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A DUAL COLUMN SCREENING METHOD
FOR SOME DRUGS OF ABUSE

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A DUAL COLUMN SCREENING METHOD
FOR SOME DRUGS OF ABUSE

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ABSTRACT

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By

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A dual column gas chromatography method to screen for drugs of abuse is described. A Shimadzu chromatograph with a SPB-1 wide-bore capillary column as a primary or screening column and a DB-1 capillary column as the second or confirmatory column both connected to a flame ionization detector is used. A library of retention indices was developed for each column on an IBM-PC XT. The results showed excellent precision, linearity, and recovery using the ultra rapid extraction method described. A comparison study on urines assayed by Syva's Urine Drugs Of Abuse kit was carried out which showed the gas chromatographic method to have great potential for drug screening. The other two benefits of this method are simultaneous confirmation of results and quantitation of drugs present. The method may prove to be rapid, inexpensive, and suitable for operation in a clinical laboratory.

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LIST OF ABBREVIATIONS

GC	gas chromatograph
FID	flame ionization detector
EMIT	enzyme multiplied immunoassay technique trademark of Syva Co., Palo Alto, Ca.
HPLC	high pressure liquid chromatography
MS	mass spectrometer
RT	retention time

INTRODUCTION

Interest in screening urine specimens for drugs of abuse is still expanding.[1,2] Not only are screening programs currently being used to monitor the success of drug rehabilitation programs, but also private industry is starting a large number of drug screening programs for the purpose of enhancing productivity by screening out potential problem employees. Many clinical laboratories are considering the addition of a drugs-of-abuse screen. A drugs-of-abuse screen could include the following: amphetamines, barbiturates, cocaine, pethidine, diazepam, hydromorphine, LSD, marijuana, mescaline, methadone, morphine, oxycodone, phencyclidine, and propoxyphene.[3]

Methods currently in use to screen for drugs-of-abuse include colorimetric, immunoassay, radio-immunoassay, and hemagglutination inhibition. Enzyme immunoassays are now available for the nine major families of drugs of abuse. Among the chromatographic methods available are thin-layer, high-pressure, and gas-liquid. Further in gas-liquid chromatography (GC) there are different detector systems available including nitrogen-phosphorus, flame ionization, electron capture, and mass spectrometry.

Colorimetric methods are available for some drugs of abuse including barbiturates, morphine and methaqualone.[4] These methods have advantages of low cost, small amount of equipment, and no need of highly-trained personnel. The disadvantages of these methods are a distinct lack of specificity and the ability to assay only one family of drugs at a time. The procedures are also labor intensive, which does not fit well into the routine of the clinical laboratory.

Thin-layer chromatography, one of the first developed broad spectrum screens for drugs, is currently the most widely used method.[5] More than one specimen can be screened simultaneously. It is an inexpensive method to screen for the presence of drugs prior to undertaking the more elaborate methods still to be described.[6] The negative aspects of thin-layer chromatography are its labor intensiveness and the many subjective aspects of interpretation based on color development. This is a method from which results must always be confirmed by another independent procedure.[7,8]

Enzyme immunoassay and radioimmunoassay techniques have been available to the clinical laboratory for many years. These techniques, particularly Enzyme Multiplied Immunoassay Technique (EMIT), have been developed for the major families of the drugs of abuse which are as follows: opiates, barbiturates, amphetamine, cocaine and metabolites, benzodiazapines, propoxyphene, methadone, phencyclidine, cannabinoids, and ethyl alcohol.[5] The precision of these techniques is adequate for routine clinical use.[7] Inexpensive automated hardware is readily available and easily operable, which makes this procedure affordable on a cost-per-test basis in terms of both labor and reagents. Since one EMIT assay is much like another and all are easily automated, training is not complex. The drawbacks to this system are tied to the antibodies developed for the assay. Laboratory scientists are dependent on the manufacturers of kits to develop the antibodies. Not all antibodies are equally specific. False positives, such as dextromethorphan reacting with the opiate assay, are an ever-present problem. Since more than one drug is commonly present, many different kits must be used. Also, only those classes of drugs that the antibody is directed at may be detected. Of the thousands of drugs available antibodies have been made to relatively few.

High-pressure-liquid chromatography (HPLC) is not the chromatographic method of choice for drug studies.[1] Its chief use in studying drugs is for use on those compounds with low volatility or compounds that are heat labile.[6] HPLC has not been used as extensively as GC in monitoring drugs due to detector systems that are not as sensitive or versatile as those used in gas chromatography.[9]

A choice that currently makes a lot of sense is gas chromatography. Its potential as a screening and confirmatory tool is almost limitless. It is a very specific methodology, and the sensitivity is acceptable for measuring clinically significant levels of drugs.[10]

Many choices exist for making use of GC. The first choice which must be considered is the specimen itself. While in forensic work or therapeutic drug monitoring the specimen of choice may be tissue or blood, urine is the commonly used specimen in drugs of abuse.[3] Since absorption into tissues or active clearance by the kidneys takes place, urine is the best place to find evidence of drugs of abuse in a screening environment. Although quantitation in the urine is not perfect, there are some relationships that can be established by measuring drugs in urine.[11]

Many methods of extracting drugs are currently used. Most involve several steps including initial extraction in solvent, centrifugation, evaporation to dryness, and solution in solvent before analysis. Simple extraction techniques involving only two steps have been successfully employed.[12] Solid phase extraction using disposable supplies is rapid and efficient.

Derivatization is sometimes necessary to make a parent compound more volatile, separable, stable, and detectable.[13] Volumes have been written about techniques and compounds such as BSTFA, a silylating agent, which is currently used. However, derivatization has the disadvantage of adding timely steps to the drug screening procedure and can be avoided. Many methods of drug screening on the GC that do not involve derivatization are currently available and will be discussed.

There is no single correct method for choosing a column for GC, but there are many considerations.[14] Capillary columns have the advantages of high reproducibility and decreased analysis time.[15] The literature is replete with studies of retention times by both standardized and nonstandardized methodologies for nonderivatized drug analysis.[6,15,16,17] Making use of literature and choosing from columns with a variety of polarities such as OV-1, SE-30, or carbowax, one can empirically rather than theoretically choose a column and analyze the results of retention time studies for peak separation, length of analysis time, and reproducibility.

A very attractive option to consider when choosing a capillary GC column is the wide-bore capillary columns. A wide-bore column is now available and licensed by the FDA for diagnostic use with drugs. Research has documented that the column is both inert and efficient enough to rival resolution provided by the narrow-bore columns.[18] A major advantage of this column is that a splitter is not needed. The splitter is run wide open, making the column much easier to use in the clinical laboratory setting by personnel who are not specially trained in gas chromatography.

For the experienced chromatographer continuing advances in capillary column technology will offer even more choices. Columns have recently been prepared which have been coated with monomers specifically designed to separate drugs of abuse such as barbiturates, benzodiazepines, and LSD.[19] This work on columns plus work presently being done with detector technology will continue to enhance the possibilities of using GC in the clinical laboratory setting for drug screening programs.

An advantage of GC is that it is widely used as a confirmatory method for drugs-of-abuse screening. Many studies report success using a dual column GC method as both a screening and confirmatory method.[20,21] Ideally, the second or confirmatory column should have a different phase and polarity.[1,22] Instrumentation is available so that a single sample injection can be entered into both columns, or the columns can be run on different GC instruments using one for screening and the other for confirmation. Each column will have its own detector and integrator.

Although drug screening by definition is principally qualitative, quantitative data is obtainable. The routine availability of quantitative data from GC is due principally to the relatively recent advances in capillary column technology.[16]

The only technique that can improve the sensitivity and selectivity of GC is coupling the GC to a mass spectrometer (MS).[17] This method yields more information at the nanogram level than any other method[23] plus it has additional benefits of the mass spectrograph, which gives the molecular weight of the parent compound from the molecular ion and the structure of the parent compound from the fragment ions. Several important factors will slow the usefulness of the GC-MS in the clinical laboratory. It is an expensive instrument to both buy and maintain.[5,23] Throughput of the instrument is quite limited. It is a difficult instrument to operate, therefore, calling for highly trained personnel. As a highly sophisticated confirmatory method in an environment in which 24 hour per day high speed throughput is not essential, GC-MS may be the instrument of choice.

The detector of choice is the flame ionization detector (FID) when using the GC for drug screening. This is due to high sensitivity, broad linear range, simple and reliable construction, and a broad general utility for organic compounds.[1,24]

It goes almost without saying that whenever large amounts of complex data are generated, such as with GC, a computer does the same things as a human interpretor with the advantages of electronic data smoothing, rapidity, and data storage.[25]

With the current interest in drugs-of-abuse screening there is an opportunity to develop a methodology to meet the needs of a hospital clinical laboratory. The method must be rapid and inexpensive when purchasing equipment, supplies, and labor. The method must be able to provide confirmation that is equivalent or better to the current standards of practice in drug screening. A dual column GC with a wide-bore capillary column for screening following a rapid extraction procedure will be developed to meet these criteria. It will include a second capillary column for confirmation, a quantitation procedure, and data reduction on an IBM PC-XT with existing software. The purpose of this study will be to assess the potential of such a system for drugs-of-abuse screening.

Materials and Methods

Standards for this work were purchased from Supelco. The drugs were diluted by the manufacturer to 1 mg/ml in methanol. Table 1 is a list of drugs which were used as standards in this study. Standards were prepared for use by diluting from 1 mg/ml to 100 ng/ul using glass distilled methanol. The standards then became the working standards used throughout the project.

Table 1 Drug Standards Used In Study

Drug	Standard	Working Standard
l-Amphetamine	1 mg/ml	100 ng/ul
Methamphetamine	1 mg/ml	100 ng/ul
Barbital	1 mg/ml	100 ng/ul
Amobarbital	1 mg/ml	100 ng/ul
Pentobarbital	1 mg/ml	100 ng/ul
Secobarbital	1 mg/ml	100 ng/ul
Hexobarbital	1 mg/ml	100 ng/ul
Mephobarbital	1 mg/ml	100 ng/ul
Phenobarbital	1 mg/ml	100 ng/ul
Methaqualone	1 mg/ml	100 ng/ul
Methadone-HCl	1 mg/ml	100 ng/ul
Codeine Sulfate	1 mg/ml	100 ng/ul
Tetrahydrocannabinol	1 mg/ml	100 ng/ul
d'Tetrahydrocannabinol	1 mg/ml	100 ng/ul
Cannabinol	1 mg/ml	100 ng/ul

The GC used for this project was a Shimadzu GC-9A. The instrument had a dual injection port, and the dual detectors were of the FID type. The single integrator on the instrument was a Chromatopac C-R2AX also by Shimadzu. It was entirely programmable, but it had no data storage capabilities.

Two columns were used on the GC. The primary or screening column was a Supelco, wide-bore SPB-1 capillary column which had a .75 mm ID and had a bonded SE-30 phase. The SE-30 phase of this column is the phase of choice for drug screening.[18] The splitter was run wide open. The carrier gas used was helium. The linear velocity of this system was 41.7 cm/sec. and the number of theoretical plates 18,050.

The second or confirmatory column was a DB-1 from Hewlett-Packard. This column was a thin-film DB-1 bonded phase .1 mm thick that is a narrow bore 60 m long with an internal diameter of 25 mm. The carrier gas used was helium and the linear velocity was 26.2 cm/sec. with a split ratio of 1:47 and a split flow of 35.9 ml/min.

The oven temperature throughout the study was programmed to begin at 115°C and increase by 6°C per minute up to 285°C. The injector temperature was set at 300°C and the detector temperature was 300°C. The run was programmed to end at 40 minutes.

The integrator was also programmed to run for 40 minutes. It was programmed for the following: width 5, attenuation 5, drift 1, slope 2000, minimum area 1000, chart speed 15 mm/min., and slope weight 100.

The extraction method used was designed to be quick and to make use of a minimum number of steps yet insure 70% recovery of the drugs in question.[12] Two milliliters of urine were first made basic with .2 ml of 5N NaOH in a very pointed 10 ml conical centrifuge tube, and 50 microliters of chloroform were added for the extraction. The tube was mixed by vortex for 30 seconds and centrifuged for 5 minutes. This procedure was repeated with the urine made acidic with 6N HCl. Injection was made directly from the solvent layer in the point of the centrifuge tube.

Injections were made by using hexanes as the solvent front, injecting a mixture of hydrocarbons C10 through C26 as markers of retention time and hexobarbital as an internal standard for quantitation. When drug standards were injected, .4 microliter aliquots were next drawn into the 10 microliter syringe. When urine specimens were used, a 1 microliter aliquot was taken from the acidic and basic extractions and injected with the hexanes, hydrocarbons, and hexobarbital.

The Shimadzu integrator was not directly interfaced to a data reduction device. The retention times and peak area which had been calculated by "area normalization-method 41" of the C-R2AX integrator were then entered into an off-line computer. The computer was an IBM PC XT which was a stand-alone system running programs designed and written by Sweeley et al. at Michigan State University for the metabolic profiling of urine. The only modification to the programs was to allow data entry by CRT rather than by interface. The computer first stores the data from each run in files for future data reduction. In metabolic profiling standards for retention time can be found in the urine, but in drug screening they are co-injected with the sample. The peaks found in the sample are then identified as belonging to the library previously built or as a significant peak not identified. The major component of identification is retention time, but in addition a confidence factor is printed in percentage which takes into account area calculations, linkage to other peaks which are the co-injected standards in this case, and concentration.[26]

Results

The first step undertaken in this project was to build a library of retention indices for each column used in the study. The drugs previously listed and hydrocarbons C10 through C26 are the individual members of this library. Table 2 is the printed output of a library built on the IBM PC which contains retention indices of drugs and standards when using the Supelco SPB-1 column and the Hewlett-Packard DB-1 column.

To obtain the values which were used to build the library each standard was run alone through the entire temperature program of the GC on the SPB-1 column. Figure 1 illustrates such a run with the methadone standard. It was run alone to guarantee that there was no chance of error in identifying the drug in question by its retention time alone and then run with mixtures of standards. The goal was to run the drugs until three values were obtained within .005 minutes of each other. This level of precision was not obtainable. However, all three values obtained for each drug were within .01 minutes. Subsequently, all three values were averaged and entered into the library. This procedure was repeated for the second column.

When building the library it became apparent that morphine sulfate and hydrocarbon 24 both came off the column at virtually the same time. Figure 2 shows two back-to-back runs on the same day with the same conditions

Table 2 Libraries of Retention Indices

		SPB-1	DB-1
1.	C-12	(321.70)	(557.70)
2.	l-Amphetamine	(333.30)	(573.50)
3.	Methamphetamine	(391.20)	(629.70)
4.	C-14	(420.50)	(657.60)
5.	C-16	(714.20)	(951.50)
6.	Barbital	(820.20)	(1070.19)
7.	C-18	(1063.00)	(1319.60)
8.	Amobarbital	(1224.30)	(1500.10)
9.	Pentobarbital	(1262.20)	(1541.20)
10.	Secobarbital	(1336.70)	(1639.30)
11.	C-20	(1410.20)	(1695.10)
12.	Hexobarbital	(1466.20)	(1762.00)
13.	Mephobarbital	(1530.20)	(1831.40)
14.	Phenobarbital	(1617.00)	(1930.10)
15.	C-22	(1734.80)	(2041.40)
16.	Methaqualone	(1928.70)	(2265.89)
17.	Methadone-HCl	(1951.00)	(2289.10)
18.	Codeine Sulfate	(2309.80)	(2634.10)
19.	Cannabidiol	(2327.50)	(-)
20.	Morphine Sulfate	(2311.00)	(2673.00)
21.	Tetrahydrocannabinol	(2379.30)	(2754.00)
22.	d'Tetrahydrocannabinol	(2406.30)	(2783.60)
23.	Cannabinol	(2492.30)	(2878.20)
24.	C-26	(2569.00)	(2948.70)

Figure 1 Chromatogram Of A Single Standard (Above) And Mixed Standards (Below)

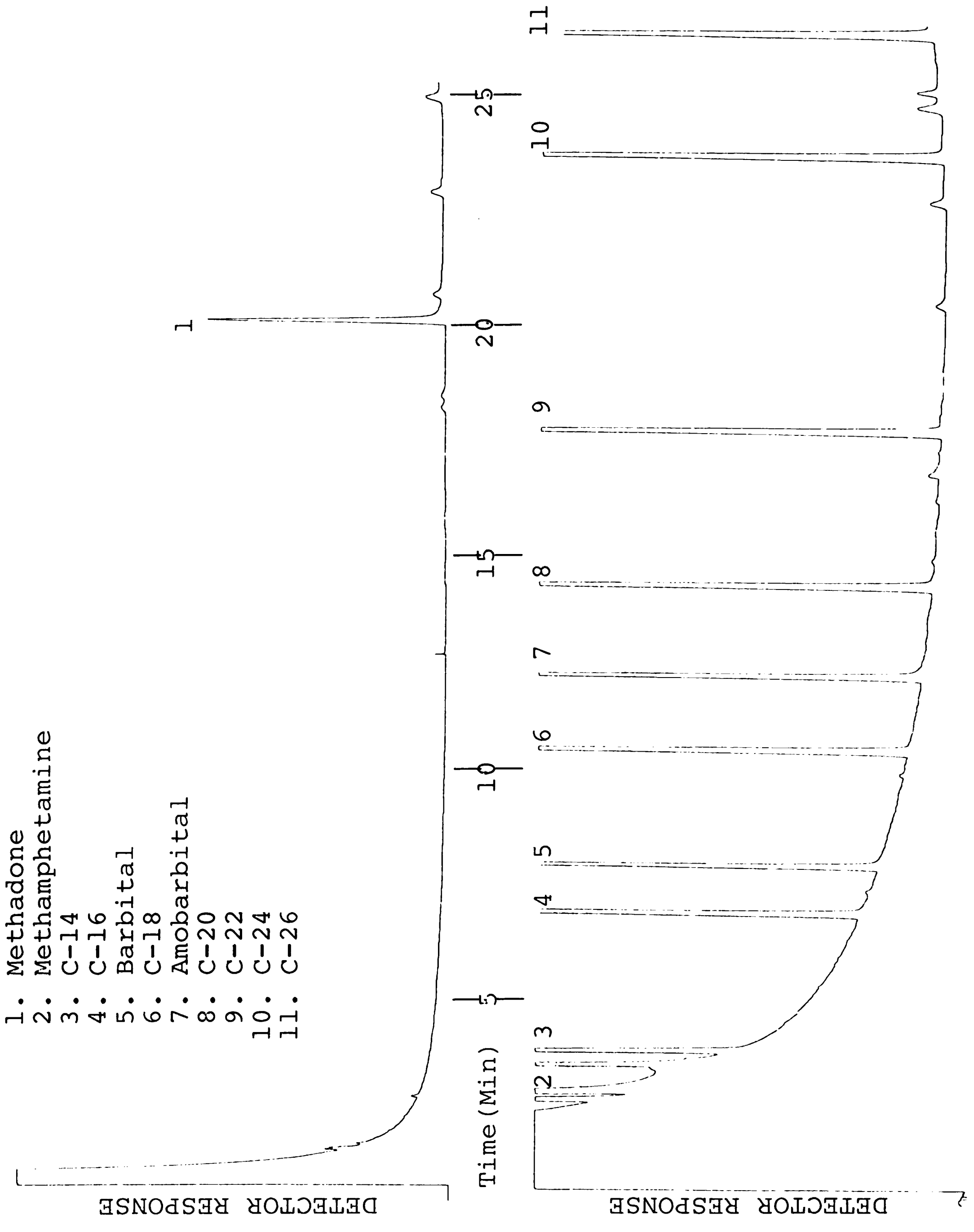
1. Methadone
2. Methamphetamine
3. C-14
4. C-16
5. Barbital
6. C-18
7. Amobarbital
8. C-20
9. C-22
10. C-24
11. C-26
DETECTOR RESPONSE
DETECTOR RESPONSE
Time (Min)
5
10
15
20
25
1
2
3
4
5
6
7
8
9
10
11

Figure 1

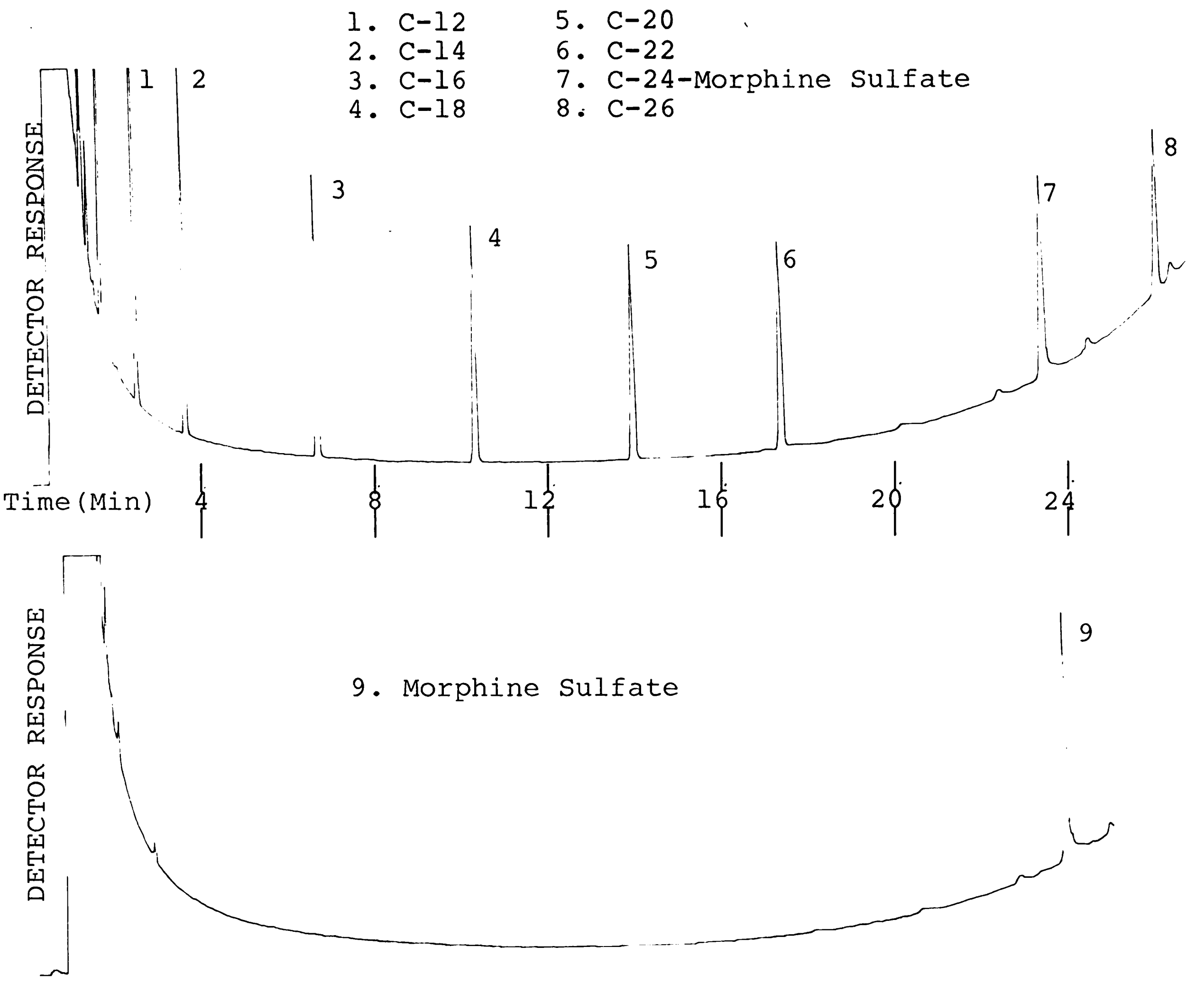

Figure 2 Chromatogram Of Morphine With Hydrocarbons (Above) And Morphine (Below)

which illustrate this problem. In order to make the entire library useful, hydrocarbon C24 must be dropped from the retention time standards. Cannabidiol was dropped from the study when it was mishandled and all standard was lost.

To test whether or not the chosen extraction method would be acceptable, a urine which was free of drugs was extracted and injected on the column. It had been decided that if too many extraneous peaks were present, the job of entering the data into the computer by hand would not be practical. The urine used was the author's and was free of drugs except possibly caffiene. Figure 3 is one of several extraction runs made using this urine specimen. In a 25 minute run, the significant time period for all the drugs in the study, only five extraneous peaks were encountered.

Acceptable recovery of drugs was determined to be 70%. Recovery studies were carried out on all drugs in the study on the Supelco SPB-1 column. All drug working standards were run in mixtures on the GC. The peak areas of each drug standard were recorded. Next the author's urine was spiked with an equal amount of the same drug mixtures. The extraction procedure was carried out on the urine specimens, and the peak areas obtained were divided by the peak areas obtained from the previous runs of unextracted standards. Figure 4 is a chromatogram of drug standards run on the GC and a chromatogram of

Figure 3 Chromatogram Of A Drug-Free Urine After Extraction

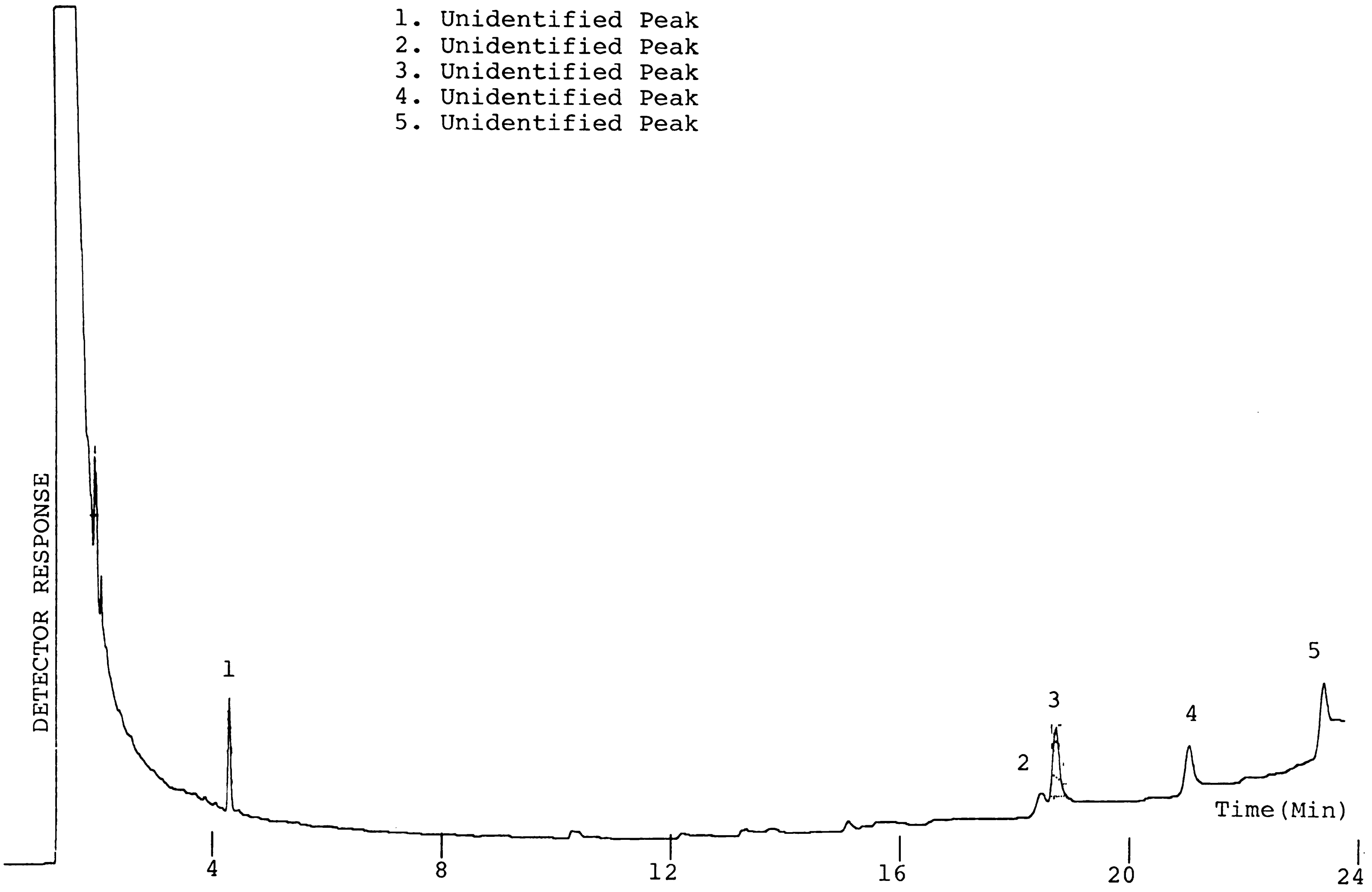
1. Unidentified Peak
2. Unidentified Peak
3. Unidentified Peak
4. Unidentified Peak
5. Unidentified Peak
DETECTOR RESPONSE
1
2
3
4
5
Time (Min)
4
8
12
16
20
24

Figure 3

Figure 4 Chromatograms Of Recovery Studies

1. C-12
2. C-14
3. C-16
4. C-18
5. C-20
6. C-22
7. C-24
8. Tetrahydrocannabinol
9. d'Tetrahydrocannabinol
10. Cannabinol
11. C-26

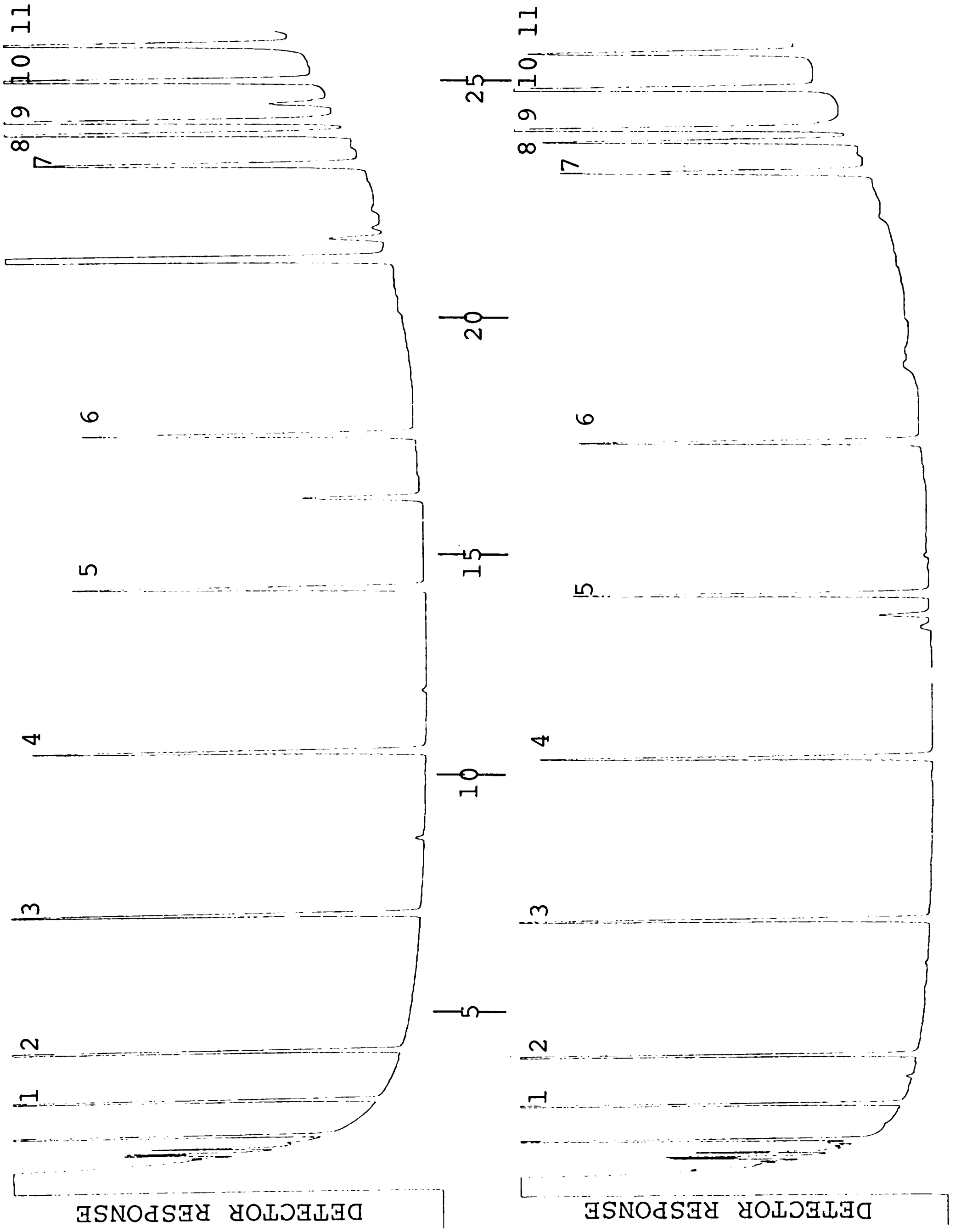

Figure 4 (cont'd)

drug-free urine spiked with an equal amount of drug standard. Both chromatograms have peak areas included.

It was evident at this time that the barbiturates and morphine sulfate were not being recovered adequately from the urine specimen. At this point the method of Ramsey et al.[12] was modified. The mixing and centrifugation steps were lengthened from 5 seconds and 2 minutes to 30 seconds and 5 minutes, respectively. This improved recovery of all drugs. Next an additional extraction with 6N HCl substituting for 5N NaOH was carried out, and the barbiturates were recovered, although phenobarbital recovery was only 55% and was not considered acceptable. Table 3 demonstrates the recovery of all drugs in the library except morphine.

Table 3 Recovery Studies on SPB-1 Column After Extraction

l-Amphetamine	125%
Methamphetamine	99.4%
Barbital	108%
Amobarbital	80.6%
Pentobarbital	81.7%
Secobarbital	120%
Hexobarbital	118%
Mephobarbital	119.7%
Phenobarbital	55%
Methaqualone	86%
Methadone-HCl	79%
Codeine Sulfate	82%
Morphine Sulfate	No Recovery
Tetrahydrocannabinol	99.7%
d'Tetrahydrocannabinol	72%
Cannabinol	105.8%

To assess the methodology for potential quantitative work it was necessary to study linearity and precision. Since hexobarbital had been chosen as the internal standard for quantitation, it was used as a standard in this study. Figure 5 is a graphic representation of a linearity study carried out on the range of concentration from 10 ng/ul to 320 ng/ul. This range is a good representation of the clinically significant range of drugs of abuse in urine. Phenobarbital was also studied between 10 ng/ul and 80 ng/ul and found to have acceptable linearity.

Finally, twenty seven urine specimens which had been screened for drugs of abuse at St. Lawrence Hospital in Lansing, Michigan were analyzed for drugs of abuse. Table 4 contains the results of these analyses. The screening method used at St. Lawrence Hospital was the Syva EMIT Urine Drug Assays which were performed on a Cobas Fara from Hoffman La-Roche Diagnostics.

Table 4 Comparison Study of 27 Urine Specimens

Drug	Cannabinoids		Barbiturates		Negative	
Method	Emit	GC	Emit	GC	Emit	GC
No. of Specimens	14	10	1	1	12	12
% Concurrence	71%		100%		100%	

The results in Table 4 show that all twelve urines screened as negative by the EMIT Urine Drug Assays were also screened as negative by the GC method. A single urine specimen contained barbiturate according to the EMIT, and the GC identified phenobarbital in this specimen. The EMIT

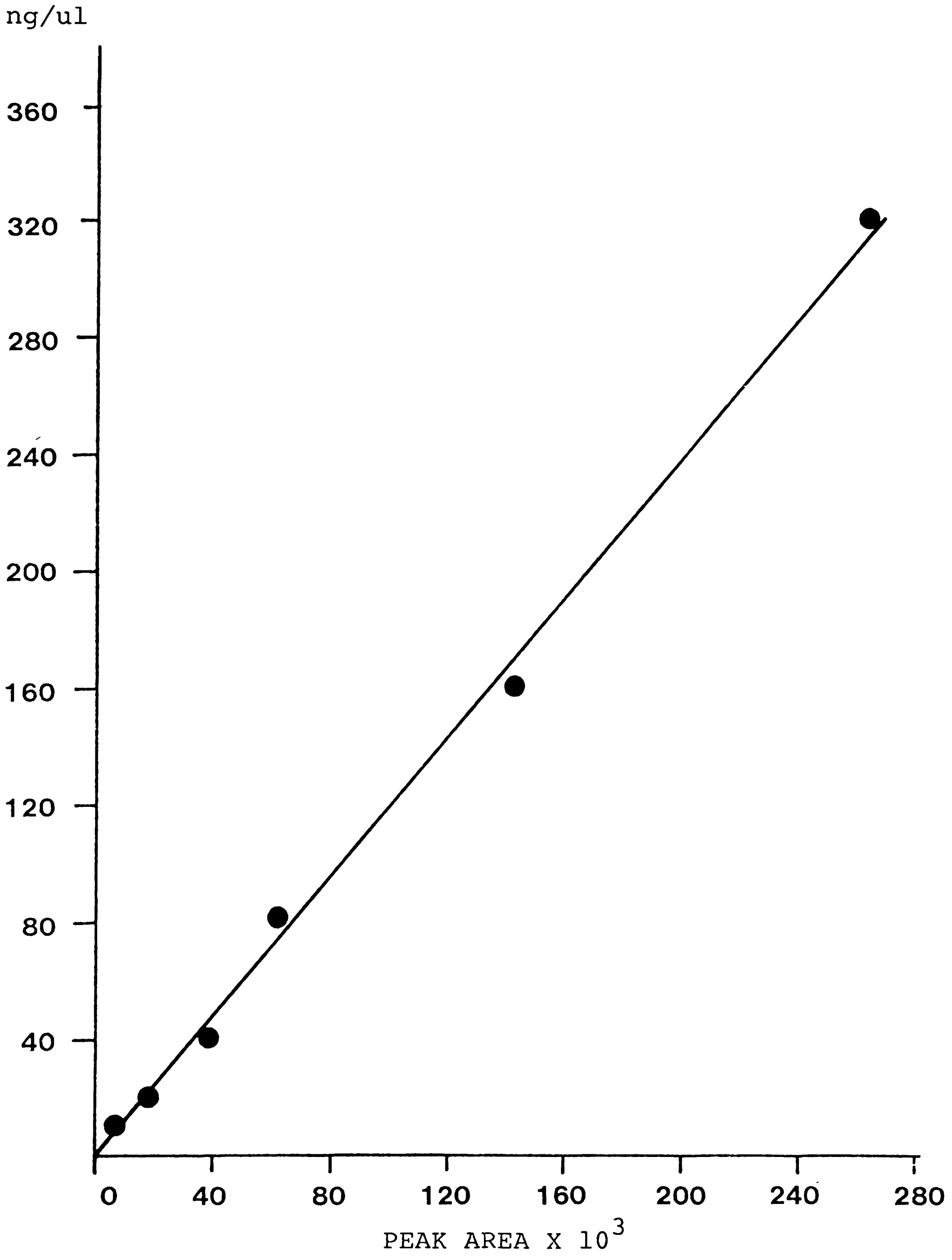

Figure 5 Linearity Study of Hexobarbital

system identified fourteen urines as being positive for cannabinoids. The GC was able to pick up ten of those same urines with a positive screen for tetrahydrocannabinol. Figure 6 is a chromatogram of a urine specimen for drug screening which was positive for tetrahydrocannabinol. As in many specimens positive for tetrahydrocannabinol, there are many small peaks detected in the same area that are unidentified.

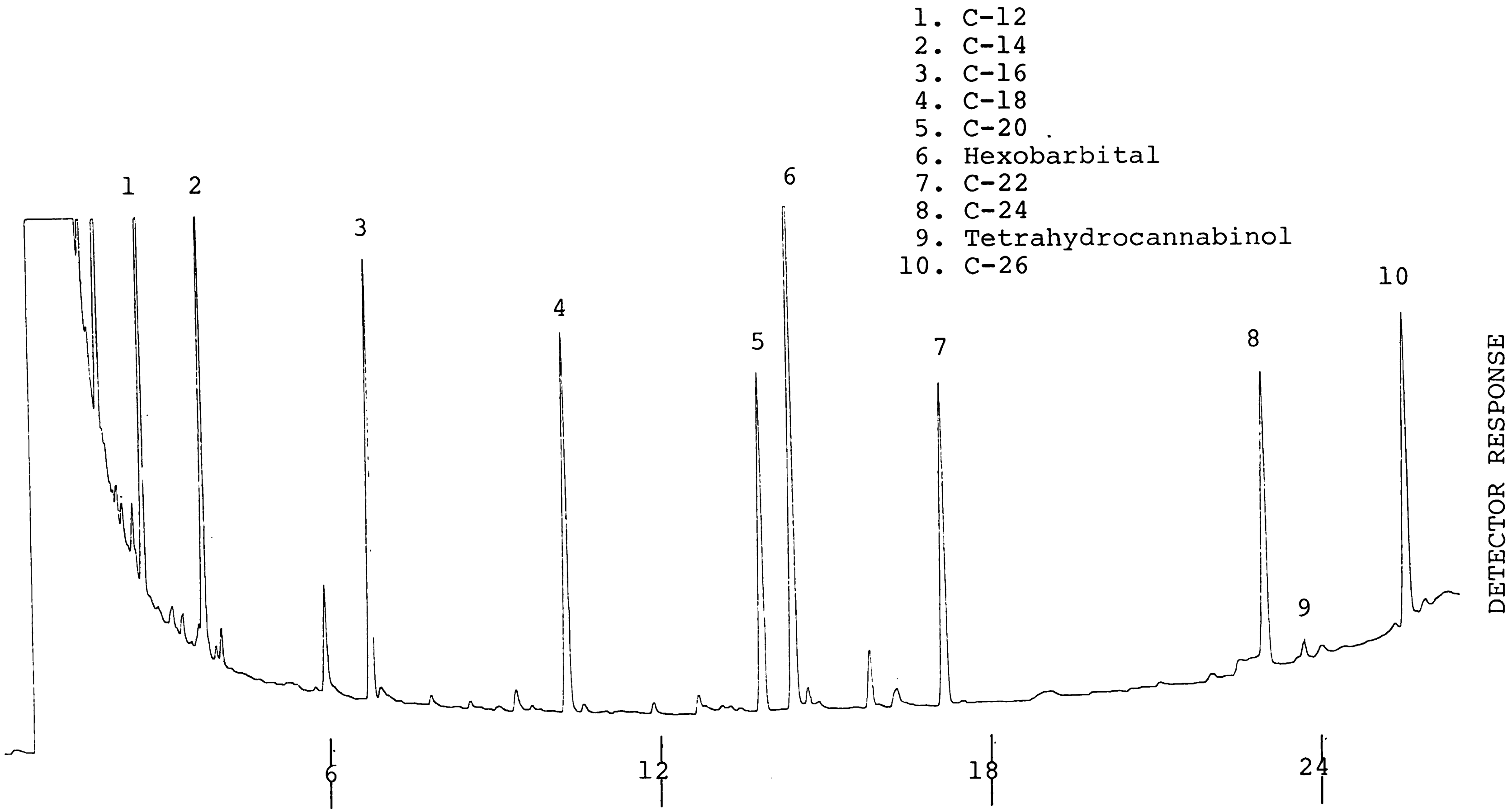

Figure 6 Chromatogram Of Urine Positive For Tetrahydrocannabinol

Discussion

Overall, the GC method appears to be adaptable to the hospital clinical laboratory as a screening method for selected drugs of abuse. By using a wide-bore capillary column with the split run wide open the technical knowledge necessary to operate the GC equipment was minimal. The techniques needed to operate the equipment properly are the same ones used to operate many other pieces of equipment in the automated clinical chemistry lab of an average hospital. The building of the libraries of data was straight forward with no real problem. By running each standard separately and then in groups a very practical type of experience is gained, and one can get a feel for the GC and its precision. Although this step is time consuming, 30 minutes per standard for 2 libraries, and expensive, $30.00 per standard, it is the best way to develop confidence in the ability of the system to identify drugs of abuse by retention time alone.

The extraction method[12] chosen was simple and rapid and would fit nicely into the routine of a stat clinical laboratory. Many other extraction methods or additional extraction steps could be used to improve recovery of drugs of abuse, but since the primary goal of the study was not to compare recovery methods, a simple goal of 70% recovery was used. The earliest extraction work in the study was an indicator that the method should be slightly modified to increase the percentage of recovery. The modification suggested by Ramsey et al.[12] as potentially necessary was to increase the mixing time on the vortex once solvent was added from 30 seconds to 1 minute and to increase the centrifugation step from 2 minutes to 5 minutes to improve the separation of the solvent front from the urine specimen. A layer of precipitate always formed at the junction, but the needle of the injection syringe was able to pass through the urine layer and precipitate with no contamination and withdraw the 1 ul specimen directly. The failure of this method to extract morphine will be addressed. An acid extraction also was added which improved recovery of the barbiturates.

The excellent precision of retention times of standards during the building of the library guarantees that acceptable reproducibility is achievable for this method in identifying drugs. A second column of different polarity will be an adequate confirmatory method.

The recovery and linearity studies done show that quantitation with this method is possible. A recovery study was done on every standard, and except for morphine and phenobarbital all results fell within the guidelines chosen for this study. Linearity studies indicate that the method for at least 2 standards is adequate in the clinically relevant ranges. Retrospectively, a linearity study for every drug standard should be done to leave no unanswered questions.

As mentioned quantitation of drugs of abuse using the GC is a relatively new development.[16] Care must be taken in the selection of GC instruments if quantitation is a consideration since not all equipment may be of necessary quality. Of course maintenance and quality control procedures will also be necessary.

As shown in the results section, the method picked up most of the drugs identified by EMIT. As a predictor of negative drug screens, it matched in all cases the EMIT system. There were few positive specimens to match most of the screen drugs on GC. However, phenobarbital was picked up. Cannabinoids were picked up in 10 of 14 positive specimens or 71%. In some cases where cannabinoids were not identified by GC there were peaks present which were similar in retention time and size to peaks which had been identified as tetrahydrocannabinol. This study employed only 4 of the major cannabinoids. The Syva EMIT Urine Drug Assays detect at least 39 of the greater than 400 THC metabolites that exist and are already prebuilt into some GC-MS libraries according to the director of the local toxicology laboratory.[27] It is very likely that unidentified peaks on the GC would turn out to be cannabinoids. It is also possible that many cannabinoid metabolites in small quantities, an area of less than 1000 on the GC, could be reacting in combination with the antibody in the Syva EMIT Urine Drug Assay.

Problems were identified with the method and several changes are recommended. First of all, it would be necessary to look carefully at the equipment used. This work was done on a single GC with one integrator. Either a second such GC should be set up for confirmation or, better, a dual column system should be used. For maximum efficiency a GC with an injector port that would split the injection evenly to 2 columns each with their own detectors and integrators is preferable. Such a system is available.[28]

As mentioned previously in this paper, the second column used for confirmation should be of a different polarity than the first. The polarity of the DB-1 column is different than that of SE-30, but it is not sufficiently different to cause the drugs to elute off the column in different order. A solution would be to use a second wide-bore capillary column coated with SPB-35.

Data reduction was tedious, and for the purposes of this work was done principally off-line by the operator. The IBM PC programs of Sweeley et al. worked, but it took too much time to enter the retention times of all standards and significant unknown peaks to really save any analysis time. The obvious solution would be to interface the integrator to the IBM PC so that data acquisition would be automatic. The interface is a standard feature of the programs, and now that the method has been shown workable this modification should be done. Also, the software should be modified to reflect predictive values of the second column confirming the findings of the first.

The extraction procedure needs modification to allow increased recovery of opiates, in particular, morphine. Morphine must be extracted first in strong acid to cleave off glucuronide groups which make these conjugates very highly water soluble.[4] Then the final extraction takes place best at pH 9.0. More control of the pH appears to be the answer to obtaining greater recovery of phenobarbital.

Since 400 cannabinoids exist, the problem of identifying these peaks cannot be taken for granted. At the very least this GC method should cover the 39 cannabinoids commonly found in urine by the Syva EMIT Urine Drug Screening kit. Further studies of potential cannabinoid metabolites will have to be undertaken and may be in process now due to the intensity of interest in this area presently.

Another problem encountered was a shift of retention times during the study. During the time that urines were being assayed and compared to EMIT results, retention times on the SPB-1 column shifted longer by as much as .7 minutes. Since this condition must be due to flow rate, the apparatus was taken apart and cleaned including washing the column. The air pressure was also adjusted, but the retention times never returned to their original values. At this time it was necessary to rerun the entire library and load it into the IBM PC. The shift in retention times made it very difficult to use the computer for peak identification. The shift could also be partly due to thermal shock of the column due to excess heating the column to 300° at greater than 25°C per minute and cooling the column by opening the oven door.[29]

Both this equipment and other GC equipment in the laboratory have been run extensively for extended periods of time with no shifts in retention time being encountered. This problem should not be considered as a detriment to the methodology. A good program of preventive maintenance and tight quality control coupled with a moderate level of experience running the equipment would entirely eliminate such shifts.

This work demonstrates the potential for this drug screening methodology in the clinical laboratory. With the modifications suggested the problems would be resolved. The method for screening drugs of abuse herein described should be considered as a starting point for the novice at drug screening with the GC.

Summary and Conclusions

There is a need to develop methods for clinical laboratories to meet the demand for drugs-of-abuse screening programs. The method must be inexpensive, rapid, accurate, and operable by clinical laboratory personnel. Key features of such a method would be confirmation of results and quantitation. Of the many methods currently available for consideration gas chromatography is one of the best choices.

This purpose of this study was to develop such a method on a gas chromatography system. Drugs of abuse can be identified and these results confirmed by the dual column gas chromatography method described. The study includes a description of excellent precision and linearity. A study in tandem with an EMIT (trademark of Syva Co.) Urine Drug Screening kit proved the method to be accurate. Data reduction on an IBM PC using programs developed by Sweeley et al. at Michigan State University for metabolic profiling is described. Also an ultra rapid extraction method which could easily fit into the routine of any clinical laboratory is used. This study shows that such a method is feasible, and it provides a starting point for the laboratory scientist who needs to develop a practical system for drugs-of-abuse screening.

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