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A MICROCOMPUTER-DIRECTED MASS SPECTROMETER AS A COMPOUND-SELECTIVE DETECTOR FOR GAS CHROMATOGRAPHY

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SUMMARY

Determination and quantitation by mass spectrometry can be difficult for compounds in complex biological mixtures where chromatographic interferences are frequently encountered. A gas chromatographic–mass spectrometric system is described which utilizes reverse spectral search and retention time screening to provide a high degree of compound specificity. Computer control of instrument operation, and of data acquisition, analysis and printout allows technologist operators to obtain highly reliable, precise quantitative results using relatively crude sample preparation procedures and short chromatographic times.

INTRODUCTION

In the past few decades, mass spectrometry (MS) has gained a reputation as a highly specific analytical technique. This well-deserved reputation is based primarily on the fact that mass spectral data are so fundamentally related to molecular structure[1].

Many organic compounds form some identical fragments under the same spectrometric conditions and, since their spectra are additive, mass spectra of even relatively simple mixtures can be difficult to interpret.

This situation led, of course, to the coupling of gas chromatography (GC) and MS. In this arrangement, it is traditionally assumed that GC separation is complete so that one pure compound at a time is introduced into the mass spectrometer. The sample spectrum is then interpreted by a spectroscopist or is compared with a computer file, of spectra of presumably pure compounds, usually taken on other instruments. The result of this *forward* search procedure is usually a list of candidate compounds and in some cases structural information as well. Quantitation is relatively difficult to obtain using this approach, and can easily be distorted when the sample is impure.

There have been recent attempts to improve analysis and quantitation by various techniques such as chemical ionization, field ionization or desorption, and selected ion monitoring[2]. In this paper, an instrument is described in which a form of selected ion monitoring, *reverse* search and retention time screening are combined in order to produce automatically highly specific quantitation of mixtures[3].

DESCRIPTION

The instrument, the so-called OLFAX II, is a microcomputer-directed gas chromatograph–mass spectrometer which functions as a highly compound-specific detector for GC. It consists of a dual-column gas chromatograph, a glass-lined transfer line to introduce the sample into the mass spectrometer, a quadrupole mass spectrometer with electron-impact source and ion-pumped vacuum system for reliability and compactness, and the microcomputer. Fig. 1 shows the functional schematic of the system, including the direct and GC inlets, the dual membrane separator which preferentially excludes solvent and carrier gas from the mass spectrometer, and the computer.

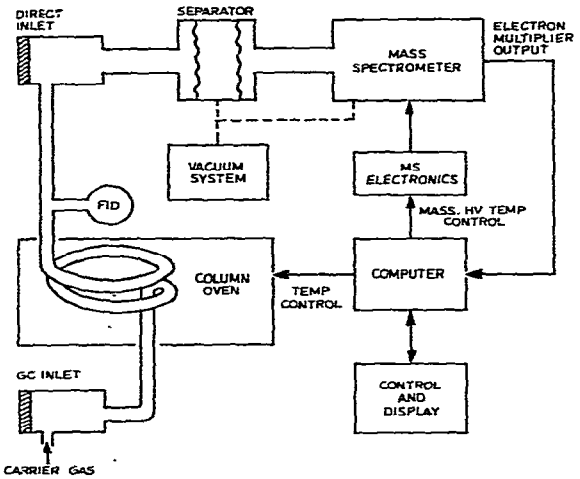

Fig. 1. Block diagram of the OLFAX II gas chromatograph–mass spectrometer–microcomputer system.

Fig. 2 indicates that the microcomputer is assigned a variety of tasks. In its housekeeping function it controls or monitors mass registration, mass scan, detector high voltage, temperatures and pressures. In an interactive mode the computer is addressed through the keyboard to control the mass spectrometer, and to acquire, store and display data in traditional MS or GC–MS fashion.

The computer also completely controls an automatic mode of operation — including temperature programming, data acquisition, and analysis and printout. It has been designed to quantitate automatically compounds in mixtures in real time. The analysis method used is a special form of reverse search and selected ion monitoring (probability based matching; PBM) developed by McLafferty and co-workers[4,5] combined with retention time screening. In reverse search the mass lines that are used to characterize a compound, and that are stored in the computer memory, are compared with data for the same lines produced by the sample, rather than by comparing independently contracted reference and sample spectra. If the lines are chosen carefully, this approach can produce an unequivocal result regarding the presence of this compound in the sample[6]. As shown in Fig. 3, up to seventeen lines of a mass

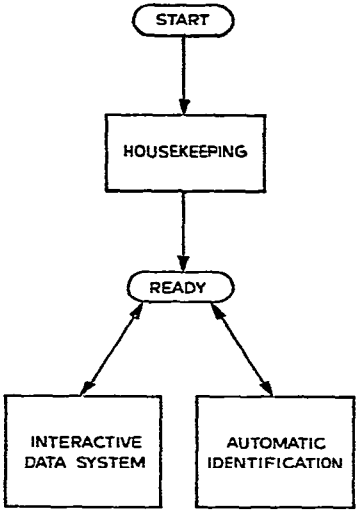

Fig. 2. Software block diagram of the microcomputer used in OLFAX II.

spectrum are chosen that best characterize the compound in the sample matrix in which it is expected to be found. (For the chemical composition of the drugs analyzed in the examples shown in this paper, see Table I.)

In this system the instrument is calibrated by injection of a pure standard. The mass lines chosen for identification, and their relative intensities, as well as quantity scale factor and nominal retention time, are stored in the computer.

The computer instructs the spectrometer to search for only the appropriate mass lines during a time near the nominal retention time for the compound. For each of the mass lines present which fits the intensity ratio pattern, there is calculated a

TABLE I

CHEMICAL COMPOSITION OF THE DRUGS MENTIONED IN THIS PAPER

Drug	*Chemical composition*
Amobarbital	5-Ethyl-5-isoamylbarbituric acid
Chlordiazepoxide	7-Chloro-2-methylamino-5-phenyl-3H-1,4-benzodiazepine 4-oxide
Diazepam	7-Chloro-1,3-dihydro-1-methyl-5-phenyl-2H-1,4-benzodiazepin-2-one
Doxepin	N,N-Dimethyl-3-[dibenz(*b*,*e*)oxepin-11(6H)-ylidene] propylamine
Flurazepam	7-Chloro-1-[2-(diethylamino)ethyl]-5-(*o*-fluorophenyl)-1,3-dihydro-2H-1,4-benzodiazepin-2-one
Glutethimide	2-Ethyl-2-phenylglutarimide
Heptabarbital	5-(1-Cyclohepten-1-yl)-5-ethylbarbituric acid
Methaqualone	2-Methyl-3-*o*-tolyl-4(3H)-quinazolinone
Phencyclidine	1-(1-Phenylcyclohexyl)piperidine
Phenobarbital	5-Ethyl-5-phenylbarbituric acid
Phenothiazine	Thiodiphenylamine
Phenytoin	Diphenylhydantoin; 5,5-diphenyl-2,4-imidazolidinedione
Primidone	2-Desoxyphenobarbital
Propoxyphene	4-Dimethylamino-3-methyl-1,2-diphenyl-2-butanol propionate
Secobarbital	5-Allyl-5-(1-methylbutyl)barbituric acid

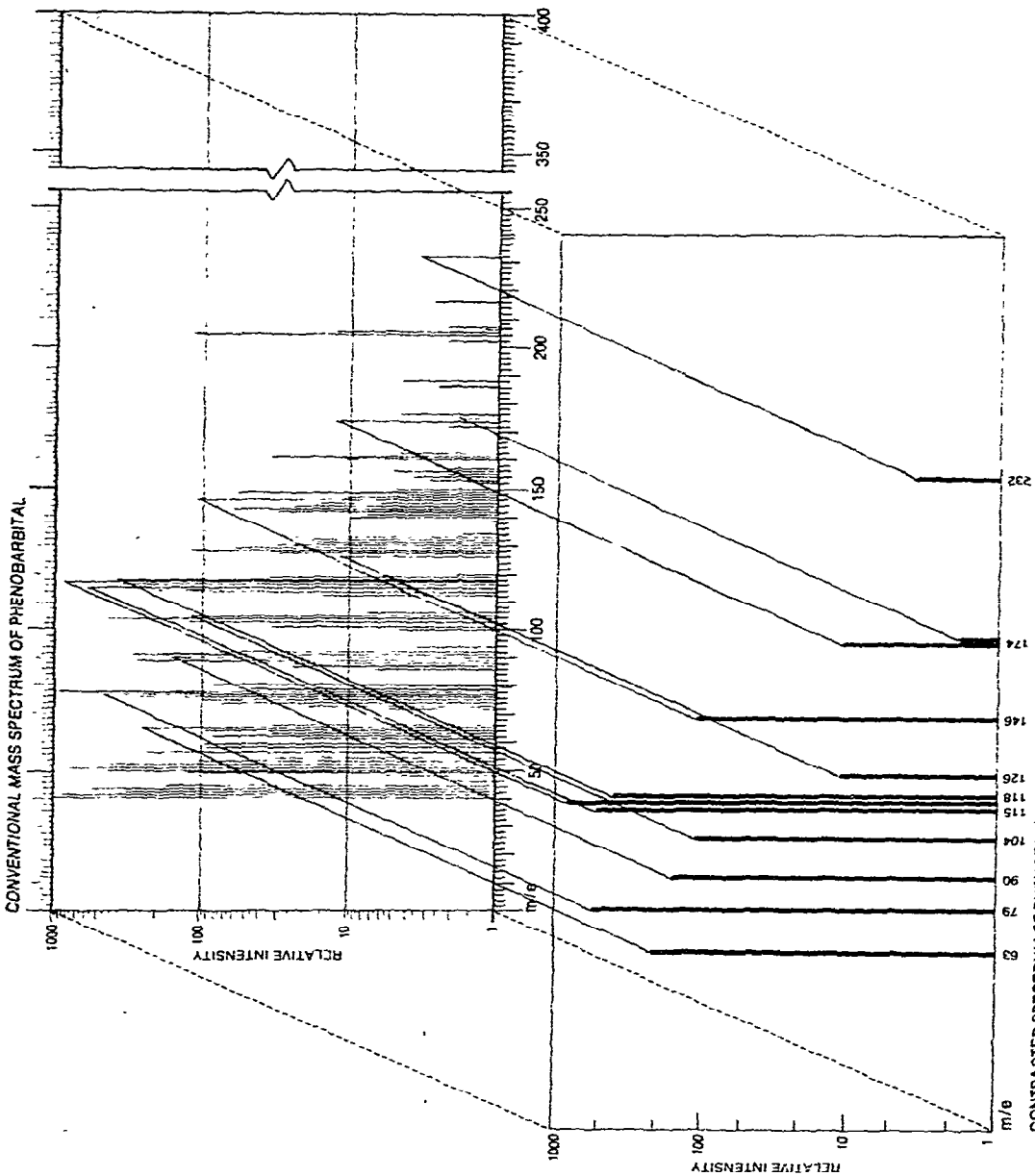

Fig. 3. Complete and contracted mass spectrum for phenobarbital.

quantity and an index K which is a measure of how well the reference and sample spectra fit. Mass lines that do not fit the pattern because of contribution from an impurity or other compound are automatically excluded by the computer from all calculations of quantity and index. PBM is, thus, a self-adapting selected ion-monitoring technique which achieves specificity by insuring that ions are used for identification which fit the reference spectrum abundances and thus are highly likely to originate only in the target compound.

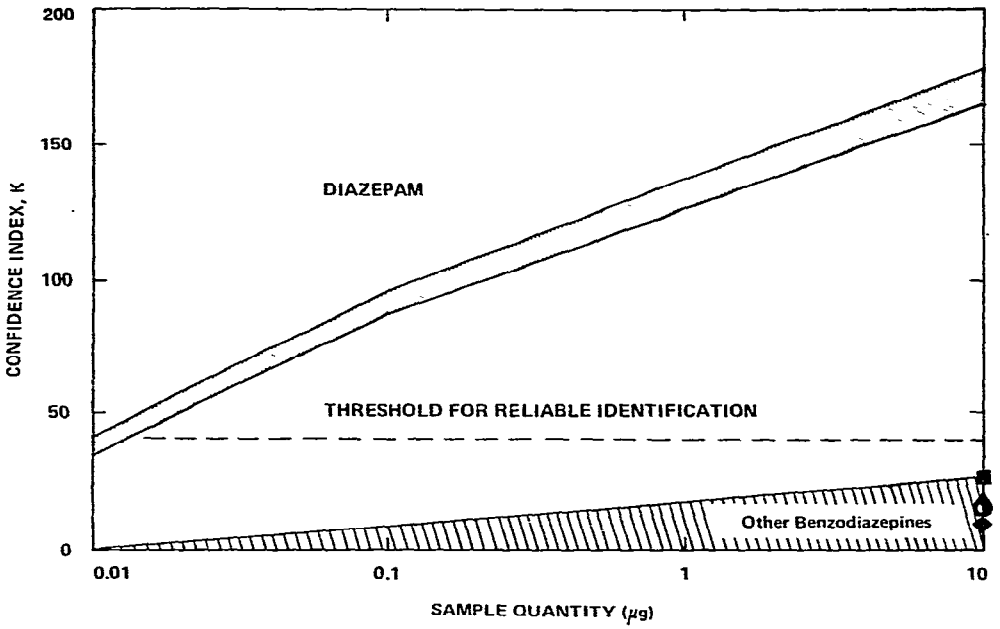

Fig. 4. Confidence index K for diazepam and challenges.

The index K calculated for an analysis as shown in Fig. 4 is the sum of the indices determined for each mass line. The high utility of K for identification derives from incorporation of several factors which reflect uniqueness and abundance of masses known to be in the compound, intensity ratios and dilution. McLafferty *et al.*[4] discuss in detail these terms and their meaning; the following relationship can be written for K:

$$\Sigma K = \Sigma(U + A + W - D)$$

where U represents uniqueness, A represents abundance, W is the tolerance required of intensity ratios and D is a factor which decreases as apparent dilution of the compound in the total sample increases.

K scores typically range from approximately 100 units for 5 μg of a compound to 50–75 units for 50 ng of the compound. Blanks usually will produce K scores of 0–10 units, and maximum K scores for similar challenging compounds such as shown

here for the benzodiazepines will be not more than 15–25 units, thus avoiding false positives. This is further illustrated in Table II for a number of drug-spiked blood samples.

TABLE II

CONFIDENCE INDEX *K vs.* DRUG CONCENTRATION IN SERUM

Drug	*Spike level (μg)*	*Confidence indices*		
		Pure drug (2 μg)	*Post-spike*	*Pre-spike*
Amobarbital	2.0	83	71	3
Secobarbital	2.0	75	75	6
Heptabarbital	2.0	96	96	—
Glutethimide	0.10	138	55	11
Phenobarbital	0.20	99	58	16
Methaqualone	0.05	141	58	13
Diazepam	0.05	147	66	12
Chlordiazepoxide	0.09	153	94	11
Flurazepam	0.20	91	55	2

The computer-produced printout includes a list of compounds searched for, a qualitative determination, quantity, index *K* and retention time. Validity of identification can be reinforced by a study of consistency of index *K*, quantity and deviation of retention time from nominal. In addition, the partial mass spectrum of each compound can be displayed, as shown in Fig. 5, compared with that for the standard, and inspected for contamination. In this case, mass lines 131, 153 and 203 in the primidone spectrum are contaminated by contributions from a compound which eluted during the primidone retention time window.

EXPERIMENTAL

Data reported are from samples that were typically obtained by simple single-step extraction procedures. Injection volumes were 1–4 μl of residue reconstituted in methanol, equivalent to approximately 20–40 μl of original serum samples, and less than 1 ml of urine samples.

The gas chromatograph was equipped with a flash vaporizer inlet operated at 230 to 280°, and the transfer line temperature was 300°. The glass column (6 ft. × 0.08 in. I.D. × 1/4 in. O.D.) was packed with 3% OV-17 on 100–120 mesh HP Gas-Chrom Q. Nitrogen carrier-gas flow-rate was nominally 30 ml/min. Post-column sample split ratio between the mass spectrometer and the flame-ionization detector was 9:1.

Quantitation under these conditions, using PBM to scale intensities of mass lines against those of stored reference data, was typically found to be linear over more than two decades. Precision using internal standards ranged from 2 to 7% relative standard deviation.

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OLFAX/GC  ANALYSIS

TS=180  T1=298
>RUN 16  3UL OF 50  ANTI SERUM EXT


PRIMIDONE      +FUS   QTY 3.40E- 1     CONF  74    RT  223


MASS  INTENSITY

103      1.20E- 8
117      2.13E- 8
131  C+M 5.84E- 9
146      9.08E- 9
153  C   1.97E- 9
158      9.08E-10
175      1.17E- 9
188      7.26E-10
203  C   1.03E- 9
218      1.92E- 9

PLOT Y OR N?Y
#OF DECADES=3

FS=2.13E- 8


      E-3                 E-2                 E-1                 E0
      +-----2-------5-----+-----2-------5-----+-----2-------5-----+
      +
      +
103   +>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>
117   +>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>
131*  +>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>
146   +>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>
153C  +>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>
158   +>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>
175   +>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>
183   +>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>
203C  +>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>
218   +>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>
      +
```

Fig. 5. Contracted spectrum of primidone in serum.

DISCUSSION

A few examples will illustrate the differences of this method when compared with usual GC procedures.

In the chromatogram of anticonvulsants in Fig. 6, although the internal standard is in the solvent peak and phenobarbital is high on its tailing edge, a satisfactory quantitative assay was obtained in four minutes. Compared with 12–14-min GC assays, the correlation coefficient for phenobarbital is 0.97, and that for phenytoin 0.98. Another anticonvulsant assay in Fig. 7 produced similar results even though the GC peaks were not well resolved.

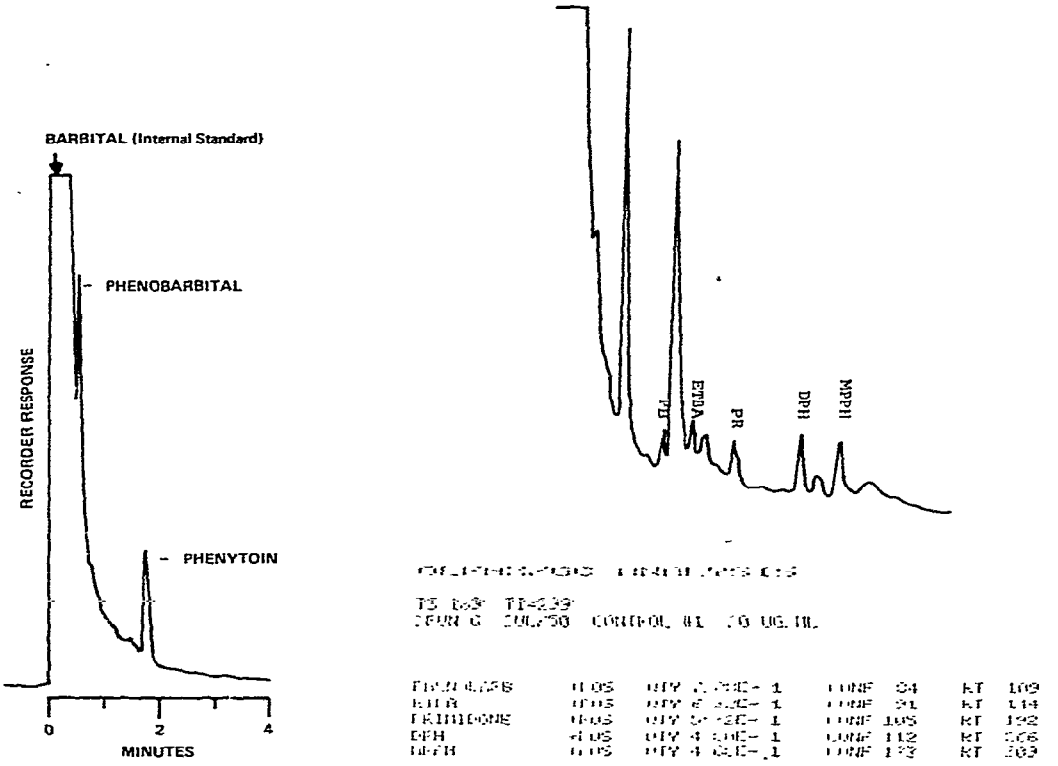

Fig. 6. FID chromatogram of a serum extract containing anticonvulsant drugs. Retention times: barbital 11 sec, phenobarbital 37 sec, phenytoin 117 sec. The drugs are present as the dimethyl acetal derivatives.

Fig. 7. Typical analysis of a serum for anticonvulsant drugs. PB = Phenobarbital, ETBA = 5-ethyl-5-p-tolylbarbituric acid (internal standard), PR = primidone, DPH = phenytoin, MPPH = 5-(p-methylphenyl)-5-phenylhydantoin (internal standard).

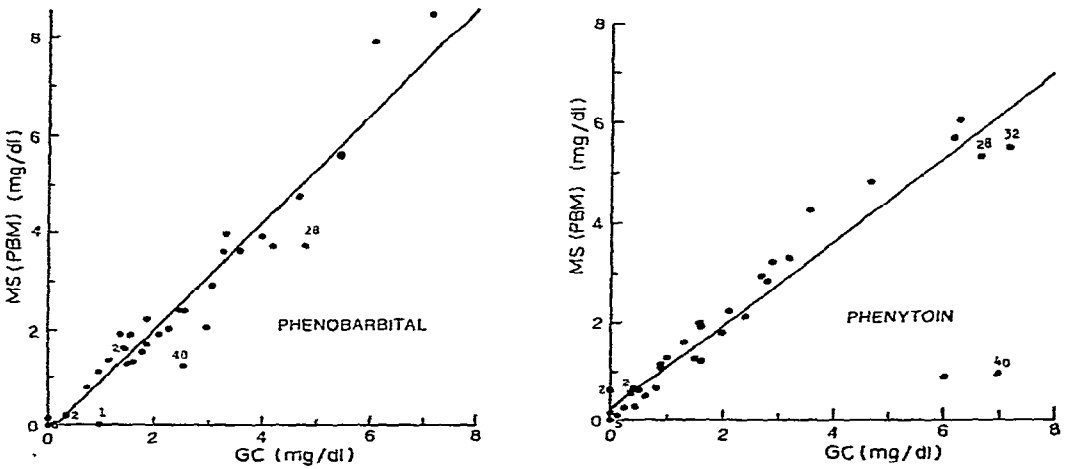

Fig. 8. Comparison of quantitation by PBM vs. GC, for phenobarbital. Correlation coefficient = 0.97; slope = 1.07; y-intercept = −0.18.

Fig. 9. Comparison of quantitation by PBM vs. GC, for phenytoin. Correlation coefficient = 0.98; slope = 0.85; y-intercept = +0.25.

Most of the data for forty samples in this assay of phenobarbital and phenytoin lie close to the regression lines, as shown in Figs. 8 and 9. Large discrepancies occur in the direction of inflation of GC values; re-investigation of the samples indicated that this effect was due to contributions from co-eluting compounds. For example, for sample number 40, GC results were twice the correct value for phenobarbital and nearly eight times higher for phenytoin.

In the theophylline assay shown in Fig. 10, existence of several nearby peaks in the chromatogram did not affect detection and quantitation by PBM. Assays of therapeutic levels of 5 to 15 μg/ml were typically performed in 10 min with 2-ml blood samples. (The procedure can be used with samples as small as 0.2 ml.) Careful comparison with GC procedures shows a correlation coefficient of 0.98. Also significant were short turn-around time, precision of 5–8%, linearity over the range studied from 1 to 60 μg/ml and no interferences from other xanthines and drugs frequently co-administered with theophylline.

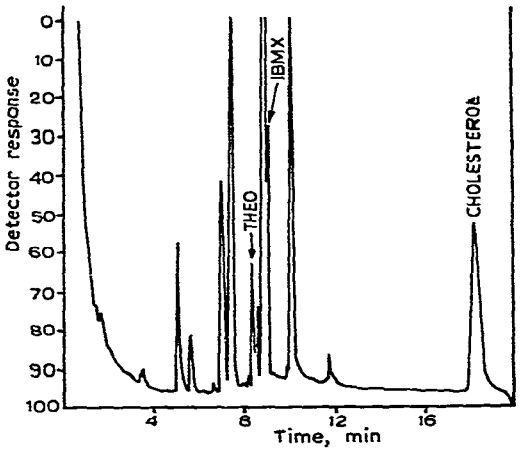

Fig. 10. FID chromatogram of a serum sample containing theophylline (THEO) and 3-isobutyl-1-methylxanthine (IBMX) as internal standard.

TABLE III

COMPARISON OF QUANTITATION BY PROBABILITY BASED MATCHING *vs.* GAS CHROMATOGRAPHIC ANALYSIS OF DRUGS IN URINE

Specimen number	*GC*		*OLFAX/GC*		
	Drug	*Conc. (µg/ml)*	*Drug*	*Conf. index*	*Conc. (µg/ml)*
6256	Phencyclidine	18.8	Phencyclidine	98**	8.85
6344	"Phenothiazine"	++++	Chlorpromazine	84**	1.85
	N.D.*	—	Doxepin	54	0.23
6347	"Phenothiazine"	+++	Chlorpromazine	84**	3.24
6375	Morphine	4.2	Morphine	55	2.63
	Propoxyphene	7.5	N.D.	—	—
	Norpropoxyphene	150	Norpropoxyphene	84**	18.5

* N.D. = Not determined.

** Maximum score possible on that assay program, *i.e.* a perfect mass spectral match.

Table III compares this technique with a GC method for a urine drug screen. The only serious discrepancy was a GC report of 150 μg/ml for norpropoxyphene *versus* 18.5 μg/ml in the MS assay. The chromatogram of this specimen shown in Fig. 11 indicates a pair of huge unresolved peaks at the retention time of unrearranged norpropoxyphene, which appear to have falsely elevated the GC concentration.

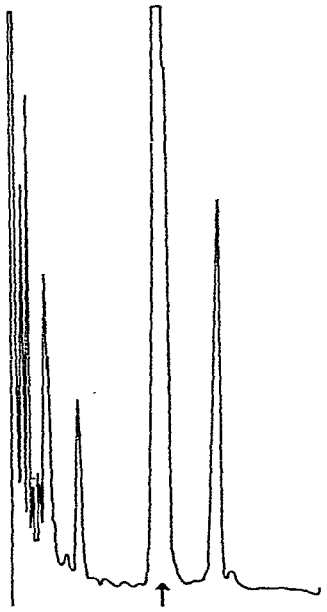

Fig. 11. FID chromatogram showing overlapped peaks at the retention time of norpropoxyphene.

CONCLUSION

In our experience, addition of PBM selected ion monitoring to GC analysis results in compound specificity and reliable, automatic quantitation even for complex biological samples. The automatic quantitation of mixtures has made the approach particularly attractive in situations in which assays are performed repetitively, highly trained personnel are not readily available for operation or interpretation, and speed of analysis is important. Compared with usual chromatographic procedures, relatively simple extractions and short, incomplete separations are employed with excellent qualitative and quantitative results. Initial applications have been in toxicology and in therapeutic monitoring of drugs.

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