Gas Chromatographic Retention Indices of Fentanyl and Analogues

Laxmi Manral, Pradeep Kumar Gupta, Kumaran Ganesan, and **Ramesh Chandra Malhotra***
Defence Research & Development Establishment (DRDE), Gwalior-474002 (M.P.) India

Abstract

Narcotic analgesics of the fentanyl class are characterized by high potency and relatively short duration of action. These compounds nowadays have become a substitute for heroine and are highly addictive for abusers. Herein, we report retention indices of fentanyl and its eighteen analogues relative to the homologous *n*-alkane series. These values are determined on a moderately polar BP-5 capillary column under programmed temperature and isothermal chromatographic conditions. The analogues differ in the substituent attached to the piperidine ring nitrogen, and retention indices are found to vary according to the nature of the substituent. The effects of chromatographic conditions like temperature programming rate, carrier gas flow rate, and oven temperature are studied. Retention indices are also determined on a non-polar BP-1 column to study the influence of stationary phase polarity. Standard deviation of all the RI values is less than one index unit.

Introduction

Fentanyl, *N*-(1-phenethyl-4-piperidinyl) propionanilide, is a representative compound of the 4-anilidopiperidine class of narcotic analgesics (1). Fentanyl and its analogues are characterized by their high anesthetic and analgesic potency, relatively short duration of action, and good overall safety margins (2). The detection and identification of fentanyl and its analogues became important because of their alleged use as drugs of abuse (3–7). The rapid rise in the number of fentanyl cases over the years, the increasing availability of fentanyl patches, and the large number of case histories indicating misuse or abuse suggest that fentanyl is rapidly becoming an additional desired opioid, similar to oxycodone and methadone. Because of the large profit involved in the trade of drugs of abuse, this compound is being synthesized and distributed clandestinely. These reasons highlight the need for easy detection and identification procedures for fentanyl and its analogues, and gas chromatographic (GC) retention index (RI) fulfills the demands for their uncomplicated screening and identification (8–11).

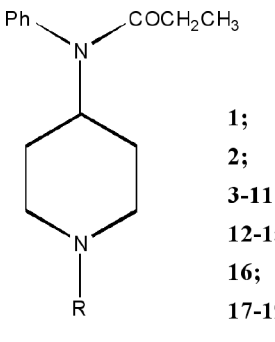
GC RI values correlate the retention time of an unknown compound to that of a reference compound, and RI value determination on two or more columns of different polarities is usually sufficient for identification purposes. For the evaluation of retention indices, a homologues series of *n*-alkanes is commonly used as a reference compound (12). Herein, we report retention indices data for fentanyl (compound **1**) and its 18 analogues (compounds **2–19**) determined under programmed temperature and isothermal GC conditions. Initially, retention indices were determined on a moderately polar BP-5 capillary column, and then the effect of stationary phase polarity was studied by determining these values on a non-polar BP-1 column. The dependence of retention indices on chromatographic conditions like temperature programming rates, carrier gas flow rates, and column temperatures was also studied.

* Author to whom correspondence should be addressed: email rcmalhotra06@yahoomail.com.

Experimental

Chemicals, reagents, and standards

The *n*-alkanes ranging from C_{21} to C_{30} were purchased from Fluka (Buchs, Switzerland) and Aldrich (St. Louis, MO). Fentanyl and its analogues were synthesized in the laboratory using a previously reported method (1), and were characterized by spectroscopic techniques (infrared, nuclear magnetic resonance, and mass spectrometry). Solutions of *n*-alkane standards and analytes were prepared in dichloromethane at the concentration level of 0.1–0.5 mg/mL.



I

1; **R**=CH_2CH_2Ph
2; **R**=CH_2Ph
3-11; **R**= $(CH_2)_nCH_3$; n =2-10
12-15; **R**= $(CH_2)_nCH{=}CH_2$, n = 1-4
16; **R**=$CH_2C{\equiv}CH$
17-19; **R**=$(CH_2)_nCN$, n = 2, 4, 5

Figure 1. Fentanyl and analogues.

Reproduction (photocopying) of editorial content of this journal is prohibited without publisher's permission.

Instrumentation

A Chemito-1000 GC equipped with flame ionization detector was used for GC analysis. Two fused-silica capillary columns (SGE, Australia), viz., BP-1 (100 % dimethyl polysiloxane, 25 m length, 0.22 mm i.d., 0.25 µm film thickness) and BP-5 (5% phenyl + 95% dimethyl polysiloxane, 25 m length, 0.22 mm i.d., 0.25 µm film thickness) were used for the determination of retention indices. For all the experiments, both injector and detector were kept at 300°C. An approximate 0.2 to 0.3 µL volume of every sample solution was injected into the GC in splitless mode. Nitrogen (> 99.5% purity) was used as carrier gas at different flow rates, while hydrogen and air flow to the detector were maintained at 40 and 30 mL/min, respectively. Three replicate analyses for every compound were performed. For programmed temperature GC analyses, oven temperature was increased from 100°C to 250°C while isothermal analyses were carried out at three different oven temperatures (viz., 220°C, 250°C, 280°C). Different temperature programming rates and carrier gas flow rates were used for GC analyses, which are specified in Tables I–V.

Results and Discussion

Gas chromatographic retention indices for fentanyl (compound **1**, Figure 1) and its 18 analogues (compounds **2–19**, Figure 1) were determined using a homologous series of *n*-alkanes (C_{21} to C_{30}) as reference. GC analyses were carried out on a moderately polar BP-5 fused silica capillary column. Every compound, along with *n*-alkane standards, was analyzed in triplicate. Electronic pneumatic control over temperature and carrier gas flow rates minimized fluctuations leading to the reproducible results. Retention indices were determined under both programmed temperature and isothermal chromatographic conditions. The effects of chromatographic conditions like temperature programming rate, carrier gas flow rate, column temperature, and change in the stationary phase polarity on retention indices were also studied.

Determination of retention indices under programmed temperature chromatographic conditions

Programmed temperature GC allowed the analysis of a number of compounds over a wide range of volatilities in a single run. Under programmed temperature conditions, a linear relationship exists between the retention time of *n*-alkanes and their carbon number. Hence, under these conditions, it is possible to calculate retention index value using retention time only. The retention indices under programmed temperature chromatographic conditions (RI_p) were calculated using the van den Dool and Kratz formula (13,14) (Equation 1).

$$RI = 100\left[\frac{t_c - t_z}{tz + 1 - t_z}\right] + 100z \qquad \text{Eq. 1}$$

Here t_c, t_z, and t_{z+1} were retention times of the analyte, alkane eluted immediately prior to the compound with z number of carbon atoms (lower alkane), and alkane eluted immediately after the compound with z + 1 number of carbon atoms (higher alkane), respectively.

The RI_p values determined on the moderately polar BP-5 column under different chromatographic conditions are presented in Tables I–III. Replacement of the phenyl group of *N*-aralkyl chain of **1** by the methyl group in analogue **3** caused a significant decrease in the RI_p value. This replacement led to a decreased interaction of the analyte molecule with the stationary phase and was manifested in a decrease in the retention time and retention index value of analogue **3**.

Successive incorporation of methylene groups in the *N*-alkyl chain of analogue **3** resulted in the increase in the RI_p values of compounds **4–11**. A similar effect of the methylene group could be observed in the RI_p value of analogue **2** in comparison to that of **1**, and also in the compounds from **12–15**. Increasing methylene content of the compounds resulted in a reduced polar character tending towards the behavior of hydrocarbons. This effect was, however, superseded by increased molecular weight and boiling point of the compound, and resulted in an increase in the RI_p values.

The introduction of different functionalities in the alkyl chain attached to the piperidine ring nitrogen of analogue **3** further affected the retention index value. The incorporation of a

Table I. Retention Indices Values under Programmed Temperature GC Conditions on BP-5 at Different Temperature Programming Rates

Compound	I; R (M Wt)	Retention indices ± S.D. (*n* = 3); Temperature programming rate*			
		20°C/min	30°C/min	40°C/min	50°C/min
1	CH_2CH_2Ph (336)	2804.0 ± 0.5	2804.0 ± 0.1	2809.1 ± 1.0	2812.0
2	CH_2Ph (322)	2690.1 ± 0.5	2692.0 ± 0.1	2693.0 ± 0.2	2699.1 ±0.1
3	$(CH_2)_2CH_3$ (274)	2180.1 ± 1.0	2188.1	2189.1 ± 1.5	2190.0 ±0.1
4	$(CH_2)_3CH_3$ (288)	2266.0 ± 0.5	2280.1 ± 0.1	2285.0 ± 0.2	2285.0 ±0.1
5	$(CH_2)_4CH_3$ (302)	2365.0 ± 0.7	2378.1 ± 0.1	2383.0 ± 0.1	2390.1 ±0.1
6	$(CH_2)_5CH_3$ (316)	2470.1 ± 0.5	2472.0 ± 0.5	2474.2	2481.1
7	$(CH_2)_6CH_3$ (330)	2561.1 ± 0.5	2573.0 ± 1.0	2576.0	2583.0
8	$(CH_2)_7CH_3$ (344)	2670.2 ± 0.5	2673.0 ± 0.5	2676.0 ± 0.1	2676.0 ±0.5
9	$(CH_2)_8CH_3$ (358)	2765.0 ± 0.5	2777.3 ± 0.1	2780.1 ± 0.1	2790.2 ±0.7
10	$(CH_2)_9CH_3$ (372)	2870.1 ± 0.5	2871.0 ± 0.1	2873.2 ± 0.1	2874.0 ±0.1
11	$(CH_2)_{10}CH_3$ (386)	2976.1 ± 0.5	2977.1	2979.1 ± 0.5	2980.0
12	$CH_2CH{=}CH_2$ (272)	2179.3 ± 0.5	2190.1 ± 0.5	2193.0 ± 1.0	2195.1 ± 0.1
13	$(CH_2)_2CH{=}CH_2$ (286)	2277.2 ± 1.1	2282.0 ± 0.1	2283.0	2287.0 ± 1.0
14	$(CH_2)_3CH{=}CH_2$ (300)	2359.2 ± 0.5	2364.0	2365.1 ± 0.1	2367.1 ± 0.1
15	$(CH_2)_4CH{=}CH_2$ (314)	2454.2 ± 1.0	2464.2 ± 0.1	2464.0 ± 0.5	2467.2 ± 1.0
16	$CH_2C{\equiv}CH$ (270)	2230.2	2232.1 ± 0.5	2235.0 ± 0.5	2239.1 ± 0.5
17	CH_2CH_2CN (285)	2508.0	2514.1 ± 0.5	2519.0 ± 0.5	2519.0
18	$(CH_2)_4CN$ (313)	2675.0	2688.1	2690.1	2691.0 ± 0.2
19	$(CH_2)_5CN$ (327)	2786.0 ± 0.5	2786.0 ± 1.0	2787.1 ± 0.2	2791.2 ± 0.2

* Carrier gas flow rate: 1.0 mL/min.

double bond in the *N*-alkyl chain did not affect RI_p values significantly, as evidenced by the RI_p values of analogues **12–15** in comparison to those of analogues **3–6**. However, the introduction of a triple bond in the *N*-alkyl chain caused a noticeable change in the RI_p value as seen in case of analogue **16** in comparison to that of analogue **3**. It might be due to the increased polar character and hence increased retention of the compound on the stationary phase. In a similar way, replacement of the alkyl group with the polar cyano group resulted in a large increase in the RI_p values (**3** v/s **17**; **5** v/s **18**; **6** v/s **19**). This increase in the RI_p values because of the polar group was principally due to strong interactions of the polar groups between the analyte and the stationary phase.

Table II. Retention Indices under Programmed Temperature GC Conditions on BP-5 at Different Carrier Gas Flow Rates

Compound	I, R	Retention indices ± S.D. (*n* = 3); Carrier gas flow rate*			
		1 mL/min	1.5 mL/min	2.0 mL/min	2.5 mL/min
1	CH_2CH_2Ph	2804.0 ± 0.5	2806.1	2808.0	2814.3 ± 1.0
2	CH_2Ph	2690.1 ± 0.5	2697.1 ± 0.1	2697.1 ± 0.1	2721.3 ± 1.0
3	$(CH_2)_2CH_3$	2180.1 ± 1.0	2181.1	2184.1	2185.0 ± 1.0
4	$(CH_2)_3CH_3$	2266.0 ± 0.5	2282.0 ± 0.1	2283.1 ± 0.1	2298.1 ± 1.1
5	$(CH_2)_4CH_3$	2365.0 ± 0.7	2378.0 ± 0.2	2378.2 ± 0.2	2386.1 ± 0.1
6	$(CH_2)_5CH_3$	2470.1 ± 0.5	2473.1	2473.0	2490.0
7	$(CH_2)_6CH_3$	2561.1 ± 0.5	2573.0	2575.1 ± 0.1	2582.1 ± 0.2
8	$(CH_2)_7CH_3$	2670.2 ± 0.5	2673.1 ± 0.1	2681.1 ± 0.5	2684.1 ± 0.2
9	$(CH_2)_8CH_3$	2765.0 ± 0.5	2776.2 ± 0.5	2778.1 ± 0.5	2789.2 ± 0.2
10	$(CH_2)_9CH_3$	2870.1 ± 0.5	2875.0	2875.0	2885.1 ± 0.1
11	$(CH_2)_{10}CH_3$	2976.1 ± 0.5	2977.1 ± 0.1	2986.1 ± 0.1	2991.1 ± 0.5
12	$CH_2CH{=}CH_2$	2179.3 ± 0.5	2184.2 ± 0.5	2196.1 ± 0.1	2195.0
13	$(CH_2)_2CH{=}CH_2$	2277.2 ± 1.1	2288.1 ± 0.1	2289.2 ± 0.2	2290.1 ± 0.1
14	$(CH_2)_3CH{=}CH_2$	2359.2 ± 0.5	2363.1 ± 0.1	2365.1 ± 0.1	2368.1 ± 0.1
15	$(CH_2)_4CH{=}CH_2$	2454.2 ± 1.0	2464.1 ± 0.1	2466.0	2467.1 ± 0.1
16	$CH_2C{\equiv}CH$	2230.2	2232.2 ± 0.5	2239.1 ± 0.1	2235.2 ± 0.5
17	CH_2CH_2CN	2508.0	2532.1 ± 0.5	2534.0	2543.1 ± 0.5
18	$(CH_2)_4CN$	2675.0	2689.1 ± 0.1	2696.1 ± 0.1	2702.1 ± 0.1
19	$(CH_2)_5CN$	2786.0 ± 0.5	2786.1 ± 0.1	2817.1 ± 0.1	2821.1 ± 0.1

* Temperature programming rate: 20.0°C/min.

The RI_p values were found to be sensitive towards chromatographic conditions like temperature programming rate and carrier gas flow rate (Figures 2 and 3, See Page 7A). The RI_p values of fentanyl (**1**) and analogues **2–19** determined at different temperature programming rates and carrier gas flow rates are shown in Tables I and II, respectively. From the data, it can be concluded that retention index values were increased with an increase in both temperature programming rate and carrier gas flow rate. However, non-linear variation in the RI_p values revealed that more than one factor was responsible for the changes which were difficult to rationalize.

Significant changes in RI_p values took place with changes in the stationary phase polarity. There was a noticeable decrease in RI_p value for each compound with decreasing column polarity (from BP-5 to BP-1 column). Retention index value differences between two columns ($\varnothing RI_p$) was most pronounced for compounds with polar groups like the cyano group. Thus, ΔRI_p value could provide information regarding the relative polarities of these compounds. The RI_p values obtained on two capillary columns (BP-5 and BP-1) are shown in Table III. The RI value obtained for **1** was found to be in good agreement with reported values (15–16).

Table III. Retention Indices Under Programmed Temperature GC Conditions on Two Different Stationary Phases (BP-1 and BP-5)

Compound	I, R	Retention indices* ± SD (*n* = 3)	
		BP-1	BP-5
1	CH_2CH_2Ph	2717.5 ± 0.7	2804.0 ± 0.5
2	CH_2Ph	2637.5 ± 0.7	2690.1 ± 0.5
3	$(CH_2)_2CH_3$	2135.5 ± 0.7	2180.1 ± 1.0
4	$(CH_2)_3CH_3$	2208.0	2266.0 ± 0.5
5	$(CH_2)_4CH_3$	2307.5 ± 0.7	2365.0 ± 0.7
6	$(CH_2)_5CH_3$	2413.5 ± 0.7	2470.1 ± 0.5
7	$(CH_2)_6CH_3$	2516.5 ± 0.7	2561.1 ± 0.5
8	$(CH_2)_7CH_3$	2614.0	2670.2 ± 0.5
9	$(CH_2)_8CH_3$	2721.5 ± 0.7	2765.0 ± 0.5
10	$(CH_2)_9CH_3$	2814.5 ± 0.7	2870.1 ± 0.5
11	$(CH_2)_{10}CH_3$	2917.0	2976.1 ± 0.5
12	$CH_2CH{=}CH_2$	2109.5 ± 0.7	2179.3 ± 0.5
13	$(CH_2)_2CH{=}CH_2$	2207.5 ± 0.7	2277.2 ± 1.1
14	$(CH_2)_3CH{=}CH_2$	2310.5 ± 1.0	2359.2 ± 0.5
15	$(CH_2)_4CH{=}CH_2$	2403.0	2454.2 ± 1.0
16	$CH_2C{\equiv}CH$	2157.5 ± 0.7	2230.2
17	CH_2CH_2CN	2434.5 ± 1.1	2508.0
18	$(CH_2)_4CN$	2591.5 ± 0.7	2675.0
19	$(CH_2)_5CN$	2696.5 ± 0.7	2786.0 ± 0.5

* Temperature programming rate: 20.0°C/min; carrier gas flow rate: 1.0 mL/min.

Determination of retention indices values under isothermal chromatographic conditions

Under isothermal conditions, unlike programmed temperature conditions, there exists a non-linear relationship between retention time and number of carbon atoms of *n*-alkanes. Therefore, for the calculation of retention indices under these conditions, logarithm of the corrected retention time was taken into account. Retention indices under isothermal experimental conditions (RI_I) are calculated using the Kovats formula (Equation 2) (17).

$$RI_I = 100\left[\frac{log\ t'_c - log\ t'_z}{log\ t'_{z+1} - log\ t'_z}\right] + 100z \qquad \text{Eq. 2}$$

Here t'_c, t'_z, and t'_{z+1} were corrected retention times of the solute, alkane eluted immediately prior to the compound with z number of carbon atoms (lower alkane), and alkane eluted immediately after the compound with z + 1 number of carbon

atoms (higher alkane), respectively. The corrected retention time is obtained by subtracting dead retention time from actual retention time. The dead retention times at three different temperatures were calculated by the graphical method as described by Zhu et al. (18).

Table IV. Retention Indices Under Isothermal GC Conditions on BP-5 at Three Different Column Temperatures

Compound	I, R	Retention indices ± S.D. (n = 3); Column temperature * 220°C	250°C	280°C
1	CH_2CH_2Ph	2757.0 ± 0.0	2804.0 ± 1.1	2854.5 ± 0.5
2	CH_2Ph	2660.0	2694.0	2736.5 ± 1.0
3	$(CH_2)_2CH_3$	2166.1 ± 0.1	2186.6 ± 0.3	2222.3 ± 0.3
4	$(CH_2)_3CH_3$	2258.1 ± 0.1	2282.3 ± 0.5	2328.1 ± 0.1
5	$(CH_2)4CH_3$	2357.0	2377.6 ± 0.5	2419.1 ± 0.1
6	$(CH_2)5CH_3$	2450.3 ± 1.0	2477.6 ± 0.5	2504.0
7	$(CH_2)6CH_3$	2550.1 ± 0.1	2569.5 ± 1.1	2613.5 ± 0.5
8	$(CH_2)7CH_3$	2651.0	2671.3 ± 0.5	2705.5 ± 0.5
9	$(CH_2)8CH_3$	2740.0	2772.6 ± 0.5	2812.1 ± 0.1
10	$(CH_2)_9CH_3$	2849.2 ± 0.5	2874.3 ± 0.5	2911.1 ± 0.1
11	$(CH_2)_{10}CH_3$	2959.3 ± 0.3	2974.0 ± 0.4	3013.0
12	$CH_2CH{=}CH_2$	2127.1 ± 0.1	2186.0	2223.0
13	$(CH_2)_2CH{=}CH_2$	2266.2 ± 0.5	2275.3 ± 0.5	2320.1 ± 0.7
14	$(CH_2)_3CH{=}CH_2$	2346.3 ± 0.7	2355.6 ± 0.3	2400.3 ± 1.0
15	$(CH_2)_4CH{=}CH_2$	2481.0	2458.3 ± 0.5	2502.0
16	$CH_2C{\equiv}CH$	2218.3 ± 0.5	2231.0 ± 1.0	2270.1 ± 0.1
17	CH_2CH_2CN	2450.3 ± 0.5	2501.0 ± 1.0	2578.1 ± 0.1
18	$(CH_2)_4CN$	2612.1 ± 0.1	2682.6 ± 0.5	2735.0
19	$(CH_2)_5CN$	2742.1 ± 0.1	2790.0	2846.0

* Carrier gas flow rate: 1.0 mL/min.

Table V. Retention Indices under Isothermal GC Conditions* on Two Different Stationary Phases (BP-1 and BP-5)

Compound	I, R	Retention indices ± SD (n = 3) BP-1	BP-5
1	CH_2CH_2Ph	2720.0 ± 0.7	2804.0 ± 1.1
2	CH_2Ph	2612.0 ± 1.1	2694.0
3	$(CH_2)_2CH_3$	2129.0 ± 1.0	2186.6 ± 0.3
4	$(CH_2)_3CH_3$	2201.3 ± 0.5	2282.3 ± 0.5
5	$(CH_2)_4CH_3$	2313.3 ± 1.1	2377.6 ± 0.5
6	$(CH_2)_5CH_3$	2413.0 ± 0.7	2477.6 ± 0.5
7	$(CH_2)_6CH_3$	2515.6 ± 1.1	2569.5 ± 1.1
8	$(CH_2)_7CH_3$	2619.6 ± 0.5	2671.3 ± 0.5
9	$(CH_2)_8CH_3$	2719.3 ± 0.5	2772.6 ± 0.5
10	$(CH_2)_9CH_3$	2818.0 ± 1.0	2874.3 ± 0.5
11	$(CH_2)_{10}CH_3$	2920.6 ± 1.0	2974.0
12	$CH_2CH{=}CH_2$	2128.0 ± 0.7	2186.0
13	$(CH_2)_2CH{=}CH_2$	2218.0 ± 1.0	2275.3 ± 0.5
14	$(CH_2)_3CH{=}CH_2$	2305.0 ± 0.7	2355.6 ± 0.3
15	$(CH_2)_4CH{=}CH_2$	2401.3 ± 0.5	2458.3 ± 0.5
16	$CH_2C{\equiv}CH$	2167.0	2231.0 ± 1.0
17	CH_2CH_2CN	2418.0 ± 1.0	2501.0 ±1.0
18	$(CH_2)_4CN$	2578.5 ± 0.7	2682.6.0 ± 0.5
19	$(CH_2)_5CN$	2692.0	2790.0

* Oven temperature: 250°C, Carrier gas flow rate: 1.0 mL/min.

The RI_I values determined under isothermal chromatographic conditions are presented in Tables IV and V. Like RI_p values, RI_I values were found to increase with an increase in the chain length of the alkyl group attached to piperidine ring nitrogen. The introduction of polar groups also increased the retention indices due to increased interaction with the stationary phase. The magnitude of the difference in the RI_I values was similar to that observed under programmed temperature conditions. The RI_I values were also found to be sensitive towards the chromatographic conditions like column temperature (Table IV) and polarity of the stationary phase (Table V). Viscosity of the mobile phase increases with the temperature, which changes the retention time of analyte and reference compounds. Hence, retention index values were influence by the change in the column temperature. From Table IV, it can be easily seen that an increase in the oven temperature led to an increase in the retention index in spite of the fact that retention time of the compounds decreases with an increase in oven temperature. Close inspection of retention time data revealed that a decrease in the retention time of higher alkanes with an increase in oven temperature was higher than that of relatively polar analytes. Because retention index is a ratio of these two terms, the net result decreased in the retention indices. An increase in polarity of the stationary phase led to an increase in the retention indices of all the compounds. Solutes containing a polar cyano group (compounds **16–19**) were more influenced by the change in the chromatographic conditions, and large increases in retention indices were observed. For all the compounds, a variation in the RI_I values was found to be nonlinear and supposed to be a combination of a number of factors.

Conclusion

The presence of different functional groups was found to affect the retention indices of fentanyl and analogues to varying degrees. Replacement of the phenyl group of the *N*-aralkyl chain of fentanyl by methyl group resulted in a large decrease in the retention index value. The introduction of the double bond on the *N*-alkyl chain only slightly influenced the retention indices, while polar cyano group caused a significant increase in retention indices in comparison to that of their alkyl counterparts. Retention indices were greatly influenced by a change in chromatographic conditions. They were found to increase with increases in the temperature programming rates, carrier gas flow rate, and column temperature. The retention indices data generated by these studies can be applied for the detection and identification of fentanyl and its analogues.

Acknowledgments

We sincerely thank Er K Sekhar, Director, Defence Research and Development Establishment, Gwalior for his keen interest and encouragement. We are also thankful to Mr. A.N. Rao, Dr. Uma Pathak, and Mr. Avik Mazumdar for their kind support.

References

1. P.A.J. Janssen, US Patent 3,164,600 (1965).
2. L.V. Kudzma, S.A. Severnak, M.J. Benvenga, E.F. Ezell, M.H. Ossipov, V.V. Knight, F.G. Rudo, H.K. Spencer, and T.C. Spaulding. 4-Phenyl- and 4-heteroaryl-4-anilidopiperidines. A novel class of analgesic and anesthetic agents. *J. Med. Chem.* **32:** 2534–2542 (1989).
3. D.-R. Hwang, A.L. Feliu, A.P. Wolf, R.R. MacGregor, J.S. Fowler, C.D. Arnett, M.J. Holland, K. Carr, and E.J. Simon. Synthesis and evaluation of fluorinated derivatives of fentanyl as candidates for opiate receptor studies using positron emission tomography. *J. Labelled Compd. Radiopharm.* **23:** 277–293 (1986).
4. N. Lalinde, J. Moliterni, D. Wright, H.K. Spencer, M.H. Ossipov, T.C. Spaulding, and F.G. Rudo. Synthesis and pharmacological evaluation of a series of new 3-methyl-1, 4-disubstituted-piperidine analgesics. *J. Med. Chem.* **33:** 2876–2882 (1990).
5. I.V. Micovic, M.D. Ivanovic, S.M. Vuckovic, M.S. Prostran, L.D. Micovic, and V.D. Kiricojevic. The synthesis and preliminary pharmacological evalution of 4-methyl fentanyl. *Bioorg. Med. Chem. Lett.* **10:** 2011–2014 (2000).
6. I.V. Micovic, G.M. Roglic, M.D. Ivanovic, L.D. Micovic, V.D. Kiricojevic, and J.B. Popovic. The synthesis of lactam analogues of fentanyl. *J. Chem. Soc., Perkin Trans.* **1:** 2041–2050 (1996).
7. S.-I. Suzuki, H. Tsuchihashi, and H. Arimoto. Studies on 1-(2-phenethyl)-4-(N-propionylanilino) piperidine (fentanyl) and related compounds. III. Effect of methyl group introduction into fentanyl on sensitivity enhancement in gas chromatography with surface ionization detection. *J. Chromatogr.* **475:** 400–403 (1989).
8. G. Tarjan, Sz. Nyiredy, M. Gyor, E.R. Lombosi, M.V. Budahegyi, S.Y. Meszaros, and J.M. Takacs. Thirtieth anniversary of the retention index according to Kovats in gas–liquid chromatography. *J. Chromatogr.* **472:** 1–92 (1989).
9. O. Farakas, K. Heberger, and I.G. Zenkevich. Quantitative structure-retention relationships XIV. Prediction of gas chromatographic retention indices for saturated O-, N-, and S-heterocyclic compounds. *Chemometr. Intell. Lab. Syst.* **72:** 173–184 (2004)
10. P.A. D'Agostino and L.R. Provost. Gas chromatographic retention indices of chemical warfare agents and stimulants. *J. Chromatogr.* **331:** 47–52 (1985).
11. E. Fernandez-Sanchez, J.A. Garcia-Dominguez, V. Menendez, and J.M. Santiuste. Programmed-temperature retention indices: a survey of calculation methods. *J. Chromatogr.* 498–506 (1990).
12. C.F. Poole and S.K. Poole. *Chromatography Today*. Elsevier, New York, NY, 1991.
13. H. Van den Dool and P.D. Kratz. A generalization of the retention index system including linear temperature programmed gas–liquid partition chromatography. *J. Chromatogr.* **11:** 463–471 (1963).
14. J.B. Pattison. A programmed introduction to gas–liquid chromatography. Heyden and Sons, Philadelphia, PA, 1973.
15. E. Marozzi, V. Gambaro, E. Salgari, R. Mariani, and F. Lodi. Use of the retention index in gas chromatographic studies of drugs. *J. Anal. Toxicol.* **6:** 185–192 (1982).
16. B.J. Perrigo, H.W Peel, and D.J. Ballantyne. Use of dual-column fused-silica capillary gas chromatography in combination with detector response factors for analytical toxicology. *J. Chromatogr.* **341:** 81–88 (1985).
17. E. Kovats. Gas chromatographic characterization of organic compounds. I. Retention indexes of aliphatic halides, alcohol, aldehyde and ketones. *Helv. Chim. Acta* **41:** 1915–32 (1958).
18. S. Zhu, X. Lu, Y. Qiu, T. Pang, H. Kong, C. Wu, and G. Xu. Determination of retention indices in constant inlet pressure mode and conversion among different column temperature conditions in comprehensive two-dimensional gas chromatography. *J. Chromatogr. A* **1150:** 28–36 (2007).

Manuscript received January 5, 2007;
revision received March 28, 2007.