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Amoxicillin sodium–potassium clavulanate: evaluation of gamma radiation induced effects by liquid chromatography on both the individual drugs and their combination

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Abstract

The effects of gamma irradiation on the stability of potassium clavulanate, amoxicillin sodium and their combination were investigated. A decrease in purity and increase in degradation products up to 30 days after the irradiation were evaluated by reversed phase HPLC. The comparison between unirradiated and irradiated amoxicillin sodium, performed within 24 h following the irradiation process, showed no significant increase in the pre-existing impurities and no evidence of newly induced degradation products. On the contrary, an appreciable increase in the content of some impurities was evidenced 30 days after the irradiation. The chromatographic profile of irradiated potassium clavulanate showed the appearance of one unidentified new product and a slight increase of one pre-existing impurity. No further change in the impurity content was noted 30 days after the irradiation. The amoxicillin sodium–potassium clavulanate combination underwent the same kind of radiation induced degradation as the single compounds.

Keywords: Amoxicillin sodium; Potassium clavulanate; Gamma irradiation; Sterilization; Degradation; Reversed-phase HPLC

1. Introduction

Gamma irradiation is frequently used to sterilize injectable drugs, as an alternative method to heat and gas exposure. The advantages of using of gamma irradiation for parentheral drug sterilization are the high penetrating power and the isothermal character of the gamma rays [1]. In addition, the drug can be sterilized in its final package, without any rise in temperature [2], avoiding the very expensive process consisting of filtration, lyophilization and filling steps under aseptic conditions. On the other hand, new potentially toxic radiolytic products could originate from the irradiation process. Consequently, ionizing radiation induced effects on the drug structure and physico-chemical characteristics should be investigated. In this context, the feasibility of gamma ray sterilization of some drugs has been evaluated by us and other authors [3–8].

The present study is concerned with the effect of gamma irradiation on potassium clavulanate,

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amoxicillin sodium and their combination. Potassium clavulanate is a potent inhibitor of β-lactamases [9], orally and parentherally used with β-lactamase sensitive penicillines to protect them against hydrolysis by the enzyme [10,11]. Amoxicillin sodium–potassium clavulanate is a broad spectrum antibiotic combination, currently also available as a powder for injection. Instability of potassium clavulanate in storage [12] as well as degradation of its combination with amoxicillin in liquid dosage forms are reported [13]. This considered, it seemed interesting to investigate eventual gamma radiation induced effects on the stability of both potassium clavulanate and amoxicillin sodium as well as on the injectable formulation comprising the two drugs. With this aim, the decrease in purity and increase in degradation products up to 30 days after the irradiation were evaluated by HPLC. As regards amoxicillin sodium and amoxicillin sodium–potassium clavulanate combination, a proper HPLC method was developed on the basis of a previously tested system [14]. As for potassium clavulanate, the method described in the European Pharmacopoeia was utilized [15].

2. Experimental

2.1. Samples

Clavulanic acid (potassium salt) and amoxicillin (sodium salt) were from SmithKline Beecham (Milan, Italy). Amoxicillin sodium–potassium clavulanate conjugated powder was from an injectable pharmaceutical preparation on the Italian market.

The following amoxicillin sodium related substances were kindly donated by Professor J. Hoogmartens who prepared most of them in his laboratory: 6-aminopenicillanic acid (**1**); amoxilloic acid (5*S*) and amoxilloic acid (5*R*) (**2 I/II**); amoxicilloic acid (5*S*, 6*R*) and amoxicilloic acid (5*R*, 6*R*) (**3 I/II**); L-amoxicillin (**4**); 2-hydroxy-3-(4-hydroxy)phenylpyrazine (**5**); 4-hydroxyphenylglycylamoxicillin (**6**); amoxicillin (5*R*) piperazine-2′,5′-dione and amoxicillin (5*S*) piperazine-2′,5′-dione (**7 I/II**); *N*-pivaloyl-4-hydroxyphenyl-glycine (**8**); amoxicillin dimer (**9**).

2.2. Irradiation

Gamma irradiation was performed at room temperature at a dose of 25 kGy on the powder samples.

A cobalt-60 plant, operating at the 'Istituto Superiore di Sanità' (Rome, Italy), was utilized. The dose rate at the sample location was 0.3 Gy/s with an uncertainty of about $\pm 3\%$.

2.3. Chemicals

HPLC-grade acetonitrile and methanol and sodium dihydrogen phosphate were supplied by Merck (Darmstadt, Germany). Trifluoroacetic acid was from Fluka (Buchs, Switzerland). Water was filtered through a 0.45-μm Nylon-66 membrane on a Millipore Milli-Q device. All other reagents were of analytical grade.

3. Methods

3.1. Sample preparation

Potassium clavulanate was dissolved in the eluent 1B (method B) at a concentration of 10 mg ml^{-1}. A reference solution was obtained by dilution in the same eluent (0.1 mg ml^{-1}).

Amoxicillin sodium solution was prepared in water adjusted to pH 4 with trifluoroacetic acid at a concentration of 5 mg ml^{-1}. A reference solution was obtained by dilution in the same solvent (0.05 mg ml^{-1}).

Impurity reference solutions were prepared in water adjusted to pH 4 with trifluoroacetic acid at a concentration of 0.05 mg ml^{-1}.

Amoxicillin sodium–potassium clavulanate combination was dissolved in water adjusted to pH 4 with trifluoroacetic acid at a concentration of 5 mg ml^{-1}.

3.2. HPLC apparatus and chromatographic conditions

HPLC analyses were performed using a PE Series 410 pump equipped with a PE LC 95 variable wavelength detector, a PE ISS 200 au-

tosampler and a PE Nelson 1020 elaboration system, all from Perkin Elmer (Norwalk, CT).

3.2.1. Method A

Amoxicillin sodium and amoxicillin sodium–potassium clavulanate combination were analysed on the basis of the method developed for the identification of amoxicillin related substances [14], opportunely modified.

The chromatographic column was a 5-μm Platinum EPS C18, 250 × 4.6 mm i.d. (Alltech Italia, Sedriano, Milan, Italy).

The eluents were: (1A) trifluoroacetic acid (0.05% v/v; pH 2.6); (2A) trifluoroacetic acid (0.05% v/v; pH 2.6)–acetonitrile (80:20 v/v).

Table 1
HPLC gradient programme (method A)

Time (min)	Eluent 1A[a]	Eluent 2A[b]	Description
0–60	100 → 25	0 → 75	Linear gradient
60–80	25	75	Isocratic elution
80–81	25 → 100	75 → 0	Switch to initial mobile phase
81–105	100	0	Equilibration

[a] Eluent 1A: trifluoroacetic acid (0.05% v/v; pH 2.6).
[b] Eluent 2A: trifluoroacetic acid (0.05% v/v; pH 2.6)–acetonitrile (80:20 v/v).

Table 2
HPLC gradient programme (method B)

Time (min)	Eluent 1B[a]	Eluent 2B[b]	Description
0–4	100	0	Isocratic elution
4–15	100 → 50	0 → 50	Linear gradient
15–18	50	50	Isocratic elution
18–19	50 → 100	50 → 0	Switch to initial mobile phase
19–35	100	0	Equilibration

[a] Eluent 1B: sodium dihydrogen phosphate (0.05 M, adjusted to pH 4 with phosphoric acid).
[b] Eluent 2B: methanol.

A gradient elution was performed as described in Table 1 at room temperature and at a flow rate of 1 ml min^{-1}. The monitoring wavelength was 230 nm and the injection volume was 20 μl.

3.2.2. Method B

HPLC analysis of potassium clavulanate was performed by the method described in the European Pharmacopoeia [15].

The chromatographic column was a 5-μm Supelcosil LC 18, 250 × 4.6 mm i.d. (Supelco, Bellefonte, PA).

The eluents were: (1B) sodium dihydrogen phosphate (0.05 M, adjusted to pH 4 with phosphoric acid); (2B) methanol

A gradient elution was performed as described in Table 2 at a temperature of 40°C and a flow rate of 1.8 ml min^{-1}. The monitoring wavelength was 230 nm and the injection volume was 20 μl.

4. Results and discussion

The chromatographic profile of unirradiated amoxicillin sodium (method A) is reported in Fig. 1. Impurities were identified by means of the impurity reference solutions and calculated as amoxicillin by comparison with the amoxicillin reference solution. The unirradiated amoxicillin sample was of a good pharmaceutical grade: it contained a small amount of total impurities (about 1%). The comparison between unirradiated and irradiated amoxicillin, performed within 24 h following the irradiation process (t_0), showed no significant increase in the pre-existing impurities and no evidence of new induced degradation products. On the contrary, an appreciable increase in the content of four impurities was evidenced 30 days after the irradiation (t_{30}). The nature and amount of the identified impurities are reported in Table 3.

Fig. 2 shows the chromatographic profile of potassium clavulanate (method B). The unirradiated sample complied with the European Pharmacopoeia requirements (total related substances $\leq 2\%$; single impurity $\leq 1\%$) [13]. In particular, it contained some impurities in a global quantity of about 1% estimated by the

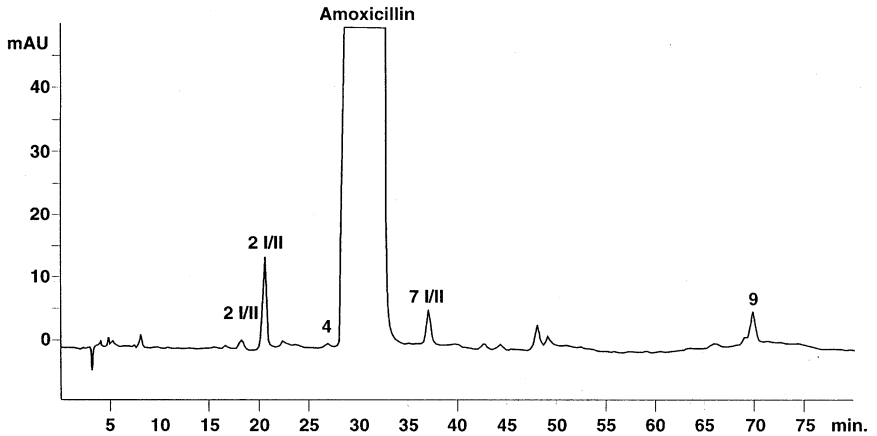

Fig. 1. Chromatographic profile of unirradiated amoxicillin sodium (method A). Irradiated amoxicillin sodium profile at t_0 was comparable. The chromatographic conditions and impurity identification are reported in Section 2.

Table 3
Impurity content increase of irradiated amoxicillin sodium

Impurity no.	% Amount* (C.V.)		
	Unirradiated sample	Irradiated sample (t_0)**	Irradiated sample (t_{30})**
2 I/II* **	0.03 (5.5)	0.03 (5.4)	0.12 (1.9)
2 I/II* **	0.33 (1.3)	0.39 (1.4)	2.21 (0.3)
4	0.01 (5.9)	0.01 (6.1)	0.01 (6.2)
7 I/II* **	0.12 (1.2)	0.13 (1.5)	0.42 (0.9)
9	0.02 (5.2)	0.02 (5.8)	0.65 (1.1)

* Mean of three replicate analyses.

** t_0 and t_{30} represent the days elapsed between the irradiation process and the analysis of the samples.

*** No definite assignment of the peak to the corresponding stereospecific structure was possible due to the unavailability of the individual stereoisomers as pure compounds.

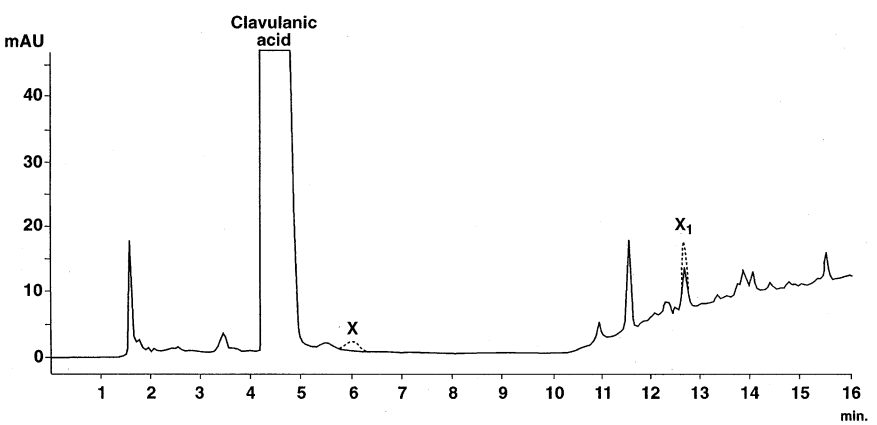

Fig. 2. Chromatographic profile (method B) of unirradiated and irradiated (t_0) potassium clavulanate (continuous and dashed line, respectively). The chromatographic conditions are reported in Section 2.

potassium clavulanate reference solution. With the lack of reference compounds, impurities could not be identified. Immediately after the irradiation (t_0), the chromatographic profile showed the appearance of one unidentified new product, even if only in traces ($X < 0.1\%$) and a slight increase of one pre-existing impurity (X_1 about 0.15%). All the other pre-existing impurities were unchanged. No further change in the impurity content was noted 30 days after the irradiation (t_{30}) (Table 4).

The chromatographic profile of amoxicillin sodium–potassium clavulanate combination is shown in Fig. 3 (method A). The impurities due to amoxicillin were identified by injecting each single potential impurity. With the lack of potassium clavulanate reference impurities, the attribution of the peaks to this compound was achieved by performing chromatographic analyses of both unirradiated and irradiated potassium clavulanate by method A and comparing the resulting chromatograms with the profile obtained with amoxicillin sodium–potassium clavulanate combination.

The chromatographic profile of irradiated amoxicillin sodium–potassium clavulanate combination at t_0 confirmed the results obtained with the single compounds separately irradiated. Also the degradation pattern 30 days after the irradiation (t_{30}) reflected the same behaviour.

5. Conclusions

The results obtained suggest that the amoxicillin sodium–potassium clavulanate combination undergoes the same kind of radiation induced degradation as the single compounds. Consequently, on the basis of the experimental evidence, the irradiation of the tested samples, as a possible

Table 4
Impurity content increase of irradiated potassium clavulanate

Impurity no.	% Amount* (C.V.)		
	Unirradiated sample	Irradiated sample (t_0)**	Irradiated sample (t_{30})**
X	–	0.07 (4.4)	0.08 (4.2)
X_1	0.20 (1.4)	0.37 (1.1)	0.40 (0.9)

* Mean of three replicate analyses.
** t_0 and t_{30} represent the days elapsed between the irradiation process and the analysis of the samples.

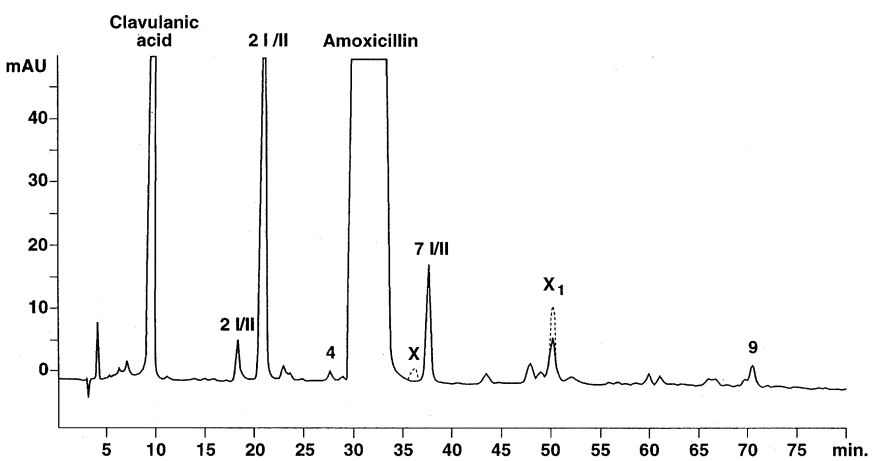

Fig. 3. Chromatographic profile (method A) of unirradiated and irradiated (t_0) amoxicillin sodium–potassium clavulanate combination (continuous and dashed line, respectively). The chromatographic conditions are reported in Section 2.

sterilization process, seems to be feasible. Nevertheless, the degradation of amoxicillin sodium 30 days after the irradiation should not be underestimated in the definition of the shelf-life of pharmaceutical preparations. Moreover, the risk of any biological damage can be excluded only after verifying the absence of induced long-lived free radicals. Studies on this matter are in progress in our laboratory.

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