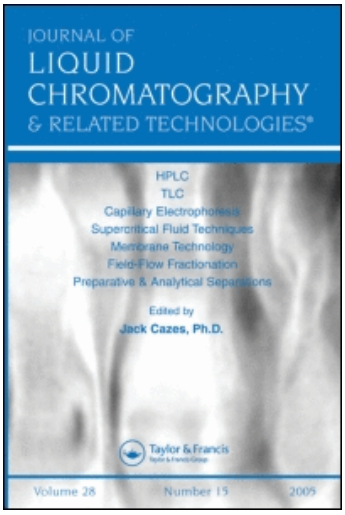

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Reversed-Phase High-Performance Liquid Chromatographic Separation of Fentanyl Homologues and Analogues. I. An Optimized Isocratic Chromatographic System Utilizing Absorbance Ratioing

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REVERSED-PHASE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC SEPARATION OF FENTANYL HOMOLOGUES AND ANALOGUES

I. AN OPTIMIZED ISOCRATIC CHROMATOGRAPHIC SYSTEM UTILIZING ABSORBANCE RATIOING*

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ABSTRACT

An optimized isocratic chromatographic system was developed using overlapping resolution mapping for the reversed-phase separation of 26 fentanyl homologues and analogues. The system consisted of a Partisil 10-ODS-3 column with a quaternary mobile phase consisting of phosphate buffer, methanol, acetonitrile and tetrahydrofuran. All 26 compounds were distinguished when UV detection at 215nm was employed in series with UV detection at 230nm.

INTRODUCTION

In the course of our work it became desirable to develop a reversed-phase high-performance liquid chromatographic separation of 26 homologues and analogues of fentanyl, a powerful narcotic analgesic. The goal was to develop an isocratic system which dis-

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tinguished among the compounds of interest in a reasonable run time, i.e., less than twenty minutes. Various close-ended methods are available for developing an optimum mobile phase. Some are based on treating retention behavior as a function of mobile phase composition or temperature (1-2), while others are based on statistical or sequential search techniques (3-6). The method utilized in this study is based on the work of Snee and employs computer generated overlapping resolution mapping (6,7).

EXPERIMENTAL

The liquid chromatograph employed consisted of the following components: Model 8800 4-solvent gradient system with oven (DuPont); Model LC85 variable UV detector set at 215nm or 254nm containing a 2.5µl flow cell, either alone or in series with a second Model LC85 variable UV detector set at 230nm and containing a 1.5µl flow cell (Perkin-Elmer); IS-100 autosampler (Perkin-Elmer); Sigma 15 Data System interfaced with a Model 3600 Data Station (Perkin-Elmer); a prepacked, 4.6mm x 25cm stainless steel column, with 10µm C18 packing material (Partisil 10-ODS-3, Whatman). Temperature was maintained at 40°C.

Materials

The following solvents were used: acetonitrile, methanol and tetrahydrofuran (Burdick and Jackson). Other chemicals used to prepare mobile phases were reagent grade. The fentanyl compounds were synthesized at the Special Testing and Research Laboratory as the hydrochloride salts, except for the 2-methyl homologue which was obtained from Dr. Thomas Riley of the University of Mississippi.

For experiments where binary or ternary solvent systems were employed solvent 1 consisted of water; solvent 2 consisted of a concentrated phosphate buffer comprised of 16 parts water, 3 parts 2N sodium hydroxide and 1 part phosphoric acid; and solvents 3 and 4 consisted of organic solvent. Solvent 2 was kept constant at 20%. For the quaternary mobile phases employed solvent 1 consisted of phosphate buffer and solvents 2-4 were either pure organic components or pre-mixed 50:50 with solvent 1. The overall phosphate concentration was kept constant for all mobile phases examined.

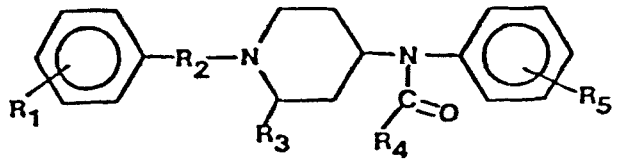
RESULTS AND DISCUSSION

The structures of the 26 homologues and analogues of fentanyl studied are presented in Table 1. In order to establish the composition of the optimum mobile phase we were required to run 7 experiments, as depicted in Figure 1, using different combinations of three organic solvents based on Snyder's selectivity triangle (8). The seven experiments were chosen to estimate the coefficients of a cubic equation which described the surface of the relationship between resolution and mobile phase composition. The composition of each of the first three mobile phases (which contained a single organic modifier) were adjusted to give k' values for fentanyl of approximately 3.5. For all seven mobile phases the approximate k' range for all compounds was between 1 and 10. Retention data for the 7 experimental runs are presented in Table 2.

A resolution map was created for all pairs of adjacent compounds in each of the seven experiments with a computer program

TABLE 1

STRUCTURE OF FENTANYL HOMOLOGUES AND ANALOGUES



	R^1	R^2	R^3	R^4	R^5
1.	. . .	CH_2	. . .	CH_3	. . .
2.	. . .	CH_2	. . .	CH_2CH_3	. . .
3.*	. . .	CH_2CH_2	. . .	CH_2CH_3	. . .
4.	. . .	CH_2CH_2	. . .	CH_3	. . .
5.	. . .	$CH_2CH_2CH_2$	. . .	CH_3	. . .
6.	. . .	$CH_2CH_2CH_2$	. . .	CH_2CH_3	. . .
7.	. . .	$CH_2CH(CH_3)$	. . .	CH_2CH_3	. . .
8.	. . .	CH_2CH_2	. . .	CH_3	o-CH_3
9.	. . .	CH_2CH_2	. . .	CH_3	m-CH_3
10.	. . .	CH_2CH_2	. . .	CH_3	p-CH_3
11.	. . .	CH_2CH_2	. . .	CH_2CH_3	o-CH_3
12.	. . .	CH_2CH_2	. . .	CH_2CH_3	m-CH_3
13.	. . .	CH_2CH_2	. . .	CH_2CH_3	p-CH_3
14.	o-CH_3	CH_2CH_2	. . .	CH_3	. . .
15.	m-CH_3	CH_2CH_2	. . .	CH_3	. . .
16.	p-CH_3	CH_2CH_2	. . .	CH_3	. . .
17.	o-CH_3	CH_2CH_2	. . .	CH_2CH_3	. . .
18.	m-CH_3	CH_2CH_2	. . .	CH_2CH_3	. . .
19.	p-CH_3	CH_2CH_2	. . .	CH_2CH_3	. . .
20.	. . .	CH_2CH_2	CH_3	CH_2CH_3	. . .
21.	. . .	CH_3CHCH_2	. . .	CH_3	. . .
22.	. . .	CH_3CHCH_2	. . .	CH_2CH_3	. . .
23.	. . .	CH_2CH_2	. . .	CH_3	o-F
24.	. . .	CH_2CH_2	. . .	CH_3	m-F
25.	. . .	CH_2CH_2	. . .	CH_2CH_3	m-F
26.	. . .	CH_2CH_2	. . .	CH_2CH_3	p-F

*Fentanyl

Buff = Buffer
MeOH = Methanol
ACN = Acetonitrile
THF = Tetrahydrofuran

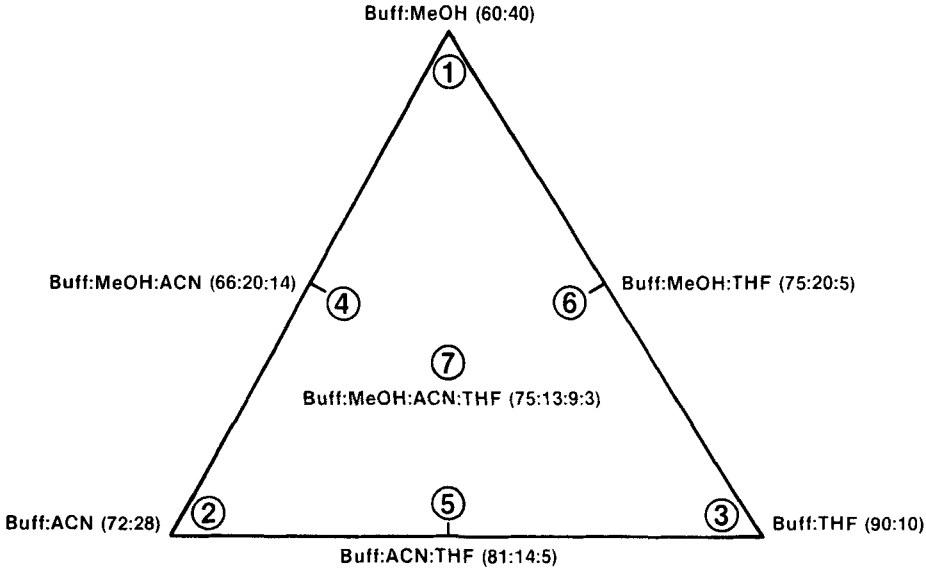


Figure 1 - Simplex experimental for mobile phase optimization utilizing experimental runs 1-7.

that corrected for peak crossover. A union of these plots, which represents overlapping resolution mapping, portrays the region where the maximum number of pairs of peaks are resolved above a preselected resolution level. Resolution can be defined by either of the following equations.

$$Rs = \tfrac{1}{4}(\alpha-1)\ (N)^{\frac{1}{2}}\ \ (k' / (k' + 1) \qquad 1)$$

$$Rs = (Rt2 - Rt1) / \tfrac{1}{2}\ (W1 + W2) \qquad 2)$$

Where α, N and k' are the selectivity factor, column efficiency and capacity factor respectively; and RT and W are retention times

TABLE 2

RETENTION TIME DATA FOR SEVEN EXPERIMENTAL RUNS

Compound No.	Retention time (minutes) Run #1	Run #2	Run #3	Run #4	Run #5	Run #6	Run #7
1	3.71	3.29	3.16	4.20	3.80	3.47	4.32
2	5.61	4.59	5.04	6.53	5.94	5.39	7.02
3	6.28	5.87	6.47	8.03	7.77	6.51	8.80
4	4.17	4.14	4.03	5.19	4.95	4.21	5.47
5	5.61	5.51	6.27	7.37	7.34	6.14	8.22
6	8.80	8.15	10.78	11.74	12.08	10.01	13.84
7	7.04	7.24	7.46	9.63	9.54	7.37	10.46
8	5.42	5.20	5.37	6.91	6.57	5.51	7.43
9	6.21	5.78	6.57	8.03	7.77	6.43	8.80
10	6.52	5.97	6.88	8.36	7.30	6.86	9.39
11	8.93	7.89	9.60	11.59	11.29	9.34	13.29
12	10.37	8.88	11.56	13.53	13.44	11.15	15.55
13	10.99	9.29	12.63	14.34	14.29	12.03	16.75
14	5.54	5.49	5.90	7.30	7.14	5.90	7.94
15	6.47	5.87	6.93	8.29	8.29	6.92	9.33
16	6.52	6.06	7.11	8.49	8.48	7.02	9.51
17	8.59	8.11	9.91	11.68	11.68	9.35	13.22
18	10.22	8.88	11.86	13.41	13.35	11.20	15.51
19	10.41	9.06	12.27	13.69	13.78	11.40	15.93
20	7.57	7.19	7.81	10.13	9.81	7.73	11.03
21	5.66	5.18	5.38	7.02	6.51	5.62	7.50
22	8.90	7.65	9.24	11.31	10.72	9.11	12.57
23	4.58	5.41	4.83	5.74	5.66	4.82	6.19
24	4.31	4.63	4.88	5.61	5.77	4.73	6.18
25	6.52	6.92	8.38	9.01	9.56	7.67	10.45
26	6.47	6.68	8.80	8.90	9.58	7.87	10.48

and peak widths of peaks designated 1 and 2. In this work, resolution was approximated by Rt2 - Rt1 because we were not interested in simultaneously separating 26 compounds, but in distinguishing the various fentanyl analogues and homologues. In this vein it is important that the retention time difference between two adjacent peaks be greater than the experimental variation in retention

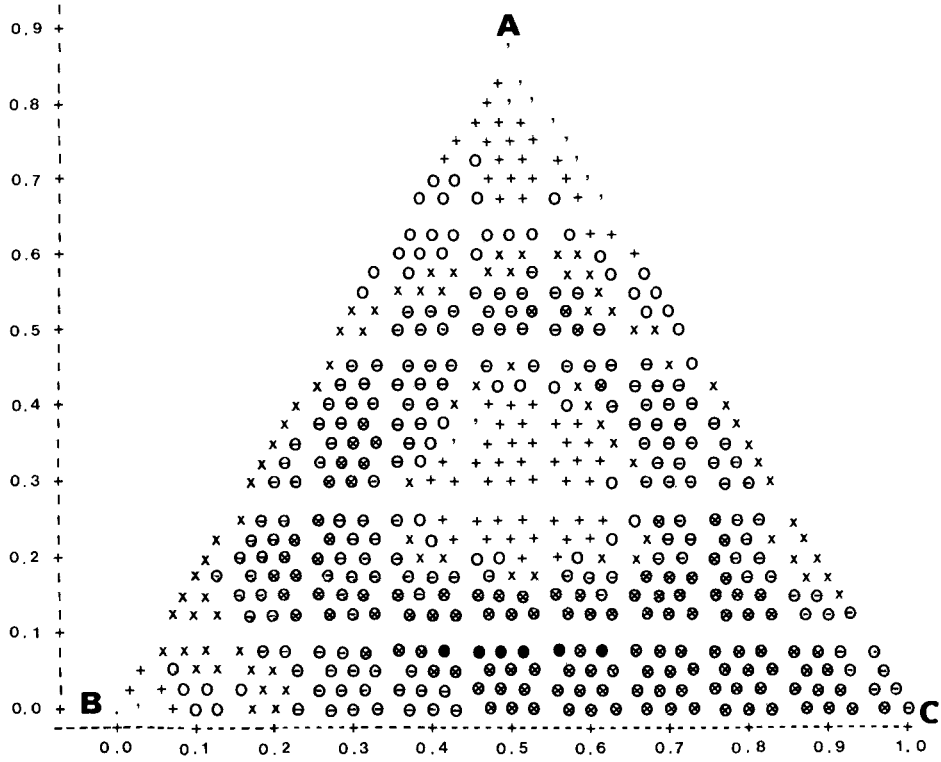


Figure 2 - Contour plot showing number of pairs of peaks where retention time difference between each pair is greater than 0.5 minutes versus mobile phase composition. Points A, B, and C refer to mobile phases depicted in the apexes of the triangle in Figure 1.

time for either peak. A retention time difference of 0.5 minutes was found to be suitable for this purpose. As illustrated in Figure 2, overlapping resolution mapping predicted that a mobile phase consisting of 11%A, 37%B and 52%C was optimum. A, B, and C refers respectively to the concentration of organic modifier which was employed in the first three experiments. Several mobile phases close to the latter solvent system were tested and were found to

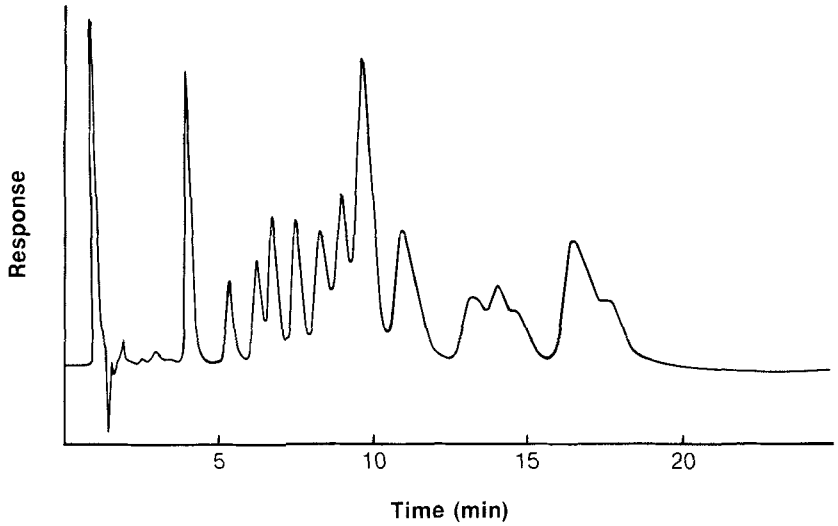


Figure 3 - Chromatogram of a mixture of 26 homologues and analogues of fentanyl utilizing optimum mobile phase predicted by overlapping resolution mapping. Mobile phase consists of 81% phosphate buffer (99 parts water, 3 parts 2 N sodium hydroxide and 1 part phosphoric acid), 4% methanol, 10% acetonitrile and 5% tetrahydrofuran.

Symbol	# of Peaks	Symbol	# of Peaks	Symbol	# of Peaks
.	274-276	@	300-305	θ	295-300
+	281-286	,	276-281	∂	305-310
X	290-295	0	286-290		

give no improvement in the separation of the compounds studied. An examination of the chromatographic run, which is depicted in Figure 3, using the predicted optimum conditions showed peak overlap for several pairs of peaks. In fact, 13 pairs of peaks were not separated by at least 0.5 minutes.

The discriminating power of our chromatographic system was tremendously improved by employing absorbance ratios obtained by using two UV detectors in series. This technique has been successfully employed for the analysis of drugs (9-11). As depicted

TABLE 3

Short Term and Long Term Relative Retention Times (RRT's) and Absorbance Ratios for Compounds in Table 1

Chromatographic Conditions Described in Figure 1
RRT's Calculated Relative to Fentanyl

CPD	RRT	215/230 Un-corrected	215/230 Corrected	RRT*	215/230* Un-corrected	215/230* Corrected
1	0.451	0.765	1.19	0.466	0.851	1.16
4	0.594	0.684	1.04	0.614	0.770	1.05
23	0.688	0.743	1.15	0.702	0.831	1.14
24	0.692	0.578	0.904	0.707	0.600	0.838
2	0.754	0.714	1.11	0.759	0.801	1.10
8	0.836	1.22	1.88	0.842	1.37	1.87
21	0.840	0.685	1.06	0.855	0.756	1.04
14	0.915	0.915	1.40	0.923	1.00	1.37
5	0.943	0.738	1.12	0.948	0.820	1.13
15	1.07	0.976	1.51	1.07	1.03	1.41
3	1.00	0.643	1.00	1.00	0.726	1.00
9	1.03	0.852	1.33	1.01	0.947	1.30
16	1.07	0.938	1.46	1.09	1.04	1.44
10	1.09	0.632	0.975	1.08	0.670	0.920
7	1.22	0.703	1.08	1.22	0.763	1.06
25	1.22	0.485	0.753	1.22	0.556	0.760
26	1.24	0.914	1.42	1.23	1.04	1.44
20	1.27	0.632	0.989	1.26	0.716	0.993
22	1.45	0.648	1.01	1.43	0.723	0.992
11	1.51	1.01	1.58	1.50	1.12	1.54
17	1.57	0.864	1.35	1.54	0.944	1.28
6	1.64	0.693	1.09	1.62	0.772	1.07
12	1.83	0.798	1.25	1.81	0.880	1.20
18	1.83	0.909	1.42	1.81	0.953	1.32
19	1.90	0.979	1.54	1.85	1.00	1.38
13	1.96	0.594	0.925	1.93	0.635	0.871

*Re data obtained after 7 weeks

in Table 3, when we employed UV detection at 215nm in series with UV detection at 230nm, the corrected absorbance ratios coupled with relative retention time distinguished all 26 compounds. The corrected absorbance ratios obtained by peak height for the individual drugs were found by dividing the absorbance ratio for

a compound by the absorbance ratio of an internal standard (fentanyl). Due to variations in wavelength accuracy and wavelength repeatability large differences in uncorrected absorbance ratios could be expected. Since the various compounds we studied, including our internal standard, have similar slopes in their UV spectrum at the wavelengths used, the corrected absorbance ratios would be expected to be considerably more reproducible. This was verified experimentally. The average long term reproducibility measured after seven weeks was 1.6% relative standard deviation for corrected ratios versus 4.7% for uncorrected ratios. However, as expected, the short term reproducibility of the corrected versus the uncorrected absorbance ratio was almost identical, 0.50% versus 0.45%. The long term average percent difference in relative retention time was 0.65% while the short term average percent difference in relative retention time was 0.10%.

Absorbance ratios, both short time and long time, are presented in Table 3.

CONCLUSION

A reversed-phase high-performance liquid chromatographic system has been developed which can distinguish between 26 analogues and homologues of fentanyl with run times less than twenty minutes.

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