R. C. Kelly,[1] *Ph.D. and D. S. Christmore,*[1] *B. S.*

Identification of Phencyclidine and Its Analogues at Low Concentrations in Urine by Selected Ion Monitoring

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ABSTRACT: This paper presents retention behavior and mass spectra of eluting peaks for twelve phenyl and thienyl analogues of phencyclidine (PCP) as well as PCP itself. An on-column decomposition product common to all thienyl analogues is described. Finally, a practical analytical procedure is given for the detection and identification of these compounds in low (under 5 ng/mL) concentrations in urine.

KEYWORDS: toxicology, phencyclidine, chemical analysis, mass spectrometry

Phencyclidine [1-(1-phenylcyclohexyl)piperidine, PCP] has been a major drug of abuse for the last several years following its withdrawal from clinical studies as an anesthetic because of bizarre side effects. Although usually not a drug of choice on its own right, PCP is one of the most common substitutes for other drugs available on the street [*1-3*]. Early clinical reports focused on the acute physical effects of PCP use [*3-7*] and the severe behavioral consequences. In various individuals and at various dosages, these have included euphoria, dysphoria, schizophreniform psychosis, disorientation, and aggressiveness, and in overdosage, hypertension, sweating, salivation, disturbances of gait, seizures, respiratory arrest, coma, and death [*7-9*].

Recent experimental studies [*10,11*] suggest that PCP has a long half-life in the body, in accord with clinical studies demonstrating the persistence of physical and psychological effects for long periods [*12-15*]. At low doses, the behavioral effects can be very difficult to distinguish from functional psychosis [*16,17*], a situation often compounded by the lack of a clear history of exposure.

As a result of governmental restrictions on PCP and its precursors, structural analogues have made their appearance; many of these substances are not regulated. These drugs possess a spectrum of pharmacological effects similar to PCP, although differing in potency in some cases [*18*].

In this report, we describe analytical techniques developed for the detection and discrimination of PCP and several of its analogues at very low concentrations in urine as an aid in the diagnosis and treatment of chemically induced psychosis. The method exploits the sensitivity

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[1]Assistant director and production chemist, respectively, Department of Clinical and Industrial Toxicology, Bio-Science Laboratories, Van Nuys, CA.

and specificity of selected ion monitoring gas-liquid chromatography/mass spectrometry (SIM) for detecting the compounds and for confirming their identity. We present and discuss the gas chromatographic retention behavior of several phenyl and thienyl analogues of PCP, describe distinctive features of their electron impact mass spectra, and outline a practical procedure for their identification at concentrations below 5 ng/mL in urine.

Experimental Procedure

Materials

Phencyclidine and its heterocyclic analogues (Compounds I to VI, Fig. 1) were purchased as crystalline hydrochloride salts from Applied Science (State College, PA). The *N*-alkylated analogues (Compounds VII to XIII, Fig. 1) were obtained as 1 mg/mL methanolic solutions from the same source. These solutions and 1 mg/mL solutions of the hydrochloride salts in methanol (calculated as the free bases) were stored at −20°C if not used immediately. Combined standards (100 ng/mL) and pools (5 ng/mL) containing Compounds I to VI were prepared weekly in fresh, drug-free urine and stored at −20°C in the dark.

Mepivacaine hydrochloride (internal standard) was obtained as a 1% aqueous solution for injection (Breon Laboratories, Inc., New York); this was diluted to 50 ng/mL with methanol for use. Other reagents used were reagent grade or equivalent.

Equipment

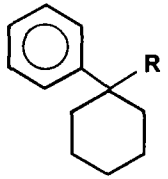
A Hewlett-Packard (Avondale, PA) Model 5992B gas chromatograph/mass spectrometer (GC/MS) was used. This instrument was equipped with a 1.2-m (4-ft) glass column (2 mm inside diameter) with GP 3% SP-2250 on 80–100 mesh Supelcoport (Supelco, Inc., catalog number 1-1767, Bellefonte, PA). The carrier gas (helium) flow rate was 30 ± 2 mL/min. The injection port temperature was maintained at 200°C, and the oven programmed at 10°C/min to 270°C after an initial hold at 180°C for 1 min. For determination of comparative retention times, an isothermal temperature of 166°C was used.

Prior to use, the mass spectrometric parameters were optimized using the instrument's AUTOTUNE routine. The electron multiplier setting was then increased to 200 V above the AUTOTUNE setting for increased sensitivity.

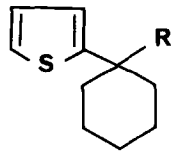
Methods

Collection of Retention Times and Mass Spectra—Two microlitres of the 1 mg/mL solutions of each drug in methanol was injected into the GC/MS at an isothermal temperature of 166°C. Mass spectral and retention time data were recorded for the eluting peaks. Each compound was injected twice and the retention times were averaged. Mass spectra were collected automatically by the instrument at the top of the total ion current peak for each compound.

Sample Preparation—Five millilitres of urine was placed in a 16- by 125-mm Teflon®-lined screw-capped culture tube. Twenty-five microlitres of concentrated ammonium hydroxide, 0.5 mL of 0.5*N* sodium hydroxide, and 10 mL of a solvent mixture (toluene/heptane/isoamyl alcohol, 78:20:2) were added. Two standards and pools were analyzed with each batch of samples. All tubes were shaken at 150 ± 20 cycles/min on an explosion-proof horizontal shaker (Eberbach Corp., Ann Arbor, MI) for 5 min, then centrifuged at 1000 rpm (300 *g*) for 1 min to separate the phases. The upper solvent layers were removed into tapered glass tubes (Concentratubes®, Laboratory Research Co., Los Angeles, CA) silanized before use with dimethylchlorosilane. The solvent was removed by careful evaporation under dry nitrogen in a heating block at 40°C.



General Structure A



General Structure B

Compound	Name	Abbreviation	General Structure	R
I	1-(1-phenylcyclohexyl)piperidine	PCP	A	$-C_5H_{10}N$
II	1-(1-[2-thienyl]cyclohexyl)piperidine	TCP	B	$-C_5H_{10}N$
III	1-(1-phenylcyclohexyl)pyrrolidine	PCPy	A	$-C_4H_8N$
IV	1-(1-[2-thienyl]cyclohexyl)pyrrolidine	TCPy	B	$-C_4H_8N$
V	1-(1-phenylcyclohexyl)morpholine	PCM	A	$-C_4H_8NO$
VI	1-(1-[2-thienyl]cyclohexyl)morpholine	TCM	B	$-C_4H_8NO$
VII	1-phenylcyclohexylmethylamine	PCMe	A	$-NHCH_3$
VIII	1-phenylcyclohexylethylamine	PCE	A	$-NHC_2H_5$
IX	1-phenylcyclohexyldimethylamine	PCDMe	A	$-N(CH_3)_2$
X	1-phenylcyclohexyldiethylamine	PCDE	A	$-N(C_2H_5)_2$
XI	1-phenylcyclohexylpropylamine	PCPr	A	$-NH\text{-}n\text{-}C_3H_7$
XII	1-phenylcyclohexylisopropylamine	PCiPr	A	$-NH\text{-}i\text{-}C_3H_7$
XIII	1-phenylcyclohexylbutylamine	PCBu	A	$-NH\text{-}n\text{-}C_4H_9$

FIG. 1—*Structures and abbreviations of PCP and its analogues.*

The tubes were removed from the heating block as soon as they were dry and cooled to room temperature. One hundred microlitres of methanol was added, and each tube was vortex-mixed (500 ± 50 cycles/min) for 10 s to reconstitute the residues. The volume in each tube prior to injection was reduced to 10 to 15 μL by heating at 40°C as above, and 2 or 3 μL was injected into the GC/MS instrument.

Identification Protocol—The 100 ng/mL standard, along with each unknown sample extract, was injected first in the scanning mass spectral mode, in which a complete mass spectrum between 40 and 500 mass units is recorded for all eluting peaks. If the quantity of drug in the sample was such that detection by this approach was feasible (for example, greater than 3 to 5 ng on-column for PCP), the combination of retention time and spectral match was sufficient to identify the compounds in the group.

If the initial injection produced no peak with a retention time within 5% of any of the compounds and an intensity adequate for a good spectrum to be collected, the extract was reanalyzed in the SIM mode, along with the standard and pool. Six masses were monitored with a dwell time of 50 ms for each: *m/e* = 243, 249, 229, 235, 245, and 251. These masses correspond to the molecular ion weights (M) for Compounds I to VI (Fig. 1), respectively. The corresponding ions for the *N*-alkylated analogues are given in Table 1.

A compound was presumptively identified by the occurrence of a peak at correct retention time in the appropriate single ion trace. This preliminary identification was then confirmed by a third injection of the sample extract and monitoring of five additional ions specific for the drug detected (Table 1) along with the *m/e* 98 ion from the internal standard mepivacaine, again using a dwell time of 50 ms for each ion. The presence of the compound in the sample was established by the appearance of the five additional specific ion peaks at the correct retention time for the compound previously detected. If desired, approximate quantitation can be obtained by relating the sum of the other monitored ion abundances to the ion at mass 98 from the internal standard.

Results and Discussion

Retention Behavior

The compounds studied are shown in Fig. 1. All of the drugs have in common the fact that they are cyclohexylamine derivatives possessing an aromatic ring, either a phenyl or thiophene (thienyl) ring. Nitrogen in the molecule is either part of a heterocyclic ring (Compounds I to VI) or is simply *N*-alkylated (Compounds VII to XIII). Aside from Compound

TABLE 1—*Characteristic ion weights for phencyclidine and analogues.*

Compound	Molecular Ion, *m/e* =	Base Ion, *m/e* =	Other Significant Ions, *m/e* =
I	243	200	242, 166, 91, 84
II	249	97	206, 165, 164, 84
III	229	186	172, 152, 91, 70
IV	235	97	192, 165, 164, 70
V	245	91	202, 188, 168, 86
VI	251	97	208, 165, 164, 123
VII	189	146	132, 117, 91, 77
VIII	203	160	146, 126, 104, 91
IX	203	160	161, 146, 126, 91
X	231	91	230, 188, 174, 154
XI	217	174	160, 117, 104, 91
XII	217	174	160, 117, 104, 91
XIII	231	188	174, 154, 104, 91

VIII, the compounds in the second group are not yet drugs of abuse; however, they were studied to extend our knowledge of the chromatographic and mass spectral properties of PCP analogues.

Table 2 presents isothermal retention times (166°C) for all of the compounds. All of the *N*-alkylated compounds eluted before the heterocyclic molecules, with the retention order being determined by molecular weight with the exception of the *N*-isopropyl derivative (XII), which co-eluted with the mono-ethylated species (VIII). All of these substances had retention times relative to PCP of 0.20 to 0.41. The heterocycles I to VI had longer retention times and the respective pyrrolidine, piperidine, and morpholine derivatives within the phenyl and thienyl series were well resolved from one another. However, on the column used, the separation between corresponding phenyl and thienyl analogues (for example, PCP and TCP) was not adequate for unambiguous identification of both drugs in a single sample. Similar conclusions have been reached by other workers using comparable column packing materials [*19-21*].

Mass Spectra

Electron impact (EI) mass spectra of PCP and several analogues obtained by direct probe insertion have been published [*22*]. A recent paper describes the sensitive determination of PCP and several hydroxylated metabolites in urine by chemical ionization (CI) mass spectrometry following derivatization [*23*]. The mass spectra of Compound VIII (PCE) and of the heterocyclic analogues in EI GC/MS have also been published recently [*20,21,24*]. Mass spectra of the eluting compounds for this study were obtained under the conditions described in the Materials and Methods section and were similar to those previously published [*19-22, 24*]. The mass spectrum of PCP itself has been interpreted in some detail, with the major fragments being further characterized by high-resolution mass spectrometry [*24*]. The spectrum has a prominent molecular ion and an M-43 ion peak resulting from the loss of $-C_3H_7$ from the cyclohexyl ring (Fig. 2) [*24,25*]. These features are shared by the other heterocyclic compounds, II to VI. In the case of the phenyl derivatives, the M-43 ion normally represents the base peak, while in the thienyl series it is simply a prominent fragment. In each spectrum this M-43 ion is much more pronounced than the M-29 and M-57 ions resulting from the loss of an ethyl or butyl group from the ring.

TABLE 2—*Retention times of phencyclidine and analogues.*

Compound	Abbreviation	Retention Time	
		Absolute, min[a]	Relative[b]
VII	PCMe	1.4	0.20
VIII	PCE	1.6	0.23
XII	PCiPr	1.6	0.23
IX	PCDMe	1.7	0.24
XI	PCPr	2.0	0.29
X	PCDE	2.3	0.33
XIII	PCBu	2.9	0.41
IV	TCPy	4.8	0.69
III	PCPy	4.9	0.71
II	TCP	6.4	0.92
I	PCP	7.0	1.00
VI	TCM	9.9	1.41
V	PCM	10.4	1.49

[a]Average of duplicates.
[b]Compared to PCP (I).

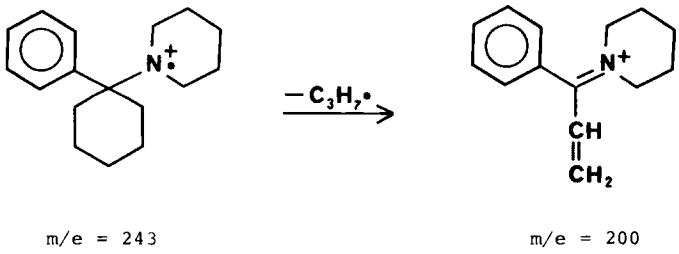


FIG. 2—*Decomposition of the molecular ion of PCP to generate the M-43 daughter ion [24].*

The loss of a phenyl radical gives rise to a significant M-77 peak, which occurs at various masses between *m/e* 154 and 168. In the thienyl series, however, the nitrogen-containing heterocycle is apparently more likely to be lost, giving rise to a common fragment (*m/e* = 165) for these compounds. Such a fragment would have an empirical formula of $C_{10}H_{13}S^+$. The existence of a fragment at *m/e* 167 (an A + 2 ion indicating the presence of sulfur [*26*]) is in support of this idea. In the phenyl compounds, a prominent tropylium ion ($C_7H_7^+$) peak is seen, a species characteristic of phenylalkyl compounds [*26*]. The thienyl compounds seem to give rise to an analogous species; indeed, the most prominent fragment at *m/e* 97 is consistent with an empirical formula of $C_5H_5S^+$.

The mass spectra for Compounds VII and IX to XIII, which have not been published elsewhere, appear in Figs. 3 and 4. These spectra display a number of features in common with the other phenyl analogues previously characterized, namely, a prominent molecular ion M (over 10% relative abundance), a base peak at M-43, minor M-29 and M-57 ions, a sizable M-77 ion, and a very prominent *m/e* = 91 peak for tropylium. Characteristic ions for all compounds are given in Table 1.

Decomposition of the Thienyl Analogues

Chromatograms of the thienyl analogues (Compounds II, IV, VI) always included a minor peak at a retention time of 0.13 relative to PCP, that is, much earlier than any of the other substances tested. The mass spectrum of this material was consistent with 1-[2-thienyl]cyclohexene [*21*], whose structure is shown in Fig. 5 (molecular weight = 164). This compound may be created in the same way that PCP is known to decompose to produce l-phenylcyclohexene. The latter compound is apparently produced by thermal degradation since its production is favored by elevated injection port temperatures. Recently, Legault [*21*] reported the production of l-thienylcyclohexene to be favored on GC columns pretreated with acid. In our experiments we did not observe the formation of l-phenylcyclohexene from PCP. The amount of decomposition product of the thienyl compounds was normally small in relation to the peak for the intact material and was not related to injection port temperature in any obvious way.

Selected Ion Monitoring Profiles

Identification of PCP and its analogues at low concentrations (that is, when insufficient material is present for a complete spectral scan) takes place in two stages (see Materials and Methods). First, the compound is detected presumptively by the presence of one of its characteristic ions, and then it is confirmed by the presence of five other specific ions. If desired, it can also be quantitated by the internal standard technique.

Typical six-ion SIM profiles from Stage one are shown in Fig. 6 for a sample extract containing the phenyl heterocyclic analogues (I, III, V). Labels (left portion of the figure) iden-

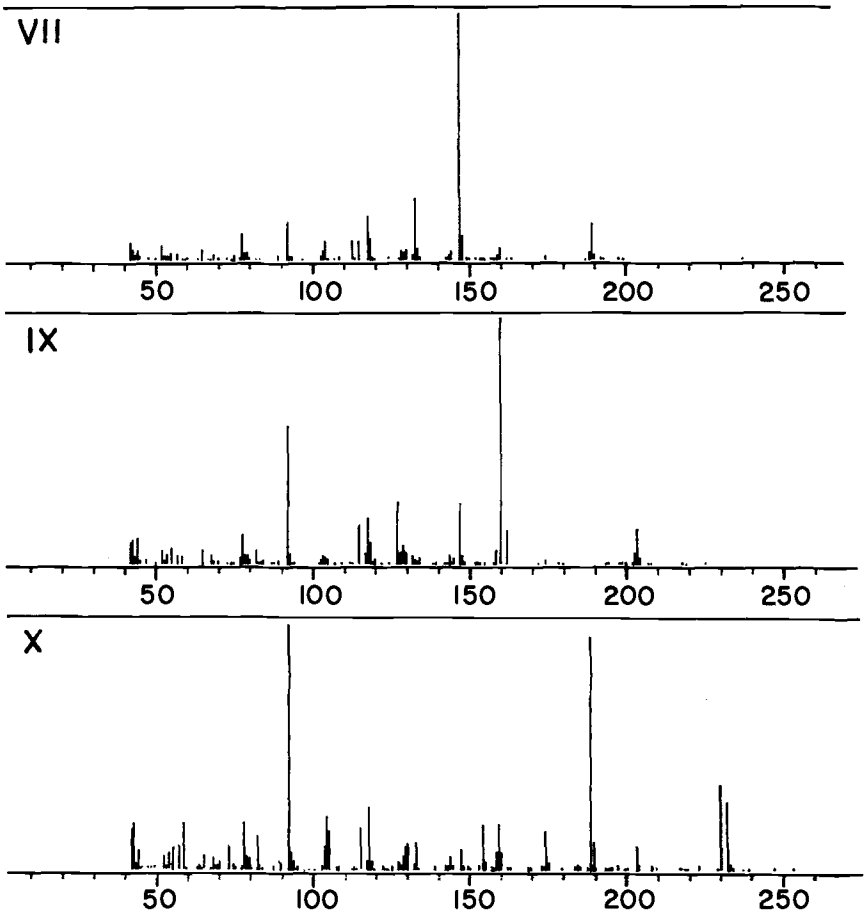


FIG. 3—*Mass spectra of the eluting gas chromatographic peaks for Compounds VII, IX, and X.*

tify the compounds whose molecular ions are being monitored. If desired, the sensitivity of the detection phase can be enhanced by the use of a more abundant (but less specific) ion than the molecular ion. For example, the M-43 ion can be used with an increase in sensitivity of threefold to fivefold for most of the compounds. Alternately, screening can be based on a two-ion SIM trace for $m/e = 91$ and 97, detecting phenyl and thienyl compounds, respectively. Both of these approaches are significantly less selective for the compounds of interest than using the molecular ion weights. In particular, the m/e 91 peak is seen in the great majority of drugs with a phenyl group in their structures. The sensitivity of the technique employed here ranges from 1 to 5 ng/mL, depending on the intensity of the molecular ion peak (Table 1).

Summary

In this paper, we have presented retention times and mass spectral data for PCP and twelve phenyl and thienyl analogues. Mass spectra for Compounds VII and IX to XIII are published here for the first time. In addition, we have described a specific and sensitive technique for the identification of phencyclidine and its analogues in concentrations as low as 1 to 5 ng/mL in urine.

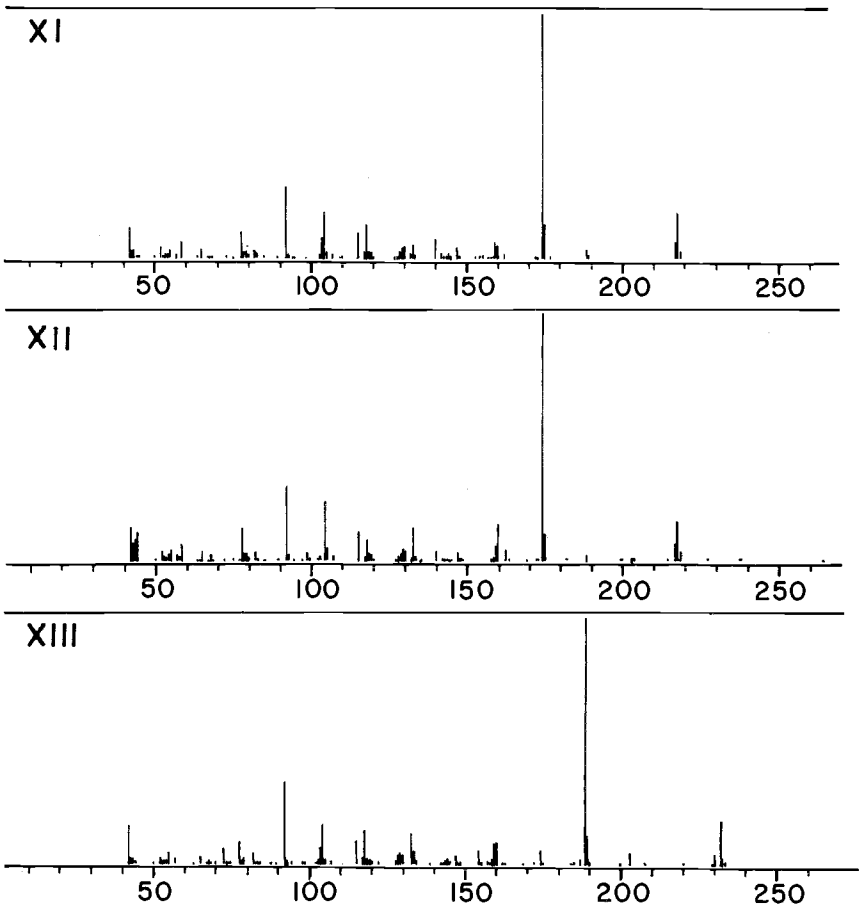


FIG. 4—*Mass spectra for the eluting gas chromatographic peaks for Compounds XI, XII, and XIII.*

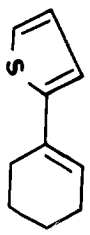


FIG. 5—*Structure of 1-[2-thienyl]cyclohexene, postulated decomposition product of the thienyl analogues (Compounds II, IV, and VI).*

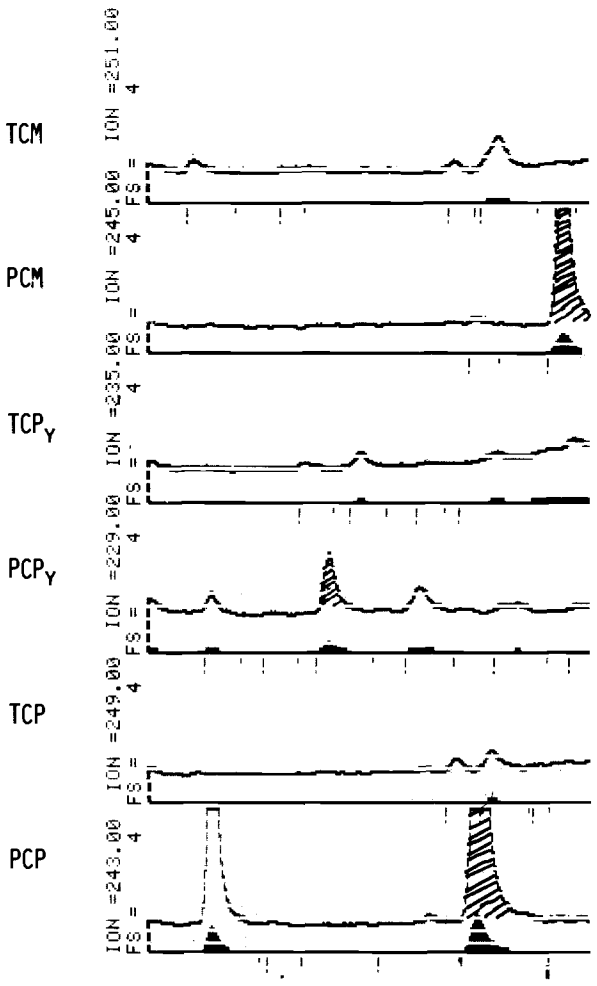


FIG. 6—*Typical six-ion selected ion monitoring profiles for Compounds I to VI on a urine extract that contained 5 ng/mL of Compounds I, III, and V. The left-hand margin is labeled to indicate the compound detected on each ion trace. Peaks for I, III, and V are cross-hatched.*

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Address requests for reprints or additional information to
Raymond C. Kelly, Ph.D.
Department of Clinical and Industrial Toxicology
Bio-Science Laboratories
7600 Tyrone Ave.
Van Nuys, CA 91405