

# Combination of Supercritical Fluid Extraction and Gas Chromatography–Mass Spectrometry: Determination of Impurities Extracted from Tablet Preparations of the Benzodiazepine Series

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**Abstract**—A method is proposed for the determination of moderately volatile organic impurities in pharmaceutical tablet preparations based on a combination of solvent-free supercritical fluid extraction (SFE) and gas chromatography–mass spectrometry. The composition of moderately volatile impurities is determined in pharmaceutical tablet preparations of the benzodiazepine series (diazepam, phenazepam, nitrazepam, and clonazepam).

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Organic impurities in drugs are most often determined by such techniques as high-performance liquid chromatography (HPLC) and high-performance thin-layer chromatography (HPTLC). Gas chromatography is used to determine residual amounts of volatile organic solvents [1, 2]. For the determination of impurities in solid substances (technical samples) and tablet forms, the main component and impurities are isolated by solvent extraction. Only a small part of the obtained extract is used in the subsequent analysis. This causes a low sensitivity of the determination of impurities and a misrepresentation of the sample composition due to a loss of some of the impurities to be determined. The use of such a technique for the separation and preconcentration of impurities as supercritical fluid extraction (SFE) can be one of the possible ways of overcoming the described limitations. This technique is widely used to separate organic impurities from various matrices such as soil, polymer materials, plant materials, biological samples, etc. [3–6]. SFE is rarely used for extraction from pharmaceutical preparations; in these cases, the compounds from the fluid flow are isolated into an organic solvent followed by a gas-chromatographic analysis of part of the obtained solution [7–11]. Articles on SFE from pharmaceutical preparations are generally devoted to the extraction of the main component. Therefore, a study of the extraction of impurities from pharmaceutical preparations by SFE without using a solvent is of great interest. In addition, the use of capillary gas chromatography with mass spectrometry for the determination of organic impurities of moderate or low volatility is also interesting.

Thus, the goal of this work was to study the isolation of moderately volatile impurities from tablet forms of pharmaceuticals of the benzodiazepine

series by solvent-free supercritical fluid extraction followed by a chromatographic–mass-spectrometric determination.

## EXPERIMENTAL

We investigated tablet forms of pharmaceutical preparations whose active components were benzodiazepine compounds, namely, diazepam, phenazepam, nitrazepam, and clonazepam.

SFE was performed using a facility containing an SFC 300 pump and an SFC 3000 series thermostat equipped with a cooling system that provides cooling down to  $-15^{\circ}\text{C}$  (Carlo Erba, Italy). A preparation tablet was ground in an agate mortar into fine powder and was placed in an 0.5-mL extraction cell. Extraction was performed with supercritical fluid (carbon dioxide or nitrous oxide) under controlled pressure and temperature ( $40^{\circ}\text{C}$ ). The flow rate of the supercritical fluid was 1.5 mL/min. The extracted compounds were trapped in a special sorption cartridge. Two sequential extractions were performed for each sample. The duration of each extraction was 5 min.

The cartridge for trapping the extract was a metal tube 10 cm in length and 4 mm in inner diameter with quartz-coated inner walls. It was filled with a 3-cm-thick layer of tenax and a 3-cm-thick layer of glass beads coated with a 5% SE-30 stationary phase. The sorbent zones were separated with a 1-cm-thick layer of quartz wool. The substances were trapped with a carrier-gas flow running from the layer of beads to the tenax layer.

The extract was analyzed using a Varian 3400 capillary gas chromatograph (United States) with an Inco-

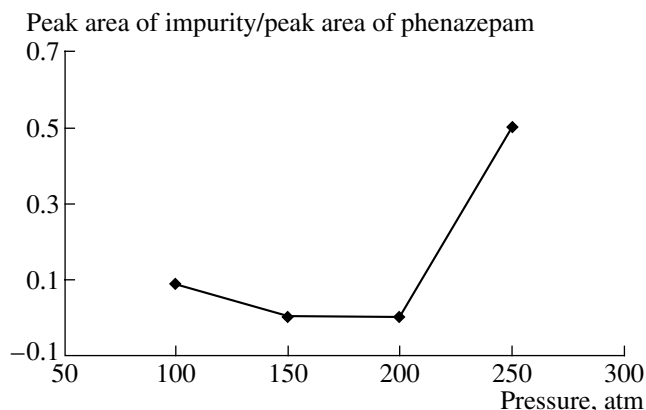
50 mass-spectrometric detector (Finnigan MAT, United States) operating in the electron impact ionization mode with the detection of positively charged ions. One microliter of a  $4 \times 10^{-9}$  g/ $\mu$ L solution of internal standards in methanol was injected in the sorption cartridge prior to the analysis. The extracted compounds were then desorbed for 10 min at 250°C with the carrier gas flow (helium, flow rate 1.5 mL/min) passing through the cartridge in the direction opposite to that at the extraction stage.

Separation was carried out using a 30 m  $\times$  0.32 mm  $\times$  0.5  $\mu$ m column with a CPSil 24 CB stationary phase. The column temperature was programmed as follows: 40°C (10 min)  $\rightarrow$  10°C/min  $\rightarrow$  290°C (30 min). The temperature of the chromatograph evaporator was 270°C. A sample was injected in the splitless mode. We used helium as the carrier gas. The temperature of the transferring line was 260°C, the temperature of the ion source was 180°C, the electron energy was 70 eV, the range of scanned masses was 50 to 500 amu, and the scan rate was 2 scan/s.

A blank experiment was carried out prior to the analysis of each sample. For this purpose, we performed SFE from an empty cell, followed by a GC/MS analysis of the obtained extract. We took into account the results of the blank experiment when treating the chromatographic–mass spectrometric data.

Unknown impurities were identified by comparing the recorded mass spectrum with the NIST database, version 1.7. We considered that the spectrum is reliably assigned to a certain structure if the convergence coefficients in both the direct and reverse library search were above 800.

The concentration of impurities was evaluated using internal standards such as 1,4-dichlorobenzene- $d_4$ , naphthalene- $d_8$ , acenaphthene- $d_4$ , phenanthrene- $d_4$ , cryzene- $d_4$ , and perylene- $d_4$ .



**Fig. 1.** Pressure dependence of the ratio of peak areas of 5-methyl-2-(1-methylethyl)cyclohexanol and phenazepam.

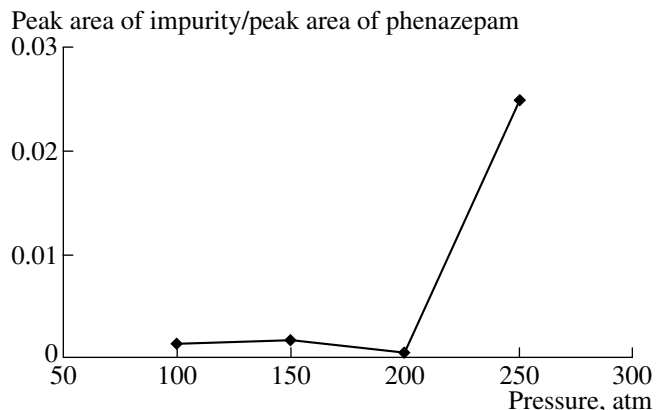
**Table 1.** Number and amount of moderately volatile organic impurities extracted from phenazepam using SFE at different pressures

Pressure in the system, atm	Total number of detected impurities	Concentration range, %
80	12	$10^{-6}$ – $10^{-4}$
100	20	$10^{-6}$ – $10^{-3}$
150	27	$10^{-6}$ – $10^{-2}$
200	36	$10^{-6}$ – $10^{-1}$
250	40	$10^{-6}$ – $10^{-1}$

## RESULTS AND DISCUSSION

We optimized the SFE conditions to provide a high degree of the recovery of moderately volatile impurities from samples of pharmaceutical preparations and the transfer of the entire extract to the gas chromatograph–mass spectrometer. The pressure values in the extraction system varied from 80 to 250 atm at a constant temperature of 40°C. Extraction was performed using supercritical carbon dioxide. Generalized data on the number and the estimated concentration of moderately volatile organic impurities extracted from phenazepam at different pressures are presented in Table 1. As seen from the presented data, the number of extracted impurities and the extraction recovery increase with a rise in pressure.

The maximization of the ratio of the impurity and the main-component peak areas was used as the criterion of optimization. Such an approach provides the conditions under which the maximum amount of impurities and the minimum amount of the main component are extracted. Figures 1 and 2 demonstrate the pressure dependences of the ratios of the peak areas of impurities (5-methyl-2-(1-methylethyl)-cyclohexanol and 5-bromo-2-aminobenzophenone) to the peak area of



**Fig. 2.** Pressure dependence of the ratio of peak areas of 5-bromo-2-aminobenzophenone and phenazepam.

**Table 2.** Sensitivity coefficients and extraction recoveries of simulators; fluid flow rate 1.5 mL/min, 40°C, 250 atm, 5 min ( $n = 5$ ,  $P = 0.95$ )

Compound	Sensitivity coefficient	Extraction recovery	
		CO <sub>2</sub>	N <sub>2</sub> O
2-Fluorophenol	1.8 ± 0.2	96 ± 11	98 ± 15
Phenol- <i>d</i> <sub>6</sub>	2.5 ± 0.3	101 ± 13	95 ± 10
Nitrobenzene- <i>d</i> <sub>6</sub>	3.8 ± 0.3	87 ± 15	83 ± 14
2-Fluorobiphenyl	1.7 ± 0.2	82 ± 12	79 ± 15
2,4,6-Tribromophenol	15 ± 1	71 ± 12	64 ± 12
Ô-Terphenyl- <i>d</i> <sub>14</sub>	2.7 ± 0.4	62 ± 9	73 ± 8

the main component. The optimal pressure value in the studied range was found to be 250 atm.

We used simulating substances (simulators) to determine the extraction recovery under the selected optimal SFE conditions (40°C, 250 atm, and 5 min). The extraction recovery was determined as the ratio of the amounts of the simulator in the extract and in 1 µL of the initial mixture

$$R_{\text{SFE}}, \% = \frac{g_{\text{sim}}^{\text{extr}}}{g_{\text{sim}}^{\text{init}}} \times 100, \quad (1)$$

where  $R_{\text{SFE}}$  is the extraction recovery,  $g_{\text{sim}}^{\text{extr}}$  is the amount of the simulator in 1 µL of the initial mixture, and  $g_{\text{sim}}^{\text{init}}$  is the amount of the simulator in the extract.

To determine the amount of the simulator in the extract, we added 1 µL of a mixture of simulators in the cell and performed SFE at a pressure of 250 atm and a temperature of 40°C. The extraction time was 5 min. Then, we introduced 1 µL of a mixture of internal standards into the cartridge with extracted compounds and carried out the thermodesorption of the cartridge content followed by a chromatographic–mass spectrometric analysis. The amount of the simulator was determined as the ratio of the peak area of the simulator to the peak area of the corresponding internal standard with the closest retention time with regard to the sensitivity coefficient obtained in a preliminary experiment

$$g_{\text{sim}}^{\text{extr}} = \frac{K S_{\text{sim}} g_{\text{st}}}{S_{\text{st}}}, \quad (2)$$

where  $S_{\text{sim}}$  is the peak area of a characteristic ion of the simulator after extraction from the cell and trapping on the cartridge followed by thermodesorption,  $S_{\text{st}}$  is the peak area of the corresponding characteristic ion of the internal standard with the closest retention time when 1 µL of the mixture of internal standards is directly introduced into the cartridge after the SFE of the simulators followed by thermodesorption,  $g_{\text{st}}$  is the amount of the

corresponding internal standard in 1 µL of the mixture, and  $K$  is the sensitivity coefficient.

We carried out a GC/MS analysis of 1 µL of a mixture of internal standards and 1 µL of a mixture of the simulators, simultaneously introducing them directly into the injector of the chromatograph. The sensitivity coefficient was calculated by the following equation:

$$K = \frac{S_{\text{st}}' g_{\text{sim}}^{\text{init}}}{S_{\text{sim}}' g_{\text{st}}}, \quad (3)$$

where  $S_{\text{st}}'$  and  $S_{\text{sim}}'$  are the peak areas of the characteristic ions of the internal standard and the simulator, respectively, when 1 µL of a mixture of internal standards and 1 µL of a mixture of simulators are simultaneously introduced directly into the injector of the chromatograph.

The sensitivity coefficients and the extraction recoveries of the simulators under optimal conditions are given in Table 2. As seen from the presented data, the extraction recoveries are 52–101% and become lower with a decrease in the volatility of substances.

Because the SFE of the simulators was carried out from the empty cell and the thermodesorption degree from the cartridge was close to 100%, the obtained extraction recovery might be expected to reflect largely the degree of trapping of the substance on the cartridge from the flow of the supercritical fluid.

When the optimal conditions for supercritical fluid extraction and thermodesorption from the cartridge had been selected, we extracted impurities from tablets of pharmaceutical preparations of the benzodiazepine series using SFE followed by the chromatographic–mass-spectrometric analysis of the isolated extracts. Supercritical carbon dioxide and nitrous oxide were used as the extracting phases. We carried out two sequential 5-min extractions of the same sample (primary and secondary extractions).

The amount of the impurity in the tablet was calculated by the following equation:

$$c_{\text{imp}}, \% = \frac{S_{\text{imp}} g_{\text{st}}}{S_{\text{st}} m_{\text{tabl}}} \times 100, \quad (4)$$

where  $c_{\text{imp}}$  is the estimated amount of the impurity,  $S_{\text{imp}}$  is the peak area of the impurity,  $S_{\text{st}}$  is the mean peak area of six internal standards,  $g_{\text{st}}$  is the amount of the internal standard in 1 µL of the solution, and  $m_{\text{tabl}}$  is the tablet weight.

Data on the chromatographic–mass spectrometric determination of moderately volatile organic impurities isolated by extraction with supercritical CO<sub>2</sub> and N<sub>2</sub>O from tablets of the studied pharmaceutical preparations are presented in Tables 3–6.

For most impurities, the main part of the impurity, namely, 75 to 100% of the total amount of the extracted (detected) impurity, is extracted within the first five minutes. The full extraction in the first five

**Table 3.** Organic impurities extracted from a diazepam tablet by SFE ( $n = 3$ ,  $P = 0.95$ ,  $RSD \leq 0.25\%$ )

Mass spectrum $m/z$ ( $I_{rel}$ )	Name (formula) of the postulated compound	Mr	Concentration, %	
			CO <sub>2</sub>	N <sub>2</sub> O
134(22), 119(14), 106(16), 105(100), 91(12), 77(19)	1-Methyl-3-propylbenzene (C <sub>10</sub> H <sub>14</sub> )	134	$5 \times 10^{-4}$	$8 \times 10^{-4}$
138(32), 123(41), 95(82), 81(96), 71(100)	5-Methyl-2-(1-methylethyl)cyclohexanol (C <sub>10</sub> H <sub>20</sub> O)	156	$2 \times 10^{-4}$	$6 \times 10^{-4}$
132(39), 117(17), 104(100), 91(35), 77(23), 65(11)	1,2,3,4-Tetrahydronaphthalene (C <sub>10</sub> H <sub>12</sub> )	132	$5 \times 10^{-5}$	$1 \times 10^{-5}$
136(35), 64(58), 61(32), 60(100), 59(33), 55(29)	[1,4,5]-Oxadithiepan (C <sub>4</sub> H <sub>8</sub> OS <sub>2</sub> )	136	$4 \times 10^{-3}$	$7 \times 10^{-3}$
132(100), 163(8), 155(5), 88(45), 73(8)	Unidentified	–	$2 \times 10^{-3}$	$4 \times 10^{-3}$
226(5), 113(8), 99(12), 85(40), 71(61), 57(100)	Hexadecane (C <sub>16</sub> H <sub>34</sub> )	226	$7 \times 10^{-5}$	$1 \times 10^{-5}$
138(100), 123(36), 95(94), 85(66), 57(86)	Butanoic acid 3-methyl-5 methyl-2-(1-methylethyl)cyclohexyl ester (C <sub>15</sub> H <sub>28</sub> O <sub>2</sub> )	240	$1 \times 10^{-4}$	$9 \times 10^{-4}$
154(100), 153(39), 152(28), 151(10), 76(30), 63(12)	Biphenyl (C <sub>12</sub> H <sub>10</sub> )	154	$2 \times 10^{-4}$	$6 \times 10^{-4}$
194(21), 163(100), 135(29), 120(9), 103(17), 76(28)	1,3-Benzenedicarboxylic acid dimethyl ester (C <sub>10</sub> H <sub>10</sub> O <sub>4</sub> )	194	$3 \times 10^{-3}$	$5 \times 10^{-3}$
156(84), 155(48), 128(100), 127(98), 126(34), 63(18)	1-Naphthalenecarboxaldehyde (C <sub>11</sub> H <sub>8</sub> O)	156	$8 \times 10^{-4}$	$5 \times 10^{-4}$
196(23), 181(20), 153(100), 74(9), 73(43)	Unidentified	–	$5 \times 10^{-4}$	$1 \times 10^{-4}$
181(97), 138(96), 110(95), 75(100), 74(73)	Unidentified	–	$7 \times 10^{-4}$	$5 \times 10^{-4}$
169(100), 168(28), 167(30), 115(10), 73(27), 51(30)	2- <i>p</i> -Tolylpyridine (C <sub>12</sub> H <sub>11</sub> N)	169	$6 \times 10^{-4}$	$8 \times 10^{-4}$
228(24), 185(27), 129(52), 83(77), 73(100), 60(79)	Tetradecanoic acid (C <sub>14</sub> H <sub>28</sub> O <sub>2</sub> )	228	$1 \times 10^{-5}$	$4 \times 10^{-5}$
256(31), 213(17), 129(49), 83(31), 73(100), 60(81)	<i>n</i> -Hexadecanoic acid (C <sub>16</sub> H <sub>32</sub> O <sub>2</sub> )	256	$1 \times 10^{-4}$	$2 \times 10^{-4}$
206(58), 205(100), 151(11), 121(20), 76(30), 57(9)	1-Phenylphthalazine (C <sub>14</sub> H <sub>10</sub> N <sub>2</sub> )	206	$8 \times 10^{-4}$	$5 \times 10^{-4}$
241(40), 239(93), 205(100), 110(28), 75(53)	Unidentified	–	$9 \times 10^{-3}$	$3 \times 10^{-3}$
245(90), 288(39), 193(39), 168(25), 105(50), 77(100)	5-Chloro-2-(methylamino)benzophenone (C <sub>14</sub> H <sub>12</sub> ClNO)	245	$7 \times 10^{-3}$	$7 \times 10^{-3}$
286(32), 285(47), 284(31), 283(40), 205(100)	Unidentified	–	$2 \times 10^{-3}$	$3 \times 10^{-3}$
259(27), 257(80), 230(26), 228(50), 222(100)	Unidentified	–	$1 \times 10^{-4}$	$2 \times 10^{-4}$
250(64), 249(85), 222(100), 221(42), 91(27), 77(37)	1,3-Dihydro-1-methyl-5-phenyl-2H-1,4-benzodiazepin-2-one (C <sub>16</sub> H <sub>14</sub> N <sub>2</sub> O)	250	$8 \times 10^{-3}$	$5 \times 10^{-3}$
316(8), 284(57), 241(100), 228(75), 222(82), 209(39)	7-Chloro-2,3-dihydro-2-hydroxy-1-methyl-2-methoxy-5-phenyl-1H-1,4-benzodiazepine (C <sub>17</sub> H <sub>17</sub> ClN <sub>2</sub> O <sub>2</sub> )	316	$7 \times 10^{-4}$	$4 \times 10^{-4}$
270(71), 242(100), 214(18), 178(14), 151(21), 103(40)	7-Chloro-1,3-dihydro-5-phenyl-2H-1,4-benzodiazepin-2-one (C <sub>15</sub> H <sub>11</sub> ClN <sub>2</sub> O)	270	$1 \times 10^{-3}$	$1 \times 10^{-3}$
284(100), 268(13), 248(11), 206(18), 151(8), 124(14)	7-Chloro-2-methoxy-5-phenyl-3H-1,4-benzodiazepine (C <sub>16</sub> H <sub>13</sub> ClN <sub>2</sub> O)	284	$5 \times 10^{-5}$	$4 \times 10^{-5}$

minutes is observed for 35% of impurities. Thus, this study of the extraction recovery of a large number of moderately volatile impurities from different pharmaceuticals by SFE in the dynamic mode showed that almost full extraction of the studied impurities from all the pharmaceutical preparations, including phenazepam and nitrazepam, is attained in 10 min.

The United States Pharmacopoeia [12] regulates the following impurities in diazepam and clonazepam: 5-chloro-2-(methylamine)benzophenone, 3-amino-6-chloro-1-methyl-4-phenylcarbostyryl [9, a], and 7-

chloro-1,3-dihydro-5-phenyl-2H-1,4-benzodiazepin-2-one (diazepam) [9, b] and 2-amino-2'-chloro-5-nitrobenzophenone and 3-amino-4-(2-chlorophenyl)-6-nitrocarbostyryl (clonazepam) [9, b].

As follows from the data in Tables 3 and 6, in the chromatographic–mass spectrometric analysis of the supercritical fluid extract using both CO<sub>2</sub> and N<sub>2</sub>O as an extracting phase, two regulated impurities were determined in the diazepam tablet and one was found in the clonazepam tablet.

**Table 4.** Organic impurities extracted from a diazepam tablet by SFE ( $n = 3$ ;  $P = 0.95$ ;  $RSD \leq 0.25\%$ )

Mass spectrum $m/z$ ( $I_{rel}$ )	Name (formula) of the postulated compound	Mr	Concentration, %	
			CO <sub>2</sub>	N <sub>2</sub> O
138(25), 123(34), 95(80), 81(98), 71(100)	5-Methyl-2-(1-methylethyl)cyclohexanol (C <sub>10</sub> H <sub>20</sub> O)	156	$4 \times 10^{-3}$	$2 \times 10^{-3}$
137(100), 110(4), 102(46), 83(17), 75(34), 58(56)	2-Chlorobenzonitrile (C <sub>7</sub> H <sub>4</sub> ClN)	137	$5 \times 10^{-5}$	$1 \times 10^{-4}$
212(5), 113(6), 99(10), 85(38), 71(61), 57(100)	Unidentified	212	$1 \times 10^{-5}$	$3 \times 10^{-5}$
172(7), 129(41), 115(12), 73(91), 60(100), 55(77)	<i>n</i> -Decanoic acid (C <sub>10</sub> H <sub>20</sub> O <sub>2</sub> )	172	$3 \times 10^{-5}$	$6 \times 10^{-5}$
226(5), 113(7), 99(12), 85(40), 71(61), 57(100)	Unidentified	226	$5 \times 10^{-5}$	$4 \times 10^{-5}$
138(93), 123(36), 95(93), 85(42), 57(100)	Butanoic acid 3-methyl-5 methyl-2-(1-methyl-ethyl)cyclohexyl ester (C <sub>15</sub> H <sub>28</sub> O <sub>2</sub> )	240	$7 \times 10^{-3}$	$3 \times 10^{-3}$
240(5), 113(8), 99(13), 85(40), 71(63), 57(100)	Unidentified	240	$2 \times 10^{-4}$	$1 \times 10^{-4}$
238(4), 111(29), 97(57), 83(67), 69(62), 55(100)	Unidentified	238	$4 \times 10^{-5}$	$3 \times 10^{-5}$
200(16), 157(23), 129(34), 73(100), 60(98), 55(74)	Dodecanoic acid (C <sub>12</sub> H <sub>24</sub> O <sub>2</sub> )	200	$5 \times 10^{-5}$	$3 \times 10^{-5}$
113(14), 99(13), 85(39), 71(76), 57(100)	Unidentified	282	$1 \times 10^{-6}$	$2 \times 10^{-6}$
254(4), 113(8), 99(13), 85(41), 71(63), 57(100)	Unidentified	254	$3 \times 10^{-5}$	$5 \times 10^{-5}$
242(8), 199(9), 143(16), 87(59), 74(100), 55(46)	Tetradecanoic acid methyl ester (C <sub>15</sub> H <sub>30</sub> O <sub>2</sub> )	242	$1 \times 10^{-5}$	$2 \times 10^{-5}$
196(31), 181(32), 154(46), 153(100), 57(85)	Unidentified	–	$8 \times 10^{-6}$	$1 \times 10^{-6}$
268(5), 113(9), 99(14), 85(42), 71(64), 57(100)	Unidentified	268	$1 \times 10^{-5}$	$2 \times 10^{-5}$
266(2), 111(31), 97(58), 83(63), 69(63), 55(100)	Unidentified	266	$4 \times 10^{-6}$	–
228(30), 185(26), 129(45), 73(100), 60(91), 55(75)	Tetradecanoic acid (C <sub>14</sub> H <sub>28</sub> O <sub>2</sub> )	228	$2 \times 10^{-3}$	$3 \times 10^{-3}$
184(7), 169(100), 168(54), 167(27), 115(11), 51(19)	1,1-Diphenylhydrazine (C <sub>12</sub> H <sub>12</sub> N <sub>2</sub> )	184	$1 \times 10^{-5}$	$1 \times 10^{-5}$
242(16), 199(13), 97(16), 73(59), 60(55), 57(100)	Pentadecanoic acid (C <sub>15</sub> H <sub>30</sub> O <sub>2</sub> )	242	$2 \times 10^{-4}$	$1 \times 10^{-4}$
270(30), 227(20), 199(15), 143(32), 87(74), 74(100)	Hexadecanoic acid methyl ester (C <sub>17</sub> H <sub>34</sub> O <sub>2</sub> )	270	$2 \times 10^{-5}$	$5 \times 10^{-5}$
256(57), 213(22), 129(40), 73(100), 60(90), 55(75)	<i>n</i> -Hexadecanoic acid (C <sub>16</sub> H <sub>32</sub> O <sub>2</sub> )	256	$7 \times 10^{-3}$	$6 \times 10^{-3}$
246(13), 243(9), 92(100), 91(70), 78(8), 60(11)	Dodecylbenzene (C <sub>18</sub> H <sub>30</sub> )	246	$2 \times 10^{-3}$	$1 \times 10^{-3}$
298(19), 255(9), 143(18), 87(61), 74(100), 55(26)	Octadecanoic acid methyl ester (C <sub>19</sub> H <sub>38</sub> O <sub>2</sub> )	298	$4 \times 10^{-4}$	$4 \times 10^{-4}$
110(10), 82(61), 68(43), 57(100), 55(81)	Tetradecanal (C <sub>14</sub> H <sub>28</sub> O)	212	$3 \times 10^{-3}$	$1 \times 10^{-3}$
206(44), 205(100), 177(10), 151(15), 76(28), 60(37)	1-Phenylphthalazine (C <sub>14</sub> H <sub>10</sub> N <sub>2</sub> )	206	$8 \times 10^{-4}$	$1 \times 10^{-3}$
284(73), 241(23), 185(30), 129(53), 73(100), 60(96)	Octadecanoic acid (C <sub>18</sub> H <sub>36</sub> O <sub>2</sub> )	284	$5 \times 10^{-2}$	$2 \times 10^{-2}$
270(23), 73(99), 71(45), 60(100), 57(99), 55(87)	Heptadecanoic acid (C <sub>17</sub> H <sub>34</sub> O <sub>2</sub> )	270	$2 \times 10^{-3}$	$3 \times 10^{-3}$
296(4), 113(10), 99(15), 85(43), 71(65), 57(100)	Unidentified	296	$1 \times 10^{-5}$	$5 \times 10^{-6}$
294(17), 111(71), 97(100), 83(72), 69(86), 55(97)	Unidentified	294	$3 \times 10^{-6}$	$5 \times 10^{-6}$
241(45), 239(87), 205(100), 110(22), 75(40)	Unidentified	–	$8 \times 10^{-3}$	$5 \times 10^{-3}$
275(53), 195(28), 105(48), 91(25), 77(100), 51(44)	5-Bromo-2-aminobenzophenone (C <sub>13</sub> H <sub>10</sub> BrNO)	275	$2 \times 10^{-4}$	$3 \times 10^{-4}$
285(47), 205(100), 177(13), 103(16), 75(29)	Unidentified	–	$1 \times 10^{-4}$	$5 \times 10^{-4}$
276(16), 150(16), 141(100), 115(12), 55(15)	Unidentified	–	$5 \times 10^{-4}$	$4 \times 10^{-4}$

**Table 4.** (Contd.)

Mass spectrum $m/z$ ( $I_{rel}$ )	Name (formula) of the postulated compound	Mr	Concentration, %	
			CO <sub>2</sub>	N <sub>2</sub> O
236(77), 235(91), 208(100), 207(89), 180(31), 77(57)	1,3-Dihydro-5-phenyl-1H-1,4-benzodiazepin-2-one (C <sub>15</sub> H <sub>12</sub> N <sub>2</sub> O)	236	$5 \times 10^{-3}$	$3 \times 10^{-3}$
270(73), 241(88), 235(100), 207(32), 178(21), 103(45)	5-(2-Chlorophenyl)-1,3-dihydro-2H-1,4-benzodiazepin-2-one (C <sub>15</sub> H <sub>11</sub> ClN <sub>2</sub> O)	270	$2 \times 10^{-3}$	$4 \times 10^{-3}$
323(100), 321(78), 294(48), 258(50), 242(48)	Unidentified	–	$2 \times 10^{-4}$	$5 \times 10^{-4}$
304(90), 275(100), 269(80), 241(33), 138(25), 120(30)	7-Chloro-5-(2-chlorophenyl)-1,3-dihydro-2H-1,4-benzodiazepin-2-one (C <sub>15</sub> H <sub>10</sub> Cl <sub>2</sub> N <sub>2</sub> O)	304	$9 \times 10^{-4}$	$2 \times 10^{-4}$
348(40), 321(50), 313(28), 102(60), 89(80), 75(100)	Unidentified	–	$5 \times 10^{-3}$	$5 \times 10^{-3}$
314(67), 286(100), 207(30), 179(26), 151(22), 103(74)	7-Bromo-2,3-dihydro-5-phenyl-1H-1,4-benzodiazepin-2-one (C <sub>15</sub> H <sub>11</sub> BrN <sub>2</sub> O)	314	$1 \times 10^{-3}$	$2 \times 10^{-3}$
392(18), 365(47), 313(69), 103(80), 89(79), 75(100)	7-Bromo-5-(3-bromophenyl)-2,3-dihydro-1H-1,4-benzodiazepin-2-one (C <sub>15</sub> H <sub>10</sub> Br <sub>2</sub> N <sub>2</sub> O)	392	$2 \times 10^{-5}$	$5 \times 10^{-5}$
286(31), 224(13), 83(46), 71(50), 57(100)	Octadecanoic acid octadecyl ester (C <sub>36</sub> H <sub>72</sub> O <sub>2</sub> )	536	$3 \times 10^{-5}$	$1 \times 10^{-5}$

Thus, a method is suggested for the determination of moderately volatile impurities in tablets of pharmaceutical preparations based on solvent-free SFE and chro-

matographic–mass spectrometric analysis of the entire extract. Using the developed method, the composition of moderately volatile impurities is determined in tab-

**Table 5.** Organic impurities extracted from a nitrazepam tablet by SFE ( $n = 3$ ;  $P = 0.95$ ;  $RSD \leq 0.25\%$ )

Mass spectrum $m/z$ ( $I_{rel}$ )	Name (formula) of the postulated compound	Mr	Concentration, %	
			CO <sub>2</sub>	N <sub>2</sub> O
103(100), 77(6), 76(40), 63(4), 51(9), 50(11)	Benzonitrile (C <sub>7</sub> H <sub>5</sub> N)	103	$9 \times 10^{-6}$	$6 \times 10^{-6}$
138(18), 123(25), 95(70), 81(94), 71(100)	5-Methyl-2-(1-methylethyl)cyclohexanol (C <sub>10</sub> H <sub>20</sub> O)	156	$1 \times 10^{-6}$	$2 \times 10^{-6}$
136(33), 106(9), 105(100), 77(74), 55(10), 51(29)	Benzoic acid methyl ester (C <sub>8</sub> H <sub>8</sub> O <sub>2</sub> )	136	$5 \times 10^{-5}$	$7 \times 10^{-5}$
198(2), 113(5), 99(7), 85(37), 71(59), 57(100)	Unidentified	198	$7 \times 10^{-6}$	$9 \times 10^{-6}$
158(2), 129(16), 115(25), 87(15), 73(100), 60(89)	Nonanoic acid (C <sub>9</sub> H <sub>18</sub> O <sub>2</sub> )	158	$3 \times 10^{-6}$	$1 \times 10^{-6}$
212(2), 113(5), 99(9), 85(35), 71(58), 57(100)	Unidentified	212	$7 \times 10^{-5}$	$7 \times 10^{-5}$
172(3), 129(39), 83(31), 73(66), 60(100), 55(78)	<i>n</i> -Decanoic acid (C <sub>10</sub> H <sub>20</sub> O <sub>2</sub> )	172	$5 \times 10^{-4}$	$3 \times 10^{-4}$
226(3), 113(6), 99(10), 85(35), 71(61), 57(100)	Unidentified	226	$8 \times 10^{-5}$	$6 \times 10^{-5}$
138(59), 123(27), 95(98), 85(75), 57(100)	Butanoic acid 3-methyl-5 methyl-2-(1-methylethyl)cyclohexyl ester (C <sub>15</sub> H <sub>28</sub> O <sub>2</sub> )	240	$7 \times 10^{-4}$	$9 \times 10^{-4}$
240(2), 113(7), 99(12), 85(38), 71(63), 57(100)	Unidentified	240	$8 \times 10^{-5}$	$6 \times 10^{-5}$
200(20), 157(15), 129(23), 102(47), 73(97), 60(100)	Dodecanoic acid (C <sub>12</sub> H <sub>24</sub> O <sub>2</sub> )	200	$1 \times 10^{-5}$	$5 \times 10^{-5}$
113(15), 99(12), 85(36), 71(77), 57(100)	Unidentified	282	$2 \times 10^{-5}$	$7 \times 10^{-6}$
254(2), 113(9), 99(13), 85(39), 71(64), 57(100)	Unidentified	254	$5 \times 10^{-5}$	$1 \times 10^{-5}$
270(3), 228(27), 129(26), 102(80), 60(100), 55(65)	Tetradecanoic acid 1-methylethyl ester (C <sub>17</sub> H <sub>34</sub> O <sub>2</sub> )	270	$7 \times 10^{-4}$	$1 \times 10^{-4}$
228(26), 185(26), 129(45), 73(100), 60(87), 55(76)	Tetradecanoic acid (C <sub>14</sub> H <sub>28</sub> O <sub>2</sub> )	228	$1 \times 10^{-5}$	$7 \times 10^{-5}$
270(3), 143(15), 87(68), 74(100), 69(23), 55(35)	Hexadecanoic acid methyl ester (C <sub>17</sub> H <sub>34</sub> O <sub>2</sub> )	270	$7 \times 10^{-5}$	$3 \times 10^{-5}$
256(46), 213(19), 129(40), 73(100), 60(93), 55(87)	<i>n</i> -Hexadecanoic acid (C <sub>16</sub> H <sub>32</sub> O <sub>2</sub> )	256	$3 \times 10^{-4}$	$4 \times 10^{-4}$
298(14), 199(7), 143(16), 87(61), 74(100), 55(28)	Octadecanoic acid methyl ester (C <sub>19</sub> H <sub>38</sub> O <sub>2</sub> )	298	$3 \times 10^{-5}$	$5 \times 10^{-5}$
284(57), 241(19), 185(25), 129(46), 73(100), 60(91)	Octadecanoic acid (C <sub>18</sub> H <sub>36</sub> O <sub>2</sub> )	284	$1 \times 10^{-3}$	$5 \times 10^{-3}$
366(4), 113(12), 99(18), 85(47), 71(70), 57(100)	Unidentified	366	$2 \times 10^{-5}$	$6 \times 10^{-5}$
128(7), 86(5), 72(41), 59(100), 55(9)	Tetradecanamide (C <sub>14</sub> H <sub>29</sub> NO)	227	–	$2 \times 10^{-5}$
340(3), 285(22), 267(4), 98(80), 70(41), 57(100)	Octadecanoic acid butyl ester (C <sub>22</sub> H <sub>44</sub> O <sub>2</sub> )	340	$5 \times 10^{-6}$	$7 \times 10^{-6}$
251(25), 234(35), 204(100), 177(25), 151(25)	Unidentified	–	$1 \times 10^{-4}$	$2 \times 10^{-4}$
242(100), 195(33), 165(24), 119(21), 105(64), 77(62)	2-Amino-5-nitrobenzophenone (C <sub>13</sub> H <sub>10</sub> N <sub>2</sub> O <sub>3</sub> )	242	$2 \times 10^{-4}$	$7 \times 10^{-4}$
251(90), 222(100), 195(23), 167(12), 144(10), 110(7)	7-Amino-2,3-dihydro-5-phenyl-1H-benzodiazepin-2-one (C <sub>15</sub> H <sub>13</sub> N <sub>3</sub> O)	251	$3 \times 10^{-3}$	$1 \times 10^{-3}$

**Table 6.** Organic impurities extracted from a clonazepam tablet by SFE ( $n = 3$ ;  $P = 0.95$ ;  $RSD \leq 0.25\%$ )

Mass spectrum $m/z$ ( $I_{rel}$ )	Name (formula) of the postulated compound	Mr	Concentration, %	
			CO <sub>2</sub>	N <sub>2</sub> O
184(3), 99(7), 85(29), 71(56), 57(100)	Unidentified	184	$7 \times 10^{-6}$	$1 \times 10^{-6}$
137(100), 102(32), 76(10), 75(19), 50(12)	4-Chlorobenzonitrile (C <sub>7</sub> H <sub>4</sub> ClN)	137	$7 \times 10^{-6}$	$1 \times 10^{-6}$
198(4), 113(4), 99(7), 85(32), 71(57), 57(100)	Unidentified	198	$1 \times 10^{-6}$	$9 \times 10^{-6}$
112(4), 113(4), 99(8), 85(33), 71(60), 57(100)	Unidentified	212	$7 \times 10^{-5}$	$2 \times 10^{-5}$
172(8), 129(34), 87(14), 73(76), 60(100), 55(47)	<i>n</i> -Decanoic acid (C <sub>10</sub> H <sub>20</sub> O <sub>2</sub> )	172	$5 \times 10^{-5}$	$9 \times 10^{-5}$
105(26), 91(100), 83(20), 69(42), 57(75)	1-Chlorododecane (C <sub>12</sub> H <sub>25</sub> Cl)	204	$2 \times 10^{-4}$	$5 \times 10^{-5}$
197(12), 99(10), 85(29), 71(48), 57(100)	Unidentified	226	$2 \times 10^{-6}$	$7 \times 10^{-6}$
111(22), 97(40), 83(63), 69(78), 55(100)	1-Dodecanol (C <sub>12</sub> H <sub>26</sub> O)	186	$1 \times 10^{-4}$	$3 \times 10^{-4}$
197(17), 99(18), 85(27), 71(73), 57(100)	Unidentified	240	$1 \times 10^{-6}$	$2 \times 10^{-6}$
240(4), 113(7), 99(12), 85(41), 71(66), 57(100)	Unidentified	240	$2 \times 10^{-5}$	$7 \times 10^{-5}$
111(22), 97(57), 83(88), 69(58), 55(100)	Unidentified	252	$6 \times 10^{-6}$	$2 \times 10^{-6}$
200(12), 157(17), 129(28), 115(13), 73(90), 60(100)	Dodecanoic acid (C <sub>12</sub> H <sub>24</sub> O <sub>2</sub> )	200	$8 \times 10^{-5}$	$2 \times 10^{-5}$
232(5), 189(10), 133(27), 119(9), 105(6), 91(100)	1-Propyloctylbenzene (C <sub>17</sub> H <sub>28</sub> )	232	$3 \times 10^{-6}$	$4 \times 10^{-6}$
113(13), 99(12), 85(37), 71(79), 57(100)	Unidentified	282	$4 \times 10^{-5}$	$1 \times 10^{-5}$
232(7), 203(12), 133(4), 119(43), 105(13), 91(100)	1-Ethylonylbenzene (C <sub>17</sub> H <sub>28</sub> )	232	$2 \times 10^{-6}$	$4 \times 10^{-6}$
254(3), 113(6), 99(11), 85(38), 71(63), 57(100)	Unidentified	254	$2 \times 10^{-5}$	$6 \times 10^{-5}$
111(25), 97(48), 83(73), 69(79), 55(100)	1-Pentadecanol (C <sub>15</sub> H <sub>32</sub> O)	228	$9 \times 10^{-6}$	$6 \times 10^{-6}$
226(3), 168(8), 96(7), 85(9), 71(35), 58(100)	2-Pentadecanone (C <sub>15</sub> H <sub>30</sub> O)	226	–	$2 \times 10^{-6}$
246(5), 189(11), 147(16), 117(4), 104(7), 91(100)	1-Butyloctylbenzene (C <sub>18</sub> H <sub>30</sub> )	246	$1 \times 10^{-6}$	$1 \times 10^{-6}$
246(5), 203(8), 133(30), 105(8), 91(100), 69(11)	1-Propylonylbenzene (C <sub>18</sub> H <sub>30</sub> )	246	$1 \times 10^{-6}$	$2 \times 10^{-6}$
268(2), 113(7), 99(11), 85(39), 71(65), 57(100)	Unidentified	268	$2 \times 10^{-5}$	$2 \times 10^{-5}$
228(28), 211(16), 102(45), 71(62), 55(100)	Tetradecanoic acid 1-methylethyl ester (C <sub>17</sub> H <sub>34</sub> O <sub>2</sub> )	270	$5 \times 10^{-6}$	$3 \times 10^{-6}$
228(18), 185(19), 129(36), 73(100), 60(82), 55(59)	Tetradecanoic acid (C <sub>14</sub> H <sub>28</sub> O <sub>2</sub> )	228	$2 \times 10^{-5}$	$5 \times 10^{-5}$
260(5), 217(8), 133(32), 119(5), 105(8), 91(100)	1-Propyldecylbenzene (C <sub>19</sub> H <sub>32</sub> )	260	$6 \times 10^{-6}$	$2 \times 10^{-6}$
119(5), 105(21), 91(51), 71(68), 57(100)	1-Chlorohexadecane (C <sub>16</sub> H <sub>33</sub> Cl)	260	$3 \times 10^{-4}$	$1 \times 10^{-4}$
242(15), 199(13), 129(24), 83(40), 60(77), 57(100)	Pentadecanoic acid (C <sub>15</sub> H <sub>30</sub> O <sub>2</sub> )	242	$7 \times 10^{-6}$	$2 \times 10^{-6}$
111(30), 97(59), 83(84), 69(78), 55(100)	1-Hexadecanol (C <sub>16</sub> H <sub>34</sub> O)	242	$4 \times 10^{-5}$	$3 \times 10^{-5}$
254(3), 111(4), 96(10), 85(15), 71(42), 58(100)	2-Heptadecanol (C <sub>17</sub> H <sub>34</sub> O)	254	$2 \times 10^{-5}$	$7 \times 10^{-5}$
270(7), 143(6), 97(11), 87(56), 74(100), 69(34)	Hexadecanoic acid methyl ester (C <sub>17</sub> H <sub>34</sub> O <sub>2</sub> )	270	$9 \times 10^{-5}$	$8 \times 10^{-5}$
284(7), 241(4), 157(10), 115(4), 101(50), 88(100)	Hexadecanoic acid ethyl ester (C <sub>18</sub> H <sub>36</sub> O <sub>2</sub> )	284	$1 \times 10^{-6}$	$2 \times 10^{-6}$
256(26), 213(13), 129(33), 83(25), 73(100), 60(86)	<i>n</i> -Hexadecanoic acid (C <sub>16</sub> H <sub>32</sub> O <sub>2</sub> )	256	$4 \times 10^{-4}$	$1 \times 10^{-4}$
119(7), 105(14), 91(41), 71(55), 57(100)	1-Chlorooctadecane (C <sub>18</sub> H <sub>37</sub> Cl)	288	$7 \times 10^{-5}$	$8 \times 10^{-5}$
298(9), 257(13), 239(14), 115(25), 102(40), 61(100)	Hexadecanoic acid propyl ester (C <sub>19</sub> H <sub>38</sub> O <sub>2</sub> )	298	$3 \times 10^{-5}$	$2 \times 10^{-5}$
111(35), 97(68), 83(85), 69(78), 55(100)	1-Heptadecanol (C <sub>17</sub> H <sub>36</sub> O)	256	$2 \times 10^{-5}$	$2 \times 10^{-5}$
110(12), 96(21), 82(40), 73(76), 57(100)	Unidentified	212	$2 \times 10^{-6}$	$3 \times 10^{-6}$
298(15), 255(5), 143(17), 87(63), 74(100), 55(45)	Octadecanoic acid methyl ester (C <sub>19</sub> H <sub>38</sub> O <sub>2</sub> )	298	$2 \times 10^{-5}$	$5 \times 10^{-5}$
113(8), 99(11), 85(41), 71(62), 57(100)	Unidentified	282	–	$4 \times 10^{-6}$
312(8), 269(4), 157(13), 102(3), 88(100), 55(16)	Octadecanoic acid ethyl ester (C <sub>20</sub> H <sub>40</sub> O <sub>2</sub> )	312	$1 \times 10^{-6}$	$4 \times 10^{-6}$
284(24), 241(7), 185(14), 129(35), 73(100), 60(75)	Octadecanoic acid (C <sub>18</sub> H <sub>36</sub> O <sub>2</sub> )	284	$8 \times 10^{-4}$	$4 \times 10^{-4}$

Table 6. (Contd.)

Mass spectrum $m/z$ ( $I_{rel}$ )	Name (formula) of the postulated compound	Mr	Concentration, %	
			CO <sub>2</sub>	N <sub>2</sub> O
326(12), 285(10), 267(11), 115(28), 102(42), 61(100)	Octadecanoic acid propyl ester (C <sub>21</sub> H <sub>42</sub> O <sub>2</sub> )	326	1 × 10 <sup>-5</sup>	4 × 10 <sup>-5</sup>
354(2), 169(19), 97(31), 85(46), 71(64), 57(100)	Didodecyl ester (C <sub>24</sub> H <sub>50</sub> O)	354	2 × 10 <sup>-5</sup>	7 × 10 <sup>-5</sup>
368(8), 201(82), 168(47), 83(50), 69(50), 57(100)	Dodecanoic acid dodecyl ester (C <sub>24</sub> H <sub>48</sub> O <sub>2</sub> )	368	1 × 10 <sup>-5</sup>	5 × 10 <sup>-5</sup>
225(9), 169(18), 85(57), 71(68), 57(100)	Unidentified	268	1 × 10 <sup>-5</sup>	2 × 10 <sup>-5</sup>
285(39), 268(23), 238(90), 205(24), 177(54), 75(100)	2-Amino-7-chloro-5-phenyl-4-oxy- 3H-benzodiazepine (C <sub>15</sub> H <sub>12</sub> ClN <sub>3</sub> O)	285	1 × 10 <sup>-4</sup>	9 × 10 <sup>-5</sup>
253(6), 169(14), 83(43), 71(64), 57(100)	2-Octadecyloxyethanol (C <sub>20</sub> H <sub>42</sub> O <sub>2</sub> )	314	7 × 10 <sup>-5</sup>	2 × 10 <sup>-5</sup>
372(28), 357(19), 217(100), 149(62), 109(58), 55(72)	Unidentified	372	2 × 10 <sup>-5</sup>	9 × 10 <sup>-5</sup>
370(34), 355(17), 301(9), 257(19), 215(35), 55(100)	Unidentified	370	5 × 10 <sup>-6</sup>	1 × 10 <sup>-5</sup>
366(49), 247(33), 143(70), 135(100), 119(62), 105(61)	Cholesta-4,6-dien-3-ol (C <sub>27</sub> H <sub>44</sub> O)	384	–	6 × 10 <sup>-6</sup>
368(35), 353(8), 260(12), 247(15), 147(48), 81(100)	Cholesta-3,5-dien (C <sub>27</sub> H <sub>44</sub> )	368	3 × 10 <sup>-5</sup>	3 × 10 <sup>-5</sup>
452(7), 285(26), 168(32), 83(45), 71(52), 57(100)	Octadecanoic acid dodecyl ester (C <sub>30</sub> H <sub>60</sub> O <sub>2</sub> )	452	1 × 10 <sup>-5</sup>	4 × 10 <sup>-5</sup>
276(43), 241(100), 195(36), 165(38), 139(74), 111(52)	2-Amino-2'-chloro-5-nitrobenzophe- none (C <sub>13</sub> H <sub>9</sub> ClN <sub>2</sub> O <sub>3</sub> )	276	2 × 10 <sup>-5</sup>	5 × 10 <sup>-5</sup>
480(5), 257(30), 224(11), 83(47), 71(52), 57(100)	Hexadecanoic acid hexadecyl ester (C <sub>32</sub> H <sub>64</sub> O <sub>2</sub> )	480	6 × 10 <sup>-6</sup>	2 × 10 <sup>-6</sup>
386(32), 316(20), 231(50), 161(35), 81(66), 55(100)	Cholestan-3-one (C <sub>27</sub> H <sub>46</sub> O)	386	2 × 10 <sup>-6</sup>	1 × 10 <sup>-5</sup>
285(17), 257(23), 83(46), 71(54), 57(100)	Unidentified	–	–	5 × 10 <sup>-5</sup>
285(30), 256(1), 97(42), 83(43), 69(65), 57(100)	Octadecanoic acid octadecyl ester (C <sub>36</sub> H <sub>72</sub> O <sub>2</sub> )	536	7 × 10 <sup>-4</sup>	7 × 10 <sup>-4</sup>
288(48), 272(100), 253(32), 226(28), 191(84)	Unidentified	–	7 × 10 <sup>-4</sup>	8 × 10 <sup>-4</sup>

lets of pharmaceutical preparations of the benzodiazepine series (diazepam, phenazepam, nitrazepam, and clonazepam).

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