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Radiation Sterilization of Ephedrine in the Solid State

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The effects of the e-beam ionising radiation of energy 9.96 MeV in doses 25–800 kGy on the stability of solid ephedrine hydrochloride (1R,2S)-(–)- 2-methylamino-1-phenyl-1--propanol hydrochloride) have been studied. These effects have been observed using the following analytical methods: organoleptic (form, colour, smell, clarity of solution), scanning electron microscope SEM, pH measurement, chirality and water content measurement (Karl Fischer method), spectrometric methods (UV, FT–IR, EPR), chromatography (TLC), and combined chromatography (TLC–UV, GC–MS). Even the standard sterilisation dose of 25 kGy has been found to cause a change in colour from white to pale yellow, the appearance of free radicals in the concentration of 3.05×10^{15} spin g^{-1}, and about 1% loss of the content. The effects of higher doses 50–800 kGy have shown that radiodegradation degree of the compound is proportional to the dose applied. The main product of radiodegradation, formed at a yield of $G = 17.17 \times 10^{-7}$ mol J^{-1}, has been identified as 2-methylamino-1 phenyl-1-propanone (methcathinone, ephedrone), a psychoactive compound of the activity similar to that of amphetamine. For the above reasons ephedrine hydrochloride can not be subjected to radiative sterilisation with a dose of 25 kGy, however, assuming sufficiently low microbiological contamination of the initial substance, lower doses could be probably used for sterilisation purposes. Our results have not confirmed the earlier reports from 1970s on the resistance of ephedrine to ionising radiation in doses up to 60 kGy.

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Przebadano wpływ jonizującego promieniowania w postaci wiązki elektronów o energii 9.96 MeV w dawkach 25–800 kGy na trwałość chlorowodorku efedryny (chlorowodorek 1R,2S)-(–)-2-metyloamino-1-fenylo-1-propanolu) w stanie stałym. Do obserwacji powstających zmian wykorzystano następujące metody analityczne: organoleptyczne (badanie postaci, zabarwienia, zapachu, klarowności roztworów), ogląd pod mikroskopem elektronowym SEM oraz pomiar pH, skręcalności optycznej i zawartości wody (metoda Karla Fischera), metody spektrometryczne (UV, FT–IR, EPR), a także chromatograficzne (TLC) oraz łączone (TLC–UV oraz GC–MS). Stwierdzono, że nawet standardowa dawka sterylizacyjna (25 kGy) powoduje zmianę koloru związku z białego na bladożółty, powstanie wolnych rodników w stężeniu 3.05×10^{15} spin g^{-1} oraz około 1%-owy ubytek zawartości. Zastosowanie wyższych dawek promieniowania 50–800 kGy pozwoliło ustalić, że proces radiodegradacji przebiega wprost proporcjonalnie do dawki zastosowanego promieniowania, a zidentyfikowanym głównym produktem rozkładu jest 2-(metyloamino)-1-fenylo-1--propanon (metkatynon, efedron), powstający z wydajnością radiacyjną $G = 17.17 \times 10^{-7}$ mol J^{-1} i będący substancją psychoaktywną o działaniu podobnym do działania amfetaminy. Z wymienionych wyżej powodów chlorowodorek efedryny nie może być poddawany sterylizacji radiacyjnej przy użyciu dawki 25 kGy, jednakże przy odpowiednio niskim zanieczyszczeniu mikrobiologicznym substancji wyjściowej niższe dawki sterylizacyjne prawdopodobnie mogą być zastosowane. Otrzymane przez nas wyniki nie potwierdzają wcześniejszych doniesień z lat 70-tych o odporności efedryny na promieniowanie jonizujące w zakresie do 60 kGy.

Ephedrine (E), a plant alkaloid known already for a long time, has been recently applied in the form of laevorotatory hydrochloride as a component of tablets, syrups, nasal drops, and injections. Drugs for parenteral use must be sterile and one of the methods of sterilisation recommended by the European Pharmacopoeia 5th as one of the most effective [1] is radiative sterilisation. Sterilisation and decontamination of a drug by irradiative ionisation has been accepted in the European Pharmacopoeias already for many years [1, 2]. This method, besides a number of advantages like the possibility of sterilisation in different forms and packages and at any temperature, brings a risk of drug degradation.

The effects of radiative sterilisation have been studied for almost 50 years [3, 4] and soon it has been found that drugs should be sterilised in the solid phase and not in aqueous solutions, because of destructive activity of free radicals formed as a result of water radiolysis and highly undesirable effect of hydrogen peroxide being a secondary product of radiolysis [5]. Although many authors have studied the effect of ionising radiation on therapeutic drugs [6–10], comparison of results is not easy because there is no uniform methodology (different doses of radiation from different sources – cobalt bomb, beam of electrons from accelerator, braking radiation; different aims of studies; different analytical techniques). The most often aim of the studies is to check if a given drug can be subjected to radiative sterilisation. However, some authors are interested in identification of the radiation-induced changes in physicochemical properties of a given drug. They often apply high doses to be able to

detect all changes [11, 12]. The aim of this type of studies can also be to explain the mechanism of radiodegradation and identification of decomposition products [13, 14]. Some authors are concerned with the use of a certain analytical method for evaluation of the radiation-induced changes [15], while some others with determination of structure and lifetime of free radicals formed upon irradiation [16–18] or the use of some new dosimetric methods [19].

The aim of our study was to establish radiochemical stability of ephedrine hydrochloride (E·HCl) in the solid state. The compound was tested by classical methods and modern analytical techniques in order to find out if it is resistant to the electron beam from an accelerator and if it can be subjected to radiative sterilisation. Our results were compared with those of earlier works [20, 21] and it was found that solid ephedrine hydrochloride is highly resistant to irradiation in contrast to its aqueous solution, which underwent degradation. As the authors of the earlier works used only titration and thin layer chromatography [21], or UV spectrophotometry and thin layer chromatography [20], its seems worthwhile to evaluate the radiochemical stability of E·HCl by the currently used analytical methods.

In order to detect all changes induced by exposition to a beam of electrons from an accelerator and to be able to identify the products of radiolysis, even those formed in trace amounts, a dose of 400 or 800 kGy was applied apart from standard doses.

EXPERIMENTAL

Materials

Ephedrine hydrochloride, 1R,2S)-(–)-2-methylamino-1-phenyl-1-propanol hydrochloride, E·HCl, molecular formula $C_{10}H_{15}NO \cdot HCl$, molecular mass 201.69 g mol^{-1}, CAS number 50-98-6. (Pharma Impas S.P.J, Gliwice, Poland) (Fig. 1). The compound satisfied the pharmacopoeia requirements [22].

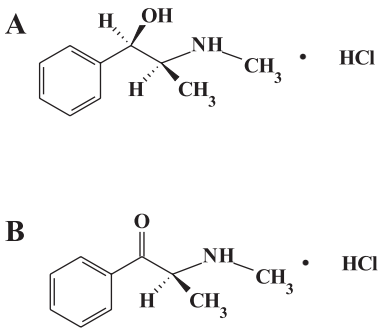

Figure 1. Structure of ephedrine hydrochloride (A) and methcathinone hydrochloride (B)

Phentermine hydrochloride, α,α-dimethylphenethylamine hydrochloride (Sigma, Poland) content 99%.

Pentafluoropropionic acid anhydride, PFP, (Aldrich, Poland) content 99%.

Reference standards

Ephedrine hydrochloride, 1R,2S)-(–)-2-methylamino-1-phenyl-1-propanol hydrochloride, 1.0 mg mL^{-1} methanolic solution and methcathinone hydrochloride, 2-methylamino-1-phenyl-1-propanone hydrochloride, M·HCl (Fig. 1.), 1.0 mg mL^{-1} methanolic solution were obtained from LGC Promochem Sp. z o.o., Poland.

Methods

e-beam irradiation. Portions of approximately 0.5 g of E·HCl were placed in colourless glass jars of 3 mL in volume, which were closed with plastic stoppers. The samples were irradiated with 25, 50, 100, 200, 400, 800 kGy with the help of a linear electron accelerator LAE 13/9 (energy of electrons – 9.96 MeV, current intensity – 6.2 μA).

Organoleptic analysis. The substance was examined before and after irradiation with respect to appearance, colour, smell, and clarity of the aqueous solution obtained according to the European Pharmacopoeia [1, 22].

pH measurements. 0.1500 g ± 0.0001 g portions of ephedrine hydrochloride were weighted before and after irradiation with doses of 25 and 200 kGy. Then, they were dissolved in 3 mL of distilled water and pH of the obtained solution was measured using a Mettler Toledo Mp 225 pH-meter.

Measurement of dispersion degree. The micrographs obtained using scanning electron microscope (SEM 515, Philips) confirmed crystalline structure of the compound. The samples were placed on specimen stubs and fixed with carbon tabs; then they were sputter-coated with gold in a sputter coater, type SCD 050 Balzers. The stubs were next placed in SEM 515 (Philips) operated at 15 kV and magnifications of 50 ×, 250 ×, 500 × and 1000 ×. Selected pictures were processed by DISS (Digital Image Scanning System). Particle size was measured manually. About 1000 particles were sampled.

Determination of water content using Karl Fisher reagent. Water content was determined in the analysed substance before and after irradiation. The procedure was as follows: carefully weighted portions of 0.03 g of ephedrine hydrochloride were dissolved in anhydrous methanol and water was titrated using the Karl Fischer reagent in a METTLER TOLEDO DL 38 Karl Fischer titrator (Switzerland). The error in water content determination was ± 0.01%.

Optical rotation measurement. Specific optical rotation was measured at 20°C using a Perkin–Elmer 243 B polarimeter in the solutions of the initial compound and of the compound after irradiation. The solutions were prepared according to the European Pharmacopoeia [1] using water as a solvent.

Infrared spectroscopy (FT–IR). A KBr disc was prepared by mixing 1.00 mg of the substance with 300 mg of KBr and compressing the mixture in a Pye Unicam mini-press. The spectra were recorded using a Bruker FT–IR spectrometer in the range 500–4000 cm^{-1} with KBr as a blank. The apparatus was calibrated using water and its resolution was 2 cm^{-1}.

Ultraviolet spectrophotometry (UV). Solutions were prepared by dissolving the substance in water to the concentration of 0.048% w/v. They were examined using a UV–VIS Perkin–Elmer Lambda 20 spectrophotometer in 1 cm cells in the range 200–400 nm using water as a blank. For the concentration range 0.024–0.12% the regression equations of the obtained calibration plots were Y= 7.28 x (λ_1 = 251 nm), Y = 9.08 x (λ_2 = 257 nm), Y= 7.05 x (λ_3 = 263 nm), with correlation coefficients of 0.9993, 0.9995, and 0.9992, respectively. Precision of the method was described by the coefficient of variation CV = 1.43, 0.92, and 2.09%.

Thin Layer Chromatography (TLC). Plates of dimensions 5.0 × 15.00 cm, covered with silica gel Kiesegel 60 F_{254} were used. The following mobile phases were applied: 2-propanol: 25% ammonia: methylene chloride (16:3:1) [1]; 2-propanol: 25% ammonia: chloroform (16:3:1) [22]; chloroform: methanol: 25% ammonia (20:3.5:0.6). 150 μL of 1% ephedrine hydrochloride solution (1.5 mg of the substance) were placed on each plate. The spots were set using a quartz lamp operated at λ = 254 nm.

Thin layer chromatography–ultraviolet spectrophotometry (TLC–UV). After the chromatographic analysis was performed as above, the chromatograms with the spots of the main radiolysis products were cut into pieces of 2 × 2 cm^2. The pieces were placed in separate conical flasks of 25 mL in volume, to which 5.0 mL of water were added. The flasks were closed with glass stoppers and the contents were shaken for 15 min and afterwards centrifuged at 400 r min^{-1} for 15 min. The UV spectra of the eluate placed in 1 cm quartz cells were recorded in the wavelength range 400–200 nm using a Perkin–Elmer instrument against the reference sample obtained by elution of a control chromatogram.

Electron paramagnetic resonance (EPR). EPR experiments were carried out for non-irradiated and irradiated samples in standard EPR quartz sample tubes from Wilmad. The measurements were performed with a Bruker EPR EMX–10 spectrometer operated at 9.4 GHz (X-band) at the room temperature (293 K) equipped with a rectangular cavity (ER 4102ST; Bruker).

Gas Chromatography–Mass Spectrometry (GC–MS). Preparation of solutions. 0.1 mg mL^{-1} methanolic solutions of ephedrine hydrochloride before and after irradiation were made. 10 μL of each solution were placed in glass vials of 2 mL in volume, then 10 μL of the internal standard solution were added (phentermine hydrochloride). The contents were evaporated to dryness in a stream of nitrogen at temp. 30°C. To the dry residue 50 μL of pentafluorpropionic acid anhydride were added and the contents were heated at 75°C for 20 min. After cooling at the room temperature, the reaction mixture was evaporated to dryness in a stream of nitrogen and the dry residue was dissolved in 100 μL of ethyl acetate. 1 μL of the obtained solution was injected into the GC–MS.

Standard solutions of ephedrine hydrochloride and methcathinone hydrochloride were prepared analogously.

Analytical conditions. The study was performed using a Claus 500 (Perkin–Elmer) gas chromatograph coupled with a quadrupole mass detector. The compounds were separated using an Elite 5 MS Perkin–Elmer of the following parameters: length 30 m, diameter 0.25 mm, bed thickness 0.25 μm.

Temperature program. Initial temperature of 75°C was kept for 1 min and then increased to 200°C at a rate 15°C min^{-1}. The temperature of the injector was 200°C, injection volume was 1 μL. The carrier gas was helium and the rate of its flow was 1 mL min^{-1} through the column, and 50 mL min^{-1} through the injector. Transfer line and ionisation source temperatures were 250°C and 180°C, respectively. Positive electron ionisation (EI) energy of 70 eV in the scan range 40–500 m/z was used. Statistical parameters of the method

were: CV = 1.9% (for ephedrine), CV = 2.3% (for methcathinone), limit of detection (LOD) = 0.3 μg mL^{-1}, limit of quantification (LOQ) 0.9 μg mL^{-1} (for both compounds).

RESULTS AND DISSCUSION

The results of the organoleptic analysis were that after irradiation ephedrine hydrochloride did not change its form and remained a fine-grained powder, but changed its colour. Already after irradiation with 25 kGy, the white colour of non-irradiated compound became pale yellow, and with the increasing dose of irradiation the intensity of the yellow colour increased. Aqueous solutions of the compound of changed colour after irradiation remained colourless and clear, only their pH increased from 6.23 (non-irradiated) to 6.67. The fact that the solutions of yellow compounds were still colourless and clear indicates that the irradiation-induced colour change is not related to the presence of free radicals. The free radicals were detected at the concentration of 3.05×10^{15} spin g^{-1} by the EPR method after irradiation with a dose of 25 kGy and their concentration was at a level of 50% of the initial concentration even after one year of substance storage (Fig. 2, Fig. 3.).

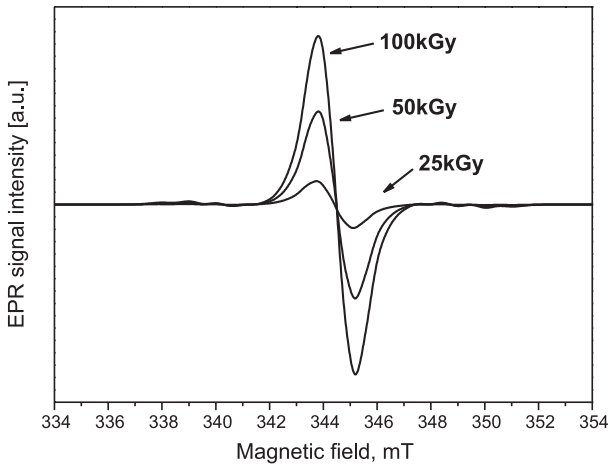

Figure 2. EPR spectra of ephedrine hydrochloride recorded 4 days after irradiation

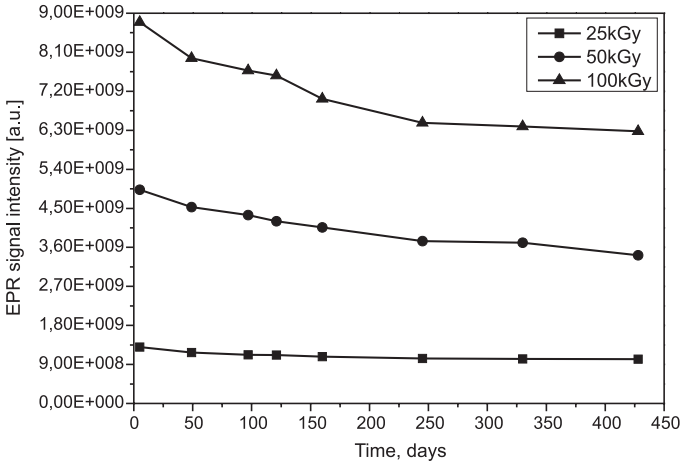

Figure 3. Decay of EPR signal intensities for ephedrine hydrochloride irradiated and stored at the room temperature

The change in colour was not accompanied by distinct changes in the SEM image or by the changes in the particle size determined in microphotographs. The results collected in Table 1 show that the absolute differences in the particle sizes were small and reached at most 3.8% (on average 1.5%). However, the analysis of relative differences has revealed that nearly 30% loss of the smallest-sized particles (100–300 μm) was accompanied by the increase in the content of larger particles (700–900 μm). This fact, accompanied by the simultaneous increase in the water content (0.23–0.31%) suggests formation of agglomerates caused by the appearance of negative charge due to the presence of free radicals. The content of water in the samples was determined before and after irradiation. After irradiation, water content was slightly increased, maximally by *ca* 0.1% for 400 kGy dose. However, the approved increase of the water content is 0.5% [1,22]. If the increase reached 25–30%, it could affect formation of agglomerates.

Table 1. Particle size distribution of ephedrine hydrochloride before and after irradiation

Particle size µm	Content, %		Difference	
	0 kGy	400 kGy	Absolute	Relative
100–199	3.2	2.0	–1.2	–37.50
200–299	11.6	7.8	–3.8	–32.76
300–399	17.6	17.7	+0.1	+0.57
400–499	20.9	18.4	–2.5	–11.96
500–599	13.9	15.6	+1.7	+12.23
600–699	10.9	12.9	+2.0	+18.35
700–799	8.3	10.2	+1.9	+22.89
800–899	6.3	7.8	+1.5	+23.81
900–999	7.3	7.6	+0.3	+4.11

Since the molecule of the investigated compound has centres of asymmetry, it was subjected to optical rotation studies. According to the Pharmacopoeia recommendation, admissible specific optical rotation of laevorotatory hydrochloride is in the range from –33.0 to –35.5° [1, 22]. The compound studied satisfied the Pharmacopoeia requirements and its specific optical rotation after irradiation varied from –34.0 to –35.5. This result means that ionising radiation does not induce changes in the spatial structure of ephedrine hydrochloride; however, the FT–IR and UV spectra of the irradiated samples did not confirm this conclusion.

The FT–IR spectrum shows the appearance of a new low-intensity band at 1695 cm^{-1} (Fig. 4), which suggests the presence of a radiodegradation product with C=O bond at the concentration of at least 3–5%.

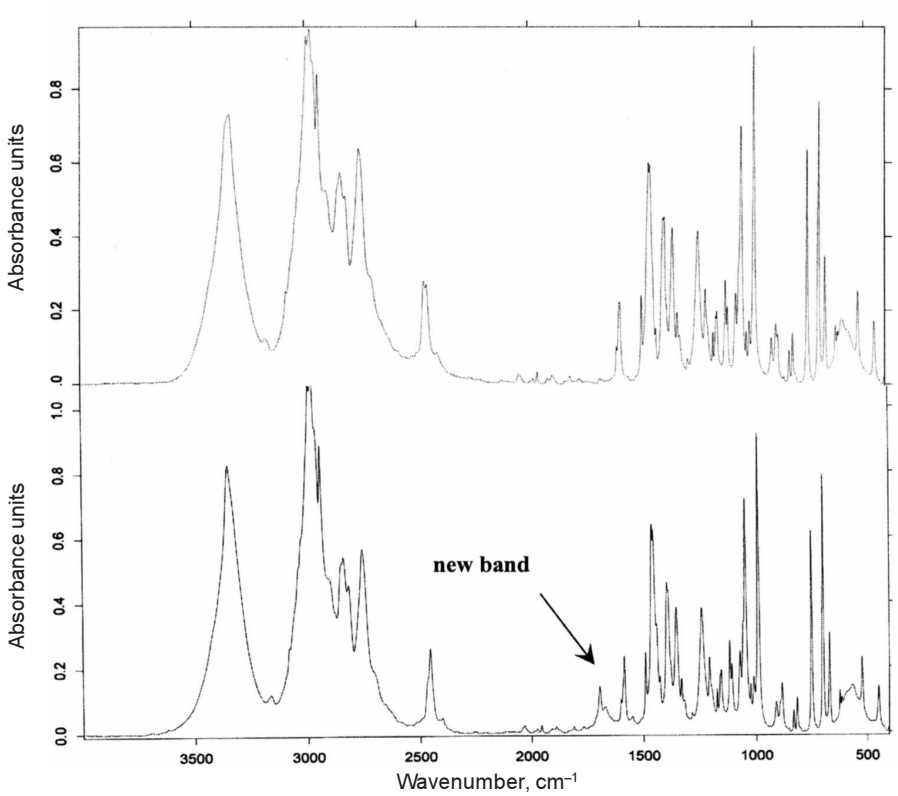

Figure 4. FT–IR spectra of ephedrine hydrochloride before and after irradiation

A comparison of the UV spectra of aqueous solutions of ephedrine hydrochloride (0.48 mg mL^{-1} m/v) before and after irradiation (Fig. 5) reveals the changes in the character of the spectrum and the increase of absorbance at λ_{max}.

The spectrum of the initial compound was in agreement with the literature spectrum [23] and showed 3 absorption maxima at λ_1 = 251 nm, λ_2 = 257 nm, and λ_3 = 263 nm (specific absorption coefficients we determined as 7.28; 9.08 and 7.05, respectively). After irradiation, absorbance of the solutions considerably increased even twice after irradiation with 400 kGy, and thrice after irradiation with 800 kGy. This increase was directly proportional to the dose for all three absorption maxima (Fig. 6). Moreover, the shape of the spectrum changed as a result of irradiation; in the range 240-265 nm the maximum intensity at π_1 (251 nm) increased becoming even higher than that at π_2 (257 nm), while the maximum at π_3 (263 nm) was broadened, which means that the absorption increase in the range 260–265 nm was less intense than in the range 240–255 nm.

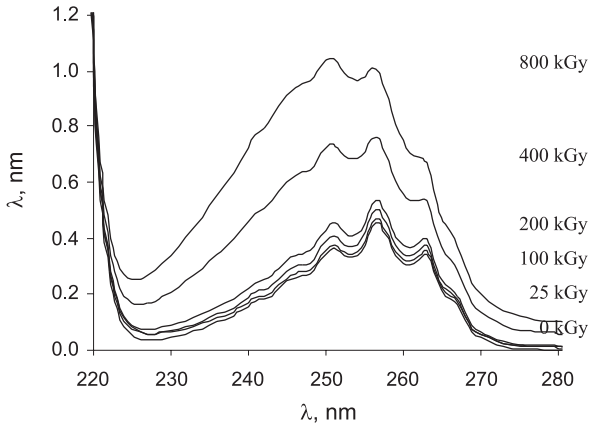

Figure 5. UV spectra of ephedrine hydrochloride before and after irradiation

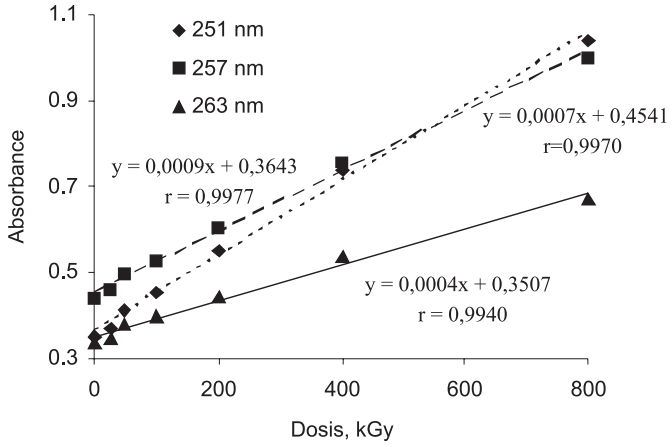

Figure 6. Absorbance of ephedrine hydrochloride *vs* irradiation dose plot

The observed increased absorption and the changes in the UV spectrum as well as the appearance of a peak at 1695 cm^{-1} in the IR spectrum indicated formation of a compound with a strong chromophore, like C=O group. This compound was detected chromatographically (TLC) with the use of three different mobile phases (Tab. 2).

Table 2. Results of TLC analysis for ephedrine hydrochloride before and after irradiation

Mobile phase	R_f coefficient					
	0 kGy	25 kGy	50 kGy	100 kGy	200 kGy	400 kGy
2-Propanol–25% ammonia–chloroform (16:3:1)	0.64	0.82* 0.64	0.81 0.65	0.89 0.65	0.83 0.67	0.84 0.66
2-Propanol–25% ammonia–methylene chloride (16:3:1)	0.56	0.84* 0.55	0.84 0.56	0.80 0.54	0.80 0.57	0.82 0.55
Methanol–ethyl acetate–chloroform–25% ammonia (10:10:5:1)	0.62	0.80* 0.62	0.81 0.64	0.80 0.64	0.78 0.60	0.81 0.63

* Trace.

The UV spectrum of this compound obtained after elution showed maximum at *ca* 250 nm, which may suggest that the structure of the radiolysis product is C_6H_5CO-R [23] (Fig. 7).

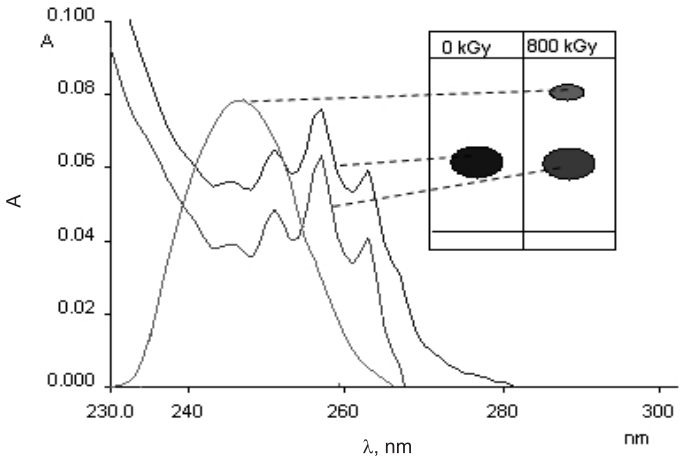

Figure 7. UV spectra of aquaeus eluates from TLC chromatograms. Stationary phase: silica gel Kiesegel 60 F_{254}. Mobile phase: methanol: ethyl acetate: chloroform: 25% ammonia (10:10:5:1)

To identify this product, the GC–MS study was performed. GC chromatograms of non-irradiated and irradiated compound show an additional peak at t_R = 7.07 min, which was assigned to the radiodegradation product 2 methylamino-1-phenyl-1--propanone (synonyms: methcathinone, ephedrone) after comparison with the standard (Fig. 8).

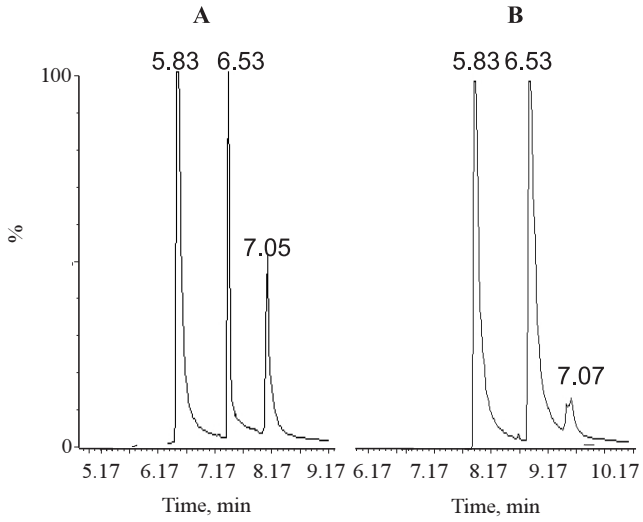

Figure 8. A – GC chromatogram of standard compounds. t_R = 5.83 min – phentermine (internal standard); t_R = 6.53 min – ephedrine; t_R = 7.05 min – methcathinone; B – GC chromatogram of ephedrine hydrochloride after irradiation with 400 kGy; t_R = 5.83 min – phentermine (internal standard); t_R = 6.53 min – ephedrine; t_R = 7.07 min – methcathinone

The most important fragment ions of the two compounds transformed into pentafluoropropionic derivatives are given in Table 3.

Table 3. Comparison of the MS spectra of ephedrine-PFP and methcathinone-PFP

Compound	$[M]^+$, m/z	Major ion, m/z	Other ions, m/z				
Ephedrine-PFP	457	204	160	119	205	56	117
Methcathinone-PFP	309	105	160	77	51	204	119

The identified product of radiolysis has been known since 1928, when it was obtained for the first time. It has a stimulating activity similar to that of amphetamine and may cause addition. Thus, it has not been approved for commercial use, although in 1940s it was used as antidepressant.

The quantitative analysis of ephedrine hydrochloride and methcathinone by GC–MS method was performed using the internal standard – phentermine hydrochloride (Fig. 8); the results are given in Table 4.

Table 4. Content of ephedrine and methcathinone in the samples before and after irradiation

Compound	Method	Dose, kGy					
		0	25	50	100	200	400
Ephedrine	GC–MS	98.50	97.60	95.80	96.57	95.36	92.46
	$HClO_4$*	99.5	98.5	97.5	97.5	96.5	99.5
Methcathinone	GC–MS	1.50	2.40	4.20	3.43	4.64	7.54

* According to FP VI [22].

The loss of E·HCl content determined by GC–MS was directly proportional to the irradiation dose (r = 0.9449), while the loss determined by titration (acidimetric) method, recommended by the Pharmacopoeia [22], did not show such a proportionality. This indicates that the method based on titration is not specific for E·HCl and the results are charged with a much greater error (~2%) than those of GC–MS (~0.8%). Moreover, the results of GC–MS studies have proved that although the initial E·HCl satisfied the requirements of Polish Pharmacopoeia [22] with respect to the content of the active substance, it was contaminated with about 1.5% of M·HCl. The content of this compound increased with the decreasing content of E·HCl, which confirmed that M·HCl was the main and only product of radiodegradation of E·HCl in the access of air (Fig. 9).

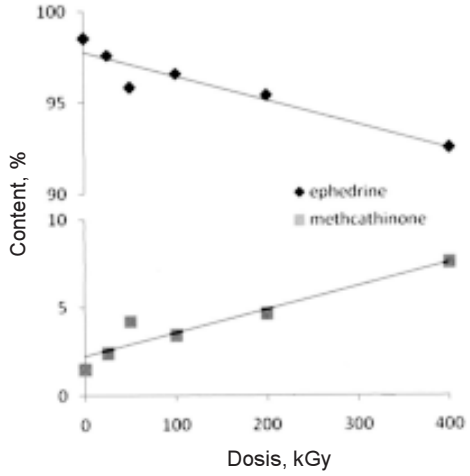

Figure 9. The dependence between the determined content (GC–MS) of ephedrine and methcathinone and irradiation dose

On the other hand, M·HCl is also a product of decomposition of ephedrine stored at the room temperature in the access of air, in other words – a product of thermal decomposition by oxidation. The radiolytic yield of ephedrine radiodegradation and radiosynthesis of methcathinone, calculated from the known relation [24] varied from 9.13×10^{-7} mol J^{-1} (400 kGy) to 32.69×10^{-7} mol J^{-1} (50 kGy) (Gephedrine), and from 9.24×10^{-7} mol J^{-1} (400 kGy) to 33.09×10^{-7} mol J^{-1} (50 kGy) (Gmethcatinone) (Tab. 5). The results of all performed analyses are presented in Table 6.

Table 5. Radiolytic yield of ephedrine and methcathinone ($G_{ephedrine}$ and $G_{methcathinone}$)

	Unit	Radiolytic yield				
		25 kGy	50 kGy	100 kGy	200 kGy	400 kGy
$G_{ephedrine}$	[1/100 eV]	21.02	31.53	11.33	9.14	8.80
	[mol/J × 10^{-7}]	21.79	32.69	11.74	9.47	9.13
$G_{methcathinone}$	[1/100 eV]	21.27	31.92	11.47	9.25	8.91
	[mol/J × 10^{-7}]	22.06	33.09	11.89	9.59	9.24

Table 6. Irradiation-induced changes in physicochemical properties of ephedrine hydrochloride and methods of their detection

Methods	0 kGy	25 kGy	400 kGy
Organoleptic analysis	white	pale yellow	yellow
pH	6.23	–	6.67 (200 kGy)
Water content	0.23%	0.23%	0.31%
Content by titration method	99.5%	98.5%	99.5%
IR	according with spectrum of reference substance	no changes	new band at 1695 cm^{-1}
UV	λ_{max} = 252 nm, 257 nm, 263 nm	no changes ↑ absorbance 3–6%	changes in spectrum course ↑ absorbance 100%
EPR	no signal	3.05×10^{15} spin g^{-1}	20.65×10^{15} spin g^{-1}
TLC*	R_f = 0.56	R_f = 0.55 R_f = 0.84 (trace of radiolysis product)	R_f = 0.55 R_f = 0.82 (product of radiolysis)
TLC–UV	–	–	radiolysis product λ_{max} = 250 nm
Content by GC–MS	1.50% methcathinone	2.40% methcathinone	7.74% methcathinone

* Mobile phase: 2-propanol: 25% ammonia: methylene chloride (16:3:1).

CONCLUSIONS

Solid ephedrine hydrochloride has been found to undergo radiolysis as a result of exposure to the high-energy e-beam. The product of radiolysis is methcathinone, formed as a result of oxidation of the secondary alcoholic group to the ketone group, which is present already in the non-irradiated compound as a contamination at the trace level. Its presence can be detected directly by chromatographic methods (TLC, GC MS) and indirectly by spectral methods (FT–IR, UV) described in this work.

Although the main changes in the physicochemical properties of ephedrine hydrochloride were observed on exposure to high doses of ionising radiation, already at the standard sterilisation dose of 25 kGy a change in the colour of the compound from white to yellow was noted, accompanied by *ca* 1% increase in the content of methcathinone at the expense of the equal decrease in the content of E·HCl and the appearance of free radicals at the concentration of 3.05×10^{15} spin g^{-1}. As follows from our results, ephedrine hydrochloride can not be sterilised by irradiation with a standard dose of 25 kGy under conditions described in the experimental section because of discolouration and appearance of the amphetamine-like product of radiolysis. Of course, on condition of sufficiently low microbiological contamination (< 10 cfu) of the initial substance, it is expected that its sterilisation with lower doses could be possible, provided that this procedure effectively eliminates microorganisms.

Our results have not confirmed the earlier reports [20, 21] on the radiochemical stability of ephedrine hydrochloride in the solid phase. However, it should be considered that the authors of the earlier reports based their conclusions on the results obtained by TLC and UV methods that were not as sensitive as they are nowadays.

Our results obtained by the GC-MS method suggest that determination method of ephedrine hydrochloride recommended by the Pharmacopoeia (FP VI, EPh 5th) should be changed because it is not selective and allows one to determine only the sum of ephedrine hydrochloride and its contaminant – methcathinone instead of ephedrine hydrochloride alone.

The identified product of radiolysis of ephedrine hydrochloride is colourless, similarly as the radicals, the presence of which was evidenced by the disappearance of discolouration of the samples after their dissolution in water or methanol. Yellow discolouration of ephedrine hydrochloride in the solid phase is caused neither by free radicals nor by the product of radiolysis. It may originate from the crystal lattice defects appearing upon irradiation and the trapped free radicals and electrons. Such an interpretation has been proposed to explain a similar phenomenon in other therapeutic drugs irradiated in the solid phase [25, 26].

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