid) (c) and T_0 + 3 months 40 °C 75%RH (freeze-dried) (d) at 300 °C.

and for different analysis times (T_0, T_0 + 3, 6, 9, 12 and 18 months): Table 1. Three cut-outs on the inside and outside of the polypropylene cartridge (around 1 mm^2 surface by 0.5 mm thickness) were studied. These cut-outs – 1–10 μg plastic container – were placed in the mass spectrometer source using a direct probe.

5. Results and discussion

5.1. Results

Under accelerated testing conditions (40 °C, 75%RH) but also over a long time (25 °C, 60%RH), yellowing of the plastic cartridge was observed. In return, this change in colour was not observed under the reference condition (5 °C). The drug substances analysed could not be used to identify the degradation products for atropine or pralidoxime under any of the conditions studied. The result was different with avizafone. For this reason, a complementary development has been initiated, as this molecule is the least well known of the three drugs. A degradation study under aggressive conditions according to International Conference on Harmonization (ICH) recommendations [5,6] has shown that this molecule mainly breaks down into diazepam which is then further broken down into MACB [7,8]. The latter, which is yellow, seems to be the final degradation product [9,10].

Three ionisation modes (EI-MS+ electronic impact, chemical ionisation with positive CI-MS ion detection and negative CI-MS ion detection) were used. The information obtained with these three modes differed somewhat. The negative CI-MS technique limits the number of peaks obtained on molecular and plastic mass spectra. It was easier to interpret these spectra than spectra obtained with positive EI-MS and positive CI-MS. Fig. 1 shows the mass spectra obtained using different ionisation methods for MACB.

Analysis of the plastic cartridge, alone or filled and stored under different conditions, gave similar results to those obtained for MACB according to the ionisation method used. This was so true that we obtained a peak at m/z = 148 for the polypropylene in negative CI-MS. Fig. 2(a) shows plastic analysis in negative CI-MS at T0, before cartridge filling:

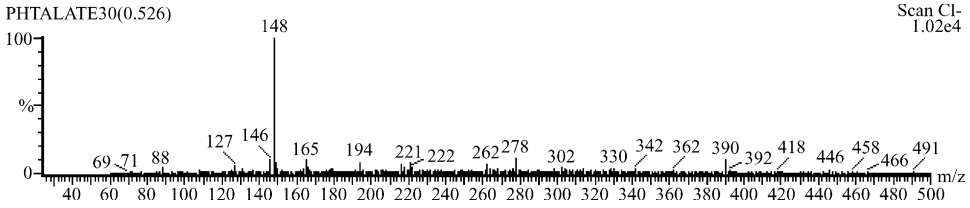

Fig. 3. Mass spectrum of dioctylphtalate in negative CI-MS at 300 °C.

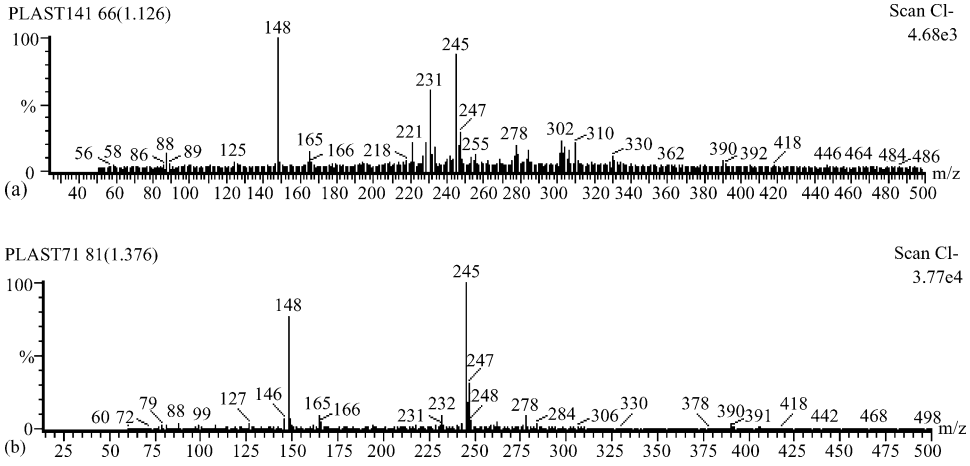

Fig. 4. Mass spectrum of polypropylene in negative CI-MS at $T_0 + 3$ months 25 °C 60%RH (a) and $T_0 + 12$ months 25 °C 60%RH (b) (liquid) at 300 °C.

only the $m/z = 148$ peak was observed on the spectrum. This $m/z = 148$ is the characteristic dioctyl phtalate peak (Fig. 3). As well as this characteristic peak, the mass spectrum of a cartridge after 18 months storage at 5 °C displays an $m/z = 231$ peak (Fig. 2(b)). Under 40 °C 75%RH testing conditions, we obtained a peak tallying with the MACB ($m/z = 245$) starting from $T_0 + 3$ months, in both liquid form (Fig. 2(c)) and lyophilisate form (Fig. 2(d)).

The results obtained at 25 °C 60%RH were more surprising. Indeed, at $T_0 + 3$ months and at $T_0 + 6$ months, 2 peaks ($m/z = 231$ and $m/z = 245$) are visible (Fig. 4(a)). However, at $T_0 + 12$ months, only the peak defined by $m/z = 245$ (MACB) is visible on the mass spectrum (Fig. 4(b)). The peak at $m/z = 231$ is practically non-existent. The dispersion of a small quantity of MACB (around 2 mg) on approximately 2 cm^2 polypropylene and storage for 1 week at 40 °C 75%RH showed effective migration of the benzophenone into the plastic (yellowing and determination of a peak at $m/z = 245$ (Fig. 5)). No $m/z = 231$ peak was observed under these conditions.

5.2. Discussion

A quantitative approach is not possible using the direct insertion method. Yet, it is clear that MACB is absorbed into the plastic cartridge filled with the mixture of three drug substances. This is valid for both the liquid and freeze-dried forms.

The appearance of an $m/z = 231$ peak for tests performed under less aggressive storage conditions (5 °C and 25 °C 60%RH) is unexpected. Analysis under conditions of negative chemical ionisation allowed us to obtain a very simple mass spectrum for all the analysed products in which the value of the characteristic peak(s) was very close to the molecular mass of the compound being studied. These peaks are: 430 for avizafone, 284 for diazepam and carbostyril, and 245 for MACB (Fig. 6). However, the appearance of an m/z 231 peak does not correspond to any of those molecules. In the presence of an oxidizing agent (H_2O_2 10%), diazepam is broken down into aminochlorobenzophenone (ACB), with a molecular mass of 231 amu (Fig. 7). Indeed, a test run in an

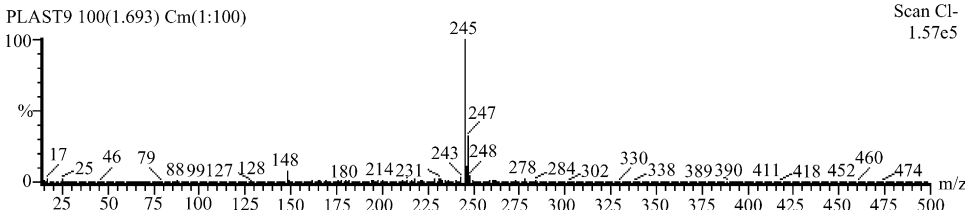

Fig. 5. Mass spectrum of polypropylene after 7 days at 40 °C 75%RH in contact with MACB at 300 °C.

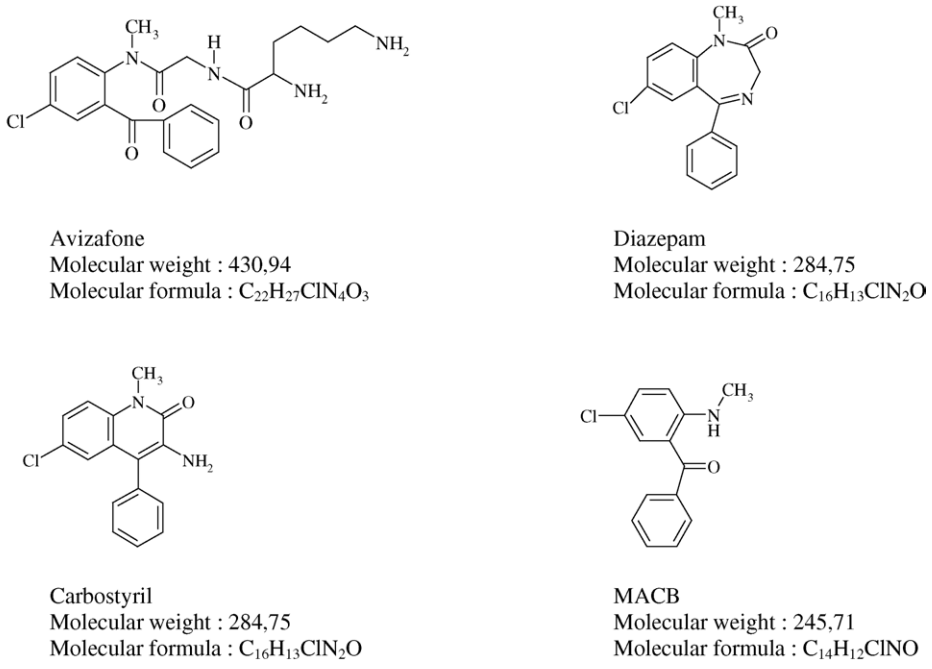

Fig. 6. Chemical formula of avizafone and the degradation drug substances it produces (diazepam, carbostyril and MACB).

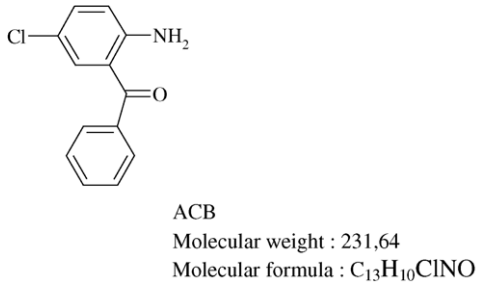

Fig. 7. Chemical formula of aminochlorobenzophenone (ACB).

aqueous oxidizing solution, made during the study to increase breakdown in accordance with ICH indications on avizafone (1 mg/10 ml) and diazepam (1 mg/10 ml) first indicates formation of MACB after about 4 days contact, then after 7 days, of ACB in the solution. Therefore, this breakdown can be observed through our mass spectrometry analysis by direct insertion (Fig. 8). The reaction kinetics show that ACB is a degradation product of MACB. This is proved by the direct oxidation of MACB into ACB by H_2O_2 10% (Fig. 9). Moreover, the dispersion of a small quantity of ACB (around 2 mg) on approximately 2 cm^2 polypropylene and its storage for 1 week at 40 °C 75%RH show that this molecule is not absorbed by the polypropylene (Fig. 10). Only the characteristic dioctylphtalate peak (m/z = 148) is observed on the mass spectrum. Under the same conditions, MACB is absorbed by the plastic (Fig. 5).

We can, therefore, assume that there is a demethylation reaction in the cartridge. It is probably due to an additive in the plastic. The company making the polypropylene has informed us that there is a limited quantity of peroxide in the cartridge composition. Therefore, it is conceivable that MACB could be demethylated by peroxidized polymerization agents. The progressive disappearance of the characteristic ACB peak supports this assumption. Indeed, MACB continues to be absorbed by the plastic even after complete consumption of the peroxidized additive. Thus, the concentration of MACB can go on increasing gradually, so that the peak attributed to ACB becomes invisible on the spectra (confused with the background noise). Spectrometry by direct insertion can only be used to view the relative intensities of the secondary peaks, compared with the main peak raised to 100%. In the case of the polypropylene analysed, it is first ACB (m/z = 231), then MACB (m/z = 245) for the long-term study condition (25 °C 60%RH).

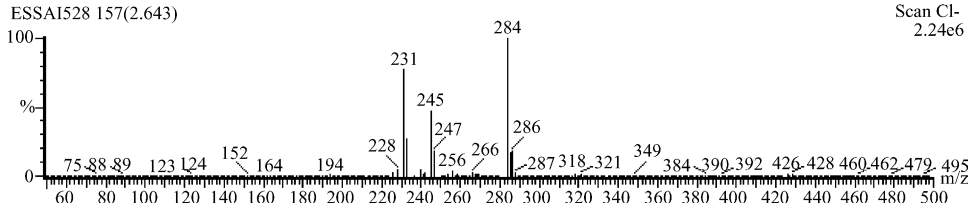

Fig. 8. Mass spectrum of diazepam in negative CI-MS after 7 days in H_2O_2 10% at 300 °C, diazepam: m/z = 284, MACB: m/z = 245 and ACB: m/z = 231.

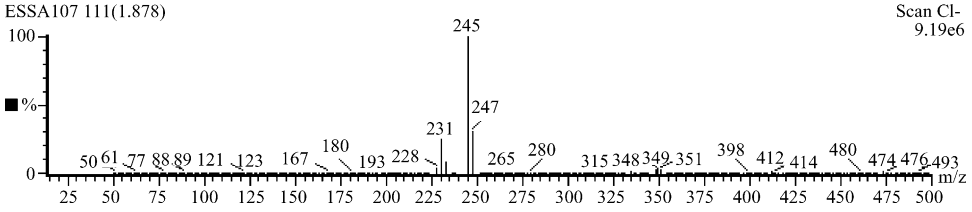

Fig. 9. Mass spectrum of MACB after 4 days in H_2O_2 10%, MACB: $m/z = 245$ and ACB: $m/z = 231$.

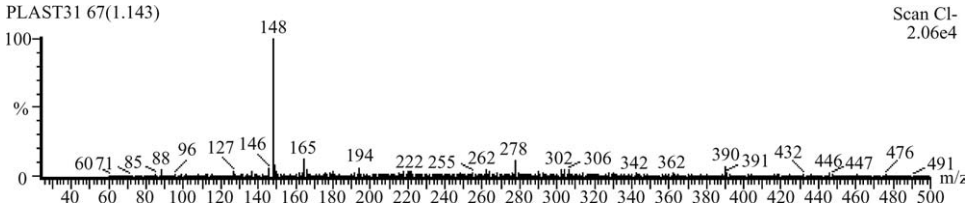

Fig. 10. Mass spectrum of polypropylene after 7 days at 40 °C 75%RH in contact with ACB at 300 °C.

The degradation study on avizafone in powder form shows that temperature is a particularly crucial factor for the production of MACB. At 5 °C, the degradation product appears very slowly, and even after 18 months, the quantity found in the active constituent remains very small. Absorption of this degradation product into the plastic must, therefore, be very slight, and peroxides are not quickly exhausted. On the contrary, at 40 °C, 75%RH, this same degradation is much quicker and peroxidized compounds are quickly exhausted. At 25 °C, 60%RH, the mechanisms used are at the intermediate stage: this explains the erroneously progressive decrease of ACB in the polypropylene. Thus, the quantity of MACB in the plastic seems to be proportional to the amount of MACB in the mixture of the three drug substances, whereas its transformation into ACB reaches a threshold due to the limited quantity of peroxides.

6. Conclusion

In conclusion, it appears that the cartridge containing the pharmaceutical formulation is partly used to store the avizafone final degradation product. Moreover, ACB is never found in the mixture of the three drug substances under the stability study conditions harmonized by the ICH. The plastic is then in the same time a site of transformation of MACB into ACB and a trap for it. This observation must be adjusted when the quantity of MACB becomes too large: migration of the plastic into the formulation would then be possible.

This original observation shows the particularly strong interactions between the pharmaceutical formulation container and the stability of the drug substance(s) which it contains. Thus, in the future, the priming packaging should be evaluated as an active compartment. In these cases, degradation products of active substances should be trapped and stored plastic. The lack of degradation product in the final pharmaceutical product may improve it shelf-live.

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