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## Migration of bisphenol A into water from polycarbonate baby bottles during microwave heating

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A study was performed to establish a comprehensive migration database for bisphenol A from polycarbonate baby bottles into water during exposure to microwave heating. Polycarbonate baby bottles from 18 different brands sold in Europe were collected. The initial residual content as well as the migration of bisphenol A after microwave heating were determined. The residual content of bisphenol A in the polycarbonate baby bottles ranged from 1.4 to 35.3 mg.kg<sup>-1</sup>. Migration of bisphenol A into water was determined by placing a polycarbonate bottle filled with water in a microwave oven and heating to 100°C. After heating, the amount of bisphenol A in the water was analysed by GC-MS. The whole procedure of microwave heating and analysis was repeated twice for the same bottle. Thus, 3 migration extracts were prepared for each test specimen. Migration of bisphenol A into water ranged from levels of < 0.1 to 0.7 µg.l<sup>-1</sup>. There was no correlation between the amount of the residual content of bisphenol A in the bottles and the migration of bisphenol A into water. Furthermore, there was also no correlation between the amount of bisphenol A in consecutive migration extracts. The data presented shows that during 3 microwave heating cycles of a baby bottle made from polycarbonate, no effects of microwave radiation on the migration of bisphenol A into water from polycarbonate can be observed. All levels found were well below the Specific Migration Limit of 0.6 mg.kg<sup>-1</sup> specified for bisphenol A in the Commission Directive 2004/19/EC.

**Keywords:** migration, residual content, bisphenol A (BPA), polycarbonate baby bottles, microwave

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## Introduction

Migration of bisphenol A from polycarbonate materials under different testing conditions applying traditional heating is well described in literature. Brede *et. al.* (2003) and Boots (2004) examined the migration of bisphenol A from polycarbonate baby bottles after dishwashing, boiling and brushing, Tan *et al.* (2003) and Biles *et. al.* (1997) investigated the leaching of bisphenol A from old and new baby bottles. Yoshida *et. al.* (2003) and Maragou *et. al.* (2007) determined the migration of bisphenol A after repeated use.

Whereas the influence of time, pH, and temperature on the migration behaviour of bisphenol A from polycarbonate is well investigated, only a few studies have examined the effects of electromagnetic radiation in microwave heating. The results of these studies by Mountfort *et. al.* (1997) and Kawamura *et. al.* (1998) indicate that migration behaviour is not influenced by electromagnetic radiation on the plastic under chosen testing conditions. The recent opinion of the European Food Safety Authority on bisphenol A (EFSA, 2006) indicated that not much information is available on the migration behaviour of bisphenol A from polycarbonate during consecutive microwave heating.

The aim of this study was to establish more information regarding migration behaviour of bisphenol A into water during microwaving of polycarbonate baby bottles. More data in the public domain will be helpful to support the safety of polycarbonate plastic. Published migration studies using conventional heating were mostly performed with water as the food simulant. To allow comparison of data from microwave heating with data of conventional heating, water was used in this study. The selection of water is in accordance with Council Directive 85/572/EEC specifying water as the simulant to be used for milk, although the recent publication of Commission Directive 2007/19/EC, amending Commission Directive 2002/72/EC and Council Directive 85/572/EEC, introduced 50% ethanol as the preferred simulant for milk.

For the purpose of the study polycarbonate baby bottles from different brands sold in Europe were collected and the initial residual content as well as the migration of bisphenol A after repeated microwave heating were determined. The overall procedure was chosen to represent typical conditions for a microwave heating procedure as derived from recommendation from the baby bottles manufacturers.

## Materials and Methods

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2  
3 Eighteen types of bottles from different brands sold in Europe were collected: ten major  
4 brands sold across Europe, 3 local brands from UK, 3 local brands from the Netherlands and  
5 2 local brands from Germany. To warrant consistent quality all bottles of each brand were  
6 purchased from a single source.  
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## 10 11 **Experimental**

### 12 *Sample characterisation*

13 One bottle of each brand was examined by means of infrared analysis to confirm that the  
14 bottles are made of polycarbonate. A piece was cut and was examined on both sides using a  
15 Perkin Elmer Spectrum GX FT-IR System. Spectra were recorded in the ATR mode (Golden  
16 Gate) from  $580\text{ cm}^{-1}$  -  $4000\text{ cm}^{-1}$ . Spectra were searched against commercial libraries to  
17 identify the polymer.  
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### 24 *Residual content of bisphenol A*

25 A sample of each brand was weighed and cut into pieces of about 2x2 cm. All pieces (the  
26 whole bottle was used) were placed in an Erlenmeyer flask. For each g of polycarbonate  
27 material 10 ml of dichloromethane was added. Bisphenol F (Aldrich) was added to the  
28 dichloromethane as internal standard at a concentration of  $0.8\text{ mg.l}^{-1}$ . The solution was  
29 refluxed until the polycarbonate was completely dissolved. A volume of methanol (0.5 x  
30 volume of dichloromethane) was slowly added to precipitate the polymer fraction, while  
31 continuing refluxing for about 2 min. The solution was filtered, and the filtrate was stored in a  
32 refrigerator for at least 6 hours. An aliquot of the clear liquid containing the residual bisphenol  
33 A was transferred into a vial and analysed by HPLC. This whole procedure was performed in  
34 duplicate.  
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### 43 *Instrumental conditions*

44 HPLC analysis was performed using a Alliance 2695 HPLC (Waters) equipped with an  
45 automatic sampler/injector and a Fluorescence 474 detector (Waters) and a PDA 996 (UV)  
46 detector (Waters). The column was a Sunfire™ C18  $3.5\text{ }\mu\text{m}$   $3.0\times 150\text{ mm}$  column (Waters),  
47 kept at  $35^{\circ}\text{C}$ . Elution was performed using a gradient as shown in table 1. Fluorescence  
48 detection was performed at an excitation wavelength set at 278 nm and emission wavelength  
49 set at 305 nm. UV spectra were recorded for identification purposes.  
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55

56 Table 1 Eluent gradient

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60 \*A=THF; B=water+0.1% TFA (trifluoroacetic acid); C=Acetonitril+0.1% TFA

### *Recovery*

One sample was dissolved in dichloromethane as described above. The dichloromethane was divided in aliquots of 30 ml which were spiked at a level of 0.3 mg.l<sup>-1</sup>. The recovery in a spiked sample extract was 103.3%. The working detection limit (WDL calculated from calibration curve according to DIN 32645) was 0.5 mg/kg.

### *Migration of bisphenol A*

#### *Sterilisation conditions*

Sterilisation conditions were derived from recommendation from the baby bottles manufacturers. For each brand 6 specimens were immersed in boiling water (water purified using a Milli-Q gradient A10 System) for 5 min. After this, the bottles were placed on a grid and left to dry for at least 3 hours.

#### *Heating and cooling conditions*

The microwave oven (Whirlpool Jet Stream JT357) was operated at maximum power (jet-mode, nominal power 1000 W). The power output of the microwave oven was determined as described in EN 14255:2002. This was carried out once before the migration studies, once in the middle of the series and once after finishing the migration tests. The average power output of the microwave oven was 728 W (RSD 3%). For one sample a temperature profile was recorded during microwave heating using a calibrated fibre-optic thermometer (950 Multichannel Fluoroptic Thermometer (Luxtron)). One probe was placed in the centre of the bottle and one probe was placed at the inside wall of the bottle. The bottle was filled with 100 ml of water and heated in the microwave oven without subsequent cooling down in the water bath. The microwaving was started after 5 sec, boiling was achieved after 1 min and 54 sec and maintained one minute till 2 min and 54 sec, after which the microwave door was opened. Figure 1 shows the temperature profiles. The temperature profiles indicate no difference between the temperature at the centre of the bottle and the temperature at the wall.

#### *Figure 1. Temperature profile*

To determine the loss of water during microwaving, the weight of a bottle filled with 103 g of water was determined before and after microwave heating. The difference in weight was 9.5 g, which means that 9 % of water was evaporated during microwave treatment. To analyse how long it takes that the microwave heated bottle content cools down to a temperature of 37°C in a water bath at 20°C, a bottle of was filled with 100 ml of water and a conventional

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3 thermometer was inserted at the centre of the bottle. After heating to 100 °C, a temperature of  
4 37 °C in a water bath at 20 °C was reached after 7 min and 44 sec.  
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#### 8 *Sample processing*

9  
10 After sterilisation, the dry bottles were filled with 100 ml of water (Sigma Aldrich, HPLC-  
11 grade) at room temperature, and placed in the centre of the microwave oven. Bottles with a  
12 volume greater than 200 ml were filled with 200 ml of water. The water in the bottle was  
13 heated until boiling, and boiling was maintained for 1 minute with unchanged power capacity.  
14 After this period the bottle was placed in a water bath at 20 °C until the water in middle of the  
15 bottle had reached a temperature of 37 °C. Then the content of the bottle was transferred into  
16 glassware to be analysed. The emptied bottle was rinsed 3 times by shaking with 50 ml  
17 water (purified using a Milli-Q gradient A10 System) at room temperature, for 15 seconds;  
18 the water was discarded after rinsing. The bottle was placed on a grid and left to dry for at  
19 least 3 hours. This rinsing and drying procedure was chosen to simulate normal use of the  
20 bottle. The procedure as described above was repeated another two times on the same  
21 bottle. Thus, 3 migration extracts were prepared for each test specimen.  
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#### 31 *Analytical method*

32 Migration extracts were fortified with deuterated ( $d_{16}$ ) bisphenol A (Aldrich) as internal  
33 standard at a concentration of 40 ng per 100 ml extract. The samples were extracted and  
34 concentrated using SPE (Solid Phase Extraction) columns (ENV+, 100 mg). The SPE  
35 columns were placed on a SPE station, and solvated with 3 ml acetone, followed by 3 ml  
36 methanol, and conditioned with 3 ml of water. The water extracts were transferred through  
37 the SPE columns by applying a vacuum. Before elution, the SPE packing was dried by  
38 applying a vacuum for about 20 min. The SPE columns were eluted with 2 ml acetone. The  
39 acetone was evaporated and the residue was re-dissolved in 10  $\mu$ l pyridine and 90  $\mu$ l MSTFA  
40 (N-Methyl-N-(trimethylsilyl) trifluoroacetamide) in a GC-vial, and heated for 45 minutes at  
41 40 °C.  
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#### 51 *Instrumental conditions*

52 GC-MS analyses were achieved using an Agilent 6890N GC coupled to a Agilent 5973N  
53 mass spectrometer. Gas Chromatographic conditions: column HP-5MS, 0.25mm x 30m  
54 0.25 $\mu$ m film thickness (Agilent); constant flow helium 1.5 ml.min<sup>-1</sup>; Injection; 1  $\mu$ l splitless  
55 injection; Injection temperature, 250 °C; oven initial temperature, 90 °C; oven initial time 4 min;  
56 oven rate, 10 °C.min<sup>-1</sup>; oven final temperature, 300 °C; oven final time 10 min. Mass  
57 spectrometer conditions: ionization mode, EI<sup>+</sup> ; scan range, 50 – 600  $m/z$ ; scan time 0.33 s  
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3 Bisphenol A and the internal standard deuterated bisphenol A were quantified using  
4 extracted ion chromatograms at m/z 357 and m/z 368 respectively.  
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#### 8 *Calibration*

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10 Calibration solutions were prepared by spiking 100 ml water (Sigma Aldrich, HPLC-grade)  
11 samples with bisphenol A (Aldrich) at a level of 0.05, 0.1, 0.2, 0.5, 1, 2 and 5  $\mu\text{g.l}^{-1}$ .  
12 Quantification was performed using calculation from regression parameters according to DIN  
13 32645. The working detection limit (WDL calculated from calibration curve according DIN  
14 32645) was 0.1  $\mu\text{g.l}^{-1}$ .  
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#### 18 *Repeatability and recovery*

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20 The repeatability and recovery of the method was determined by spiking 5 aliquots of water  
21 (Sigma Aldrich, HPLC-grade) at a level of 1  $\mu\text{g.l}^{-1}$ . The mean recovery of 5 spiked samples  
22 was 95%. The standard deviation was 2 %.  
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#### 27 *Recovery in migration extracts*

28  
29 Twelve specimens of one sample were sterilised and migration extracts were obtained. The  
30 contents of the 12 specimens were combined and divided in aliquots of 90 ml. Three aliquots  
31 were spiked at a level of 0.1  $\mu\text{g.l}^{-1}$  and 3 aliquots were spiked at a level of 0.2  $\mu\text{g.l}^{-1}$ . The  
32 recovery of spiked migration extracts was 113.7% for the extracts spiked at 0.1  $\mu\text{g.l}^{-1}$  and  
33 91.4% for the extracts spiked at 0.2  $\mu\text{g.l}^{-1}$ . The concentration of the non-spiked extracts was  
34 0.11  $\mu\text{g.l}^{-1}$ .  
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## 41 **Results and discussion**

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43 The FT-IR analysis confirmed that all 18 samples were made of polycarbonate. Figure 2  
44 shows a typical FT-IR spectrum of a polycarbonate baby bottle. Characteristic wavelengths  
45 are 1768  $\text{cm}^{-1}$ , 1503  $\text{cm}^{-1}$ , 1244  $\text{cm}^{-1}$  and 3040  $\text{cm}^{-1}$   
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50 *Figure 2. FT-IR spectrum of a polycarbonate baby bottle*  
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#### 53 *Residual content of bisphenol A*

54  
55 Table 2 shows the results of the residual content of bisphenol A in the polycarbonate baby  
56 bottles. This shows that the residual content of bisphenol A in polycarbonate baby bottles is  
57 in range of 1.4 to 35.3  $\text{mg.kg}^{-1}$ . Figure 3 and 4 present an HPLC chromatogram for the  
58 determination of the residual content of bisphenol A in the polycarbonate.  
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1  
2  
3 Table 2. Results of the residual content of  
4 bisphenol A in polycarbonate baby bottles.  
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7  
8 *Figure 3. HPLC chromatogram of a sample  $R_{t_{\text{bisphenol A}}}$  15.2 min*  
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11 *Figure 4. HPLC chromatogram of a sample with an addition of  $0.3 \text{ mg.l}^{-1}$  of bisphenol A.*  
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#### 14 *Migration of bisphenol A*

15  
16 Table 3 gives the average results of the migration of bisphenol A from the polycarbonate  
17 baby bottles into water after 3 microwave heating cycles, indicating that the migration of  
18 bisphenol A from the polycarbonate baby bottles into water ranged from  $< 0.1$  to  $0.7 \text{ }\mu\text{g.l}^{-1}$ .  
19 Figure 5 - 7 present GC-MS chromatograms for the determination of migration of bisphenol A  
20 into water after microwave heating.  
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25  
26 Table 3. Average results of the migration of bisphenol A into water after 3 microwave heating  
27 cycles.  
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31 *Figure 5. GC-MS chromatogram of a standard bisphenol A at a level of  $1 \text{ }\mu\text{g.l}^{-1}$ .*  
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34 *Figure 6. GC-MS chromatogram of a sample*  
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36

37 *Figure 7. GC-MS chromatogram of a sample with an addition of  $0.1 \text{ }\mu\text{g.l}^{-1}$  of bisphenol A.*  
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#### 40 *Statistical analysis*

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42 To determine if there is a correlation between the amount of the residual content of bisphenol  
43 A in the bottles and the migration of bisphenol A, Spearman's correlation coefficients were  
44 calculated. This coefficient is a non-parametric version of Pearson's correlation coefficient, in  
45 which the actual data is converted to rankings before calculating the coefficient. The  
46 Spearman's correlation coefficients reveal that there is no correlation between the residual  
47 content and the migration of bisphenol A.  
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#### 52 Spearman's correlations

53  
54 (r; p-value is given between brackets)

55 Residual content versus 1<sup>st</sup> migration extract:  $r=0.005$  ( $p=0.9833$ )

56 Residual content versus 2<sup>nd</sup> migration extract:  $r=0.055$  ( $p=0.8271$ )

57 Residual content versus 3<sup>rd</sup> migration extract:

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60  $r=-0.104$  ( $p=0.6803$ )

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5 Furthermore it was tested whether the residual content of bisphenol A differs between  
6 migration extracts. This was done using the Kruskal-Wallis test, which is a one-way analysis  
7 of variance by ranks. The p-value for this test was 0.3690, so there was no difference in  
8 bisphenol A between migration extracts.  
9  
10

### 11 12 13 **Conclusions**

14 The residual content of bisphenol A in the polycarbonate baby bottles ranged from 1.4 to  
15 35.3 mg.kg<sup>-1</sup>. Migration of bisphenol A into water was found to be in the range of < 0.1 to  
16 0.7 µg.l<sup>-1</sup>. The data presented shows that during 3 microwave heating cycles of a baby bottle  
17 made from polycarbonate, no effects of electromagnetic radiation on the migration of  
18 bisphenol A into water from polycarbonate was observed. Statistical analysis (Spearman  
19 correlations) showed there is no correlation between the amount of the residual content of  
20 bisphenol A in the bottles and the migration of bisphenol A. Furthermore, there was no  
21 statistical difference (Kruskal-Wallis correlation) in the amount of bisphenol A in the  
22 consecutive migration extracts. All levels found were well below the specific migration limit of  
23 0.6 mg/kg specified for bisphenol A (Commission Directive 2004/19/EC)  
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38 paper.  
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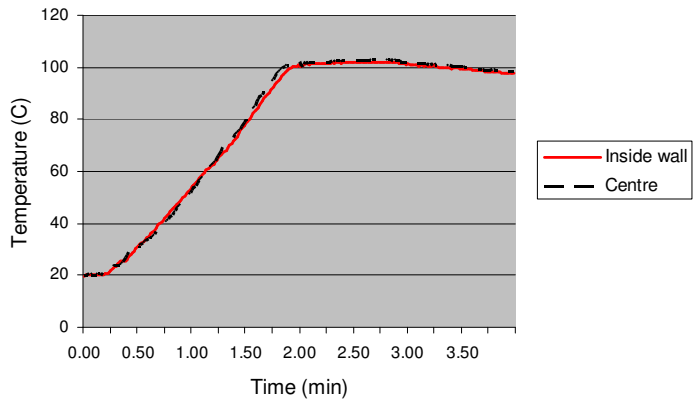
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Time (min)	Flow (ml.min <sup>-1</sup> )	%A*	%B*	%C*
0	0.4	0	95	5
2	0.4	0	95	5
20	0.4	0	0	100
25	0.4	80	0	20
35	0.4	80	0	20
37	0.4	0	0	100
38	0.4	0	95	5
45	0.4	0	95	5

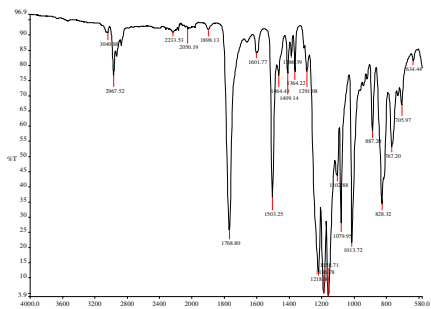
Sample Number	Residual content (mg.kg <sup>-1</sup> )		
	A	B	Average
1	2,6	1,1	1,9
2	1,8	2,8	2,3
3	2,9	3,2	3,1
4	3,5	4,5	4,0
5	2,4	2,5	2,5
6	27,2	30,0	28,6
7	4,6	6,7	5,7
8	7,2	4,0	5,6
9	23,4	26,5	25,0
10	4,1	5,9	5,0
11	4,7	3,8	4,3
12	33,8	36,7	35,3
13	14,4	14,3	14,4
14	3,4	4,2	3,8
15	1,4	1,4	1,4
16	2,5	2,7	2,6
17	2,5	3,1	2,8
18	25,6	16,9	21,3

Sample number	Migration ( $\mu\text{g.l}^{-1}$ )		
	First cycle	Second cycle	Third cycle
1	0.10	<0.10	<0.10
2	0.12	0.11	0.14
3	0.22	0.16	0.30
4	0.26	0.17	0.13
5	0.13	0.11	0.18
6	<0.10	<0.10	<0.10
7	0.10	0.10	0.10
8	<0.10	0.11	<0.10
9	<0.10	<0.10	<0.10
10	0.19	0.12	0.11
11	0.13	<0.10	<0.10
12	0.49	0.73	0.28
13	0.13	<0.10	<0.10
14	0.17	<0.10	0.10
15	0.32	0.11	0.11
16	0.10	<0.10	<0.10
17	<0.10	0.12	<0.10
18	0.21	0.26	0.14

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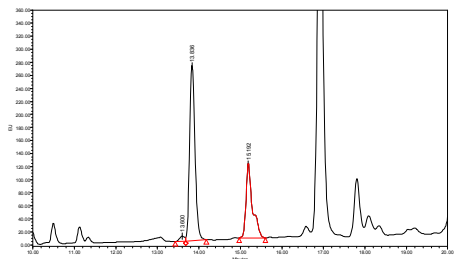


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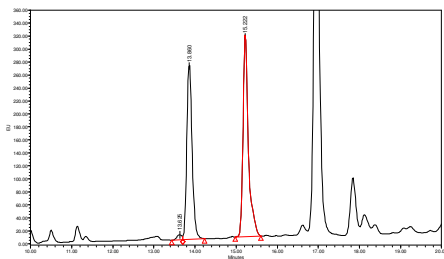
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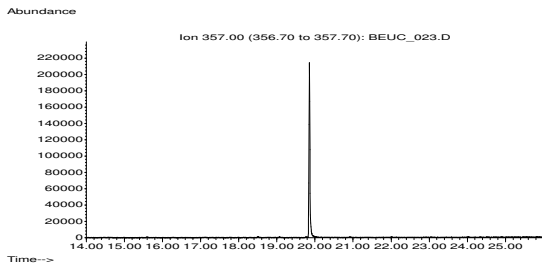


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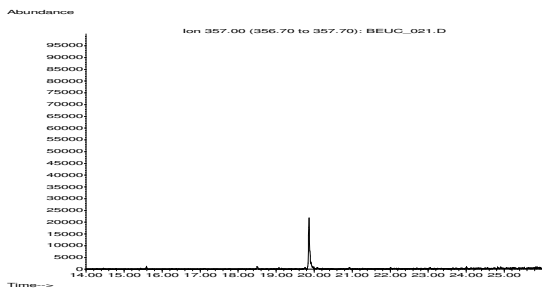
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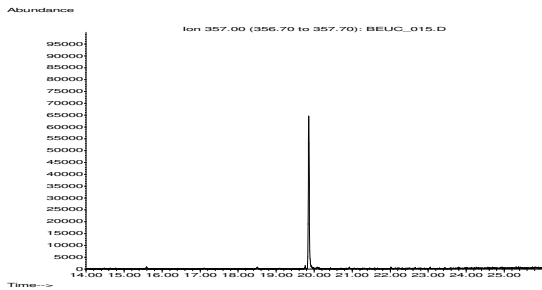
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