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Radiolysis characterization of cetostearyl alcohol by gas chromatography-mass spectrometry

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Abstract

Methods for sample preparation, assay test and impurity test were established. Degrees of cetostearyl alcohol (CSA) radiolyses in pure state, ointment base and in chloramphenicol eye ointment (CAPEO) were determined at doses of 25 and 50 kGy. Radiolyses of CSA occur in all cases. The degrees are directly proportional to the irradiation dose in each case. Forty-two impurities and radiolysis products were identified using gas chromatography-mass spectrometry. The radiolysis products were assigned to be *n*-alkane, *n*-aldehyde and 2-methyl-1-alcohol. Accordingly, the degradation pathways of cetosteary alcohol were elucidated. Radiolysis behaviors of CSA in pure state, eye ointment base and CAPEO were studied by assay and impurity analyses. The influence of eye ointment matrixes is modest and chloramphenicol molecule exhibits slight scavenger function for cetostearyl. Both qualitative and quantitative data confirm that the radiolysis products of CSA do not cause safety concerns for human use.

Keywords: GC-MS; Cetostearyl alcohol; Gamma radiation

1. Introduction

Cetostearyl alcohol (CSA) is a mixture of solid aliphatic alcohols, consisting mainly of cetyl alcohol (1-hexadecanol, $C_{16}H_{34}O$) and stearyl alcohol (1-octadecanol, $C_{18}H_{38}O$). It contains not $<40.0\%$ of stearyl alcohol and not less 90.0% of both alcohol substances according to the requirements of European Pharmacopoeia and the United States Pharmacopoeia [1,2]. CSA is a material widely used as a component of cream, ointment and emulsifying waxes in the pharmaceutical industry. It functions as an emollient and emulsifying agent, which improves the emollient properties of petrolatum ointments and imparts emollient properties without being greasy.

Historically and currently, there has been being great industrial interest to use gamma radiation for sterilization of petrolatum ointment pharmaceutical products, such as chloramphenicol eye ointment (CAPEO). However, γ-rays could induce the degradation of the irradiated objects and cause the formation of radiolysis products in such quantities that toxicity must be considered. Radiation sterilization of a pharmaceutical product is

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permissible only when the absence of deleterious effects on the product has been confirmed experimentally. As an ingredient of CAPEO, therefore, CSA has to be investigated to ensure that its radiolysis products are definitely safe for the pharmaceutical use. Knowing qualitative and quantitative chemical changes is a must to validate gamma sterilization process on CSA and related products.

Previous studies were focused on the physical changes, especially changes in pH value [3–5]. No literature was available either on the radiolysis degree of CSA and its radiolysis products, or on the relevant analysis methods.

The aim of the present work was to develop analytical methods for sample preparation, assay test and impurity test, to determine the radiolysis degrees of CSA, and to assess the safety risk of γ-irradiation treatment on CSA with qualitative and quantitative data.

2. Experimental

2.1. Reagent and materials

CSA is of research grade from SERVA (Germany). Eye ointment base (EOB, a petrolatum oily base without active ingredient), and CAPEO were supplied by CIBA Vision AG (Switzerland). Reference compounds, solvents and reagents are of analytical reagent grade or better from Fluka Chemie GmbH (Switzerland).

Samples were irradiated with ^{60}Co gamma rays at 25 and 50 kGy, respectively in a radiation sterilization plant of Studer Draht-und Kabelwerk AG (Däniken, Switzerland).

2.2. Apparatus

2.2.1. GC and GC-MS

Instrumental conditions are shown in Table 1. GC-MS chromatograms were recorded by monitoring the total ion current in the range of 30–400 u. Transfer line and ion trap manifold were maintained at 220 and 170 °C, respectively. Mass spectra were obtained by electron impact at 70 eV and molecular weights were obtained by chemical ionization (CI, with methane reagent gas) at 15 eV.

Identification analyses were performed by matching their mass spectra with those recorded in the National Institute of Standards and Technology (NIST) instrument library, and referring to their molecular weights from CI mass spectra. Confirmation was performed using authentic reference, retention time order and two analytical columns with different polarities.

2.2.2. Headspace-GC-MS

The analysis was carried out on an instrument of Varian 3400cx Saturn 4D/GC-MS-MS equipped with a headspace autosampler as described in our former work [6]. A column of Rtx®-624 (30×0.32 m I.D., 1.8 μm, from Restek Corporation) was used for analysis on the conditions: 100 °C (hold 5 min) to 200 °C at 5 °C min^{-1} (hold 55 min), Helium carrier gas (5.0 grade), and flow rate 1.1 ml min^{-1}. Headspace platen temperature was 110 °C for 1 h. Mass spectrometry conditions were the same as above in GC-MS.

2.3. Sample preparation

2.3.1. Cetostearyl alcohol

CSA and 1-heptadecanol (Internal Standard) were prepared in hexane for assay test. To reach better dissolution at high concentrations, CSA was prepared in cyclohexane for impurity test (see details in Table 2).

2.3.2. Eye ointment base

According to Table 2, EOB was accurately weighed and dissolved in hexane with vortexing and warming in a 45 °C water bath. To remove hydrophobic ingredients from the solution, methanol was used, following by vigorously shaking, cooling down in ice bath for ≈10 min, then filtering. The filtrate was used for gas chromatography injection of assay test. For impurity test, all filtrate and washings were further treated by vacuum evaporation, and then the dry residues was dissolved in cyclohexane.

Table 1
Experimental conditions for assay and impurity analysis

	Assay test	Impurity test, GC and GC-MS
Instrument	Varian 3400cx	Varian 3400cx, Saturn 4D/GCMS/MS
Column[a]	GBG 1, 30 × 0.53 m 1. D., 2 μm	(1) Rtx®-624, 30 × 0.32 m I.D., 1.8 μm (2) Rtx®-5MS, 30 × 0.25 m I.D., 0.25 μm
Temperature	100 °C (hold 2 min) to 250 °C at 8 °C min^{-1} (hold 5 min)	(1) 90 °C (hold 15 min) to 220 °C at 15 °C min^{-1} (hold 75 min) (2) 50 °C (hold 1 min) to 200 °C at 5 °C min^{-1} (hold 100 min)
Carrier gas	He (5.0 grade), 6.0 ml min^{-1}	He (5.0 grade), 1.1 and 1.4 ml min^{-1}
Detector	FID (340 °C)	FID(340 °C), MSD
Injection	SPI, 60 °C (hold 0.5 min) to 250 °C at 200 C min^{-1} (hold 20 min), 1.0 μl	SPI, 50 °C (hold 0.5 min) to 250 °C at 100 °C min^{-1} (hold 70 min), 1.0 μl
Sample	0.2 mg ml^{-1} (CSA)	25 mg ml^{-1} (CSA)

[a] GBG 1 capillary column, a column with polydimethylsiloxane stationary phase, is from GBG Analytik RG, and Rtx®-624 and Rtx®-5ms are from Restek Corporation.

2.3.3. Chloramphenicol eye ointment

An equivalent amount of CAPEO was accurately weighed into a 15-ml glass centrifuge tube, followed by adding 10 ml internal standard solution (for assay test) or hexane (for impurity test). The sample was warmed in a 45 °C water bath for 5 min with stirring twice for 20 s, and centrifugated at 3000 rpm min^{-1} for 2 min. The supernatant liquid was quantitatively collected together with washings. Then the above-described procedure for EOB was followed.

2.3.4. Samples for headspace-GC-MS

Five hundred milligrams of sample was weighed in a headspace vial and capped air-tightly for the chromatographic procedure.

3. Results and discussion

3.1. Assay test

3.1.1. Method suitability

To determine accurately the degree of radiolysis, a valid assay test (a quantitative analysis of main content) has to be established first. A typical chromatogram from CAPEO sample is presented in Fig. 1, which indicates that good resolutions were fulfilled among the cetyl alcohol, stearyl alcohol, internal standard compound, and trace impurities.

Six replicate injections of a CSA solution were performed to determine the GC method precision. For precision of sample preparation method, six independent CAPEO samples were prepared according to the described sample preparation pro-

Table 2
CSA extraction from ointment base for essay and impurity tests

Step	Assay test	Impurity test
1	Weigh equivalent 25 mg CSA in 25-ml volumetric flask	Weigh equivalent 100 mg CSA in 250-ml conical flask
2	Add 10 ml IS solution[a], dilute with hexane	Add 30 ml hexane
3	10 ml of above solution in 50-ml volumetric flask, dilute with methanol	Add 75 ml methanol
4	Filter	Filter, and wash with 10 ml 0 °C methanol, three times
5	None	Evaporate all filtrates to dryness
6	None	Dissolve in 2 ml cyclohexane

[a] Internal standard solution (1- heptadecanol. 1.25 mg ml^{-1} in *n*-hexane).

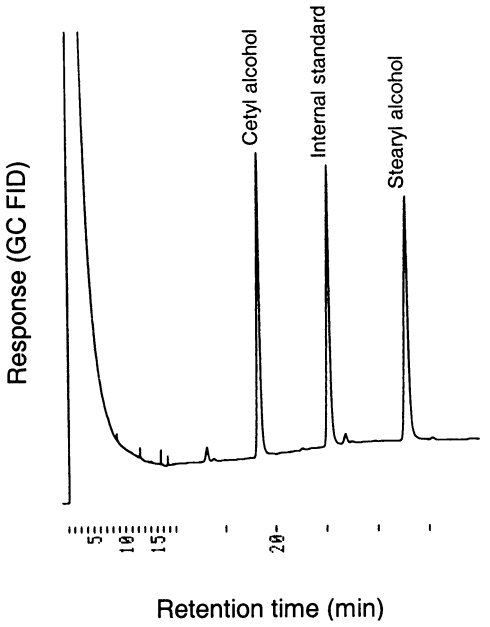

Fig. 1. GC chromatogram of assay test, internal standard = 1-heptadecanol (sample: extraction from CAPEO).

cedures, and followed by three successive GC injections for each sample. This precision of sample preparation method covers the errors from GC determination and sample preparation procedure. These results were summarized in Table 3. Both GC and sample preparation methods have a precision well below 1.0%, the criteria of international requirements [7].

The linearity of peak area responses versus concentrations was studied for the sample preparation method at five levels over a range of 80–120% of the nominal sample active concentration. CAPEO samples were used to perform the linearity measurement and four replicates were taken at each level. Good linearities for both cetyl alcohol and stearyl alcohol over the range were attested by least squares regression and statistical analysis (Fig. 2).

The recovery of 96.9 and 97.3% was obtained for cetyl alcohol and stearyl alcohol, respectively, which were calculated with the area averages of the target compounds in pure state and in CAPEO (Table 3). Although a recovery of 100% did not reach by this assay method, the excellent precision can ensure accurate determination after relevant calibration.

These results demonstrate that the sample preparation procedure and the GC processing are suitable for CSA quality control and their stability studies.

3.1.2. Radiolysis degree in different matrices

Radiolytic behaviors of components in a formulated product may be different from those occurring when these components are exposed to irradiation individually. Thus, investigations for the radiolysis of CSA were carried out in pure state, EOB and CAPEO, respectively.

Although irradiated pure CSA showed no changes in color and appearance at doses of 25 and 50 kGy, content losses of both cetyl alcohol and stearyl alcohol were detected by the assay test in all studied cases. At irradiation dose of 25 kGy, the standard irradiation dose for pharmaceuticals, 1.40% of cetyl alcohol and 1.94% of stearyl alcohol degraded in pure state, 2.15 and 1.86% in EOB, and 1.10 and 1.55% in CAPEO (Fig. 3). The losses are directly proportional to the irradiation doses.

Table 3
Precision of GC method and sample preparation method for CSA assay test (n = 6)

Compounds	GC method			Sample preparation method			Recovery (%)
	Mean[a]	S.D.	R.S.D. (%)	Mean[a]	S.D.	R.S.D. (%)	
Cetyl alcohol	1.013	0.005	0.48	0.982	0.0031	0.31	96.9
Stearyl alcohol	1.083	0.005	0.47	1.054	0.0031	0.30	97.3

Pure CSA samples were used for GC method measurement, and CAPEO samples were used for sample preparation method measurement.

[a] Mean of GC relative responses versus internal standard.

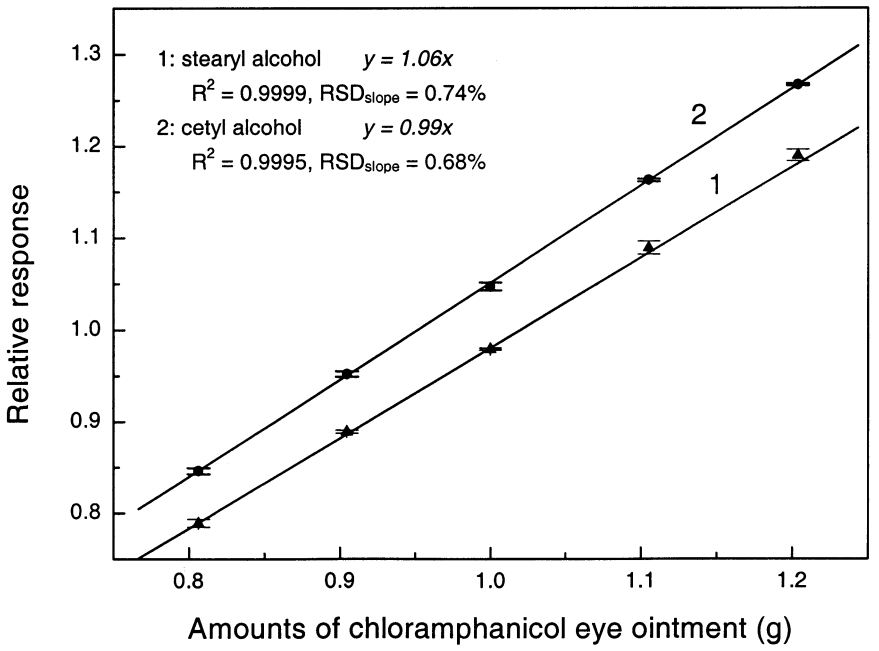

Fig. 2. Linearity of assay test for cetyl alcohol and stearyl alcohol (samples: extractions from CAPEO, $n = 4$ at each points).

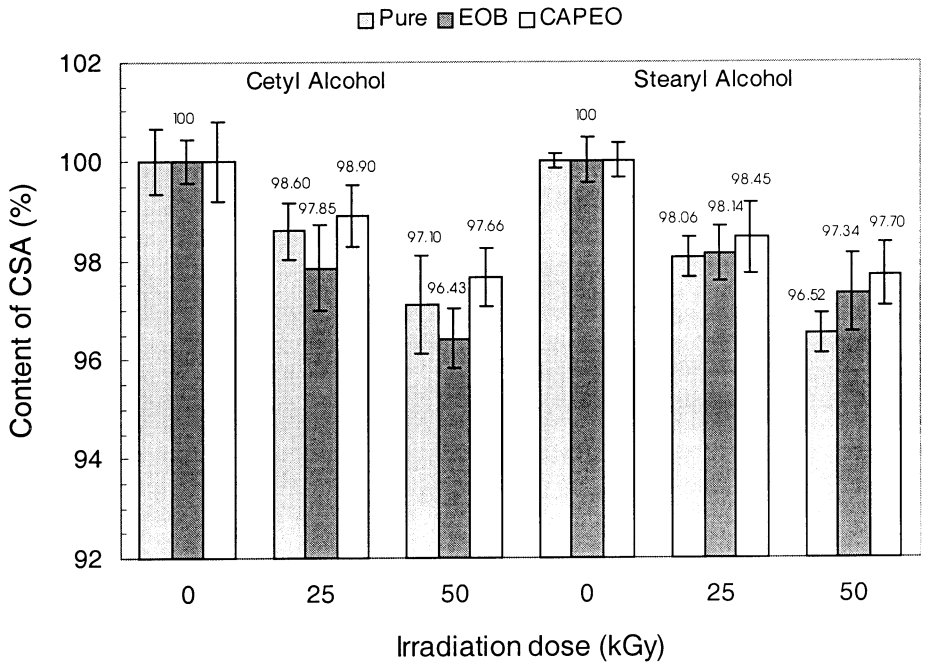

Fig. 3. Determination of gamma radiation effect on CSA content by assay test in different matrices (Pure, pure CSA; EOB, eye ointment base without chloramphenicol; CAPEO, chloramphenicol eye ointment; $n = 6$).

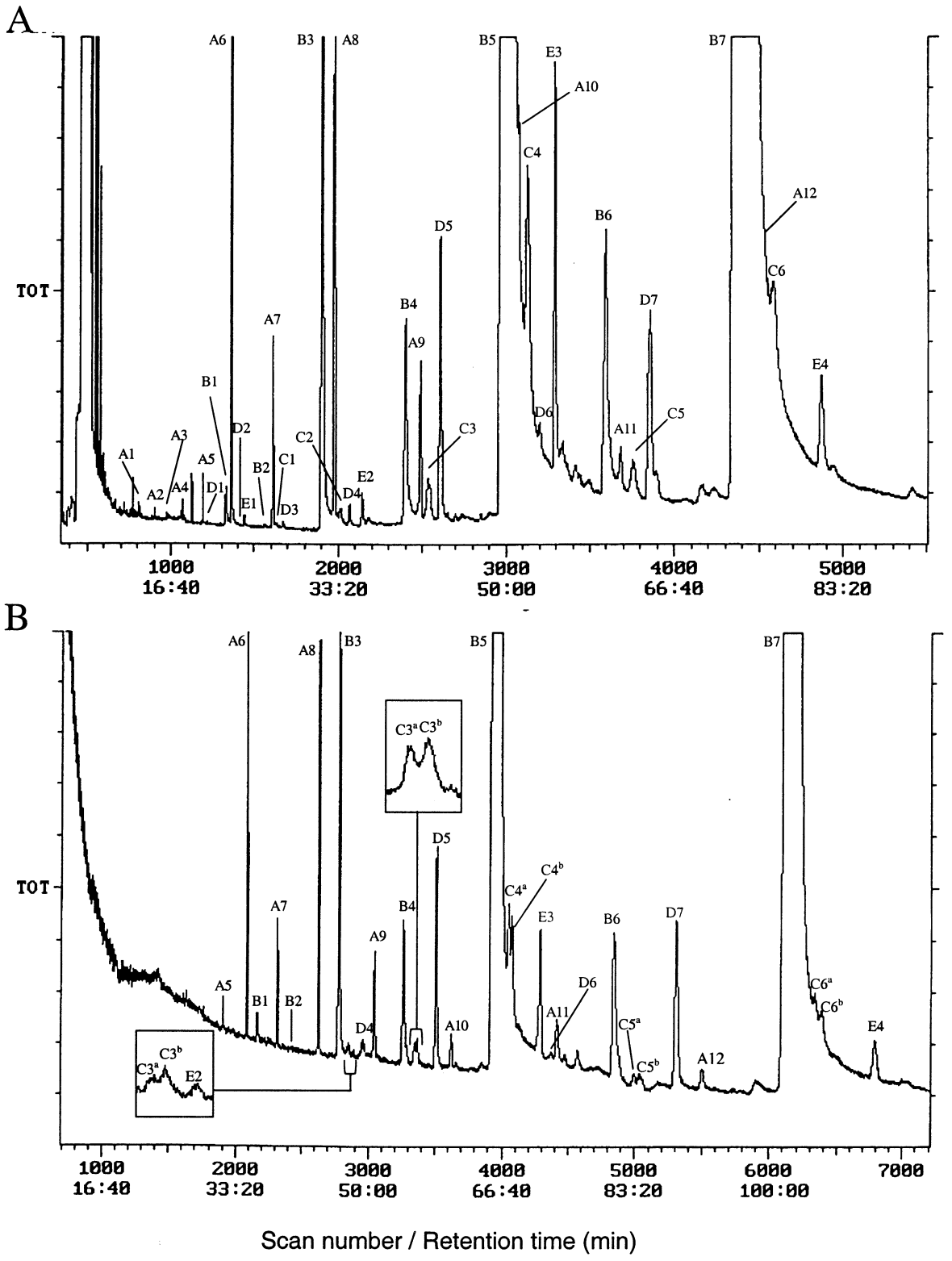
A
TOT
A1
A2
A3
A4
A5
A6
B1
D1
D2
E1
A7
B2
C1
D3
B3
A8
C2
D4
E2
B4
A9
D5
C3
B5
E3
A10
C4
D6
B6
A11
D7
C5
B7
A12
C6
E4
1000
16:40
2000
33:20
3000
50:00
4000
66:40
5000
83:20
B
TOT
A6
A8
B3
B5
B7
C3a C3b
D5
C4b
C4a
A7
B4
E3
B6
D7
A9
A5
B1
B2
D6
C6a
C6b
D4
A10
A11
C5a
E4
C3b
C3a
E2
C5b
A12
1000
16:40
2000
33:20
3000
50:00
4000
66:40
5000
83:20
6000
100:00
7000
Scan number / Retention time (min)

Fig. 4

Table 4
Statistic analysis of t-test between different groups at 50 kGy

$n = 6$	Pure state		EOB		CAPEO		t = test ($t_{0.05,5} = 2.57$)	
	Mean	S.D.	Mean	S.D.	Mean	S.D.	Pure/EOB	EOB/CAPEO
Cetyl alcohol	97.10	0.954	96.43	0.588	97.66	0.562	1.47	3.71
Stearyl alcohol	96.52	0.383	97.34	0.755	97.70	0.628	2.37	0.9

EOB, eye ointment base; CAPEO, chloramphenicol eye ointment; S.D., standard deviation.

Table 5
Radiolytic products and original impurities of CSA

Peak no.	Compounds	Products	Peak no.	Compounds	RPs
A1	Decane	+	**C2**[a]	3-Pentadecanol	Δ
A2	Undecane	+	**C2**[b]	2-Pentadecanol	Δ
A3	Dodecane	+	**C3**[a]	3-Hexadecanol	Δ
A4	Tridecane	+	**C3**[b]	2-Hexadecanol	Δ
A5	Tetradecane	+	**C4**[a]	3-Heptadecanol	Δ
A6	Pentadecane	+	**C4**[b]	2-Heptadecanol	Δ
A7	Hexadecane	+*	**C5**[a]	3-Octadecanol	Δ
A8	Heptadecane	+*	**C5**[b]	2-Octadecanol	Δ
A9	Octadecane	+*, Δ	**C6**[a]	3-Nonadecanol	Δ
A10	Nonadecane	Δ	**C6**[b]	2-Nonadecanol	Δ
A11	Eicosane	Δ	**D1**	Dodecanal	+, ?Δ
A12	Henicosane	Δ	**D2**	Tridecanal	+, ?Δ
B1	1-Dodecanol	Δ	**D3**	Tetradecanal	+, ?Δ
B2	1-Tridecanol	Δ	**D4**	Pentadecanal	+, ?Δ
B3	1-Tetradecanol	Δ	**D5**	Hexadecanal	+*, Δ
B4	1-Pentadecanol	+, Δ	**D6**	Heptadecanal	+, Δ
B5	1-Hexadecanol	Δ	**D7**	Octadecanal	+*, Δ
B6	1-Heptadecanol	+, Δ	**E1**	2-Methyl-1-dodecanol	+
B7	1-Octadecanal	Δ	**E2**	2-Methyl-1-tetradecanol	+
C1[a]	3-Tetradecanol	Δ	**E3**	2-Methyl-1-hexadecanol	+*
C1[b]	2-Tetradecanol	Δ	**E4**	2-Methyl-1-octadecanol	+*

Products; +, radiolytic product; +*, main radiolytic product; Δ, original impurity; ?Δ, possible original impurity. Peak no. C(1-6)[a], C(1-6)[b], isomeric forms of n-alkanols.

This is different from the previous report that cetyl alcohol remained unchanged at 25 kGy [8].

Assay test also shows that the influence of surrounding media on CSA content is modest during γ processing although the degradation degrees are different among pure state, CAPEO and EOB. EOB (without active ingredient chloramphenicol) played a little role during irradiation. Statistic analysis of t-test shows that differences between pure state and EOB are insignificant because the calculated t-values are < 2.57, the critical value of $t_{0.05,5} = 2.57$ (Table 4). The degradation degrees of both cetyl alcohol and stearyl alcohol in CAPEO are slightly lower than those in both pure state and EOB. This suggests that chloramphenicol molecule functions somehow as a scavenger for CSA during γ processing. This slight scavenger function was clearly detected

Fig. 4. Total ion current chromatograms of GC-Ms of irradiated CSA at 50 kGy (A, using a non-polar column of Rtx-5MS®; B, using a mid-polar column of Rtx-624®).

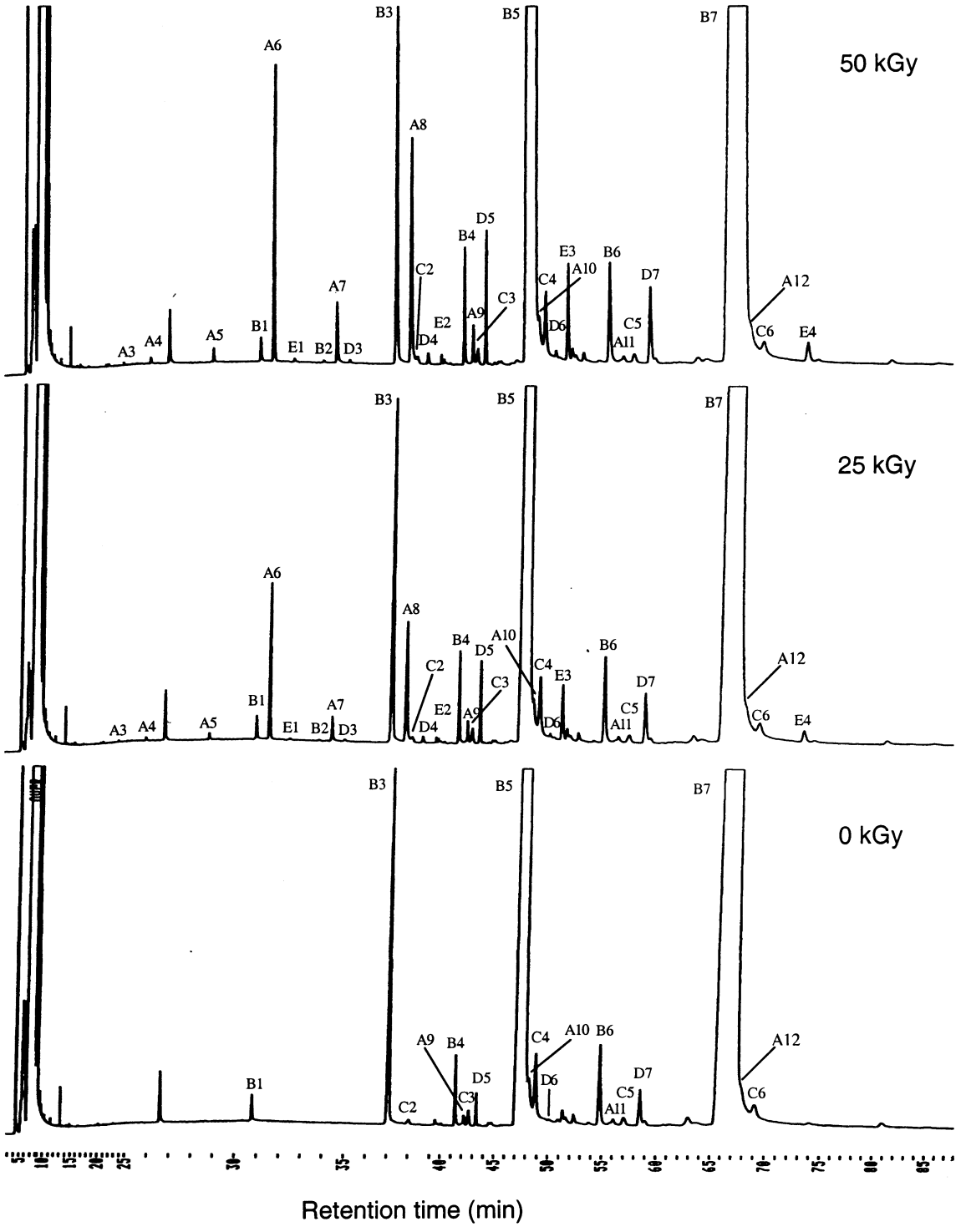

Fig. 5. Comparison of GC chromatogram between irradiated and non-irradiated CSA (using a non-polar column of Rtx-5®).

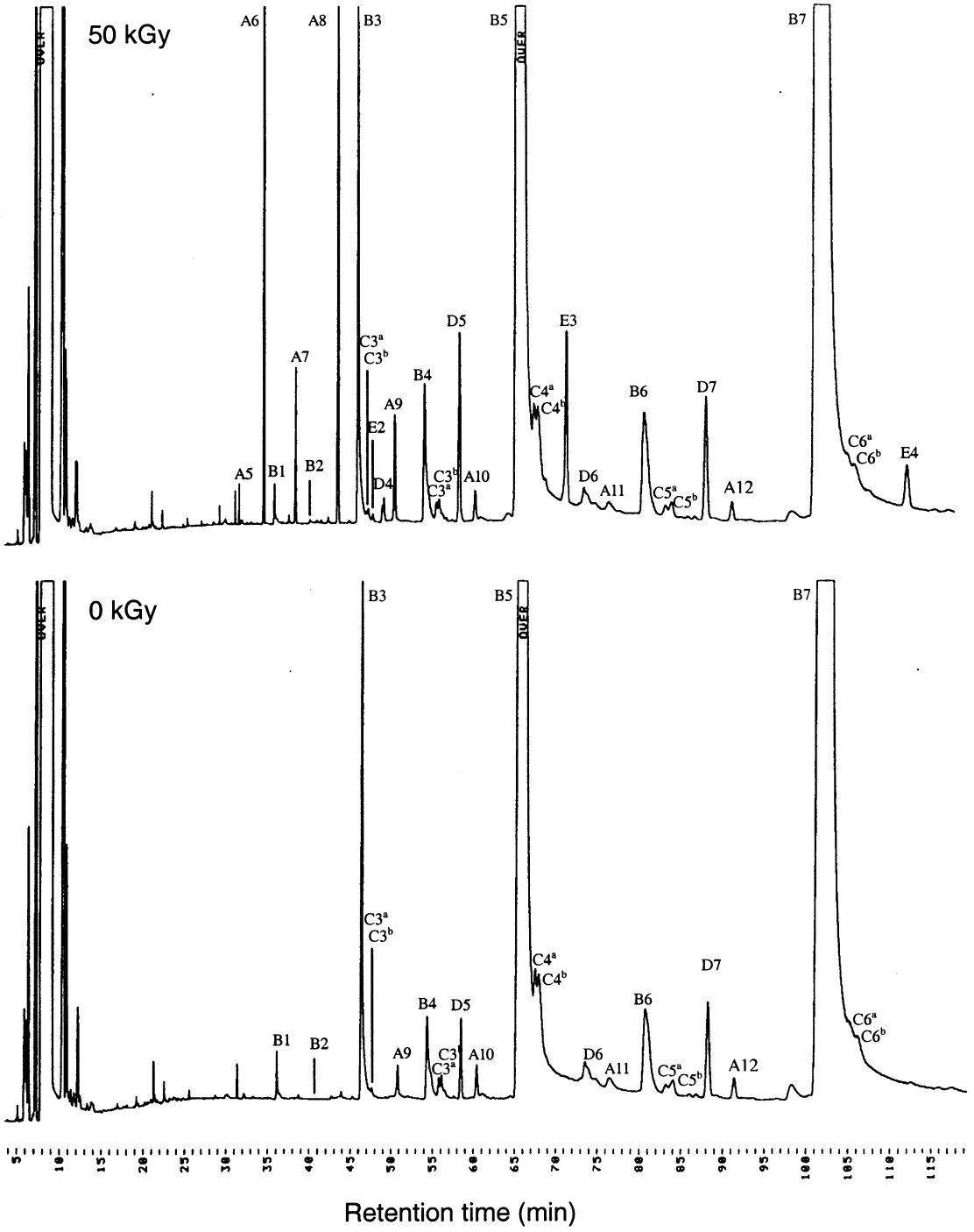

Fig. 6. Comparison of GC chromatogram between irradiated and non-irradiated CSA (using a mid-polar column of Rtx-624®).

in the case of cetyl alcohol by *t*-test as well. The *t*-value calculated between EOB and CAPEO is 3.71, higher than 2.57 (Table 4). Chloramphenicol molecule might somewhat alleviate the radiolysis of CSA, or its exciting energy could be lower than those of cetyl alcohol and stearyl alcohol [9,10].

3.2. Radiolysis products

3.2.1. Identification

Identification of impurities and radiolytic products in irradiated CSA was carried out using GC-MS. Typical total ion current chromatograms describes impurity profile of a CSA sample irradiated at 50 kGy in Fig. 4, in which chromatogram (A) impurity profile of CSA using a non-polar column of Rtx-5®. In order to verify the identification result and especially to improve the resolution to the peaks **A10**, **A12** and C*i* series, the same experiment was performed with a mid-polar column of Rtx-624 as shown in chromatogram (B). The assigned compounds were found in both cases and gave the same mass spectra. With column Rtx-624, all peaks of 'C' series in chromatogram (A) are separated into two peaks and assigned to the compounds of series of 'C^a' and 'C^b'. The presence of compounds of **A10** and **A12** in chromatogram (A) was clearly confirmed in chromatogram (B).

The mass spectrum analyses show that homologues with long straight carbon chain give similar mass spectra, which was independent on the carbon chain numbers of the homologues when the carbon chain numbers are >13. The original impurities and radiolytic products in the irradiated CSA (Fig. 4) can be sorted into six series of 'A', 'B', 'C^a', 'C^b', 'D', and 'E'. By using the mass spectra and the molecular weights, which were determined by GC-MS-CI analyses, 42 compounds in irradiated CSA were assigned and listed in Table 5. The 'A' series was assigned as *n*-alkane, 'B' *n*-alkan-1-ol, 'C^a' *n*-alkan-2-ol, 'C^b' *n*-alkan-3-ol, 'D' *n*-aldehyde, and 'E' 2-methyl-1-alcohol, respectively.

By comparing the chromatograms of the non-irradiated and irradiated CSA in Fig. 5, the radiolytic products were distinguished from the original impurities (the results were summarized in Table 5). Compounds **A6**, **A7**, **A8**, **D5**, **D7**, **E3** and

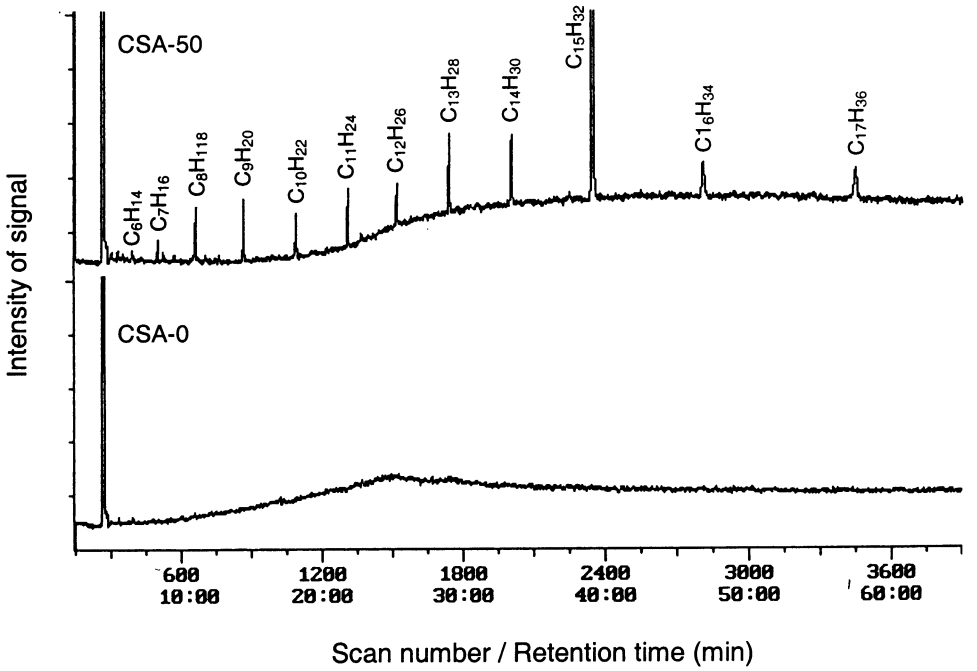

Fig. 7. Total iron chromatograms of Headspace-GC-MS of CSA non-irradiated (CSA-0) and irradiated at 50 kGy (CSA-50).

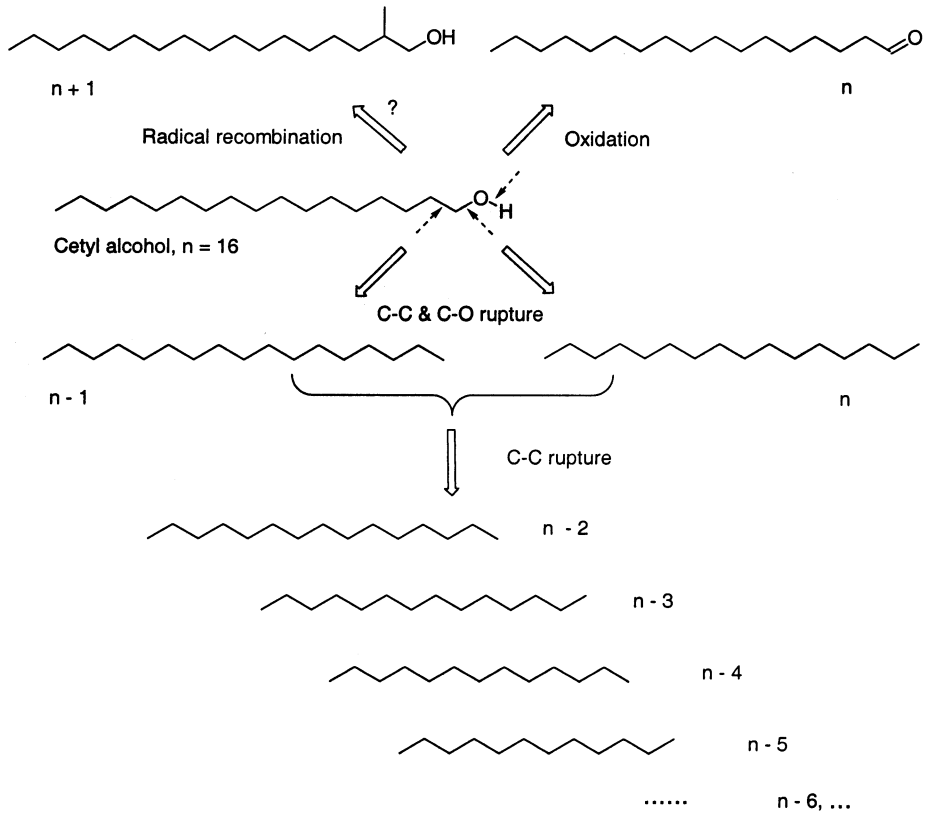

Fig. 8. Radiolysis pathway of cetyl alcohol (n, the carbon number of cetyl alcohol).

E4 (pentadecane, hexadecane, heptadecane, hexadecanal, octadecanal, 2-methyl-1-hexadecanol and 2-methyl-1-octadecanol) are main radiolytic products, which appear or increase dramatically after irradiation and proportionally to irradiation doses.

Compounds in 'D' series were not unique radiolytic products, and they are also original impurities, which exist in non-irradiated CSA and their amounts increase after irradiation. As oxidation products from alcohol, they could be produced by spontaneous oxidation under air and oxidized by radical oxidants $^{\bullet}OH$, $HO_2^{\bullet}$ and peroxide during gamma irradiation. These radical oxidants would be easily derived from water molecule radiolysis during the gamma processing because the presence of moisture is unavoidable in the given samples [11].

Compounds of series 'E' are unique γ radiation products, and they are not detected before irradiation. In contrast, compounds of series C^a and C^b are definitely non-radiolysis products, and they exist in non-irradiated CSA and remain unchanged after irradiation. The case of alkane compounds is special. Compounds of **A1**–**A8** are unique radiolytic products and they are present only in irradiated CSA. Octadecane (**A9**) is both radiolytic product and original impurity. Nonadecane (**A10**), and henicosane (**A12**) are difficult to recognize with column Rtx-5®. However, the chromatograms by using the mid-polar column of Rtx-624® show that they are only original impurities because they keep unchanged before and after irradiation (Fig. 6), the same as eicosane (**A11**) .

Chromatogram impurity profiles (at 0, 25 and 50 kGy) shown in Fig. 5 also shows that the

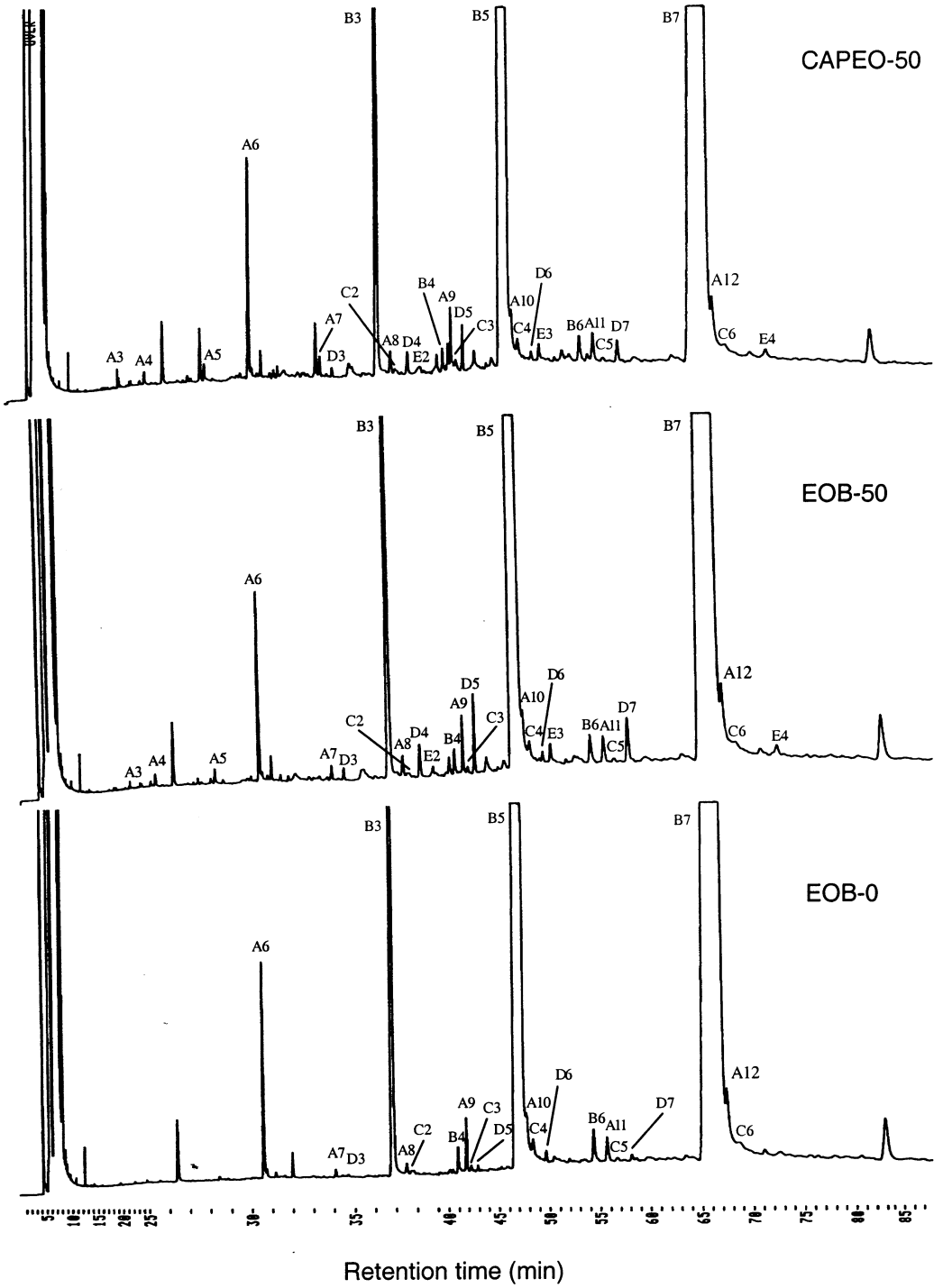

Fig. 9. Impurity profiles of irradiated chloramphenicol eye ointment (CAPEO-50) and eye ointment base (EOB-50) as well as non-irradiated eye ointment (EOB-0), using Rtx-5® column.

amount of all radiolysis products increased proportionally with the irradiation dose.

3.2.2. Radiolysis pathways

As described above, whether or not the alkane compounds were radiolysis products depended on the number of their carbon chain. When their carbon chain numbers are $\leqslant 18$, they are radiolysis products, and otherwise they are non-radiolytic products. Fig. 5 shows that the concentration level of peaks **D5** (hexadecanal) and **D7** (octadecanal) are approximately identical and they are two highest peaks amongst their homologues, while the concentration of **D6** (heptadecanal) is much lower. It is also true for **A6** (pentadecane) and **A8** (heptadecane), **A7** (hexadecane) and **A9** (octadecane), as well as E3 (2-methyl-1-hexadecanaol) and E4 (2-methyl-1-octadecanol).

In the irradiated CSA system, cetyl alcohol (1-hexadecanol) and stearyl alcohol (1-octadecanol) are the main components at approximately identical concentration levels, while 1-heptadecanol is minor or impurity component. During the irradiation processing, the energy deposited by the gamma rays to a component of a mixture, to a first approximation, is proportional to the fraction of the molecules of each compound. Therefore, it could be inferred that **D5**, **A6**, **A7** and **E3** are the radiolysis products derived from cetyl alcohol, **D7**, **A8**, **A9** and **E4** are the products from stearyl alcohol, and **D6** is the product from 1-heptadecanol.

In addition, Headspace-GC-MS analysis reveals a profile of volatile radiolysis products of irradiated CSA (Fig. 7), showing the presence of lower molecular weight *n*-alkene compounds. This indicates the occurrence of degradation of long carbon chain radicals.

Accordingly, the main radiolysis pathways in irradiated cetyl alcohol can be elucidated as shown in Fig. 8. The degradation pathways of stearyl are similar, but carbon chain number $n = 18$. The principal effect of irradiation on the straight long chain alcohols is cleavage of α-carbon–carbon bonds, resulting in the formation of *n*-alkanes, pentadecane from cetyl alcohol and heptadecane from stearyl alcohol. Both are formed in the greatest yield amongst radiolysis products of CSA, indicating that α-carbon–carbon bonds are the most readily broken bonds in the alcohol with long chain. Octadecane and hexadecane are also main radiolysis products, meaning that scission of the carbon–oxygen bond occurred.

All the long carbon chain *n*-alkanes or radicals may be further fragmented, producing alkane (C_{n-2}, C_{n-3}, C_{n-4}, C_{n-5}, etc., n = carbon atom of cetyl alcohol or stearyl alcohol). These bond rupture reactions could be promoted by ionization process (the primary process of gamma irradiation), and by radical reductants $H^{\bullet}$ and $HO_2^{\bullet}$ through $^{-}e_{solv}$, produced when the radiation energy is absorbed by the target substance [11].

Another important effect is oxidation of hydroxyl group, which results in the formation of aldehydes. The formation of 'E' series compounds could involve bond cleavage and recombination of radicals, but the details were not clear and should be further studied.

3.2.3. Radiolytic products in different matrices

The impurity analysis of CSA was also carried out for irradiated EOB and CAPEO because the radiolytical behavior of the same compounds could be different in different matrix. Chromatogram impurity profiles of CSA in irradiated CAPEO, irradiated EOB and non-irradiated EOB were compared in Fig. 9. Similar radiolysis product profiles were found in both CAPEO and EOB. Radiolysis products that exist in pure CSA appear in irradiated CAPEO and irradiated EOB, and no large amounts of new radiolysis products are found. Three new small peaks were found in the vicinity of peaks **A5**, **A7** and **A9** in the chromatogram of irradiated CAPEO sample, however, they are original impurities because of their appearance in non-irradiated CAPEO (from our unpublished data).

The peaks of most radiolysis products in irradiated EOB are slight higher than those in irradiated CAPEO under identical conditions, suggesting the stronger degradation in EOB. This is in agreement with the assay result in Fig. 3 and demonstrates the scavenger function of chloramphenicol molecule again.

This strongly suggests that CSA in pure state, EOB and CAPEO degrade through similar pathways. Both assay and impurity tests show that the influence of EOB on radiolysis of CSA is negligible.

3.2.4. Safety Assess of radiolysis products

Amongst the radiolysis products, *n*-alkane series are definitely the compounds safe for human use. 2-methyl-1-hexadecanol and 2-methyl-1-octadecanol are the compounds of low toxicity. Although low molecular weight aldehydes, e.g., formaldehyde and acetaldehyde, have sharp, unpleasant odors, the higher molecular weight aldehydes have pleasant, often fragrant odors, which are found in the essential oils of certain plants. Pentadecanal, hexadecanal, and heptadecanal were reported to be found in the essential oil of citrus limon, a traditional medicinal plant [12]. Hexadecanal is listed as a register of flavoring substance used in or on foodstuffs [13]. They are therefore low toxic compounds and safe for human use.

Furthermore, CAPEO contains about 2.5% of CSA and the radiolytical amounts of cetyl alcohol and stearyl alcohol in CAPEO are 1.10 and 1.55% at 25 kGy, respectively (Fig. 3). This means the absolute percentage of radiolysis products from CSA in CAPEO will not be more than 0.066%, which is at a safe concentration level for pharmaceutical products [14,15]. Thus, both qualitative and quantitative data of CSA radiolysis products ensure that gamma process imposes no unsafety effect on CAPEO at the reference radiation dose of 25 kGy.

4. Conclusion

The present assay test method is accurate and suitable, and the impurity test method is specific for the determination of radiolysis products. Gamma sterilization processing causes the radiolysis of CSA in pure state, EOB, and CAPEO. The influence of eye ointment composition on the radiolysis of CSA is modest.

Radiolysis products can be sorted into three series, *n*-alkane, *n*-aldehyde, 2-methyl-1-alcohol. Oxidation of hydroxyl group, cleavages of α-carbon–carbon bonds and carbon–oxygen bond are the main radiolytical reactions. Both assay and impurity tests show that chloramphenicol molecule has a very slight scavenger function for CSA. All the radiolysis products belong to low toxicity compounds and they are at safe concentration levels for pharmaceutical products. Gamma process is feasible for sterilization of CSA in petrolatum eye ointment and pure state.

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