Clinical Corner

Column Selection for Toxicological Analyses

Testing for the presence of drugs in biological fluids is a technique that is commonly used in a variety of applications. These applications are broken down into two main categories. The first includes drug overdose screening for clinical and forensic purposes. The second covers drug abuse screening for drug addiction clinics, employee screening and athletic testing. In all cases an analyst screens for as many substances as possible within a limited time frame. Capillary gas chromatography offers the greatest flexibility among the various methods for drug screening. A large number of compounds can be screened in a single chromatographic analysis within a relatively short time period. Confirmation of a positive result is usually achieved by re-analyzing the specimen by a second, more specific technique, usually GC-Mass Spectrometry. GC-MS instrument time can be a valuable commodity in laboratories with limited budgets. Therefore, confirmational analyses of suspected positive results are performed only on those samples that have a clear indication of the presence of a positive result. Reliability of identification of an initial positive result can be increased without actdmg additional testing time through dual column analysis. Intms mode, one injection can be split between two columns that-e attached to separate detectors. By using columns with dissimilar stationary phases, retention time shifts and elution order changes can be demonstrated for most analytes.

Capillary gas chromatography offers the greatest flexibilty among the various methods for drug screening.

Several factors should be considered in the selection of appropriate stationary phases for screening and confirming positive results. Overall resolution and peak shape, analysis time, inertness, and thermal stability are all factors that are influenced by the composition of the stationary phase. Comparative values obtained for retention time and elution order also depend on phase composition. Previous studies employing dual column systems have compared methyl silicone and phenylmethyl silicone stationary phases. In this article we have compared these phases along with another intermediate polarity phase, the trifluoropropyl methyl silicone phase (Rtx-200).

Tabie I (page 8) shows the retention times for a group of drugs on a methyl silicone, several phenylmethyl silicones and the trifluoropropyl stationary phase. The polarity of the stationary phase can be changed by adding increasing percentages of phenyl groups to the silicone polymer. The resulting increase in polarity yields longer retention times for many analytes. This shifting of retention times when columns are run parallel can be used to tentatively identify an unknown substance. Substantial increases in polarity can also cause changes in the elution order for a given set of substances. However, increasing the polarity of columns to change separations can bring diminished returns in extended analysis times and poor peak shape.

Retention time shifts and elution order changes are also accomplished by incorporating different functional groups into the stationary phase. The Rtx-200 exhibits unique selectivity for specific drugs based upon its affinity for compounds that are nucleophilic in nature. Figures l-3 (page 9) show the analysis of a select group of drugs on the Rtx-5, Rtx-35 and the Rtx-200 columns. Large changes in elution order can be attributed to the presence of specific functional groups in the analyte. Peaks 2 and 4, cotinine and caffeine, are retained significantly longer than the other early eluting compounds. This is due to the presence of a carbonyl group on the ring of both compounds. Morphine and diazepam, peaks 19 and 20, which had been difficult to separate on the phenyl columns are well resolved on the R&"-200 column. Diazepam and the other benzodiazepines exhibit a strong affinity for the trifluoropropyl stationary phase because of a carbonyl group and an azo group in the molecule. The triazolo benzodiazepines alprazolam and triazolam, peaks 28 and 29, are particularly affected because of the large number of azo groups in each compound. Flunitrazepam, peak 24, also shows a large elution order change relative to the other benzodiazepines due to the nitro group in the molecule. Compounds with electron donating groups (carbonyl, azo and nitro) will be preferentially retained when compared to compounds with similar base structure but do not contain these groups.

The Rtx"-200 column can be used in combination with any of the methyl silicone or phenylmethyl silicone stationary phases for a dual column screening and confirmation system for drug analysis. All of the columns have comparable temperature ranges and exhibit excellent inertness. The unique selectivity can be used to improve specific separations of hard to resolve compounds or to offer an alternative phase for confirmation of results by altering retention times and elution order.

Table I - Retention times for common drugs on various polarity stationary phases.

Compound	Retention Times				
	Rtx-1 Cat.#10123	Rtx-5 Cat.#10223	Rtx-35 Cat.#l0042:	Rtx-50 Cat.#10523	Rtx-200 Cat.#15023
ethosuximide	6.06	7.49	10.02	11.92	9.96
barbital	12.96	14.87	18.27	20.31	16.29
benzocaine	14.32	16.77	21.46	23.55	17.08
aprobarbital	16.4	18.45	21.74	23.75	18.86
butabarbital	17.55	19.67	22.92	24.81	20.35
butalbital	17.79	19.84	22.73	24.58	19.98
cotinine	17.53	20.55	26.35	28.62	25.02
amobarbital	19.13	21.2	23.84	25.53	21.6
methyl phenidate	19.56	21.76	25.4	26.72	18.3
pentobarbital	19.7	21.86	24.84	26.59	22.12
meperidine	19.58	21.95	24.7 1	25.86	18.49
secobarbitai	21.08	23.25	26.09	27.86	22.9
caffeine	21.27	24.33	30.18	33.01	25.05
ketamine	22.12	24.93	29.4	31.09	23.71
diphenhydramine	22.76	25.3 1	28.4	29.41	20.04
lidocaine	22.97	25.58	28.68	30.06	27.7 1
phencyclidine	23.38	25.8	28.08	28.82	19.05
mephobarbital	23.66	26.29	30.7	32.66	25.58
doxylamine	24.01	26.64	30.05	31.2	20.76
phenyltoloxamine	24.7	27.34	30.47	31.61	22.32
phenobarbital	25.36	28	32.97	35.43	27.64
tripelennamine	25.67	28.39	31.98	33.21	23.07
methapyriline	25.8	28.56	32.4	33.73	23.44
chlorpheniramine	26.27	28.96	32.09	33.3	23.9
procaine	26.77	29.61	33.84	35.65	27.91
brompheniramine	28.58	31.4	34.86	36.18	26.03
dextromethorphan	29.34	32.08	35.52	36.84	26.37
methadone	29.59	32.32	35.18	36.2	26.95
propoxyphene	30.59	33.31	35.93	36.8	28.09
amitriptytine	30.7 1	33.55	36.93	38.06	27.6
atropine	30.86	33.74	37.83	39.41	30.61
nortriptyline	31.09	33.99	38.01	39.11	28.5
trimipramine	31.27	34.1	37.21	38.18	28.3
imipramine	31.27	34.25	37.79	38.93	28.66
tetracaine	31.43	34.33	37.55	39.07	32.39
doxepine	31.46	34.8	38.17	39.5	29.46
pyrilamine	31.59	34.56	38.54	40.04	29.37
medazepam	31.54	34.71	39.71	41.6	29.95
desipramine	31.83	34.82	38.97	40.13	29.79
procainamide	31.87	34.99	40.1	42.32	35.93

Compound	Retention Times				
	Rtx-1 Cat.#10123	Rtx-5 Cat.#10223	Rtx-35 Cat.#10423	Rtx-50 Cat.#10523	Rtx-200 Cat.#15023
primidone	31.76	35.2	42.83	45.88	35.15
promethazine	32.46	35.43	39.62	41.12	30.28
bupivacaine	32.7	35.57	38.57	39.86	34.92
pentazocine	32.91	35.57	38.74	40.13	30
promazine	33.46	36.6	40.9	42.46	31.64
sCOpOiUlline	33.4	36.6	41.62	43.48	35.49
carbamazepine	33.23	36.64	43.76	46.4	36.79
maprotylme	33.89	36.88	40.91	42.03	32.12
diphenyihydantoin	34.05	37.27	43.76	46.5	36.39
codeine	34.68	37.96	43.32	45.4	34.18
clomipramine	35.38	38.41	41.83	43.04	33
lolazepam	35.26	38.65	44.07	46.3	37.34
dipam	35.87	39.26	44.8	47	38.63
morphine	36.17	39.26	44.75	47.05	36.09
hydrocodone	35.95	39.47	45.48	47.7	37.89
hydromorphone	36.5 1	39.9	45.93	48.29	38.95
chlorpromazine	37.1	40.31	44.29	45.86	34.99
chlorprothixene	37.1	40.3 1	44.29	45.86	34.4
nordiazepam	37.27	40.81	47.05	49.53	40.13
oxycodone	37.55	41.13	47.2	—	40.11
clobazam	38.29	41.84	47.97	50.3	44.24
nalorphine	38.68	41.96	47.2	—	37.99
temazepam	39.02	42.6	48.5 1	50.9	42.74
flutlitrazepam	39.36	43.02	48.88	51.26	45.33
bromazepam	39.48	43.37	50.77	53.56	41.68
pt=epam	40.11	43.56	48.62	50.62	41.68
trifluoperazine	40.5 1	43.73	46.68	47.79	38.86
dibucaine	41.03	44.28	47.43	48.9	41.85
acetopromazine	41.18	44.64	49.32	51.23	41.32
flurazepam	42.44	45.81	49.96	51.61	43.45
papaverine	43.43	47.06	53.22	55.76	43.51
clonazepam	43.6	47.61	54.68	57.58	49.6
haloperidol	45.3 1	49.07	53.72	55.58	46.61
alprazolam	45.46	49.34	56.24	59.6	52.67
prochlorperazine	45.79	49.41	53.98	55.72	43.21
tliaZOliXll	47.12	51.04	58.39	62.37	54.15
thioridazine	48.45	52.16	57.55	60.09	45.81
strychnine	48.84	52.95	61.53	—	49.69
verapamil	49.17	52.89	57.49	59.8	49.95

Run Conditions and Peak List for Figures 1 - 3.

30m. 0.25mm ID, 0.25um
1.0ul split injection of 100ug/ml (2ng on-column)
Oven temp.: 100°C to 320°C @ 4C/min. (hold 10 min.)
Inj. temp.: 225'C
Det. temp.: 300'C
Carrier gas: helium
Linear velocity: 30cm/sec. @ 100°C
TSD sensitivity: 4 x 10-10 AFS
Split ratio: 50:l

COMPOUNDS					
1	benzocaine	12	amitriptyline	23	clobazam
2	cotinine	13	trimipramine	24	flunitrazepam
3	meperidine	14	imipramine	25	prazepan
4	caffeine	15	medazepam	26	flurazepm
5	lidocaine	16	pentazocine	27	haloperidol
6	phencyclidine	17	promazine	28	alprazolam
7	doxylamine	18	codeine	29	triazolam
8	phenyltoloxamine	19	morphine	30	thioridazine
9	chlorphenhamine	20	diazepam	31	verapamil
10	daxtromethorphan	21	chlorpromazine	32	strychnine
11	methadone	22	clorprothixene		